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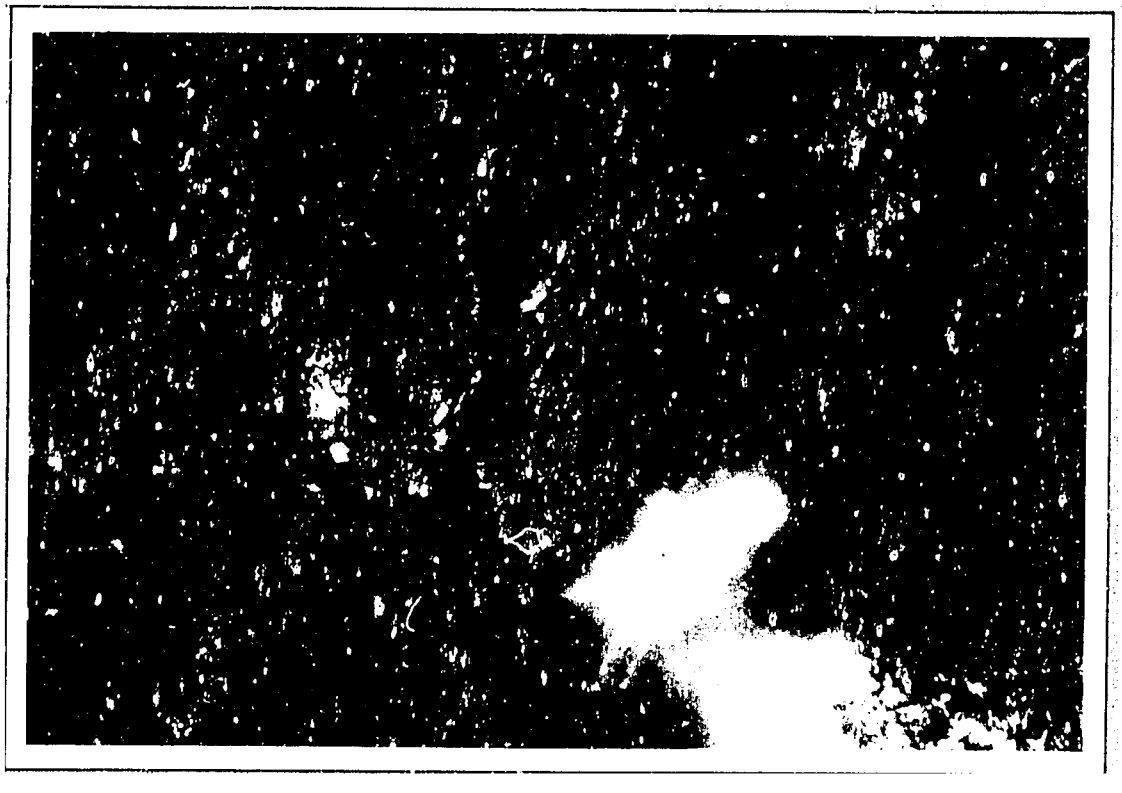
Detritus and Microbial Ecology in Aquaculture

Edited by
D. J. W. Moriarty
R. S. V. Pullin



INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT

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Proceedings of the Conference on Detrital
Systems for Aquaculture
26-31 August 1985
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ICLARM
INTERNATIONAL CENTER FOR LIVING AQUATIC
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Cover: Decomposing plant detritus, with associated colonies of filamentous bacteria and a fungal hypha (red). Photomicrograph by D.J.W. Moriarty.

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Preface

The application of biological principles to aquaculture in the disciplines of nutrition, physiology, reproduction and pathology has allowed for rapid expansion and intensification of production from aquacultural systems. A great deal of this research has been done in the temperate zone and has been focused on carnivorous freshwater and marine fish species. Little effort has been devoted to the controlled use of nutrients from decomposing organic matter, particularly in tropical systems. Some extremely successful culture systems are based in large part on microbial-detrital production, notably pond fertilization with livestock manure. The potential for utilizing such detrital systems to reduce or eliminate the need for costly supplementary feed in pond aquaculture is excellent. Full development of this potential is dependent upon more complete knowledge of the systems and assessment of methods for their manipulation.

The term 'detritus', as used here, is defined as nonliving organic matter, usually in a particulate form, but also including dissolved organic matter. Inorganic matter and various biota (bacteria, fungi, Protozoa, meiofauna and larger invertebrates) may occur in association with detritus, but they are not detritus *per se*.

Detritus develops when organic material is decomposed, particularly in soils and aquatic ecosystems. Microbial ecologists have long recognized its contribution to aquatic productivity. Studies of aquatic detrital food chains, however, have been largely limited to fundamental research focused on natural waters; little effort has been directed to their importance and manipulation in aquaculture systems. Microbial production in detritus affords a rich source of nutrients for fish and a mechanism for rapid recyling of nutrients released upon death of plants or breakdown of waste products. However, the interrelationships between such heterotrophic food chains and autotrophic food chains (fuelled by light and dissolved nutrients and based on phytoplankton) are poorly understood. Because detritus is a complex mixture of different types of organic matter, its composition is very variable, depending upon its derivation. Thus we have to be cautious when discussing the role of detritus in food chains, not to generalize from particular situations without adequate knowledge of whether the processes occurring in those situations are generally applicable.

Many of the fish recognized as most suitable in tropical developing countries for large-scale culture with a low input of energy feed on detritus (e.g., tilapias, carps and mullets) and it is clear that much of the current production of traditional wastefed Asian aquaculture derives from detrital food chains. Shrimp are also

detritivores. However, detrital food chains in aquaculture have been poorly investigated. The wastefed fishpond, for example, is commonly treated as a black box in experimental aquaculture. The constraints to production, such as water quality, dissolved oxygen and feed availability, are partially understood but the prospects for channeling more nutrients through detrital food chains into fish flesh have scarcely been considered.

There have been major advances recently in the methodology available for the study of detritus and its associated biota. Determining the composition of detrital aggregates and particularly the relative proportions of microorganisms and detritus *per se* is difficult, but methods are now available and are being used to study this in natural ecosystems. Microbiologists have also been working with processes utilizing microorganisms in solid waste conversion and waste water treatment.

The prospect of applying new techniques to aquaculture systems is exciting because in these systems the environment can be modified, e.g., by supplying detritus of known composition at known rates, varying nutrient supply, and altering grazing pressures, fish species composition, circulation patterns and flushing rates. Manipulating detrital food chains has far-reaching implications for the utilization of wastes in aquaculture. The vast quantities of agricultural wastes and low value byproducts (straws, sugarcane bagasse, coffee residues, banana wastes, rice hulls, other food processing wastes, trash vegetation and aquatic weeds generated annually in the tropics) may be useable as supplementary detritus, added to culture ponds as composts and microbial substrates either alone or mixed with fertilizers such as livestock manure. This could greatly reduce the need for inputs of high quality feeds for aquaculture: an important innovation particularly where feedstuffs are expensive or scarce. The development of such techniques appears feasible and would constitute a major advance in aquaculture, but firstly, additional information must be collected and research gaps must be filled.

ICLARM decided to convene this conference to analyze and summarize available information on detrital food chains and the means for their manipulation in aquaculture. By bringing together workers in aquaculture, aquatic microbial ecology and organic waste utilization, ICLARM sought to bring new perspectives to bear on the future of wastefed aquaculture and to catalyze new research initiatives and cooperative programs.

The conference structure was devised so as to group contributions under the Session headings: Microbial Ecology in Aquaculture; Production and Characteristics of Detritus; Productivity and Food Chains; and Manipulation of Detrital Systems for Aquaculture. Because these broad topics are all interlinked, there was considerable overlap in the discussions following the papers. All the various discussion themes and viewpoints were then drawn together in a final discussion session, from which a consensus statement on priorities for further work was formulated.

That this unique conference became a reality is due to the generous support of two agencies. First, the Rockefeller Foundation offered the use of its superb Bellagio

Study and Conference Center to hold the conference. Second, the German Agency for Technical Cooperation (GTZ), GmBH, provided a generous grant to cover ICLARM's organizational expenses and the costs of publishing the proceedings. We are most grateful for this generous support. We also wish to acknowledge the excellent work of the indexer, Dr. Roy Harden Jones.

The review papers and records of discussions published here show how well this fusion of ideas from different disciplines worked out. It was agreed that a great deal more research is needed to define the processes occurring in detrital-based food chains and the roles of the various organisms involved. More information is also needed on the actual food items of the cultured animals, e.g., the relative proportions of algae, bacteria and detritus. Only after gaining such knowledge can we suggest mechanisms for manipulating detrital food chains and improving the yield of the desired animals. The papers in this conference discuss some of these topics and point the way towards increasing aquaculture yields, or decreasing costs (feeding costs especially). The conference has posed many more questions than it has answered, but we believe that it will be recognized as a major event in progress towards energy-efficient waste recycling through aquaculture and that the further research and technology development which it has stimulated will have a major impact on aquatic food production, especially in developing countries.

D.J.W. Moriarty
R.S.V. Pullin

Microbial Ecology in Aquaculture

CHAIRMAN'S OVERVIEW

D.J.W. Moriarty

Extensive or semi-intensive aquaculture systems depend on microbial food webs of which there are two principal types: those dependent on microalgae (i.e., directly utilizing primary production) and those dependent on detritus (dead organic matter). The detritus is converted by bacteria and other microorganisms into nutritionally useful organic matter. In this conference we will be discussing chiefly the second food web, and the biogeochemical processes affected by it. This topic cannot be completely separated from that dealing with the generation of primary production and its utilization by animals. Many animals feed on both algae and detritus with its associated microorganisms. Furthermore, inorganic nutrients released from decomposing detritus stimulate primary production and so we will also discuss aspects of primary production in ponds.

One question that we need to consider is whether aquaculture systems that depend solely on detritus added to ponds from an external source (allochthonous systems) are more productive than those dependent on *in situ* primary production that is eaten either directly or via a detrital food chain (autochthonous systems). To answer this question, we will need to consider the biochemical composition of detritus; whether some or any of the detritus can be digested by animals or by bacteria; whether inorganic nutrients are generated from the detritus or need to be added to enhance decomposition; the types, biomass and productivity of microbes and the efficiencies of energy transfer through the food web; and the effect of detritus decomposition on water quality, especially oxygen concentration.

Considerable advances have been made in microbial ecology recently, particularly in the study of microbial activities in biogeochemical processes. The activities of bacteria in aquatic carbon and nitrogen cycles can now be quantified and this has a direct application to aquaculture. Until about 10 years ago, the role of bacteria in the ocean had been neglected by many marine biologists because bacteria were not considered to have an important function in food chains. New techniques for determining biomass and production have shown that bacteria are more numerous and grow much faster than was thought to be the case and thus do play a very important part in aquatic food chains and nutrient cycles. Thus, methodology is

important in the analysis of the role of microbes and is the subject of my review in this session.

The methods that are used must be checked critically to ensure that results obtained are indeed accurate. Some of the methods used or developed recently are discussed in this session. These are the best that are available at present for determining biomass and growth rates of microorganisms, but they are not perfect and all results need to be interpreted with caution. Improvements and new procedures are being published frequently in the aquatic and microbial ecology literature. Thus, workers new to this field will need adequate access to this literature. Some of the new techniques (e.g., lipid analysis) require expensive instruments and the assistance of trained chemists. Such methods will not be widely used, but if they were applied in aquaculture, problems could be tackled that would otherwise be difficult to study. For example, studies on the composition of detritus are needed to determine what proportions of organic matter in detritus of a given origin (e.g., phytoplankton or straw) is detritus and what proportion is the biomass of microorganisms. Furthermore, how these proportions change as detritus ages and successions of microbes occur, leading to different community structures, could also be studied. The nature and composition of detritus can be very varied and is often unknown and difficult to analyze. In particular, methods are needed to determine the amounts of nutritionally valuable compounds, e.g., protein, that are present as detritus rather than in microorganisms attached to the detritus. Accurate analyses of microbial biomass are necessary for such studies.

The production of bacteria can now be estimated with reasonable accuracy in aquatic systems, including sediment and detritus. This is particularly relevant to the theme of this conference because bacteria have a central role in the detrital carbon cycle, converting detritus in aquatic systems into useable energy, protein and vitamins for animals. A problem to be resolved in studying bacterial functions in the carbon cycle is the growth efficiency of bacteria. Growth efficiencies can be very variable, and are difficult to determine in natural systems such as the open ocean. Aquaculture ponds, where inputs and outputs are controlled and measurable, would be useful semi-natural model systems to complement laboratory microcosm studies on this problem. If the growth efficiency of bacteria can be measured or predicted for given conditions (e.g., detritus composition), then accurate estimates can be made of bacterial respiration in the presence of algae. Better estimates of net primary production, which are critical to productivity studies in aquaculture, can then be made.

Processes such as the turnover of carbon and nitrogen can be studied on a broad scale with data on the biomass and growth rates of the bacterial community as a whole. Dr. Anderson will be discussing utilization of detritus from this viewpoint. If more detailed analyses of the role of bacteria in different processes are to be made, then information on the principal types or species of bacteria and their ecophysiology is needed. Dr. Fry will discuss the role of particular bacteria in the main elemental cycles. From knowledge of environmental factors affecting bacterial species distribution and activity, it should be feasible to determine the best conditions for establish-

ing detrital food chains in aquaculture ponds. In the terrestrial sphere, fungi are the principal decomposers of detritus and it has been shown that a succession of species is involved as the decomposition proceeds. Bacteria are more important as decomposers than fungi in aquatic systems and as Dr. Fry points out, successional changes also occur, but these have not been studied at the species level. Seasonal changes in activity, and presumably species composition, of bacteria do occur. Bacterial activity varies during diel periods in some environments. Whether these changes in activity reflect changes in species composition also is not known, but it is possible if protozoan grazing activity is intense and bacterial doubling times are fast.

Anaerobic processes, discussed by Dr. Blackburn, occur in pond sediments and are significant in the decomposition of detritus. The initial decomposition of particulate material in sediment is carried out by fermentative bacteria, but little detailed information is available on this part of the process or the bacteria involved. As it is likely that much added detritus will settle to the pond bottom and be decomposed in an anaerobic environment, more research in this area would be valuable.

The second stage of anaerobic decomposition wherein small molecules are fermented, and various electron acceptors are used as electron sinks, is well studied. The flux of nutrients, oxygen and toxic products of anaerobic processes between sediment and water needs to be studied. As Dr. Blackburn points out, this is not a simple procedure, but requires detailed studies on the rates of the many interacting processes.

The activity of bacteria on particulate detritus in the water column is the subject of Dr. Kirchman's review. In contrast to sediments, most if not all, processes are aerobic. The activity of bacteria on particles is not easy to study and distinguish from that of bacteria free in the water column. In fact, some unattached bacteria may be loosely associated with particles, but separated by the filtration techniques that are used to study them. Dr. Kirchman argues that in the sea free bacteria have a more important role in the carbon cycle than attached bacteria. In ponds with much detritus that is kept in suspension by mixing or aerating equipment, bacterial activity on particles can be very intense. Dr. Schroeder, in a later session, will discuss the importance of bacteria in pond processes.

Decomposer pathways are important in aquatic food webs, as Dr. Anderson points out. Decomposition rates and production of organic matter that is useful or necessary for aquaculture depend on the nature of the detritus, microbes and environment. Through a detailed understanding of the various factors, we can formulate hypotheses concerning pond dynamics and suggest practical approaches for maximizing pond production.

Methodology for Determining Biomass and Productivity of Microorganisms in Detrital Food Webs

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Moriarty, D.J.W. 1987. Methodology for determining biomass and productivity of microorganisms in detrital food webs, p. 4-31. *In* D.J.W. Moriarty and R.S.V. Pullin (eds.) Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings 14, 420 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Bacteria and algae in water, detritus and sediments can be counted most easily with an epifluorescent microscope after they are stained with acridine orange. Cell volume can be determined by microscopy, but may be subject to considerable error. Biomass can then be calculated using conversion factors for carbon content. Alternatively, the biomass can be determined by chemical methods, such as muramic acid and lipid analyses. Bacterial productivity can be determined from the rate at which tritiated thymidine is incorporated into DNA. Productivity values for bacteria are essential in studies on their role in food chains, because they can double their biomass in a few hours; values for biomass alone are not sufficient. More research is needed to determine the efficiencies with which various bacteria convert organic matter into biomass; values of 25% to 50% are commonly reported. If growth efficiencies are known, the rate at which oxygen is removed from the water by bacteria can be estimated. Bacterial respiration can be substantial in detrital systems. The transfer efficiencies of organic matter via bacteria to higher trophic levels are discussed.

Introduction

Bacteria decompose organic compounds that cannot be digested by animals and thereby increase the nutritive value of the organic matter. This has led many people to suggest that bacteria must play an important role in aquatic detrital food webs (for reviews see Mann 1972; Fenchel and Jørgensen 1977), but quantitative studies have been hampered until recently by lack of adequate methods to measure the biomass, growth rates and production of bacteria; such methods are now available (van Es and Meyer-Reil 1983). This paper reviews the appropriate methods for

analysis of detrital food chain dynamics in aquaculture ponds. The methods discussed are mainly applicable to heterotrophic bacteria, which may be considered as a single trophic group utilizing the primary production that has entered the detritus pool. In fact, because there are many different functional groups of bacteria, there are many trophic pathways within the bacterial component of the food web. For simplicity, all heterotrophic bacteria shall be considered as one group linking detritus with higher trophic levels.

Fungi are also decomposers of plant material, and certainly play a part in aquatic systems, especially where C:N ratios are high and complex structural compounds in higher plant leaves are degraded (Barlocher and Kendrick 1974; Suberkropp and Klug 1976). However, the methods for studying their biomass and growth in natural systems are less well developed than are those for bacteria. Fortunately, bacterial decomposition seems in general to be more important than fungal decomposition in aquatic systems (in contrast to terrestrial systems), and thus the poorer methodology is not a real drawback to studying detrital food chains.

Methods for determining primary production will be discussed briefly. Although this conference is concerned primarily with food chains based on detritus, such food webs cannot be separated entirely from those dependent on algal production in aquaculture ponds. Decomposition of detritus having a moderate to low C:N ratio will lead to mineralization of nitrogen and phosphorus and thus promote algal production in ponds. In fact, concomitant algal production may be necessary in extensive or semi-intensive aquaculture systems that make use of detrital food chains. Algae may not only improve productivity and ecological efficiency via herbivores in ponds, but also raise oxygen concentrations and perhaps supply essential dietary components that are not available from bacteria or detritus (e.g., linoleic acid).

The productivity of the cultured animals in ponds largely depends on the efficiency with which detritus and primary production are converted into biomass by the various organisms in the food chain or food web. For detrital systems that depend on bacterial decomposition, the efficiency with which heterotrophic bacteria convert their organic nutrient supply into bacterial biomass (i.e., their growth efficiency) is an important factor that may control productivity. Growth efficiencies of bacteria seem to vary widely in aquatic systems, but are difficult to measure. Growth efficiencies may be studied more easily in aquaculture ponds, where input and outputs of organic matter can be measured. This will be discussed here as it is relevant to measurements of production in ponds and to estimates of bacterial respiration, which can be so intense in ponds with large amounts of detritus that the resulting low oxygen concentrations may limit productivity.

Biomass of Microorganisms and Composition of Detritus

Bacteria

Two different strategies are available for determining bacterial biomass: 1. bacteria can be counted and their average volume estimated by microscopy; 2. a biochemical constituent that is correlated with biomass may be analyzed.

Direct counting of bacteria stained with a fluorescent dye is a quick and accurate method for enumerating bacteria in the water column or sediments (Francisco et al. 1973; Zimmerman and Meyer-Reil 1974; Hobbie et al. 1977). The dyed bacteria are clearly visible against the black background of the filter surface (Plate 1). The bacteria are also easily distinguished when they are enveloped in detritus particles (Plate 2). In my experience, acridine orange gives particularly good contrast between bacteria and detritus. Contrast is best and the image fades only slightly if the microscope is equipped for epifluorescence with a narrow-band blue excitation filter assembly (e.g., as for fluorescein isothiocyanate; Moriarty 1980). If much detritus is present, fading may be rapid and contrast poor. The concentration of acridine orange should be increased if this happens (2-5 μg/ml is usually adequate; the stained filter should not be washed). Other dyes that are supposedly more specific for DNA have been recommended for use with detritus (Colman 1980; Porter and Feig 1980; Paul 1982). All of these dyes do fluoresce with some plant compounds and I have found that bacteria in detritus are more difficult to count with them than with acridine orange.

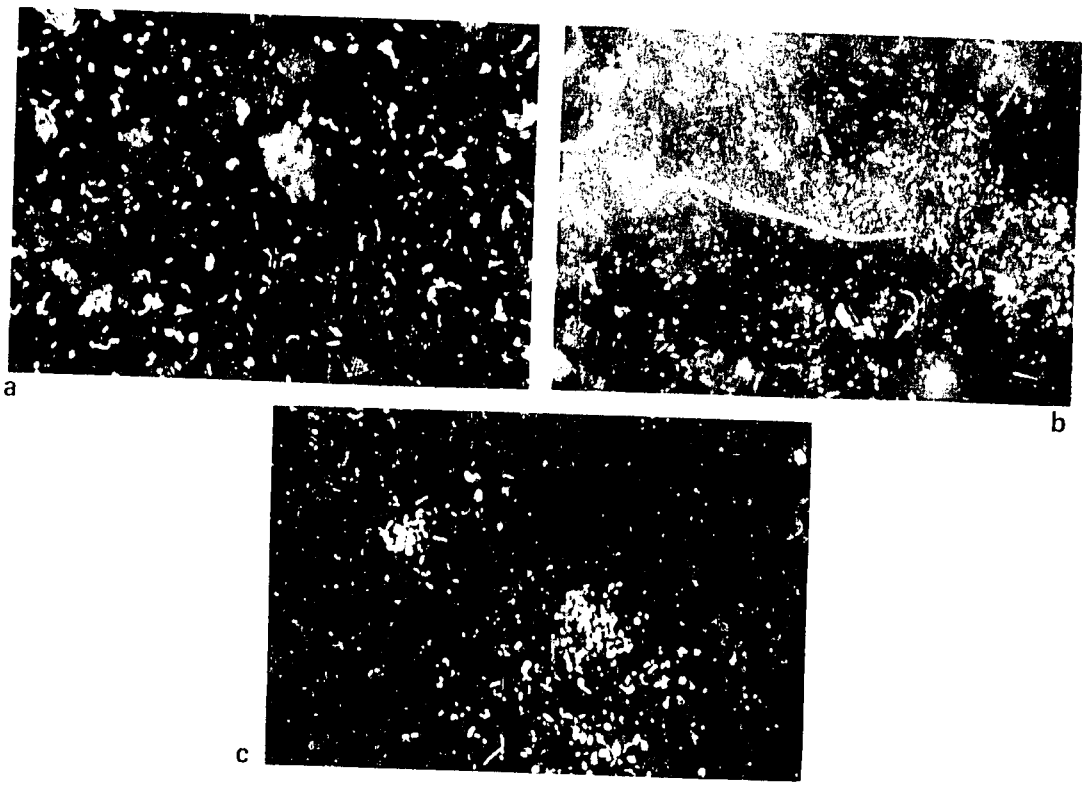

Plate 1. Bacteria from the water column stained with acridine orange and viewed with epifluorescence microscopy. a: Bacteria from water containing algal detritus. b: Bacteria from water with large amounts of algal and seagrass detritus; note the large size and different morphologies of the cells. c: Bacteria from an aquaculture pond, with particles containing attached bacteria. Scale: 1 cm = 8.6 μm.

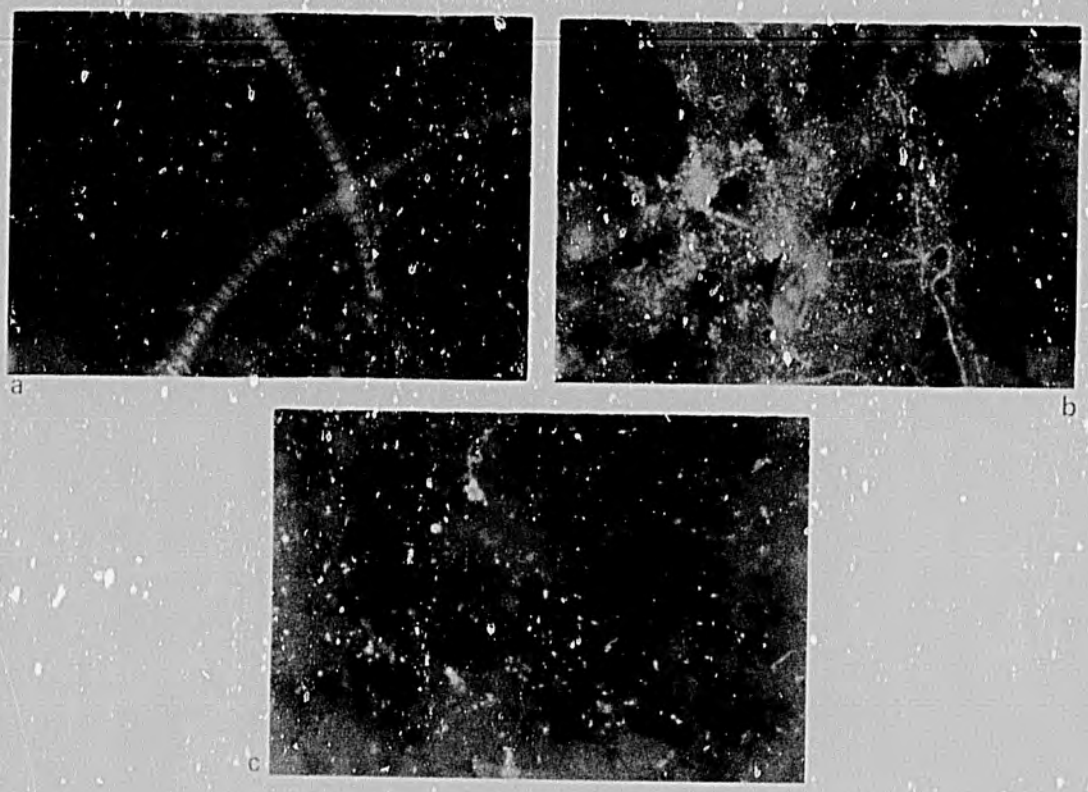

Plate 2. Microbes associated with detritus in sediments, stained with acridine orange. a: Heterotrophic bacteria (green) enveloped in slime (red) and two filaments of a cyanobacterium. Scale: 1 cm = 17.1 μm. b: Bacteria showing many different morphological forms, together with a diatom, enveloped in slime. Scale: 1 cm = 8.6 μm. c: Bacteria (green) with detritus (red) in the stomach of a tilapia from an aquaculture pond.

There are problems in counting bacteria that are patchily distributed in detritus. Particles with attached bacteria are common in aquaculture ponds and if the particles are small, bacteria can be counted (an example is shown in Plate 1c), but if the particles are large or dense counting is difficult.

Once the number of bacteria is known, their average volume must be calculated to determine the biomass. Cell volumes can be calculated from measurements of cell dimensions made under the microscope, or on negatives of photographs projected onto calibrated graph paper or by image analysis with a video monitor and computer. If an image analysis system is available, volumes can be very quickly and easily determined (Fry and Davies 1985). The other methods are, unfortunately, very tedious. Note that very small errors in measuring cell dimensions cause large errors in calculated volumes.

Although electron microscopy gives more precise values for dimensions, Fuhrman (1981) recommends epifluorescence microscopy as being more accurate, because the shrinkage of bacterial cells during fixation is less variable. Some shrinkage may occur, however, if bacteria are fixed with formaldehyde for epifluorescence microscopy and thus volumes may be underestimated. Volumes may also be underestimated with epifluorescence microscopy because acridine orange stains nucleic acids with much greater intensity than other cell constituents. Thus, the cell wall may not be easily distinguished from detritus. In the sea, many bacteria are small, which makes it difficult to measure their size accurately. In aquaculture ponds, however, they are generally larger (Plate 1). Other factors that may cause bacterial cell volumes to be underestimated have been discussed by Bratbak (1985). Bakken (1985) found that the volumes of soil bacteria were underestimated by 25% with epifluorescence microscopy compared to phase contrast microscopy.

To convert volumes to biomass, the carbon content of bacterial cells must be known. Revised values for carbon content, which are about double those previously used, have recently been published (Bratbak and Dundas 1984). The revised values support the finding of Robinson et al. (1982) that the carbon content of a population of bacteria growing on detritus was actually three times higher than values calculated previously.

Errors in estimating biomass from microscopical observations arise from the difficulty in measuring cell size and carbon content accurately. Thus accurate estimates of bacterial biomass are difficult to achieve, which affects not only conclusions about the nutritive value of detritus, but also values for bacterial production and thus ecosystem dynamics. For trophic studies, a further problem is estimating the importance of capsular and slime material excreted by bacteria (Paerl 1974; 1978). The products of bacterial cells are not included in biomass estimates, but may be significant as food for animals (Paerl 1974; Moriarty and Hayward 1982). Many bacteria in sediments or detritus produce large amounts of slime (Plate 3).

Chemical methods for determining bacterial biomass have been sought, because microscopical methods are tedious and subject to many errors. Of the various biochemical constituents of bacterial cells that have been proposed or used in the estimation of biomass, muramic acid is the most useful. An approximate value for the proportions of Gram-negative and Gram-positive bacteria is needed for calculating biomass from muramic acid concentration (Moriarty 1980; Moriarty and Hayward 1982). Good correlations were observed between numbers of bacteria, counted with acridine orange, and muramic acid content of surface marine sediments (Moriarty 1980). A close correlation between muramic acid concentration and direct counts was found for bacteria in the water column of an aquaculture pond (Moriarty 1986b). Muramic acid is not useful if cyanobacteria (blue-green algae) are abundant because they also contain muramic acid

Earlier colorimetric or biochemical methods for determining muramic acid have been replaced by simple procedures using high performance liquid chromatography (HPLC) (Mimura and Delmas 1983; Moriarty 1983). Although the equipment needed for HPLC is expensive, it is now so widely used in biochemical and analytical

Plate 3. Electron micrographs showing single bacteria and colonies of bacteria surrounded by slime layers (glycocalyx) and mineral particles in sediment. Scale bars are 0.2 μm.

chemistry laboratories, that it is probably accessible to many researchers in the aquaculture field.

Lipopolysaccharides, components of Gram-negative bacterial cell walls, have been used to estimate biomass of bacteria in seawater (Watson et al. 1977). There are problems in calibrating the technique and these would be compounded where lipopolysaccharides are bound to detritus. Lipopolysaccharides do not persist for long in sediments once bacteria die (Saddler and Wardlaw 1980). The analytical procedures and interpretation of results in terms of biomass for bacteria bound in sediment and detritus are more complicated than for muramic acid (Saddler and Wardlaw 1980; Parker et al. 1982). Because only Gram-negative bacteria contain lipopolysaccharides, and Gram-positive bacteria are common in sediments and probably detritus also, this method is not useful for studying detrital systems.

Adenosine triphosphate (ATP) has been widely used to estimate microbial biomass since 1966 when the method was proposed by Holm-Hansen and Booth. But because all organisms contain ATP, the method cannot be used to distinguish the various groups of microbes in detritus Furthermore, it may not be useful even as a measure of total microbial biomass, because firstly ATP concentrations per cell vary considerably with environmental conditions and secondly large organisms have so much more ATP than bacteria, that most measurable ATP would be due to them. Aquaculture systems contain a complex array of organisms and we need to study the different types of organisms and the processes in which they participate. Thus ATP is not useful as a measure of "microbial" biomass, when "microbial" could mean bacteria, Protozoa, algae, fungi, meiofauna, macrofauna, zooplankton, etc. We need to be able to determine the composition of microbial communities with more precision and accuracy than is possible with ATP. Thus it is not a useful method for studying detrital food chains.

Many lipid compounds can be used as markers for various groups of microorganisms (Perry et al. 1979; Volkman et al. 1980; White 1983; Gillan and Hogg 1984). They could be used in aquaculture studies to provide estimates of biomass for bacteria, microalgae and other microbes from fatty acid composition (Gillan and Hogg 1984). The analytical procedures are complex, time-consuming and require expensive equipment. If such facilities are available, however, some of these analyses may prove to be very useful, particularly if simpler procedures are developed. For example, the growth rates of bacteria and microalgae in sediments have been measured separately with a simplified measure of phospholipid and sulfolipid synthesis (Moriarty et al. 1985b). Similarly, the proportion of anaerobic fermenting bacteria on seagrass detritus in sediments has been measured (Moriarty et al. 1985a).

Techniques for counting numbers of bacteria that depend on culturing them (agar plates, most probable number dilution series) cannot be used for obtaining the numbers of the whole bacterial community. One of many sources of error with such techniques arises when the detritus contains an aggregation of bacteria, which produces results that are likely to be several orders of magnitude too low (van Es and Meyer-Reil 1983). Culture techniques may be useful if information is needed about a particular population of bacteria.

The direct microscopical and biochemical techniques discussed above do not distinguish dormant or dead bacteria from living or metabolically active bacteria. For trophic studies this is not important, but for studies on pond ecosystem dynamics it may be necessary to know how many bacteria are respiring or growing. There is no simple or accurate technique for doing this, even for free-living bacteria in the water column, which are the easiest to study. Respiring bacteria can be detected microscopically with a tetrazolium dye that is reduced to an insoluble formazan and deposited in the cell (Zimmerman et al. 1978). This technique does not work well with sediments, where nonbiological reduction occurs, nor does it work well with bacteria in detrital aggregates if the bacteria cannot be dispersed and separated from the aggregates (pers. obs.).

Many workers have combined autoradiography with microscopy, particularly fluorescence microscopy, to study bacteria that actively take up radioactive organic compounds (van Es and Meyer-Reil 1983). Because not all active bacteria may take up a particular organic compound at equal rates, there may be problems in interpreting the results. The techniques work reasonably well for water column bacteria although there are problems (e.g., see Fuhrman and Azam 1982). In sediments or detritus, however, the bacteria have to be well dispersed and separated from aggregates. Microautoradiography with tritiated thymidine and transmission electron microscopy could be an informative method for determining the proportion of growing bacteria in detritus.

Actively-growing bacteria in water were detected by incubating water samples with yeast extract and naladixic acid which inhibits cell division (Kogure et al. 1979). The elongated forms of bacteria were easily recognizable under the microscope.

Fungi

The fungal biomass in detritus cannot be accurately determined with currently available techniques. Direct microscopy techniques, which are probably the simplest, are discussed by Newell and Hicks (1982). Two biochemical compounds have been used: ergosterol (Seitz et al. 1979; Lee et al. 1980) and glucosamine (Ride and Drysdale 1972). As glucosamine is present in many organisms, including bacteria, it is not useful unless fungi are present in high proportions. Glucosamine can be determined by HPLC, together with muramic acid (Moriarty 1983).

Protozoa

No useful chemical methods for determining protozoan biomass in a mixed assemblage of microorganisms are available. Fluorescent dyes have been used to count flagellates in the water column (Fenchel 1982; Sherr and Sherr 1983), but fragile organisms may break apart during fixation. In sediments and detritus, it is best to extract and count flagellates and ciliates alive as many of them lyse easily during fixation. Also, I have observed that ciliates lyse when exposed to blue light

after being stained with acridine orange. Techniques for extracting Protozoa are similar to those used for meiofauna (Uhlig 1964; Schwinghamer 1981). Protozoan biomass will be underestimated if methods for examining fixed specimens, such as those used by rumen microbiologists, are used. Amoebae have not been studied extensively in natural aquatic systems, so it is difficult to recommend a method for determining their numbers or biomass, though the method of Singh (1946) might be applicable. Sieburth (1979) has discussed the distribution and types of Protozoa in marine systems and the methods of studying them.

Algae

Microscopy techniques for counting algae and determining biomass give the most accurate results. The techniques that preceded the introduction of epifluorescent microscopy are described in an IBP Handbook (Vollenweider 1969). Epifluorescent microscopy is a better technique for examining algae if they are embedded in detritus or sediment (references in section 1 above). Acridine orange is probably the most useful stain, because cell walls, as well as nuclear material, lightly fluoresce with it and cell sizes can be determined. Algae are easily recognized by the autofluorescence of their photosynthetic pigments. The color is generally a deep red where chlorophyll *a* predominates, but it may vary where other pigments predominate. For example, some cyanobacteria (blue-green algae) and coralline red algae fluoresce orange.

Filamentous algae can be counted using the procedures of Olson (1950) as described by Brock (1978). Brock recommended the dye primuline yellow, but this does not work well in detritus because the detritus fluoresces with the same color as the algae.

As with bacteria, measurements of biomass from numbers and size of cells are time consuming and liable to subjective errors in volume determinations, so chemical methods have been sought. Pigment analysis, and in particular the determination of chlorophyll *a* is the preferred technique for algae. Details of methods for extracting pigment and determining chlorophyll *a* by spectrophotometry are given by Vollenweider (1969) and Parsons et al. (1984).

If large amounts of chlorophyll degradation products (e.g., phaeophytins and chlorophyllides) are present, as may be the case in detritus, spectrophotometric techniques do not give accurate results. It is better to separate the pigments by chromatography. A number of techniques have been described for doing this with HPLC (e.g., Mantoura and Llewellyn 1983; Wright and Shearer 1984). An advantage of this procedure is that some indication of algal community structure may be obtained from the pigment analysis. The factors for converting pigment data to biomass are variable, and may give inaccurate values for biomass (Banse 1977). For benthic diatoms, conversion factors ranging from 10 to 154 have been reported (de Jonge 1980). An overall mean value that could be used is 50 (from data of de Jonge 1980).

Fatty acid composition may also be used to obtain estimates of algal biomass (Perry et al. 1979; Volkman et al. 1980; Gillan and Hogg 1984).

Microbial Composition of Detrital Systems

For each type of detrital system or even each pond or set of ponds the following questions are important: (i) what is the proportion of biomass to detritus (nonliving organic matter)? (ii) what organisms are present and how do their biomasses change with time? (iii) what is the composition of the detritus and how does it affect the community structure and succession of microorganisms associated with it? (iv) what is the nutritive value of the detritus in terms of protein, digestible carbohydrate, essential amino acids, fatty acids, vitamins, etc. These topics are discussed by Bowen (this vol.), but attention should be drawn to some points of methodology here.

The methods described above cannot give a complete description of the composition of detritus, but only an estimate of the biomass of some of the principal organisms. The size of organisms ranges so widely in detritus, from very small bacteria (0.2 μm diameter) to meiofauna, that general techniques such as ATP content are not likely to yield useful information on composition. Yet biochemical studies of biomass and community composition may be necessary to complement more direct microscopical studies. Many organisms (e.g., some Protozoa and microalgae) are very fragile and cannot be easily separated from detritus or fixed for microscopical study. Thus direct microscopical methods may underestimate the biomass of some groups. The newer techniques involving lipid composition (fatty acids, phospholipids, sterols, etc.), although requiring complex equipment and facilities, will be useful tools in microbial ecology once the biochemical composition of the various microbes is better known. When this work is undertaken, our knowledge of the detrital food chains will be enhanced. Microbial ecologists will be able to use aquaculture ponds as semi-natural model systems, where successional studies on microbial community structure may be carried out with detritus of known composition.

The question of whether detritus *per se* or the microorganisms associated with it are utilized as food by deposit or detrital feeders has been discussed in the literature; the nature of detritus is so variable that no generalization can be made. For example, neither detritus derived from vascular plants with a high C:N ratio, nor much of the organic matter present in indigestible carbohydrates will be useful directly, whereas detritus from algae may be much more digestible without microbial decomposition (Findlay and Tenore 1982; Tenore 1983).

Productivity and Growth Rates of Microorganisms

Bacteria

Growth Rates and Production

Techniques for measuring the activity and growth of heterotrophic bacteria have been reviewed by van Es and Meyer-Reil (1983), but only recently has it become

possible to measure the growth rates of heterotrophic bacteria in aquatic environments satisfactorily. Methods requiring counts of bacteria at intervals of time do not work in natural systems, due to the effects of predation as well as the difficulties associated with counting bacteria. Such methods may be used in the laboratory or in microcosms of natural systems where predators have been excluded in order to check or calibrate the technique described below (Fuhrman and Azam 1982; Kirchman et al. 1982).

A widely accepted method for measuring bacterial growth rates and production in natural environments is based on the measurement of rates with which tritiated thymidine is incorporated into DNA (Fuhrman and Azam 1980, 1982; Moriarty and Pollard 1981, 1982). It is reviewed in detail elsewhere (Moriarty 1986a). Such measurements are particularly important in studies on detrital aquaculture systems, because bacteria provide the main mechanism for transforming detritus with a low food quality into useful food material. As the method is new, and is promising for aquaculture, some details will be given here.

In principle, the thymidine method for measuring bacterial growth is simple, as there is a direct correlation between rates of DNA synthesis and cell division. Bacteria contain one chromosome, so when a cell grows and divides, every new chromosome that is synthesized represents a new bacterial cell. The rate of synthesis is calculated from the rate of incorporation of thymidine into DNA. Thymidine is one of the four nucleosides of which DNA is composed. By labelling thymidine with a radioactive isotope (tritium), this process can be simply and conveniently measured. Thymidine has the advantage that it is used in cells almost entirely for DNA synthesis; excess thymidine is not incorporated into any other macromolecules.

Methods based on adenine and adenosine triphosphate (ATP) have been developed by Karl and his colleagues for determining microbial biomass and growth rates, e.g., Karl (1982). All methods for such studies have deficiencies. The deficiencies of the adenine methods, however, are so severe, that I do not consider these methods to be useful for aquaculture studies and thus have not discussed them in detail. For a critical appraisal of these methods, see Fuhrman and Azam (1980), Fuhrman et al. (1986a, 1986b), Moriarty (1986a).

The chief disadvantages of the methods are:

1. Adenine is not taken up by all organisms, but its use in methods for measuring growth rates depends on measurements of ATP concentrations. Large errors result because ATP is in all organisms.
2. Adenine is taken up by some algae as well as bacteria and is incorporated into nucleic acids, but at probably quite different rates. The complexities of adenine metabolism make it very difficult, if not impossible, to determine meaningful growth rates in natural ecosystems from rates of labelled adenine incorporation (Moriarty 1986a).

In practice, there are some problems in the use of tritiated thymidine that need to be considered if accurate and ecologically meaningful results are to be obtained. In

detritus and sediment, thymidine should be added at very much higher concentrations than would be added to the water column, otherwise insufficient thymidine will penetrate to the growing bacteria. Problems may arise from the effects of isotope dilution if the concentration of thymidine supplied is too low because it adsorbs to detritus (Pollard and Moriarty 1984). Thymidine may be degraded within the cell and the tritium distributed into other macromolecules if bacteria are incubated for too long with the labelled thymidine. Short-term assays (generally 10 to 30 min.) are necessary to avoid the effects of predation and containment of natural samples in bottles.

Conversion factors are needed to calculate rates of cell division from rates of thymidine incorporation, and of production from rates of cell division. Rates of cell division can be calculated with reasonable accuracy (Moriarty 1986a). Estimates of production, however, may be inaccurate if the size of the growing bacterial cells is not known. The errors that apply to biomass determinations also affect productivity when cell sizes and carbon content are not known. More research is needed in this area. Aquaculture ponds are suitable experimental systems for this type of work, because it is easier here than in uncontrolled natural environments to check estimates of production from data on known rates of input and from successional studies.

The growth of most types of aerobic and anaerobic heterotrophic bacteria is specifically measured by the thymidine method. Cyanobacteria, eukaryotic algae and fungi lack thymidine kinase, the enzyme that is needed to incorporate thymidine into the biosynthetic pathway leading to DNA. Some bacteria, particularly those with strict nutritional requirements such as chemoautotrophs, are unable to use thymidine, which may be due to their lack of uptake mechanisms, rather than of thymidine kinase. Thus the method may underestimate bacterial production, but apparently not severely. Protozoa, which have thymidine kinase, lack uptake mechanisms equivalent to those of bacteria, and thus their DNA is not labelled in short-term experiments with nanomolar concentrations of thymidine. The specificity of the thymidine method for bacterial growth rates is discussed in more detail elsewhere (Moriarty 1986a).

Other proposed methods of estimating bacterial production in aquatic systems include those that are based on measurements of the dark fixation of $^{14}CO_2$, the frequency of dividing cells and the uptake of small compounds such as amino acids or sulfate. These are more difficult than the thymidine method to calibrate or interpret, and are not suitable for use with detrital systems. For methods that measure the synthesis of cellular constituents other than DNA, the bacteria must be in a state of balanced growth or conversion factors will be invalid. Balanced growth occurs when all components of the cell (e.g., DNA, RNA, protein, lipid) increase at the same rate, which is unlikely to be the case in natural systems.

The fixation of $^{14}CO_2$ in the dark was proposed by Romanenko (1964) as a method for measuring heterotrophic bacterial growth. Its use has been reviewed by van Es and Meyer-Reil (1983). Results obtained with the method were generally up to an order of magnitude too high. It is unlikely that accurate values can be obtained in natural systems because the method requires long incubation times, all

organisms fix small amounts of CO_2 in the dark and the conversion factors are variable.

The frequency of dividing cells was suggested as a measure of bacterial growth rates in the water column (Hagström et al. 1979). Seasonal changes in the frequency of dividing cells were covariant with the uptake by bacteria of phytoplankton exudates. The method is, however, difficult to calibrate (Hagström 1984), requiring culture studies that match laboratory values with field estimates (Riemann et al. 1984). Problems with interpretation of the method have been discussed by Newell and Christian (1981). The method would not work with detritus or sediments, where filamentous bacteria abound and cannot be distinguished from dividing cells. Fallon et al. (1983) found that the frequency of dividing cells method gave values for bacterial production in sediments that were over an order or magnitude too high.

Protein Synthesis

The microorganisms associated with detritus are usually the main source of protein for detritivores, as detritus itself generally has a low protein content if it is aged or is derived from vascular plants (Fenchel and Jørgensen 1977). In studies of detrital decomposition, the production of microbial biomass, measured using techniques described above, can be used to estimate protein production. If growth is not balanced, the rates of protein synthesis will not be equivalent to the rates of cell division. Under starvation conditions, bacteria may still synthesize DNA and divide, but they become smaller and their protein content declines (Kjelleberg et al. 1982). Alternatively, where nutrients are plentiful, the bacteria increase in size and synthesize protein faster than DNA.

For studies of trophic dynamics in detrital systems, direct measurement of the rates with which bacteria synthesize protein would be preferable. Protein synthesis is often determined in the laboratory from rates of ^{3}H-leucine or ^{14}C-leucine incorporation. This technique is also useful as an index of rates of protein synthesis of bacteria in natural aquatic systems (Kirchman et al. 1985). There are problems in determining the specific radioactivity of ^{14}C-leucine within the bacteria, and thus obtaining an accurate rate of synthesis; however, further work should resolve these problems.

Kirchman and Hodson (1984) have discussed the relationships between protein synthesis and the uptake and utilization of dissolved organic carbon compounds. The turnover of organic carbon in aquatic systems is not necessarily directly correlated with rates of bacterial growth. For a fuller understanding of the carbon cycle and trophic dynamics in ponds, processes such as protein and lipid synthesis, bacterial respiration and bacterial growth rates need to be studied.

ALGAE

The two principal and well-known methods for measuring primary production depend on oxygen production or $^{14}CO_2$ fixation (Vollenweider 1969; Parsons et al.

1984). For quantitative studies on detrital food chains, net primary production must be measured. True net primary production is much more difficult to measure than gross production, because the respiration of algae and other organisms in the community is difficult to measure during photosynthesis. There has been considerable discussion in the literature over the last few years on measurements of net primary production and the accuracy of $^{14}CO_2$ method (Peterson 1980; Davies and Williams 1984). Davies and Williams (1984) concluded that the $^{14}CO_2$ method can give values for primary production that agree with gross production measured by the oxygen method, provided that certain conditions are met. Much of the respiration in planktonic communities is probably due to bacteria (e.g., see Williams 1981), so unless bacterial respiration is measured or estimated separately from algal respiration, calculations of net primary production will not be accurate. In aerobic environments, estimates of bacterial respiration can be obtained from growth rates (thymidine technique), but a value for growth efficiency has to be assumed or chosen from published values. Aquaculture pond systems would be useful model environments for studying this problem.

Growth or Ecological Efficiencies and Food Chains

In the fields of aquaculture and microbial ecology in general we need to know how much detrital organic matter is converted into bacterial biomass and how much is respired or mineralized. In other words, what is the growth efficiency of the bacteria? And furthermore, what is the overall ecological efficiency of the pond, that is, how much of the organic matter added to the pond is converted into biomass in the end product?

Biomass Production

In a grazing food chain where a herbivore feeds on plants, the plant biomass is usually very high in proportion to the biomass of the herbivore. Biomass values alone are sufficient to determine whether the animals are food limited and to compare one food chain with another. With food chains that are based on bacteria, biomass values are not sufficient. Bacteria can double very rapidly and so production values are essential for a full understanding of the importance of bacteria in food chains. Consider a case where bacteria have a doubling time of 12 hours and are grazed at a rate that keeps the biomass constant. A measurement of biomass would underestimate by twofold the amount of food available each day to animals. The rate of bacterial biomass production must also be measured.

When bacterial production is measured with the thymidine method, the conversion efficiency must be known to assess the relative amounts of detritus consumed and mineralized. Conversion or growth efficiencies of bacteria have been extensively studied in culture, where an average value of 60% was commonly found (Payne 1970). Conversion efficiencies vary with such factors as temperature, pH and nutrient

consumption (Payne and Wiebe 1978). Quite high values for carbon assimilation have been found (e.g., 85% for *Enterobacter aerogenes*), usually when nutrient complexity is high (Payne and Wiebe 1978). Where simple or structural carbohydrates are supplied with inorganic nitrogen, efficiencies are generally low. Because so many different interacting factors affect growth yields, culture studies cannot be extrapolated to the natural environment.

Payne and Wiebe (1978) pointed out that growth efficiencies have to be determined *in situ*. One technique they discussed measures growth and respiration with ^{14}C-labelled substrates having a low molecular weight. They make an important point; in any work with radioisotopes one must be aware that isotope dilution can be variable and changeable in short-term experiments. For respiration studies, it is necessary to check that the specific radioactivity of the respired $^{14}CO_2$ is the same as that of the substrate. Although the problems of isotope dilution are recognized by biochemists, it seems that aquatic microbial ecologists have overlooked them (King and Berman 1984).

Microcosm studies where mass balances can be determined may be a better approach for studying growth efficiency. Koop et al. (1982) calculated that about 30% of carbon from a decomposing kelp *(Ecklonia)* was converted into bacterial carbon over a 9-day period. Their values were based on bacterial biomass conversion factors that have since been reported as being too low (Bratbak and Dundas 1984). If the newer conversion factor is accepted, the growth efficiency of bacteria on the kelp would be around 50 to 60%. In that case, twice as much food (bacterial biomass) would be available to higher trophic levels.

Robinson et al. (1982) measured bacterial conversion efficiencies during degradation of another kelp *(Laminaria)*. They found that bacterial biomass measured directly was about 3 times higher than that calculated from measurements of cell size. Conversion efficiencies were about 45% for the first 2 to 3 days of degradation, but declined to about 20% after 36 days, as the percentage of refractory detritus increased with the depletion of the readily digestible material.

Lower values for conversion efficiencies of bacteria that were degrading phytoplankton detritus have been reported (Bauerfeind 1985). Respiration was measured directly (i.e., O_2 uptake which avoids isotope dilution problems with $^{14}CO_2$) and was found to account for 73 to 83% of carbon uptake. Bauerfeind's (1985) values were calculated on the basis that bacterial carbon was 10% of the cell mass, but as mentioned above, Bratbak and Dundas (1984) have reported that carbon content is higher (around 20%). Using this higher value, the respiration was 57 to 72% or, in other words, conversion efficiencies were around 35%. From these studies, it seems that bacteria may be marginally more important as mineralizers of organic matter than as converters into useful biomass, but there is much variation, depending on the nature of the organic substrates and the availability of nitrogen and phosphorus. Williams (1984) has discussed this problem with reference to marine water columns and points out that a single conversion efficiency for all conditions is too simplistic.

Conversion efficiencies need to be measured for each environment. Thus, it is not possible to give a general answer to the question posed above, namely, what propor-

tion of detrital organic matter is mineralized by bacteria, and what proportion is made available to the rest of the food web? Research programs in aquaculture will need to include experimental studies to determine conversion efficiencies in each system, and to determine whether these efficiencies can be increased by manipulating nutrient concentrations.

The need for accurate conversion efficiencies in understanding pond ecosystem dynamics is illustrated by some studies on ponds used for the culture of penaeid shrimp, where an excess supply of pelleted food became a high quality detritus for a bacterial-based food chain (Moriarty 1986b; Moriarty et al. 1987). The bacterial production in the water column was very high compared to natural marine systems, and production in the sediment was also high, but not as great as in the water (Table 1). The production values were clearly correlated with food input.

To determine how closely correlated they were, conversion efficiencies are needed to calculate the total amount of organic matter utilized by the bacteria. Production values for the control pond were subtracted, and then total organic matter utilized was calculated, assuming conversion efficiencies of 25 and 50% (Table 2). It can be seen that, with an efficiency of 50%, rates of food input and utilization agree closely in ponds and 11 and 23, whereas with 25% there is a large discrepancy. In pond 29, a conversion efficiency of 42% is necessary to balance the two. A conversion efficiency of 40 to 50% is quite possible in these ponds, because the detritus, being pelleted food, was nutritionally of high quality.

Table 1. Bacterial production in shrimp aquaculture ponds supplied with pelletized food (from Moriarty 1986b). Units: food and production: g C m^{-2} day^{-1}; doubling time: days.

Pond	Food input	Water column		Sediment (0-10 mm)	
		Production	Doubling time	Production	Doubling time
Control	0	0.43	0.25	0.26	4
11	1	1.07	0.17	0.24	9
29	3.2	1.32	0.17	0.41	4
23	4.0	2.10	0.34	0.50	8

Table 2. Comparison of pelletized food input and organic matter utilization by bacteria in shrimp aquaculture ponds. Bacterial production was measured, and values for utilization are calculated, assuming either 25 or 50% bacterial growth efficiency. Values are g C m^{-2} day^{-1}. Values for a control pond have been subtracted: from Moriarty (1986b).

Pond	Input	Water column		Sediment	
		25%	50%	25%	50%
11	1.0	2.6	1.3	0	0
29	3.2	3.6	1.8	1.0	0.5
23	4.0	6.6	3.3	1.4	0.7

Oxygen Uptake

Besides the direct relevance to biomass production, studies of conversion efficiencies are important in other ways. Aerobic bacterial growth is, of course, accompanied by oxygen utilization; the more efficient the conversion, the less oxygen is used. Bacteria may account for the bulk of oxygen uptake in ponds where detrital food chains operate. Their respiration, simply by lowering oxygen concentration in the water, may be the most important factor that limits the production of fish or prawns on detrital food chains.

An example of such effects is seen in the data from the shrimp aquaculture ponds discussed above. In all ponds that were studied, respiration was greater than gross production; gross production ranged from 0.8 to 1.6 g C m^{-2} day^{-1} and respiration from 2.3 to 3.6 g C m^{-2} day^{-1} (Moriarty et al. 1987). Bacterial respiration, estimated from production measurements, accounted for a considerable proportion of the total respiration (Table 3). In pond 29, for example, bacterial respiration was about 70% of the total. With the bacterial respiration known, a maximum value for net primary production can be calculated; in this case, 0.5 g C m^{-2} day^{-1} (Table 3). The large respiratory activity in this pond was reflected in the low oxygen concentration in the water, particularly the bottom waters (Table 4). Such low values stress animals and limit their production. The amount of detritus that can be added each day to ponds would be limited by the degree of aeration.

Table 3. Primary production and respiration in shrimp aquaculture ponds. Values for bacterial respiration are calculated assuming a 42% conversion efficiency. Values are g C m^{-2} day^{-1} (from Moriarty et al. 1987).

Pond	Gross production	Respiration		Net primary production
		Total	Bacterial	
29	1.2	2.5	1.8	0.5

Table 4. Oxygen production, respiration and concentration in a shrimp aquaculture pond (from Moriarty et al. 1987).

Depth cm	Gross production mg l^{-1} h^{-1}	Respiration mg l^{-1} h^{-1}	Concentration mg l^{-1}
10	0.49	0.34	4.61
60	0.43	0.39	3.38
80	0.08	0.20	0.92

Higher Trophic Levels

Now that the biomass and production of bacteria can be measured with some assurance of accuracy, it is possible to study the efficiency with which that production is transferred to higher trophic levels. The control of bacterial growth by grazers can also be studied.

The ecological efficiency of a pond depends on the feeding strategy of the cultured species and the number of levels in the food chain to those species. Organisms that feed on bacteria and microalgae, such as tilapia, should be produced more efficiently than carnivores (e.g., penaeid shrimp). Even so, there are likely to be variations in the efficiency of tilapia production because a complex food web operates at the microbial level. Protozoa, microzooplankton and meiofauna compete with larger "detrital" feeders for bacteria and microalgae and can have a considerable effect on the ecological efficiency. The rates of grazing on bacteria by Protozoa and meiofauna in sediments are unknown, but need to be determined, because a large proportion of bacterial production may be removed by them. An extension of such studies would be to determine whether bacterial production and detritus decomposition are stimulated by grazing on the bacteria.

To illustrate the effects of multiple trophic levels and controls on pond production by productivity of autotrophs and bacteria, consider a hypothetical example of a food web in a pond (Fig. 1). A pond used for the culture of penaeid shrimp in

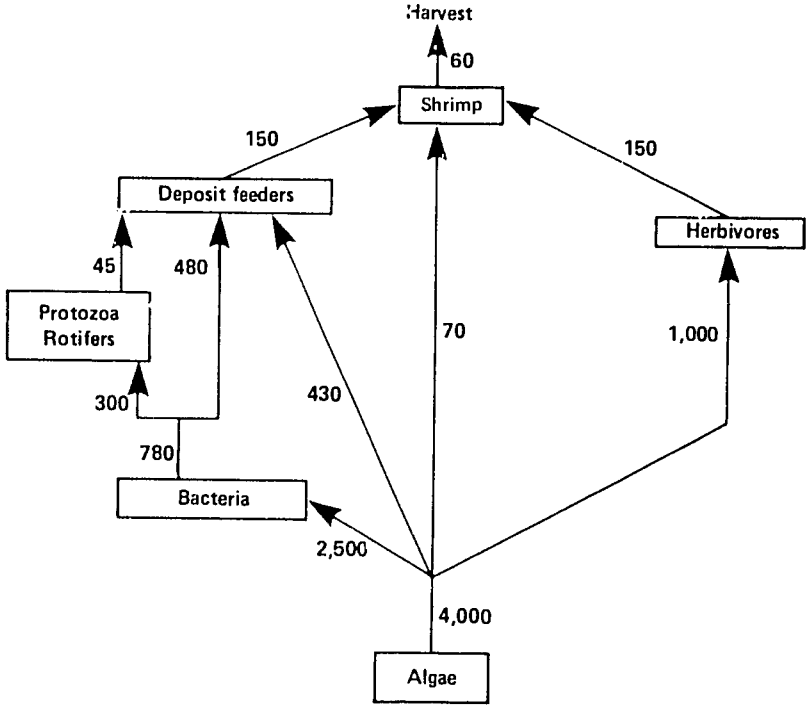

Fig. 1. Hypothetical food web for a penaeid shrimp pond based on known production of fish and shrimp of 60 mg C m^{-2} day^{-1}. Values are mg C m^{-2} day^{-1}.

Thailand has no inputs except for 30% water exchange each day from a productive estuary. It produces about 1,800 kg wet weight of shrimp and 1,000 kg of fish per month. Extensive mats of benthic algae grow in the pond and are one source of production, either directly, or as detritus when they decay. The monthly production of fish and shrimp is approximately equivalent to 60 mg C m^{-2} day^{-1}. The hypothetical food web and values constructed for this pond indicate that primary production would need to be about 4 g C m^{-2} day^{-1}, which is high, but not unrealistic for the tropical environment concerned. Some organic matter would have come from the estuary also. It has been assumed that a little over half the primary production enters the detrital food web directly, and that growth efficiencies are 30% for bacteria, 20% for Protozoa and rotifers and 15% for other organisms. The shrimp are assumed to feed mainly on deposit feeders and herbivores and also to some extent on algae. Only a small fraction of the original primary production entering the detrital food web would be needed to increase prawn production significantly based on deposit feeders. In other words, detrital food chains are much less efficient than those with herbivores for feeding carnivores.

The population density of bacteria in water columns is relatively constant, and yet the bacteria are known to grow rapidly. Zooflagellates are the main grazers on bacteria in the water column, and their density and grazing rates are sufficient to control the bacterial density (Fenchel 1982; Andersen and Fenchel 1985). Larger Protozoa, e.g., ciliates and metazoans, control the flagellate populations.

In sediments and detritus, the relationships between bacteria and their growth substrates on the one hand and bacterial grazers on the other are more complex. A much greater array of animals, ranging in size from small Protozoa to large fish, feed on bacteria attached to detrital or sedimentary particles. It has been suggested that Protozoa may be the most important grazers on bacteria in sediments, but more detailed studies are needed (Fenchel and Jørgensen 1977).

Bacterial biomass is positively correlated with food supply in sediment. Dale (1974) showed that, in intertidal sediments, bacterial biomass depended on organic carbon and nitrogen content. He suggested that food supply might limit bacterial numbers, a hypothesis for which there is strong evidence. For example, in seagrass sediments, bacterial densities were about fourfold greater around seagrasses, where organic carbon and nitrogen concentrations were also four times higher (Moriarty 1980). Bacterial density was high in aquaculture pond sediments where pelleted food was provided (Moriarty 1986b). The bacterial biomass in detritus depends not simply on the amount of organic carbon and nitrogen present, but on the composition of the detritus. It is the availability of readily digestible energy substrates and nitrogen sources that governs biomass (Lopez et al. 1977; Hanson 1982; Tenore et al. 1982; Alongi and Hanson 1985).

Bacterial growth rates or production may not be correlated with amounts of detritus present in the same way that biomass is. In an artificial system, no obvious correlations of bacterial growth rates with detritus supply were seen (Alongi and Hanson 1985). Diel changes in bacterial growth rates in seagrass sediments have been observed, which may be related to exudation of readily available organic compounds

by the seagrass (Moriarty and Pollard 1982). Thus growth rates of some bacteria in sediments may be influenced by food supply. Many bacteria may be dormant until the correct substrate or environmental conditions are provided. Others may grow slowly on degrading particulate matter and thus would not easily be detected by the thymidine method. With the thymidine method it is possible to carry out more incisive experiments on the relationships of bacteria to detritus supply and composition than was previously possible.

The biomass and growth rates of bacteria in detritus are influenced by animals grazing on them. Rates of decomposition of detritus are often increased by the effects of grazing animals (Fenchel 1970; Welsh 1975; Harrison 1977). Bacterial activity, and presumably growth rates, are increased by grazing, although only general studies have been carried out (see Fenchel and Jørgensen 1977). If bacterial growth rates are substantially increased by grazing activities of animals, values solely for biomass will not be useful in determining the role of bacteria in food chains. Production must also be measured.

In some aquaculture ponds in Malaysia, the effect of added chicken manure on microbial populations was examined (Moriarty 1986b). After one week, biomass increased and doubling times slowed. After two and three weeks, bacterial biomass fell markedly, while the meiofauna population density increased (Table 5). The bacterial doubling times also fell markedly, which supports the suggestion that bacterial growth is stimulated by the effects of grazing animals. The mechanisms by which this occurs may be various.

Table 5. Effect of chicken manure on bacteria and meiofauna in pond sediments.

Treatment	Bacteria		Meiofauna
	Biomass g C m^{-2}	Doubling time (days)	No. 10 cm^{-2}
Control	3.4	5	140
Manure, 1 week	4.3	8	300
Manure, 2 weeks	1.9	7	500
Manure, 3 weeks	0.8	3	1,500

It is now possible to investigate some of these mechanisms with the tritiated thymidine method to measure changes in instantaneous growth rates of bacteria in short time periods. A study of this type has been conducted by Alongi and Hanson (1985). They found that the polychaete *Capitella capitata* stimulated bacterial growth to some extent when detritus was in low supply, but had no effect when detritus was present in large quantities and bacteria were growing very rapidly. Alongi (1985) suggested that faster bacterial growth rates in the presence of *Capitella capitata* may be due to the effect of protozoan grazing on bacteria attached to the tube walls of the polychaete.

It has long been accepted that bacteria have a role in sediments as food for deposit feeders, but the significance of that role has been debated. With the improvement in methods for determining biomass and growth rates of microbes, and the increased values for carbon content of bacteria, it is likely that, at least in sediments and detritus, bacteria will be confirmed as having a very significant role. It has been shown, for example, that 40% of daily bacterial production was eaten by holothurians on a coral reef flat (Moriarty et al. 1985c). One of the important areas for further research is in determining growth efficiencies for the various groups of microbes, and in particular whether food chains can be manipulated to improve overall ecological efficiency.

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Discussion

MORIARTY: Regarding the efficiency of conversion by bacteria, one of the big problems in microbial ecology is what is the conversion efficiency of bacteria in a natural system? We can measure bacterial production but we don't know how much organic matter the bacteria need to take in to achieve that production. We can make hypotheses, for example, their conversion efficiency may be around 30 to 50%.

SCHROEDER: I doubt that they are that efficient. A 50% conversion efficiency is almost perfec' They are probably closer to 20%. I am assuming here that the bacteria in a pond are achieving the same efficiency as we might observe in the laboratory in a test tube or fermentation vessel.

MORIARTY: Bacterial conversion efficiencies of up to 80% have been recorded. It all depends very much on the energy value and C:N ratio of what the bacteria take in and their respiration.

SRINIVASAN: It depends on the substrate. In fermentation, if we feed 100 g of glucose to the bacteria, 50 g should be fixed as biomass and 50 g will go off as CO_2. For other substrates like amino acids and proteins, the maximum conversion efficiency to biomass is about 40 to 45%.

SCHROEDER: But it is unlikely that the conditions we are discussing are optimal conditions.

MORIARTY: The only point I am trying to make is that we do not have yet a single reliable measure of conversion efficiency. However, now that we can measure bacterial production we at least have some idea of what is going on. If we can now proceed to measure food inputs and particularly if we can measure oxygen changes as well, then we can make some better estimates of conversion efficiency. Conversion efficiency is likely to be higher in the water column than the sediment.

SCHROEDER: Why?

MORIARTY: Because in the water column aerobic processes predominate. These are more efficient than the anaerobic processes which predominate on the sediment.

SCHROEDER: But anaerobic processes are probably more efficient in producing total biomass, that is slime plus bacterial cells. Aerobic processes are only more efficient than anaerobic processes for cell production.

MORIARTY: Slime production is controlled by many factors, especially C:N ratios. Slime is also produced during aerobic processes.

PRUDER: This is a very important point which we should discuss further.

ANDERSON: If anyone is planning to use redox dyes to look for anaerobic microsites then do ask a good chemist to assist. One of the problems with these dyes is the phenomenon of poisoning. A dye itself has an oxygen demand. The best way to proceed is to reduce the dye and then let the reaction go back the other way. If you put in a dye which has to be reduced to get a color change, it can take up oxygen from the microsite and still not change color. There are some ghastly artefacts in the literature resulting from misuses of redox dyes.

COLMAN: When using the labelled-thymidine method for estimating bacterial production, could the addition of the thymidine actually increase the rate of production, because the bacteria would not have to synthesize it for themselves?

MORIARTY: No. It has no such effect. This is a fundamental aspect of the method. If it did, when we studied bacterial production over time using isotope dilution experiments, we would see curves that were not linear. The control of DNA synthesis is very much more than just the control of thymidine synthesis. Four bases go into DNA and its synthesis is controlled by the whole status of the cell with respect to energy supply and nutrient conditions. If the cell is not getting enough thymidine from an external source, it switches on some enzymes and makes some more. If it is getting too much thymidine it switches off these enzymes. The same applies to adenine, cytosine, etc. All these are rigidly controlled—in fact the concentrations of their precursors are controlled, so that more of any one has no effect on the overall rate of synthesis.

BOWEN: You mentioned that staining with acridine orange was a good method for counting, but that there were some problems with bacteria attached to particles. I have used a sonic probe to strip most of the bacteria off the particles. This is much more efficient than a blender. It also reduces the particle size and disaggregates the particles. After this, it is possible, by focusing up and down on a particle, to count the bacteria on the front and back surfaces. I am confident that by counting in this way we are not missing many bacteria. Have you or others present tried this?

MORIARTY: I have used both methods. I have used a blender (Ultra-Turrax) which is a very high speed blender. It is not like a Waring blender. It fragments particles like sand grains. I have also used a sonic probe. The problem with this is that as you give it more and more energy for a longer and longer time, you get less and less particles and everything becomes spread more evenly. If you do total counts during this process, your numbers go down and down, so it is obviously disrupting cells. In fact biochemists use sonic probes to break up bacterial cells to get the enzymes out.

BOWEN: But you can adjust the energy to an appropriate level.

MORIARTY: Yes, I agree; using a low energy for 30 seconds is very efficient, and better than a blender for sand grains. Even this does not give complete particle breakup and even distribution. I now use an ultrasonic probe! The main exception is for coral reef sediments. Coral reef sediments are difficult because, once you have removed the calcium carbonate, what is left is bound up with a lot of slime. In any situation where you have a lot of slime, neither blenders nor sonic probes can easily disperse the particles and bacteria. In these situations, if you need to do a lot of estimations, then muramic acid estimations are probably better. There is no really good technique and the techniques that we have, depend to a large extent on the artistry of researchers and fine-tuning in their respective laboratories.

BOWEN: You also mentioned the tedium of making measurements directly on images seen through the microscope and you referred to an image analyzer.

MORIARTY: Yes, these are being used in a number of laboratories. Perhaps John Fry would like to comment? I haven't got one in my laboratory.

FRY: We use an image analyzer for measuring the size of bacteria (Fry and Davies*) but we have not yet been successful in adopting this technique for making total counts. We use a Cambridge Instruments Quantimet 800, which unfortunately is no longer made. I have also used a Cambridge Instruments Quantimet Q10 which is much cheaper and is still on the market. This works equally well. We do conventional acridine orange staining and then photograph the preparation and measure the sizes of the organisms directly from the photograph. This is a reasonably quick method.

BOWEN: I have used a camera lucida to trace the images of detritus particles, bacteria and algae using different magnifications. These drawings can then be analyzed with a digitizer. A tablet costs only about two to three thousand dollars whereas image analyzers cost more than ten thousand.

We simply use the pen on the digitizer to mark the long and short axes and indicate which of several geometric shapes best approximate to the drawings. Using this approach you can quantify the volumes of a whole range of particles with reasonable precision. To guarantee accuracy, we take pure samples of bacteria or algae or sand grains and determine a conversion from measured volume to directly determined weight. With the aid of a microcomputer, this method is very quick and not at all tedious. For me this method provided something of a breakthrough. At reasonable speed and cost we can now make direct measurement not only of the biomass of various microorganisms (autotrophs and heterotrophs) but we can also quantify the amount of detritus. Formerly we had to rely on using estimates of differences for such data and 'difference' methods are always hazardous.

Finally I have a question about muramic acid determinations. What is the half-life of muramic acid in a dead organism in the natural environment?

MORIARTY: From work that I have done it is less than 24 hours in mangrove sediments. David White has done similar work in estuarine systems. I think his values were around 12 hours. It doesn't last long because it is associated with glucosamine and amino acids. Muramic acid is just one component of peptidoglycan—the macromolecule wrapped around the bacterial cell. It is very nutritious. Muramic acid from dead bacteria will be utilized rapidly in the presence of other live, actively growing bacteria. However, all these comments apply to surface sediments. In deeper sediments, it can persist for much longer. So muramic acid determination is a useful technique if you need to look at turnover in surface sediments. It is not a useful technique for deeper sediments.

MORIARTY: Should we include slime in biomass or should we call it detritus? Slime is important when we consider productivity, particularly when we compare work by different people. My view is that the term biomass should be restricted to actual living cellular material, which is shown clearly by epifluorescence microscopy. This technique does not show all slime. Slime can be seen by acridine orange staining but is very difficult to quantify. Slime is also extremely variable both in chemical composition and in its structure around the cells. You can find well-structured slime layers around some microorganisms, whereas in others, its structure can be very loose and it tends to dissociate from the microorganisms and become part of the general organic matter in the water body. Therefore, the term biomass should be restricted to living cells and biomass production to their protein and other internal biosynthetic activities. Slime production should only be considered in the definition of total organic matter production.

SCHROEDER: I agree. Slime may have an important function in making food available to the target animals. The slime can act as an ion exchange column and the large amounts of dissolved organic matter produced by phytoplankton may be absorbed on to slime. Therefore, the slime may be nutritious even though its own fiber molecules are unavailable to a target organism. It is these ionic exchange properties which allow the slime to provide organic molecules for the slime-producing bacteria. We can therefore regard slime as really having the property of changing dissolved organic matter into fixed organic matter.

GRAY: This reminds me of the long standing debate about mucus production in animals. Mucus is generally regarded as an excretory product. It is not included in standing crop estimations, so this agrees with Dr. Moriarty's definition. However there are many organisms - meiofauna, for example—which produce mucus to trap bacteria and then reingest the mucus. This has been called the 'gardening' phenomenon.

PRUDER: Regarding our definition of primary production, which refers to fixed particulate carbon, how can we frame some definitions to account for the large quantities of dissolved organic carbon which are released?

BOWEN: Ecologists generally treat dissolved organic carbon as a part of detritus, even though it is not particulate matter.

MORIARTY: Well, the definitions I have suggested here were meant to refer to bacterial production. However, as has just been pointed out, there are similar difficulties with defining terms for

the phytoplankton. The main point is to be clear about what we mean by production. If we wish to work out total net production with ^{14}C methods then, of course, we should include all the bacteria and slime, the phytoplankton and dissolved organic matter, leaching away from all organisms, that is labelled. But this would not be a production of biomass. These definitions are merely for our convenience in trying to understand these systems.

WOHLFARTH: I think we have said enough about slime. We can surely all agree that it is not alive.

SCHROEDER: Yes, biomass is cellular production—matter that is within a cell.

MORIARTY: A good definition.

ANDERSON: However many of these slimes which are mucopolysaccharide matrices do contain free enzymes. The work of Lock et al.** on epibenthic communities has shown that these are not simply microorganisms in an inert matrix. There is a holistic function served by the presence of free enzymes in that matrix. Therefore, while I agree with your definition of biomass we should not forget the importance of slime.

BOWEN: The same applies to the water column. There are many free enzymes in the water. They are concentrated on surfaces, for example, plant surfaces and roots. They have an important function in production of detritus by acting upon dissolved organic matter in the water column.

ANDERSON: Do they also act on colloidal material? The importance of colloidal material is not well understood. It is outside the conventional definitions of dissolved and particulate organic matter. Perhaps these free enzymes are important in colloidal degradation? They tend to floc on surfaces.

BOWEN: Right, the colloids tend to aggregate and precipitate. Biologists have not considered colloids to any significant extent.

SRINIVASAN: Are these free enzymes of microbial origin?

ANDERSON: I imagine so, almost exclusively. Where free enzymes are produced by bacteria in the aqueous medium, how can they control these enzymes?

KIRCHMAN: There is no good evidence for the production of free enzymes away from the cell wall.

MORIARTY: These enzymes are found with the Gram-negative bacteria which have two membranes around the cell with a space between them. The inner cytoplasmic membrane bounds the main cell, but there is a much looser outer membrane. Many of the enzymes associated with digestion of polymers are found in the space between the membranes. Thus the enzymes are in contact with the bacterial cell and the substrate and there are gradients of substrate and products. The Gram-positive bacteria lack this arrangement (Costerton and Cheng***) but again the enzymes do seem to be associated with the cell wall. One reason put forward for the production of slime by a lot of bacteria on particles is that it provides a microenvironment around the bacterial cells in which the enzymes responsible for hydrolysis can operate without being dispersed to the aqueous medium. The slime polymers provide a site for the enzymes and also help to hold their products. However, some of the enzymes are lost to the surrounding medium. There can be complex situations in which bacteria live 'on the backs' of other bacteria and make use of their enzymes. This is very difficult to study.

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***Costerton, J.W. and K.J. Cheng. 1975. The role of the bacterial cell envelope in antibiotic resistance. J. Antimicrob. Chemotherapy 1: 363-377.

Role and Impact of Anaerobic Microbial Processes in Aquatic Systems

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Abstract

The penetration of oxygen into sediments is very limited, and as a result sediments are dominated by anaerobic processes, particularly when the organic loading is heavy. Increasing sediment depth is matched by processes that occur at low redox potential. A nitrate zone is followed by sulfate and methane zones, in both of which substrates are supplied to the sulfate-reducing and to the methane-producting bacteria by fermentative bacteria. It is these fermentative bacteria that are the active hydrolyzers of organic detritus. Newly isolated strains of fermentative, sulfate-reducing and methane-producing bacteria are discussed. Knowledge of the range of substrates utilized by the fermenters and the sulfate-reducers has increased greatly in recent years. The methane-producing bacteria appear to be limited in their choice of substrates. Measurement of the rates of carbon mineralization in the nitrate, sulfate, and methane zones is discussed, followed by a short discussion on N-mineralization. The effect benthic animals have is to increase the rate of nutrient flux between sediment and overlying water.

Introduction

In the context of aquaculture it may be assumed that anaerobic processes are restricted to the sediment underlying the water column, and that because this water is aerated, there is some penetration of oxygen into the sediment. The depth of oxygen penetration will depend on the organic input to the sediment, and on the degree of water aeration and mixing, but the depth of penetration will normally be a few millimeters. This is in large measure due to the low solubility of oxygen in water. With moderate mixing, oxygen penetration was between 2 and 3 mm; vigorous mixing increased the penetration down to 3 to 4 mm (Fig. 1). This is typical of a

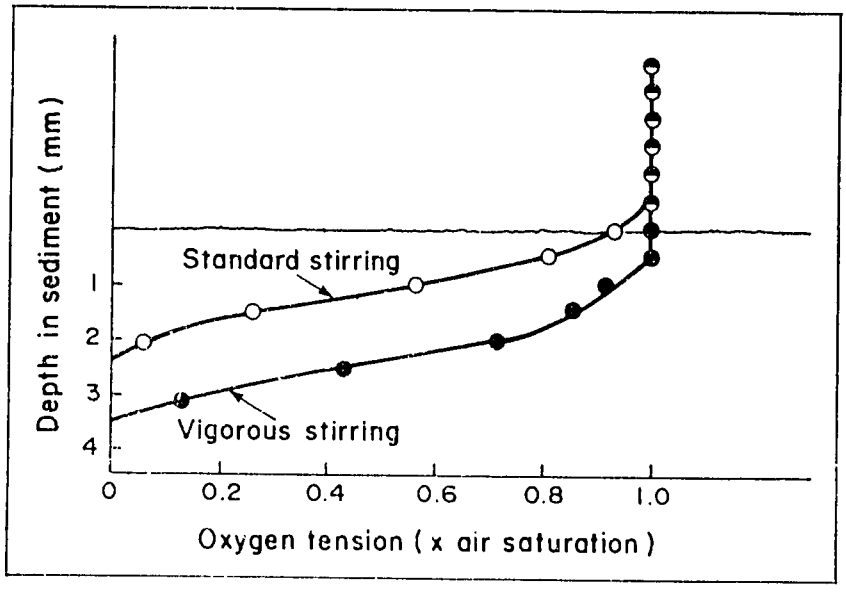

Fig. 1. Depth of oxygen penetration into marine sediment, with moderate and with vigorous stirring. Air saturation of surface water corresponded to 235 μM O_2 at *in situ* temperature of 20°C (redrawn from Revsbech et al. 1980).

sediment receiving a modest organic input and containing approximately 1% organic detritus. The higher organic loading in an eutrophic aquaculture system might be expected to restrict even further the depth of the oxidized layer, due to the increased rate of oxygen consumption. If the organic loading and oxygen consumption are sufficiently high, the anaerobic layer can move up into the water column, particularly if the latter is poorly mixed, as can occur in stratified water systems. Strictly anaerobic photosynthetic bacteria can, however, be found in oxidized water although it is unlikely that they grow under these conditions (Caumette 1984). There is a slight possibility that anaerobic microniches may occur in particles in the water column, but there is little doubt that the major site of anoxic metabolism is in the sediment.

Algal cells proliferate in water when phosphate, nitrogen and light are not limited. In the ocean, when grazing is limited in the algal spring bloom, the algal cells sediment and fuel the anaerobic processes in the sediment (Smetacek 1980). There can be a similarly high algal productivity in fishponds, and if the algae sink due to under grazing or other causes, the products of anaerobic metabolism may not be consumed at the sediment/water interface, resulting in the buildup in the water of products (ammonia, high pH, sulfide) toxic to fish. The composition of the products will mostly depend on whether the water contains sulfate (e.g., marine ponds) and on the composition of the sediment detritus. The presence of algae in aquaculture systems may, therefore, be dangerous because they can result in excessive anaerobic

activity in the sediments, but they have obvious advantages in that they produce oxygen, thus helping to keep the water oxygenated, at least during daylight hours.

When the reduced products of mineralization processes in anoxic sediment diffuse to the oxic interface, they are utilized by aerobic bacteria, whose biomass increases. This biomass is used as a substrate for detritus- and filter-feeding animals. When these animals are located within the sediment, the sediment surrounding the animals is usually aerated by burrowing and irrigation activities. These animal activities have profound effects on the exchange of nutrients between the sediment and the overlying water phase, and the animals themselves can be a source of food to the fish population. Bacterial cell production as a result of anaerobic processes is meagre and may be disconnected from higher trophic levels. Anaerobic processes are not inherently slower than those occurring in aerobic environments, as is demonstrated by the fast rates of organic degradation in the rumen (Hungate 1963). There is, however, a general consensus of opinion that aerobic decomposition of organic detritus proceeds more rapidly and more completely than if the same substrate were decomposed anaerobically. There may be some truth in this supposition, but there is little evidence to substantiate it, and there is no evidence for the accumulation of any specific organic substances in anoxic environments. This suggests that almost anything can be decomposed in the absence of oxygen, although some specific compounds, e.g., lignin, resins and waxes appear to be decomposed more readily by aerobic microbial populations (Fenchel and Blackburn 1979).

In this review, the different types of anoxic environments that exist in sediments were first examined. It will be seen that there is a change in environment with sediment depth, corresponding to a decrease in redox potential (E_h). Some general characteristics of these depth zones are discussed, mostly in relation to carbon mineralization. A survey of some of the important types of bacteria associated with these mineralizations is made, and is followed by a discussion of the actual rates of mineralization, how they are measured and how they are interrelated. There is no discussion on P-mineralization, because many of the processes are not biological.

Processes

The vertical stratification of the main zones of C-mineralization, as they occur in a typical sediment, are illustrated in Fig. 2. This stratification is principally a result of the increased energy that is available through the oxidation of reduced carbon compounds or of hydrogen, as the following electron acceptors are utilized: oxygen, nitrate, sulfate and carbon dioxide, in that order (Mechalas 1974). The zones are not totally distinct from each other; some overlap occurs, and there are probably other electron acceptors involved (e.g., Mn and Fe), that are not included in this scheme. It is a characteristic of this type of stratified system that the reduced endproducts of anoxic degradation (mainly ammonium, sulfide and methane) will diffuse to a more oxidized zone, where they will be oxidized. Methane from the lowest layer will be

oxidized by sulfate or oxygen; sulfide will be oxidized by nitrate or oxygen, and ammonium only by oxygen.

These individual zones will be discussed in more detail later, but it is important to emphasize here some of the more important characteristics of the zones. The bacteria involved in the mineralization of organic detritus in the nitrate zone are generally aerobic species, that can utilize nitrate as an alternative electron acceptor to oxygen, when the latter has been depleted (Fig. 2). It is a characteristic of these aerobic

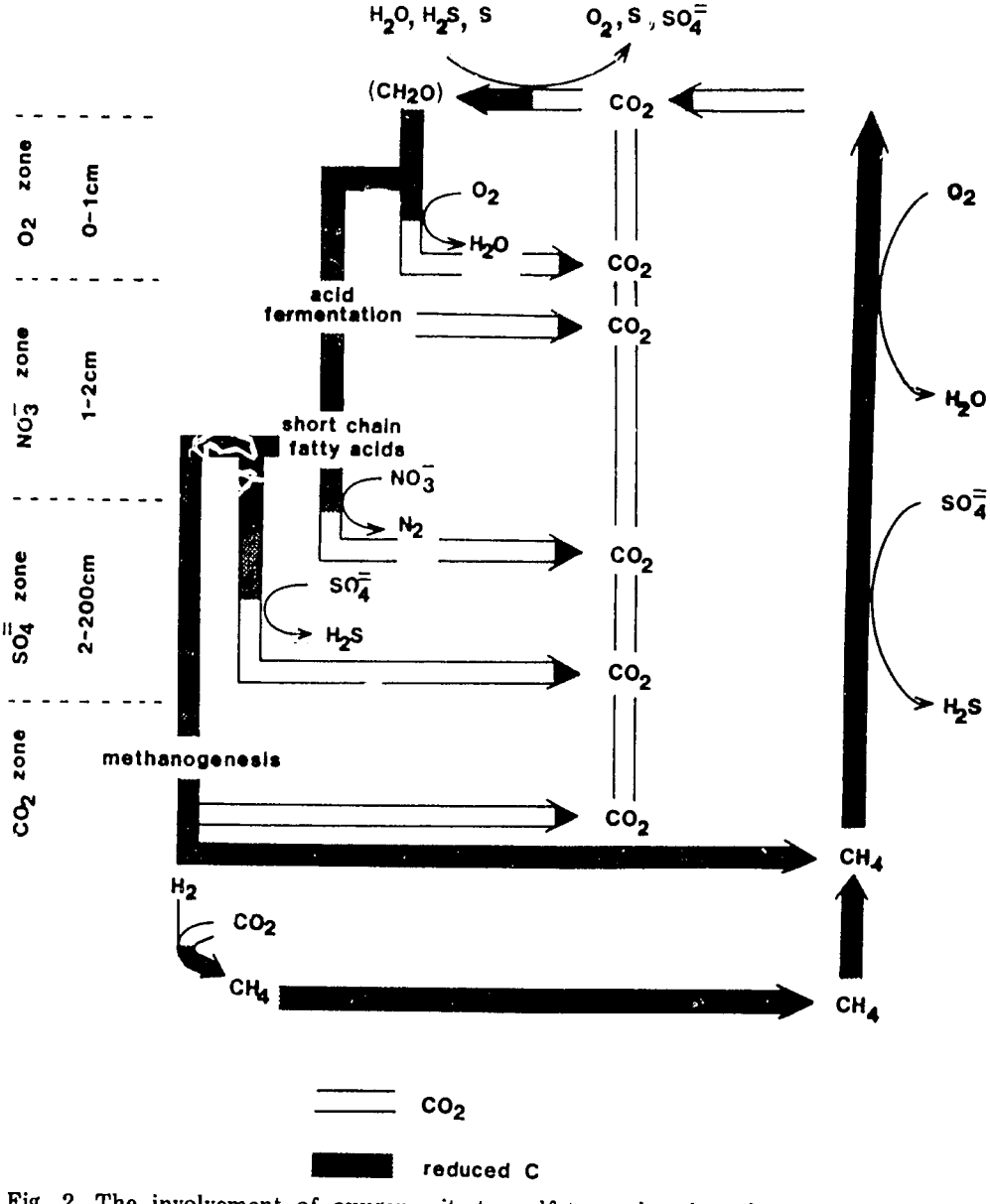

Fig. 2. The involvement of oxygen, nitrate, sulfate, and carbon dioxide as electron acceptors in the oxidation of detrital carbon, as a function of sediment depth and decreasing E_h (redrawn from Fenchel and Blackburn 1979).

bacteria that they can hydrolyze complex, polymeric, organic detritus and can also utilize the hydrolytic products, oxidizing the reduced carbon completely to carbon dioxide. It is a characteristic of the sulfate-reducing bacteria and of the methane-producing bacteria that they can neither hydrolyze the complex organic detritus, nor directly oxidize the initial hydrolytic products. Fermentative bacteria must first perform the hydrolysis and, in general, ferment the monomers to a variety of fermentation products before the detrital carbon can be used by the strictly anaerobic sulfate-reducing bacteria and methane-producing bacteria. The situation is made more complex by a tight coupling that exists between the fermenters and the anaerobic respirers (methane-producing bacteria, that oxidize hydrogen by carbon dioxide reduction, perform a type of respiration). This coupling is based on the necessity that the hydrogen, which is produced as an inevitable consequence of fermentation reactions, be removed otherwise it would inhibit further fermentation. This is the concept of interspecies hydrogen transfer (Wolin 1974). This involvement of hydrogen occurs at the level of NADH:

$$NADH + H^+ \leftrightarrow NAD^+ + H_2$$

At low hydrogen partial pressure, NADH can be reoxidized by the formation of hydrogen and fermentation can proceed, utilizing the oxidized NAD, otherwise the process stops. Obviously some sink for hydrogen has to be present in a situation where the hydrogen cannot be removed rapidly enough by diffusion. Several examples of the coupling of reactions producing hydrogen to those consuming hydrogen are quoted in Fenchel and Blackburn (1979).

In anaerobic sediment, the degradation of detrital carbon can proceed by two different mechanisms, depending on whether an oxidant (e.g., sulfate) is present (Fig. 3). The two alternative mechanisms are dependent on the production of fermentation intermediates, some of which will be directly oxidized by sulfate, otherwise they will be converted to acetate, carbon dioxide and hydrogen. If sulfate is present, the oxidation will go completely to carbon dioxide. In the absence of sulfate, the only possibility for further degradation is via methane production, which results in the production of equimolar amounts of carbon dioxide and methane from carbohydrate (CH_2O). It is also seen in Fig. 3 that twice as much methane would be predicted to originate from acetate as from the reduction of carbon dioxide by hydrogen.

Bacteria

The main groups of bacteria that are involved in the anaerobic degradation of sediment detritus are: hydrolytic, fermentative, sulfate-reducing and methane-producing bacteria.

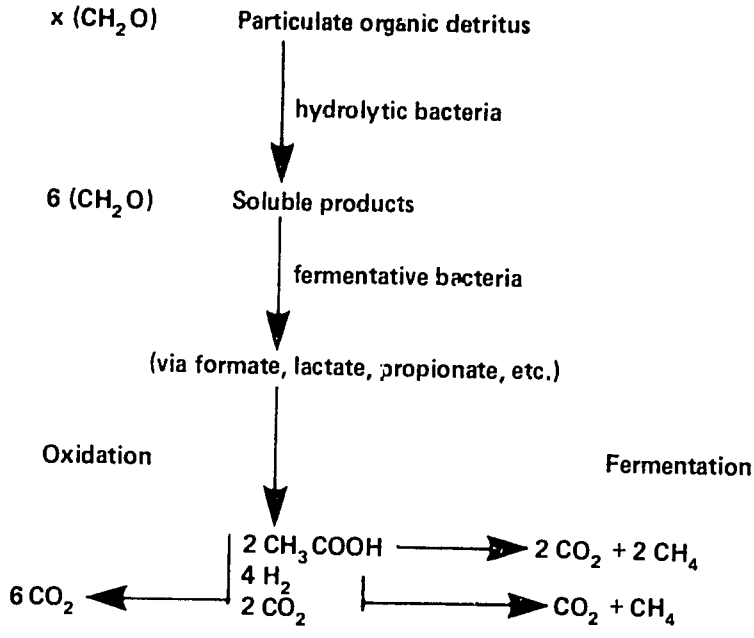

Fig. 3. The anaerobic degradation of detrital-C in an oxidative and in a fermentative environment.

Hydrolytic Bacteria

Almost nothing is known about this important group of bacteria that perform the vital function of making particulate organic matter available to themselves and to other bacteria in the form of soluble hydrolytic products. Part of the difficulty in studying this process is that the substrate is so ill defined. The organic detritus in the sediment, particularly in the anoxic layers, consists of a mixture of primary sedimenting material that could not be readily degraded and of complex bacterial residues, also recalcitrant to decomposition. It would seem likely that most of the hydrolytic bacteria will themselves be fermenters. They probably belong to a variety of genera, and by inference from ruminant studies, they will belong to genera such as *Bacteroides*, *Ruminococcus* and *Clostridium* (Hungate 1963). Several of these types of fermenting bacteria have been isolated, but there is no indication that they can hydrolyze complex polymers.

Fermentative Bacteria

Although there has been more success in isolation of fermentative bacteria in anoxic sediment, the numbers that have been studied in pure culture are not impressive. There are two main types of fermentative bacteria, those that are involved in the fermentation of the first soluble products of hydrolysis and those that ferment further the products of the first group. The initial fermenters may also be the

hydrolytic bacteria themselves, and there is the same problem for these bacteria in choosing the correct substrate on which to enrich. Just as the composition of the complex polymers is not known, the composition of the hydrolytic products in unknown. Virtually nothing is known in detail about individual species of these bacteria, but it is assumed that they, collectively, decompose almost any organic material reaching the sediment, because there is little evidence that any particular organic material accumulates in an unmodified form.

There has been more success in the isolation and study of the second group of bacteria, those that ferment the relatively reduced products (lactate, formate, propionate, etc.) that are produced by the primary fermenters. The technical difficulties associated with these studies are mostly due to the fact that the bacteria work together cooperatively. One bacterium will ferment a reduced substrate e.g., propionate (Bryant et al. 1967), producing acetate and hydrogen. If the hydrogen is not removed by another organism (e.g., sulfate-reducing bacteria or methane-producing bacteria), the fermentation is inhibited, and the bacterium cannot grow. Bacteriologists, who traditionally like their culture to be pure, have shown some reluctance to bow to the necessity of using consortia and have not evolved an alternative method for hydrogen removal. A group of microbiologists at the University of Constance has had considerable success in the isolation of some types of fermentative bacteria. *Clostridium magnum* is a sporeforming, anaerobic bacterium that produces only acetic acid from the fermentation of glucose, sucrose, xylose, malate, citrate, acetoin and 2, 3-butanediol, on which it was first isolated (Schink 1984a).

A number of bacteria capable of fermenting L-, D- and M-tartrate isomers have been isolated and identified as belonging to the genera *Bacteroides*, *Acetovibrio*, *Ruminococcus* and *Ilyobacter* (Schink 1984b). Highest numbers of tartrate-fermenting bacteria were isolated from freshwater creek sediments, and L-tartrate fermenters were most prevalent. Tartrate, citrate, pyruvate, and oxaloacetate were fermented to acetate, formate and carbon dioxide. Fructose and glucose were fermented also to ethanol.

Ilyobacter, a new genus of bacteria was first isolated from anoxic marine sediment, using 3-hydroxybutyrate as substrate, which was fermented to acetate and butyrate. Pyruvate and citrate yielded acetate and formate; glycerol gave 1, 3-propanediol and 3-hydroxypropionate; glucose and fructose gave acetate, formate and ethanol; and malate and fumurate gave acetate, formate and propionate (Steib and Schink 1984).

Propionigenium modestum is another species of fermentative bacterium isolated from marine and freshwater muds by the Constance researchers (Schink and Pfennig 1982). Succinate was fermented to propionate; fumurate, L-malate, oxaloacetate and pyruvate were fermented to propionate and acetate. These reduced endproducts are characteristic of fermentations by a pure culture, where instead of hydrogen being produced, the NADH is reoxidized by the reduction of some more oxidized intermediate products.

Strains of *Acetobacterium carbinolicum* were isolated from freshwater sediments and sludge; glucose, fructose, pyruvate, lactate, ethylene glycol, C1 to C5 primary

alcohols and methoxylated benzoates were among the substrates fermented, principally to acetate (Eichler and Schink 1984). Another new type of anaerobic fermenter, *Acidaminobacter hydrogenformans*, has recently been isolated from black mud (Stams and Hansen 1984). This bacterium ferments glutamate, but the fermentation products are altered by growing in mixed culture with sulfate-reducing bacteria or methane-producing bacteria, which act as hydrogen sinks.

Sulfate-reducing Bacteria

The Constance research group has also been responsible for extending our knowledge about the range of sulfur- and sulfate-reducing bacteria, starting with the isolation by Pfennig and Biebl (1976) of *Desulfuromonas acetoxidans*, which oxidized acetate at the expense of sulfur. Previous to this date no bacterium had been found to oxidize acetate at the expense of a sulfur compound, although complete oxidation of organic carbon is known to occur at the expense of sulfate (Jørgensen 1977) and sulfate oxidation of acetate is considered to be an obligatory step in the process (Fenchel and Blackburn 1979; Ansbek and Blackburn 1980). A sulfate-reducing *Desulfotomaculum acetoxidans* was isolated from a variety of sources, but its high optimum temperature and other characteristics suggested that it was of more significance in the oxidation of acetate in animal intestines than in marine sediments (Widdel and Pfennig 1977, 1981c). The marine sediment sulfate-reducing, acetate-oxidizing bacteria had different characteristics, including a requirement for salt, which was different from *Desulfotomaculum acetoxidans* (Laanbroek and Pfennig 1981). *Desulfobacter postgatei* was a true marine bacterium, one strain could use only acetate, whereas others oxidized ethanol and/or lactate completely to carbon dioxide (Widdel and Pfennig 1981a; Brandis-Heep et al. 1983; Gebhardt et al. 1983). Another new bacterium *Desulfobulbus propionicus* was isolated, which oxidized propionate to acetate, using sulfate, sulfite or thiosulfate as oxidant; lactate, ethanol, propanol and pyruvate were also utilized (Widdel and Pfennig 1981b). In the absence of sulfate, ethanol was fermented to propionate and acetate, and 1-butanol to propionate and butyrate by *D. propionicus* Lindhorst (Laanbroek et al. 1982).

Sulfate-reducing bacteria can use substrates other than hydrogen, short chain fatty acids and short chain carboxylic acids; they can also use amino acids as carbon sources and act as electron donors in sulfate reduction, as has recently been demonstrated for strains of *Desulfovibrio* that had been isolated from marine sediments (Stams et al. 1985). Some of these strains oxidized alanine, glycine, serine and other amino acids completely to carbon dioxide; other strains gave acetate as an endproduct. Other sulfate-reducing bacteria can also oxidize long chain fatty acids to carbon dioxide. *Desulfococcus niacini* is a sulfate-reducing bacterium that degrades nicotinic acid (Imhoff-Stuckle and Pfennig 1983).

Although sulfate-reducing bacteria that can oxidize methane have not been isolated, there is very good circumstantial evidence that methane in marine sediments is oxidized by the reduction of sulfate (Reeburgh 1980; Iversen and Blackburn 1981).

METHANE-PRODUCING BACTERIA

Methane-producing bacteria are a diverse group, with a variety of nutritional requirements (Balch et al. 1979). Most can oxidize hydrogen with carbon dioxide, and many (e.g., *Methanobacterium* species) were thought to be obligately autotrophic, using sulfide and ammonium as sole sources of S and N (Taylor and Pirt 1977). The picture now seems to be more complicated, and *Methanobacterium* species have been shown to assimilate organic nitrogen, sulfur and carbon sources (Bhatnagar et al. 1984). In addition to producing methane from hydrogen/carbon dioxide and C1 compounds, it has long been recognized that methane is produced from acetate (Mah et al. 1976). It is now well established that methane-producing bacteria can utilize other more complex substrates; *Methanococcoides methylutens* (Sowers and Ferry 1983), *Methanolobus tindarius* (Konig and Stetter 1982) and *Methanosarcina barkeri* (Hippe et al. 1979) all use methylamines. It seems unlikely, however, that methane-producing bacteria exist that can utilize the range of substrates that sulfate-reducing bacteria can oxidize. Because sulfate-reducing bacteria and methane-producing bacteria both compete for hydrogen and acetate, which are the most important products of fermentation, most ecological interest centers on their competition for these substrates.

Rates

It should be remembered, in discussing the rates of C-mineralization in the different zones, that hydrolysis and fermentation are almost obligatory steps, except perhaps in the nitrate zone, where the facultative nitrate-respirers can hydrolyze detritus and directly utilize the hydrolytic products.

In many situations, possibly in fishpond management, what is required is information regarding the reduced products that may come from the sediment and cause water quality problems. It would seem that the simplest solution to monitoring this potential problem would be to measure the input to the sediment and the rate of accumulation in the sediment; the difference between the two rates would be the rate of exit from the sediment. In fact this simplistic approach can seldom provide a solution, although input/output budgets are an important aspect of ecological budgets. A more realistic methodology, to define sediment/water interactions, is to measure individual rate processes in the sediment in order to define C-, N-, and S-cycles in the sediment and their interactions. This approach allows cross-checks to be made between the individual rates, to ensure that the different methodologies are reliable. Reeburgh (1983) has recently written an excellent review of the rates of biogeochemical processes in anoxic sediments.

In this present review, it is not possible to cover in detail the actual results of investigations in a wide variety of freshwater and marine sediments; more emphasis is placed on a survey of the types of methodology that have been employed.

C-Mineralization in the Nitrate Zone

The oxidation of C-compounds by nitrate obviously depends not only on the availability of nitrate, but also on the absence of oxygen. Oxygen, which inhibits the process, probably does so by competing for the carbon substrate (Payne 1973). Nitrate can be supplied from the overlying water or may be generated within the sediment by ammonium oxidation. It is unlikely that much oxidation of ammonium could occur in the anoxic sediments of fishponds, unless there is a seasonal fall in temperature and sediment respiration (Sørensen 1984). Fig. 4 illustrates how nitrate reduction and denitrification are dependent on seasonal effects, the peak of nitrate reduction occurred at the time of maximum nitrate concentration in the overlying water, as well as on the time of maximum denitrification. It is seen that the rate of nitrate reduction to ammonium is considerably higher than the rate of denitrification.

The most practical method for measuring denitrification is by acetylene blockage in intact sediment cores, where *in situ* gradients of oxygen and nitrate are maintained (Sørensen 1978; Andersen et al. 1984). Alternatively, Nishio et al. (1982, 1983) describe an elegant ^{15}N-procedure, which again demonstrates the importance of the reduction of nitrate to ammonium.

It is probably only in the most unusual circumstances that nitrate reduction plays a significant role in the oxidation of carbon; it is always of significance as a nitrogen sink.

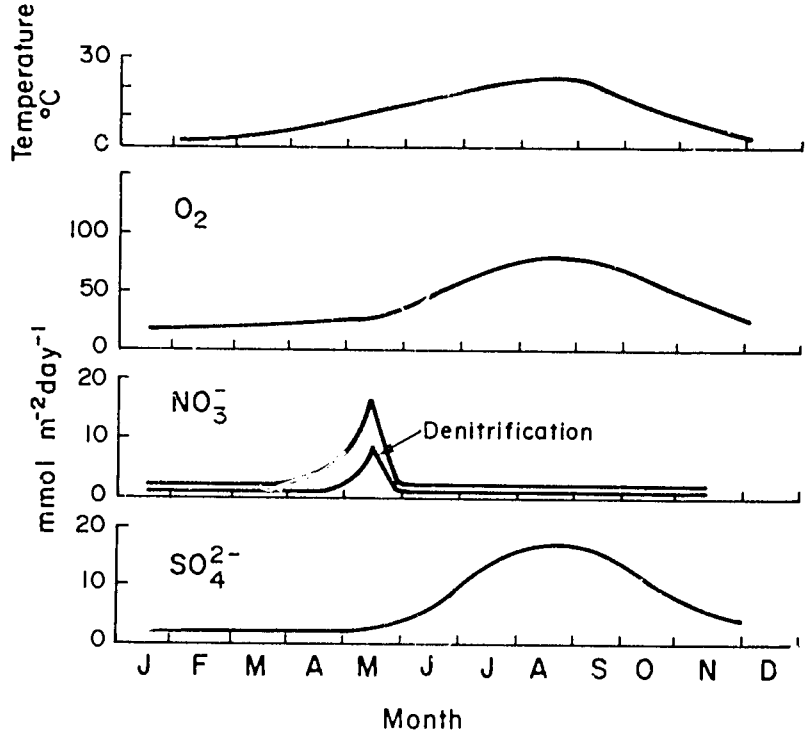

Fig. 4. Seasonal variation in temperature and the rates of reduction of oxygen, nitrate (to ammonium and by denitrification), and sulfate in Kysing Fjord (redrawn from Sørensen 1984).

Carbon Mineralization in the Sulfate Zone

There is a very large difference between the sulfate concentrations in fresh and in marine saltwater. In the latter, the concentration ranges from low values in brackish-water up to >30 mmol in oceanic water, whereas freshwater might typically have a maximum value of 0.2 mmol in Lake Vechten (Hordijk et al., in press) and Wintergreen Lake (Lovley et al. 1982). This does not mean that sulfate reduction is not an important oxidant of organic carbon in freshwater systems, but it is quantitatively less important than methane production.

Typically, in the past, sulfate reduction and hence carbon oxidation coupled to this reduction, has been measured by injecting ^{35}S-sulfate into sediment cores, which were then incubated under *in situ* conditions. The acid-volatile sulfide was distilled, and its radioactivity determined (Jórgensen 1977). There is evidence that a portion of the radioactive sulfide is converted to pyrite in salt marsh sediments. Because pyrite is not acid-volatile, it is not recovered by the normal distillation procedure and the rate of sulfate reduction is underestimated (Howarth and Merkel 1984). Some radioactive sulfide may also be converted to S and also lost, again due to the nonvolatility of S. In a marine sediment, the error involved in not measuring the label in S and in pyrite was 24 to 34% (Howarth and Jórgensen 1984). Sulfate reduction has been calculated to account for approximately 50% of the organic carbon oxidation in a Danish coastal sediment, uncorrected for pyrite and S effects (Jórgensen 1977). This calculation was made on the assumption that most of the sulfide diffused to the sediment oxic surface and was oxidized by oxygen; oxygen was thus used to oxidize reduced sulfur compounds and reduced carbon compounds. In order to derive the amount of oxygen used for carbon oxidation, the oxygen used for sulfur oxidation was subtracted. There is now evidence that oxygen consumption, in this type of sediment, is not balanced by carbon dioxide production (Hargrave and Phillips 1981; Andersen and Hargrave 1984). The implication is that all sulfide is not immediately reoxidized by oxygen, at least in the small cores that are used, and that no correction should be made for sulfide reoxidation, in calculating carbon oxidation by oxygen. Presumably, the sulfide is oxidized when the sediment is disturbed by storm action or by bioturbation. This point is important because the contribution to total carbon mineralization of sulfur compound reduction can be calculated from the total carbon dioxide flux minus the carbon dioxide calculated to be produced from oxygen consumption.

^{35}S-sulfate has also been used to measure the rate of sulfate reduction in lake sediments (Smith and Klug 1981; Lovley et al. 1982; Hordijk et al. 1985). Some problems are encountered because sulfate concentrations are generally low, and more accurate measurements may be made from measuring the decrease in sulfate concentration with time (Hordijk et al. 1985). The seasonal changes in sulfate concentration in the sediment of Lake Vechten are shown in Fig. 5. The sulfate concentration decreased to very low values, even in the surface sediment, during the summer months. The contribution of sulfate reduction to the total carbon mineralization was, however, quite significant. This is presumably because there was rapid

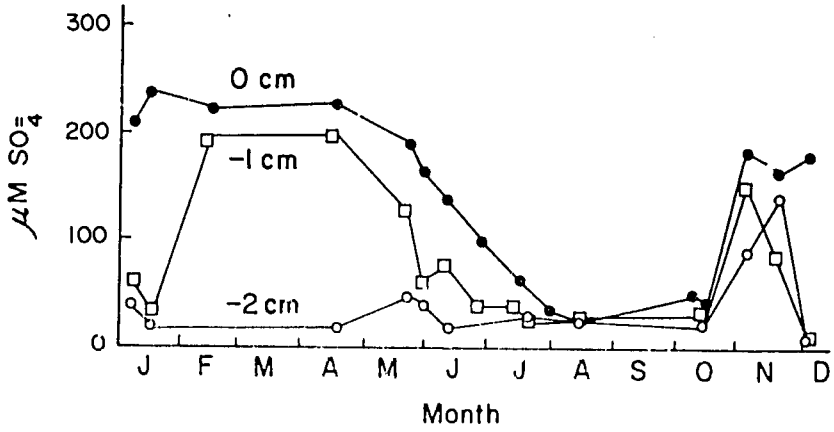

Fig. 5. Seasonal changes in pore water sulfate concentration in Lake Vechten sediments (redrawn from Hordijk et al. 1985).

reoxidation to sulfate; turnover rate constants for the sulfate pool of two/day were measured in Lake Vechten (Hordijk et al. 1985).

A more direct method for the measurement of sulfate reduction rates involves the inhibition of sulfate reduction by molybdenum, and the measurement of the rates of accumulation of the substrates that would have been oxidized by the sulfate. These substrates have included hydrogen, acetate, propionate, butyrate and methane, but they probably should also include amino acids (Stams et al. 1985) and long chain fatty acids. This approach has been used, mainly with the object of determining some of the substrates that are of importance in sulfate reduction in marine sediments (Oremland and Taylor 1978; Sørensen et al. 1981; Winfrey and Ward 1983), salt marsh sediments (Banat et al. 1981; Nedwell and Banat 1981) and in lake sediments (Lovley et al. 1982).

Radioactive tracer methods may also be employed to measure the rate of oxidation of key intermediates, such as acetate, in the sulfate zone. Rates of sulfate reduction may be made in parallel, or the rate of total carbon oxidation in this zone determined by other procedures, to be compared with the measured acetate oxidation rate. This approach avoids the use of inhibitors, in this case Mo, which can have nonspecific effects on processes other than that which is targeted. The radiotracer methodology is not without its problems, mostly due to difficulties in determining the pool of available acetate. In principle, tracer quantities of labelled acetate are injected into intact cores or sediment slurries (Ansbek and Blackburn 1980). The kinetics of the appearance of label in carbon dioxide are measured and the rate of turnover of the acetate pool to carbon dioxide, multiplied by the pool size, gives the rate of acetate oxidation. In general there is evidence that the acetate concentration that is measured in the pore water does not accurately represent the concentration of the acetate that is available to the sediment bacteria, due to adsorption of acetate

to sediment particles and complexing of dissolved acetate (Ansbek and Blackburn 1980; Christensen and Blackburn 1982; Shaw et al. 1984; Parkes et al. 1984). No adsorption was observed in autoclaved (and possibly denatured) lake sediments (Jones and Simon 1984) or in marine sediments (Sansone 1982), but the actual availability of dissolved acetate was not reported in these studies. Jones and Simon (1984) present evidence that diffusion of the labelled substrate may have a marked effect on the observed kinetics. Many different methods have been used to measure acetate and other short chain fatty acids in sediment pore water, but the most satisfactory procedure is to use high-pressure, liquid chromatography (Hordijk and Cappenberg 1983). This method has the advantage that short chain carboxylic acids and formate are also separated and measured. The concentrations of short chain acids in the pore water of Lake Vechten sediments have been measured with this method (Fig. 6b). There is a point source for lactate production at 3 cm, with sinks for lactate above and below this point. The upper sink may be attributed to the presence of sulfate-reducing bacteria close to the sediment/water interface (Fig. 6a).

There has been considerable interest in the production of methane in the sulfate zone (Mountfort et al. 1980) which is reviewed by Reeburgh (1983). Available evidence points to the competitive inhibition of methane-producing bacteria by sulfate-reducing bacteria, the latter having a lower Ks for both hydrogen and acetate (Lovley et al. 1982; Schonheit et al. 1982; Ingvorsen et al. 1984). Some methane may be produced in the sulfate zone from hydrogen, but probably little is produced from acetate; the methylamines may act as "noncompetitive" substrates for methane-producing bacteria (see review by King 1984). In terms of carbon flow, methane production in this zone is insignificant.

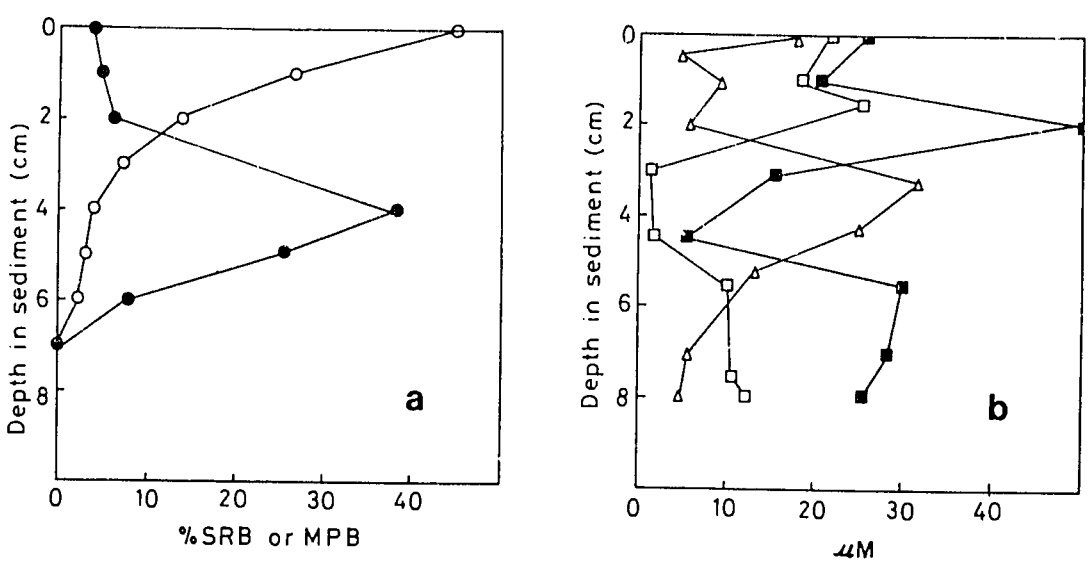

Fig. 6. Profiles of bacterial numbers, and of short chain acids in Lake Vechten sediments. a: The percentage of the total population of sulfate-reducing bacteria (SRB) (○), and of methane-producing bacteria (MPB) (●). b: The concentration of lactate (△), formate (□), and of acetate (■) in pore water (redrawn from Hordijk and Cappenberg 1983).

Carbon Mineralization in the Methane Zone

In relation to other mineralization processes, methane production in most marine sediments is quantitatively unimportant and no methane reaches the sediment surface. It is, however, a mechanism whereby some organic mineralization can proceed in the absence of sulfate. The processes that occur in the methane zone must be analogous to those occurring in the rumen and in sludge, but at very much slower rates. Possibly, methane-producing bacteria in deep sea sediment may be adapted to growth at low temperatures. In marine sediments that receive a high input of organic matter, e.g., Cape Lookout Bight, where sulfate rapidly becomes depleted (Martens and Klump 1980), very high rates of methane production occur, and bubbles of methane reach the sediment surface (Crill and Martens 1983).

Methane production is the terminal process for the mineralization of organic material in lake sediments and the rates are analogous to those for sulfate reduction in marine sediments. A high proportion of the organic carbon that enters lake sediment is processed through methane-producing bacteria (Cappenberg et al. 1982).

Methane production is measured by plotting the increase in methane concentration against incubation time. This apparently simple system may have some dangers, as differences in rate may be observed, depending on the presence or absence of a head space (N. Iversen, pers. comm.). Chloroform is an efficient inhibitor of methane production and chloroform inhibition has been used to determine the precursors of methane in lake sediments and their rate of convertion to methane (Lovley and Klug 1982).

N-Mineralization

As with the other rate determinations that have been discussed, the rate of most interest in relation to sediment/water interactions is the rate at which mineralized nitrogen leaves the sediment (Billen 1978). This determines to some extent the rate of primary production in the overlying water and at the sediment surface. The simplest method for measuring this net rate of ammonium production is to incubate sediment slurries anaerobically and to measure the increase in ammonium concentration with time (Blackburn 1979a). Alternatively intact sediment cores may be incubated (Blackburn and Henriksen 1983). More information on the mineralization processes may be gained by adding $^{15}N\text{-}NH_4^+$ to the sediment. In this way the total rate of organic nitrogen mineralization (d) is determined from the $^{15}N\text{-}NH_4^+$ dilution, and the rate of incorporation of ammonium into bacteria (i) is derived from d and the net rate of production (d-i). Both the slurry and intact core methods yield similar rates (Fig. 7a).

A plot of d and i against sediment depth (Fig. 7b) showed that both rates decreased with depth except for the stations in the Skagerrak where there was a peak in both activities at 6 to 8 cm, and where ammonium uptake was greater than ammonium production. This could be correlated with a high C:N ratio in the organic matter

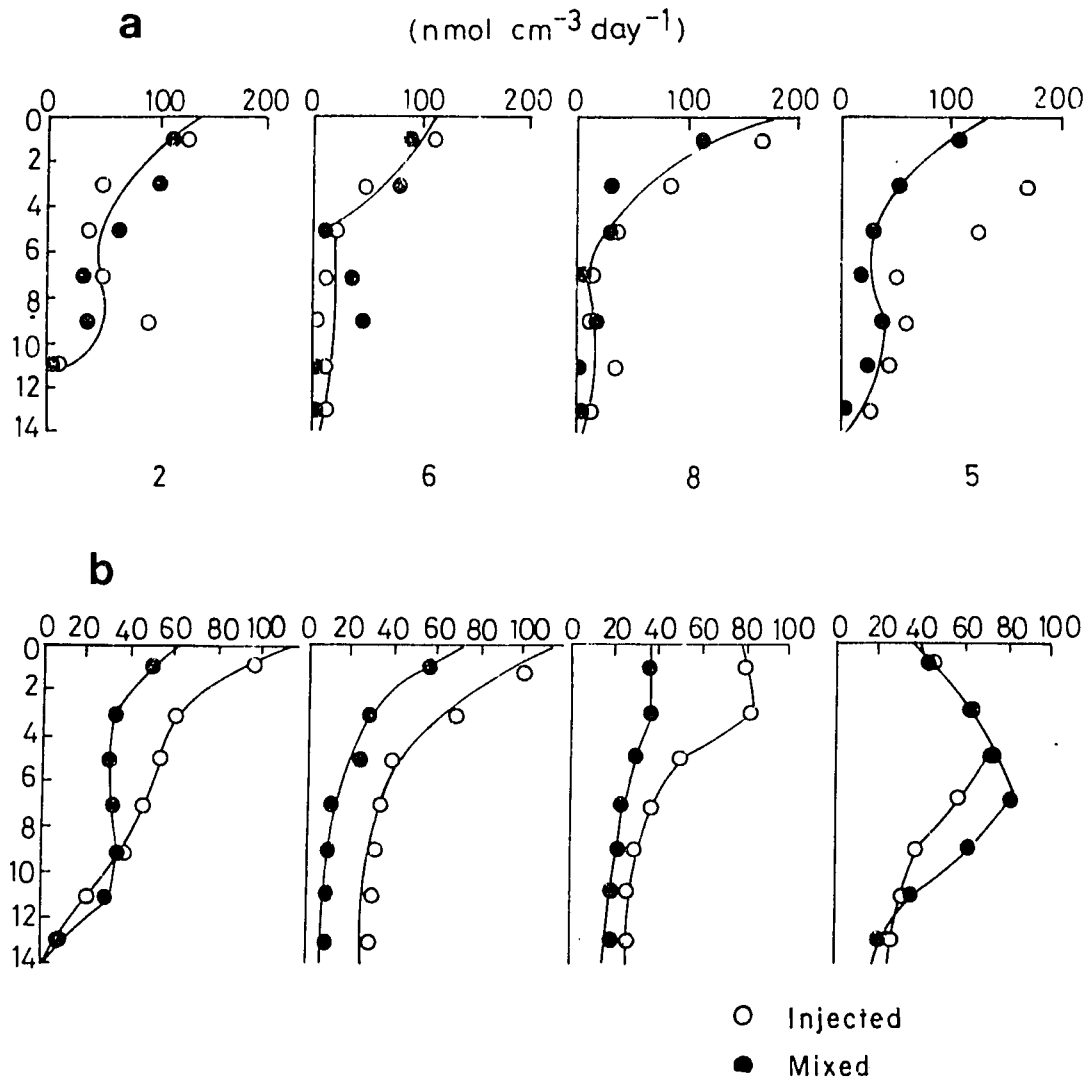

Fig. 7. Rates of N-cycling in Linfjorden, Denmark, plotted against sediment depth in cm (redrawn from Blackburn and Henriksen 1983). a: Rates of organic-N mineralization measured by ^{15}N-NH_4 + dilution with mixed (○) and injected (●) procedures at stations 2, 6, 8 and 5. b: Mean rates of organic-N mineralization (○) and bacterial-N synthesis (●) for some sediments of different types in Danish waters.

in these sediments. Models may be used to relate N:C ratios to rates (Blackburn 1979b; Blackburn 1980; Blackburn 1983) to make the comparison illustrated in Fig. 8. There is a discrepancy between the carbon oxidation calculated from the consumption of oxygen and sulfate, and carbon oxidation calculated as corresponding to the ammonium mineralization. The latter was almost twice the former for Danish and for Greenland sediments (Fig. 8). One interpretation of these data is that other electron acceptors are involved, possibly Fe or Mn. The advantage of making

this type of rate measurement, in addition to measuring fluxes across the sediment interface, is that cross checks may be made to ensure that the rates measured are accurate.

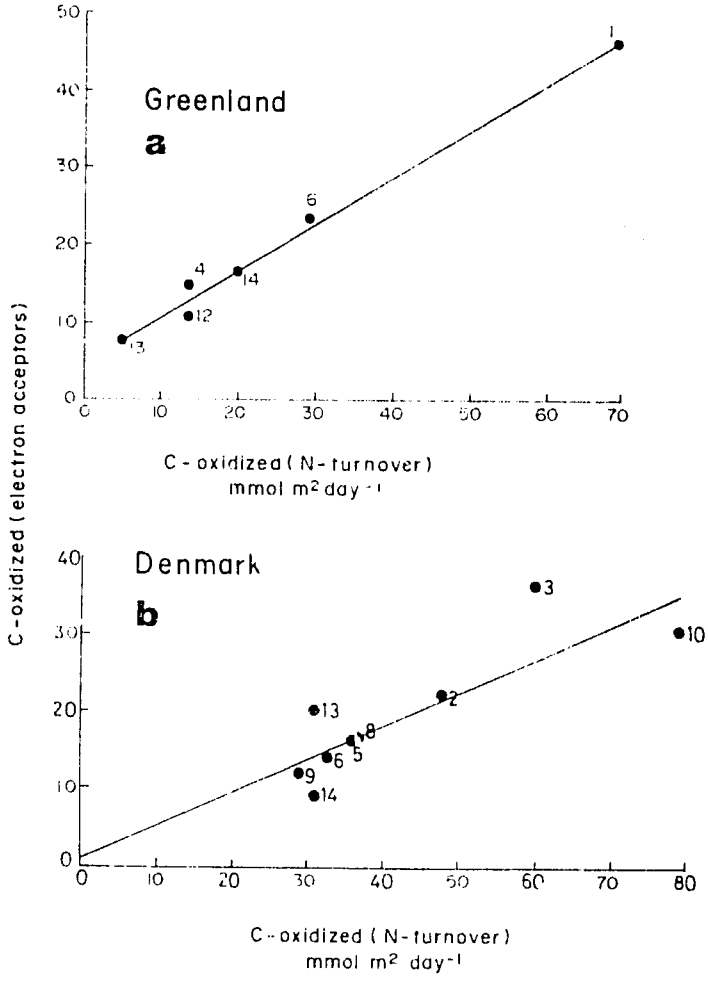

Fig. 8. Plots of C-oxidized, as calculated from the rates of reduction of oxygen and of sulfate, against the C-oxidized as calculated from the N:C ratio and the rate of ammonium mineralization. a: Greenland. b: Denmark (T.H. Blackburn, unpublished data).

Effect of Benthic Animals

The benthic fauna can increase the flux of ammonium from the sediment by 50% (Blackburn and Henriksen 1983). The bottom fauna, by building burrows, can have a marked effect on transfer rates between the sediment and the overlying water (Aller 1980; Aller and Yingst 1980). The effects of macrofauna can be quite complex, and the end result of their activities depends greatly on the concentration of nutrient in the overlying water. When the concentration of nitrate is high, denitrifi-

(Kristensen 1984). Benthic macrofauna increase the rate of oxygen consumption and detrital decomposition, but strangely, there is also a greater coupling between oxygen uptake and carbon dioxide production than in nonfaunated sediments (Kristensen and Blackburn, unpublished data). This means that the ratio of oxygen uptake/carbon dioxide production is greater when animals are present in the sediment. There is the unexpected implication that more anaerobic oxidation occurs in the presence of animals such as polychaetes than in their absence.

It must be concluded that animals have a marked effect on sediment decomposition, but not always what might be predicted.

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Discussion

SCHROEDER: When you discussed oxygen concentrations at different depths in the sediment your inference was that with standard stirring, anaerobic conditions were found 1 or 2 mm into the sediments (which had about 1% organic matter) and also that within particles of less than 1 or 2 mm diameter in the water column, anaerobic conditions would not occur. Of course large particles of 1 or 2 mm diameter or longer would sink to the bottom. Therefore you concluded that anaerobic conditions in particles are unlikely in the water column. I wonder if this is a valid conclusion. When you made your measurements in sediment you were measuring the oxygen concentration in pore water in between particles which had little organic content. Oxygen diffusion in such pore water must be happening or it would go anaerobic. Now consider particles of very high organic content (say 300 μm diameter) in the water column or on the surface of the sediment. If we could measure the oxygen concentration within such particles with a suitable microelectrode, I suspect that anaerobic conditions would occur inside them say 100 μm from their surfaces. This is because diffusion of oxygen through such a particle would be much slower than through pore water and also because there would be a lot of microbial activity within the organic particles. Therefore, there may well be anaerobic microenvironments associated with organic particles in the water column and in say the first mm of sediment in the presence of aerobic pore water.

BLACKBURN: The resolution of oxygen microelectrodes is about 5 μm. I have never known of such electrodes hitting anoxic microsites.

MORIARTY: But were your electrodes pushed through a slime layer around particles. This is a critical point.

BLACKBURN: Obviously you cannot see exactly what is happening at this resolution, so you don't know, but in thousands of trials I have never seen any anoxic 'pockets'. It is reasonable to assume that some of these trials did traverse slime surrounding particles. I suppose that you could envisage a sort of 'micro-cannon ball' with very active bacteria near the center around which there could be a lack of oxygen, but I wonder whether this would have any significance. If oxygen can diffuse in, even slowly, then other things can diffuse out. On the other hand, if oxygen cannot diffuse in, then H_2S for example cannot diffuse out. I doubt therefore whether this debate means very much in relation to the possible production of H_2S in the water column.

BOWEN: In the 1983 Symposium on Detrital Foodchains in Savannah, Georgia,* the relative importance of these processes in particles, whether suspended or in the sediment, was discussed in some detail. Joel Goldman showed that, based on calculation of diffusion coefficients, anaerobic microsites could be expected to occur even within relatively small particles. Hans Paerl went on to demonstrate such microsites in suspended organic matter in the Sargasso Sea and other oligotrophic environments by using indicator dyes. So it seems that anaerobic microsites in particles are abundant and probably important.

MORIARTY: David White** and his colleagues in Florida have used detailed lipid analysis to demonstrate the same thing. Lipids characteristic of anaerobes were found to be quite abundant in aerated sediments. This supports the view that anaerobic microsites within aerobic zones are probably quite common.

BLACKBURN: I stand corrected. I made the mistake of getting too far into the water column!

SCHROEDER: I have one brief comment concerning diffusion in and out of particles. In a nutrient-starved system, anaerobic conditions will help to maintain microbial activity because the need for N and P is about an order of magnitude less than in aerobic conditions. Therefore if oxygen and N and P diffusion into a particle falls off, anaerobic processes can still continue. The end products will be slimes, however, not bacterial cells.

BLACKBURN: A good point.

PIEDRAHITA: When you spoke about oxygen tension decreasing the further you go into the sediment, you showed a saturation concentration in the water itself. How do you think that the depth of penetration by oxygen into the sediment will change if there is less than saturation in the water?

BLACKBURN: Well, that situation is of course common and the curve just gives as we would expect much lower oxygen concentration.

PIEDRAHITA: Could that be an explanation for some localized anoxic conditions in fishponds?

BLACKBURN: Subsaturation values do not have a dramatic effect on this until you get down to about 10% saturation. Of course fish would not be too happy in water like this. What really determines whether conditions in particles are going to be anoxic is the rate of respiration within the particles, their quality and degradable energy. You can get growth on your teeth in less than a millimeter of scum because of the high energy quality of the substrates.

PULLIN: Can you comment further on the effects of temperature on microbial production, especially in tropical ponds where temperatures approaching or even exceeding 30°C are common and processes would seem to be very rapid?

BLACKBURN: It depends upon what regulates the system. Observations can be misleading. For example, you can get effects which are largely due to the amount of organic inputs rather than temperature. There is temperature sensitivity of course which we can demonstrate by raising the temperature during winter. However for most of the systems that we are interested in, it is the organic detrital inputs which are most important. Whether the temperature goes much above or below 30°C in the temperature range used for fish culture is probably irrelevant for the bacteria.

EDWARDS: How important are sediments in detritus-fed fishponds? There are large amounts of materials put into intensive ponds but there may be greater scope for microbial activity in the water column, the space for which is determined by a cubic relationship, rather than on the pond bottom which is determined by a square relationship. We cannot get rid of the sediment during a culture period, but can we just regard it as a physical feature and more or less ignore it as an important contribution to production?

BLACKBURN: In coastal ecosystems, we find a primary productivity of about 100 to 150 g C/m^2/year. With regard to N, probably about 30 to 70% of the primarily-fixed N is mineralized in sediments. Therefore, we cannot ignore the sediment processes.

EDWARDS: But perhaps this is not relevant to highly eutrophic waste-fed fishponds. These systems have inputs of up to 10 kg of N/ha/day. In some systems, there are bottom-foraging fish which stir up the sediment, but I wonder if we compare two systems, one, a pond with a conventional mud bottom and the other, a pond with a plastic bottom (on which sediment processes could scarcely operate) how their productivities would compare.

MORIARTY: In Malaysian prawn ponds which I have studied, the majority of the bacterial productivity occurred in the water column, even though materials settled to the bottom fairly quickly.*** If we compare productivities on a per m^2 basis in the water column and on the bottom, the former is much greater. This is not just because of the volume effect. We studied say the top 1 cm layer of the sediment. When considerable quantities of organic inputs like composts settle out, then anaerobic conditions predominate here. The water column is the better site for productivity. This difference was found even when we included the activities of anaerobic fermentative bacteria as well as aerobic processes. We did not include the activities of the sulphate-reducers, but these were probably unimportant.

PRUDER: In polyculture of carp and *Macrobrachium* in Hawaiian ponds, it has been shown by Dr. Laws, Professor Oceanography, University of Hawaii (personal comment) that only 10% of the N which is added as feed pellets is incorporated into fish and crustacean biomass.

Regarding the differences between plastic-lined and earthen ponds, Dr. Ed McSweeney, Amorient International, Kahuku, Hawaii, lined ponds with a plastic liner to prevent seepage and found drastically reduced productivity as compared to unlined earthen ponds. Similarly, in the highly intensive shrimp culture systems in Taiwan, the highest yields come from those with earthen bottoms. According to Dr. I-C. Liao, the yields from ponds with concrete bottoms are lower. So there seems to be some kind of a positive effect from an earthen bottom.

EDWARDS: Yes, but you are referring here to systems in which bottom feeders are being cultured. I was referring to systems in which plankton-feeding fish are cultured.

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Trophic Dynamics of Particle-Bound Bacteria in Pelagic Ecosystems: A Review

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Abstract

Although free-living bacteria have been shown to be important in microbial trophic dynamics of pelagic ecosystems, the role of bacteria attached to suspended detritus is unclear. The studies that have examined the biomass and production of attached bacteria and their contribution to detritus decomposition and grazer nutrition in the pelagic zone are reviewed here. The biomass and production of bacteria attached to detritus are relatively low and limited, as a first approximation, by the concentration of particulate matter. Rates of metabolic activity (cell size and uptake of organic compounds) of particle-bound bacteria are higher than rates of free-living bacteria. But there is little evidence that growth rates differ significantly. In spite of their low abundance, particles may be important microniches of high organic matter concentrations, which may allow selected species of bacteria and bacterivorous grazers to exist that otherwise would not in the bulk-phase. Because their biomass and production rates are low, particle-bound bacteria do not degrade detritus significantly, and appear not to be a major food source for grazers, although few direct measurements are available. Perhaps the most important role of attached bacteria is to transform detritus to useful food items for grazers. Although more work is needed on the relationship between grazers and bacteria-particle complexes, studies on particle-bound bacteria already provide information as to how aquaculture yields may be increased by stimulating the production of bacteria attached to detritus.

Introduction

Recent studies have shown that bacteria are important in mediating the flux of carbon and nitrogen from the pool of dissolved organic matter to organisms in higher trophic levels. Azam et al. (1983) termed this pathway for primary production, the "microbial loop". Estimates of the proportion of primary production consumed by bacteria and the microbial loop range from 10 to 50% (see reviews by Williams 1981; Azam et al. 1983; Ducklow 1983). Most recent studies on microbial trophic dynamics in pelagic environments have focused on free-living bacteria not attached to suspended particles. Microbial trophic dynamics include the production of biomass by bacteria assimilating organic compounds and the grazing on bacteria by larger organisms. In spite of much research, the role of particle-bound bacteria in microbial trophic dynamics is not as well understood as that of free-living bacteria.

Our goal here is to review what is known about the trophic dynamics of bacteria attached to naturally-occurring particulate detritus suspended in pelagic environments. We will try to point out generalizations and conclusions that are common to particle-bound bacteria in both freshwater and marine systems. Three general questions guide the organization of this review: i) How can comparing the metabolic activity of attached and free-living bacteria give insight into what controls bacterial growth and mineralization rates in pelagic environments? ii) What organisms, if any, are the predominant mineralizers of particulate detritus? iii) Are particle-bound bacteria and detritus significant food sources for higher trophic levels?

Particle Colonization by Bacteria

The early perception of pelagic systems, especially oceanic environments with relatively low rates of primary production, was that free-living bacteria were dormant and unable to grow on low concentrations of dissolved organic compounds. Bacteria attached to particles, on the other hand, were believed to be able to utilize not only the organic compounds that made up the particle, but also the dissolved compounds concentrated at the particle's surface (Jannasch and Pritchard 1972). Early measurements of bacteria numbers confirmed this view, as most bacteria that could be sampled by plate counts in pelagic systems were attached to particles. We now know that, although the number of particle-bound bacteria is probably being underestimated by current methods, most of the bacterial assemblage is usually not attached and is actively assimilating organic compounds and producing new cells and biomass.

Free-living and particle-bound bacteria have been enumerated in many pelagic environments by staining the bacteria with fluorochrome dyes and then counting the stained bacteria by epifluorescence microscopy (Zimmermann and Meyer-Reil 1974; Hobbie et al. 1977). At times bacteria attached to particles can account for as much as 90% of total bacterial abundance in some freshwater lakes and estuaries. However, particle-bound bacteria usually make up <10% of the total assemblage. This has been demonstrated repeatedly in many aquatic environments as summarized in Table 1. Because attached bacteria are usually larger than free-living bacteria (discussed in

more detail below), attached bacteria account for a larger proportion of total bacterial biomass than indicated by numbers alone. Still, bacterial biomass attached to particles is usually relatively small.

Table 1. Summary of reports on the proportion of total bacterial abundance attached to particles in pelagic environments.

Environment	% attached	Comments	Reference
Freshwater			
Ice House Pond	3 0.1	summer winter	Kirchman (1983)
F[illegible]er River	82		Bell and Albright (1981)
Lake Constance	4	annual average	M. Simon (pers. comm.)
Lake Mosso	17		Riemann (1978)
Lake Mendota	32 1	summer winter	Pedros-Alio and Brock (1983)
Estuarine			
Kiel Bight	3.1		Zimmerman (1978)
North Inlet Marsh	35		Wilson and Stevenson (1980)
Great Sippewissett Salt Marsh	1.9		Kirchman et al. (1984)
Little Sippewissett Salt Marsh	1.7		Kirchman and Mitchell (1982)
Eel and Mill Ponds	1.3		Kirchman and Mitchell (1982)
Nobska Pond	6.0		Kirchman and Mitchell (1982)
Fraser River Plume	69		Bell and Albright (1981)
Many sites	44	overall mean	Bell and Albright (1982)
Bay of Fundy	10	range = 1-90%	Cammen and Walker (1982)
Humber estuary	87	high particle concentration	Goulder (1977)
Newport River	3.3		Palumbo et al. (1984)
Palo Alto Salt Marsh	22		Harvey and Young (1980)
York River	85 10	stratified destratified	Ducklow (1982)
Marine			
Strait of Georgia	24		Bell and Albright (1981)
North Carolina coast	15		Ferguson and Rublee (1976)
South California coast	1-3	marine snow	Alldredge et al. (1986)
Gulf Stream	0.1-4.4	marine snow	Alldredge et al. (1986)
New York Bight	13		Ducklow and Kirchman (1983)

Microscopic examination has revealed that many particles suspended in pelagic systems are not colonized by bacteria (Wiebe and Pomeroy 1972; Kirchman 1983). In a study of several freshwater and estuarine systems, 30 to 80% of the particles were colonized by less than three bacteria (Kirchman and Mitchell 1982). In lakes most particles colonized by bacteria appear to be organic whereas those particles that appear to be inorganic are devoid of bacteria (Kirchman 1983; Palumbo et al. 1984; Simon 1985). In one lake there was a weak but significant correlation between the extent of bacterial colonization and amount of detrital carbon per particle, which is a rough index of particle quality (Fig. 1; Kirchman 1983). On the other hand, Cammen and Walker (1982) did not find a significant correlation between the number of attached bacteria and the organic carbon content of the detritus. Most of the particles examined in these studies had no definite shape and their origin could not be identified.

The particles examined in the survey studies just mentioned were sampled by standard methods, such as by Niskin bottle casts. In oceanic environments, there are at least two other classes of rare particles, which are hand-captured by scuba divers or sampled by sediment traps. The hand-captured particles include the houses of larvaceans (Alldredge 1972), feeding nets cast off by gelatinous zooplankton (Pomeroy and Deibel 1980; Pomeroy et al. 1984), and amorphous marine snow (Alldredge et al. 1986). Fecal pellets from calanoid zooplankton are sampled adequately only by sediment traps (Honjo and Roman 1978). The biomass of bacteria and other microorganisms associated with these particles can be quite high. Karl et al. (1984) suggested that bacteria associated with particulate matter contributed to a zone of high microbial production in mesopelagic waters. However, although some particles, such as marine snow, may harbor greater numbers of microorganisms, the abundance of these particles is so low that their impact on total biomass production and the turnover of organic compounds appears to be minimal. Alldredge et al. (1986) found this to be the case in their study of bacterial production associated with marine snow. Furthermore, most particles collected in sediment traps are not colonized by bacteria (Ducklow et al. 1985). It seems likely that freshwater ecosystems also have particles that are sometimes colonized by many microorganisms, but are relatively rare and not sampled adequately by standard methods.

It has been argued that current methodology underestimates the total number of bacteria associated with particles in pelagic systems. Filtration of samples, which is necessary for enumerating bacteria by epifluorescence microscopy, may dislodge bacteria reversibly attached to particles. Even if all the bacteria remain firmly adhered to the particle, attached bacterial numbers may be underestimated by around 45% because some bacteria underneath particles cannot be counted (Kirchman and Mitchell 1982). However, in many environments and especially oceanic waters, the number of particle-bound bacteria is so low that, even if our estimates are two to three times too low, particle-bound bacteria would still account for <25% of total bacterial biomass.

There are several factors that may limit the number of attached bacteria in a pelagic ecosystem. Two important factors are probably predation by bacterivores

and competition for organic carbon, both which of course also affect the population size of free-living bacteria. In particular, pelagic grazers may feed more heavily on particle-bound bacteria because these cells and the bacteria-particle complexes are larger prey than free-living bacteria (Ferguson and Rublee 1976). Prey size is an important factor in determining feeding rates by pelagic grazers.

The detritus concentration usually accounts for most of the variation in the number of attached bacteria. Several studies have found strong positive correlations between the number of attached bacteria and the concentration of particulate matter or particle numbers (Bell and Albright 1982; Cammen and Walker 1982; Kirchman and Mitchell 1982; Ducklow and Kirchman 1983), as illustrated in Fig. 2.

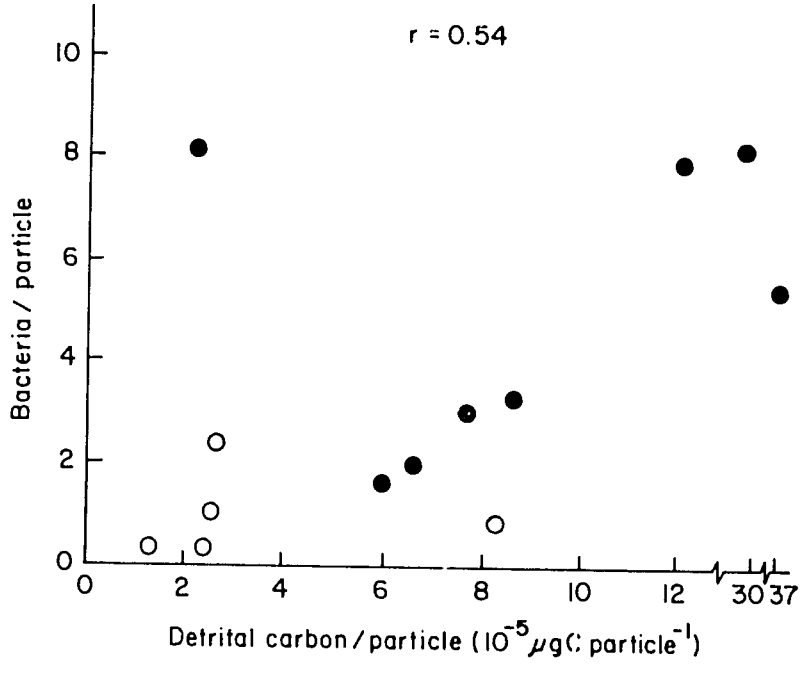

Fig. 1. Comparison between average number of bacteria per particle and amount of detrital carbon per particle in the surface layer of Ice House Pond. Spearman's correlation coefficient of $r = 0.54$ significant at $p < 0.05$. Samples from November to March = open symbols; remaining months = closed symbols (from Kirchman 1983).

However, it is not clear why the number of particles should be so important. Surface area on particles is not limiting because relatively few particles are actually colonized (see above), and much of the surface area of those particles with attached bacteria is not colonized by bacteria (Marsh and Odum 1979). There are at least two explanations (not mutually exclusive) for the observed dependence of attached bacterial numbers on the concentration of particulate detritus. Firstly, the number of attached

bacteria is directly related to the probability of a bacterium encountering a particle, which increases if the number of particles increases. Secondly, not all of the surface area on a particle is utilizable by bacteria, and thus the presence of more particles increases the utilizable surface area. There have been several laboratory studies on how physical properties of surfaces influence bacterial attachment (see review by Daniel 1980; see also Paul 1984), but more work is needed before these studies can be applied to understanding bacteria/particle interactions in natural aquatic ecosystems.

The proportion of the bacterial assemblage associated with detritus is not important for determining the role of attached bacteria in trophic dynamics. We argue below that the production of attached bacteria is the most important factor to measure. Data on the high numbers of free-living bacteria not associated with detritus have helped to disprove the hypothesis that free-living bacteria are dormant. The hypothesis that bacteria not associated with particles can grow and divide is directly supported by two lines of evidence. Firstly, bacterial abundance increases over time even when particles are removed (Fuhrman and Azam 1980). Secondly, a high proportion of unattached bacteria incorporate thymidine, which is only incorporated by actively dividing cells (Fuhrman and Azam 1982). Even so, the cellular metabolism of attached bacteria differs from free-living bacteria, as will be discussed below, and the survival of some bacterial species may depend on attachment to detrital surfaces (reviewed by Kjelleberg 1984).

Metabolic Activity and Growth Rates of Attached and Unattached Bacteria

Bacterial numbers alone do not indicate rates of bacterial metabolism, which includes the uptake of organic compounds and the production of new cells and biomass. It is entirely possible that bacteria found on particles have higher rates of metabolism and are growing faster than free-living bacteria and that the low numbers of bacteria typically observed on particles is a result of grazing or detachment of bacteria from the particle (Ferguson and Rublee 1976). Two types of data indicate that attached bacteria have higher metabolic rates than free-living bacteria.

Firstly, the rate at which radioactive organic compounds are taken up by attached bacteria is faster than the rate by free-living bacteria, when those rates are expressed' on a per cell basis. Uptake rates per cell for glucose, glutamate (Kirchman and Mitchell 1982), a mixture of amino acids (Simon 1985), dissolved ATP (Hodson et al. 1981) and phosphate (Paerl and Merkel 1982) are all faster for attached bacteria. Data on uptake rates for glucose, glutamate, and acetate by free-living and attached bacteria are given in Fig. 3.

In most of these studies, the uptake of a single added amount of the radioactive compound was examined. In their study of the Bay of Fundy, Cammen and Walker (1982) pointed out that whether the rates of uptake by attached bacteria were higher than rates by free-living bacteria, depended on the relationships among the

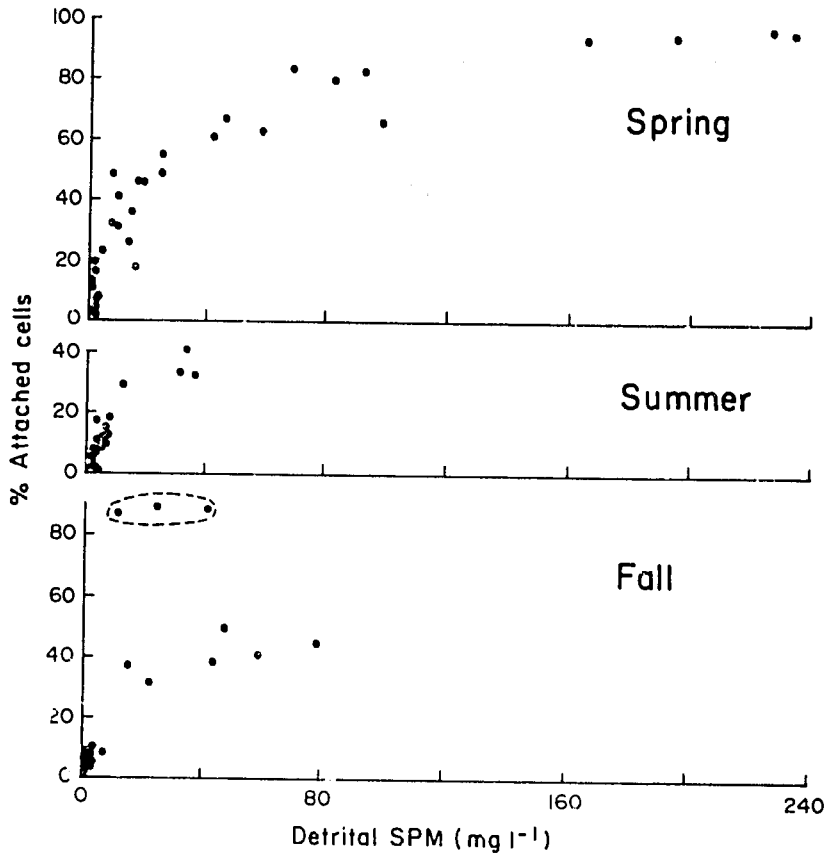

Fig. 2. Comparison of proportion of attached bacteria with concentration of detrital suspended particulate matter (SPM) in the Bay of Fundy (from Cammen and Walker 1982).

kinetic parameters of uptake (the maximum uptake rate [V_{max}] and the half-saturation constant [K]; see Wright and Hobbie 1965), the *in situ* concentration of the organic compound and the added concentration. They found that uptake rates per cell of compounds near the *in situ* concentration were faster for free-living bacteria than for attached bacteria in the summer, but not in autumn. However, V_{max} per cell for attached bacteria was always greater than that for free-living bacteria (Cammen and Walker 1982). Likewise, Goulder (1977) found that V_{max} of glucose uptake per bacterial cell was higher for attached bacteria than for free-living bacteria in an estuary, but at some locations and in winter the reverse was true (Bent and Goulder 1981).

Simon (1985) found that uptake rates for amino acids per attached bacterial cell were faster than for free-living cells in a freshwater lake, as determined by direct microscopic counts, but were similar to uptake rates per active bacterial cell, as determined by microautoradiography. Apparently, a greater proportion of attached

bacteria were assimilating amino acids compared with free-living bacteria. It is technically difficult to examine directly by microautoradiography the number of active attached bacteria, but this is needed to demonstrate conclusively that a greater proportion of attached bacteria than free-living bacteria assimilate compounds such as amino acids.

Kato (1984) recently pointed out another possible difference in the metabolic activity of attached and free-living bacteria (see also the review by Hoppe 1984). Kato (1984) found that attached bacteria mineralized ^{14}C-labeled particulate matter from algae faster than free-living bacteria, when rates were expressed on a per cell basis. Unattached bacteria probably do not synthesize hydrolytic enzymes to degrade polymers in particulate matter.

The second type of data indicating that bacteria attached to particles are metabolically more active than free-living bacteria is based on comparing cell sizes of these assemblages. In laboratory cultures of single species, cell size increases with growth rate (Pierucci 1978; Larsson and Hagström 1982). In several marine and freshwater environments, attached bacteria have been observed to be larger by several-fold than free-living bacteria. These studies have been summarized in Table 2. It should be noted that during some seasons and in some environments the average cell size of attached and free-living bacteria do not differ (Table 2). The difference in cell size explains much, but not all of the difference in uptake rates. That is, attached bacteria

Table 2. Summary of reports[a] on the cell size (volume) of bacteria free-living or attached to particulate detritus.

Environment	Cell size (μm^3) Attached	Free-living	Comments	Reference
Lake Constance	0.16	0.054		Simon (1985)
Lake Mendota	0.067 0.462	0.072 0.129	August October	Pedros-Alio and Brock (1983)
Ice House Pond	0.29 0.12	0.16 0.11	July February	Kirchman (1983)
Bay of Fundy	0.12	0.09	much variation	Cammen and Walker (1982)
coast of Georgia	1.5	0.02		Hodson et al. (1981)
coast of South California	1.02	0.18	marine snow	Alldredge et al. (1986)
Gulf Stream rings and slope waters	0.83	0.033	marine snow	Alldredge et al. (1986)

[a]Linley and Field (1982) and Zimmermann (1978) mentioned that attached bacteria were larger than free-living bacteria in the waters they examined, but average cell volumes cannot be calculated from their data.

have higher uptake rates than free-living bacteria even when normalized per unit biomass (Hodson et al. 1981; Cammen and Walker 1982; Palumbo et al. 1984). Based on the correlation between growth rate and cell size noted in laboratory studies, it would seem that attached bacteria do grow faster (shorter doubling times) than free-living bacteria.

There is little direct evidence, however, that attached bacteria do grow faster than free-living bacteria. Rates of thymidine incorporation into DNA per cell (which is a measure of bacterial growth, Furhman and Azam (1980); see Moriarty, this vol.), were similar for attached and free-living bacteria in a freshwater lake (Kirchman 1983) and in the New York Bight (Ducklow and Kirchman 1983). Jacobsen and Azam (1984) noted that bacteria in fecal pellets of zooplankton *(Calanus pacificus)* grew slower than bacteria in the surrounding seawater. Alldredge et al. (1986) came to the same conclusion based on their study of bacteria in marine snow.

The thymidine method was originally developed for measuring biomass production by free-living bacteria in the water column (Fuhrman and Azam 1980) and total bacterial production in sediments (Moriarty and Pollard 1981). The method has been tested extensively (Fuhrman and Azam 1982; Kirchman et al. 1982; Pollard and Moriarty 1984), but there are some problems with applying the thymidine method to particle-bound bacteria. For example, it is not clear whether bacteria embedded in particles would be exposed to a sufficient concentration of thymidine when the thymidine is added to the bulk-phase (see Moriarty, this vol.). However, in the study by Alldredge et al. (1986), thymidine incorporation per cell was actually much lower for attached bacteria than for free-living bacteria. Thus, thymidine incorporation by attached bacteria would have to be greatly underestimated, if in fact attached bacteria were growing faster and incorporation more thymidine per cell than free-living bacteria.

Using an entirely different method, Pedros-Alio and Brock (1983) also found that growth rates of attached and free-living bacteria were often very similar. They noted that the frequency of dividing cells (FDC) for attached and free-living bacteria did not differ significantly on 9 out of 13 sampling days during a year-long survey of Lake Mendota. Hagström et al. (1979) showed that FDC is correlated with bacterial growth rates. In Lake Mendota, FDC for attached bacteria was significantly higher than FDC of free-living bacteria on three dates, and on one day FDC for free-living bacteria was significantly higher than FDC for attached bacteria. Newell and Christian (1981) noted that the FDC for attached bacteria was significantly higher than the FDC for free-living bacteria in two samples from a salt marsh estuary.

Net bacterial production on detritus may be underestimated because bacteria may detach from the particle as cells divide (Jacobsen and Azam 1985). Even though the "parent" cell may be firmly attached to the particle, the daughter cell may not have the necessary extracellular polymers to remain attached to the particle. This is illustrated in Fig. 4. Although Jacobsen and Azam (1984) found that bacterial growth was relatively slow on fecal pellets compared with free-living bacteria, subsequent work showed that bacteria produced on fecal pellets became free-living as the fecal pellet was washed (Jacobsen and Azam 1985). The rate of washing was

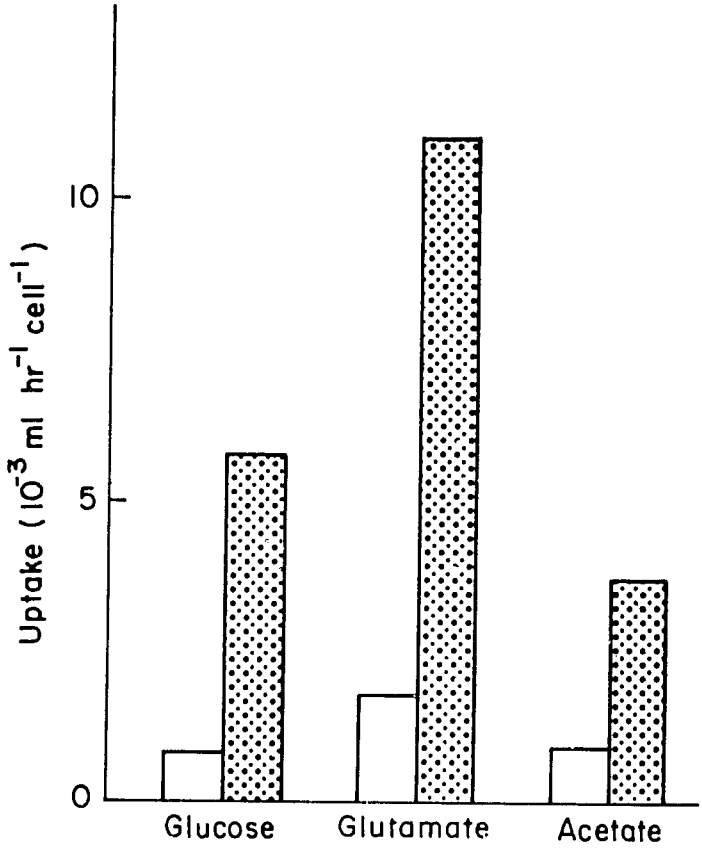

Fig. 3. Uptake of ^{14}C-labelled compounds by attached (spotted bars) and unattached bacteria in the Ice House Pond in summer (Kirchman, unpublished data).

equivalent to the sinking rate of fecal pellets. A similar process may apply to other forms of detritus.

Fast rates for uptake of organic compounds such as glucose, coupled with relatively low growth rates, imply that particle-bound bacteria have a lower growth efficiency than free-living bacteria (Kirchman 1983). Growth efficiency is the amount of bacterial biomass produced per total amount of organic compounds taken up. The growth efficiency of bacteria utilizing particulate detritus from marsh grass or macroalgae is in fact lower than bacteria utilizing dissolved organic carbon compounds (Newell et al. 1981; Linley and Newell 1984). Pomeroy et al. (1984) found that efficiency of bacteria utilizing tunicate feces was 10-20% which is low compared to the 50% usually assumed to be the growth efficiency of free-living bacteria.

In a study of particle-bound bacteria in a freshwater lake, Kirchman (1983) noted that the relative uptake rate of glucose was higher than of glutamate and acetate. He

hypothesized that glucose was being used by particle-bound bacteria for the production of extracellular polymers which are often composed of glucose (Sutherland 1972). Bacteria use extracellular polymers to attach to surfaces (Marshall et al. 1972). It is conceivable that polymers also protect the bacterial cell from predation. Thus, particle-bound bacteria may have to devote substantial amounts of carbon and energy to extracellular polymer production. High energetic cost of extracellular polymer production by attached bacteria could lead to low growth efficiencies.

The calculations of Jarman and Pace (1984) show that extracellular polymer production can account for a large proportion of total cell ATP demand. The synthesis of two polymers, xanthan from *Xanthomonas campestris* and alginate from *Pseudomonas aeruginosa*, required approximately 45% of the total ATP demand during growth in continuous cultures. Because both species produced large amounts of polymer, the energetic cost of polymer production by particle-bound bacteria in natural aquatic environments may not be as high. Still, the work of Paerl (1973) has demonstrated that even in natural systems attached bacteria are often embedded in a complex matrix of extracellular polymers.

Biomass Production of Attached Bacteria

Attached bacteria are often dismissed as being unimportant as a food source because the biomass of attached bacteria is small compared with total detrital carbon (see Bowen, this vol.). However, the potential contribution of attached bacteria to the nutrition of a grazer can only be critically examined by comparing the biomass production of attached bacteria with the nutritional needs of the grazer. The fluxes (production and consumption) of a particular substance are more important parameters than the standing stock in examining trophic dynamics of food webs.

Nevertheless, estimates of biomass production by attached bacteria suggest that attached bacteria are relatively unimportant as a food source, although some organisms appear to be specialized grazers of attached bacteria (see below). But no study has compared grazing rates with rates of biomass production by attached bacteria. What is clear is that the production of attached bacteria is relatively low in most marine and freshwater environments. Results from several studies on the production of attached bacteria are summarized in Table 3. These results indicate that, like the relatively low amounts of bacterial biomass associated with particulate detritus, the production of attached bacteria is usually <20% of total bacterial production. The fastest rates of attached bacterial production were observed in an estuary and over a coral reef. These results do not prove that attached bacteria are not an important food source, but they do indicate that the supply rate of free-living bacteria, phytoplankton and probably detritus is greater than that of attached bacteria.

The estimates of biomass production summarized in Table 3 are mainly from studies on suspended particles collected by standard methods. There is still great uncertainty as to the abundance and production of bacteria on rare large particles. Even though the contribution of bacteria attached to these particles to total bacterial

Table 3. Summary of reports on biomass production of attached bacteria.

Environment	Production on particles (μg C liter^{-1} day^{-1})	% Total bacterial production[a]	Reference
Crooked Lake	—[b]	0.3 — 29[c]	Lovell and Konopka (1985)
Lake Constance	0.36 — 2.7	15[c]	M. Simon (pers. comm.)
Ice House Pond	0.01 — 4.8	1.2 — 6[c]	Kirchman (1983)
Rhode River Estuary	1.0 — 13[d]	7 — 22[e]	Rublee et al. (1984)
Hudson River Plume	0.31[d]	10	Ducklow and Kirchman (1983)
New York Bight	0.034 — 0.061	5	
Atlantic Ocean	0.007 — 0.086	2.3 — 26[f]	Alldredge et al. (1986)
Pacific Ocean	0.050 — 0.74	1.3 — 13.4	Alldredge et al. (1986)
Antarctic	0.13	9	Fuhrman and Azam (1980)
Scripps Pier	0.91	7	
Coral Reef	1.0 — 32[g]	30 — 50	Moriarty et al. (1985)

[a]% Total production is the proportion of total bacterial production associated with particulate detritus as measured by thymidine incorporation and size fractionation.
[b]Not given.
[c]Range reflects variation over a year-long study and different depths.
[d]Assumed a conversion factor of 2.0×10^{18} cells per mole of thymidine incorporated and a volume to weight conversion of 1.21×10^{-13} gC μm^{-3} for a bacterial cell 0.1 μm^3.
[e]Range reflects variation over a year-long study and in different water masses in the estuary.
[f]Range from several sites. Biomass production on marine snow only was measured.
[g]Calculated using a biomass value of 15 fg C cell^{-1}

biomass and production may be small (see above), they may be important micro-niches for specialized grazers as discussed below.

Particles as Microenvironments

Presumably, attached bacteria differ from free-living bacteria in cell size and rates of organic compound uptake, because higher concentrations of organic compounds and inorganic nutrients are available to attached bacteria than to free-living bacteria. In addition to particulate detritus, attached bacteria can potentially use dissolved compounds concentrated at detrital surfaces (Marshall 1976; Shanks and Trent 1979). For example, organic compounds such as amino acids, peptides and proteins, probably adhere to clays suspended in pelagic environments; Dashman and Stotzky (1984) examined this in soils, but it has not been thoroughly examined in aquatic

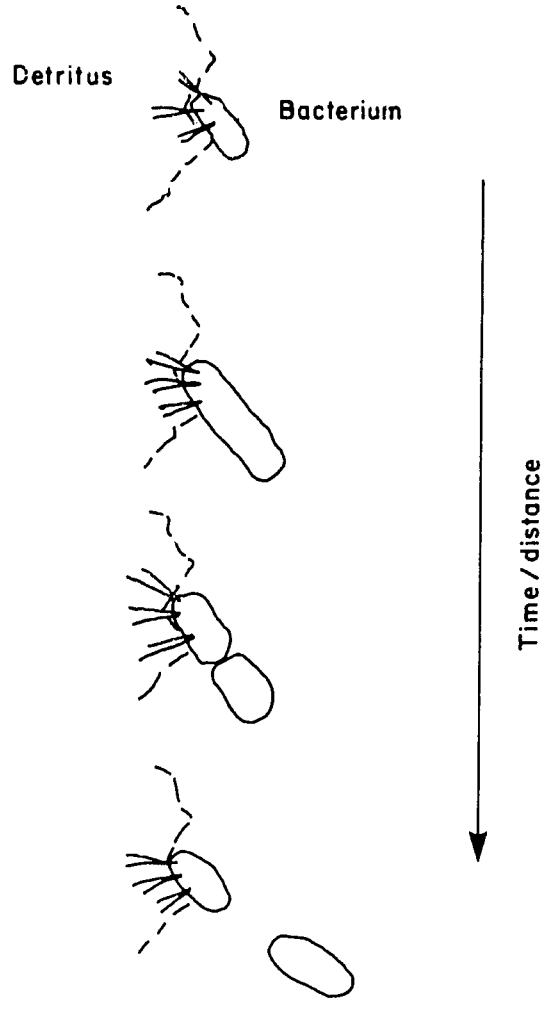

Fig. 4. Schematic illustration of how bacteria may become unattached from particles during cell division. The strands from the bacterium to the detrital particle are extracellular polymers. The particle is falling through the water column, hence the vertical axis is both time and distance.

environments. An important question to consider is whether or not the high concentrations of organic and inorganic compounds associated with the detritus have an impact on organisms not firmly attached to the particle. If so, detritus may support substantial amounts of bacterial production, even though production by attached bacteria is low when measured by conventional techniques.

Bacteria, which appear to be free-living by standard direct count methods, may attach to particles for short periods, utilize the carbon associated with the particle and then detach. Reversible attachment of bacteria to surfaces is well known (Marshall 1976). Bacteria can temporarily attach to surfaces through electrostatic interactions and need not produce extracellular polymers, which would in fact irreversibly attach the bacteria to the surface (Marshall et al. 1972). Hermansson and Marshall

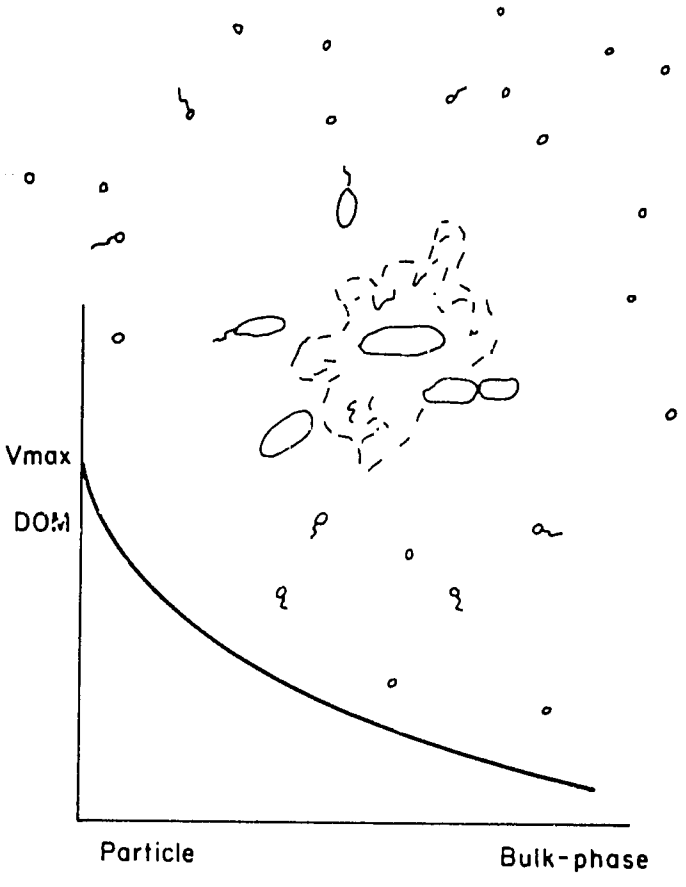

Fig. 5. Schematic illustration of how detrital particles may influence the metabolism of unattached bacteria. The bacteria attached to the particle and directly adjacent to the particle are larger and have a higher V_{max}, because of the higher dissolved organic matter concentrations (DOM), than bacteria in the bulk-phase where DOM concentrations are low.

(1985) demonstrated that stearic acid, a C_{18} fatty acid, could be mineralized by bacteria even when the fatty acid was adsorbed to glass. The fatty acid was apparently assimilated by reversibly-attaching bacteria, because Hermansson and Marshall (1985) observed few bacteria firmly attached to the glass. Bacteria in aquatic environments may use a similar mechanism to utilize compounds adsorbed to particles suspended in aquatic systems.

Reversibly-attaching bacteria have not been examined in natural pelagic ecosystems, but it seems unlikely that at any given time they are numerically abundant compared with bacteria unassociated with particles. Few bacteria could be in contact with a particle during any given sampling time, because the numbers of particles range from 10^3 to 10^4/ml whereas the total number of bacteria ranges from 10^5 to 10^6 cells/ml in pelagic systems. We may expect reversibly-attaching bacteria to have some characteristics, most importantly the large cell size, of the irreversibly-attached bacteria typically sampled in pelagic systems. Because the apparently free-living

bacteria are small, it seems that few of them would, therefore, be reversibly-bound. Thus reversibly-bound bacteria are probably not abundant in natural aquatic ecosystems. In aquaculture ponds with high detritus concentrations, however, bacteria may be considerably larger than those in natural aquatic ecosystems and many may be associated with particles (Moriarty 1986).

Another mechanism by which particles may influence the metabolism of surrounding free-living organisms is through the release of dissolved compounds. An important step in the degradation of phytoplankton detritus is the leaching of dissolved compounds from the particulate detritus (Newell et al. 1981; Cole et al. 1984). Leaching could create localized high concentrations of dissolved compounds around particulate detritus as illustrated in Fig. 5. The difference between the high concentrations around a particle and the low bulk-phase concentrations would create a gradient, which may be detected by bacteria (Bell and Mitchell 1972). Azam and Ammerman (1984) suggested that chemical gradients should also result in gradients in bacterial abundance, with the highest abundance near the source of the inorganic or organic nutrients. In turn, high abundances of bacteria would attract more bacterivores. Localized high organic carbon concentrations associated with particles may allow some bacterial species or metabolic forms to exist that otherwise would not be able to grow at the low organic carbon concentrations found in the bulk water phase (Azam and Hodson 1981).

Although it is certainly true that dissolved compounds are released from particulate detritus, it is not clear whether the half-life of the gradient or patch of high concentrations would be long enough for bacteria and other organisms to detect and utilize the organic matter (Williams and Muir 1981). At the submicron scale, diffusion is the main mechanism by which the gradient or patch of dissolved organic matter would disperse, and it is likely to occur too rapidly for bacteria to respond by chemotaxis. The relative importance of micropatches in supplying ammonium for phytoplankton growth (McCarthy and Goldman 1979) has been examined much more thoroughly than the relative amount of bacterial growth supported by micropatches.

Several studies appear to show that the bacterial species associated with detritus differ from free-living species, as determined by growth on solid media (see the review of Sieburth 1979). However, only about 1% of the bacterial assemblage can be cultured on solid media and thus can be identified. Our current methods are too primitive to allow any conclusions about species composition of bacterial assemblages associated or unassociated with detritus.

The microenvironment associated with detrital particles may be important in elemental cycles. Compounds that are byproducts of anaerobic processes, such as methane (Burke et al. 1983), have been found in aerobic waters. One possible explanation is that oxygen concentrations in particles could be low enough to allow anaerobic bacterial metabolism. Jørgensen (1977) has compared oxygen diffusion and particle size in regard to sulfur cycling in sediments. Other reviewers have discussed this topic more thoroughly than is possible here (Anderson and Meadows 1978; Paerl 1984).

Organic Particle Decomposition in Pelagic Ecosystems

The possible role of bacteria in the degradation of particulate detritus in pelagic systems is obviously important in carbon and inorganic nutrient cycles. It is perhaps equally important that bacterial degradation of detritus to CO_2 and inorganic nitrogen represents a loss of particulate carbon and nitrogen that could have been an important food source for higher trophic levels. On the other hand, bacterial biomass produced at the expense of particulate detritus may be an even more important food source than the detritus itself. Grazing on detritus and bacteria will be considered in the next section.

There is no doubt that bacteria can degrade organic detritus to CO_2 and inorganic nutrients and that this process is important in several environments. A question that needs answering is whether bacterial degradation is rapid compared to the sinking rate of the particle out of the pelagic zone and the grazing rate by zooplankton to maximize the utilization of the particulate matter. Recent studies indicate that bacterial degradation of particulate detritus is relatively slow in the pelagic zone.

Some investigators have calculated rates of particle decomposition from the disappearance of particulate carbon (Fallon and Brock 1980; Stuart et al. 1981) or the appearance of $^{14}CO_2$ derived from ^{14}C-labelled particles (Cole and Likens 1979). The contribution of attached bacteria to particle decomposition is overestimated by these methods because the calculated decomposition rates include the loss of particulate detritus due to abiotic leaching of dissolved organic compounds, which are utilized by free-living bacteria.

Alternatively, rates of particle decomposition due to the activities of attached bacteria can be estimated from biomass production rates of attached bacteria (Kirchman 1983; Ducklow and Kirchman 1983; Ducklow et al. 1985). These decomposition rates will be overestimates because some biomass production will be at the expense of dissolved compounds, not the particulate detritus. In a freshwater lake and in the New York Bight, turnover of detrital carbon due to bacterial decomposition was on the order of months (Ducklow et al. 1982; Ducklow and Kirchman 1983; Kirchman 1983; Ducklow et al. 1985) and thus is slow compared to other sinks of particulate detritus.

Jacobsen and Azam (1984) reached a similar conclusion based on their study of fecal pellet decomposition. They fed ^{14}C-labelled algae to the copepod *Calanus pacificus* and collected ^{14}C-labelled fecal pellets. The rate of $^{14}CO_2$ evolution by bacteria attached to the fecal pellets was relatively slow. Jacobsen and Azam (1984) hypothesized that bacterial degradation of sinking fecal pellets was not significant in the euphotic zone of marine systems.

If direct bacterial decomposition is relatively unimportant, how is particulate detritus degraded? In lakes and in coastal environments during some seasons (e.g., Malone et al. 1983), a significant proportion of particular matter may sink out of the water column to the benthos without being decomposed (Walsh 1983). Bacterial decomposition of detritus in sediments is of course important. However, in open oceanic environments only about 10-20% of primary production is exported out of

the euphotic zone (Eppley and Peterson 1979). Another important process in the decomposition of detritus is the leaching of dissolved organic matter from the detritus (Cole et al. 1984). In his study of the decomposition of phytoplankton detritus, Cole (1982) showed that direct decomposition by attached bacteria was relatively unimportant. The principal pathway for the degradation of phytoplankton detritus was via physical leaching of dissolved compounds from the particulate detritus and subsequent bacterial mineralization of the dissolved compounds (Cole et al. 1984). One final pathway of detritus decomposition is the ingestion and mineralization of detritus by grazers.

Grazing on Particle-Bound Bacteria and Detritus

Grazing is the mechanism by which bacterial biomass is transferred to higher trophic levels. It is clear that the biomass production of free-living bacteria is roughly balanced by grazing. Bacterial production rates are much higher than the *in situ* change in bacterial abundance, which is relatively constant over time. This has been observed for both freshwater (Pedros-Alio and Brock 1982) and marine systems (Ducklow and Kirchman 1983). Methods for measuring grazing on bacteria are only now becoming available, and these confirm that production rates are approximately equal to grazing rates (Fuhrman and McManus 1984). Thus, in those aquatic ecosystems where bacterial production is high relative to primary production, bacterial biomass is potentially an important food source for bacterivorous grazers, which in turn may be consumed by other organisms (Berk et al. 1977; Porter et al. 1979).

Most reports on bacterivory have focused on the consumption of bacteria not attached to detritus, and there are several recent reviews (e.g., Sieburth 1984; Sherr and Sherr 1984; Porter 1984). Although microflagellates are thought to consume most of bacterial production in pelagic zones of aquatic ecosystems (Azam et al. 1983), a wide variety of organisms are in fact capable of feeding on bacteria, free-living or attached to detritus (Table 4). It was once thought that the distance between setae (hair-like structures on feeding appendices) was too wide to allow some organisms, such as macrozooplankton, to feed on bacteria (e.g., see Marshall 1973). However, more recent models envision the feeding appendages acting more like paddles than sieves, which sweep food particles to the mouth in a boundary layer of water (e.g., Koehl and Strickler 1981). Only recently have the techniques for identifying potential grazers of bacteria in pelagic systems been developed (Caron 1983; Sherr and Sherr 1983).

Bacteria attached to detritus may be specialized food items for some grazers. The high density of bacteria on these particles may support bacterivores such as ciliates which otherwise could not survive on the low density of bacteria existing free in the water column (Fenchel 1980). Some bacterivores may specialize on bacteria attached to particles. Schoenberg and Maccubbin (1985) found that the feeding rates of *Acantholeberis*, *Chydorus* and *Eubosmina* (freshwater cladocerans) were higher on particle-bound than on free-living bacteria. Two other cladocerans, *Pseudosida*

Table 4. Examples of pelagic organisms known to consume free-living bacteria.

Organism	Size (mm)	Reference
Microflagellates		
Monas sp.	0.005	Sherr et al. (1983)
Ciliates		
Many species	0.1 – 0.2	Jackson and Berger (1984) Fenchel (1980)
Rotifers		
Brachionus calyciflorus	0.05[a]	Startweather et al (1979)
Larvacea		
Oikopleura dioica	0.2 – 1.5	King et al. (1980) Hollibaugh et al. (1980)
Cladoceran zooplankton (many species)	0.4 – 2.9	Pace et al. (1983) Peterson et al. (1978)
Calanoid copepods		
Eucytemora sp.	1.0[a]	Boak and Goulder (1983)

[a]The sizes listed here are approximate as the actual size was not given in the original report.

and *Ceriodaphnia*, showed no feeding preference (Schoenberg and Maccubin 1985). Boak and Goulder (1983) showed that the calanoid copepod *Eurytemora* sp. grazed faster on attached than on free-living bacteria. In oceanic waters, amorphous, floc-like particles are often heavily colonized by bacteria (Caron et al. 1982).

To date, investigators of pelagic bacterivory have examined the contribution of bacterial carbon or nitrogen to grazer nutrition and have ignored the contribution—or lack thereof—of specific compounds required for grazer growth. Phillips (1984) pointed out this problem in his review of benthic invertebrate nutrition. For example, bacteria do not have sterols nor long chain polyunsaturated fatty acids, which are necessary for growth of eukaryotic cells. The linolenic series of fatty acids, which are used in membranes, cannot be synthesized from short-chain fatty acids by metazoans (Phillips 1984). Phillips (1984) suggested that methionine is low in bacteria compared with the molar fraction in marine invertebrates, but this methionine deficiency is not substantial. The average methionine content of bacterial protein is 2.2% (S.E. = 0.1%; Reeck 1983) compared with the average of 2.5% for six invertebrates (Phillips 1984).

Bacterial biomass associated with particles is a small proportion of total detrital carbon and nitrogen (see Bowen, this vol.). However, Hobbie and Lee (1980) and Paerl (1978) pointed out that bacteria may contribute to grazer nutrition by producing extracellular polymers, which would not be included in most measures of

bacterial biomass and production. Polymer production by attached bacteria can be quite high (e.g., Paerl 1973, 1978) and may be a significant source of carbon for grazers. But the chemical composition of extracellular polymers indicates that they are not necessarily a good food source. The C:N ratio of extracellular polymers is usually quite high, as they are mainly composed of polysaccharides and only a few proteins (Sutherland 1972). Furthermore, some polymers are probably relatively recalcitrant to degradation by grazer digestion enzymes. Harvey and Luoma (1984) found that a deposit-feeding clam did not digest one bacterial extracellular polymer.

The role of detritus in grazer nutrition is controversial and interactions among bacteria, inert detritus and grazers are difficult to study. Zooplankton ecologists have shown that detritus could be a significant food source for zooplankton (Heinle et al. 1977; Chervin 1978). But these studies did not consider the role of bacteria in supplying carbon and nitrogen to grazer nutrition (see Roman 1984). On the other hand, benthic ecologists at first hypothesized that benthic invertebrates derived all their carbon and nitrogen by ingesting bacteria and other microorganisms colonizing detritus; the detritus itself was thought to be indigestible by invertebrates (Newell 1965; Levinton et al. 1984). More recent work has recognized the importance of both microorganisms and the detritus (Moriarty 1982; Levinton et al. 1984). The exact amount contributed to grazer nutrition by microorganisms versus detritus will depend on the grazer, and on the source and age of the detritus (Findlay and Tenore 1982; Tenore et al. 1984). Generally, detritivores can digest detritus from macroalgae and phytoplankton but not from vascular plants. Utilization of detritus by benthic invertebrates has been examined extensively and would require an entire review to discuss thoroughly. A few pertinent points are mentioned here.

The direct contribution of detritus to grazer nutrition probably depends largely on the relative amount of complex recalcitrant polymers in the detritus, a factor which is most obvious in freshwater and estuarine environments with high inputs of detritus from vascular plants. Important examples of complex polymers include cellulose, lignin and lignocellulose, which are the most abundant organic compounds in nature and are used in vascular plant structures (Hodson et al. 1984). Few organisms other than fungi and bacteria have the necessary enzymes to degrade compounds such as cellulose. Two exceptions appear to be a wood boring isopod (Boyle and Mitchell 1978) and a freshwater zooplankter (Schoenberg et al. 1984), which apparently have their own cellulases. Dall and Moriarty (1983) discuss the occurrence and action of cellulases in crustaceans. No organisms other than fungi and bacteria can degrade lignin and lignocellulose (Crawford 1981) and most of the degradation of these compounds in aquatic systems is by bacteria (Benner et al. 1984). Some pelagic grazers may have cellulolytic gut microflora analogous to terrestrial herbivores. Preliminary examination of one pelagic grazer, the copepod *Calanus pacificus*, did not reveal such a microflora (Jacobsen and Azam 1984). Because the degradation of cellulose, lignin, and lignocellulose is relatively slow and the gut passage time of grazers is short (<5 hours), digestion of complex polymers by pelagic grazers is probably insignificant.

An important role for bacteria may be to degrade or transform complex polymers to compounds that are digestible by pelagic grazers. In addition, bacterial degradation may release utilizable compounds that were previously bound in a complex matrix. These roles have not been extensively examined. In short, bacteria attached to detritus may be essential for grazer nutrition even though the biomass and production by attached bacteria are relatively low and the direct ingestion of bacterial biomass is insufficient to meet the respiratory needs of grazers.

The relative importance of different sinks for detritus in pelagic environments has been summarized in Fig. 6, at the risk of obvious oversimplification. Two important points are illustrated. The first is that the direct decomposition of detritus by bacteria is probably relatively unimportant. As shown in Fig. 6, sedimentation is the most important process for removing detritus from the surface layer, but this may not be true for all kinds of detritus and for many oceanic systems with deep mixed layers and relatively little sedimentation (Eppley and Peterson 1979). Detritus loses a substantial amount of organic matter through the leaching of dissolved compounds (Newell et al. 1981), but leaching decreases with detritus age and water temperature (Cole et al. 1984). Even though direct decomposition of detritus may be low, bacteria are indirectly important in detritus degradation because they utilize dissolved organic matter leached from detritus. Direct ingestion of detritus by macrograzers (e.g., calanoid copepods) is probably more important than degradation by micrograzers, which cannot prey on large particles such as marine snow or fecal pellets. What is not illustrated by Fig. 6 is the indirect role of bacteria in transforming detritus to digestible food for grazers.

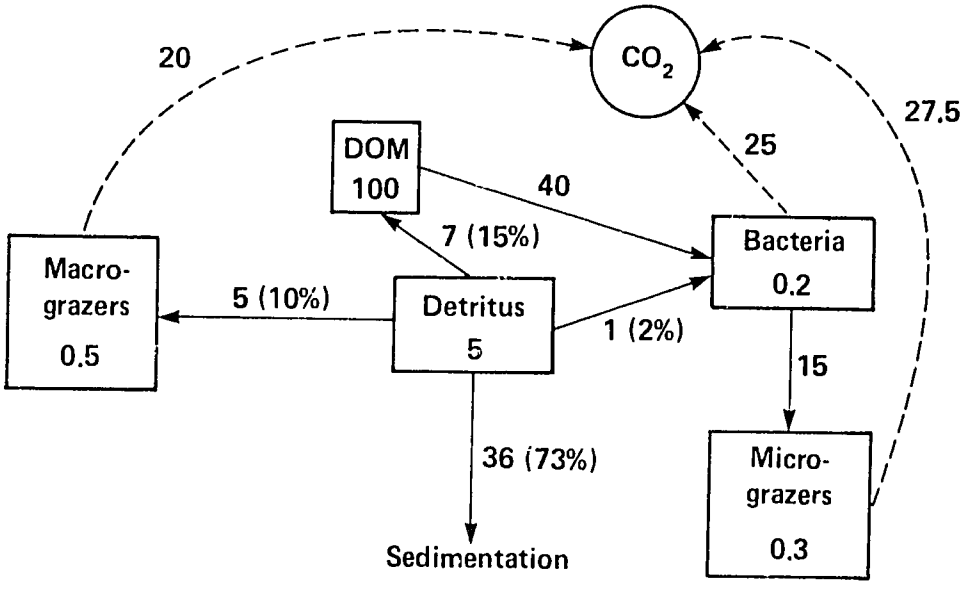

Fig. 6. Partial budget of the fate of detritus in pelagic ecosystems. Only sinks of detritus are listed, although sources and sinks of bacterial carbon are all drawn in. The values in the boxes are standing stocks with units of gC m^{-2}, assuming a 100 m mixed layer. The values outside the boxes are fluxes with units of g C m^{-2} $year^{-1}$. The percentages given represent the relative importance of various sinks of detritus. Based mainly on Peterson (1984).

The second important point illustrated by Fig. 6 is that the contribution of bacteria and the microbial loop to total heterotrophic respiration is large. Bacterial respiration, supported both by dissolved matter and particulate detritus, accounts for 35% of total heterotrophic respiration. The proportion due to microbial loop respiration, i.e., bacterial and micrograzer respiration, is 73%.

Detritus and Attached Bacteria in Aquaculture

In this review we have considered particle-bound relationships in the context of a two-layered pelagic zone such as a stratified lake or the open sea, in which the upper, illuminated layer is isolated from the lower, less productive layer by a thermocline. In such systems, particles and their attached microflora will sink to the lower layer and cease to influence processes in the upper layer. This model may not be appropriate for aquaculture systems. In aquaculture ponds particles may sink to the bottom, but are likely to remain available to organisms in the entire water column of the pond. Not only do bottom-feeding fish graze at the water-sediment interface but particles and their attached microflora can be resuspended into the water column and become available to pelagic feeders. The resuspended particles may be densely colonized by microorganisms, because the high concentration of particles and microorganisms at the water-sediment interface may stimulate attachment and growth on particles. Thus, it seems conceivable that at least in shallow habitats, including aquaculture ponds, attached bacteria will be more important than we have suggested above. Research on attached bacteria and detrital complexes should be carried out in specific aquaculture habitats.

In spite of the differences between aquacultural and natural aquatic systems, we can make a few suggestions as to how one may increase the production of free-living and attached bacteria and thus increase yields of aquaculture products.

Because bacterial production is dependent on phytoplankton production in pelagic systems, treatments that increase phytoplankton production, such as the addition of inorganic nitrogen and phosphorus, would also increase bacterial production (Hobbie and Cole 1984). Of course, the production of free-living as well as attached bacteria will increase, which may be more important than stimulating the production of attached bacteria alone

The production of attached bacteria would increase with the addition of some types of particulate matter. It seems highly unlikely that the addition of inorganic particles such as clay would stimulate bacterial production, although inorganic nutrients associated with clay may have an indirect effect. In contrast, the addition of organic detritus could stimulate attached bacterial production and may prove to be a direct food source for grazers. The detritus could be added directly without any pretreatment or it could be pretreated to increase availability of organic nutrients, at least for bacterial growth, if not for grazer nutrition. Other authors in this volume discuss how some pretreatments like composting may prove effective. Kerr et al. (1984) found that treating lignocellulose material with nitric acid followed by exposure to a lignin-degrading bacterium (*Arthrobacter* sp. KB-1) decreased lignin content and increased digestibility.

Conclusions

New techniques and approaches for examining bacterial growth have greatly increased our knowledge about the role of bacteria in pelagic ecosystems. It is clear that free-living bacteria can grow relatively rapidly on lower dissolved organic carbon concentrations than was previously imagined. It is also clear that bacteria attached to detrital particles are larger and have higher rates of metabolic activity than free-living bacteria, suggesting that free-living bacterial growth is limited at least in part by organic carbon concentrations. Most recent reports have tended to emphasize the importance of grazing in limiting bacterial growth, but both competition for organic compounds and grazing are probably important. Particulate detritus may be an important source of organic compounds for bacterial growth, even though the numbers of attached bacteria are usually relatively small. We know little about how the growth of apparently free-living bacteria (assuming it occurs) is supported by organic compounds from particulate detritus. More research is also needed on the relationship between bacteria and the rare but large particles not sampled by standard methods.

Attached bacteria probably are not directly important as food sources for pelagic grazers, except for those specialized for grazing on particles. The biomass and production of attached bacteria is low compared with the biomass and production of free-living bacteria and phytoplankton. As a consequence, the direct bacterial decomposition of detritus to inorganic nutrients is relatively slow. However, bacteria may be very important in transforming detritus to a digestible food source for pelagic grazers. Furthe work is needed on this question. Information about the digestion of detritus by bacteria may prove to be essential not only for understanding the nutrition of natural detritivores, but also for improving the productivity of aquaculture systems.

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Discussion

EDWARDS: I wonder if your viewpoint on the importance of attached bacteria would be different if you were used to working with tropical fishponds. You said that the numbers of attached bacteria increase with increasing food quality of particles from silt to organic particles. You have worked in natural ecosystems where, I assume, much of the organic matter is highly refractory

compared to that which we add to tropical fish ponds, such as livestock manures and decomposing macrophytes.

KIRCHMAN: That's right. Both the quality and the amounts will be higher in tropical fishponds.

PULLIN: Bacteria attached to particles should be easier for fish to eat than free bacteria. I would like to know more about the diversity of bacterial species which attach to particles. Are there many different kinds?

KIRCHMAN: We have identified less than 1% of the bacteria present. There are difficulties in separating those species which are more likely to attach from those free-living in the water column. I feel that it does not really matter whether the attached bacteria are pseudomonads or vibrios or other groups with regard to their food value for higher organisms.

Your point about attached bacteria being more available to filter feeders is interesting. Particle size is obviously important but I wonder whether our concept of filter feeding needs to be revised. Koel and Strickler (1981)* suggested that filter feeders do not "sieve" out food particles, but rely on viscous boundary layers along filtering appendages to trap food particles. It may be that bacteria do not need to be attached to particles of a given size range or attached at all to be harvested by aquaculture organisms. Again my bias is towards natural systems where most activity does not concern the particles. The situation in fishponds could be very different.

ANDERSON: With regard to the relationship between bacteria and the carbon content of particles, a high carbon content does not mean high carbon availability. For example, particles that have a high lignin content have high carbon and energy contents but these are extremely unavailable to bacteria and indeed to grazing animals. So one has to be careful about statements equating carbon content with availability, just as one does for Kjeldahl N content.

Also I think we must be careful to not classify particulate organic matter as a single class of compounds. It includes a range of particle composition from highly refractory compounds to those that are readily utilizable by zooplankton, for example.

KIRCHMAN: I agree. The carbon analysis is poor measure of quality. It was just the first step I was able to make.

ANDERSON: It is also dangerous to make assumptions about food availability to grazing animals by extrapolation from laboratory studies to field situations. The concentrations of bacteria, the sizes of bacteria and the size and composition of particles are highly variable. In the Antarctic, there have been some questionable extrapolations with regard to potential krill production. In fact, the food available to the krill is patchy. There are localized concentrations formed by currents. It is unwise to make generalizations and extrapolations, say from freshwater to marine systems or from oligotrophic to eutrophic systems about food availability because of all these variables.

KIRCHMAN: I quite agree.

FRY: Have you looked at the flux of bacterial populations associated with particles? You seem to have found few differences in the parameters which you measured for attached and free-living bacteria. I have been studying bacteria on aerobic sediments and looking at cell division (Davidson and Fry 1987**). One might expect that a bacterium attached for a long time would divide into two, four, eight, etc. I found very few examples of this. We used a mathematical model to investigate bacterial movement on and off the sediment particles and we found that such movements were extremely great. This was unexpected. Our conclusions are that there can be a high state of flux between free-living and attached bacteria. The latter are just at higher concentrations.

KIRCHMAN: I think that there are qualitative differences between the populations attached to sediment particles and those free in the water column rather than just differences in concentration. The point here is that the pelagic zone is a very different environment from a sediment/pore water system. We should not extrapolate from one to the other, but I think that there is considerable flux of bacteria on and off particles in the pelagic zone, as you have described for your sediment system.

MORIARTY: The methodologies available for such studies are worth commenting on here. Kevin Marshall*** and his colleagues have done a lot of work on this. The methods available to determine whether bacteria are attached or free in the water column are open to considerable doubt. There are shear forces, even under gentle filtration with virtually zero pressure difference, which are still great enough to separate bacteria that Marshall terms 'loosely associated' (not firmly bound). He refers to different stages of binding. Loosely associated bacteria and particles can even be separated by water collection techniques, let alone filtration. Therefore, many bacteria observed to be free-living in our samples may have been formerly associated with particles. This is an important point when we think of dissolved organic matter leaching out of particles. We don't know whether the enzymes responsible for this have been pushed out by bacteria into the particle or whether they are still closely associated with bacterial cell walls. The methodological difficulties here are very great.

KIRCHMAN: I wonder whether the bacteria attached to particles are available to grazers. This is an important question. If we have to use sonic probes, for example, to separate them, how available are they?

EDWARDS: There is a paper by Kuznetzov (1977)**** on fish like the silver carp that use mucus production to trap individual bacteria. This is in contrast to the traditional view of silver carp as narrowly specialized phytoplankton feeder.

MORIARTY: Of course the availability of attached bacteria depends on the target animal. Small zooplankton may not be able to use bacteria attached to particles that are quite large in relation to the size of the consumers. However, for some tilapias this makes no difference because the bacteria are lysed in a strongly acidic stomach. Digestion then follows in the tilapia intestine. Therefore, in order to determine availability we must consider whether ingestion and digestion are possible. The microphagous tilapias can take particulate matter down to 5 to 10 μm diameter. This raises the important question—exactly how do different animals digest and utilize bacteria? There are many aspects to such digestive processes. A particular problem is how to cope with cellulose and other molecules which are not normally digested by animal enzymes. There are some fish, for example, two marine kyphosids in Australia, which have a fermentative organ at the end of their intestine. They utilize red and brown algae through their fermentative gut flora. All animals of course have a bacterial flora on their gut walls derived from the bacteria they ingest. However, fermentation of celluloses normally takes a long time whereas the residence time of ingested material in the fish gut is normally rather short. I suspect, therefore, that aside from the few marine species with specialized fermentative organs, most species do not derive significant benefits from the processing of celluloses by their gut flora. This is why the external processes of fermentation in the fishponds are so important.

BILIO: What about mullets?

MORIARTY: Mullets do not have fermentative organs. They have a slightly acidic foregut and then a long intestine in which presumably the normal processes of digestion by enzymes take place.

PULLIN: Returning to the question of which foods can be utilized by which fish, some cultured fish have a stomach, others do not. I wonder whether in some culture systems we have fish which are ingesting potentially valuable food resources but not utilizing them efficiently. This may also apply to crustaceans.

MORIARTY: We will return to this in later discussions.

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*Koel, M.A.R. and J.R. Strickler. 1981. Copepod feeding currents: food capture at low Reynolds number. Limnol. Oceanogr. 26: 1062-1073.

**Davidson, A.M. and J.C. Fry. 1987. A mathematical model for the growth of bacterial microcolonies on marine sediment. Microb. Ecol. 13: 31-45.

***See Hermansson, M. and K.C. Marshall, 1985. Utilization of surface localized substrate by non-adhesive marine bacteria. Microb. Ecol. 11: 91-105.

****Kuznetzov, Y.A. 1977. Consumption of bacteria by the silver carp *(Hypophthalmichthys molitrix)*. J. Ichthyol. 17: 398-403.

Functional Roles of the Major Groups of Bacteria Associated with Detritus

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Abstract

The main physical and chemical features of a series of freshwater and marine habitats are described, with particular reference to how temperature, dissolved oxygen and redox potential change with depth. The natural cycles of carbon, sulfur and nitrogen are then considered by discussing those steps in the cycles that are predominantly controlled by bacteria and the relative importance of each step in aquatic habitats. The morphology and physiology of the main genera of bacteria involved in each process are then discussed, followed by the effect these factors have on the depth distribution of bacteria and the chemical environments in a range of habitats. The prime environmental features controlling the distribution of many bacteria appear to be the availability of organic carbon, the redox potential, the position of the H_2S/O_2 inteface and the light profile. In the last section of the review, temporal changes in some examples of bacterially dominated communities are discussed in relation to the physiology of the bacteria in the community. Especially considered are the diurnal movement of the H_2S/O_2 interface in stratified waters, the role of temperature in controlling seasonal population changes, the effect of deoxygenation in the water column and sediment and the successional changes in bacterial populations after herbicide-induced death of aquatic macrophytes.

Introduction

Aquatic habitats contain a great diversity of bacteria, from many different taxonomic and physiological groups. These bacteria play a wide variety of different roles in nature and their occurrence and distribution are controlled mainly by the environmental conditions in a particular habitat. Thus, a review of the functional roles of

bacteria must consider the organisms, the habitats, the physical and chemical conditions in the habitats and the processes carried out by the bacteria. As the subject matter of this review is broad, only a summary will be attempted. The literature quoted will give examples of relevant work and the review will attempt to show the breadth of existing knowledge, rather than give exhaustive coverage. Papers have been chosen to illustrate the main points made and are not intended to indicate the major contributions to the subject.

Organic detritus enters aquatic habitats from external or internal sources. Externally generated or allochthonous detritus might enter an aquaculture system from sources such as leaf litter, an inflowing stream, sewage effluent or added waste material. On the other hand, autochthonous detritus is internally generated and might come from an algal bloom or a decomposing stand of macrophytes. Most detritus enters the water column near the surface and sinks to the bottom sediments. Thus, to understand the functional roles of bacteria in relation to detritus it is important to understand how bacterial populations change with depth and time in the water column and sediment. The latter part of this review will cover these aspects of the subject. As aquaculture systems are usually rich in organic carbon the main emphasis here will be on eutrophic or nutritionally enriched waters rather than oligotrophic or nutritionally poor waters.

Most detritus-fed aquaculture systems are shallow and contain large amounts of dissolved and particulate organic carbon and other nutrients. Thus, they support dense growths of algae, at least in the surface waters. Additionally, some aquaculture ponds will have marginal stands of aquatic macrophytes or may even be fed with macrophytes or other plant matter. As little is known about the bacteriology of these systems, it is necessary to compare them with other waters about which more bacteriological information is available. A lot is known about the bacteria in lakes, ponds and coastal waters. These habitats have many environmental features in common with the oxidation ponds used for sewage treatment in tropical regions and these ponds are structurally and chemically quite similar to many detritus-fed aquaculture systems. Thus, aspects of the bacteriology of lakes, ponds and coastal waters are likely to be similar to aquaculture ponds.

Habitats and Environmental Conditions

The Water Column

Shallow waters in hot climates are rapidly heated during daytime and this can lead to temperature stratification with a pronounced thermocline. This condition is found in many oxidation ponds. Bacterial activity in the lower water layers, which are not open to the atmosphere, causes deoxygenation and so induces chemical stratification. These stratifications have been extensively studied in oxidation ponds (Marais 1966, 1970; Edwards and Sinchumpasak 1981; Ellis 1983) and may be stable under calm conditions or broken up and re-established daily in windy periods.

Similar stratified conditions are likely to be common in aquaculture ponds as dissolved oxygen concentrations as low 0 mg l^{-1} are not uncommon at dawn (Edwards et al. 1981).

Other types of fresh waters are also stratified (Wetzel 1983). Meromictic lakes are permanently stratified; the high conductivity waters at the bottom of these lakes (the monimolimnion) is separated from the low conductivity surface water (the mixolimnion) by a steep density gradient (the chemocline). The mixolimnion is kept mixed, aerated and warm by wind induced currents and sunshine. The monimolimnion is static, cold and isolated from the atmosphere. Hence bacterial activity removes all oxygen and increases concentrations of hydrogen sulfide, ammonia and total iron. Thus, steep gradients of all these chemical types are found at the chemocline. Deep dimictic eutrophic lakes normally have much lower conductivity throughout the water column but become thermally stratified in the spring. Deoxygenation begins in early summer in the deepest parts of the lake (Jones 1976) and the oxycline slowly moves vertically upwards in the hypolimnion, which is the water layer below the thermocline. Eventually the oxycline will reach the thermocline in late summer and cannot move any further upwards as the surface water layer or epilimnion is well mixed and aerobic. Chemical conditions in the anaerobic water below the oxycline are similar to those in the monimolimnion of the meromictic lake. Stratification in these eutrophic lakes is broken down in autumn when cool, windy conditions occur. This type of stratification can even occur in shallow waters if they are protected from wind (Moss 1969a).

Other types of stratification can occur in stands of aquatic macrophytes in ponds or the littoral regions of lakes. Permanently anaerobic water is often found at the base of stands of macrophytes (Goulder 1969; Rich et al. 1971) and below floating plants such as *Lemna* spp. (Duffield 1981). Surface water in such stands is often supersaturated with dissolved oxygen by day and anaerobic at night; this is caused by a large photosynthesizing plant biomass at the surface (Adams et al. 1974). Although temperature stratification can occur in coastal waters and sometimes persist throughout the summer (Fogg et al. 1985), chemical stratification does not occur.

Sediments

The bacteriology and environmental conditions are basically similar in most aquatic sediments. Much higher numbers of bacteria are found than in the water column (van Es and Meyer-Reil 1982; Meyer-Reil 1984). The population density depends on the grain size and organic carbon content of the sediment (Dale 1974); sandy sediments with large grains and low organic carbon have lower bacterial populations than silty sediments with small grains and high organic carbon contents. These two factors also determine the rate at which chemical determinants change with depth (Billen 1982). The surface layer of sediment is normally aerobic if oxygen is present in the overlying water, but the oxygen concentration decreases rapidly with depth in the sediment. In nutrient rich waters, oxygen is detectable

in only the surface few millimeters of sediment (Revsbech et al. 1980). The sediment E_h also decreases with depth, usually showing a sharp redoxycline somewhat below the limit of oxygen penetration. In the deeper anaerobic sediments reduced chemical species, such as ammonia and sulfides, accumulate. The surface sediment is often light brown in color due to oxidized species of iron whereas metal sulfides color the deeper layers black: this transition normally occurs quite sharply at an E_h of about 100 mV. If the water overlying the sediment is deoxygenated, the surface layer of sediment will not contain oxygen and the redox discontinuity layer will be at the sediment surface.

Thus the environmental changes, which occur with depth in sediments, are similar to those that occur vertically in the water column of eutrophic and meromictic lakes. However, as populations of bacteria are much larger in sediments the changes occur over a few centimeters rather than over several meters.

Processes Involving Bacteria

Bacteria are very important in the cycling of most of the major elements in aquatic habitats because they are responsible for many of the key steps in these cycles. In this section microbial aspects of the carbon, sulfur and nitrogen cycles will be considered briefly; more extensive coverage can be found elsewhere (Fenchel and Blackburn 1979; Krumbein 1983).

The Carbon Cycle

This cycle is very complex in water because many organisms are involved and many pools of different carbon compounds can be envisaged. Consequently, many very complex representations of this cycle have been presented (Wetzel 1983); however, a simple version will suffice here (Fig. 1). In this representation all higher organisms dependent upon the primary producers have been excluded. Algae and macrophytes exude soluble organic material as they photosynthesize, which enters the dissolved organic carbon pool. When they die these primary producers will be decomposed either directly by microorganisms, which are mainly bacteria in water and sediment, or by the combined shredding action of invertebrates and bacteria. The decomposition contributes to both the dissolved organic carbon and particulate organic carbon pools. In most aquatic habitats, there is much more dissolved organic carbon than particulate organic carbon. For example, the average values from over 500 lakes in Wisconsin, USA, were about 15.1 and 1.4 mg l^{-1}, respectively (Wetzel 1983). Heterotrophic bacteria grow on the particulate organic carbon and secrete exoenzymes that decompose it. The decomposition products enter the dissolved organic carbon pool and are taken up by these bacteria or other heterotrophs. The rate of particulate organic carbon decomposition is slow, and the turnover time is often in the order of tens of days. The dissolved organic carbon pool contains both refractory and labile materials. The refractory dissolved organic carbon contributes

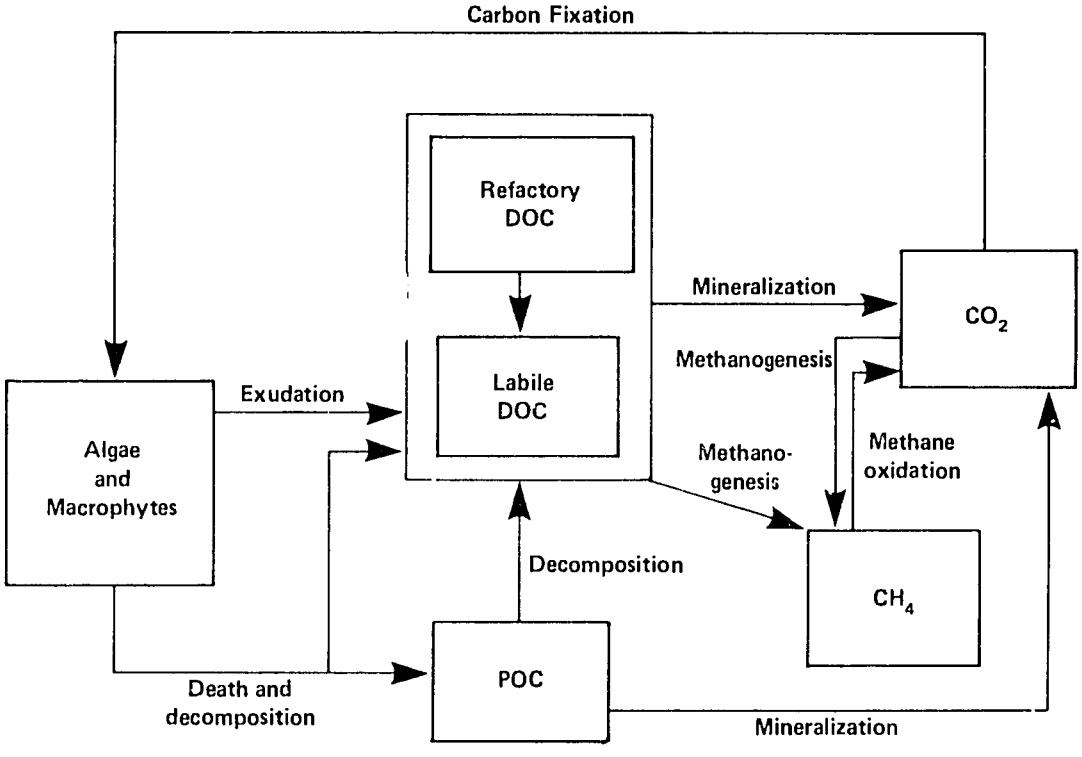

Fig. 1. Microbial aspects of the carbon cycle. DOC = dissolved organic carbon; POC = particulate organic carbon.

up to 99% of the total pool, is slowly broken down and consists of such substances as humic and fulvic acids and complex carbohydrates. The labile dissolved organic carbon pool consists mainly of simple sugars, fatty and amino acids that are present at very low concentrations (10-50 $\mu g\ l^{-1}$), but are rapidly mineralized. The flux through this pool is very high; the turnover time for these materials can be less than 1 hour in summer in nutrient-rich waters. A proportion of the dissolved organic carbon pool will be converted to methane, probably mainly through acetate, but most is respired to carbon dioxide. Some of the carbon dioxide is used for methanogenesis and some for photosynthesis by algae, macrophytes and photosynthetic bacteria. Carbon dioxide is also fixed by autotrophic bacteria and some heterotrophs.

A simple model for carbon flow in a marine planktonic ecosystem has been proposed by Williams (1981). This model includes herbivores and carnivores but excludes methanogenesis. The model shows that 56% of the phytoplankton production is taken up by planktonic heterotrophic bacteria (Fig. 2a). In an ecosystem where macrophytes dominate, this model would not be a fair representation because macrophyte biomass would enter the particulate organic carbon pool directly by death and decomposition processes. Decomposition directly to particulate organic carbon

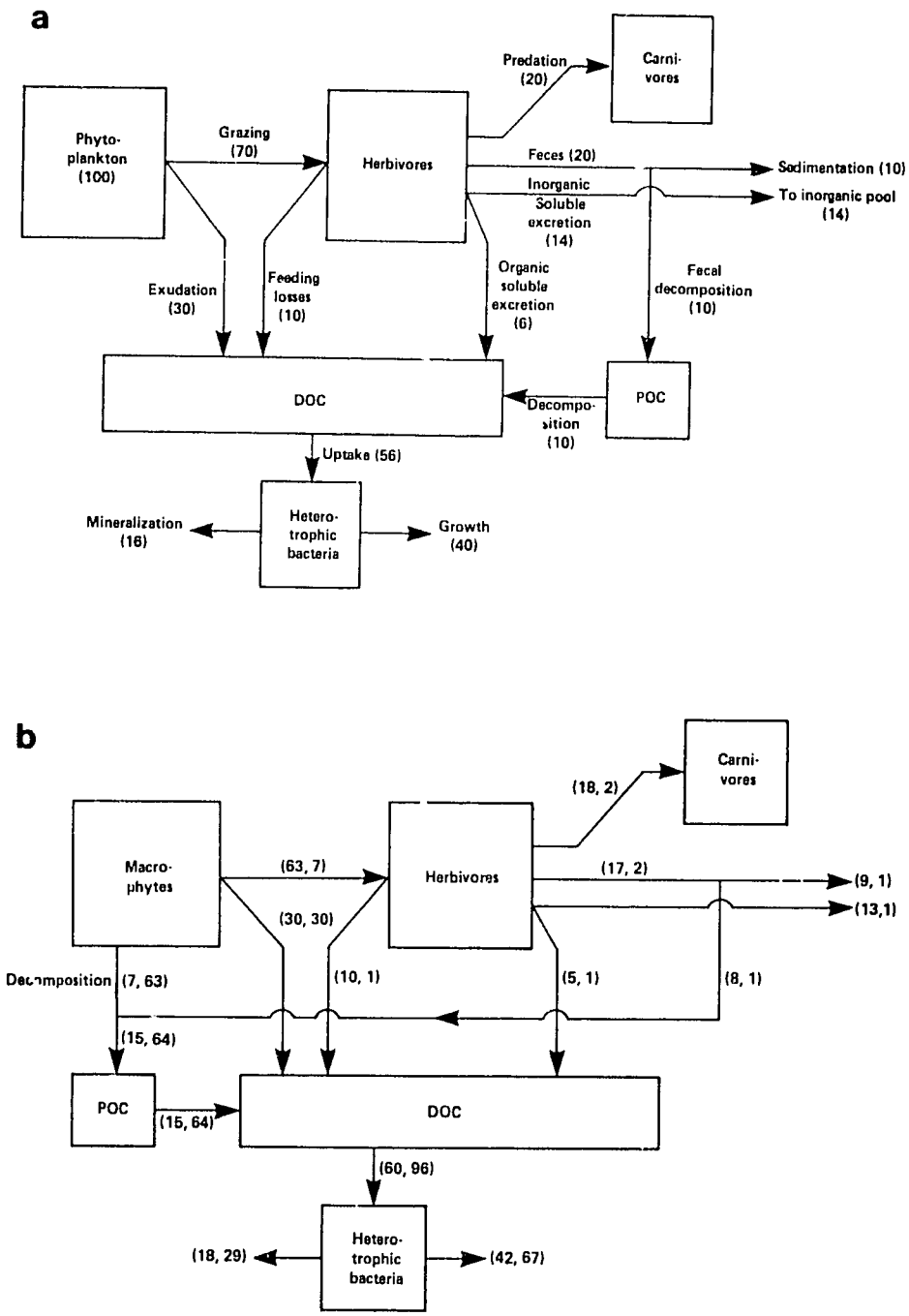

Fig. 2. Carbon flow in two aquatic ecosystems: (a) a planktonic ecosystem (modified from Williams 1981) dominated by phytoplankton and (b) an ecosystem dominated by aquatic macrophytes. Numbers are the percentages of phytoplankton or macrophyte production passing along the specified route. In (b) the first and second numbers represent 90% and 10%, respectively, of macrophyte consumption by herbivores. Route labels given in (a) also apply to (b). DOC = dissolved organic carbon; POC = particulate organic carbon.

could also occur in an ecosystem where herbivores did not completely consume all the phytoplankton. The carbon flow has been recalculated for a macrophyte dominated ecosystem (Fig. 2b), assuming 10 and 90% of the macrophyte biomass is consumed by herbivores. In these cases, 60 and 96% of the production is taken up by heterotrophic bacteria. Although all fixed carbon is eventually taken up by heterotrophic bacteria (Fig. 1), these calculations show that the majority of green plant production will be mineralized by heterotrophic bacteria within a relatively short time.

Carbon is mineralized by both aerobic and anaerobic respiratory mechanisms as well as by methanogenesis (Nedwell 1984). The relative importance of these mechanisms varies between habitats but has been estimated for both marine and freshwater sediments (Table 1). It is clear that aerobic respiration is always of major importance, but that denitrification and methanogenesis are also important in freshwater, but not in marine sediments; the reverse is true for sulfate reduction. The reasons for these differences will be discussed in later sections.

Table 1. The percentage of organic carbon mineralized by various mechanisms in aquatic sediments.

	Organic carbon mineralized (%) by:				
	Aerobic respiration	Denitrification	Sulfate reduction	Methanogenesis	Reference
Eutrophic lake sediment	43	22	3	23	Jones (1985)
Coastal marine sediment	47	3	50	0	Jørgensen (1980)

The Sulfur Cycle

There is almost as much energy and reducing power to be gained from oxidation/reduction reactions involving the sulphur cycle intermediates as there is from the carbon cycle intermediates. Hence this cycle is very important in aquatic systems. The sulfur cycle is basically very simple (Fig. 3). Sulfate is the commonest form of sulfur found in most habitats. It is assimilated by bacteria and the primary producers as they grow and incorporated mainly into the sulfur amino acids of proteins. When algae and macrophytes die they decompose either directly or through the particulate organic carbon pool. This process results in production of H_2S by heterotrophic bacteria and is often called putrefaction. Sulfide is also produced

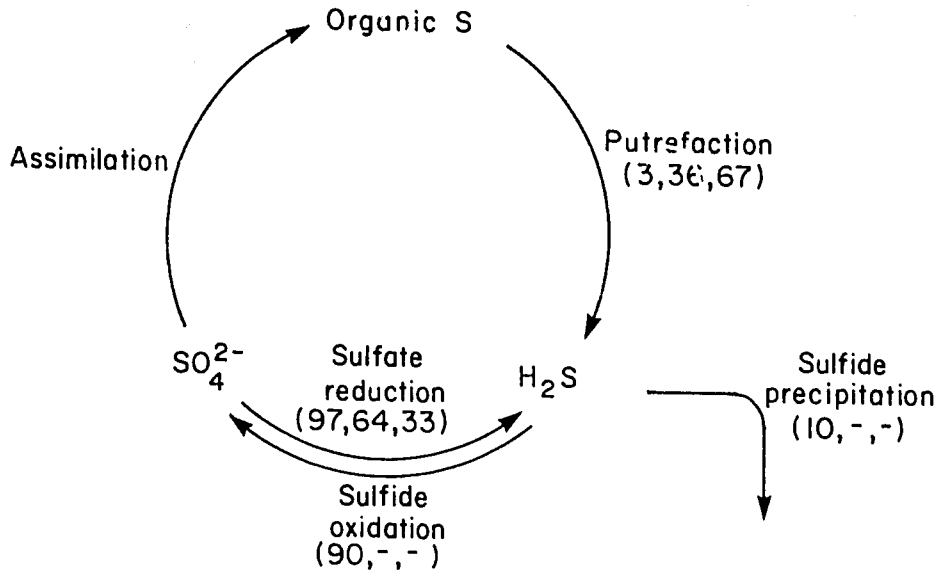

Fig. 3. The sulfur cycle. Numbers are the percentages of sulfide produced or turned-over by the specified route. The three percentages given are for, respectively, a coastal marine sediment (Jørgensen 1983), the profundal and the littoral zones of the sediment from a eutrophic la' (Jones et al. 1982c). Maximal putrefaction is assumed for the values for the freshwater sediments.

directly from sulfate by specialized bacteria. Once formed, the H_2S is either reoxidized to sulfate or precipitated with iron to form insoluble iron sulfides. Reoxidation of sulfide is carried out either chemically in oxygenated water or biologically by a wide range of sulfide oxidizing bacteria.

The relative importance of the different parts of the sulfur cycle varies from habitat to habitat (Fig. 3) although anaerobic conditions are needed for the complete cycle to operate. It is clear that in marine sediments sulfate and H_2S are constantly recycled between the oxidation and reduction steps. In this environment, putrefaction is an insignificant source of sulfide and that, despite the large amount found in sediments, iron sulfides are a minor sink for H_2S. The situation in freshwater sediments is different as putrefaction is a much more important source of sulfide; this is particularly true in the littoral region of eutrophic lakes and in hypereutrophic lakes (Molongoski and Klug 1980), where particulate organic carbon is very important to the carbon budget. The probable reason for this difference between marine and freshwater habitats is the relatively low sulfate concentrations found in freshwaters, which limits sulfate reduction. Thus the turnover times of sulfate to sulfide are long (4-5 months) in marine sediments (Jørgensen 1983) and shorter in the sediments of eutrophic (8-17 hours; Jones et al. 1982c) and hypereutrophic (1.5 hours; Smith and Klug 1981) lakes.

The Nitrogen Cycle

The main processes that make up the nitrogen cycle and some rates for the processes in freshwater and marine sediments are shown in Fig. 4. Most of the nitrogen present in aquatic habitats is in the organic nitrogen pool (Wetzel 1983), which is derived directly from all kinds of organisms by processes such as cell lysis, death and exudation. The bulk of the available complex nitrogen is proteinaceous and is converted to free ammonia or ammonium ions, depending on the pH. This process, called ammonification, is the dominant mechanism for ammonia production in most aquatic habitats (Fig. 4; Molongoski and Klug 1980). Ammonia is also produced from nitrate by nitrate dissimilation, which is probably important only in strongly anaerobic habitats like the profundal sediments in eutrophic lakes, which generate the ammonia found in the anaerobic hypolimnion. In an organically rich coastal sediment, 52% of the nitrate reduced was converted to ammonia by nitrate dissimilation (Herbert 1982). In aerobic habitats nitrification converts ammonia to nitrate; this process uses oxygen and can account for 15-20% of the oxygen removal from the hypolimnion (Hall and Jeffries 1984). Nitrate is reduced to nitrogen gas by denitrification, which together with nitrate dissimilation causes the hypolimnetic nitrate depletion in eutrophic lakes. Nitrogen fixation is rarely of crucial importance in aquatic habitats as the annual rate is low compared with many of the other steps in the cycle.

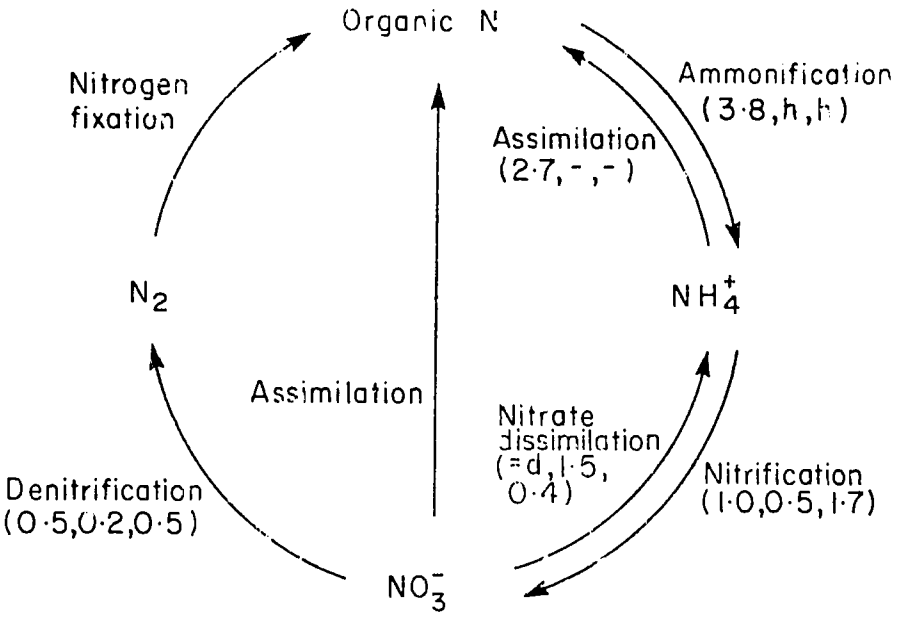

Fig. 4. The nitrogen cycle. Numbers are rates (mmol m^{-2} day^{-1}) for the specified process from, respectively, the aerobic layer of a coastal marine sediment (Blackburn 1983), the sediment in the profundal and the littoral zones of a eutrophic lake (Jones and Simon 1981; Jones et al. 1982a). The symbol h specifies a high rate and =d specifies the rate is approximately equal to the rate of denitrification for that sediment.

The relative importance of the sediment and water column varies for the different steps of the nitrogen cycle. Values from four lakes compiled by Wetzel (1983) show that on average denitrification in the water column was only 10% of that in the sediment. Sometimes, however, the water column is of equal importance as shown by nitrification rates in Grasmere, a mesotrophic lake in the English Lake district (Hall and Jeffries 1984). Most of the nitrogen cycle steps are carried out solely by bacteria. However, nitrate and ammonia are assimilated by eukaryotic plants and ammonia is produced as an excretory product by some animals.

The Main Functional Groups of Bacteria and Their Distribution with Depth

The structure and physiology of the different groups of aquatic bacteria are discussed in relation to their occurrence vertically in the water column and sediment. Details of the relevant biochemistry can be found elsewhere (Jones 1982a). The organisms are divided into groups mainly based on their roles in the cycles described earlier. Very similar distributions of organisms are found in the water column of meromictic and eutrophic lakes in late summer, hence these distributions will be used as a model for the water column in general. Marine and freshwater sediments also have similar populations of bacteria, the main differences in depth distributions are dependent on the amount of oxygen in the overlying water, the sediment grain size and its organic content. These variables affect the rate of change with depth rather than the order of different organisms with depth. Hence a sediment with an oxygenated surface layer will be used as a model. Many articles and texts show plots of actual data to illustrate changes with depth (Gorlenko et al. 1983), but in this review, idealized diagrams will be presented without values, so that the patterns of change with depth are not complicated by the differences in magnitude that occur between habitats. Further details of the organisms described can be found in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974) and The Prokaryotes (Starr et al. 1981).

Morphological Diversity

Most bacteria that occur in aquatic habitats are straight rods, curved rods or cocci (Fig. 5a, 5d, 5e). They tend to be fairly small, being typically about 0.4 x 2 μm. The size appears to depend on the nutritional enrichment or amount of dissolved organic carbon in the water (Fig. 6). Mean volumes range from about 0.1 μm^3 in oligotrophic waters to 1.0 μm^3 in sewage (Fry and Zia 1982; Fry and Davies 1985). Very small bacteria are commonly found in all aquatic habitats; all small bacteria tend to be narrow and narrow cells (<0.3 μm) are always present even in sewage which is rich in dissolved organic carbon compounds. Spirally-shaped bacteria (Fig. 5b, 5c)

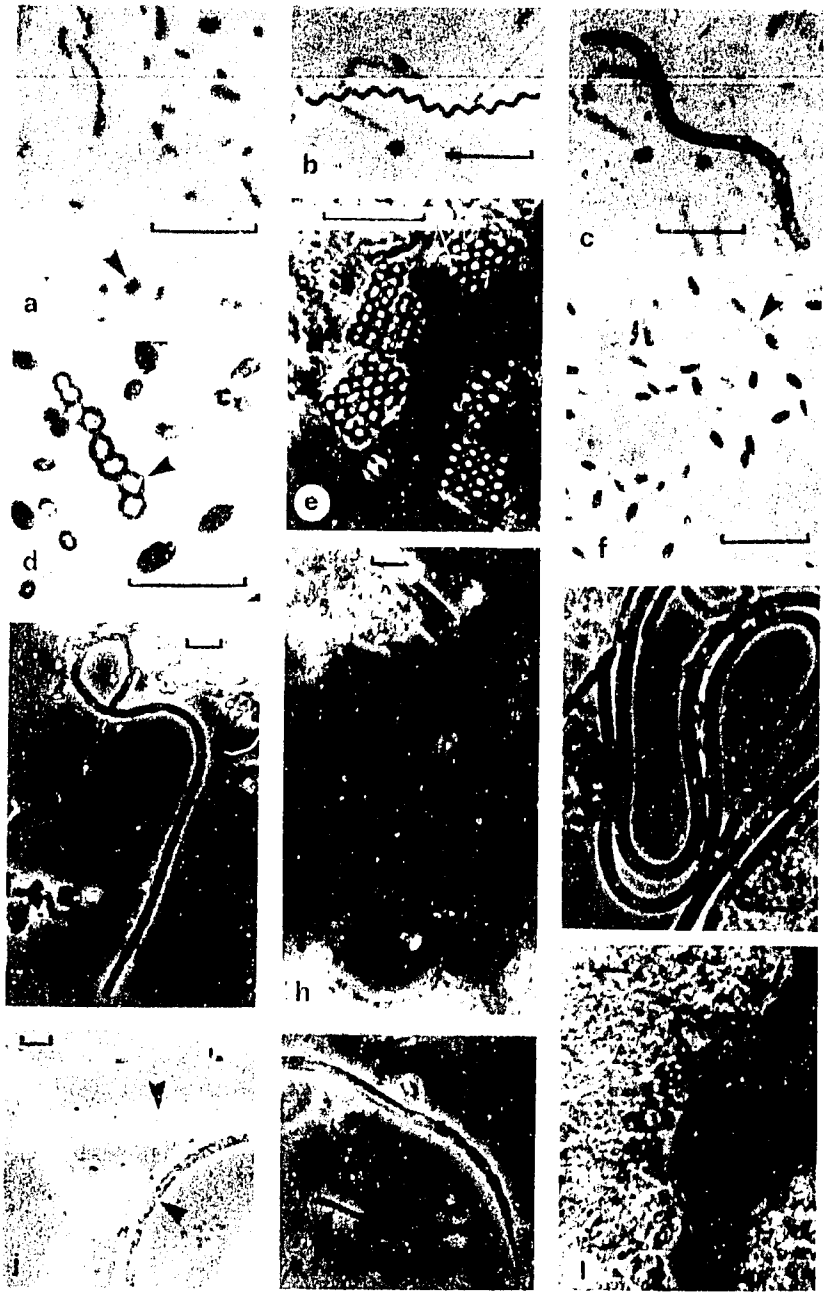

Fig. 5. Morphological diversity of aquatic bacteria: (a) sewage bacteria, pair of dividing cells arrowed; (b) unidentified spiral bacterium, enriched from R. Taff, Wales; (c) *Spirillum volutans* enriched from canal sediment; (d) *Azotobacter chroococcum*, enriched from canal sediments, with dense rod-shaped vegetative cells and coccoid, thick-walled cysts (arrowed); (e) *Thiopedia rosea*, enriched from reservoir water; (f) *Hyphomicrobium* sp., enriched from canal sediment, with cells budded at ends of prosthecae (arrowed); (g) *Vitreoscilla* sp., (h) *Pelonema* sp., with bright gas vacuoles (arrowed); (i) *Achroonema* sp.; (j) *Beggiatoa* spp., with bright sulfur granules (arrowed); (k) *Leptothrix* sp., with iron encrusted sheat (arrowed); (l) unidentified sheathed bacterium sheath arrowed. Organisms g to l were isolated with agar-coated slides (Godinho-Orlandi and Jones 1981a) from pond sediment. Scale bar = 10 μm.

are less commonly observed directly in water or sediment but are present as they can always be grown by simple enrichment procedures. The bacteria with simple shapes characteristically divide by binary fission forming pairs (Fig. 5a) or rafts of cells (Fig. 5e). Prosthecate bacteria have cells joined by thin processes, called prosthecae, which are extensions of the rigid cell wall without cell contents. Many of these bacteria grow by budding new cells from the ends of the prosthecae (Fig. 5f).

Filamentous bacteria (Fig. 5g to 5i) are either trichomes of single cells, or very long, undivided cells. Although they commonly occur in sediments and on submerged surfaces they make up only about 1% of the bacterial population numerically. Many are very large, however, often 1 to 5 μm in diameter by up to several hundred micrometers long and so can make up over 50% of the bacterial biomass in sediment (Jørgensen 1977a; Godinho-Orlandi and Jones 1981a). Gliding motility is a common feature amongst filamentous bacteria and some filaments are enclosed in sheaths that may (Fig. 5k) or may not (Fig. 5i) be encrusted with iron or manganese salts. Cell inclusions are common in the cells and may be gas vacuoles (Fig. 5h), sulfur granules (Fig. 5j) or volutin (polyphosphate) granules. Some filaments attach by a hold-fast and may form rosettes.

Carbon Cycle Organisms

Carbon-fixing bacteria

Cyanobacteria, or blue-green algae as they used to be called, are oxygenic photosynthesizing bacteria because they use sunlight to fix carbon dioxide aerobically. They all contain chlorophyll *a* as the major photosynthetic pigment and are very similar in physiology to the green algae and macrophytes. These organisms are predominantly aerobic and so are mainly found in the epilimnion or mixolimnion. The rate of photosynthesis may, however, be maximal below the surface due to photoinhibition (Fig. 7). Some cyanobacteria can function anaerobically and so are sometimes found in sediments or deoxygenated waters. Anoxygenic photosynthesis is carried out by specialized anaerobic photosynthetic bacteria and will be considered in the section on sulfide oxidation. Autotrophic bacteria are those that can fix carbon dioxide as a sole carbon source. Several specialized groups of bacteria are able to do this in the dark, such as some methylotrophs, iron-oxidizing bacteria, thiobacilli and the nitrifying bacteria (for details see later sections). Some heterotrophs are able to fix carbon dioxide in the dark and some predominantly autotrophic bacteria can use organic compounds as sources of energy and carbon; these types are often called mixotrophs. Most autotrophic carbon dioxide fixation occurs at the thermocline and near the sediment because special factors limit the growth of autotrophic bacteria to this zone. However, heterotrophic carbon dioxide fixation can occur wherever large populations of heterotrophs are found, so it is often high in the epilimnion as well as the thermocline (Fig. 7).

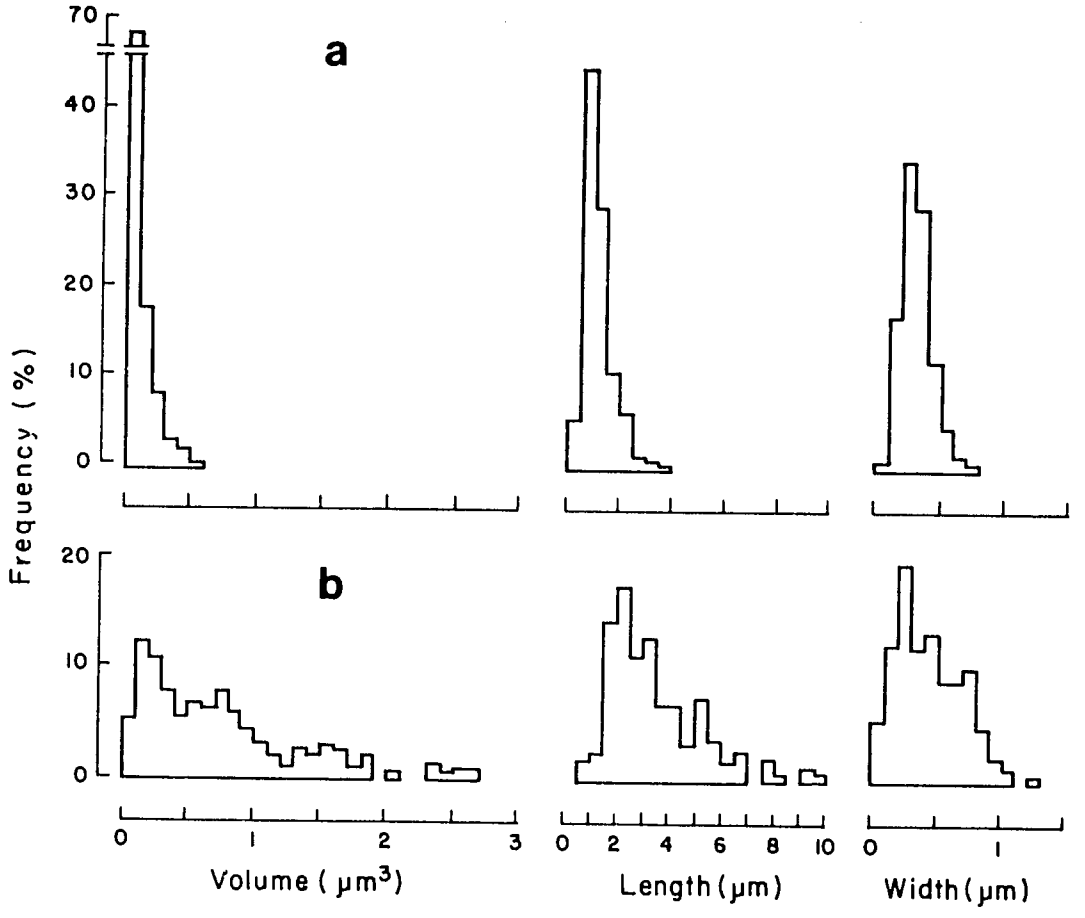

Fig. 6. Frequency distributions of volumes, lengths and widths of bacteria in (a) an oligotrophic reservoir (from Fry and Davies 1985) and (b) sewage.

Carbon-mineralizing bacteria

A very wide range of heterotrophic bacteria are capable of mineralizing dissolved organic carbon to CO_2 as well as some more specialized bacteria (Table 1) such as sulfate-reducing and denitrifying bacteria, which will be considered later. Taxonomic studies of aquatic bacteria able to grow as colonies on organic-based nutrient media have shown that there is a wide variety of different genera (Table 2). In over half of these studies, the presence of *Flavobacterium*, *Pseudomonas*, *Vibrio*, *Aeromonas* and *Alkaligenes* has been demonstrated, so these can be considered as typical aquatic heterotrophs. It is likely that most bacteria routinely seen in water by microscopic examination are heterotrophs. Some of these bacteria are copiotrophic (grow best at high concentrations of dissolved organic carbon), and some are oligotrophs

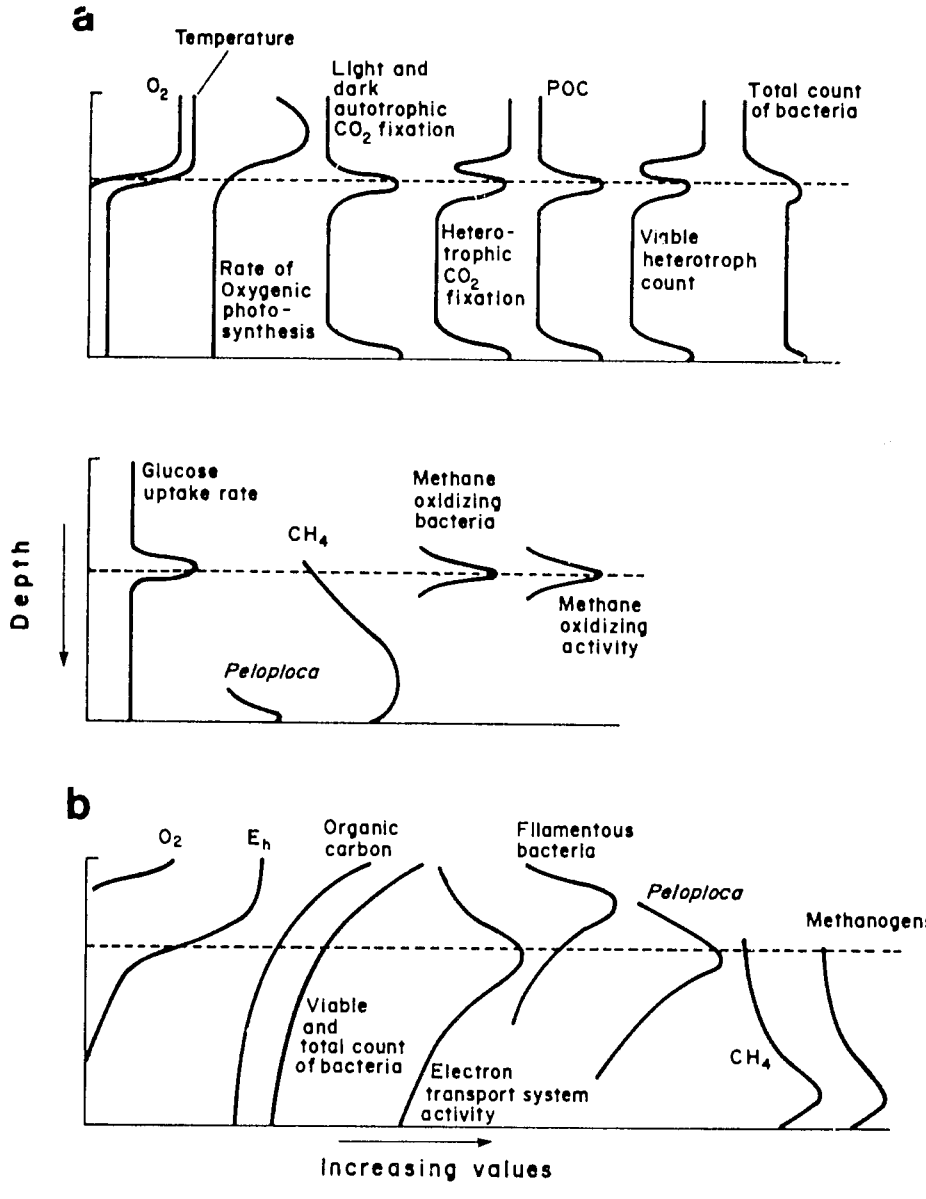

Fig. 7. Generalized diagram of the distribution of carbon-cycle bacteria and other variables with depth in (a) a typical meromictic lake or a eutrophic lake in late summer and (b) aquatic sediments. The dotted lines indicate the thermocline or chemocline (a) and redoxycline (b).

(grow best at low concentrations of dissolved organic carbon, <5 mg l^{-1}). Both of these types contain similar genera, but some more specialized oliogotrophs are also seen (Mallory et al. 1977). It is likely that bacteria found in detritus-fed aquaculture ponds will be similar to those found in other aquatic systems because Pike (1975) reports that 90 to 95% of the bacteria in oxidation ponds are *Pseudomonas*, *Flavobacterium* or *Achromobacter* and Gloyna (1971) claims that *Pseudomonas*, *Alkaligenes* and *Flavobacterium* are widely distributed or predominant in these systems.

As populations of algae in the aerobic surface waters die and become replaced by others, a large amount of particulate organic carbon is generated which falls through the water column. When the particulate organic carbon reaches the thermocline or chemocline the physical resistance there slows it down and concentrations are increased. Once past the thermocline, sinking rates again increase and particulate organic carbon concentrations do not increase again until just above the sediment surface where the organic matter accumulates. For this reason both the numbers of heterotrophs obtained by plate counts and total bacteria by direct counts are often highest at the thermocline, although such peaks are sometimes transitory (Fig. 7; Jones 1977).

This increase in numbers at the thermocline or chemocline is also associated with increases in mineralization rates for simple organic materials like acetate and glucose. For similar reasons, large numbers of bacteria are sometimes found at the interface between the salt- and freshwater in estuaries (Rheinheimer 1980). Large numbers of heterotrophs (plate counts) can also be found in the epilimnion due to growth on exudation products of algae. The number of total bacteria is sometimes large in the hypolimnion or monimolimnion, due to increases in numbers of other more specialized groups of bacteria. The large numbers of bacteria near the sediment are also due to the high concentrations of both particulate organic carbon and dissolved organic carbon found there.

The organic matter deposited on the sediment surface from either allochthonous or autochthonous sources is decomposed mainly in these surface layers by aerobic activity. Hence concentrations of organic carbon are highest at the sediment surface and decrease with depth (Billen 1982). Numbers of bacteria are also highest in the surface layers and thereafter decrease (Fig. 7b). Although more anaerobic bacteria can be grown from the sediment surface, their proportion of the heterotroph plate count increases with depth (Bell and Dutka 1972). These changes coincide with the decreases of oxygen concentration and redox potential that occur with depth in sediments. The zone near the surface containing the redoxycline is often the site of most bacterial activity when gross measures are used, such as with the electron transport system method (Jones 1982b).

Most filamentous bacteria are heterotrophic and aerobic or microaerophilic; many exhibit gliding motility. These features ideally suit them to life in the surface layers of sediment where they are commonly found. Godinho-Orlandi and Jones (1981a, 1981b) clearly showed this; most of the 11 genera of filamentous bacteria they examined were concentrated in the surface layers of lake sediments (Fig. 7). Filamentous bacteria are also found in the aerobic surface layers of marine coastal sediments (Jørgensen 1977a) and are sometimes found as macroscopic whitish growths on the sediment surface; this was the case with *Thiothrix* in some road drainage ditches (Jones et al. 1982a). Although most abundant in sediments some filamentous bacteria move into the water column when conditions become suitable for them (Fig. 7). This happens with the iron-oxidizing bacterium, *Leptothrix* (Fig. 5k), when iron concentrations in the hypolimnion increase as it becomes anaerobic (Jones 1975) *Peloploca*, an organism growing in helical bundles of filaments, is

normally found in the upper anaerobic regions of sediments and moves into the hypolimnium once it is thoroughly anaerobic in late summer (Maiden and Jones 1984).

Methanogens

Methane-producing bacteria are a morphologically diverse but physiologically similar group (Mah and Smith 1981). They are now all thought to be taxonomically related and, together with others, form the "archaebacteria", a group of very primitive bacteria. Most of the seven genera are rod-shaped (e.g., *Methanobacterium*) or coccoid (e.g., *Methanogenium*) but one genus *(Methanospirillum)* has spirally-shaped members. All are anaerobic and grow best at an E_h of −200 mV or below, although some are obligately anaerobic others can tolerate oxygen for short periods. All the organisms that have been obtained in pure culture can form methane from hydrogen and carbon dioxide and most can produce it from formate. Only one methanogen can use organic materials and this organism, *Methanosarcina barkeri*, can use methanol, methylamines and acetate for methane generation.

Methanogens are widespread in both freshwater and marine aquatic sediments and as would be expected are found deep in the sediment where E_h is very low and oxygen is totally absent (Fig. 7). Their growth is limited, however, by sulfate-reducing bacteria because they compete with sulfate-reducing bacteria for acetate and hydrogen (Nedwell 1982, 1984). When sulfate is at limiting concentrations, as in many freshwater sediments, methanogens are most successful. The reverse is true when sulfate concentrations are high, as in marine sediments. For these reasons sulfate-reducing bacteria and methanogens rarely grow together in the same layer of sediment. Methanogens are normally found below the sulfate-reducing bacteria and their presence is indicated by high methane concentrations in the pore water, large quantities of coenzyme F_{420} (a fluorescent methanogen-specific cofactor) and by viable counts. The depth distribution of methanogens might also be limited by the hydrogen sulfide, produced by sulfate-reducing bacteria, which is toxic to methanogens. These interactions also account for the relative importance of sulfate reduction and methanogenesis noted earlier (Table 1) in freshwater and marine sediments.

Methane-oxidizing bacteria

Methane produced in sediments by methanogens is not oxidized anaerobically and consequently rises into the water column by bubbling and diffusion. While the water is anaerobic the methane remains in solution, however once the oxycline is reached it is rapidly oxidized by methanotrophic bacteria. The taxonomy of these bacteria is not at present clear, although *Methylomonas*, *Methylococcus* and *Methylosinus* might represent major groups of similar strains (Whittenbury and Dalton 1981). All methanotrophs are Gram-negative rods or cocci which form a resting stage or cyst, like that of *Azotobacter* (Fig. 5d). They can use methane and some other 1-carbon

compounds as sole carbon and energy sources. They can also use carbon dioxide, acetate and some amino acids as supplementary carbon sources and can oxidize some other compounds in the presence of methane. They are aerobic, but many strains are sensitive to oxygen at atmospheric oxygen tensions, thus they can be classed as microaerophilic. For these reasons methanotrophs are most abundant in the water column at the oxycline where both methane and low concentrations of oxygen are present (Cappenberg 1972; Rudd and Hamilton 1975). They are most active in a tight band in this region of the water column because it is only here that conditions are optimal for their growth. These bacteria are most abundant in nutritionally enriched water bodies where methane production in the sediment is high. In some cases up to 40% of the dissolved oxygen in the water can be used for the bacterial oxidation of methane.

Sulfur Cycle Organisms

Hydrogen sulfide producers: putrefying bacteria

Many heterotrophic bacteria are able to produce hydrogen sulfide from organic materials such as proteins, both aerobically and anaerobically. However, in the presence of oxygen, hydrogen sulfide will quickly be chemically oxidized. Protease-producing bacteria are abundant in all aquatic habitats; for example in one study 45 to 48% of freshwater isolates that were found degraded casein (Nuttall 1982a). So from these and other results it would appear that hydrogen sulfide production from putrefying bacteria will be found wherever organic matter and heterotrophic bacteria are found. Some genera have been specifically implicated as important putrefiers in lakes. These are: *Proteus*, *Mycobacterium*, *Chromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium* and *Vibrio* (Wetzel 1983). Many of these genera have been found in aquatic habitats in more general taxonomic studies (Table 2).

Hydrogen sulfide producers: sulfate-reducing bacteria

This group of bacteria has generated a lot of scientific interest recently, after Widdel (Pfennig et al. 1981) used a wide range of ingenious isolation techniques to enlarge the number of known organisms from two genera to eight. The sulfate-reducing bacteria are now known to be a morphologically and metabolically diverse group. However, they are all strict anaerobes and use sulfate as a terminal electron acceptor to oxidize organic compounds. They are heterotrophs, although some can grow autotrophically (e.g., *Desulfosarcina*). Biochemically there are two groups oxidizing carbon compounds such as long-chain fatty acids, propionate, lactate and benzoate either completely to carbon dioxide or incompletely to acetate. *Desulfovibrio* (rod, curved-rod or spiral shaped) and *Desulfobulbus* (lemon shaped) are examples of the complete oxidizers, whilst *Desulfonema* (filamentous), *Desulfotomaculum* (a spore-forming rod) and *Desulfococcus* (coccoid) are examples of the

Table 2. Genera of heterotrophic bacteria isolated from different aquatic habitats during taxonomic studies using various criteria for the primary isolation. The genera are listed alphabetically in order of the number of occurrences.

	Presence (+) of bacteria in the following studies, grouped by type of bacteria isolated and source of isolation:								
	Copiotrophic bacteria							Oligotrophic bacteria	
	Planktonic				Planktonic and very small		Epiphytic	Planktonic	
	Marine		Freshwater		Estuarine	Deep-sea	Marine	Estuarine	Marine
Genus	Baltic Sea[a]	Gulf of Alaska[b]	R. Dee, UK[c]	R. St. John, Canada[d]	Perdido Bay Florida, USA[e]	Puerto Rico Trench, Atlantic Ocean[f]	Beach, Hong Kong[g]	Chesapeake Bay and Baltimore Harbor[h]	Coastal Water Japan[i]
Flavobacterium	+	+	+	+		+	+		+
Pseudomonas	+	+	+	+	+	+	+		
Vibrio	+	+		+	+	+	+		+
Aeromonas		+	+	+	+	+			+
Alkaligenes		+	+		+	+		+	
Corynebacterium	+	+						+	+
Micrococcus	+	+					+		+
Moraxella		+	+			+			+
Acinetobacter		+	+						+
Cytophaga			+	+					+
Xanthomonas		+		+			+		
Achromobacter	+					+	+		
Agrobacterium	+		+						
Bacillus	+	+							
Chromobacterium		+							+
Flexibacter			+	+					
Alteromonas							+		
Azotobacter				+					
Beneckea		+							
Caulobacter									+
Erwinia				+					
Escherichia			+						
Hyphomirobium								+	
Hyphomonas								+	
Listeria								+	
Microcyclus		+							
Norcardia									
Pedomicrobium								+	
Planococcus								+	
Sphaerotilus								+	
Streptomyces								+	
Streptothrix								+	

a-i, References: a = Bolter (1977); b = Hauxhurst et al. (1980); c = Nuttall (1982a); d = Holder-Franklin et al. (1978); e = MacDonell and Hood (1982); f = Tabor et al. (1981); g = Kong and Chan (1979); h = Mallory et al. (1977); i = Akagi et al. (1980).

incomplete oxidizers. As well as being anaerobes they also require reducing conditions, growing optimally at redox potentials between 0 and −100 mV.

The primary habitat for sulfate-reducing bacteria is the sediment. In a typical sediment (Fig. 8b) their activities are responsible for the depletion of sulfate which occurs below the redoxycline. They are most active in this zone because the redox potential is low enough for their optimal growth and activity. They produce hydrogen sulfide from the sulfate and this accumulates below the redoxycline. This pattern is observed in both marine (Jorgensen 1983) and freshwater (Jones 1982b) sediments. It might appear unexpected that both sulfate-reducing bacteria and their

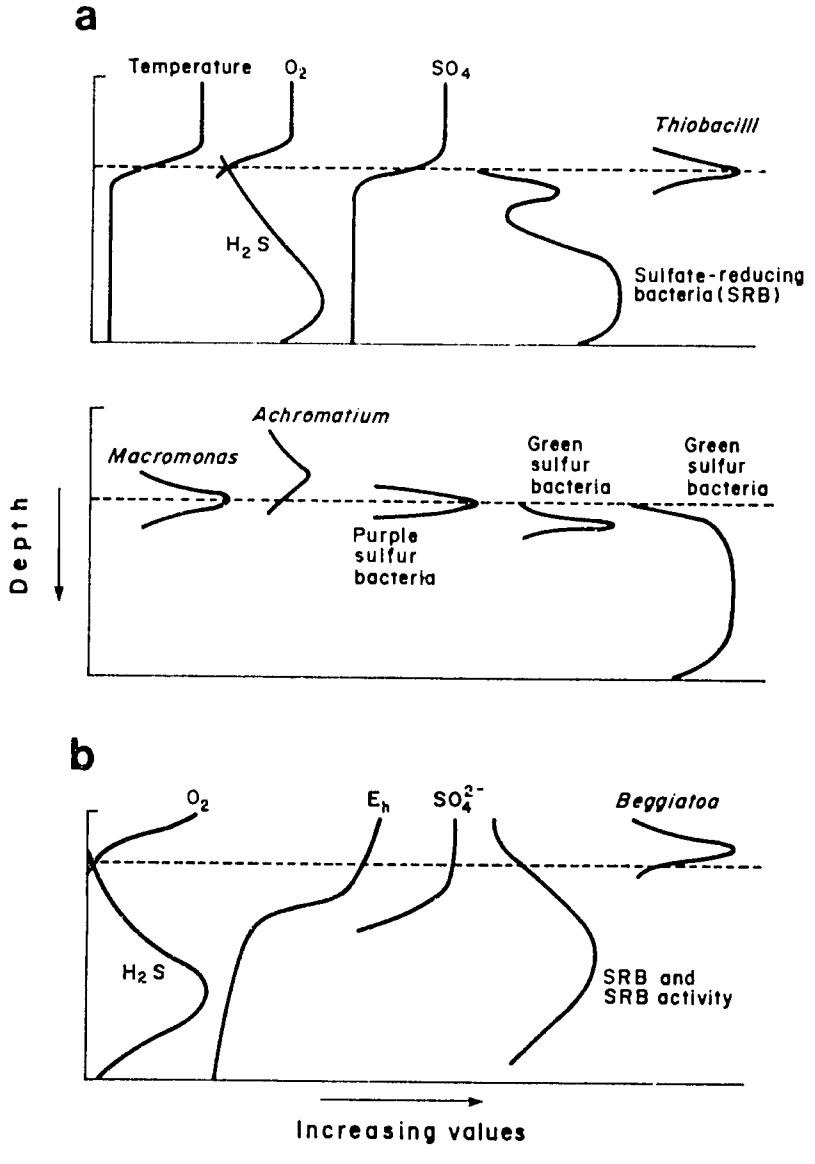

Fig. 8. Generalized diagram of the distribution of sulfur cycle bacteria and other variables with depth in (a) a typical meromictic lake or a eutrophic lake in late summer and (b) aquatic sediments. The dotted lines represent the thermocline or chemocline (a) and the H_2S/O_2 interface (b). S.R.B. = sulfate-reducing bacteria.

activity can be detected above the redoxycline in the more oxidized layers of sediment (Fig. 8b), but this has been shown to be due to the occurrence of reduced microniches in sediment particles with diameters of 50 to 200 μm. In these particles aerobic heterotrophs consume oxygen and so keep the center of the particle anaerobic, allowing sulfate-reducing bacteria to flourish (Jørgensen 1977b). Hydrogen sulfide is not present in detectable amounts in these upper sediment layers because it is rapidly oxidized both chemically and microbiologically.

In meromictic lakes, and eutrophic ones in late summer, the surface sediment is anaerobic and highly reduced as is the monimolimnetic and hypolimnetic water. This enables the sulfate-reducing bacterial populations to move out of the sediment into the anaerobic water. In meromictic lakes these bacteria are active right up to the chemocline (Jørgensen et al. 1979b) but in eutrophic ones they only just manage to creep into the lower layers of the hypolimnion (Cappenberg 1972). In some meromictic waters, peaks of sulfate reduction are observed just below the chemocline where organic matter concentrates and also near the organically rich littoral regions.

Hydrogen sulfide oxidizers: colorless sulfur bacteria

Thiobacilli

Under this heading will be considered the genera *Thiobacillus* (rod shaped) and *Thiomicrospira* (spiral cells); they oxidize hydrogen sulfide and other inorganic sulfur compounds to produce energy and form sulfate. Their carbon requirements are very varied; some are obligate autotrophs, but others are mixotrophic or heterotrophic (Kuenen et al. 1985). Almost all the species are strict aerobes, but both *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* can grown anaerobically by converting nitrate, as terminal electron acceptor, to nitrogen by denitrification. The latter organism can also grow microaerophilically at very low oxygen tensions.

Thiobacilli can be readily isolated from almost any freshwater or marine sediment or water, hence they are probably ubiquitous in aquatic habitats (Kuenen and Tuovinen 1981). However, from the very limited number of ecological studies undertaken, it seems that their distribution and activity is largely restricted to the hydrogen sulfide/oxygen interface that exists in the water column and in aerobic sediments. The evidence for this comes from the many studies in which dark CO_2 fixation has been measured in meromictic and eutrophic lakes (Fig. 7). As these bacteria are not easy to identify from morphology or pigments, studies of their numbers are rare. In several Russian lakes, peaks of numbers as well as chemosynthetic activity regularly occur at the H_2S/O_2 interface (Fig. 8; Gorlenko et al. 1983). Autotrophic thiobacilli alone would be detected by these methods, but in one or two Russian lakes appreciable numbers of thiobacilli were found higher in the water column and this was attributed to heterotrophic growth. However, their source of reduced sulfur has

not been suggested. Thiobacilli often compete with phototrophic bacteria for H_2S and are sometimes found in the same tight band as pigmented bacteria, so numbers are restricted to about 10^3 ml^{-1}. In one study, a tight band of dark CO_2 fixation attributed to thiobacilli was found only 10 cm above a band of photosynthetic bacteria. In lakes where light does not penetrate to the H_2S/O_2 interface, thiobacilli do not have to compete for H_2S and can grow at high densities, about 10^6 ml^{-1} Sometimes a zone of dark CO_2 fixation is found close to the sediment in the anaerobic region of lakes (Fig. 7) and this is attributed to the denitrifying thiobacilli. These bacteria are also thought to be important H_2S oxidizers in sediments where numbers ranging from 10^4 to 10^6 g^{-1} have been found (Jørgensen 1983). In a recent study Kepkay et al. (1979) also reported high rates of aerobic CO_2 fixation into bacterial cells in sediment, which was consistent with the activity of *Thiobacillus* spp. As *Thiobacillus denitrificans* can readily be isolated from many anaerobic sediments this organism is thought to oxidize H_2S under these conditions when nitrate is also present.

Colorless bacteria storing sulfur

This group of bacteria are morphologically very diverse and interesting (La Riviére and Schmidt 1981). Unfortunately, as only one genus, *Beggiatoa* (Fig. 5j), has been obtained in pure culture little is known of their physiology. They are all aerobic or microaerophilic and oxidize H_2S to sulfur, which they store as sulfur globules within their cells and they can use this sulfur later to obtain energy when H_2S is unavailable. It is thought that they are heterotrophic, but some might be autotrophic. Some genera contain organisms that are nonmotile or motile by flagellae. *Macromonas* has rod or bean-shaped cells (9 x 20 μm) with a large tuft of polar-flagellae. *Thiovulum* is ovoid (about 20 μm diameter) with peritrichous flagellae; the cells of this genus aggregate together to form dense 'veils' of organisms. *Thiospira* is spiral (2 x 50 or 10 μm) with polar flagellae and *Thiobacterium* is a nonmotile rod (1 x 2 μm), growing in gelatinous masses that float. Another group show gliding motility or are filamentous. *Beggiatoa* cells form long, gliding filaments (1-55 μm wide), whereas *Thioploca* is similar but sheathed and *Thiothrix* is also similar, but forms attached rosettes of filaments. *Achromatium* is a very large ovoid organism (about 30 x 50 μm), which is sometimes packed with calcium carbonate crystals.

The distribution of these organisms has very rarely been studied. They seem to occur in habitats containing both H_2S and oxygen (Fig. 8) so they are common in aerobic sediments with a lot of organic detrital decomposition. Some have been found as macroscopic surface mats, such as *Beggiatoa* (Jørgensen 1983) and *Thiothrix* (Jones et al. 1982b). *Beggiatoa* also grows in small numbers within marine sediments at the H_2S/O_2 interface (Jørgensen 1977a). *Macromonas* has been found at this interface in the water of a eutrophic lake in large numbers (2 x 10^4 ml^{-1}; Gorlenko et al. 1983). *Achromatium* cells are very large and dense, due to $CaCO_3$ crystals,

and so are normally found at the sediment surface. Surprisingly, however, *Achromatium* has been found as a dense band (1.5×10^4 ml^{-1}) just above the H_2S/O_2 interface in a meromictic lake in Tasmania (Croome and Tyler 1984).

Hydrogen sulfide oxidizers: photosynthetic bacteria

There are four families of phototrophic sulfur bacteria. In most habitats, two of the families, the Chloroflexaceae and the Rhodospirillaceae, are of little ecological importance and so will not be considered. The remaining families, the Chromatiaceae (purple sulfur bacteria) and the Chlorobiaceae (green sulfur bacteria), are of great importance in many habitats. The two families are differentiated by the bacteriochlorophylls and carotenoids they contain. The purple bacteria contain bacteriochlorophyll *a* or *b*, the main absorption peak of which is around 850 nm, whilst their carotenoids absorb light maximally at about 370 nm. The equivalent peaks for the green bacteria are 740 nm due to bacteriochlorophyll *a* and either *c*, *d* or *e*, and 460 nm, due to carotenoids. These peaks are all distinct from those of green algae which are at 665 nm and 430 nm, respectively. These pigments make the Chromatiaceae appear purple and the Chlorobiaceae green or brown, hence their common names. Morphologically these families have rod, coccoid, spiral or star-shaped cells and are classified into 15 genera. The purple bacteria are normally rather large cells; two examples are *Chromatium* (polar-flagellated ovoid-rod, 1 to 6 μm x 2 to 15 μm with no gas vacuoles) and *Thiopedia* (Fig. 5e; nonmotile, gas vacuolated, ovoid cells, 1.5 x 2 μm, forming flat plates with up to 64 cells). The green bacteria are smaller and include *Chlorobium* (nonmotile rod-shaped cells, 0.3 to 1.1 μm x 0.7 to 2.7 μm, without gas vacuoles) and *Pelodictyon* (gas-vacuolate, rod-shaped cells, 0.8 x 1.8 μm, forming three dimensional nets).

The physiology of these two groups of bacteria is similar, but distinct and this leads to differences in their distribution (van Gemerden and Beeftink 1983; Jørgensen 1983; Kuenen et al. 1985). Both groups use H_2S or sulfur as electron donors to fix carbon dioxide phototrophically. Because H_2S is converted to sulfur more quickly than sulfur is oxidized to sulfate, free sulfur accumulates inside the cells of the purple bacteria and outside the cells of the green bacteria. Hydrogen sulfide is toxic to both groups of bacteria, but the Chlorobiaceae are far more resistant than are the Chromataciae. Both groups can grow at much lower light intensities than can either green algae or cyanobacteria. The green bacteria can, however, grow at even lower light intensities than the purple bacteria, for example when light is too dim for the purple bacterium *Thiocapsa* to grow, *Chlorobium* can still grow with a generation time of about 40 hours (Kuenen et al. 1985). Although both groups are anaerobic, the purple bacteria can tolerate very small amounts of free oxygen but the green bacteria cannot. Both groups can assimilate acetate and some other low molecular weight carbon compounds whilst photosynthesizing. The purple bacteria can do this much better than the green bacteria, growing mixtrophically on acetate with no diminution of the rate of CO_2 fixation.

Phototrophic bacteria are found growing in the water column in many different habitats, where light reaches the H_2S/O_2 interface. They have been found in meromictic and eutrophic lakes, seawater lagoons and oxidation ponds treating sewage (Pike 1975; Biebl and Pfennig 1979) as well as in small natural ponds (Moss 1969b). Their growth is dependent on precise mixtures of conditions found on the gradients of light, oxygen and H_2S. So they often grow as dense populations in very thin layers (Fig. 8), perhaps only a few centimeters thick: such growth forms the so-called 'bacterial plate'. Light almost always limits their growth. The green bacteria grow at the lowest light intensities, cannot tolerate oxygen but can tolerate high H_2S concentrations, so they grow from the bottom of the oxygen gradient until light stops penetrating the water. Thus if light penetrates a long way down into the water, they might extend right down to the sediment surface. Alternatively, if their growth is dense enough to exclude light they may grow as a thin 'plate'. The purple bacteria need more light, are oxygen tolerant and H_2S sensitive so they always grow in a thin band just above the green bacteria and may even penetrate the oxygenated part of the H_2S/O_2 interface. Conditions are often ideal in this zone because if this interface coincides with the thermocline or chemocline then higher concentrations of organic carbon will be present from surface algal production which will aid mixotrophic growth.

The green and purple sulfur bacteria do not always grow together and may be present in some waters on their own. Most species seem to react in the general manner described (Gorlenko et al. 1983), the reasons for the slight differences that exist between species is sometimes explainable by mixed culture studies (Kuenen et al. 1985) but are occasionally obscure.

There will be little competition for light with algae in the surface waters because the absorption peaks of the two types of phototrophs do not overlap; this also precludes direct competition for light between the purple and green bacteria. The photosynthetic bacteria absorb light at long wavelengths, but these do not penetrate water very well, so the bacteria often rely on the carotenoid absorption peaks, which absorb light at shorter wavelengths that penetrate water better.

In some waters, these bacteria are so successful that they can account for more carbon fixation than the phytoplankton. Biebl and Pfennig (1979) report that on a daily basis bacterial photosynthetic production varied between 3 and 90% of the total photosynthetic production and that on a yearly basis this proportion varied between 3 and 82.5%. They do say, however, that the highest figures are rather atypical of most waters.

Phototrophic bacteria are also found in sediments. They are only found in sediments rich in organic matter as only in these sediments will the zones of light penetration and H_2S accumulation meet. Light can normally only penetrate about 3 mm into sediment so purple and green bacteria will be found here. Some sediments receive a lot of organic matter from decomposing plant material and are anaerobic nearly right up to the surface. Such sediments reduce a lot of sulfate due to high sulfate-reducing bacterial populations and so a lot of H_2S is generated, thus dense

growths of green and purple bacteria can occur. Such sediments are called 'sulfureta' and are common in productive coastal regions. Purple and green bacteria are once again situated in tight bands close to the H_2S/O_2 interface for similar reasons to those discussed for the water column. In some sediments green bacteria are most active (Blackburn et al. 1975), in others purple forms predominate and in some a mixture occurs (Jørgensen 1983).

Phototrophic bacteria can be an important food source for zooplankton and ciliates Several studies have found peak concentrations of these predators just above the bacterial plate and evidence suggests that the predators dive from the oxygenated water in which they live into the anaerobic water containing the phototrophs to feed on the bacteria (Sorokin 1970; Matsuyama and Shirozu 1978, Biebl and Pfennig 1979).

Nitrogen Cycle Organisms

There is a lot of information in the literature about the taxonomy, morphology and biochemistry of the bacteria that mediate the transformations in the nitrogen cycle. However, information on the ecology of the organisms, their distribution and the processes that they carry out is lacking. Much more is known about the ecology of the carbon and sulfur cycles in water and sediment than is known of the nitrogen cycle. The information given in this section reflects this paucity of knowledge.

Ammonifying bacteria

Sepers (1981) examined the diversity of aerobic ammonifying bacteria from a freshwater basin in the Netherlands. He used plating, batch culture and continuous culture to isolate 169 strains of different ammonifying bacteria with 19 different amino acids. His examination of the utilization of 41 organic compounds as sole carbon and energy sources by 68 of the isolates showed all the bacteria to have a very wide substrate specificity. No isolate could utilize all the compounds but 83% of the strains could use 50 to 83% of the substrates. The great similarity of the strains in their ability to use the organic compounds was confirmed by cluster analysis. From this work it appears that aquatic heterotrophic bacteria are capable of growing on almost any substrate they encounter and ammonifying most amino acids. Thus ammonification is unlikely to be limited by lack of organisms or the right kind of substrate. The bacteria capable of ammonification are heterotrophs, so they are distributed throughout the water column and sediment. Ammonification can take place either aerobically or anaerobically, and so it will continue in either oxic or anoxic water or sediment. However, because more organic matter and bacteria are present at the surface of sediment than elsewhere, it is not surprising that most ammonification is at the sediment surface (Fig. 9; Blackburn 1979, 1983). Although a lot of ammonia is produced in lakes it is also rapidly assimilated by algae (Wetzel 1983) and used for nitrification by bacteria (see next section). Both these processes

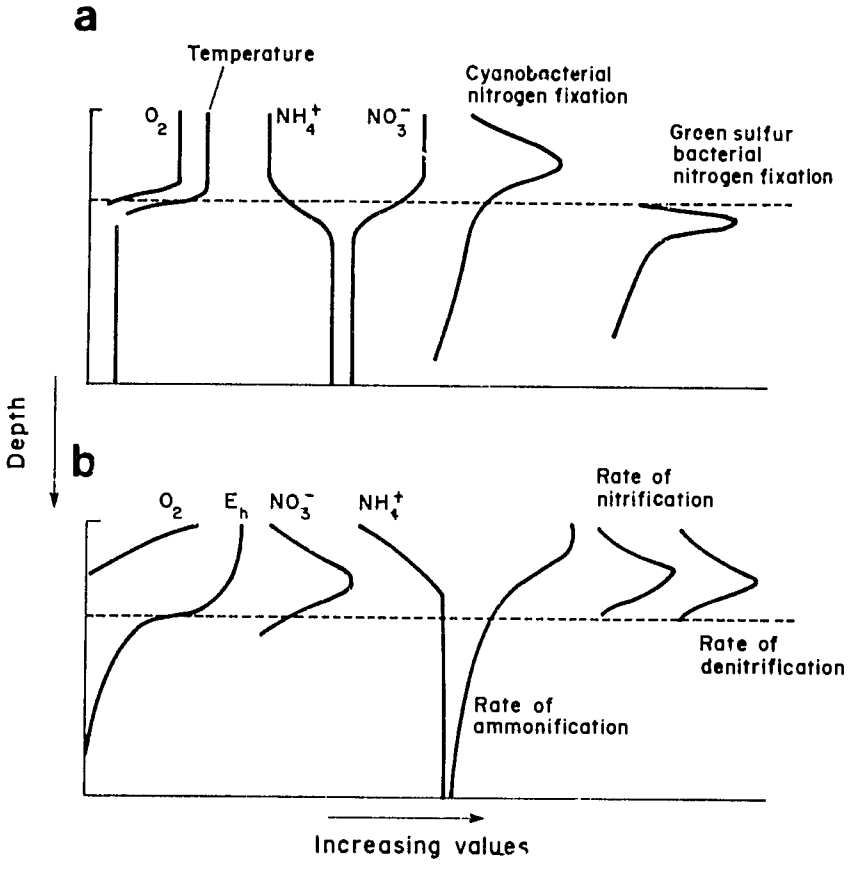

Fig. 9. Generalized diagram of the distribution of different forms of nitrogen, the activity of nitrogen cycle bacteria and other variables in (a) a typical meromictic lake or a eutrophic lake in late summer. The dotted lines represent the thermocline or chemocline (a) and the redoxycline (b).

are more active in oxygenated water; thus, ammonia concentrations are low in aerobic waters or sediment and high in anaerobic regions (Fig. 9).

Nitrifying bacteria

Heterotrophic nitrification is thought to be insignificant in aquatic habitats, and so all the important nitrifiers in water are the autotrophic nitrifiers or Nitrobacteriaceae. These organisms have been fully described recently (Watson et al. 1981) and consist of two groups of genera that use different respiratory mechanisms. The ammonia-oxidizers convert ammonia to nitrate; there are five genera, most of which have been isolated only from soil, but *Nitrosomonas* (rod-shaped, 1 x 1.5 μm) and *Nitrosococcus* (coccoid, 1.5-2.2 μm diameter) are aquatic. The nitrite-oxidizers

convert nitrite to nitrate and are all aquatic, although only *Nitrobacter* (pear-shaped rod, 0.7 x 1.5 μm) is found in freshwater; *Nitrococcus* (coccoid, 1.7 μm diameter) and *Nitrospina* (rod-shaped, 0.35 x 5 μm) are obligately marine. All the genera are highly aerobic, containing numerous cytomembranes that are rich in cytochromes.

The Nitrobacteriaceae are found in both water and sediment and, although their distribution has not been studied directly, their activities have. The distribution of nitrification with depth in sediments has been well researched (Billen 1982; Blackburn 1983). Nitrification is maximal when both oxygen and ammonium ions are present and so occurs in the oxygenated surface layers of the sediment only (Fig. 9). This results in utilization of ammonium ions and production of nitrate, hence ammonia is depleted and nitrate accumulated in the surface sediment (Fig. 9).

Denitrifying bacteria

Bacteria capable of denitrification are predominantly facultative anaerobes. Denitrification is an anaerobic respiratory process by which bacteria use nitrate as a terminal electron acceptor and produce nitrogen gas via the intermediates nitrite, nitric and nitrous oxides. Jetter and Ingraham (1981) list 73 genera capable of denitrification. These include common aquatic heterotrophs such as *Pseudomonas*, *Alkaligenes* and *Vibrio* and other less widely distributed genera like *Hyphomicrobium* (Fig. 5f), *Leptothrix* (Fig. 5k) and *Thiobacillus*. Because denitrification is a respiratory process carried out mostly by heterotrophs, a source of organic carbon is normally required and if insufficient carbon is available only nitrite will be produced.

Denitrification rates should be high whenever there are anaerobic conditions and plentiful supplies of nitrate and organic carbon. In eutrophic lakes, denitrification rates are highest in early summer in freshly anaerobic water. These rates decrease as nitrate becomes depleted. Hence denitrification is responsible for nitrate depletion in the hypolimnion (Wetzel 1983). It is thought that in some North Sea sediments the same principles hold; denitrification occurs in the top layer of anaerobic sediment immediately below the redoxycline in a zone quite distinct from the aerobic nitrification zone (Billen 1982). However, in some other marine and freshwater sediments (Sørensen 1978; Jones 1982b) denitrification rates are maximal in the oxidized surface layer of sediment. Jones (1982b) says that the denitrification zone in a eutrophic lake sediment occurred at an E_h of about +200 mV in a similar place to the nitrification layer. He argues that this is because nitrate, the substrate for denitrification, is produced in this zone only by nitrification and that denitrification can occur in the anaerobic centers of sediment particles. The high rates of denitrification in oxidized littoral sediments are also explained by this argument.

Nitrate dissimilatory bacteria

Reduction of nitrate to ammonia is another anaerobic process carried out by heterotrophic bacteria. However, it has not been very intensively investigated in

sediments. Herbert (1982) reports a series of attempts to isolate nitrate-dissimilating heterotrophs using plating and continuous culture techniques. This work showed *Aeromonas*, *Vibrio*, *Klebsiella*, *Escherichia* and *Clostridium* to be the most important organisms, but *Pseudomonas* and *Acinetobacter* were also isolated. Although nitrate dissimilation is an anaerobic process it can also be carried out under microaerophilic conditions (Herbert 1982). Little is known of the depth distribution of the process in sediments. Jones (1985) says that it only occurs in the deeper layers of anaerobic freshwater sediments, but others working on organically rich coastal sites have shown it to occur in aerobic sediments (Herbert 1982).

Nitrogen-fixing bacteria

Gordon (1981) lists 59 genera of nitrogen-fixing bacteria and these consist of many different taxonomic and physiological types. The largest group are the cyanobacteria (28 genera) and although nitrogen-fixing occurs in the heterocysts of heterocystous types, it can also occur in the vegetative cells of nonheterocystous cyanobacteria. Several genera of the anoxygenic photosynthetic bacteria (6 genera) are also said by Gordon (1981) to be nitrogen-fixing although this list might well not be comprehensive (Gorlenko et al. 1983). *Azotobacter* (Fig. 5d) and *Clostridium* are often said to be the most important free-living heterotrophic nitrogen-fixers although many other types may also be important such as methanotrophs, the enterobacteria and some sulfate-reducing bacteria and methanogens. The nitrogenase enzymes that mediate nitrogen fixation are very sensitive to oxygen. Aerobic nitrogen-fixing bacteria have developed mechanisms for protecting their nitrogenase; thus, nitrogen fixation can occur aerobically or anaerobically.

Sediments do not seem to be very important sites of nitrogen fixation (Blackburn 1983) and the distribution of the process with sediment depth does not follow a regular pattern (Jones et al. 1980). In the water column, nitrogen fixation is often associated predominantly with cyanobacteria (Wetzel 1983) and the vertical distribution of nitrogen fixing activity follows the biomass of the cyanobacteria. Thus most nitrogen is fixed in the upper well illuminated and oxygenated layers of the water column. In one study in a freshwater lake, cyanobacterial nitrogen fixation was undetectable (Jones et al. 1980) and only a low rate of heterotrophic fixation was observed. This was accounted for by sewage input into the lake, which resulted in increased ammonia concentrations that suppressed nitrogen fixation. Whether or not this is a general phenomenon has yet to be demonstrated. The phototrophic sulfur bacteria often fix nitrogen and in one study (Wetzel 1983) the green sulfur bacterium *Pelodictyon* caused a peak in nitrogen fixation activity just below the oxycline.

Temporal Variation in Bacterial Populations and Their Activities

The size and activity of populations of bacteria in water and sediment do not remain constant but are in dynamic equilibrium with the environment and so change with time. These changes occur both because environmental changes affect the bacteria and the bacteria affect the environment. Such changes seem to occur on both annual and daily time scales. Apart from these regular seasonal and diurnal variations successional changes will also occur after detritus is added to an aquatic habitat. The principles which have been discussed in the preceding sections normally apply to the temporal variations in a similar manner to the ways in which they control vertical variations in the bacterial populations and activities. Consequently, in this section, the subject matter is not covered exhaustively, but a few examples are given to illustrate the types of temporal changes that occur.

Diurnal Changes

Studies on the sulfur cycle have provided some good examples of the diurnal changes that occur in aquatic habitats. One such study (Hansen et al. 1978) has examined the release of H_2S from two coastal sediments. Although one was richly supplied with organic matter from decomposing seagrass and the other was sandy and less organically rich, the general changes that occurred were similar (Fig. 10). There was very little aerobic layer in these sediments as the 0 mV layer, which crudely separates the oxidized and reduced sediment zones, was never deeper than 2 mm from the surface. Consequently, sulfate-reducing bacteria were constantly active in the top 1 cm of sediment, producing H_2S. During the daytime, benthic microalgae photosynthesized, producing oxygen that diffused into the overlying water and maintaining a high concentration of dissolved oxygen. Purple photosynthetic bacteria were also able to photosynthesize at the H_2S/O_2 interface, because it was within the top 1 to 3 mm of surface sediment, which was illuminated. Thus these purple bacteria oxidized some of the H_2S produced by the sulfate-reducing bacteria during the day, whilst the rest of the H_2S was oxidized chemically by the oxygen produced by the benthic microalgae. Thus no H_2S was released into the overlying water in daylight. As dusk approached and oxygenic photosynthesis stopped, the oxygen concentrations in the sediment and overlying water decreased, and H_2S was no longer chemically oxidized. Purple-bacterial photosynthesis also stopped at dusk and so their utilization of H_2S also stopped. For these two reasons H_2S produced by the sulfate-reducing bacteria was not removed at night and accumulated in the sediment and overlying water. Once the pH had dropped sufficiently, H_2S was also released into the atmosphere. At dawn the reverse of these processes occurred and once again the stable daytime situation was reached. Very similar cycles of events have been observed in other marine sites (Ingvorsen and Jørgensen 1979) and in the benthic cyanobacterial mats of a hypersaline lake (Jørgensen et al. 1979b).

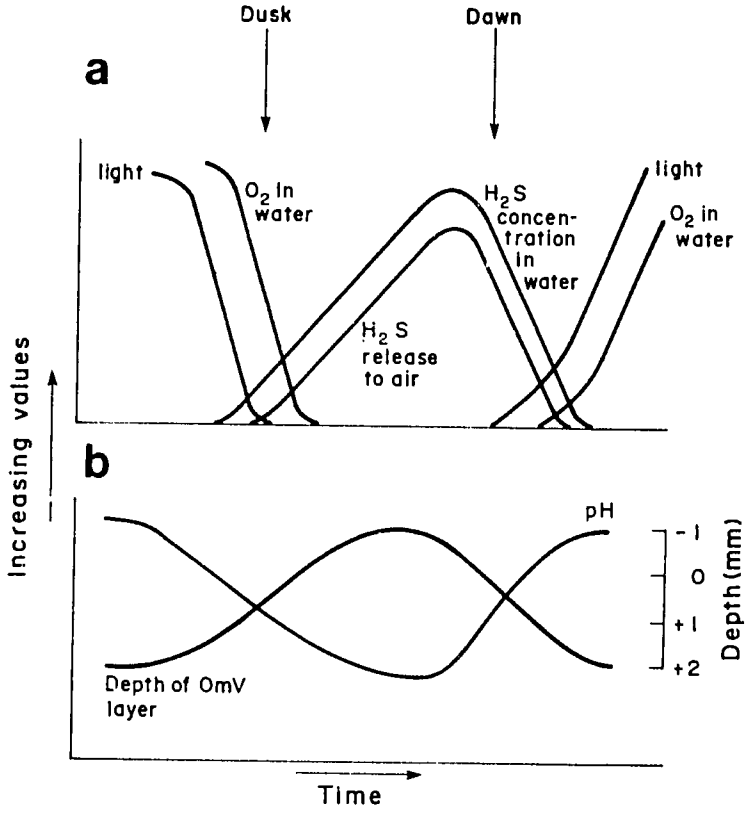

Fig. 10. Generalized diagram of diurnal changes for (a) several variables important in the release of H_2S from coastal sediment overlain by 0-10 cm of water and (b) the depth of the O mV layer in the sediment and pH changes in the overlying water (based on data presented by Hansen et al. 1978).

Other similar types of diurnal changes involving sulfur cycle organisms have been observed in the water column of some aquatic habitats. In the hypersaline lake mentioned earlier, such changes have been well documented (Jørgensen et al. 1979a). In that lake, the H_2S interface, which occurred near the chemocline, was forced downwards in the water column during the day and rose at night. This was caused by the oxygenic photosynthesis of cyanobacteria in the daytime producing more oxygen to oxidize the H_2S produced by sulfate-reducing bacteria from below. These aerobic conditions also encouraged H_2S oxidation by thiobacilli thus further helping to depress the H_2S/O_2 interface. These processes caused the H_2S/O_2 interface, the layer of dark CO_2 fixation and the layer of light CO_2 fixation by anoxygenic cyanobacterial photosynthesis, to move about 10 cm vertically during a diurnal cycle.

It is likely that diurnal changes in bacterial populations can be very important in even very shallow waters receiving large amounts of detritus. For example, waste

stabilization ponds in tropical countries used for sewage treatment undergo very large physical and chemical diurnal changes (Mara 1976; Hawkes 1983) although they are only about 1.5 m deep. At night these ponds are fully mixed but very low in dissolved oxygen due to the high rates of respiration by heterotrophic bacteria and algae. In the daytime they are fully stratified like a eutrophic lake, the surface water being supersaturated with dissolved oxygen from algal photosynthesis and the lower water being nearly deoxygenated. Thus, although these ponds have not been studied in detail microbiologically, it is quite likely that large diurnal changes in the populations and activity of carbon, sulfur and nitrogen cycle organisms occur.

Seasonal Changes

Temperature is one of the major environmental factors affecting populations of aquatic bacteria seasonally. Many workers have shown that the uptake of dissolved organic compounds by planktonic heterotrophic bacteria varies seasonally with maximum values in summer and minimum values in winter (Hobbie 1971; Hobbie and Rublee 1977; Gillespie and Spencer 1980). Similar changes have also been documented for bacterial sediment oyxgen uptake (Wetzel 1983). These changes are strongly correlated with temperature (Gocke 1977; Fry and Humphrey 1978; Nuttall 1982b; Wetzel 1983) and closely follow a sinusoidal pattern (Fry et al. 1981) that is also consistent with the pattern of temperature change. Consequently, there is little doubt that temperature is the major driving force behind these changes, particularly as there do not seem to be any large seasonal changes in total numbers of bacteria in aquatic habitats (Fry et al. 1981; Quinn et al. 1985). Temperature also affects many other bacterially mediated processes. For example sulfate reduction, methanogenesis (Senior et al. 1982) and denitrification (King and Nedwell 1984) are all highest in summer and lowest in winter in salt-marsh sediment as a result of temperature changes. In the case of denitrification the denitrifying bacteria also change with the seasons (King and Nedwell 1984). Mesophilic denitrifiers were present all the year but were most developed in late summer, whilst psychrophilic denitrifiers were only present during winter. In this study the mesophiles were predominantly *Pseudomonas* spp. and the psychrophiles were mainly *Vibrio* spp.

Temperature is not always the driving influence for population changes of heterotrophic bacteria. Sometimes the supply of organic carbon is most important. An example of this is with pectinolytic anaerobes in the sediments of eutrophic Lake Mendota where the highest numbers were recorded in autumn when the deposition of algae and leaf detritus was maximal (Schink and Zeikus 1982)

In stratified lakes, many seasonal changes in bacterial populations are controlled by the extent of deoxygenation of the hypolimnion or monimolimnion. In eutrophic lakes, seasonal variations are great because stratification and hypolimnetic oxygen depletion only occurs during the summer. In meromictic lakes, seasonal changes are less because the chemocline is permanently present. Changes in populations of

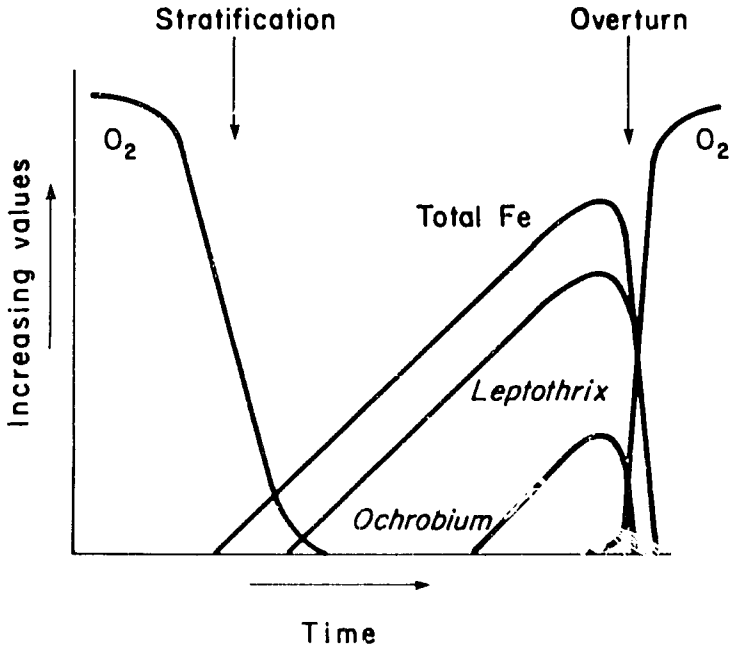

Fig. 11. Generalized diagram of the seasonal changes in iron-oxidizing bacteria and related variables in the hypolimnion of a eutrophic lake.

iron-oxidizing bacteria in a eutrophic lake will be used as an example (Fig. 11). In oxygenated water iron occurs mainly in its ferric form, which is insoluble; so little iron is in solution. In deoxygenated water iron occurs mainly in the ferrous form, which is soluble, and thus insoluble iron in the sediments dissolves in the anaerobic water increasing soluble iron concentrations. Consequently in spring when thermal stratification occurs and the hypolimnion starts to become anoxic iron concentrations in the hypolimnion increase. Hydrogen sulfide which is also present in the hypolimnetic water then reacts with the dissolved iron and forms a black precipitate of iron sulfide. More iron then moves from the sediment into the water to compensate for the loss of soluble iron as the sulfide precipitates. Thus total iron concentrations in the hypolimnion also increase with the onset of stratification. The increase of iron concentrations in the hypolimnion then makes it possible for iron-oxidizing bacteria to move from the sediment, where they grow during the winter, to the water column. The first organisms to start growing in the hypolimnetic water are those that grow best under microaerophilic conditions and are found at the oxycline. *Leptothrix* (Fig. 5k; Jones 1975) and *Metallogenium* (Gorlenko et al. 1983) behave in this way. Later, bacteria that grow best under anaerobic conditions colonize the lower layers of the anoxic hypolimnion; *Ochrobium* seems to be an example of this

type of organism (Jones 1981). At overturn in the autumn, when the deoxygenated water in the hypolimnion is dispersed, iron concentrations decrease rapidly and the iron-oxidizing bacteria return to the sediment. Many different groups of bacteria show seasonal changes of this sort. Some other examples have been reported by Cappenberg (1972) for sulfate-reducing bacteria and methanotrophs and by Hall (1982) for nitrifying bacteria.

Seasonal changes also occur in the bacterial populations of sediments. Billen (1982) reports a study of seasonal changes in nitrification and denitrification in two sediments from a marine lagoon near the coast of Belgium. The rates of these two processes were controlled mainly by temperature, with high rates in summer and low rates in winter. The depth of the nitrification layer also varied; it was near the sediment surface in summer and further from it in winter. This movement corresponded to the movement of the redoxycline in the sediments as would be expected from previous discussions. The relative magnitude of nitrification and denitrification in sandy and muddy sediments had a great effect on nitrate flux. In the sandy, aerobic sediment nitrification was always higher than denitrification and nitrate always diffused into the water column. However, in the more anaerobic, muddy sediments the relative importance of the two processes was such that nitrate diffused into the water column in winter but was required to drive denitrification during the summer; thus nitrate concentration in the overlying water was lowered at this time of year.

Seasonal changes of bacterial populations over a two and a half year period have been studied in one set of aquaculture ponds in India (Jana and Roy 1985a, 1985b). These studies showed marked seasonal changes in numbers of proteolytic, ammonifying and nitrifying bacteria in water and sediment. Largest numbers of all types were found in winter and smallest numbers in summer. Seasonal changes were accounted for by changes in the available substrates for these bacteria. Differences between numbers in the different fishponds studied were, however, thought to be related to the amount of organic carbon entering the ponds.

Successional Changes

Studies in my laboratory to investigate the effect of herbicides on heterotrophic bacteria in freshwater habitats give some idea of the changes that will occur when a large amount of detritus is added to an aquatic ecosystem. This is because the main effect of the herbicide is to kill the aquatic plants and the bacterial changes (Fig. 12) are almost all due to the death and decomposition of the plants rather than direct effects of the herbicide (Fry et al. 1973; Ramsay and Fry 1976; Fry and Ramsay 1977). Most of the work has been done with paraquat, but diquat and terbutryn show similar effects (Cragg and Fry 1984) as all are quick-acting herbicides.

The plants were killed very quickly after addition of the herbicide and photosynthesis stopped. Thus, the diurnal oscillations in dissolved oxygen concentration observable before treatment, caused by photosynthesis of the macrophytes, stopped after treatment. Oxygen concentrations declined as the continuing plant respiration

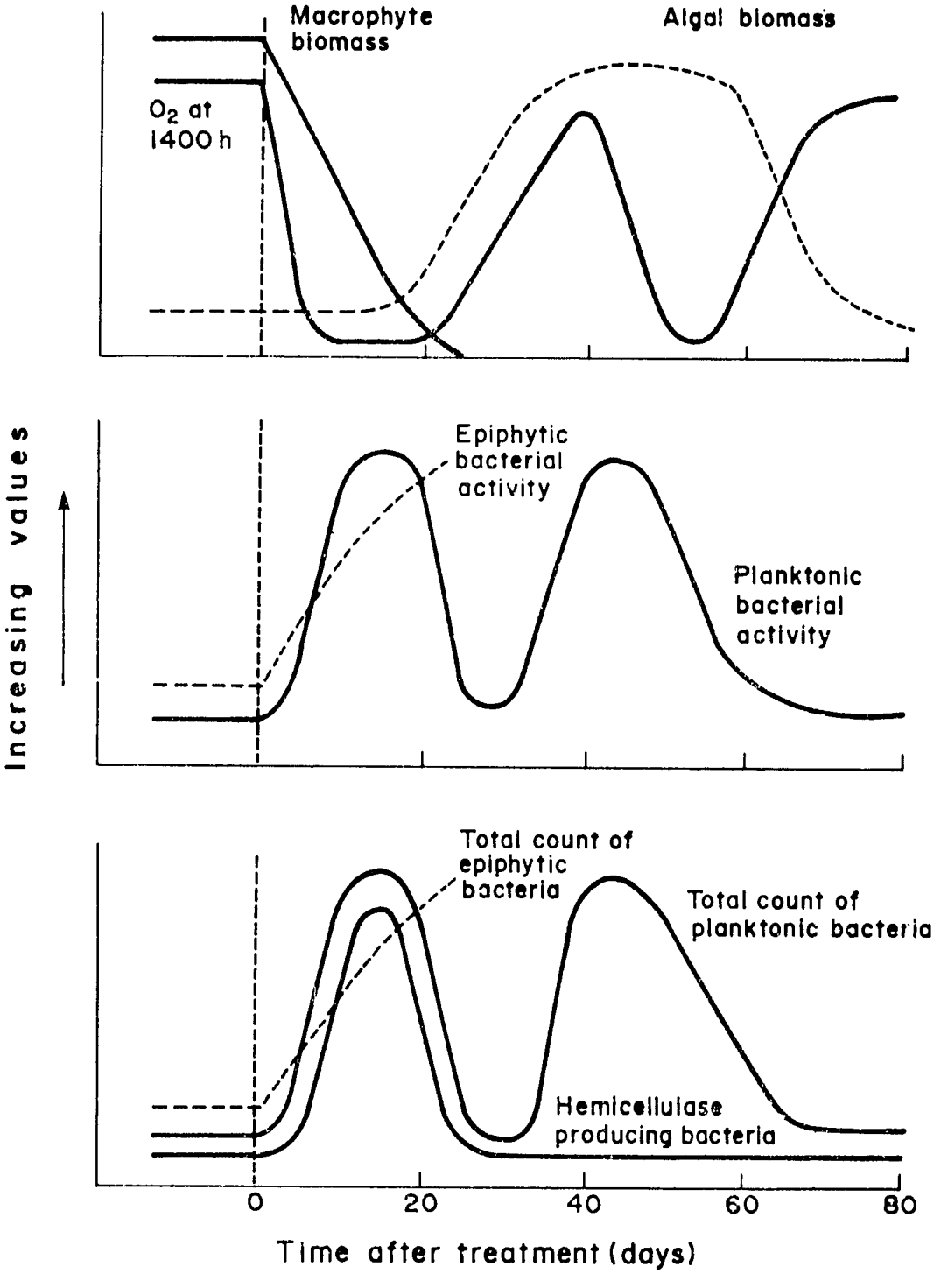

Fig. 12. Generalized diagram of the changes in several bacterial variables, algal and macrophyte biomass and water chemistry that occur after treatment of a weedy pond with the herbicide paraquat (based on data presented by Fry et al. 1973; Humphrey 1977; Fry and Humphrey 1978; Cragg and Fry 1984).

and oxygen consumption by the increasing populations of heterotrophic bacteria removed dissolved oxygen. By about 5 to 10 days after treatment oxygen concentrations reached a minimum close to zero; the precise value depended on the biomass of plants present before treatment (Brooker 1974). The macrophyte biomass also decreased and normally by 20 to 25 days all standing macrophytes had gone. While these changes occurred total and viable numbers of bacteria in the water, on the plants and in the sediment (Fry et al. 1973) increased, reaching maximum numbers by about 4 to 10 days. These population increases corresponded with similar increases in rates of uptake of labile organic materials such as glucose, acetate and glycollate. Autoradiography has also shown similar maxima in the proportion of the bacteria taking up glucose (Ramsay and Fry 1976). Bacterial populations usually returned to pretreatment values by about four weeks after treatment, when all the plant material had decomposed. However, during one study (Fig. 12; Fry and Humphrey 1978) a bloom of an alga (*Euglena* sp.) started to appear 17 days after treatment. Algal photosynthesis then caused the dissolved oxygen concentration in the water to increase, at about the time that the bacterial numbers and their activity decreased. Once the *Euglena* sp. had nearly reached maximum biomass, bacterial numbers and activity started to increase once more, to reach maxima by about six weeks after treatment, and dissolved oxygen again decreased to a minimum of 0.1 mg l^{-1}. The bacteria were presumably growing on exudates and particulate organic carbon from the algae. Thereafter, values of all variables returned to normal by about 10 weeks after treatment. This second maximum in the values for the bacterial variables was not due to macrophyte decomposition because numbers of hemicellulase-producing bacteria did not increase at that time. This indicated that macrophyte cell walls, which probably contain large amounts of hemicellulose, were not being decomposed. These changes show that the primary effects that occur when large amounts of detritus are decomposed in water are straightforward. However, secondary changes may be complex and at present it is not possible to predict when they will occur. No long-term effects on the aquatic bacteria in the ecosystem were detectable after the herbicide treatment (Fry et al. 1981).

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Discussion

WOHLFARTH: Regarding the carbon cycle, what is the source of your figures?

FRY: Williams (1981). I have adapted his figures in the text of my paper.

WOHLFARTH: The other point is that, as you have presented it, this is not strictly speaking a cycle because it comes to an end.

FRY: Yes. I have done this to illustrate the way the carbon is incorporated into the bacteria; hence their great importance. Even the carnivores, like some fish, can decompose and contribute to bacterial carbon, but this is a very minor source. I have not tried to explain everything in detail but rather to indicate the overall importance of bacteria in carbon mineralization.

WOHLFARTH: But what happens to the carbon fixed in heterotrophic bacteria?

FRY: The bacteria die. The carbon is released when they decompose and other heterotrophic bacteria live on their decomposition products. Therefore, you have sort of 'heterotrophic shunt': live bacteria living off dead bacteria.

EDWARDS: I was intrigued by your revelation that some bacteria can be as large as 35 μm and could be mistaken for blue-green algae (otherwise called Cyanobacteria). In a series of ponds at AIT fed with human excreta, we had a dominant organism—which I tentatively identified as the blue-green alga *Romeria*. However, it was brownish in color and filamentous. Perhaps it was one of these large bacteria. It was persistent for several months but has not been seen since.

FRY: That is possible. I would say that to label something as a blue-green alga, one should be able to demonstrate the presence of chlorophyll, for example, by fluorescence microscopy. However, there are blue-green algal taxonomists who refer to 'colorless' blue-greens. We are in a grey area of taxonomy here but the Cyanobacteria are well-separated by taxonomists from other major groups of bacteria.

EDWARDS: You said that 96% of detritus was taken up by heterotrophic bacteria. I presume that by the term detritus you mean here decomposing plant matter as opposed to manure, which has a considerable fertilization effect in stimulating phytoplankton growth in fishponds.

FRY: That is correct. In my paper, it is regarded as material derived from macrophytes. However, detritus derived from manure would follow a similar route. The fact that phytoplankton are produced does not change the fact that their ultimate consumers are bacteria.

EDWARDS: Yes, this applies to the phytoplankton which die, but large quantities are cropped by the fish which are then harvested.

Production and Decomposition in Aquatic Ecosystems and Implications for Aquaculture

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Abstract

Secondary (total heterotrophic) production in terrestrial and most aquatic ecosystems is largely sustained by recycling dead organic matter rather than by direct consumption of net primary production. On land, little of this production can be exploited by man but in aquatic systems, mainly because water is a support and transport medium, the decomposer and herbivore systems have closer functional integration resulting in the effective transfer of microbial production to higher trophic levels. The component processes of decomposition and the links between particular organic matter, dissolved organic matter and inorganic nutrient fluxes are keys to the potential for manipulating these transfers.

All stages in the processing of organic matter involve the interaction of resource quality (Q), organisms (O) and physical environmental conditions (P). The net effect of this complex is manifested in terms of the component processes of decomposition: catabolism (K), comminution (C) and leaching (L). Thus OPQ can be seen as a module effecting all state changes in organic matter with the particulate organic matter or dissolved organic matter products forming inputs to other modules with different suites of organisms and environmental conditions. The products, including mineral elements, can also enhance or inhibit the decomposition of other resources.

Cases are reviewed in which resource quality, organism activities (particularly animal/microbial interactions) and physical/chemical environmental conditions interact in determining the rate and pathways of detritus decomposition, and the input/output balances of nutrients and organic matter for the systems.

The extension of these ecological principles to detritus exploitation in aquaculture is considered in terms of experimental approaches and testable hypotheses.

Introduction

More than 99% of plant biomass and 67% of global net primary production occurs on land (Leith 1975). The oceans, which comprise about 90% of the marine biome and cover 70% of the globe, have a mean net primary production of about 125 g C m^{-2} $year^{-1}$; a similar figure to tundra or semi-desert conditions on land. The primary production of the continental shelves and upwelling areas of 200-1,000 g C m^{-2} $year^{-1}$ is comparable to temperate woodlands and grasslands, and only limited areas of aquatic ecosystems and fringing communities (Teal 1980; Brinson et al. 1981) approach the burgeoning NPP (2,000 to 3,000 g m^{-2} $year^{-1}$) of the extensive tracts of humid tropical rain forests. Nevertheless, aquatic ecosystems are estimated to contain around half of the animal biomass in the biosphere and contribute almost all of the secondary production harvested by man from natural systems; mostly as fish from high trophic levels.

Many ecological and socioeconomic factors underlie this disparity of harvestable secondary production between terrestrial and aquatic systems (Odum 1980). But a key factor is the nature of water as a support and transport medium because this affects the quality of food resources available to herbivores and serves to integrate the decomposer and herbivore systems, which largely function as discrete entities in terrestrial ecosystems. These contrasting patterns of secondary production are briefly outlined for terrestrial and aquatic systems and the decomposition of plant materials is discussed in terms of the effect of water on the component processes and rate determinants. Finally, the role of detritus is considered in the context of aquaculture systems.

Secondary Production in Terrestrial and Aquatic Systems

A simple model of energy flow pathways in terrestrial ecosystems is shown in Fig. 1. The model is based on an extensive review of ecological efficiencies within or between trophic levels but the key parameters governing energy partitioning between the herbivore and decomposer systems are the consumption efficiencies (C_n/P_{n-1}). This is a measure of the consumption by trophic level 'n' of production by the previous trophic level. Mean consumptions of net primary production by animals has been estimated at 1% in cultivated land, 4 to 7% in forests and 10 to 25% in grasslands (consumption of above ground production may approach 50% but a major proportion of net primary production is allocated to roots and is unavailable to most herbivores). The consumption efficiencies of higher trophic levels (shown in Fig. 1) are more uncertain but have little direct effect on the energy fluxes through the herbivore system. Thus 80 to 90% of terrestrial net primary production, together with the excreta and animal residues, is transferred to the decomposer system. Here only an average of 10% of the energy is utilized by animals, mostly small invertebrates, and the remaining 90% is utilized by microorganisms. However, nonutilized materials, including the products of higher trophic levels are reutilized in the decom-

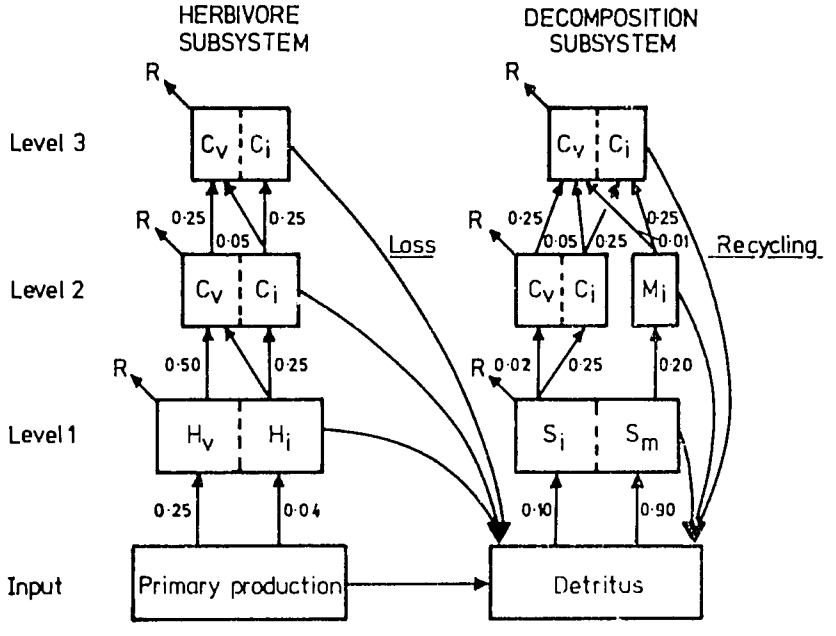


Fig. 1. A simple trophic model comparing the organisms of the herbivore and decomposition subsystems. The symbols are those of the original paper: S = saprovore decomposers; M = microbivore; H = herbivore; C = carnivore; v = vertebrate; i = invertebrate; m = microorganism; R = loss to respiration. The transfers linking the compartments are the fractions transferred at any one linear run of the model. (After Heal and MacLean 1975).

poser subsystem in contrast to the single passage through the herbivore subsystem. As a consequence of this recycling, Heal and Maclean (1975) showed that even in an intensively grazed grassland only 1.6% of secondary production occurred in the herbivore subsystem and 94% of total heterotroph production was by fungi and bacteria. The model was applied to data from a number of sites in the International Biological Programme and predicted the empirically determined level of secondary production within fairly close limits.

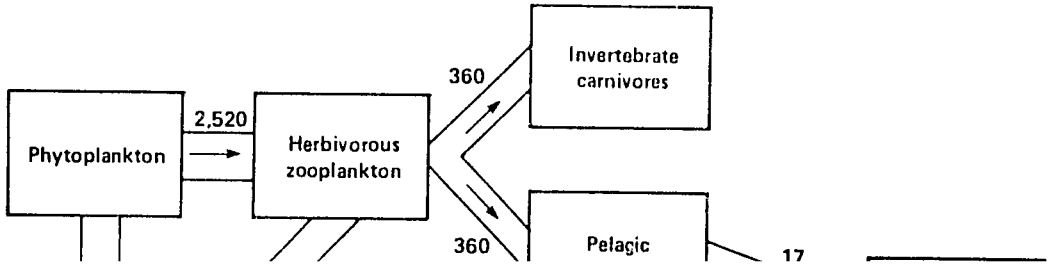
Hence terrestrial secondary production is limited by the efficiency with which net primary production is utilized by herbivores and the spatial isolation of the herbivore and decomposer systems imposed by the effects of gravity. Most secondary production is located in the soil where it cannot be exploited by man as a food resource and the predator links from the decomposer to the herbivore subsystem are negligible in relation to the total fluxes. Input of surface net primary production to the benthos in lakes and marine systems varies between 64 and 0.5% according to turbulence and the depth of the water column (Hargrave 1973). In mid-ocean with surface productivity around 50 g C m^{-2} $year^{-1}$ only 1 to 2% of net primary production reaches the benthos compared with an eutrophic lake with a production of 500 g C m^{-2} $year^{-1}$, and a mixed layer depth of less than 5 m, where 200 g C m^{-2}

may reach the bottom each year. In deep waters, therefore, production, consumption and decomposition reach approximate equilibrium in the photic zone and the rate of production at higher trophic levels can be estimated as:

$$\text{Production} = BE^n$$

where B is phytoplankton production, E the transfer efficiency and n the number of trophic links between the producers and consumers (Parsons 1976).

Consumption of net primary production is generally higher in marine systems than on land; perhaps 40% in open oceans, 35% in upwelling areas and 30% on the continental shelves; in algal beds and estuaries macrophyte consumption of 10-15% is similar to terrestrial systems; on average 63% of marine net primary production enters the decomposer subsystem. But in contrast to the situation in terrestrial ecosystems, the energy budget for the North Sea (Fig. 2), constructed by Steele (1974), shows fish production supported by the pelagic food chain and detrital resources reaching the sea floor. Implicit in this model are very high consumption efficiencies (75%) for zooplankton and negligible recycling of dissolved organic matter to zooplankton via planktonic bacteria. This pathway has been invoked by various authorities to account for seasonal discrepancies between net phytoplankton production and zooplankton consumption (Crisp 1975; Hollibaugh et al. 1980) but is discounted by Porter (1984) on the grounds of bacterial densities, filtration efficiencies and nutritional status of oligotrophic bacteria as a food resource.



However, these criteria for dismissing the importance of bacteriophagy need reappraisal in the light of evidence reviewed by Azam et al. (1983) that microflagellates (3 to 10 μm) can regulate bacterial populations and hence promote rapid recycling of potentially limiting nutrients to phytoplankton in the photic zone. But although the bacteria may utilize 10 to 50% of the photosynthetically fixed carbon to scavenge nitrogen for protein synthesis, the returns to the main food chain through the 'microbial loop' of bacteria—microflagellates—microzooplankton to zooplankton are probably small because of the number of trophic links involved.

A similar situation pertains in lakes though there is little doubt that zooplankton, and particularly Cladocera, can effectively exploit the larger bacteria generally found in freshwater systems (Fenchel and Jørgensen 1977; Peterson et al. 1978; Coveney et al. 1978). Pedros-Alio and Brock (1983) showed that zooplankton in Lake Mendota consumed up to 60% of bacterial biomass when cell densities increased above the threshold concentration of 10^6 ml^{-1} (below which they do not feed on bacteria). But because the production to biomass ratio for the bacteria was about 3:1, the animals only consumed about 1 to 10% of bacterial production; a major component of the remainder settled to the bottom on particulate organic matter. In Lawrence Lake, Wetzel et al. (1972) found that out of 171 g m^{-2} photosynthetically fixed carbon, 20 g m^{-2} was respired by bacteria in the pelagic zone and 118 g m^{-2} was respired in the sediment; thus 80% of the primary production was utilized by the detritus food chain and approximately 70% in the benthos.

Freshwater ecosystems display this full range of functional organization. On one hand there are the head streams with low secondary production at higher trophic levels and food chains based almost entirely on terrestrial detritus, and on the other, highly productive lakes and carp ponds supported by indigenous algal and macrophyte production.

Bear Brook (Fig. 3a) is heavily shaded by forest trees and thus no attached algae and few macrophytes can survive. Allochthonous inputs are from three sources: 44% as tree litter (leaves, fruits, twigs and branches), 25% as dissolved organic matter from ground water drainage and the remaining 31% from upstream (22% dissolved and 9% particulate matter). The inputs of leaves or branches and stores within the system did not vary much from year to year. Assuming steady state conditions, Fisher and Likens (1973) calculated turnover times of 1 year for leaves and 4.2 years for branches. Heterotrophic respiration (34% of incoming energy) was largely by fungi and bacteria: there was negligible production of invertebrates or fish, and 66% of the incoming energy was exported downstream (46% as dissolved organic matter and 20% as particulate organic matter).

The River Thames, which is dammed at many sites, has significant phytoplankton production and, although there are major throughputs of dissolved and particulate organic matter, it is more functionally analogous to an eutrophic lake than a river (Fig. 3b). The transfer efficiency from gross primary production of fish ranges from between 0.1 and 1.6% for lakes and reservoirs to around 4% for carp ponds (Morgan 1980). Mann et al. (1972) suggest that the transfer efficiency of the Thames is as high as 10% because roach are directly utilizing detritus as a food resource. But this

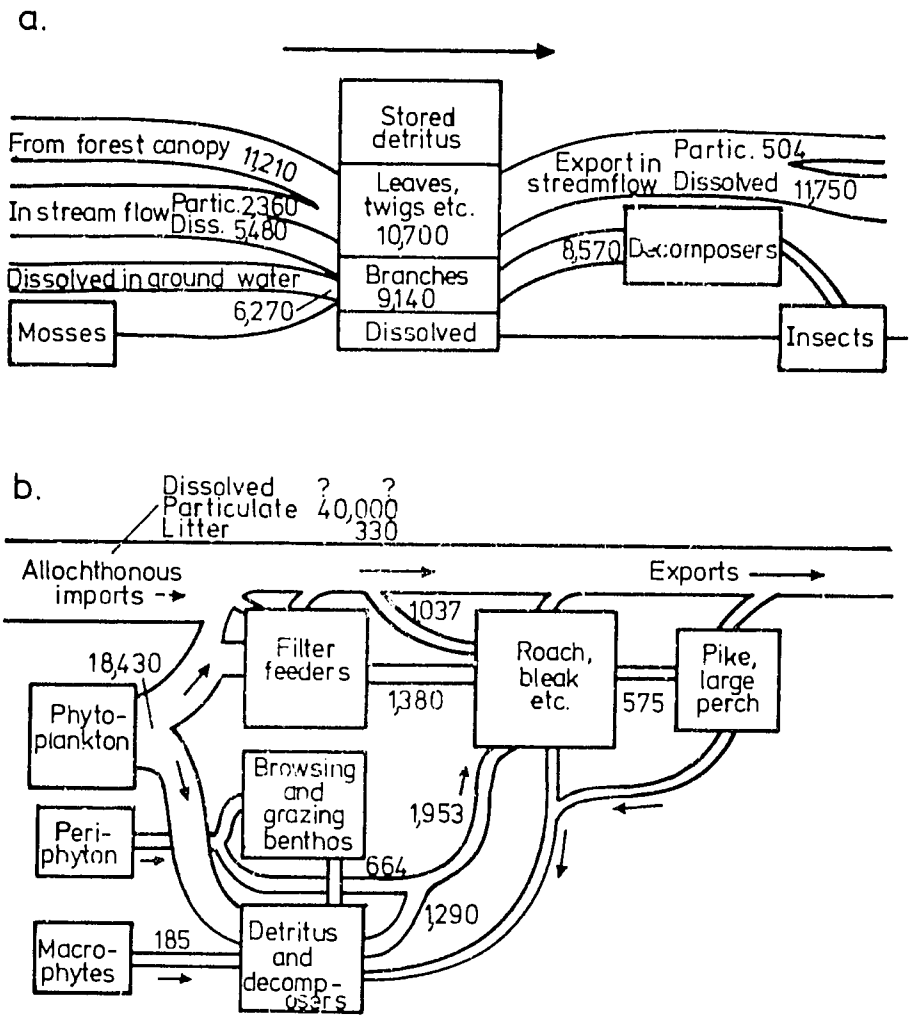


Fig. 3. Patterns of energy fluxes in (a) Bear Brook (Fisher and Likens 1973) and (b) River Thames (Mann et al. 1972).

figure is challenged by Morgan (1980) on the basis that the transfer efficiency has little meaning for an open system with detritus inputs and losses. For example, the energy budget for a lowland chalk stream determined by Westlake et al. (1972) shows that fish production had a transfer efficiency of about 0.001% because the amount of dissolved and particulate material entering and leaving the system was many times higher than primary production.

It is now increasingly recognized that stream and river systems must be seen as a functional continuum of systems integrated not only from source to sea but with

the surrounding catchment (Vannote et al. 1980; Minshall et al. 1985). The River Continuum Concept has important implications for the understanding of aquatic decomposition processes because not only does the relative mass and quality of allochthonous and autochthonous inputs vary seasonally, and with the position in the river gradient, but the environment for decomposition of dissolved organic matter and particulate organic matter changes as the material moves downstream (Fig. 4). This environmental complex includes physical parameters (P) of temperature and oxygen which regulate community metabolism as well as current speed which structures the community and differentially transports materials according to their mass, the organisms (O) particularly the invertebrate fauna which regulate the transport of particulate organic matter and the quality of the plant resources (Q) on which the organisms are acting. This concept of a complex of OPQ variables regulating decomposition has been developed by Swift et al. (1979) for terrestrial systems and is here considered in the context of aquatic decomposition processes.

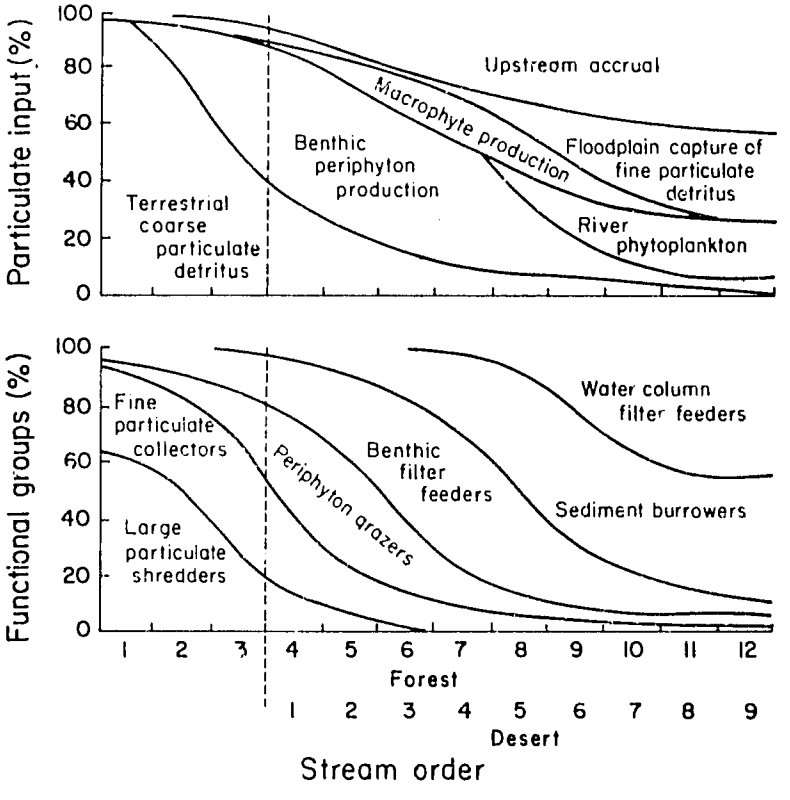


Fig. 4. Expected changes in particulate organic matter inputs and functional feeding group relationships along a river system. The abscissa is shown as a 'sliding scale' to emphasize the fact that different streams enter the continuum at different points. In the two cases illustrated, forest streams begin with a strong terrestrial influence (reflected by a predominance of allochthonous organic matter and detrital processors) whereas desert streams, due to the lack of shading and reduced influx of allochthonous detritus, enter the sequence at a point displaced to the right and equivalent to a more downstream position of the forest stream. (After Minshall et al. 1985).

Decomposition in Aquatic Ecosystems

The Component Processes

All changes in the state of a resource from intact plant or heterotroph tissues to the final processes of carbon and nutrient mineralization are governed by the interaction of O, P and Q and can be represented by the simple module shown in Fig. 5a. The state changes produced by each module involve fragmentation by biotic and abiotic processes, enzyme action and the loss of water soluble materials. Decomposition is thus the sum of catabolism (K), comminution (C) and leaching (L) (Swift et al. 1979).

Catabolism is the enzymic degradation of a substrate (chemical compound) such as depolymerization (cellulose to oligo- and monosaccharides) or to its mineral constituents (glucose to CO_2 and H_2O). The rate of the reaction will depend upon the nature of the substrate: glucose is readily metabolized, cellulose is more recalcitrant and lignin refractory to most organisms.

Comminution is a reduction of resource or particle size and differs from catabolism in being predominantly a physical rather than a chemical process. It is largely brought about by animal feeding activities in terrestrial systems but the action of currents and waves are also important in aquatic systems. The particular groups or organisms producing the resource will determine the nature of the products, their availability to other organisms, and sedimentation or transport rates.

Leaching is the removal of soluble materials by water and is entirely a physical process. The contribution of leaching to mass losses largely depends on resource composition and physical environmental factors but is very much influenced by comminution and catabolism. Conversely, leaching can enhance or inhibit organism activities (by removing toxins or readily metabolized carbon compounds) and thus feedback to the processes of catabolism and comminution. Leaching also increases the availability of low molecular weight compounds to other organisms.

Decomposition processes can therefore be visualized as a cascade of steps with the products of catabolism, comminution and leaching, as well as organism tissues forming inputs to other modules, as shown in Fig. 5b. Products of different modules can also combine as in flocculation of dissolved organic matter or microbial immobilization of exogenous nitrogen sources in low quality litters. Biochemical and physical complexity of the resource will initially increase with time associated with a corresponding increase in species, some of which may be responsible for specific transformations, while others are functionally nonspecific or simply utilizing particulate organic matter as an attachment site. Then progressively, as the most readily catabolized fractions of the resource are mineralized, the rate of decomposition and the diversity of resource types decrease until theoretically only the constituent elements remain. But in practice the most recalcitrant fractions, including the products of microbial synthesis, become incorporated into sediments after being reprocessed by a large number of modules along the stream gradient or down the lake profile.

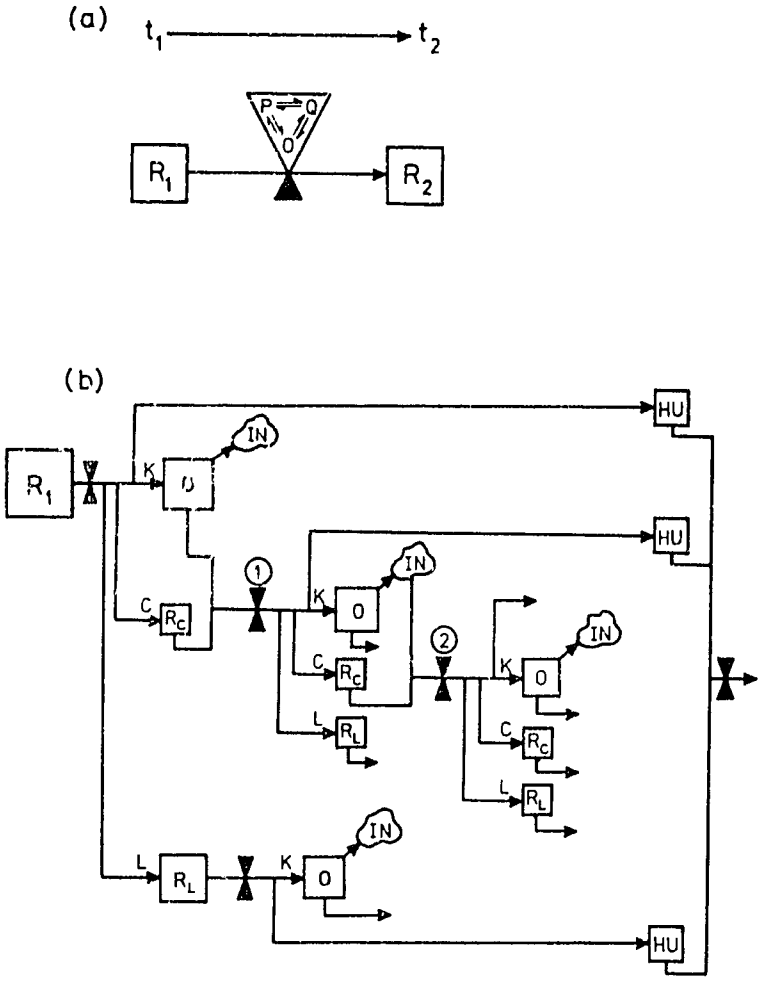


Fig. 5. Resource model of the decomposition subsystem and the driving variables. (a) The basic decomposition module illustrating the regulation of changes in resource state (R_1 to R_2) over a short period of time (t_1 to t_2) by the driving variables: the organisms (O), the physicochemical environment (P) and resource quality (Q). (b) A simplified model of the cascade processes whereby a resource is dissociated by the processes of catabolism (K), comminution (C) and leaching (L) and the substrate components are mineralized (IN), or resynthesized into tissue (O) and humus (HU). Soluble materials (R_L) and particulate organic matter (R_C) may be transported in unchanged form to other sites. Outputs of modules processing the same or different cohorts of materials may combine; e.g., ingestion of microbial tissues and fecal material (1) or the immobilization of inorganic nitrogen sources (2).

The relative decomposition rates of different classes of dissolved and particulate material are indicated in Table 1. In practice fine particulate matter will be composed of a spectrum of these materials (higher plant residues, remains of algae, animal tissues, microbial biomass and products and so forth) which have been subject to different degrees of reprocessing by terrestrial and aquatic modules. The resource quality of fine particulate organic matter is then very variable and generally poorly defined in the literature. Similarly, dissolved organic matter contains low molecular weight

Table 1. Relative rates of decomposition of organic materials in productive lake and stream systems. The same materials produced or decomposed in less productive water bodies may decompose more slowly. (After Saunders 1980).

Resource class	Decomposition (% day^{-1})	Substrates and resources
Dissolved organic matter	500-1,000	Low molecular weight compounds, e.g., volatile fatty acids
	25	Leaf leachate (labile fraction); extracellular algal and bacterial products. Simple sugars.
	2.5	Refractory component of extracellular product
	1	Refractory component of leaf leachate, e.g., tannins.
Particulate organic matter	10	Phytoplankton, zooplankton, insect exuviae, algae in littoral zone, submerged macrophytes.
	1	Tree leaves in streams and littoral zone, residual components of zooplankton.
	0.03	Refractory residues of primary and secondary resources.

materials that are rapidly decomposed, as well as a spectrum of aromatic and higher molecular weight compounds, such as humic and fulvic compounds of terrestrial and aquatic origins, together with phenolic compounds from allochthonous litter.

Consideration of the decomposition of these particulate and dissolved organic matter fractions lies outside the context of this review. The operation of the regulatory variables O, P and Q is therefore mainly discussed in terms of the processes operating on the original plant resource.

Decomposition Rate Determinants

The resource is the medium through which the effects of O and P are expressed and is therefore considered first.

Resource quality

Resource Quality is a composite definition of the value of a food resource to an organism and embodies both physical and chemical criteria.

The physical attributes of the resource include particle size, surface properties and texture which influence the group of animals or microorganisms exploiting the

resource. The influence of physical resource parameters on colonization and ingestion are considered in the section on organisms.

The chemical attributes of the resource can be classified into three main groups of compounds: the carbon and energy sources, nutrients and modifiers.

Carbon and energy sources

The carbon compounds differ widely in their biodegradability. They range from single sugars, starch and hemicelluloses, which most saprotrophs can catabolize to cellulose, and lignins that are more resistant. The resistance of lignin to depolymerization is conferred not only by the stability of the phenyl rings and covalent bonding between side chains but also by its overall hydrophobic nature. These resistant properties of lignin can also result in an inhibition of enzyme attacks on cellulose when the two polymers are in close proximity. The theoretical decomposition rates of these compounds in woodland leaf litter is shown in Fig. 6 and range from about 10% per annum for phenolic compounds to 99% for sugars. The total mass

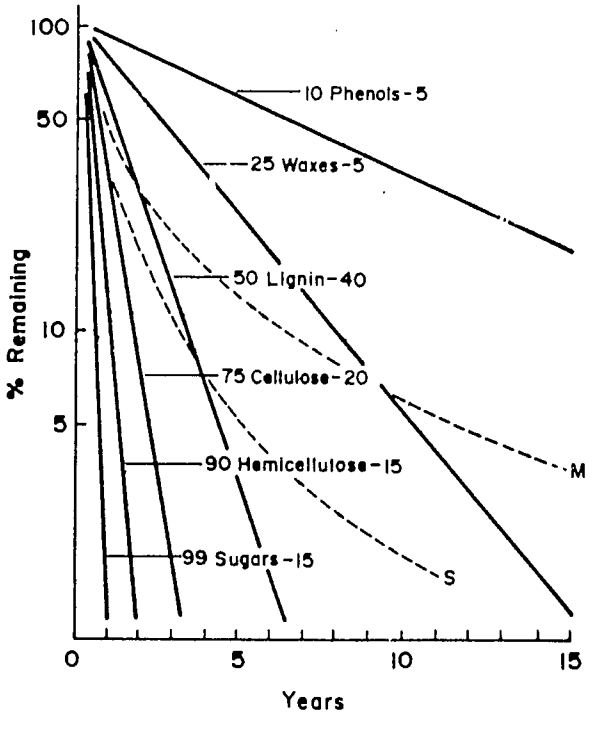


Fig. 6. The decomposition curves of the various groups of constituents, if their decomposition could be represented by a logarithmic function (the straight lines from the point 100%). The number in front of the name of the constituent indicates the loss after one year. The number after the constituent represents its percentage in weight of the original litter (these values are rough averages and they do not represent a specific analysis). The line S shows the summation curve obtained by annual summation of the residual values of the separate components. The line M gives an approximation, based on some analyses, of the probable course of the decomposition of similar resources in the mor-type forest soil at Hackfort. (Redrawn from Minderman 1968).

loss might be expected to be a summation of these curves which could be represented by a simple negative exponential decay curve of the form:

$$M_t = M_o e^{-kt}$$

where M_o and M_t are the resource mass at the start of the experiment or after time t, respectively (Swift et al. 1979). As has already been mentioned, however, the time course of decomposition is usually a series of curves of different slopes due to the initial effects of leaching and the accumulation of recalcitrant secondary products of microbial metabolism in the resource with time. Mass changes in various chemical components of leaf litter during decomposition in a stream are shown in Fig. 7. This illustrates the point that leaching rapidly removes the labile carbohydrates and the more recalcitrant phenols from the leaves so that little of these compounds is

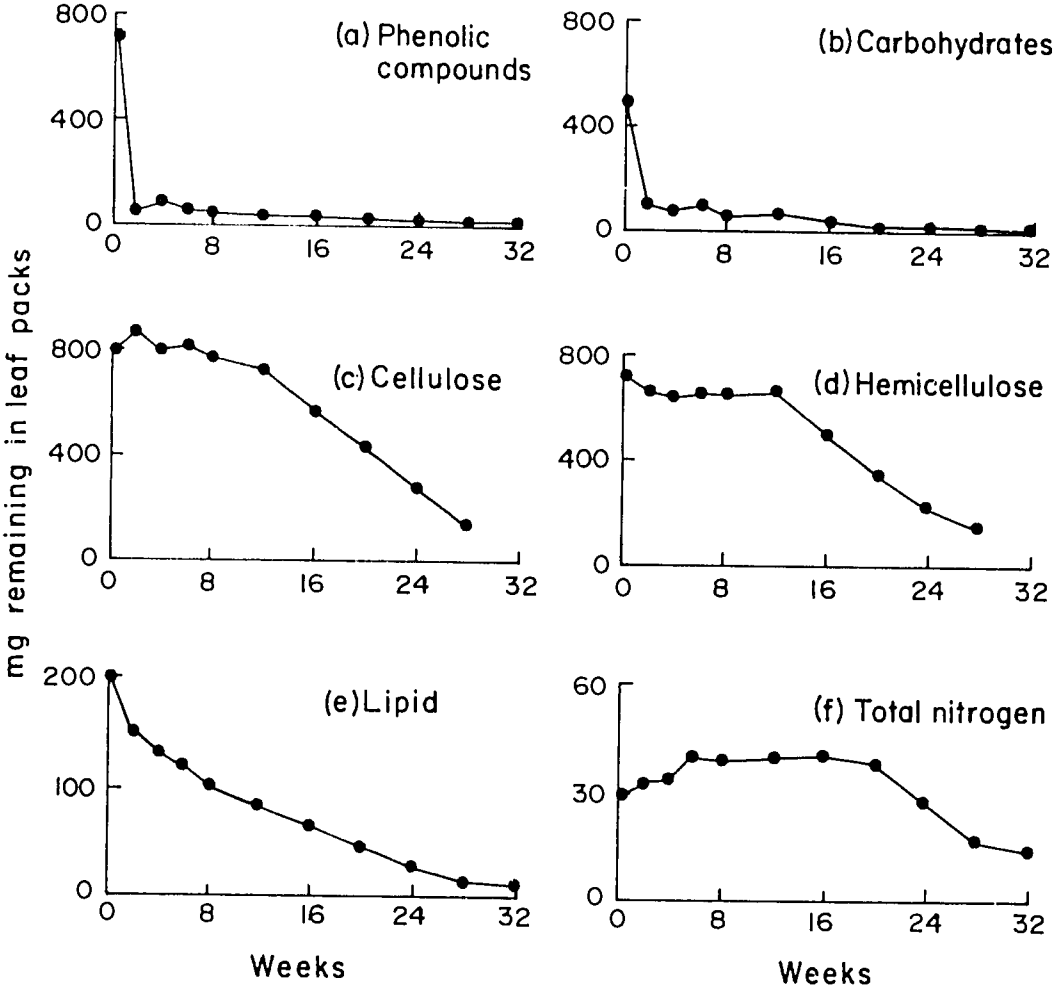


Fig. 7. Weights of various chemical components remaining in white oak leaf-packs at various times during decomposition. (After Suberkropp et al. 1976).

decomposed *in situ*. Dead leaves lose 5 to 30% of the initial mass through leaching within one or two days in temperate streams and then the remaining leaf material decomposes at rates ranging from 0.5% day^{-1} for low quality resources such as oak and beech leaves to 1.5% day^{-1} for high quality elm and ash leaves (Petersen and Cummings 1974). Emergent macrophytes have broadly comparable decomposition rates to tree litter (Polunin 1984). The decomposition rates of the leached fractions have already been considered.

Nutrients

Theoretically, deficiencies in any elements required for heterotroph nutrition could limit decomposition, but in practice nitrogen is generally found to be the key element. Concentrations of N in plant materials are generally inversely correlated with lignin so that the decomposition rates of tree leaves in terrestrial (Melillo et al. 1982) or freshwater (Kaushik and Hynes 1971) habitats generally decrease with increasing lignin and decreasing N concentrations. For woody materials, lignin alone may show a significant correlation with decomposition rates in streams (Melillo et al. 1983), whereas N alone may be an adequate indicator of resource quality in materials with lower lignin concentrations. Marinucci et al. (1983), for example, carried out a laboratory study on the decomposition of *Spartina alterniflora* stems and leaves from plants grown under different fertilization regimes and found a linear relationship between decomposition rates and initial N concentrations. Resources with low N and high lignin concentrations often have a rate of N immobilization from exogenous sources that is negatively correlated with decomposition rates (Aber and Melillo 1982). In some cases this results in increased rates of litter decomposition although in other studies no effects of N enrichment have been recorded. Interpretation of these effects is complicated because some workers failed to distinguish between absolute increase in N and increases of N relative to carbon losses, which are characteristic of the initial phases of decomposition of most litter materials (see Fig. 7).

The study of *Spartina* decomposition by Marinucci et al. (1983) also illustrates the point that the composition of plant tissues (including microorganisms) varies with the growth conditions. This is generally recognized for agricultural crop residues and is also a feature of natural vegetation. But, in addition, it has been shown for forest trees (Vitousek 1982; Melillo and Gosz 1983) as well as marsh vegetation (Shaver and Melillo 1984) that the efficiency of nutrient withdrawal from senescent tissues is higher under conditions of nutrient stress. This nutrient translocation further reduces the resource quality inputs to the decomposer system and may effect a negative feedback on nutrient availability through slower litter decomposition rates and immobilization of nutrients in soil organic matter (Melillo and Gosz 1983).

The presence of high concentrations of polyphenols in the leaves of woody plants growing on low fertility soils probably reflects the sequestering of fixed carbon in

compounds that are not osmotically damaging to the cell when growth is nutrient limited. Phytoplankton apparently show a similar phenomenon, where the release of soluble carbohydrate relative to potential production varies from less than 1% in eutrophic waters to 40% under oligotrophic conditions (Fogg 1980).

Modifiers

The final group of resource quality parameters, the modifiers, are compounds that influence the rate or timing of animal or microbial activity on other resource components. Tannins, for example, are frequently invoked as modifiers because they inhibit enzymes and deter feeding. But there is little good evidence for their role as major rate determinants in terrestrial or aquatic systems. This is mainly because high concentrations of secondary compounds are often correlated with low resource quality set by other parameters (Swift et al. 1979). Some evidence showing that phenolic compounds may deter feeding is provided by Cameron and La Point (1978) who showed that although tannins leached from Chinese tallow *(Sapium sebiferum)* in temperate grassland ponds were not toxic to litter-feeding macro-arthropods, the animals suffered high mortality through starvation unless the leaves had been subjected to an extended period of leaching and microbial conditioning. Valiella et al. (1979) have also shown that cinnamic acids in the cell walls of *Spartina alterniflora* inhibited feeding by salt marsh amphipods and snails until the material had been conditioned in the field for up to nine months. In both cases it is difficult to isolate the effects of the phenolic compounds from other biochemical and physical changes in the resource during the conditioning period.

Quality of Major Resource Types

From the preceding discussion it is possible to infer the relative decomposition rates of the plant tissues shown in Table 2, which form allochthonous and autochthonous inputs to aquatic systems.

Table 2. Major organic components (substrates) of some plant resource inputs to aquatic systems.

	Deciduous tree wood	Deciduous tree leaf (*Quercus* sp.) (living)	(dead)	Straw	Grass (dead)	Emergent macrophytes *Scirpus/Typha*	Submerged macrophytes	Benthic algae	Phytoplankton
Lipid (ether soluble)	2-6	8	4	1-3	2	0.5	–	0.7-2	1.5-5.0
Carbohydrate (water soluble)	1-2	22	15	4-6	13	53	30-70	36-44	30-50
Cell wall: hemicellulose	19-24	13	16	36-39	24				
cellulose	45-48	16	18	40-43	33	33	15-20	9-17	18
lignin	17-26	21	30	13-14	14				
Protein (N x 6.3)	< 0.3	9	3	1	2	7	5-18	5-18	17-30
Ash	0.3-1	6	5	–	–	6.5	–	–	5-14

Woody litter has the lowest quality but decomposition rates are also affected by the mass of the resource as well as the chemical composition. The size of woody resources also affects the overall chemical composition because the cambium tissues have much higher concentrations of N and available carbohydrate than heartwood. Twigs therefore decompose faster than branches.

Terrestrial litter inputs to aquatic systems are generally in the form of dead materials that have lower resource quality than living tissues. Living tree leaves have a chemical composition closer to that of emergent macrophytes and decompose faster than litter from the same plant.

Emergent macrophytes have a higher proportion of cell-wall material than submerged forms, reflecting the requirement of support tissues in the aerial environment. Polisini and Boyd (1972) showed that in the progression from macrophytes with low to high standing crops (and more terrestrial life forms) there was a proportional decrease in noncell-wall material and protein content that reduced the potential food quality of the plant to herbivores. Aerial leaves of macrophytes also contained higher tannin concentrations than terrestrial plants. The alkaloid concentrations of some aquatic plants, however, are within a range which has been shown to be pharmacologically active in terrestrial plants and serve as potential deterrents to herbivory (Ostrofsky and Zettler 1986). Phytoplankton and submerged macrophytes have the highest resource quality of these plant types. Anderson and Seddell (1979) recorded decomposition rates for macrophytes of 50% per week. Both macrophytes and phytoplankton lose 30 to 50% of their mass as soluble carbohydrates. However, particularly for phytoplankton, the mechanisms and timing of the release of this material will be very different to the leaching of terrestrial litter discussed earlier.

Organisms

A functional classification of aquatic communities is shown on Fig. 8. This illustrates the integration of decomposer and herbivore subsystems and the role of different groups of detritivores in determining the pathways of energy and nutrient fluxes through the system.

Microbial colonization initiates the processes of decomposition. The relative importance of fungi and bacteria is mainly determined by the physical characteristics of the resource.

The filamentous growth form of aero-aquatic hyphomycetes is adapted for growth on the surfaces, invasion of, and ramification through large plant resources such as leaf litter, whereas the unicellular thallus of many aquatic bacteria is more effective at exploiting particulate resources of less than 1 mm. Mason (1976) found that the decomposition of *Phragmites* leaf discs was dominated by fungal activity in the early stages but after 122 days bacterial activity was higher than that of fungi. In contrast, fungal respiration was a negligible component of microbial activity in homogenized *Phragmites* leaves and bacterial respiration in leaf detritus and lake sediments increased as an inverse function of particle size (Hargrave 1972).

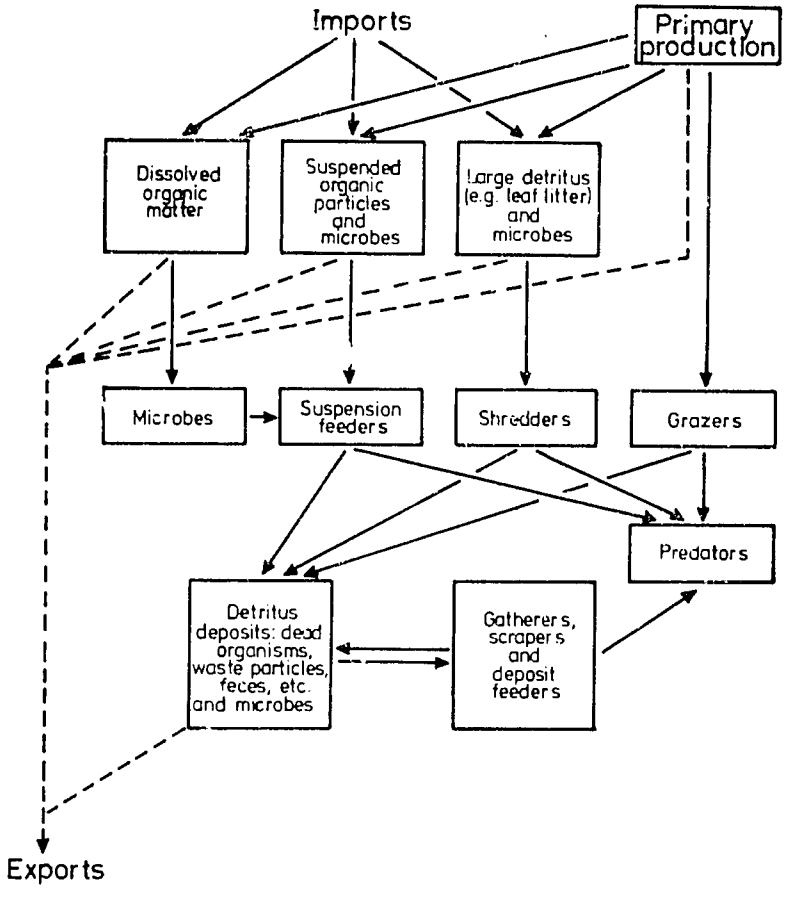


Fig. 8. Food resources and their utilization in aquatic ecosystems. (After Berrie 1976).

Litter comminution influences, therefore, the subsequent pattern of microbial activity, but the species of aquatic hyphomycetes and resource quality also determine the extent and timing of animal attack. Consumption of leaf litter by larval Trichoptera (shredders) was shown by Arsuffi and Suberkropp (1984) to be unrelated to the fungal species on leaves or conditioning time alone, but was related to the interaction of these two factors. The most palatable leaves were those colonized by particular fungi and showing maximum relative weight losses, softening of leaf tissues and a doubling of N concentrations in relation to uninoculated leaves. Leaves colonized by *Lemonneira aquatica* were unpalatable irrespective of the degree of conditioning and *Alatosposa acuminata* was the most palatable. Some fungi are unsuitable as a sole food resource for *Gammarus* whereas other species promote rapid growth (Bärlocher and Kendrick 1973). The assimilation efficiency of *Gammarus* fed on unconditioned elm or maple leaves is about 10% (Bärlocher and Kendrick 1975) compared with 20 to 35% on conditioned leaves (Berrie 1976).

The pattern of fungal colonization, therefore, influences the proportion of slow decomposing leaves which remain in the stream empoundment, and the amount and size of particulate organic matter which is exported downstream. This regulatory role of consumers for detritus production has been experimentally demonstrated by the use of pesticides in streams (Wallace et al. 1982) and also by manipulating the invertebrate predators on the populations of shredders (Oberndorfer et al. 1984).

Litter comminution by soil fauna (Anderson and Ineson 1984) or aquatic invertebrates (Hargrave 1976; Fenchel and Jørgensen 1977) enhances carbon mineralization rates by microorganisms, but this appears to be a transient phenomenon. The overall effect of fauna on carbon mineralization by microorganisms has yet to be demonstrated in the context of total C fluxes. The most significant effect in streams may be that transport of litter to more or less favorable environments for microbial decomposition and N uptake is facilitated.

Similar considerations apply to mineral cycling. Macro-arthropod feeding can disrupt the time course of microbial N mineralization and results in net N release from litter at a time when microbial immobilization of N occurs in the absence of fauna (Anderson et al. 1985). This may represent only a small proportion of the total of N pool in the resource, but the location and timing of this mineral N release might be important for the functioning of the system, as in the case of N and phosphorus release by zooplankton for sustained phytoplankton production. The subsequent rates of C and N mineralization by microorganisms from particulate organic matter may depend upon the availability of exogenous N sources; though this has yet to be demonstrated in terrestrial or aquatic systems.

What is generally lacking is the integration of these microsite effects into the context of ecosystem processes.

Physical Controls

The main physical determinants of decomposition processes and nutrient cycling are temperature and oxygen. Water movements are the dominant physical factors influencing the location of the resource and modifying physical environmental conditions (Goldshaulk et al. 1977).

Many species of aero-aquatic hyphomycetes have high lignolytic activity (Fisher et al. 1983) but lignin depolymerization proceeds more slowly by strains of *Pseudomonas*, *Flavobacterium* and *Achromobacter* (Ferjingstad 1975). These are all obligate aerobes and if particulate organic matter enters anaerobic bottom sediments lignin decomposition is inhibited. Hence lignin constitutes 30 to 40% of these sediments, but cellulose continues to be decomposed by hydrolytic reactions. Conversely, in lakes comminution may slow settlement rates and facilitate the reworking of detritus particles.

In stratified lakes, the decomposition rates of algal cells and macrophyte detritus are slower when they settle into the anaerobic hypolimnetic water (Goldshaulk et al. 1977; Fallon and Brock 1979). Lake turnover returns dissolved organic matter to an aerobic environment and suspended organic matter for further reprocessing. As a

consequence of this recirculation, virtually all the primary production in mesotrophic temperate lakes is decomposed with little permanent sedimentation of undegraded particulate organic matter. As lakes (and rivers) become more productive, the efficiency or recycling the resistant fractions of particulate organic matter is overridden and permanent sedimentation rates then increase (Goldschaulk et al. 1977).

Discussion: Detritus in Aquaculture

Aquaculture systems can be broadly classified into extensive and intensive systems.

The extensive systems involve exploitation of natural food resources at various trophic levels and the main parameters that can be manipulated are the choice of the organism and the mode of placement; for example, fish cages in still or running water and the transplantation of shellfish to favorable growing areas. The food resources are not usually manipulated directly in extensive systems, but access to new feeding areas can be controlled by flooding. There is enormous aquaculture potential in wetlands (Brinson et al. 1981) where water levels could be controlled to simulate the productive inundation forests (varzea) of Amazonia.

The intensive systems range from the ranching of salmonids, caged in clean natural waters and intensively fed with high quality foods, to the culture of carp, mullet, tilapia and other herbivorous or polyphagous fish in highly eutrophic ponds. This discussion of options for manipulating the detritus resource inputs to aquaculture is mainly concerned with these pond systems and is based on the following conclusions from the previous sections:

1. Yields of natural fisheries are sustained by the high efficiency of trophic links in the planktonic food chain and the complex web of trophic interactions channelling carbon and nutrients in detritus through to exploitable trophic levels. In pond aquaculture systems, the food chains are shortened to the algal-fish or detritus-fish links since zooplankton and macrofauna are largely eliminated by intense predation (Schroeder 1978).
2. Microbial exploitation of dissolved organic matter represents a major carbon flux that drives algal production through carbon and mineral nutrient regeneration. There is negligible carbon transfer to higher trophic levels via bacterial production in the water column.
3. Microbial biomass is not available to fish unless attached to particulate organic matter.
4. Detritus is an inadequately defined resource comprising dissolved, colloidal and arbitrarily defined size fractions of organic matter that vary according to the parent material from which they were derived and the extent of animal and microbial reprocessing.
5. The distinction between living material and detritus is arbitrary in many cases; for example, senescing algal cells or green manures/feeds decomposing in pools,

impose an artificial distinction between herbivores and detritus feeders. Thus microbial decomposition rates and the assimilation efficiencies of herbivores and detrivores will broadly show the same ranking algae > macrophytes > terrestrial forces > woody vegetation with respect to quality of the resources as defined by carbon and nutrient availability.

Fish production in ponds is most commonly maintained by frequent inputs of terrestrial plant material, stock manure or human feces/sewage as nutrient sources for algal production, or fish feed, or both (see papers by Edwards; Wohlfarth and Hulata, this vol.). But despite the multiplicity of different fish culture systems and the vast cumulative wisdom of traditional management practices, there appear to be no farming systems in which the attributes of the detritus are specifically manipulated as a fish food resource. The practical reason for this is that the ponds are operated as 'black box' systems where the effects of inputs are assessed in terms of yields. Hence the relative contributions of algal and 'detrital' carbon and nutrients to fish production are not well known (Schroeder, this vol.). It is not known at what stage of lysis or decomposition the added plant materials are preferred as food, and the extent of detritus reprocessing by animals before residues are incorporated into sediments.

The intensificaton of fish production, or any stock, involves increasing the food intake rates, consumption efficiencies (reducing food wastage) and assimilation of carbon and nitrogen from the food. In addition to nutritional quality the food must be readily available to the fish. The following considerations apply to food quality because availability is more a function of behavior that can be manipulated by presentation of food and pond architecture. The first priority is for carefully controlled experiments to quantify carbon and nutrient flux pathways in aquaculture ponds. Until this is done the options for manipulating detritus pathways for fish production appear limited. But in the meantime it is necessary to consider some practical options for utilizing plant materials as a food resource, which are expressed as testable experimental hypotheses.

Microbial Preconditioning of High Quality Plant Feeds Improves Nitrogen Conservation in the Resource and Nitrogen Availability to the Fish

Substantial soluble nitrogen and carbon losses occur from high quality food materials in the initial leaching phase of dead materials or during the death and lysis of living plants added to the ponds. Controlled aerobic microbial preconditioning for a few days could convert carbon and nitrogen into available microbial biomass which would otherwise occur in the water column. Preconditioning can be combined with wilting treatments that are required to reduce the water content of aquatic macrophytes used as feeds, such as water hyacinth (P. Edwards, pers. comm.). Microbial growth shows three characteristic phases: lag, exponential and plateau. Microbial respiration declines after the lag growth phase and feeds should be harvested before

peak respiration to avoid the formation of recalcitrant carbon and nitrogen complexes that occurs with time during composting (Biddlestone and Gray, this vol.).

Controlled Composting of Low Quality Resources Increases Available Nitrogen and Carbon in Feeds

Extensive research has been carried out screening basidiomycetes and ascomycetes to convert low quality food materials such as rice and wheat straw into edible protein for humans (fungal fruiting bodies) and higher quality ruminant feed that can also be used for monogastric animals. The yield and conversion properties depend upon species but 5 to 20% (w/w) of the straw can be converted into harvestable fruiting bodies and the remaining straw material can show an increase in *in vitro* digestibility of 40 to 80% against unconditioned straw (Zadrazil 1979). In Southeast Asia, *Volvariella volvacea* could be used for rice straw processing and to provide harvestable yields of up to 10 kg fresh weight of fruiting bodies per 100 kg straw. Further details of the biotechnology of straw utilization are reviewed in Grossbard (1979).

Microorganisms Conditioning Low Quality Resources Will Immobilize Exogenous Sources of Nitrogen and Carbon

This principle is commonly employed in ensilement or composting and could be extended to conditioning straw or other low quality resources in hypereutrophic ponds. The nitrogen enriched material would ultimately provide feed if left *in situ* but could be removed at an earlier stage for composting, as considered above. Various options could be explored for adding crop residues to the pond to improve microbial colonization and uptake of nitrogen from solution, but a better knowledge of aquaculture systems is required to ensure that such practices improve feed utilization by the fish.

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Discussion

GRAY: With regard to Steele's (1974)* model, this is now 15 years old and we have progressed a long way in this time. The problem with his model is that he assumed that all phytoplankton are consumed by grazers, whereas we know now that in most ecosystems only about 50% of phytoplankton goes to the grazers and the rest settles out. The general trend, moving from the tropic zone to the poles, seems to be a lack of coupling between primary production and secondary production. An example of this is that up in the Barents Sea at about 2,000 m depth, there are polychaetes full of green phytoplankton which has settled out. The presence of phytoplankton in the deep sea is puzzling because it shouldn't sink fast enough to be there. If we look at C:N ratios, we assume that plankton normally has a ratio of about 9-10 C:N; we have found in the spring that this rises to 11 and 12 and the in the summer it goes up to 19 or 20. The N is being siphoned off and the C is almost an unutilized waste product. We then get a little C:N pulse in autumn after which the benthic system is sustained. So the benthic system in fact mobilizes and keeps going on these inputs. This is not the case in Steele's model.

ANDERSON: Yes, Steele assumed that all non-utilized material was an input into the benthic system. However, I think the work that people like Golterman have done on stratified lakes and some of the work on permanently stratified areas of the oceans has shown that you do get equilibria between production and recycling nutrient through grazing and decomposition in the euphotic zone.

One of the major areas of controversy is the fate of the dissolved organic carbon, the production of which appears to be a positive function of the oligotrophic status of the system. Phytoplankton produce more soluble carbohydrates under oligotrophic conditions than eutrophic conditions, presumably because of N and P limitations to reproduction. Porter** has suggested that it is impossible for this dissolved organic carbon to be processed via micro-zooplankton whereas others feel that this is a major route. Many of the assumptions made in these models have not been tested. I accept the points which you are making, but the controversies remain despite a long period of investigations since Steele's model was put forward.

KIRCHMAN: I have a comment about lignin degradation. It has been assumed that fungi are the predominate organisms degrading lignin. However, Ron Benner and Bob Hodson have been looking at this and they found surprisingly that bacteria were more important than fungi (Benner et al. 1986).*** They have been doing experiments in both a salt marsh and an acidic freshwater swamp. They use ^{14}C-labelled lignin prepared from various grasses—only the lignin is labelled.

ANDERSON: Well from the limited literature I have seen, the complement of lignin-degrading bacteria seems to be fairly small in aquatic systems. One should be careful in lignin degradation assays of the problems of the properties of different lignins and of specifying the criteria for degradation. All the synthetic lignins have a two-phase degradation pathway. There are some

relatively accessible components of these molecules and some extremely recalcitrant ones. It's the same as end-labelled cellulose as opposed to homogeneously-labelled cellulose.

I'm sure you're right about the relative importance of bacteria and fungi. However, the aero-aquatic hyphomycetes do not suffer from many of the problems which you put forward as affecting degradation by bacteria. So I'm really promoting them as a possible means of exploiting agricultural residues rather than as a natural pathway.

SRINIVASAN: I agree with David Kirchman that bacteria are very important in lignin degradation. However, they require an alkaline environment. In acidic environments the fungi take over.

WOHLFARTH: I was interested in your mention of a lack of 'target animals' to utilize detritus. Could you please explain this?

ANDERSON: I was thinking mainly of fish or molluscs or shrimps.

WOLFARTH: But some of these can utilize detritus.

ANDERSON: My point is that while there are indeed large numbers of organisms that can do this, they may not be the ones which the aquaculturists are choosing to culture. There may be fish, for example, which can utilize detritus better than the common carp. The traditionally cultured fishes were probably chosen for other reasons than their intrinsic physiological characteristics.

WOHLFARTH: Well, the common carp is regarded as more of a benthos feeder than a detritus feeder, but some of the tilapias are very good at utilizing detritus.

SCHROEDER: Yes, they have a very acidic stomach.

ANDERSON: Well, perhaps I should ask you the question. Do you think that culturists have already chosen the best species to utilize detritus?

WOHLFARTH: Well, the Chinese have chosen to culture mullets close to the coast. These are good detritus feeders. In more inland situations, they use the mud carp *(Cirrhinus molitorella)* Also, some of the tilapias are such versatile feeders that they will eat almost anything. They certainly utilize detritus.

SCHROEDER: The tilapias can vary their feeding pattern according to the food that is available.

PULLIN: The species in current use in aquaculture have been fairly well-chosen. We have available a good range of herbivores, detritivores and carnivores This is not to say that additioned useful species will not emerge in the future. The crux of the matter is, of course, the choice of the ultimate consumer which is man. You could identify a superb new detritivore, but if nobody wanted to eat it, then it would not be cultured.

I would like to stress again the feeding versatility of the tilapias. They will cross feeding niches. They will feed on plankton, detritus and a huge range of natural and supplemental foods. These are tremendous attributes given our task of pumping more N into fish flesh by the most cost-efficient routes.

BILIO: I would still like to know: When you speak of fish feeding on detritus, do you mean they are utilizing the detritus itself or the organisms associated with it?

MORIARTY: We will consider this question after Dr. Bowen's paper.

EDWARDS: You presented a table showing the major inorganic constituents of different sorts of plants in which there was a decline in the ratio between protein and structural carbohydrates from the aquatic to the terrestrial environment. The protein value you gave to phytoplankton was rather

low (17%). In view of your comment that N is so important and that we need to increase N inputs to boost aquaculture production, I would like to point out that plants growing in highly eutrophic situations normally have a much higher protein content; for example, it is usually about 50% for phytoplankton and 25 to 40% for floating aquatic macrophytes.

Also in your suggestions for strategies to improve aquaculture production, you mentioned the use of rice straw as a fishpond input. This is used as a pond input in China. However, we should bear in mind the opportunity cost of inputs like this. Putting them into ponds may not be the best way to use them. In most Asian aquaculture, the fishpond inputs come from other agricultural systems. Therefore, we must not just think of the fish and what they need to eat but rather of the whole integrated farming system; for example, a crop-livestock-fish system. A better use for rice-straw would probably be to feed it to ruminant livestock, perhaps mixed with urea, and then to use the livestock manure as a pond input.

ANDERSON: It still all comes down to N balance. You cannot put all your terrestrial N into an aquatic system unless the gains from raising fish are much better than those for raising crops and livestock. In any case, you cannot just feed rice straw. There has to be N source as well. One other consideration for such integrated farming systems is how to channel N back from the aquatic components to the land. Are such feedbacks used?

EDWARDS: There are myriads of interactions in integrated farming systems. Regarding N pathways, it all depends on local economic conditions, but from the point of view of maximizing biological efficiency for the total integrated systems, it may be better to put the N on to vegetable crops and then put vegetable waste into the fishponds.

SRINIVASAN: Unfortunately, rice straw and urea is not a particularly good feed for ruminants. Many people have tried feeding ruminants with urea-treated rice straw. It satisfied the animals' hunger but was not very efficient. As Preston pointed out in a study about five years ago in Guatemala, it is essential to have a bypass protein along with the straw. The idea persists that straw treated with alkali and urea is a good ruminant feed, but really it is not.

PRUDER: Most discussions about aquaculture tend to discuss these possibilities of putting residues such as straw and manure into ponds. However, we still know very little of what happens when we put a perfectly good feed such as a chicken feed or a fish meal-based feed into a pond. How are such inputs processed by the detrital food web and the grazing food web? This lack of understanding is particularly serious in marine shrimp production where large quantities of feed are given without knowing how these are partitioned and processed. So let us not try to tackle too many problems here, but rather consider the situation of high quality organic material, which we can select, coming into the pond. I don't think we even have the knowledge at present to manage effectively a straightforward corn and soybean-enriched feed input to ponds. If we could first work out how to use corn and soybean inputs to maximum advantage, this would have a major impact on aquaculture.

MORIARTY: Well, let us leave these discussions on culture systems for later and get back to the more specific aspects of microbial ecology.

ANDERSON: Do the microbial ecologists have any suggestions for improving conversion efficiencies? How can we manipulate the processes involved in the context of aquaculture?

BOWEN: We should consider the relationship between microbial production and fish production. I suspect that there is not often a one to one relationship.

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*Steele, J.H. 1974. The structure of marine ecosystems. Harvard University Press, Cambridge, Massachusetts. 128 p.

**Porter, K.G. 1944. Natural bacteria as food resources for bacteria, p. 340-345. *In* M.J. Klug and C.A. Reddy (eds.) Current perspectives in microbial ecology. American Society of Microbiology, Washington, DC.

***Benner, R., M.A. Moran and R.E. Hodson. 1986. Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. Limnol. Oceanogr. 31(1): 89-100.

Session on Production and Characteristics of Detritus

CHAIRMAN'S OVERVIEW

M. Bilio

In the earlier sessions, we have seen how much still has to be done in order to arrive at a basic understanding of the organisms and factors involved in the decomposition and recirculation of organic matter in the aquatic environment. We learnt about the advantages that the aquaculture environment offers to the natural recycling of dead organic matter, as compared with the terrestrial one. The topics of the present session are intended to enlarge further the basis of an interdisciplinary understanding of the potential role of detritus as fertilizer and feed in aquaculture.

It is useful to distinguish the possible origins of organic detritus in aquaculture. Table 1 distinguishes between "autochthonous" material derived from auto- and heterotrophic organisms growing in the aquaculture environment itself, and "allochthonous" material derived from organisms produced outside the aquaculture environment; and further between material introduced in an uncontrolled (natural forces) or controlled manner (human forces).

The first paper by A.J. Biddlestone and K.R. Gray, on "Production of organic fertilizers by composting", includes a wide variety of compostible items introduced to the pond by humans (category B.2 of Table 1). The paper reviews all aspects of converting organic detritus of different origin into an easily utilizable conditioner and fertilizer of agricultural soil, improving above all soil structure and water-holding capacity. This synopsis of controlled terrestrial decomposition of organic matter is of double interest:

- for comparison with the corresponding processes occurring under aquatic conditions;
- for the application of compost as fertilizer and feed in aquaculture.

However, while the effect of introducing compost into a terrestrial system is well known, this is hardly the case with its introduction into aquatic systems. So this remains a major point for further discussion.

The paper by V.R. Srinivasan on "Conversion of cellulosic and other organic wastes into microbial protein" draws upon very specific experience, as was considered desirable in an earlier session. It concentrates on the controlled production of

Table 1. Origin of organic detritus in aquaculture. There is an increasingly "alien" character of the materials from top to bottom of the list below, and increasing need for their pretreatment to allow better utilization of nutrients and to avoid side effects.

A. Autochthonous material (plant and animal debris)

B. Allochthonous material

1. Introduced by natural forces (wind, water, animals)

   — from aquatic ecosystems
   — from terrestrial ecosystems

2. Introduced by man (as fertilizer or feed)

   — from natural aquatic or terrestrial environments
   — from agricultural production systems
   — from human settlements
   — from industrial production

single cell protein (SCP) from several types of organic compounds that are usually among the last to be decomposed under natural conditions. These substances are mainly derived from agricultural byproducts or residues. The products obtained can be included in artificially prepared feeds.

S.H. Bowen's paper on "Composition and nutritive value of detritus" discusses the nature of detritus and how it is decomposed by microbes. Bowen reviews 82 papers on the subject and gives very useful summary tables.

In SCP production, the process of deriving utilizable protein from a detritus source takes place in a controlled system outside the aquaculture environment. The paper by G.L. Schroeder on "Carbon pathways in aquatic detrital systems" elaborates on microbial production utilizing agricultural detritus (manure) introduced directly into fishponds. The most controversial point of this paper will probably be the statement of the author that anaerobic microbes produce mainly energy-rich extracellular fermentation products, whereas aerobic microbes produce mainly cell biomass.

At this point it seems appropriate to draw attention to some further aspects of the utilization in aquaculture of detritus from various sources, thus providing reference points for pertinent discussion at this symposium and perhaps in the future:

- There are a number of factors which can influence the results of experiments on detritus use (Table 2).
- Controversial issues remain concerning the problem of dealing with anaerobic conditions due to excessive detritus inputs into ponds (Table 3).
- Various economic considerations must be taken into account (Table 4).
- There are potential health hazards, not only to humans but also to the aquaculture organisms and the microbes themselves, from detritus use. Characteristics of these three levels of hazards are given in Table 5.

Table 2. Sources of diversity influencing the use of detritus in aquaculture.

1. Detritus: origin, composition, treatment
2. Environment of application: temperature, salinity, pH, O_2, nutrient concentration
3. Microbial flora and microfauna present on detritus and in the aquaculture environment
4. Feeding habits of aquaculture organisms
5. Production procedure: intensity, frequency of feed or fertilizer administration, water movement and exchange, aeration and oxygenation

Table 3. Excess detritus in aquaculture ponds: controversial solutions to avoid or alleviate anaerobic conditions.

1. Ploughing and harrowing the pond bottom in submerged conditions
2. Draining and drying the pond bottom in brackishwater conditions
3. Removal of pond deposits for fertilizer in agriculture

Table 4. Economic considerations of detritus use in aquaculture as a new production procedure.

1. Economics of the procedure, including costs of material, transport, storage, pretreatment, application, and removal of residues. Such costs should be monitored during the research and development phases
2. Economic advantages in the context of rural development
3. Acceptance of the procedure and product by target groups
4. Economic potential for scaling-up

Table 5. Potential hazards from detritus use in aquaculture.

Level 1: Threat to vitality of microbial populations

- from microbial contamination
- from inorganic contamination (heavy metals, etc.)
- from changing environmental conditions

Level 2: Threat to growth and survival of aquaculture organisms from the same sources as for Level 1

Level 3: Health concerns regarding consumers of aquaculture products

- from microbial contamination
- from inorganic contamination

Production of Organic Fertilizers by Composting

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Abstract

Composting is a process of microbial degradation that is of importance in the recycling of organic matter. A wide range of organic materials, crop residues, animal manures, sewage sludge, nightsoil and the organic part of urban refuse can be converted into a useful endproduct.

The principles of composting are outlined and the chemical and physical parameters that need consideration in the design of a practical process are reviewed. A summary of the important design parameters is given. The differing practical processes in use are described from the very simple heap system to the highly sophisticated mechanized urban system.

Composting provides a means of obtaining a stable humified product that quickly equilibrates with the ecosystem in which it is placed without causing the major disruption associated with raw materials.

Introduction

Composting is an exothermic process of biological oxidation in which organic matter is decomposed by a mixed population of microorganisms in a warm, moist, aerobic environment. During the process putrescible organic substrate undergoes chemical and physical transformation to give a stable humified endproduct. The product is of value in agriculture both as an organic fertilizer and as a soil improver.

Wastes amenable to composting vary from the heterogeneous organic/inorganic mixture in urban refuse to the more homogeneous farm manures, crop residues, sewage sludges and nightsoil. During the composting process most of the oxygen demand of the wastes is met, the organic materials are converted to more stable products, carbon dioxide and water are released and heat is evolved. Under natural

conditions the degradation process takes place slowly, on the surface of the ground at ambient temperature and mainly under aerobic conditions. The natural process of breakdown can be accelerated by gathering the material into heaps to conserve part of the heat of fermentation so that the temperature of the mass rises and faster reaction rates are obtained. This accelerated process is composting.

Principles of Composting

The process of composting is a complex interaction between the organic waste, microorganisms, moisture and oxygen. The waste material will normally have an indigenous mixed population of microorganisms present. When the moisture content and oxygen concentration are brou[illegible] a suitable level microbial action increases. In addition to oxygen and moisture, the microorganisms require for their growth and reproduction a source of carbon, macronutrients such as nitrogen, phosphorus and potassium, and certain trace elements. These additional requirements are usually provided by the waste materials. In using the organic matter as a food source the microorganisms reproduce themselves and release carbon dioxide, water, other organic products and energy. Some of the energy released by the biological oxidation of carbon is used in metabolism, the remainder is given off as heat.

The final product, compost, comprises the more resistant residues of the organic matter, breakdown products, the biomass of dead microorganisms and some living microorganisms, together with products from further chemical reaction between these materials.

The overall process is illustrated in Fig. 1.

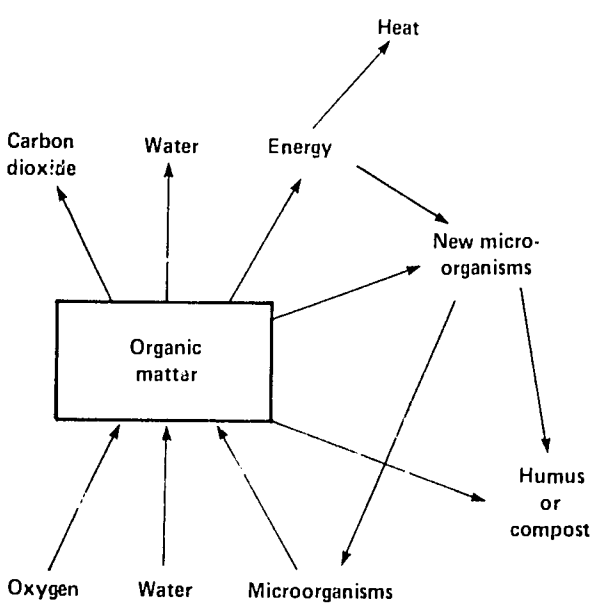

Fig. 1. The composting process.

Biochemical Aspects

Organic waste materials, whether of industrial, urban or agricultural origin, are mixtures of sugars, proteins, fats, hemicelluloses, cellulose, lignin and minerals in a wide range of concentrations as shown in Table 1. The fractions contained in plant material will depend upon the age of the plant, its type and environment. Fresh green material contains much water soluble matter, proteins and minerals. As the plant ages, minerals tend to return to the soil and low molecular weight compounds are converted to higher molecular weight compounds especially the hemicelluloses, cellulose and lignin. The composition of animal detritus will depend upon the type of animal and its feed.

Table 1. Composition of organic matter.

Fraction	% in dry weight
Hot/cold water soluble (sugars, starches, amino acids, urea, ammonium salts)	2 to 30
Ether/alcohol soluble (fats, oils, waxes)	1 to 15
Protein	5 to 40
Hemicelluloses	10 to 30
Cellulose	15 to 60
Lignin	5 to 30
Volatile matter	70 to 95

Composting is both a building up process and a breaking down process. The cell wall of the microorganism attacking the organic matter is the significant factor. Simple, low molecular weight carbon compounds, such as soluble sugars and organic acids, can pass through the cell wall easily and be metabolized and mineralized, providing energy and being built up into larger polymers. The longer chain components of the organic wastes cannot pass through the cell wall and cannot be used without being broken down into simpler compounds. This is accomplished by some of the microorganisms exuding extracellular enzymes that hydrolyze the long chain polymers into simpler compounds. Virtually all microorganisms present in composting masses can assimilate the resulting fragments but only certain organisms can carry out the hydrolysis.

The extent of the biochemical changes taking place during composting is indicated by the results of Yung Chang and Hudson (1967) who composted wheat straw with added ammonium nitrate. The straw had lost 50% of its dry weight in 60 days with the majority of the loss in the first 34 days. The loss of total dry weight could be accounted for almost entirely by the loss in hemicelluloses and cellulose. The greater rate of loss occurred over the first five days, averaging 2.7% day, as compared with an average of about 1.3% day over the following 30 days. The hemicellulose content declined steadily over the 34-day period from 37 to 18% of the initial dry weight. Cellulose degradation slowed down during the middle of the cycle, presumably because the fungal population declined as the temperature rose above 55°C. The cellulose content was 46% at commencement, falling to 12% of the initial dry weight after 34 days of composting. The ethanol soluble fraction, which contains the simpler c[illegible]on compounds, decreased very little being probably continually replaced by the breakdown of longer chain polymers. The most resistant fraction, lignin, is extremely resistant to enzyme attack, the degradation being restricted to a limited microbial group of higher fungi. However, during composting the lignin molecule does become modified, losing some methoxy groups and aliphatic side chains and gaining carboxyl and phenolic hydroxyl groups (Alexander 1977).

Microbiology

Composting is a dynamic microbial process brought about by the activities of a succession of various microbial groups each of which is appropriate to an environment of relatively limited duration. A list of the main classes of organisms involved in the composting process is given in Table 2. These organisms represent both the plant and animal kingdoms.

There are many different species, possibly 2,000 of bacteria and at least 50 of fungi, within each genus. The species can be subdivided according to the temperature ranges of their activity. Psychrophiles prefer temperatures below 20°C, mesophiles

Table 2. Organisms in composting.

Microflora (very small plants)	Bacteria, actinomycetes, fungi, moulds, yeasts, algae, viruses
Microfauna (very small animals)	Protozoa
Macroflora (larger plants)	Fungi
Macrofauna (small soil animals)	Mites, springtails, ants, termites, millipedes, centipedes, spiders, beetles, worms

20 to 40°C and thermophiles above 40°C. The organisms that flourish during the final stage of composting are essentially mesophiles.

Although the bacteria are present in large numbers, 10^8 to 10^9 per gram of moist compost, they are of very small size (1 to 8 μm) and form less than half of the total microbial protoplasm. Some species form endospores, which can withstand considerable heat and dessication.

The actinomycetes develop far more slowly than most bacteria and fungi and are ineffective competitors in the early stages of composting. They are more prominent in the later stages of the process when they can become abundant and the white or grey color typical of these organisms is clearly visible some 10 cm below the surface of the composting mass. They are numerically less prominent than bacteria, being of the order of 10^5 to 10^8 per gram of moist compost.

Fungi are important in the decomposition of cellulose and the environment of composting masses should be adjusted to optimize the activities of these organisms. Temperature is an important consideration as the fungi will die out as the temperature rises above 55 to 60°C, reinvading from cooler zones as the temperature falls.

Studies on the populations of bacteria, actinomycetes and fungi during composting have been made by a number of workers. Yung Chang and Hudson (1967) give data on the changes in numbers of all of these organisms during the composting of wheat straw. Hayes and Lim (1979) also give experimental data on changes in the populations of these organisms during the composting of wheat and rice straw for mushroom production. At the commencement of composting they found that the aerobic thermophilic bacteria dominate, while in the later stages bacterial numbers decline and the actinomycetes population increases. Fermor and Wood (1979) and Atkey and Wood (1983) also provide information on the microbial succession of a wide range of species of mesophilic and thermophilic bacteria, actinomycetes and fungi during the composting of wheat straw to provide a mushroom substrate. De Bertoldi et al. (1983) have reported the microbial changes during the composting of urban solid waste (60%) with sewage sludge (40%) in a static heap system with forced aeration. Over 50 days of composting they found an increase in cellulolytic fungi and actinomycetes with a corresponding decrease in the numbers of bacteria. Fungi isolated from the static heap system included 14 thermophilic strains (at 50°C) and 43 mesophilic strains (at 28°C). The mesophilic strains were characterized according to their enzymatic activity; cellulolytic (35), pectinolytic (3), amylolytic (13) and ligninolytic (14). An increase in ligninolytic fungi was also reported and it is suggested that lignin decomposition is enhanced in static systems. Agitation of the material for other purposes disturbs the growth of hyphae within the mass.

Viruses are organisms of considerable importance because they are responsible for diseases of plants, animals and humans. They are noncellular organisms that are far smaller than the cells or filaments of bacteria and Protozoa. The virus particle requires for its reproduction a viable host organism and various strains are specific to certain hosts. When diseased material is passed through a composting process the numbers of pathogenic viruses are greatly reduced, a predominantly temperature effect.

The Protozoa are the simplest form of animal life and are unicellular. Most soil Protozoa feed upon other microorganisms such as bacteria, algae and other strains of Protozoa. Only certain strains of bacteria are susceptible to attack; others are entirely unsuitable as are actinomycetes and yeasts. It is suspected that the Protozoa maintain a control on the expansion of the bacterial population. When environmental conditions, such as moisture and temperature, become unsuitable for growth, the Protozoa can enter a cyst form and withstand adverse conditions for a considerable time.

As the compost mass cools from its peak temperature it is accessible to a wide range of the soil macrofauna. These feed upon other animals, animal excreta and the plant remains. They normally require well aerated conditions, adequate moisture and prefer temperatures in the range of 7 to 13°C. Many of the soil animals make a major contribution to breakdown in the composting mass due to physical maceration; breaking the material into smaller particles exposes greater surface area for subsequent attack by microflora. They also make a contribution to the mixing of the various constituents. In temperate climates the earthworm plays a major role in the final stages of composting and in the subsequent incorporation of organic matter into the soil; in arid and semiarid climates this function is usually undertaken by the termite (Edwards 1974). The macrofauna build up tissues that are rich in nitrogen and are easily decomposed. With fairly short lives, their mass is a reservoir of nitrogenous matter that is continuously replenished and broken down.

Temperature-Time Pattern

When organic wastes are gathered together for composting, the insulating effect of the materials conserves the heat released by biological activity and causes a rise in temperature. The subsequent composting process may conveniently be divided into four stages: mesophilic, thermophilic, cooling down and maturing (Fig. 2).

At the commencement of composting the wastes are at ambient temperature and are slightly acidic. In the first or mesophilic stage, the indigenous microorganisms multiply rapidly, the temperature rises to about 40°C and the mass becomes increasingly acidic. The thermophilic strains take over as the temperature continues to rise; the pH turns alkaline as ammonia is liberated during the breakdown of protein molecules. When the temperature reaches 60°C the thermophilic fungi cease activity and the reaction is continued by the actinomycetes and spore-forming strains of bacteria. The rate of reaction falls and the temperature peak is reached; the rate of heat generation then becomes equal to the rate of heat loss from the mass. This marks the end of the thermophilic stage. Very frequently, high temperatures have been considered a necessary condition for good composting. In fact decomposition is suppressed at excessively high temperatures because the growth of the microorganisms is inhibited; only a few species show metabolic activity above 70°C. The threshold of suppression is approximately 60°C. Thus for rapid composting high temperatures for long periods should be avoided. A peak temperature of the order of

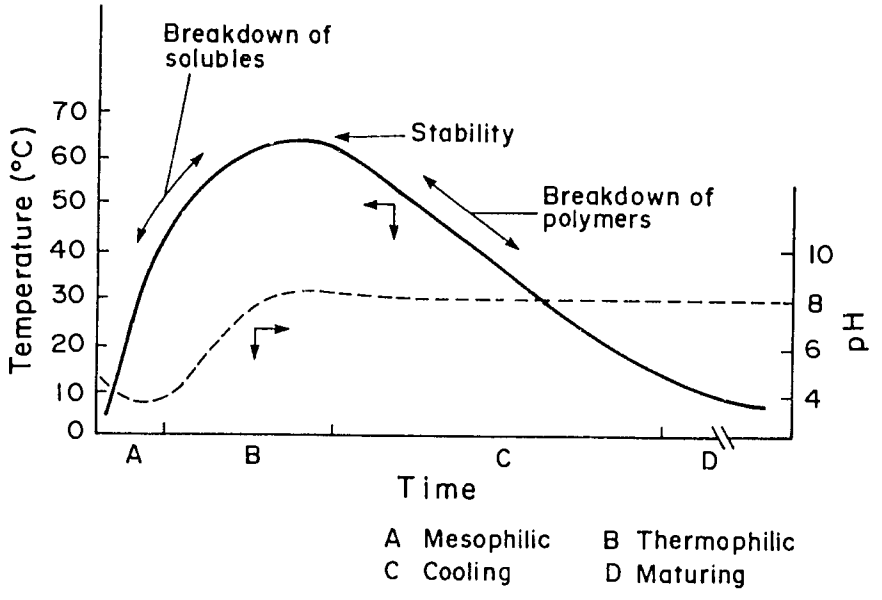

Fig. 2. Temperature and pH variations in a compost heap.

60°C is useful in controlling thermosensitive pathogenic organisms but the temperature should be kept to levels which encourage those organisms that are the main decomposers of long chain polymers. An optimum of 55°C is recommended. It has been suggested (Finstein et al. 1983) that the problem of temperature control can be solved by using forced pressure ventilation throughout the process. A forced aeration system removes heat by evaporative cooling and Finstein et al. (1983) recommend a temperature feed back control system in which the air blow time is linked to a temperature sensor placed in the composting mass. Such a system facilitates water removal in high moisture content situations.

When the composting mass has passed through the thermophilic stage the wastes have reached stability because the easily converted materials, carbohydrates, fats and proteins, have been degraded and most of the high rate of oxygen uptake has been met. The wastes are no longer attractive to flies and vermin and should not give off bad odors; the material may now be put into external heaps without causing major environmental pollution.

During the cooling down stage, which follows the temperature peak, the pH drops slightly but remains alkaline. When the temperature falls below 60°C the thermophilic fungi reinvade the mass and together with the actinomycetes attack the long chain polysaccharides, hemicelluloses and cellulose, breaking them down into simpler sugars, which may then be utilized by a wider range of microorganisms. There may be some limited attack upon the lignin fraction. Following the breakdown of these polysaccharides the rate of energy release becomes very small and the temperature of the mass falls to ambient.

The process now enters the maturing stage in which mass loss and heat evolution are small. The macroflora and macrofauna now invade the mass. As the food supply becomes exhausted antagonism between the organisms develops and antibiotics are produced. Complex chemical reactions occur between the lignin residues of the original waste and the proteins from dead microorganisms to form humic acids, the wastes will not heat up on turning, nor become anaerobic in storage, nor rob nitrogen from the soil when incorporated. The material has become humus or compost.

Process Factors

The decomposition of organic matter during the composting process is a dynamic and complex ecological process in which temperature, pH and food availability are constantly changing. The numbers and species of organisms present change markedly during the process. The rate of progress towards the mature endproduct is dependent on several interrelated process factors. These include nutrient supply, particle size, moisture level, structural strength, aeration, agitation, pH and size of heap. It is desirable to adopt the most suitable operating conditions within the prevailing economic constraints. The complexity of the processing plant, and the quality of the final product, will depend upon the nature of the organic matter to be processed and the level of investment available.

Separation

It is in the interest of the satisfactory end use of a compost product that it should contain as high an organic matter content as possible and a minimum of nonorganic debris. This is of particular importance when processing some wastes, particularly from urban sources, the composts from which can contain significant amounts of trace metals such as copper, lead, nickel and zinc. Thus, with such wastes, it is desirable to remove as much glass, metal, plastic and nonorganic debris as is economically possible. On a very small scale such separation may be manually undertaken. On a large scale a variety of devices are available for such separations; air classifiers, rotodisc separators for plastics and ballistic separators for heavy particles. Where sewage sludge is used it should be mainly from domestic and not industrial sources to avoid heavy metal contamination.

Particle Size

The smaller the size of the particles of the organic matter, the greater is the surface area exposed to microbial attack, thus theoretically allowing a higher rate of reaction. Very small particles, however, pack tightly together giving material with a high bulk density having narrow pores and channels within it.

This restricts the diffusion of air into the mass and carbon dioxide out of the mass, thereby reducing the rate of reaction. The high bulk density may cause excessive loads on mechanized turning equipment, particularly when the materials have a high moisture content.

A compromise on particle size is therefore necessary. For mechanized plants with agitation and forced aeration the particle size may be as low as 12.5 mm after shredding. For naturally aerated static heaps and windrows a particle size of approximately 50 mm is appropriate. In large-scale plants particle size reduction may be achieved using hammer mills, raspers or by self abrasion in rotary drums.

Nutrients

The composting process depends upon the activity of microorganisms that require a source of carbon to provide energy and material for new cells, together with a supply of nitrogen for cell proteins. There is also a lesser requirement for phosphorus, potassium, calcium, sodium, magnesium, sulfur, iron and traces of other elements such as cobalt and zinc. In most composting situations the requirement for these nutrients is adequately met from the original organic matter; only the carbon:nitrogen (C:N) ratio and occasionally the phosphorus concentration may need adjustment.

Chemical analysis of microorganisms established that on average they contained 50% carbon, 5% nitrogen and 0.25 to 1.0% phosphorus on a dry weight basis (Alexander 1977). Approximately 50 to 60% of the organic carbon in the composting materials is converted to carbon dioxide and thus an initial C:N ratio of about 25:1 should be optimum if no nitrogen is lost. A higher ratio involves the oxidation of excess carbon, the organisms passing through many life cycles to achieve a final C:N ratio of 10:1. With C:N ratios lower than 25:1, as in the case of sewage sludge and manures, nitrogen will be lost as ammonia, often in considerable amounts. The loss of nitrogen by volatilization of ammonia can be partially offset by the activity of nitrogen-fixing bacteria. De Bertoldi et al. (1983) report the presence of N-fixing bacteria during the composting of solid urban waste, mainly in association with mesophilic temperatures in the later stages of decomposition. It is suggested that biological nitrogen fixation is inhibited by the presence of ammonia and by high temperatures (De Bertoldi et al. 1982) and is thus associated with the later stages of the process. The uncertainty of nitrogen losses makes accurate prediction of initial C:N requirements difficult, but in practice a ratio in the range 25:1 to 30:1 is recommended. For low initial C:N ratios the loss of nitrogen as ammonia may be partially suppressed by the addition of extra phosphate; this may not be practicable on cost considerations.

Additives

Various claims have been made as to the effectiveness of adding chemical, herbal or bacterial supplements to increase the rate of composting. Apart from the possible

need for extra nitrogen, most wastes amenable to composting contain a wide range of microorganisms and all the nutrients required. There is some evidence that the onset of the thermophilic phase could be speeded up by recycling some product compost to the feed. This was the case with a large-scale rotary drum system where no separation of material, except for removal of ferrous scrap and some textiles, was carried out. In a composting plant where pulverization was carried out prior to composting, the recycling of actively composting materials did not have any significant effect.

Bulking agents are normally necessary to ensure an open matrix for air diffusion when composting finely divided organic solids, such as sewage sludge and animal manure slurries. Wood chips have been favored as the bulking agent in aerated pile systems for sewage sludge (Epstein et al. 1976; MacGregor et al. 1981; Higgins 1983b) proposed pulverized tyres as an alternative; Biddlestone et al. (1985) used straw when composting manure slurries, sewage sludge and vegetable wastes.

Moisture Content

Water is essential to the composting process as the nutrients for the microorganisms must dissolve in water before they can be assimilated. At moisture content below 30% on a fresh weight basis the biological reactions slow down markedly. At too high a water content the voids within the matrix become waterlogged, limiting access of oxygen to the microorganisms. Some materials, such as paper, readily lose structural strength when very wet, collapsing into an impervious mass. Straw type materials, however, can tolerate high moisture content. Thus, optimal moisture content varies and depends upon the physical state and size of the particles. For urban refuse the optimum moisture content lies in the range 50 to 60%.

Water is produced during the composting process by microbial action and is lost by evaporation into the air stream. Where forced aeration is applied moisture loss can be excessive and it may be necessary to supply additional water to the composting mass. This can be supplied by the addition of sewage sludge or other liquid wastes. Problems of water loss are naturally more severe in hot climates.

Moisture content, particle size and aeration are interrelated in terms of the movement of air within the interstices of the composting matrix. Some materials amenable to composting contain lipids (fats, oils and waxes), that are liquid at composting temperatures and affect the void space. Wiley (1957) has suggested that the total liquid content should be used as a factor in place of the water content. This liquid content is given by:

$$\% \text{ liquid} = \frac{100\,(\% \text{ moisture} + \% \text{ lipids})}{(100 - \% \text{ ash})} \qquad \ldots 1)$$

Lipids are determined by solvent extraction with diethyl ether, and ash by incinerating a dry sample at 450°C.

Free Air Space

A composting mass can simplistically be considered as a three phase matrix of solids, water and gas. The matrix is a network of solid particles that contains voids and interstices of varying size. The voids between particles are filled with gas (oxygen, nitrogen, carbon dioxide), water or a mixture of gas and water. If the voids are completely filled with water then oxygen transfer is greatly restricted. It is an over-simplification to assume that there is a discrete water volume and gas volume within the void space but this is the traditional approach of soil mechanics and can be used to define a porosity and free air space for a composting matrix (Haug 1980). The porosity of the composting mass is defined as the ratio of the void volume to the total volume and the free air space within the matrix is defined as the ratio of the gas volume to total volume.

The optimum moisture content for a particular composting mass varies and depends upon the physical state and particle size; thus, different materials can hold different moisture levels whilst still maintaining an adequate free air space. However, experimental work by Jeris and Regan (1973) on the effect of moisture content in various feed materials on free air space and on the oxygen consumption rate of mixed refuse samples suggests that a minimum free air space of 30% should be maintained for a wide variety of composting situations.

Aeration Requirements

Oxygen is essential for the metabolism of the aerobic species of microorganisms responsible for composting. Aeration is possible by natural gaseous diffusion into the composting mass, by turning the material regularly by hand or with a machine, or by forced aeration from a fan. Natural diffusion frequently fails to supply adequate oxygen in the early stages of the process, leading to anaerobic conditions in the lower central regions of the mass of material.

Aeration has other functions in the composting process. A flow of air removes the carbon dioxide and moisture produced in the microbial reaction and also removes heat by evaporative heat transfer. The latter is particularly significant in high rate, mechanized, composting systems. Oxygen requirements vary throughout the process, being low in the mesophilic stage, increasing to a maximum in the thermophilic stage and decreasing towards zero through the cooling down and maturing stages.

The stoichiometric oxygen requirement can be determined if the chemical composition of the organic matter and the extent of degradation during the process are known. For example, the oxidation of proteinaceous material may be represented by the following equation:

$$\underset{352}{C_{16}H_{24}O_5N_4} + \underset{528}{16.5\,O_2} \rightarrow 16\,CO_2 + 6\,H_2O + 4NH_3 + \text{HEAT released} \qquad \ldots 2)$$

Thus, based on this equation 1.5 g oxygen will be required per gram of the material oxidized. This theoretical requirement will vary from about 1.0 g oxygen/g organic material for highly oxygenated wastes such as cellulose, to 4.0 g/g for saturated hydrocarbons. In practice the composting mass will comprise a mixture of materials with differing theoretical oxygen demands and varying degradability such that typically only 40% of the organic matter may be oxidized. Also in practice more air than the stoichiometric level should be supplied to ensure aerobic conditions throughout the mass. There may also be a controlling requirement in relation to heat and water removal in some composting situations.

Wiley and Pearce (1955) recommended aeration rates to supply 6 to 19 mg oxygen/hour/g volatile solids in the composting mass. An alternative recommendation is that the oxygen concentration in the air flow within the mass should be maintained between 10 and 18%. *In situ* measurement of oxygen concentration presents difficulties and in practice it is more realistic to use an indirect factor to indicate that aerobic conditions are being maintained. In forced aeration systems temperature feed back control of the air supply is a possibility.

Agitation

In natural aeration composting systems the lower central regions of the composting mass may be deficient in oxygen because the rate of diffusion of oxygen into the mass is too low for metabolic requirements. In such cases turning the material by hand or by machine allows air to reach these deficient regions. Agitation also helps to break up larger pieces of material, exposing fresh surfaces to microbial attack. Control of the agitation process ensures that most of the material is subjected to the high temperature of the thermophilic phase. However, too much agitation can lead to excessive cooling and drying of the composting mass and shearing of actinomycetes and fungal mycelia. Turning heaps of material can be expensive in machine or labor costs and the frequency of turning is a compromise between economics and the requirements of the process. Flintoff (1976) considers that turning a windrow heap three or four times should be sufficient.

Agitation in mechanized plants is usually achieved by means of slowly rotating drums, augers, or specially designed arms similar to those in multihearth incinerators. These move through the materials, turning and mixing. Excess agitation may also destroy the physical structure of the materials creating a wet impervious mass. For example, agitation of water melon waste for composting in tropical countries may have to be restricted to avoid this problem.

Drums are generally rotated at 0.5 to 1.0 rev/min. continuously. Gray et al. (1971) suggest that in mechanized plants short periods of vigorous agitation should be alternated with periods of no agitation.

pH Control

Addition of chemicals to control the pH in a composting mass has generally proved uneconomic and is not normally recommended. The pH changes from acid to alkaline during composting as shown in Fig. 2. The initial low pH value is a consequence of the activity of acid-forming bacteria which break down complex carbonaceous material to organic acid intermediates. As the temperature rises the pH increases because of release of ammonia.

Heat Production and Heap Size

The various organic compounds present in composting masses each have a different value for the heat of combustion. Three materials commonly found, proteins, carbohydrates and lipids, have heats of combustion within the range 9 to 40 kJ/g. Lipids generally contain about twice the energy per gram as proteins or carbohydrates. This energy is released during the biological oxidation of the composting process. The stoichiometric chemical oxygen demand (COD) for this oxidation, if the composition of the composting is known, can be determined from the chemical equations. For example, if the composting mass was proteinaceous, as represented by Equation (2) the chemical oxygen demand would be 1.5 g COD/g organic material. The heat release per gram of material can then be estimated because most organic compounds have a heat of combustion of about 14.2 kJ/g COD of the organic material. The total heat release would then depend upon the amount of material oxidized.

It is difficult to estimate heats of reaction for composting masses because there is a heterogeneous mixture comprising the mass. However, if there is some knowledge of the major constituents, a chemical oxygen demand calculation could estimate possible levels of heat release.

Heat production may also be determined directly by experimentation. Wiley (1957) studied the heat release when pulverized refuse was composted and concluded that, over 8 to 10 day cycles, it amounted to approximately 7×10^3 kJ/kg of initial volatile solids. Mote and Griffis (1982) determined heat production rates from composting two organic materials, obtaining values in the range 20 to 28 W/kg of initial dry mass.

The amount of heat produced is sufficient such that in large composting masses high temperatures in the range 80 to 90°C can be reached (Spohn 1970). This is well beyond the optimum temperature of 55°C and forced aeration evaporative cooling may well be necessary in such cases. Small masses of material have high surface/volume ratios and hence much of the material has to act as insulation. It is preferable to have at least 1 tonne of material to ensure that a reasonable proportion of the mass reaches a satisfactory temperature. For heaps composting under natural aeration conditions the material should not be piled over 1.5 m high or 2.5 m wide, otherwise diffusion of oxygen to the center will be impeded. The heap can be elongated into a windrow of any convenient length.

Optimum Process Conditions

There are now sufficient experimentally determined data in the literature on the microbiological, chemical and physical parameters of composting for reasonable process design of composting systems. A summary of the optimum values of the important parameters is given in Table 3.

Table 3. Optimum composting parameters.

Parameter	Value
C/N ratio of feed	25 to 30/1
Particle size	12.5 mm for agitated systems and forced aeration, 50 mm for windrows and natural aeration
Moisture content	50 to 60% (higher values possible when using bulking agents)
Free air space	about 30%
Air flow	0.6 to 1.8 m^3 air day^{-1} kg^{-1}; volatile solids during thermophilic stage, or maintain oxygen level at 10 to 18%
Temperature	55°C
Agitation	No agitation to periodic turning in simple systems; short bursts of vigorous agitation in mechanized systems
pH control	Normally none desirable
Heap size	Any length, 1.5 m high and 2.5 m wide for heaps and windrows using natural aeration, with forced aeration heap size depends on need to avoid overheating

historically practiced by the Chinese, is essentially a small-scale batch operation in heaps (King 1927; FAO 1977). Interest in the scientific aspects of composting followed from the visit by King (1927) to the Far East and the subsequent development of the Indore method of heap composting (Howard and Wad 1931; Howard 1943).

Recent interest in composting has been in response to the need to deal hygienically with large quantities of urban refuse and sewage sludge and the increasing need to recycle crop residues and animal manures in agriculture.

For the composting of agricultural, horticultural and garden wastes relatively simple processing schemes are still employed. Agitation is rare but forced aeration is sometimes used. Widely varying inputs of capital investment, running costs and labor are to be found in a variety of processing arrangements. Because of the fairly low monetary value of composts, sophisticated processes cannot be afforded in most agricultural situations and process conditions frequently fall short of the optimum levels listed in Table 3.

During the past 40 years some 30 different processing schemes have been introduced for composting urban wastes, with varying success. Equipment for feed preparation and compost product finishing are similar to many of these processes. The decomposition stage, however, has varied widely, being attempted in pits, cells, silos, digesters and drums. Very recently large-scale mechanized systems with capacities in the range 200 to 500 t of refuse/day have been installed in various countries. Tenders for 750 t/day systems are currently being invited and enquiries have been instituted regarding a 1,000 t/day operation.

Materials for Composting

A wide variety of organic materials that are suitable for compost production are produced by human communities and agriculture. Some of these materials are listed in Table 4, with very approximate values for their carbon/nitrogen (C:N) ratios. The C:N ratio of some materials, such as manures, is below the optimum (Table 3); this can lead to excessive ammonia loss during composting. These are best mixed with materials having a high C:N ratio such as straws and woody type wastes. Materials that have high moisture contents should be mixed with materials of low moisture content to give a material which will compost readily. This occurs with animal manures and straw, sewage sludge and straw or woodchips, sewage sludge and refuse.

In practice not all of the materials in Table 4 will be available in any given locality. In tropical situations fresh green material is short lived and is frequently used for forage, while dung is sometimes burnt for fuel. As a result, the available wastes tend to be those with rather high C:N ratios. It is important, therefore, to conserve liquid manure as this supplies nitrogen; it also contains potash and trace elements. If facilities are not provided for the collection of liquid manure, soil from the floors in animal housing should be collected from time to time and used for composting. A little soil is in fact a useful component of a compost heap; clay is preferable to sand. Clay has a high base exchange capacity and will help to hold any liberated ammonia

within the heap until the microorganisms can immobilize it, thus reducing nitrogen losses. Although the availability of differing materials will vary, virtually any material which has once lived will compost, but crockery, glass, metal and plastics and man-made fibers should not be used.

Typical analyses or urban refuse from cities in different regions of the world are given in Table 5; considerable local variations are to be expected. In refuse only the

Table 4. Approximate composition of materials suitable for composting.

Material	C:N ratio
Urine	0.8
Dried blood	3
Nightsoil, dung, sewage sludge	8
Bone meal	8
Coffee pulp	8
Farmyard manure	14
Brewers wastes	15
Water hyacinths	16
Grass, weeds	20
Refuse	35
Leaves	60
Pigeon pea stalks, millet stalks	70
Wheat straw	80
Rice straw	100
Rotted sawdust	200
Coconut fiber waste	300
Fresh sawdust	500
Paper	Infinity

Table 5. Composition of urban refuse from different sources.

	% by weight (fresh weight basis)		
	Far East	South America	Europe
Vegetable and putrescible	75	55	16
Paper	2	15	43
Plastics and textiles	4	10	7
Glass	0	4	10
Metals	0	6	10
Miscellaneous	7	10	1
Inerts and rubble	12	0	13
Approximate moisture content within the total	60	45	18

vegetable/putrescible and paper fractions are of value in compost, the other materials being inorganic detritus. In the design of urban waste compost systems it is essential that a thorough analysis be made of the local refuse (Flintoff 1976).

Vast quantities of urban wastes, agricultural wastes and low value byproducts are available for composting. On a global scale some 4,000 x 10^6 t of cellulosic and livestock wastes are available annually. On a town/city scale 100,000 to 250,000 t of urban waste may be generated annually by a single town/city.

Preparation of Materials

The pretreatment available in preparing the materials for the composting reaction will vary and will depend on whether the composting system is essentially small-scale and manual or large-scale and mechanical.

In manually intensive systems various types of material can be differently prepared. Fresh green material such as weeds and vegetable trimmings can be used without pretreatment. If there is a rainy season some withering should be allowed. Coarse materials such as stalks of cotton, maize, millet and the pulses are best broken before use. This can be done by chopping with sickles or in a chaff cutter. It can also be achieved by using the materials as bedding in an animal house or laying them on farm roads to be broken up by passing transport. Leaves, rice bran and straws can be used without pretreatment. Woody materials such as sugar cane trash, treebark and sawdust should be steeped in water for several days or placed in a pit with moist soil for several weeks.

Urban refuse is suitable for composting after some degree of pretreatment. This usually takes the form of salvage recovery, a pulverization stage, magnetic recovery of ferrous metals, removal of plastics and textiles and adjustments to moisture content and C:N ratio. The order and degree of pretreatment varies according to the type of composting system used and the nature of the refuse.

Small-scale Systems

The composting of garden, small holding or nursery wastes is widely practiced, the system usually being a series of batch heaps handling 0.5 to 1.0 t of material/batch. The major problem with such small batches is that of relatively high heat loss because of the large surface to volume ratio. It is preferable at this scale (Gray and Biddlestone 1976) to compost:

(i) within a walled enclosure with some degree of insulating value;
(ii) to cover the wastes with an insulating but permeable top blanket that sinks with the wastes;
(iii) to arrange for air to permeate underneath the heap and percolate upwards by the 'chimney effect'; and
(iv) to prevent rain falling on the wastes, thereby causing cooling and leaching of nutrients.

It is also recommended that the materials be collected and saved to build up the heap to a full height of 1.0 to 1.25 m in one operation, rather than in thin layers at perhaps weekly intervals. By construction in one operation, much higher temperatures are achieved and a greater degree of weed seed kill and pasteurization results. Whenever possible the materials to be composted should be premixed thoroughly before assembling the heap.

Where temperatures of about 60°C are achieved and the heat permeates to the edges of the heap because the side wall is well insulated, then turning is not necessary. In two to three weeks the materials should have broken down significantly, although maturing will take much longer.

Simple Heap and Windrow Systems

In simple heap systems the prepared material is formed into long heaps known as windrows, either manually or using equipment such as tipper lorries or front end loaders. The height and width of the approximately triangular sectioned heaps can vary, but it is recommended as a general guide that simple heaps using natural aeration should be approximately 1.5 m high and 2.5 m wide. The windrows may be any length and are usually arranged according to the shape of the site. The windrow area should preferably be concreted in order to withstand the movement of vehicles; however, such concreting may be expensive for low cost installations. The windrows may be left standing for several months, until the temperature begins to fall, or they may be occasionally turned. The aims of turning the heaps are to provide aeration, reduce particle size, and to ensure that all material is subjected to the high temperature of the thermophilic stage. The latter requirement is achieved by turning the outer parts of the heap into the center of the reconstructed heap. Turning of windrows may be achieved in several ways with differing effectiveness. For very small installations manual labor may be used, but more frequently tractor mounted bucket loaders are used. For larger installations specially designed mobile processors that straddle the heap and turn, aerate, and, if necessary, add moisture may be used. Turning is a time consuming operation and in some simple systems stakes or bamboo poles have been used to create channels within the heaps to improve natural convective aeration.

Several simple, heap composting systems have been reported, for a variety of organic materials. In Europe and the USA, mixed farm systems involve the use of front end loaders with forks and in some cases flat bed manure spreaders. The organic material, usually from animal houses, is loaded into a flat bed manure spreader that shreds the wastes, aerates them and throws them into a pile about 1.25 m high. The machine is gradually moved along to form a long heap. A series of vertical holes to ground level and 1 m apart are next made through the mass with a 75 mm diameter stake. Another section of heap is then made and aeration holes created. Where possible, some green vegetable material is added to the material in the manure spreader. Such heaps have reached 70°C within a week and then cooled to 30°C

after a month, when they are turned with a front end loader. After two to three months the compost is ready for use. In the USA, Demmel (1980) reports the composting of animal manure bedding in windrows about 2 m wide by 1.25 m high, with a turning machine passing through the heap several times a month. It is a method that gives a good product, but consumes a lot of energy and space; the turning machine handles 400 to 500 t/hour. Farmers in Europe are also using a 'moving windrow' technique. A flat bed manure spreader with side belt elevator is used to lay out a windrow up to 2 m high alongside the moving machine. In practice a small triangular shaped windrow is laid out first; this is readily permeated by adequate air. After three to four days, when most of the high rate of oxygen demand has been met, a layer of new material is added to the side. The heap is not subsequently turned.

In the tropics the improved Indore process is still remarkably useful. In this technique the organic materials are composted in pits approximately 9 x 4.5 x 1.2 m deep. Vegetable wastes are put on in 150 mm layers, followed by manure in 50 mm layers and a sprinkling of earth, wood ashes and water. Layering is continued to a height of 1.2 m and vertical aeration vents are made. The heap is turned by hand two or three times and further water is added as necessary. The method has been used in recent years with marked success by Dalzell at Medak Agricultural Centre, India (Dalzell et al. 1979).

Organic waste is recycled in China by several techniques of composting (FAO 1977). Human and animal wastes are layered into a heap with chopped plant stalks, similar to the Indore process. A typical heap comprises 40% crop stalks, 30% agricultural wastes/refuse and 30% animal manures, excreta or nightsoil. The dimensions of the heap are 1 m high, 2 to 3 m wide at the top, 4 m wide at the bottom and 6 to 7 m long. When the heap is built, sets of bamboo poles, 100 mm in diameter, are laid horizontally every 1.5 to 2 m along the heap at a height of about 300 mm above the base. Each set is connected with bamboo poles standing vertically to provide aeration channels. When completed the heap is covered with mud about 30 mm thick. The bamboos are removed after 24 hours leaving airways for ventilation. After four to five days a temperature of 60 to 70°C is reached. Turning of the heap is practiced after two weeks and the compost is used after two to four months. A similar approach is reported for the composting of a mixture of nightsoil, water hyacinths and leaves in Central Thailand (Polprasert et al. 1980). A method described as 'ground surface continuous aerobic composting' is used in which the three feed materials are mixed and formed into heaps on a 2 x 2 m square base to a height of about 0.5 m. Bamboo poles are used for aeration channels and rice straw as a covering to control heat loss from the heap. The rate of composting is reported as being most rapid when sufficient leaves are added to the nightsoil and water hyacinth mix to give an initial C:N ratio of 30.

Many examples of simple heap systems have been reported in the literature. Shuval et al. (1978) specifically considered nightsoil composting. Shea et al. (1979) reported the composting of dewatered sewage sludge with compost product. Paatero (1979) used a mobile mixing machine to compost dewatered sewage sludge with

wood chips, bark, peat or dried leaves; a double screw conveyor discharged the mixture to form a windrow. Hyde and Consolazio (1982) composted the wastes from various food manufacturing processes. Large particle materials from fruit and vegetable canning operations may possibly be composted alone whereas sludges need the addition of a bulking agent. Gaur (1984) reviewed composting in Southeast Asia; materials such as maize straw, rice straw, chicken manure and nightsoil have been used. Biddlestone et al. (1985) described experiments on the composting of animal manure slurries, dewatered sewage sludge and vegetable wastes when intimately mixed with straw, the straw being incorporated at a level of 5 to 10% of the total mass.

Accelerated Windrow Systems

In order to increase the rate of decomposition and to reduce or remove the need for turning, some windrow systems forcibly aerate the heaps from air channels or pipes beneath the windrows. The air may be sucked through the heap and into the channels, or blown from the channels, through the heap to the atmosphere. There are advantages and disadvantages to both these methods of aeration.

In the case of a suction system the air is drawn downwards through the windrow, into the channels, and through the fan. It may be discharged directly to the atmosphere or pass through some form of odor control, usually a bed of mature compost. The disadvantages arise because the air being drawn through the heap becomes saturated with moisture. This may condense in the lower regions of the heap causing anaerobic conditions and producing a very offensive leachate. A moisture trap must, therefore, be incorporated in the suction line. Other compounds such as ammonia and carbon dioxide may also be drawn into the suction line causing corrosion. The outer layers of the heap may dry out, particularly in dry warm climates. The advantage is that the gases from the windrow converge into one outlet, enabling odor removal, ammonia recovery or heat recovery to be carried out.

Blown aeration systems move air under pressure from the base of the heap to the atmosphere. This avoids the problem of leachate and anaerobic conditions at the bottom center of the heap, but in this case, the moisture in the saturated air may condense in the outer layers of the windrow. This effect may be reduced to some extent by covering the windrows, when possible, with a layer of mature compost as insulation. In this method of air supply odor control cannot be carried out.

Several forced aeration windrow processes have been installed in the USA for the composting of dewatered sewage sludge with wood chips as bulking agent. The Beltsville process (Epstein et al. 1976) uses sludge of 78% moisture content with wood chips in the volume ratio of 1 to 2. The combined materials are laid out in windrows on top of perforated pipes, covered with a layer of finished compost, and air is drawn through the perforated pipe. The heap is composted for four weeks, then removed and stored for a further four weeks. The wood chips are then recovered by screening. In order to achieve effective screening the moisture content of the compost needs to have been reduced to about 40%. Singley et al. (1982) have

produced a detailed design and operating manual for such a system. Finstein et al. (1983) compare two 36-t heaps of sludge and woodchips, one with suction aeration, the other with blown aeration. Their recommendation is that a blown air system with interactive feed back temperature control of the blower should be used as the best means for preventing excessive temperatures being reached.

De Bertoldi et al. (1982) composted a mixture of sewage sludge and the organic fraction of urban refuse in three 2-t heaps that were identical apart from their aeration systems. The urban waste and sludge were mixed in the proportion of 60 and 40% (w/w), respectively. The initial moisture content was 67%. Heap 1 was turned twice weekly. A vacuum induced aeration system was used for heap 2, the blower being activated by a timer providing 40 sec. suction every 13 min. Heap 3 was a forced pressure-blown system with temperature feedback; the blower operated for 40 sec. every 13 min. below 55°C and operated continuously above 55°C. The conclusion reached from the experiment was that the forced pressure-blown system was the most rapid of the three processes tested, and led to the production of the best product in terms of lower moisture content and a higher degree of humification and stabilization.

In the absence of a forced aeration system, oxygen supply can become the limiting factor. We have carried out experiments on the composting of a variety of organic materials with straw. Fig. 3 shows the temperature profile of two 5-t heaps comprising vegetable waste (leeks) with 5% by weight of straw. The forced aeration heap was blown for 7 min. every hour by a blower delivering 2.8 m^3/min. at 160 mm water head. The heat release in the heap relying on natural aeration is significantly less than the forced aeration heap.

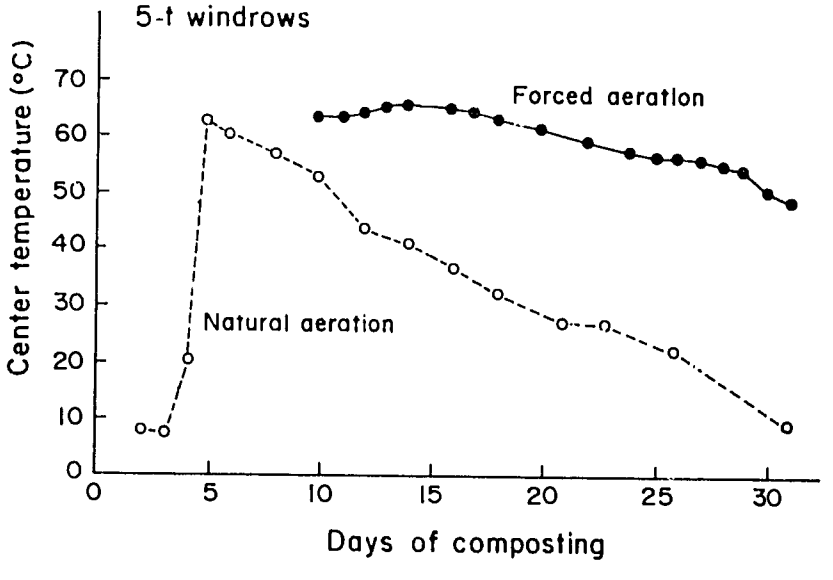

Fig. 3. Temperature time pattern when composting vegetable wastes (leeks) with 5% straw.

Large-scale Mechanized Systems

The modern large-scale urban composting system usually comprises refuse storage facilities, feed preparation equipment, a biological degradation stage and final product upgrading. Refuse collection vehicles discharge their refuse into deep hoppers or onto flat concreted areas from where it is conveyed by moving floors, overhead grabs or front-end loaders. The material is then prepared by size reduction, separation of unwanted and salvageable materials and then adjustment of moisture. Size reduction can be carried out in wet pulverizers that consist of a slightly inclined drum, typically 3 m diameter and up to 10 m long, rotating at 3 to 10 rpm with a throughput of about 10 t/hour. Alternatively, dry pulverizers can be used and these are either rasping or hammer mill types, although ball mills have been used in a few installations. The power requirements of pulverizers depend mainly upon the final size of the outlet material; for reduction to 50 mm about 8 kWh/t. After size reduction magnetic separators are used to remove ferrous metals, sheet plastics and rags are removed by rotodisc machines and screens remove oversized material. Prior to composting the moisture content of the material is adjusted, if necessary, by the addition of water or sewage sludge.

The biological degradation stage is carried out in windrows/accelerated windrows or in more sophisticated mechanized units. Mechanized units vary from automated windrow systems with continuous automatic turning equipment, to totally enclosed silos which achieve extensive breakdown of the material within a few days.

In automated windrow systems the material is usually placed in troughs or between walls. The base of the composting area has provision for blowing or sucking air. Agitation is provided by a turning device that moves along the walls on rails or wheels. The turning devices vary in design; some consist of large paddle wheels 4 m in diameter that pick up the compost and place it into a central conveyor that ejects the material to form a new parallel heap. Other designs have screw conveyors or augers that gradually move the compost horizontally towards the final processing stage. The residence time in the heaps varies from 4 to 12 days depending upon the particular design.

Rotating drum units basically consist of a cylinder up to 4 m in diameter and 40 m long, inclined slightly to the horizontal. Refuse, usually without pulverization, is fed to the higher end. The refuse is physically broken down by attrition and abrasion as the drum rotates continuously at about 1 to 2 rpm. Various sizes of screen may be incorporated into the sides or the end of the drum to separate the material. The rejects may be separately disposed of or returned to the inlet of the drum. Some designs of drum include fans to provide forced aeration. The residence time in the drums is typically two to three days after which the material is usually put into windrow heaps for several weeks.

Vertical silo systems may be circular or rectangular in cross section and may have more than one floor. The material passes through on a continuous batch principle. The simplest form of circular silo is a single floor unit that holds one day's batch of refuse. A number of such towers are provided to give the required residence time of

four to five days. Air is usually supplied through a perforated floor. Agitation may be provided either by vertical screw or auger devices, or by horizontal rotating arms or ploughs.

In multifloored silos, of typical size 8 m diameter by 12 m high, the refuse is fed in at the top in approximately 50 t batches and gradually moves downwards. The floors may be in segments that are hinged to open and discharge the material to the floor below. Some designs have perforated floors and rotating arms that force the composting material through the perforations to the floor below. A more sophisticated design has hinged trap doors in each floor that open at predetermined times to allow rotating arms to push the material through to the floor below. Aeration is provided either through the floor or through the rotating arms.

A single silo may contain up to 200 t of material that can release substantial amounts of heat; this may raise the temperature beyond the optimum of 55 to 60°C. Excess air is often provided to effect cooling by evaporation of water into the air stream and, in order to prevent the materials from becoming too dry, provision for water addition on each floor is usually made. Aeration rate may be controlled from floor to floor. The extent of instrumentation and automation provided for silo systems varies according to the design. Residence times are in the range 4 to 20 days, eight days being most common. Some systems make provision for subsequent windrowing while others have a simple storage area of two to three weeks capacity.

In multidecked rectangular shaped compost houses material is loaded into one end of the top floor and is moved along the floor either by a moving conveyor or by augers. The material then drops to the floor below making another pass in the opposite direction. Aeration is provided through the floors. An alternative design uses pivoted sections of floor that turn over and drop the material to the floor below. Aeration is achieved as the material falls from floor to floor. Residence times are typically four to eight days usually followed by a maturing period.

Some recent schemes are offering a highly mechanized forced aerated composting unit with a short residence time of one to two days followed by a simple windrowing period of two to three weeks. The initial temperature peak with its high demand for oxygen and agitation occurs in the mechanized section. The cooling down period, which requires less aeration and agitation, takes place in the windrows, which can be made higher than normally recommended without the onset of anaerobic conditions. These windrows are not normally turned. Such a combination of mechanical and windrow composting reduces the land area requirement without the high capital investment of a completely mechanized process.

Product Upgrading

Depending upon the scale of operation and the type of material being composted, there may be some degree of treatment before final use. Simple small-scale and windrow systems appropriate to small communities are likely to use the product after breakdown without further processing. Large-scale mechanized units processing mixed refuse are likely to have some form of upgrading before sale of the product.

In upgrading operations, rotary or vibrating screens of 25 mm mesh remove most of the remaining plastic and textiles that were not broken down during composting. Large glass particles may be removed, together with any inert material. The material less than 25 mm may be further screened, the finest compost below 8 mm being classed as horticultural grade and the coarser being agricultural grade. This latter, coarser product, is also suitable for covering tips or for land reclamation.

The most common form of final treatment, after coarse screening, is to finely pulverize the compost in a hammer mill. In this way glass fragments, often a cause of complaint, are reduced to sand like particles.

Experiments are being conducted by various manufacturers into the effectiveness of a variety of separation devices. Ballistic separators use the different elastic properties of the materials to achieve separation. Density separation employs a current of air to remove light materials, a most recent example being the formation of a fluidized bed on a vibrating screen. It has also been shown recently that electrically heating the wire meshes of screens allows finer screening; this is because the moisture content of the compost product is critical to screening and the heated screens cause surface drying of the compost particles thereby allowing them to fall through more easily.

Compost Yield

Yield is the concept used to identify how much material leaves the end of a process in relation to how much material entered the beginning of the process. It is a ratio of quantities and may be calculated on a wet weight basis or a dry weight basis. The dry weight basis calculation has the advantage of avoiding the complication of varying water content in feed and product.

The breakdown of organic material during composting results in the loss of approximately 30 to 40% of the organic matter to carbon dioxide and water. Thus the mass of compost product produced is significantly less than that of the raw materials supplied. There may be additional losses from any separation processes. Predicting yield is difficult. Only part of the organic matter will be degraded; any nondegradable fraction in the feed will pass through the process into the product unless separated. The water content is difficult to predict. Water is required by the microorganisms and is also lost by evaporation into the air stream. In dry climates, it is sometimes necessary to add water to maintain a sufficient level for microbial action.

The fraction of organic matter that will actually degrade will depend upon the proportions of different compounds in the materials that are being broken down. Simple sugars have a high level of degradability, but lignified materials have a very low level of degradability. The mixture of low and high molecular weight compounds in the materials for a given composting situation will have an intermediate degradability.

An indication of yield for specific operations can be obtained from past experience. A simple pit system in the tropics for composting a mixture of crop/veget-

able wastes, manure, weeds, wood ashes and urine earth is likely to have yield of about 40% (on a wet basis). A single pit charged with approximately 18 t will provide about 7 t of product. It is quite likely that water will have been added during the turning operations. An urban refuse composting plant in Europe is likely to produce 40 t of product from 100 t refuse input, on a wet weight basis, but some organic matter in the form of sewage sludge is likely to have been added at the degradation stage. Urban refuse plants in semi-arid and tropical areas tend to have a higher proportion of degradable materials than European plants and can achieve 50% yields on a wet weight basis. Simple garden compost boxes containing garden and kitchen wastes typically have yields of 40-50%.

This paper has been concerned exclusively with compost as the only product of value when organic matter is degraded. However, as shown in Fig. 1 and Equation (2), the degradation of organic matter also gives rise to considerable amounts of CO_2, some NH_3, much low grade heat and moisture. In recent years several research teams have started to study the recovery of these other products, with CO_2 being used for supplementing the atmosphere in greenhouses and heat from composting 'heat heaps' being used for warming buildings and water masses.

Compost Product Composition

The composition of compost products varies widely and reflects mainly the composition of the organic materials used The range of compositions normally encountered is indicated in Table 6. Composts prepared from urban wastes tend to be lower in organic matter and the major plant nutrients than those made from garden/farm wastes. The concentration of the major plant nutrients, nitrogen, phosphorus and potassium, in composts produced from different starting materials is indicated in Table 7. The relatively high concentration of potassium in the first five examples is for compost produced in tropical conditions with careful conservation and inclusion of animal urine.

Table 6. Composition range of matured composts.

Substance	Composition range % by weight, dry basis
Organic matter	25.0 to 80.0
Carbon	8.0 to 50.0
Nitrogen (as N)	0.4 to 3.5
Phosphorous (as P)	0.1 to 1.6
Potassium (as K)	0.4 to 1.6
Calcium (as CaO)	7.0 to 1.5

Table 7. Composition of various composts.

Raw materials	% by weight, fresh basis N	P	K
Cotton stalks and dung	0.40	0.13	1.4
Vegetable wastes	0.49	0.12	0.9
Vegetable wastes and dung	0.43	0.10	1.0
Mixed weeds and san hemp	0.41	0.11	1.7
Mixed weeds	0.40	0.12	1.3
Poultry dung and straw	1.1	0.9	0.8
Garden compost	0.4 — 3.5	0.3 — 1.0	0.2 — 0.3
Straw and sewage sludge	0.5	0.15	0.12
Municipal compost	0.4 — 1.6	0.1 — 0.4	0.2 — 0.6
Poultry manure and sawdust	1.0	0.4	0.46
Pig slurry and straw	0.53	0.37	0.33

Composts from urban wastes can also contain significant quantities of trace metals such as copper, nickel, lead and zinc. The uptake by crops of these metals from soils treated with such composts has been examined in several field trials (Gray and Biddlestone 1980). Although some of these trace metals are essential to plant life, the concentration of heavy metal trace elements should be monitored to prevent a buildup of toxicity in the soil.

Pathogens

Pathogens are organisms that cause infections in man, animals and plants; most organic wastes are contaminated to some degree with pathogens. They may belong to any of the main classes of microorganisms: bacteria, fungi, viruses and Protozoa, to the macrofauna: helminths, nematodes and intestinal worms. Most of these pathogenic organisms are adapted to the body temperatures of man, animals and plants and will die if exposed to higher temperatures for a long enough time. In a composting process, there are several barriers to the survival of pathogenic organisms; the temperature/time relationship is a barrier and pathogens are also destroyed or controlled by competition for food with other organisms, antagonistic relationships and antibiotic or inhibiting substances such as ammonia.

It is important to note that above a certain temperature level, thermal death is time dependent. A higher temperature for a short period of time or a lower temperature for a longer duration can be equally effective. Based on known time/temperature relationships, heat inactivation of most pathogens is normally effective under the conditions common to composting. Temperatures of 55 to 60°C for periods of a few minutes to a few days are typically effective. However, although most disease and parasitic organisms are killed, a few spore-forming bacteria, if present, can survive

temperatures in excess of 100°C. Examples are the aerobic spore-forming *Bacillus* species, notably that causing Anthrax, and the anaerobic-spore forming *Clostridium* species, which cause tetanus, botulism and gas gangrene. These organisms are likely to survive the composting process.

It is quite clear that it is impossible to guarantee that a particular composting process will produce a product that is entirely free of pathogens. However, if composting is correctly effected a sufficiently hygienic product should be obtained. Totally enclosed mechanically agitated systems may be more desirable in this respect than the occasionally turned windrow heap. In a mechanically agitated, totally enclosed digester the heat is spread fairly evenly throughout the mass, and thus there should be no major cool static pockets. In a windrow heap, however, there is a large variation in temperature and aeration. There are cool outer layers, cool pockets at the lower edges, possibly an anaerobic region at the center bottom and a high temperature region just above the middle. By periodic turning of the heap cooler areas may be brought into the hot center, but a number of turnings may be necessary to obtain the required degree of sterilization.

A composting mass contains a very large population of indigenous natural organisms carrying out the process. There may be in addition pathogens present, but they will represent a numerically insignificant fraction of the total microbial population. Air passing through the mass will pick up organisms and their spores and eject them into the atmosphere. This is true of both pathogenic organisms and of the normally innocuous organisms. Under certain conditions, usually very high microbial concentrations, some of the latter can cause allergic lung disease. Consequently, in composting plants it is advisable not to release dusty spore-laden air into enclosed working areas nor to carry out windrow turning operations in roofed buildings.

A number of tests of pathogen survival in composting systems have been reported. It is evident that, in a composting mass that is well agitated or is turned fairly often or is well insulated, so that all the material is exposed to the higher temperatures for a period of several hours, all the common pathogenic organisms investigated are destroyed. Composting material is not the natural environment for pathogens and they will tend to be eliminated in such an ecosystem. In those situations in which highly resistant spore-forming pathogens may be found, the organisms are very likely to be present in the soil and there is no point in having a composting process that yields a sterile product, or lower numbers of those organisms than are present in the soil to which the product is to be applied.

The literature on the presence and survival of pathogens in composting systems has been reviewed recently (Finstein et al. 1982; Higgins 1983a; Golueke 1983).

Advantages of Composting

The importance of recycling organic wastes is being increasingly recognized. At present many of the wastes available in tropical situations are poorly used. The addition of fresh organic wastes to any ecosystem can create problems because there is a high oxygen demand from the wastes, or because there is competition for

nitrogen, or there may be effects of intermediate compounds, or the release of ammonia.

Composting provides a means for obtaining a stable product by biological oxidative transformation. The humified product comes quickly into equilibrium with the ecosystem into which it is placed without causing the major disruption associated with raw wastes. It provides a means for combining together low value straw wastes with human and animal wastes that pose a problem of hygienic disposal. At the temperatures achieved in composting there is a significant kill of pathogenic organisms.

Compost is primarily a soil conditioner and to some extent a fertilizer. When added to the soil, it improves soil structure and water holding capacity. As a fertilizer, it may be added to an ecosystem as a means of supplying major nutrients as well as minor and trace elements and a range of micro- and macroflora and fauna.

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Discussion

SCHROEDER: It is interesting that your analyses showed continued presence of solubles. Even though the microbial concentration and activity was high, the microbes did not immediately consume the solubles. A higher animal, e.g., a fish, could feed on the compost and benefit from these solubles. You had about 10% wheat solubles and that remained constant. I would have thought that such solubles would be highly digestible.

BIDDLESTONE: They are. Of course, they are consumed but during the composting process hemicelluloses and celluloses are being broken down and constantly replacing them. Yung Chang and Hudson* found there was always a measurable level.

SCHROEDER: This is very similar to the situation in a rumen. The amounts of solubles remain rather constant.

BIDDLESTONE: But these would be different solubles.

SCHROEDER: Yes.

FRY: Wouldn't it be better to conserve nutrients instead of losing some in lengthy composting processes and to have your microbial production as close to the fish as possible.

BIDDLESTONE: Yes, I suppose so. Peter Edwards will talk later about feeding composts directly to fish, but I would like to make two points here. First, there is the question of acceptable BOD of pond inputs. Composts have quite low BODs. Second, and a point I have not addressed very fully, is the question of pathogens. *Salmonella* and the infective stages of intestinal worms, for example, are dealt with very effectively by the temperatures achieved in compost heaps. Therefore, public health problems could be reduced.

PULLIN: What about heavy metals and other toxic materials in composts?

BIDDLESTONE: There has been a lot of work on heavy metals in composts. It is difficult to generalize on this. If your starting material contained heavy metals, for example, sewage from an industrial area, they will be in the composts. A lot of work has been done on land application of composts and heavy metal uptake by crops. The more alkaline the soil conditions, the less is taken up however, if not taken up they build up in the soil and could eventually be released if acidic conditions were to occur. There is a sort of barrier between soil concentrations of heavy metals and the amounts taken up by plants quite apart from pH.

WOHLFARTH: To return to the question of whether we should compost organic materials at all for pond application because of the losses of valuable nutrients, the Chinese use a whole range of practices from having latrines over ponds and putting up in other raw organic matter to making compost heaps. I suppose we could consider the pond itself as a type of compost pit when raw material is put in.

BIDDLESTONE: You could call this liquid phase composting.

WOHLFARTH: But why do you think the Chinese do all these things?

BILIO: Let's postpone this general question for later discussion.

COLMAN: I suppose there are two possible benefits from composting, an increase in mineralization and an increase in the protein content of the material. Also the BOD of pond inputs is very important. If it is too high because of microbial activity, it can deplete the oxygen in the water. But does the protein content increase with the composting process or is there just an increase in N.

BIDDLESTONE: I am not sure how to answer. There is microbial protein certainly.

BILIO: We can return to this question.

KIRCHMAN: Concerning your comments on the importance of temperature in composting and the inhibitory effects of high temperatures, would such temperatures tend also to reduce the activities of other predator organisms grazing on bacteria such as Protozoa? If this grazing pressure is reduced, it may also inhibit bacterial production.

BIDDLESTONE: Yes, I believe so.

PRUDER: Is industrial composting a consistent process which produces materials of fairly constant composition from place to place given similar starting materials?

BIDDLESTONE: Yes, there is consistency between the material produced from piles of similar material because ultimately you are getting down to the C:N relationship of the microorganisms plus the nondegradable part.

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*Yung Chang and H.J. Hudson. 1967. The fungi of wheat straw compost: ecological studies. Trans. Br. Mycol. Soc. 50(4): 649-666.

Conversion of Cellulosic and Other Organic Wastes into Microbial Proteins

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Abstract

Some microorganisms among the bacteria and fungi have the potential for converting organic wastes with a high C:N ratio into animal feed with a high protein content at a rapid rate. Recent advances in the knowledge of basic physiology of these organisms have led to optimization and control of the environmental conditions, resulting in increased productivity from fermentations. Carbohydrate wastes, especially of agricultural origin, provide a plentiful and renewable resource for the production of microbial protein. Most of the wastes require a physical or chemical pretreatment for making them suitable for microbial fermentation. Higher productivity of microbial biomass is generally obtained in submerged fermentation than in solid state fermentation because the environment can be easily controlled in submerged systems. Further studies on the modification of controlled instrumented fermenters to suit the appropriate technology, and development of inexpensive techniques for immobilization of cells to inert materials, will make microbial proteins a highly desirable feed for intensive aquaculture.

Introduction

Aquatic animal husbandry may be practiced as extensive, semi-intensive or intensive cultivation. Extensive aquaculture depends mostly on the growth of a few compatible species (polyculture) to make use of the nutrients present in a body of natural or managed water in a pond. It has been shown that fertilization of the pond with inorganic nutrients as well as organic residues increase the productivity (Van der Lingen 1959). Intensive aquaculture presently in the form of cage culture

(aquatic counterpart of cattle feed lots or chicken batteries) is more capital intensive and requires a knowledge of the nutritional needs of the aquatic animal to be cultivated in order to supply the animal with the necessary feed. Until recently, the protein ingredients in the feed consisted mainly of plant or fish origin. However, partial replacement or supplementation of algal or fungal proteins in the feed for fish has shown favorable results (Yu and Sinnhuber 1983). Yeast has been included in several test formulations in pelleted fish feed (Windell et al. 1974). "Pruteen"—trade name for bacteria *(Methylophilus methylotrophus)* grown on methanol, which has been found to be an efficient protein feed for calves, has also been investigated for its inclusion into diets of aquatic animals (Nell 1985). There is an additional advantage in the use of single cell proteins (bacteria, fungi) as ingredients in aquatic feed formulations, for microorganisms contain vitamins and other supplementary growth factors. Thus microbial proteins may become more important as the development of intensive aquaculture progresses. In this report is first a discussion of the "state of the art" in the production of single cell proteins from lignocellulose and other organic wastes and then how present-day fermentation processes can be modified to appropriate technology for their use in aquaculture.

Substrates for Microbial Protein Production

Production of microbial protein involves certain basic steps: (i) addition of an easily assimilable carbon source; sometimes it is necessary to pretreat the available substrates physically or chemically to make the substrates susceptible to attack by microorganisms; (ii) a suitable medium has to be prepared combining the substrate with sources of nitrogen, phosphorus and other factors for growth of organisms under reasonably optimized conditions and (iii) harvesting the organisms and post-harvesting steps such as drying, pelletizing etc.

Although microbial proteins may be produced from a variety of carbon sources, carbohydrate raw materials, especially of agricultural origin, are more pertinent to our discussion. Such raw materials fall into two major classes, mono- or disaccharides and polysaccharides. The polysaccharides may be classified further into two types: starch and cellulose. Sources of available carbohydrates are presented in Table 1.

Table 1. Carbohydrate substrates and sources for production of single cell proteins (from Gaden 1974).

Mono- or disaccharides :	Molasses	— cane, beet, corn, citrus
	Whey	
	Sulfite liquor wastes	
	Fruit/vegetable packing wastes.	
Polysaccharides :	Starch	— grains, cassava, potato processing waste
	Cellulose	— rice straw, wheat straw, corn cobs, sugarcane bagasse, feed lot waste.

Organisms

A number of different organisms have been investigated for their use in the production of microbial proteins (Porter 1979). Both pure cultures of yeast and mixed cultures of yeast, fungi and bacteria have been used to produce single cell proteins. Yeasts such as *Saccharomyces* or *Candida utilis* have been known to utilize mono- and disaccharides very efficiently. Special strains of yeasts (suitable for food industries) have been developed and they have been grown on molasses as substrates in large quantities for food and feed supplements. World production of such yeasts exceeds 300,000 t/year. Substances rich in starch can be converted to microbial biomass by pure organisms having enzymes (amylases) to hydrolyze starch into mono- or disaccharides. A few examples of organisms that are generally used belong to the genus *Aspergillus*—*A. oryzae*, *A. niger*, and *A. fumigatus*. A commercially developed process in Sweden for utilization of potato waste is known as the "Symba" process which makes use of two yeasts—*Endomycopsis filbuliger*, an amylase producing strain, and a *Candida*. Residues from agro-industrial activities consist of mixtures of several compounds, some soluble and others insoluble. Soluble substrates are easier to handle whereas the insoluble residue, which is usually lignocellulose, is generally recalcitrant to microbial attack. However, a pretreatment of such residues makes cellulose susceptible to enzymatic hydrolysis and facilitates the growth of a number of organisms. When the naturally occurring cellulose is pretreated to make it susceptible to biodegradation, there are several approaches to the production of microbial protein. A few of the approaches are shown schematically in Fig. 1. In addition, the lignocellulosic compounds may be hydrolyzed by acids completely to monosaccharides; the well-known industrial processes can then be used to produce microbial protein as yeasts. Several excellent reviews on the microbial conversion of lignocelluloses have been recently published (Tong and Cannell 1983; Phillips and Humphrey 1983). A few selected organisms that have applications in the production of single cell proteins are presented in Table 2.

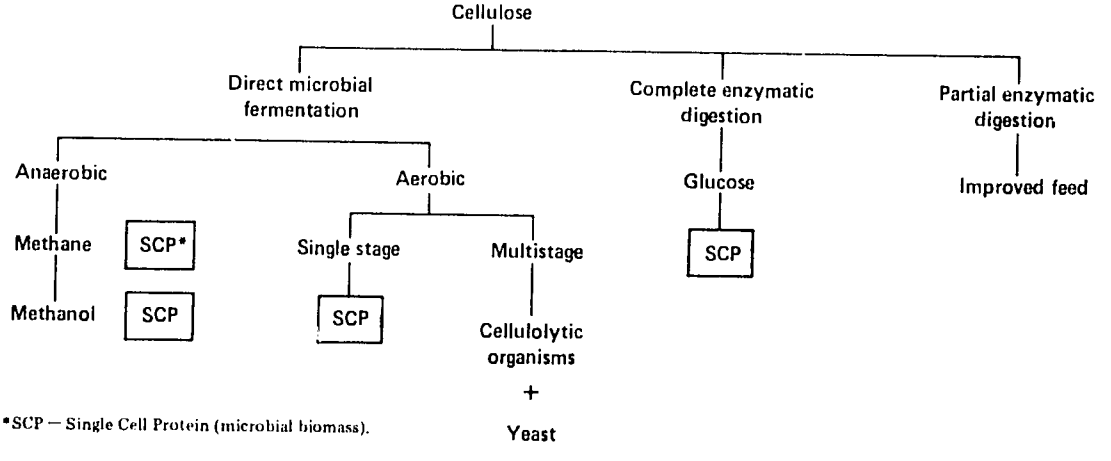

Fig. 1. Proposed methods for production of microbial protein from cellulose.

Table 2. A few selected cellulolytic microorganisms (from Srinivasan 1979).

Fungus	:	*Trichoderma (viride) reesii*
		Trichoderma lignorum
		Penicillium janthinellum
		Penicillium vericulosum
		Aspergillus terreus
		Aspergillus oryzae
		Chaetomium cellulolyticum
		Sporotrichum pulverulentum
Actinomycetes	:	*Actinomucor* sp.
		Streptomyces
		Thermomonospora
Bacteria	:	*Cellulomonas*
		Pseudomonas
		Celluvibrio
		Cytophaga

Pretreatment of Substrates

It is only the insoluble lignocellulosic residues that require rather extensive pretreatment, although raw materials such as molasses are clarified by adjustment of the pH and removal of deleterious ions before they are utilized as substrates for fermentation. It is essential to lower the lignin content of the residues, either by physical or by chemical pretreatments, to increase the rate of bioconversion. Lignin surrounding the cellulose forms a physical barrier for microbial or enzymatic attack. Pretreatments disrupt the lignin structure and increase the accessibility of cellulose to enzymatic hydrolysis and enhance the rate of biodegradation. The different methods of pretreatment are summarized in Table 3. Milling and radiation treatments have been extensively studied and found to decrease the particle size, decrease the degree of polymerization, increase the surface area, and thereby increase the susceptibility of cellulose to microbial attack. Steam explosion of lignocellulose has been developed as an efficient method for pretreatment by Saddler and his collaborators (Brownell and Saddler 1984). Treatments with sodium hydroxide or ammonia swell the fibers, solubilize lignin and hemicellulose and increase the digestibility of cellulose to microorganisms (Wilson and Pigden 1964). Several methods for pretreatment of agricultural residues for efficient enzymatic conversion have been experimentally evaluated using wheat straw as a model substrate (Fan et al. 1981). It was found that physical pretreatments alone were ineffective in enhancing the rate of hydrolysis of cellulose. "Caustic soda pre-treatment is a promising candidate for large-scale application because of its excellent capability to increase the hydrolysis rate and its low cost" (Fan et al. 1981). Our research activities centered around the use of nonwood plant residues such as sugarcane bagasse as raw material and we have used primarily alkali pretreatment in our studies on the production of microbial proteins.

Table 3. Pretreatment methods to increase the rate of biodegradation of cellulose.

Physical methods :	Photolysis and radiation		
	Ball milling (pot milling)		
	Hammer milling		
	Fiber explosion	:	Steam explosion
			Freeze explosion
Chemical methods :	Acid treatment	:	Sulfur dioxide
			Sulfuric acid
			Phosphoric acid
	Alkali treatment	:	Sodium hydroxide
			Ammonia
	Organic solvents for lignin removal	:	Ethylene diamine
			Ethanol
			Phenol

Fermentation

Submerged fermentations lend themselves to better optimization of the environmental conditions and thereby result in increased productivity. However, there have been several studies reported on the application of solid state fermentation to produce microbial biomass from feed-lot waste fibers and in crop residues such as wheat straw (Ulmer et al. 1981; Laukevics et al. 1984). Two examples of microbial biomass production will be reviewed here briefly: one from feed lot waste and another from corn stover, before a discussion of our investigations on the bioconversion of lignocellulose.

Ulmer et al. (1981) conducted a series of investigations to improve the nutritional value and digestibility of pretreated feed lot fiber waste by solid state fermentation. Initially, the waste contained 25 to 30% w/w dry matter. The fibers were subjected to steam treatment by keeping them at 170°C for 10 min. under pressurized steam. Then they were supplemented with ammonium sulfate and urea to adjust the C:N ratio (by weight) to 9:1 to obtain maximal protein production. The substrate was inoculated with a cellulolytic mold *Chaetomium cellulosum* and incubated at 37°C for seven days. The results of a typical experiment are presented in Table 4. It is important to note that aeration during fermentation decreased the cellulose content by 28% in untreated fibers and 38% in steam treated wastes.

A second example of a process of single cell protein production from waste biomass is the one developed by Moo-Young et al. (1979) as a submerged fermentation. The raw material was pretreated with hot water or dilute alkali (NaOH or NH_4OH), which enhanced the susceptibility of solids to attack by microorganisms by causing swelling and/or partial delignification. The solid substrate was introduced in the form of a slurry into the fermenter with the addition of necessary nutrient

Table 4. Solid state fermentation of feed lot waste fibers.

Treatment	% dry weight of the product		
	Crude protein	TCA protein	Cellulose
Untreated	7.9	6.8	33.8
Untreated and fermented	11.7	8.1	35.4
Untreated and fermented with aeration	14.3	10.6	24.5
Treated	7.8	5.9	32.2
Treated and fermented	14.5	8.6	38.6
Treated and fermented with aeration	20.0	10.9	20.1

supplements (N, P, K, etc.) derived from commercially available fertilizer blends. Fermentation was carried out by a new strain of *Chaetomium cellulolyticum* at a temperature of 37°C with adequate aeration. The pH of the medium was maintained at 5.5 with a pH controller. The authors claim that the organism had a specific growth rate of 0.24 h^{-1}, which is one of the highest known for cellulolytic fungi. The amino acid composition of the single cell protein is similar to any other single cell protein and the nutritional value of the product is comparable to fodder yeast.

We have been interested in the production of single cell protein from lignocellulose for the past several years, mainly from the point of developing a byproduct industry for sugar refineries. If fermentation processes have to be developed on native lignocellulose one has to deal with three different carbon substrates, namely short-chain pentose polymer, a complex aromatic phenyl propane polymer of lignin, and a large molecular weight, linear polymer of cellulose. In general, microbial growth on more than one substrate tends to lower the overall growth rate. Thus the productivity of the fermentation may in all probability be lower on mixed substrates with a single organism than on different components used singly (Eriksson and Larsson 1975). However, the productivity on mixed substrates may be increased with a system of several microbes whose populations can coexist commensally or neutrally (Thayer and David 1978). In early studies, we concentrated on the optimization of bacterial degradation of alkali-pretreated cellulose by *Cellulomonas*, an isolate from the sugarcane fields of the University Agricultural Experiment Station. Initially, the isolate was found to double its population two weeks after inoculation on a cellulose substrate. The experiments were designed to increase the productivity of biomass on cellulose in the following manner: (i) symbiotic growth of the organism with other cellobiose utilizing strains; (ii) mutation of the parent strain by chemical mutagenesis; (iii) optimization of the environmental conditions for the organism under continuous cultivation in order to promote maximum growth. These experiments led to a simple technique known as gradient feed method for growing microbial cultures with high cell densities (Srinivasan et al. 1977). The experimental results are summarized in Table 5.

Table 5. Production of biomass during growth of *Cellulomonas* on cellulose in a batch fermenter (Srinivasan 1979).

Microorganisms	Carbon source	Volume of cultures (liters)	Fermentation time (hours)	Biomass g/l D.W.*
Cellulomonas ATCC 21399	Cellulose powder	1	96	4.5
Cellulomonas + *Candida*	Cellulose powder	1	96	10.2
LC-10 (mutant)	Cellulose powder	5	36	15.0
LC-10	Bagasse	5	36	18.0

*D.W. — dry weight.

On the basis of the methodology during our studies with *Cellulomonas*, we extended our investigations on the production of biomass by a thermotolerant strain of *Aspergillus terreus*. The advantages of using fungi are: (i) they can be grown at low pH, thereby minimizing the problem of contamination; (ii) they can be harvested more economically from the fermenter by filtration because they are large.

Aspergillus terreus was grown in batch as well as continuous mode. A semi-continuous fermentation (draw and replace) technique, which may be readily adaptable to a labor-intensive and low investment technology, was also investigated. Results of the continuous mode of operation as well as a semi-continuous mode are presented in Tables 6 and 7.

Basic studies on the physiology of growth of microorganisms have led to the development of a rapid rate of bioconversion of cellulose under aerobic conditions. Optimization of nutrient environment is of paramount importance in the development of any successful fermentation as shown by the *A. terreus* system, which exhibits high cellulose utilization coupled with high productivity of protein due to rapid growth rates. At the present "state of the art" in lignocellulose conversion to proteins, submerged fermentation is the method of choice. Although several attempts have been made to develop solid state fermentation, there are a number of inherent disadvantages in the technique itself. Biomass generation by solid state fermentation is severely limited by the difficulty of regulating fermentation parameters such as moisture and aeration. A considerable portion of the substrate is spent on maintenance energy for the organisms in solid state fermentation, and the overall efficiency of conversion to protein is rather low. In spite of all these shortcomings, solid state fermentation has been thought attractive as a low level farm technology. However, it gives a false sense of economy. Village or low level technology would be better

Table 6. Continuous cultivation of *Aspergillus terreus* on cellulose (Miller and Srinivasan 1983).

Experiment	Dilution rate hours^{-1}	% utilization	% protein content in biomass
1	0.10	78.0	35.3
2	0.11	83.3	32.2
3	0.14	82.0	35.0
4	0.14	84.2	34.7

Table 7. Cellulose utilization and protein content of the dried product during fermentation by *Aspergillus terreus* in a semi-continuous mode (Miller and Srinivasan 1983).

Experiment	Fermentation duration (hours)	% cellulose consumed	% protein in biomass
1	30	84.3	30.0
2	24	83.7	32.0
3	24	83.5	32.5

Fermentations were carried out with 5% carbon source. In Experiments 2 and 3, 10% of the biomass was used as the inoculum from the previous experiment.

served by adopting the basic design of the process developed by researchers at Tate and Lyle Research for the production of protein-rich product by submerged fermentation for incorporation into aquaculture feeds. The plant consists of a substrate preparation vessel, a polyethylene fermenter of 10,000 liter capacity, a filter and drier (Righelato 1974). The original plant was designed for the use of soluble substrates. Nevertheless, with modification of the plant to accommodate its use for insoluble substrates and introduction of the technique of gradient feed for the growth of organisms, bioconversion of cellulose to microbial protein may be developed as an economical process for the production of supplementary feed protein for aquaculture.

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Discussion

EDWARDS: Rice straw is such a widespread resource. Do you see any scope for improving its quality as a feed for livestock or fish by any of the methods you have described, particularly for use on small-scale farms?

SRINIVASAN: I would say that this would not be economical for separate small farms. If, however, farmers could use together a plant of 10,000 to 40,000 liters capacity then this could be possible. If you are going to use about 20 g/liter you must have available 20 t/day; that is about 80 t of raw material.

PRUDER: We have a process which we call semi-continuous harvesting, that is we harvest daily about 90% of the algae produced in a reactor. We find that our cultures tend to lose vitality after 7-8 days even when we maintain good nutrient inputs. We cannot get beyond 14 days without having to start up the reactor again. Have you had similar experiences?

SRINIVASAN: No, most cultures can go on indefinitely. Unfortunately, however, they usually do not, because of contamination. If you have near-optimal nutrient media and a 10% inoculum you can keep a culture going for a long time even under nonsterile conditions. However after 3 to 4 transfers you do get contaminants appearing, particularly flavobacteria. Interestingly, these flavobacteria may not grow at the pH of the medium (pH 5.0 in our case), but can grow in close proximity to the cultured organisms. There is probably a pH gradient between the cultured organism and the medium. Therefore, contamination is the problem, not 'vitality' of the cultured organism.

PRUDER: From what you say, our problems seem to be due to contamination.

WOHLFARTH: Following up Peter Edwards' question on the scale of economically viable units. In Szechuan Province, there are said to be 5 million domestic scale biogas units (Shian et al 1979)*. These are often run by a single family and provide gas for light and cooking. The inputs are human wastes and pig manure. This seems very efficient since little or no transportation is involved.

SRINIVASAN: I am familiar with these. Their rate of production would be considered too low for use in a developed country. They suit the family circumstances in their locality, rather like growing garden vegetables in America. I prefer to conceptualize larger centralized fermentor units for feed production. If good quality feeds are produced to satisfy a demand, the costs of installation and operation are not prohibitive.

PULLIN: Your original objective was to manufacture a feed to be competitive with soy protein?

SRINIVASAN: Yes.

PULLIN: How successful have you been?

SRINIVASAN: We have reached a competitive stage. We can now grow the organism and with a 100,000 liter fermentor as an auxiliary unit to a sugar cane refinery we can produce fungal biomass at $180 to $200/t. It is 30-35% protein. We had achieved this 4-5 years ago, but were faced with the problems of the costs of disposing of the alkaline effluent. Hence, we have now developed an anaerobic process and now we can run an anaerobic fermentation on hemicellulose with 65% methane coming out every four hours.

PIEDRAHITA: Were the figures you gave primarily for pure cellulose fermentation?

SRINIVASAN: For bagasse, too.

PIEDRAHITA: How much pretreatment is needed for a straw or bagasse?

SRINIVASAN: First, it must be comminuted to a small size, then treated with alkali: 0.5 N sodium hydroxide. Treatment must be at over 95°C. Below 90°C, it doesn't work so well. Treatment should be for about 1 hour.

SCHROEDER: ICI, working with microbial proteins, found some acceptability problems with certain target animals. Have you found similar problems with fungal protein?

SRINIVASAN: We have tested our fungal protein as 10% substitute for poultry rations. There were no toxic effects. The fungi are rich in vitamins, so vitamin addition to the ration is not needed. We also tested it on tadpoles. That was my introduction to aquaculture!

EDWARDS: Dr. Tim Flegel is working in Thailand using water hyacinth as a substrate. He adds urea. He has isolated a whole range of cellulose and lignin-splitting fungi. He claims to be able to increase the protein content of the substrate quite significantly by this relatively simple technology, without pretreatment.

SRINIVASAN: I have strong reservations about this type of technology. Many people have tried this kind of solid state fermentation. It's just like composting. The problem here can be mycotoxins. These are produced by fungi, particularly at sporulation. They are not such a problem if the fungi remain in the phase of vegetative multiplication. Moreoever, such systems allow the organisms to grow for a long time. This produces a lot of protein initially, but then you get species differentiation of the fungi and a lot of protein is turned over (degraded) by these changes. So, your initial 30-35% protein content can go down to 10-12%.

EDWARDS: But couldn't you stop the process by drying?

SRINIVASAN: No, because the organisms will differentiate and form spores when you do this. This is a feature of solid state fermentation and is why I have avoided it.

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*Shian, S.T., M.L. Chang, Y.T. Ye and W. Chang. 1979. The construction of simple biogas digesters in the province of Szechwan, China. Agr. Wastes 1: 247-258.

Composition and Nutritional Value of Detritus

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Abstract

Beginning as soon as plants senesce or die, two different pathways are involved in their progressive decomposition and modification. Soluble components lost from the plants are acted upon by various physical, chemical and microbial agents, many of which convert dissolved organic matter back into particles. Insoluble plant tissues are progressively fragmented by the interaction of microorganisms, invertebrates and mechnical abrasion. In the course of this detritus processing, the more labile components are lost or bound into recalcitrant organic complexes, while the more refractory organic components accumulate. In consequence, detritus contains less digestible protein (amino acids) and digestible energy than other foods consumed by aquatic animals. Animals that feed successfully as detritivores possess various behavioral and physiological adaptations that allow them to select the most nutritious detritus and efficiently digest and assimilate its nutrients.

Introduction

Detritus is dead organic matter. Although dead animals may make some small contribution, most detritus appears to be derived from plants (Parsons and Tinsley 1975). Individual detritus particles range in size from stumps and tree trunks that are measured in meters, to ultrafine particles with maximum dimensions of < 0.5 μm. Detritus is found in all aquatic habitats. It accumulates as conspicuous organic debris, makes up much of lake and stream sediments and is found as thin layers on the surfaces of stones and living plants. In addition, a large portion of the particulate matter suspended in both standing and flowing waters is detritus. Ecologists now believe that particulate detritus is the food resource that supports (directly or indirectly) well over half the animal production in most ecosystems. Although

herbivores in pelagic waters and grasslands are important exceptions, most animals wait until plants die and partly decompose before they use this material as food (Wetzel 1975).

During the decomposition process, plant detritus is acted upon by physical, chemical and biological agents that continuously alter its composition and thus its nutritional value. The effects of decomposition processes on nutritional value are complex and depend on the type of detritus and the digestive abilities of the detritivore. Early in the process, the nutritional value may be increased by the decomposition of compounds that are the remnants of plant defenses against herbivory. Later, selective removal of the more labile organic fraction may render the detritus less nutritional to detritivores that lack special adaptations for digestion of the refractory residuum. On a biological time scale, the ultimate product of decomposition must have extremely limited nutritional value because detritus accumulates as sedimentary layers tens of meters deep in lakes and oceans.

In this paper, the pathways of detritus processing as a context for the subsequent discussion of detritus chemical composition are reviewed. Against this background is also a review of current understanding of variables that affect the nutritional value of detritus and adaptations that detritivores have for using detritus as their principal food source.

Pathways of Detritus Processing

Detritus is processed along two pathways that diverge as soon as a unit of plant tissue begins to senesce and die (Fig. 1). Structural compounds such as cellulose and lignin are left behind in particulate form while soluble proteins and carbohydrates rapidly dissolve (Suberkropp et al. 1976; Goldshaulk and Wetzel 1978; Whyte et al. 1981). Insoluble particles are gradually broken up to produce progressively smaller

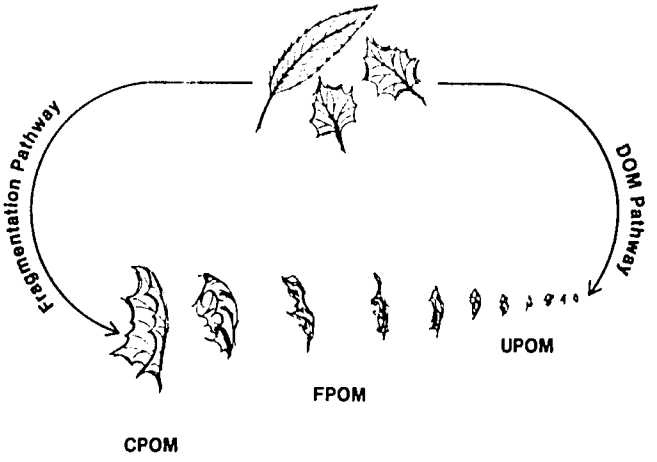

Fig. 1. Diagrammatic representation of the two pathways of detritus processing, and their relationship to particle size. CPOM = coarse particulate organic matter; FPOM = fine particulate organic matter; UPOM = ultrafine particulate organic matter; DOM = dissolved organic matter.

fragments (fragmentation pathway). Soluble organic matter lost from autumn shed leaves in temperate streams ranges from 5 to 30% of total organic weight after only 24 hours (Petersen and Cummins 1974; Lush and Hynes 1978). Dissolved organic matter is acted upon by a variety of physical and chemical agents and microorganisms that convert it to a particulate form (dissolved organic matter pathway) (Dahm 1981; see Wooton 1984 for review). Thus, both pathways of detritus processing produce particles that are available as food for detritivores.

The pathway in which insoluble plant structural compounds are gradually fragmented to make progressively smaller particles is the more studied. Most of this work concerns decomposition of deciduous leaves in low-order streams, and mangrove leaves and saltmarsh grasses in coastal/marine habitats. Similar trends are seen in each habitat. Dissolution of labile components begins immediately and is soon followed by microbial colonization. In aerobic environments, aquatic hyphomycetes are dominant during the early stages of decomposition and may reach maximum biomass and activity in about 20 days (Arsuffi and Suberkropp 1984). The fungal hyphae invade the structure of the plant matter and fungal exoenzymes break down the structural cellulose. This dramatically weakens the plant tissue and thus renders it more friable. Subsequent fragmentation may be due to the mechanical effects of turbulent water movement, abrasion by other particulate matter and the feeding of "shredder" invertebrates that specialize in feeding on large particle size plant detritus (Cummins and Klug 1979). The microorganisms on fragments are typically bacteria. Some types of bacteria may utilize some of the remaining particle, but others may depend on dissolved organic matter as their major nutrient/energy source and use the particle solely as a surface for attachement (see Kirchman and Ducklow, this vol.).

The pathway in which particulate detritus is formed from dissolved organic matter is less understood. Researchers have identified many different agents that convert dissolved organic matter to particulate organic matter. Bacteria appear to be some of the most important. In addition to production of new bacterial cells, bacteria use dissolved organic matter to produce an extracellular organic matrix (Paerl 1974, 1978; Hobbie and Lee 1980; Costerton et al. 1981). The mass of this organic matrix can exceed the mass of bacteria many fold (Parsons and Dugan 1971; Paerl 1978; Rice and Hanson 1984). A host of physical and chemical processes may also convert dissolved organic matter to particles. Adsorption to both mineral and other organic particles, formation at the air-water interface, and precipitation by multivalent ions, pH shifts and salinity changes have all been suggested as at least locally important in production of particulate detritus (reviews in Dahm 1981; Bowen 1984c; Wooton 1984). Although most workers seem to have concluded that bacteria play the dominant role in processing dissolved organic matter, abiotic formation of particles can be so rapid that bacteria have little opportunity to utilize this material (Jensen and Sondergaard 1982). The relative importance of abiotic agents in processing dissolved organic matter probably differs from habitat to habitat, but little is known about this at present.

Samples of detritus collected from natural aquatic habitats typically contain particles from both pathways (Fig. 2). The larger size classes (roughly 2,500 to

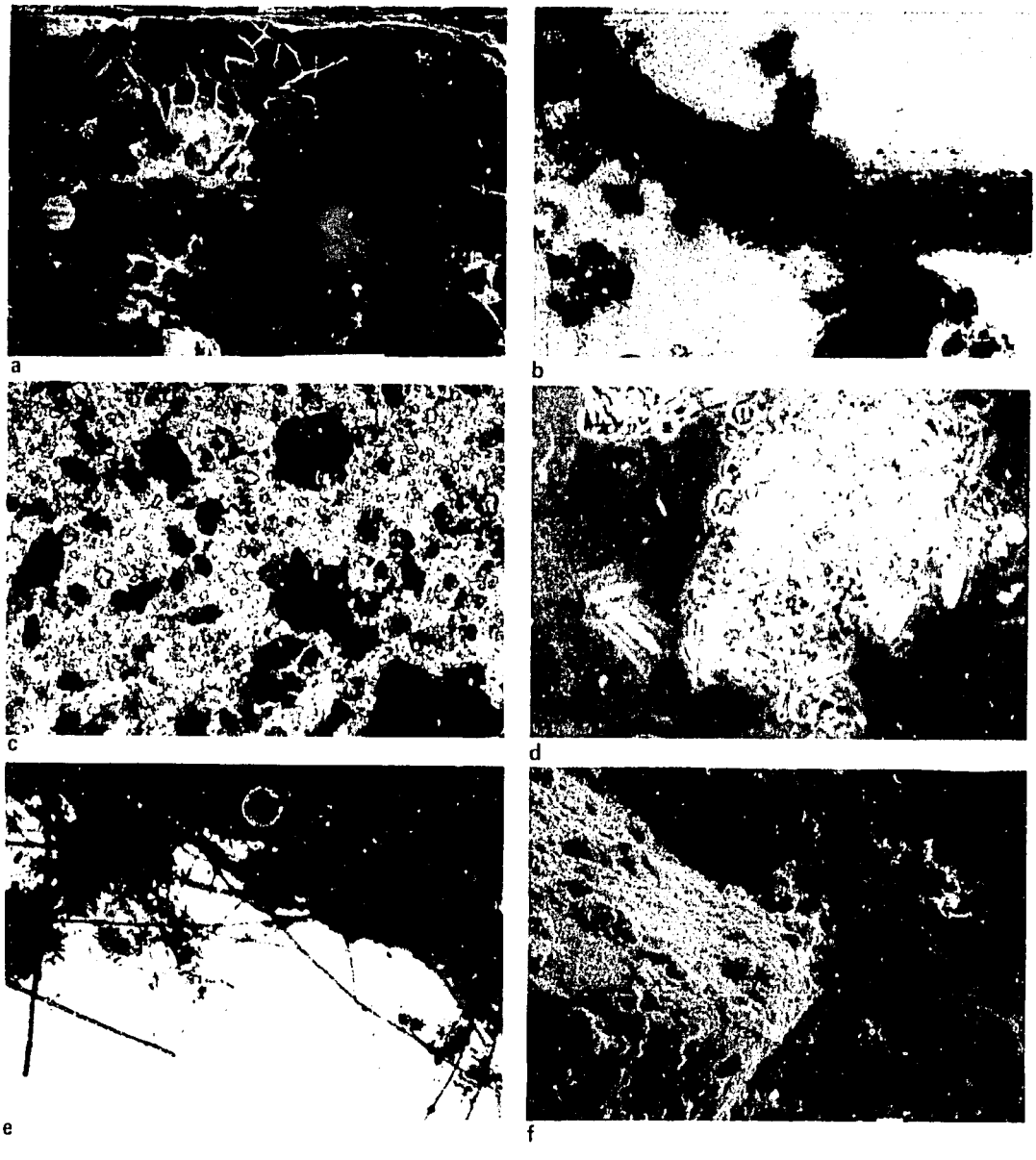

Fig. 2. Light and scanning electron micrographs of detritus. a. Morphous detritus particles separated from finer particles using a 100 μm sieve. These are clearly fragments of vascular plants. SEM, Portage Lake, Houghton Co., Michigan, USA. b. Vascular plant detritus coated with amorphous detritus. Light micrograph (160x), Traprock River, Houghton Co., Michigan, USA. c. Amorphous detritus and sand grains from the pyloric stomach of the detritivorous fish *Prochilodus platensis*. Light micrograph (160x), Riachuelo backwater, Corrientes, Argentina. d. Detrital aggregate: the aggregated mixture of amorphous detritus, algae and bacteria. Light micrograph (160x), Portage Lake, Houghton Co., Michigan, USA. e. Detrital aggregate as it commonly occurs enmeshed in filamentous algae growing on the surface of a vascular aquatic plant. Light micrograph (160x), Lake Valencia, Venezuela. f. Detrital aggregate coating the surface of a sand grain from the pyloric stomach of *Prochilodus platensis*. SEM, Riachuelo backwater, Corrientes, Argentina.

250 μm) are dominated by fragments of insoluble plant tissues, whereas smaller size classes (250 to 0.5 μm) are usually amorphous and appear to be derived from dissolved organic matter (Bowen 1984c). Extracellular organic matter produced by both bacteria and algae adhere to other particles and tend to bind particles together in heterogeneous aggregates of algae, bacteria, detritus and mineral particles (Seki 1972; Bowen 1978; Paerl 1978). On stable substrata such as stones and macrophytes, this process results in organic coatings that are several millimeters thick (Allanson 1973; Lock et al. 1984). Detritus in these coatings is almost exclusively of the amorphous type. Elsewhere, small amorphous detritus particles are found attached to larger plant fragments.

Water movements and the effects of substratum result in a nonuniform distribution of different detritus types. In low order streams, stones, roots and wood in the channel retain large particles such as leaves and twigs whereas smaller particles are transported downstream (Wallace et al. 1982). Gradients of flow velocity and turbulence further separate particles in flowing waters according to size and density (Hynes 1970). In standing waters, the littoral zone is a site where much detritus enters the lake ecosystem as allochthonus or autochthonous vascular plant debris, and as fine particles formed from dissolved organic matter in interflow. Because resuspension by wave action becomes less frequent with increasing depth, wave action tends to transport littoral detritus down-slope (Hargrave and Kamp Nielsen 1977). In littoral zones with relatively steep slopes, this process results in a gradient of particles separated according to age: the mean age of particles increases with increasing depth (Bowen, unpublished data). Thus, interactions among the processes of detritus formation, detritus aggregation and detritus transport result in a complex distribution of heterogeneous detritus mixtures in the natural environment.

Quantities of Microorganisms Associated with Detritus

Detritus is rarely found without attached or embedded microorganisms. Before discussing the chemical composition of detritus, it may be useful to consider what contribution the microorganisms make to the organic matter of microorganism-detritus aggregates. Although there are several problems concerning the accuracy of various techniques, this problem has been studied from enough different perspectives that two consistent relationships are apparent. Firstly, fungal biomass is greatest during the early stages of coarse particle (> 1 mm) processing in the fragmentation pathway. For example, Lee et al. (1980) report that fungi colonizing coarse particles from *Spartina alterniflora* leaves increased in dry biomass to a maximum of 2.5% of the organic dry weight after two months, and then declined to less than 0.3% after a total of seven months. Bacteria were much less important in this material comprising about 1% of organic dry weight. Nearly identical results were obtained in independent studies of *Spartina alterniflora* by Marinucci et al. (1983) and Valiela (1984). In contrast, bacteria predominate on fine particles (< 1 mm) and tend to be most abundant on the smallest particles (Wallace et al. 1982). Secondly, microorganisms make up a relatively small proportion of the organic matter in detrital aggre-

gates (Linley and Newell 1984). Most estimates place bacterial contributions to fine particle weight at less than 1% (Table 1). Similarly, algae made up of less than 5% of organic matter in detrital aggregates on living macrophytes (Bowen 1979b), and less than 1% of organic matter in benthic detrital aggregates (Bowen 1979a). Thus, the chemical composition of detritus is due, in large part, to the detritus itself rather than the associated microbial biomass.

Table 1. Recent literature values for microbial biomass associated with detritus from a variety of aquatic and marine habitats.

Biomass estimated	Sample source	Estimate as % organic mass	Method	Authority
Total microbial	Stream, suspended POM[b] – Georgia	0.9[a]	ATP	Wallace et al. (1982)
Total microbial	Stream, benthic POM – Michigan	0.03[a]	ATP	Ward and Cummins (1979)
Total microbial	Stream, leaf packs – Michigan	<5.0[a]	ATP	Suberkropp et al. (1976)
Total microbial (including meiofauna)	Salt marsh sediment – North Carolina	<4.0	ATP	Rublee (1982)
Bacterial	– North Carolina	<0.6	Direct counts	Rublee (1982)
Algal	– North Carolina	<0.4	Direct counts	Rublee (1982)
Bacterial	Marine sediment	0.7	Direct counts	Kepkay et al. (1979)
Bacterial	Suspended river POM	<0.5	Direct counts	Baker and Bradnam (1976)

[a]Calculated from authors ATP values (corrected for extraction efficiency) based on 286 mg biomass per mg ATP (Holm-Hanson et al. 1966).
[b]POM = particulate organic matter.

Nutrients in Detritus—Amino Acids and Energy

The chemical composition of detritus has been examined to some degree on several scales, ranging from specific isotopes of a given element (e.g., ^{12}C and ^{13}C), to colloids that make up the finest particles. A few of these studies have involved a search for very specific compounds that can be used as tracers in biogeochemical cycles. In most cases, the compounds studied have no identifiable connection to detritivore nutrition and thus these studies are not considered here. The

present review will focus on those molecules that supply detritivores with amino acids and energy—the macronutrients that appear to limit detritivore growth (see "Nutritional Value" below).

Amino Acids and Other Forms of Organic Nitrogen in Detritus

Qualitative Aspects

Because nitrogen has a widespread importance as a limiting nutrient for the growth of both plants and animals, nitrogen dynamics are the most studied aspect of detritus processing. Plant amino acids are believed to be the original source of detrital nitrogen (de la Cruz and Poe 1975). Nearly all the organic nitrogen in living plants is in the form of amino acids (Parsons and Tinsley 1975). Other nitrogen-containing compounds such as organic bases and amino sugars are functionally important but make up a small proportion of the total organic nitrogen present in plants. In the process of decomposition, plant proteins are variously modified and react with other compounds in the organic milieu, especially with phenolic compounds (Rice and Hanson 1984). As a result, it is the gradual modification of parent plant proteins that determines the chemical form(s) taken by organic nitrogen in detritus. As plant detritus is processed via the fragmentation pathway, much of the original protein that is not lost to solution is gradually degraded to smaller amino acid polymers (polypeptides), individual amino acids, and nonamino acid organic nitrogen (Odum et al. 1979). Nonamino acid nitrogen is frequently greater than 30% of the total nitrogen present (Godshalk and Wetzel 1978). These decomposition products form relatively refractory complexes with lignin, humin and other lignin-like compounds formed in diagenesis (Suberkropp et al. 1976; Odum et al. 1979; Rice 1982). Because they are refractory, these nitrogen-rich complexes accumulate in detritus relative to other more labile components with the result that the *percentage* organic nitrogen increases over time (review in Rice 1982).

The fate of proteins and amino acids that dissolve into solution is less well known. Results of studies reported to date suggest competition for dissolved amino acids between the process of microbial mineralization, and processes leading to formation of particles. These compounds undergo complex chemical reactions similar to those described for particulate amino acids and tend to accumulate in large molecular weight "heteropolycondensates" (Degens 1970). Freshly extracted dissolved organic matter and dissolved organic nitrogen can be mineralized by microorganisms (Dahm 1981), but somewhat older, complexed dissolved organic matter and dissolved organic nitrogen are relatively refractory (Wetzel and Manny 1972). Thus, amino acids make up a very small part of the total dissolved organic matter (e.g., 0.7%, Bott et al. 1984), but make up a relatively large fraction of the complexed collodial fraction (12 to 22%, Sigleo et al. 1983; 4 to 13%, Means and Wijayaratne 1984). Experiments conducted in streams and anaerobic marine sediments suggest that conversion to particulate form rather than mineralization by microorganisms is the

more common fate of dissolved amino acids (Lush and Hynes 1978; Rosenfeld 1979), but more study of these two competing processes is necessary before generalizations can be made. In addition to their roles as mineralizers, microorganisms also contribute to the formation of particles from dissolved amino acids. The *absolute* amount of nitrogen frequently increases during decomposition via the fragmentation pathway. This additional nitrogen must be derived from dissolved nitrogenous compounds by chemical condensation with particulate detritus (Rice 1982), or from deposition of nitrogen-rich exopolymers that bacteria and fungi produce from dissolved organic matter (Fell et al. 1979; Rice and Hanson 1984). Thus, the effects of dissolution, decomposition and condensation result in a diverse array of nitrogen-rich organic compounds in particulate detritus.

Quantitative Aspects

The concentrations of protein or total amino acids in different types of detritus are given in Table 2. This table contains data only from studies in which protein or total amino acids were determined directly. Many protein values reported for detritus are based on the assumption that all nitrogen in detritus is amino acid nitrogen, and thus those estimates cannot be included here. In addition, the many estimates of protein or amino acid concentrations in suspended organic matter have not been included because it is not clear how much of the material is detritus and how much is living plankton.

The available data indicate that detritus contains less protein and less amino acid than other foods. The 17 types of detritus in Table 2 average about 10% amino acid or protein as a percentage of ash free dry weight. Comparable values reported for other materials used as food by aquatic consumers include 17% for 32 species of aquatic vascular plants, 23% for 7 species of microalgae, 27% for phytoplankton, 50% for zooplankton and 61% for 12 aquatic macroinvertebrate species (Boyd 1968; Mayzaud and Martin 1975; Yurkowski and Tabachek 1979). The low concentration of amino acids in detritus is due to the more rapid loss of amino acids relative to other organic compounds from detrital particles during the early stages of processing (Tenore et al. 1984; Wakeham et al. 1984). Among samples of detritus formed in the fragmentation pathway, the concentration of amino acid in the parent plant matter appears to affect directly the amino acid concentration of the subsequent detritus: protein-rich plants make protein-rich detritus (Rice 1982; Rice and Hanson 1984).

The specific amino acid compositions of various types of detritus and living plants and animals are compared in Table 3. As concluded by several other authors, comparisons across broad taxa show that the specific amino acid composition of diverse plants, the animals that feed on them (directly or indirectly) and detritus derived from them are remarkably uniform (Boyd 1970, 1973; Cruz and Poe 1975; Tenore et al. 1984).

Table 2. Concentrations of amino acids and protein in detritus as percentage ash-free dry weight.

Detritus type	Location	Amino acids	Protein	Authority
Marine sediment (top 1 cm)	Long Island Sound, USA	17.0[a,b]		Rosenfeld (1979)
Marine sediment (top 1 cm)	Florida Bay, Florida, USA	12.1[a,b]		
Plant fragments aged/50 days	Savannah, Georgia, USA			Rice (1982)
Gracilaria foliifera			18.1	
Spatoglossum schroederi			24.2	
Thalassia testudinum			11.5	
Spartina alterniflora			2.9	
Rhizophora mangle			7.7	
Freshwater epilithic detritus (6 samples)	England		0-8.6%	Calow (1975)
Freshwater sediment (39 samples)	L. Sibaya, South Africa		5.9% (1.8-14.2)	Bowen (1979a)
Periphytic detrital aggregate (45 samples)	Lake Valencia, Venezuela		2.6% (0.5-5.8)	Bowen (1979b)
(30 samples)		14.6% (6.9-23.6)		Bowen (1980)
Deciduous leaves from Riparian trees aged 1-180 + days	Switzerland		6.3% (0-15)	Barlocher (1983)
Benthic detritus (total of 34 samples from 3 lakes)	Houghton County, Michigan, USA	6.9 (2.6-11.3)		Bowen (unpublished data)
Benthic detritus from 5 streams	Houghton County, Michigan, USA	8.6 (5.5-12.8)		Bowen (unpublished data)
Detritus formed *in situ* from salt marsh plants	Mississippi, USA			de la Cruz and Poe (1975)
Juncus roemerianus		9.2		
Spartina cynosuroides		4.8		
Scirpus americanus		6.0		
Distichlis spicata		7.2		

Assumptions: [a]Amino acids are 16% N by weight, [b]Organic matter is 50% by weight, [c]Leaf dry weight 80% ash-free dry weight.

Energy in Detritus

The energy density of biological materials is frequently reported using one of two expressions: energy units per total sample weight (fresh weight or dry weight), and energy units per ash-free dry weight (AFDW). The ash-free dry weight basis is of interest because it provides some insight into the basic biochemical makeup of the sample. On average, lipids contain about 35.7 kJ/g AFDW, proteins contain about 23.3 kJ/g AFDW and carbohydrates contain about 16.9 kJ/g AFDW (Brett and Groves 1979). Thus, samples with high energy densities, say greater than 24 kJ/g

Table 3. Concentrations of specific amino acids (mg/100 mg total amino acid) in detritus, plants and animals.

	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
Periphytic detritus L. Valencie, Venezuela (Bowen 1980)	14.5	5.8	6.2	15.9	5.6	7.6	8.9	4.2	1.5	3.2	7.2	3.1	2.7	1.7	5.4	6.9
Benthic detritus Portage L., MI, USA (Bowen, unpublished data)	16.7	7.4	6.0	16.5	5.2	8.9	8.5	5.1	1.6	3.3	5.9	2.0	2.1	1.3	5.5	4.1
Estuarine colloids	11.6	7.3	6.4	11.4	3.0	7.9	9.3	6.3	1.6	4.5	7.9	3.6	5.4	4.3	4.6	5.0
Estuarine seston (Sigleo et al. 1983)	10.7	4.9	4.4	12.2	2.7	6.3	7.2	5.5	2.1	4.4	7.7	2.6	5.0	3.0	6.1	5.9
Salt marsh plants	15.6	4.9	5.1	14.3	9.8	4.8	6.0	6.2	1.9	4.3	7.3	2.7	7.8	2.3	5.4	4.9
Salt marsh detritus (Cruz and Poe 1975)	12.1	6.7	5.9	12.3	5.9	7.9	7.8	6.7	2.2	4.8	8.2	2.0	5.0	1.6	4.5	4.9
Freshwater algae, 13 sp. (Boyd 1973)	12.2	5.0	5.0	13.3	4.4	6.1	8.7	5.8	1.8	4.5	8.6	3.8	4.9	2.1	7.0	6.2
Mean of 16 freshwater invert. sp. (Yurkowski and Tubachek 1979)	10.5	5.4	5.1	14.3	5.2	5.7	6.8	5.8	1.6	4.5	7.7	5.1	4.5	3.4	7.4	6.8
Aquatic vascular plants, 11 sp. (Boyd 1970)	13.1	5.2	5.4	12.2	5.1	6.4	6.5	6.0	1.4	5.1	9.8	3.9	5.7	2.3	5.5	6.1
Marine invertebrates, 6 sp. (Phillips 1984)	—	5.2	—	—	—	—	—	5.4	2.5	4.6	7.3	—	5.1	2.4	6.4	7.0

AFDW, are likely to contain a large proportion of lipid. Samples with energy densities below 17.5 kJ/g AFDW usually contain significant quantities of plant structural materials such as cellulose and lignin. Energy densities expressed as kJ/g total weight are of interest because they show the weight of material a consumer must handle in order to acquire a given quantity of energy.

Energy densities for detritus and other organic materials used as food by aquatic consumers are listed in Table 4. In units of kJ/AFDW, the energy density of detritus is slightly less than that of living aquatic plants. Most values for detritus are less than the average energy density of plant structural compounds, implying that decomposition processes have released some of the original energy. On a kJ/g dry weight basis, vascular plant fragments have values similar to those reported for plants, but fine

Table 4. Energy density measured for detritus and comparable materials used as food by aquatic consumers.

Material analyzed	Number of observations	Mean kJ 1 g dry wt.	Mean kJ 1 g AFDW*	Authority
Benthic detritus Lake Sibaya, S. Africa	39	7.3 (4.0-9.6)	17.0 (9.2-22.3)	Bowen (1979a)
Benthic detritus 44 Lakes, Poland	44		(5.0-28.4)	Rybak (1969)
Epilithic detritus One Lake, England	6		(14.3-19.7)	Calow (1975)
Benthic detritus Gratiot Lake, MI, USA	14	5.2 (3.8-6.1)	16.8 (12.2-19.8)	Bowen (unpublished data)
Spartina detritus	9	14.8	16.5	Tenore et al. (1984)
Gracilaria detritus	9	14.4	15.4	Tenore et al. (1984)
Aquatic vascular plants 32 sp.	32	15.5 (10.2-17.7)	17.8 (16.3-19.8)	Boyd (1968)
Benthic detritus Lake sediments	20	9.2	18.4	Cummins and Wuycheck (1971)
Marine macroalgae 70 species	74	12.7 (2.7-18.8)	18.6 (13.5-22.4)	Paine and Vadas (1969)
Aquatic algae	93	13.5	19.1	Cummins and Wuycheck (1971)
Aquatic invertebrates	142	17.4	22.5	Cummins and Wuycheck (1971)
Fish	13	20.9	21.8	Cummins and Wuycheck (1971)

*AFDW = Ash-free dry weight.

benthic detritus that is mixed with inorganic sediment has a much lower energy density. Plants use carbohydrates for both structural and storage functions, whereas animals use energy rich proteins and lipids for these purposes. Thus, energy densities reported for aquatic invertebrates and fishes are much higher than for plants and detritus.

The classes of compounds in detritus that contain energy change during processing. Concentrations of proteins, lipids and soluble carbohydrates decrease, while concentrations of lignocellulose and refractory products of diagenesis increase (Suberkropp et al. 1976; Bowen 1984c). This appears to apply to detritus formed by both pathways. In consequence, most detritus is significantly less digestible than other foods (Fig. 3). Even though the energy density of detritus differs little from that of the parent material, availability of the energy to detritivores changes significantly during processing (Tenore et al. 1984).

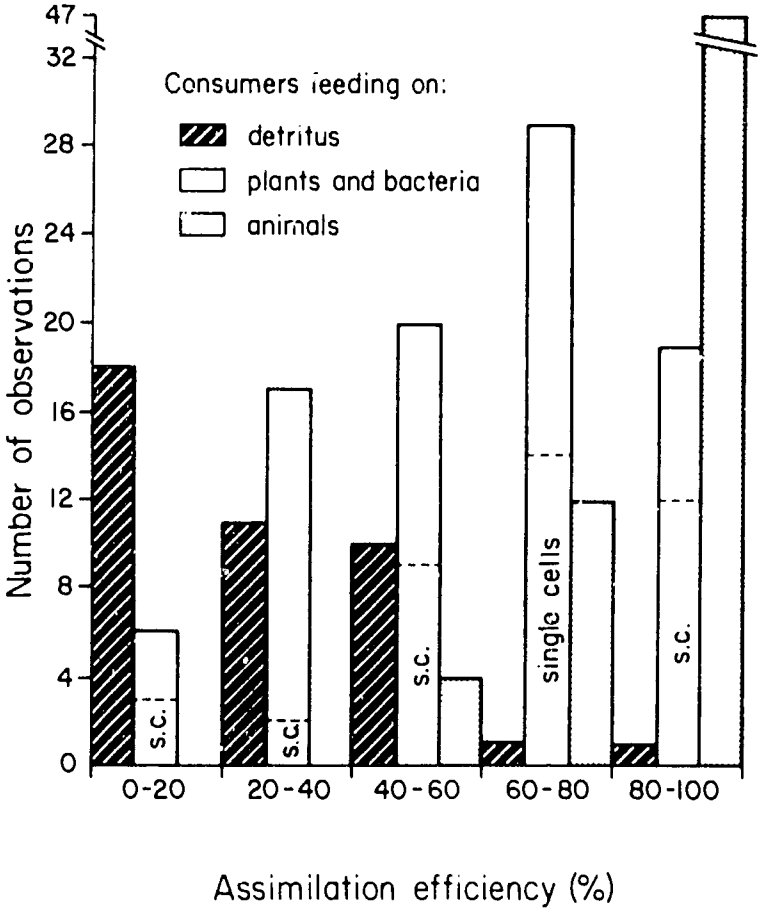

Fig. 3. Frequency distribution of assimilation efficiencies reported for animals feeding on diets of animal prey, plant matter and detritus compiled from the recent literature. Herbivores that feed on single-celled algae, bacteria or yeasts are grouped in the lower portion of the open bars. From Valiela (1984) with permission from Springer-Verlag, New York.

Nutritional Value of Detritus

Definition of Nutritional Value

The measure of general nutritional value used here will be the growth rate that food is able to support when available *ad libitum*. Growth rate is a good approximation of the contribution made by diet to adaptation fitness (*sensu* Calow and Townsend 1981; Werner and Hall 1976), and thus we might reasonably expect natural selection to favor feeding behavior that tends to increase the nutritional value of the diet. This provides a firm ecological basis for interpretation of selective feeding by detritivores. In addition, growth rate is immediately relevant to the goals of aquaculturists.

The effect of diet on the growth rate of a detritivore depends on the quantity and quality of nutrients obtained per unit time. This, in turn, depends (1) on the form and concentration of nutrients in the detritus, and (2) on the abilities of the detritivore for handling, digestion and assimilation of those nutrients. Thus, consequences of both nutrient content and detritivore trophic abilities are discussed below.

General Concepts of Detritus Nutritional Value

General concepts of detritus nutritional value have undergone considerable development since this question first became of interest to ecologists some 50 years ago (Baier 1935). Three distinct concepts have emerged, and all three are potentially useful in guiding development of detritus food chains in aquaculture. These concepts have alternatively focused on microorganisms, energy and amino acids as the factors determining detritus nutritional value.

Some investigators concluded detritus serves primarily as a carrier for attached microorganisms, and that these microorganisms are the nutritional resource supporting detritivores (Newell 1965; Ward and Cummins 1979). According to this perspective, the detritus itself is considered largely invulnerable to animal digestive systems, but the microbes are efficiently digested and provide a nutrient rich diet. Thus detritus nutritional value is proportional to the attached microorganism biomass. Application of this concept must be limited to situations where detritivores consume detritus exceptionally rich in attached microorganisms. The low biomass of microorganisms in most natural detritus was discussed above. Recently, researchers have concluded that the microorganism biomass consumed by detritivores accounts for less than 10% of the detritivore's growth and thus microorganisms are not the principal food resource utilized (Baker and Bradnam 1976; Cummins and Klug 1979; Rublee 1982; Bowen et al. 1984; Findlay et al. 1984). In aquaculture, however, it may well be feasible to manipulate conditions such that microbial biomass or the sum of microbial biomass and microbial excudates represents a major nutritional resource (Schroeder 1983; Ahlgren and Bowen, unpublished data). In this capacity, microorganisms may be considered sources of energy and amino acids within the framework of other nutritional value concepts discussed below.

A second concept of detritus nutritional value has emerged from studies of detritivores that feed on sediment (Odum 1968; Mattingly et al. 1981; Taghon 1982; Miller et al. 1984; Taghon and Jumars 1984). In addition to organic detritus, sediment contains 80 to 99% inorganic matter in the form of clays, mineral grains, carbonates, etc., which are expected to make no direct contribution to detritivores' nutrition. Because the rate at which a detritivore can pass food through its gut is limited (Brett 1971), inorganic matter in sediment reduces the consumers' energy assimilation rate and subsequent growth (Mattingly et al. 1981). Thus, the nutritional value of sediment may be expressed in terms of kJ digestible energy/g, and benefit to the consumer may be expressed as an energy assimilation rate (kJ assimilated/g consumer/time). This concept is equally applicable to evaluation of detritus that contains a large proportion of indigestible organic matter such as cellulose, lignin, chitin and refractory products of organic diagenesis. Measures of detritus organic matter content and detritus digestibility have been used as approximations to energy assimilation rate (Odum 1968; Tenore et al. 1982; Lopez and Cheng 1982, 1983). Although this concept of detritus nutritional value has been very useful in explaining detritivore food selection and growth in some cases, results in other cases have been equivocal (Lopez and Cheng 1982; Tenore et al. 1982). Either energy assimilation rate is a correlate of detritus nutritional value, or additional factor(s) combine with energy assimilation rate to determine detritus nutritional value.

A third concept of detritus nutritional value is based on detritus amino acid or protein content. Amino acids linked together as proteins are the fundamental structural components of animal bodies. Unlike other nutrients, amino acids must comprise a large portion of the diet for the consumer to achieve maximum growth rate. Fishes, for example require about 45% of their diet as amino acids for maximum growth (National Research Council 1977). At lower concentrations of amino acids in the diet, growth is proportional to amino acid content (Fig. 4). Although different experimental approaches yield somewhat different estimates, it is believed that all animals have similar total amino acid requirements (Russell-Hunter 1970; Hainsworth 1981). In view of the fundamental similarity of animal biochemical structure, this conclusion is not surprising. The growth limiting effects of suboptimal amino acid levels in the diet have been studied extensively in connection with human nutrition and animal husbandry, but application of these principles to wild populations has been limited to studies of primates and a few ungulates (Coe 1983), terrestrial insects (Mattson 1980), and detritivorous fishes (Bowen 1979a, 1980, 1984a, 1984b, 1984c).

Energy-Amino Acid Interactions in Determination of Nutritional Value

Although biologists have usually treated these two variables as if they act independently (Tenore 1983 *inter alia*) amino acid concentration and energy assimilation rate must interact directly in determining the growth of detritivores. According to a simple yet accurate analogy, dietary amino acids provide the material whereas dietary

energy does the work of animal growth. In consequence, either may limit growth, and thus determine nutritional value. As shown above, detritus is typically low in both energy and amino acids, and both nutrients tend to be bound in complexes that are difficult to digest.

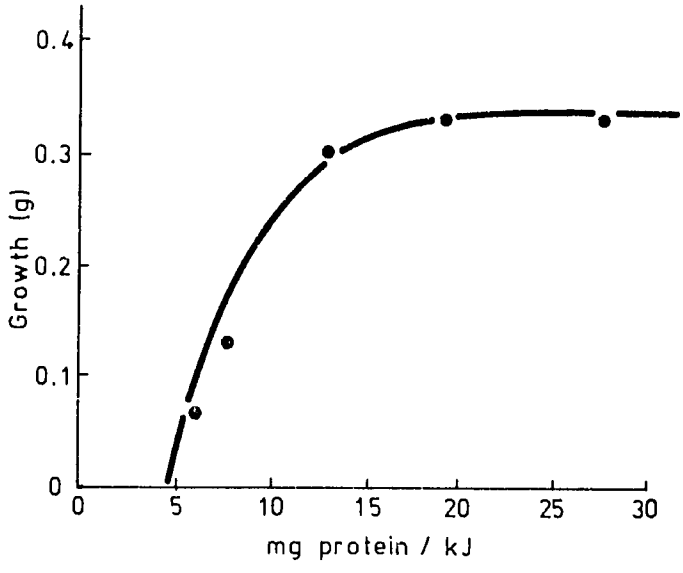

Fig. 4. Growth response of groups of goldfish *(Carassius auratus)* to diets of different protein richness fed *ad libitum* in aquaria. From Bowen (1984a) with permission.

At the physiological level, interaction between amino acids and energy in determining growth rate is complex. Amino acids can be catabolized to provide energy, but energy cannot be used by animals to synthesize the 10 essential amino acids (Hainsworth 1981). Due to the metabolic complexities of this interaction, physiologists are far from a general mechanistic explanation that can serve as the basis for a nutritional value concept (Sedinger 1984). As an alternative, interactions between energy and amino acids in a given diet can be evaluated experimentally. To simplify analyses, the apparent effects of interaction are reduced by description of the diet in terms of its protein:energy ratio. Thus, the protein:energy ratio provides a useful measure of diet nutritional quality (Fig. 4) (Bowen 1979b, 1984a).

The protein:energy ratio may be especially useful in nutritional evaluation of plant matter being processed in the fragmentation pathway. With many plants, amino acid concentration increases while available energy decreases (Suberkropp et al. 1976; Tenore et al. 1984). Thus, there is likely to be some stage in the process at which the energy-amino acid balance has maximum nutritional value.

There is no *a priori* reason why other nutrients (e.g., vitamins, minerals, essential fatty acids) may not also affect detritus nutritional value (Phillips 1984). Preliminary

tests with several fatty acids failed to show these were limiting the growth of a stream detritivore (Cargill et al., unpublished data), but a comprehensive series of tests involving vitamins and minerals has not been attempted. In contrast to protein, vitamins are required at very low concentrations in the diet: typically in the range 2×10^{-6} to 6×10^{-2} by weight (National Research Council 1977). Compared to higher plants and animals, microorganisms are very rich sources of vitamins. As suggested by Cummins and Klug (1979), the small quantities of microorganisms invariably attached to detritus may supply significant quantities of these nutrients. In addition, detritus itself may acquire significant quantities of vitamins through adsorption from overlying water (Wetzel 1975). At present, I am unaware of any evidence to suggest that a specific nutrient other than amino acids or energy limits the growth of an aquatic animal feeding on a natural diet. If a new specific nutrient deficiency should be discovered, it will necessarily operate within the constraints imposed by protein and energy in the diet.

Adaptations for Digestion of Detritus

Adaptations that enable detritivores to increase the efficiency with which they extract nutrients from detritus are described for both aquatic invertebrates and for fishes. Several invertebrates that graze on marine sediments increase the energy density of their diets by selectively ingesting the organic fraction of the sediment (Connor and Edgar 1982; Deans et al. 1982; Lopez and Cheng 1983). Mullet process sediment in their buccal cavities and selectively reject some of the inorganic fraction (Odum 1968; Payne 1976). *Oreochromis mossambicus* in alkaline Lake Valencia secretes gastric acid, which decomposes mineral matter in the diet of periphytic detrital aggregate, and this increases energy density by about 20% (Bowen 1981). Other adaptations increase the efficiency of amino acid digestion. The larvae of the cranefly *Tipula abdominalis* feed on large fragments of leaf detritus in streams. These insects have a gut pH as high as 11, which frees much of the detrital protein from phenolic complexes. Unusual proteolytic enzymes with pH optima of > 11 are responsible for subsequent digestions (Martin et al. 1980). In *O. mossambicus* gastric acidification similarly liberates complexed amino acids. In many cases, adaptations that increase the availability of one nutrient are likely to have a similar effect on the other. The extremely long digestive tract in *O. mossambicus*, which is necessary for complete digestion of amino acids, but does not further the digestion of those compounds that provide dietary energy, is perhaps an exception (Bowen 1981).

Behavioral adaptations are also important in allowing detritivores to exploit detritus as a food resource. In many habitats, detritus of relatively high nutritional value is consistently found at certain locations. This makes it possible for large, mobile detritivores like fishes to select feeding areas that maximize their rate of growth (Bowen 1979a, 1979b, 1984b). Smaller invertebrate detritivores may be able to distinguish between individual particles on the basis of nutritional value, but this has not yet been tested. Other consumers may complement low protein detritus

with protein-rich animal prey. One stream detritivore, *Gammarus minimus*, feeds almost exclusively on detritus when protein-rich detritus is available, but supplements its diet with animal prey when protein concentrations are low (Barlocher 1983). Another species, *Clistoronia magnifica*, feeds extensively on detritus but requires animal prey to complete its life cycle (Anderson 1976).

Conclusion

The chemical composition and nutritional value of detritus change during diagenetic processing. Nutrients important for animal growth are lost more rapidly than other components of the organic milieu. Over time, those nutrients that remain are progressively more tightly bound into refractory organic complexes which are difficult for detritivores to digest. Digestibility of nutrients in detritus depends on both the form of the nutrients, and the abilities of a given detritivore to liberate them. Thus, it is not possible to write a single equation that describes the nutritional value of detritus in general for all potential consumers. Nonetheless, predictible changes in digestible amino acids and energy provide a valuable perspective to guide future trials with specific combinations of detritus and detritivores.

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Discussion

GRAY: I don't think a measured low number or low biomass of bacteria means very much in terms of their importance as food for detritivores. Because the bacteria are consumed, we must measure production.

BOWEN: I understand your argument which is usually applied to food chains with different trophic levels. However, an individual organism does not eat 'production', it eats standing crop. It will consume, for example, a piece of detritus and the associated microorganisms. Moreover, it can only consume so much per day. Therefore, if we know the amount of material it consumes and the microbial contribution to that amount, the energy content of the microorganisms and the metabolic rate of the consumer, we can then determine what proportion of the consumer's metabolism could be accounted for if it digested and assimilated 100% of the microorganisms. This is how we get our figures for 10% or less of nutrients.

GRAY: To look at it the other way round, if you just determine (as most of us do) numbers or biomass of bacteria and then try to interpret the data, you can come to false conclusions. We find, for example, in Oslo fjord, that there is no change in biomass or numbers throughout the year but in spring there is a tremendous increase in production which is when detritus and the bacteria on detritus are getting eaten in large quantities.

BOWEN: That's certainly true. In such circumstances, there is a great increase in the transfer of energy from bacteria to their consumers. But in all cases where this has been measured, it is a minute fraction of the total energy transfer being made. We have also measured, in a few cases, the digestion and assimilation of the detrital component, compared to which those of the bacterial component are insignificant.

MORIARTY: Whether you should measure bacterial blomass or production depends to large extent on the nature of the consumers. For relatively small organisms like meiofauna, which consume bacteria that are often dividing rapidly, then it is important to know the turnover rate of the bacteria. However, for a larger consumer like a tilapia, which takes in large pieces of material, then biomass measurements can be adequate.

Regarding techniques, any preparative steps—for example, to get good clear images for scanning electron microscopy (SEM) or electron microscopy (EM)—involve great losses of materials from the surface. If you try to avoid this, you get an image that is very cluttered by slime, etc. With ordinary light microscopy and acridine orange staining, you can see lots of bacteria embedded in slime. For SEM/EM work you can, therefore, get a rather amorphous mess if the specimen is little disturbed or a very clear image from which much has been lost. I recommend the acridine orange technique, not EM.

I also think that ATP determinations are useless. There is a large variation in ATP content between organisms. There are also great difficulties in extracting ATP quantitatively from natural systems like this. ATP determinations are only useful for pure cultures in which you know its growth rate. ATP content varies tremendously with growth rate. We can forget ATP determination as an ecological technique and discount the data which have been published using it.

ANDERSON: I agree.

BOWEN: I am well aware of the multiple criticisms of ATP as measure, but I disagree that it should just be thrown out. It can be an indicative measure. Chlorophyll is by comparison even less reliable. There are greater variations in chlorophyll:biomass ratios than in ATP:biomass ratios. ATP can be a useful cross-check. There is a tendency to overestimate rather than underestimate ATP. Corrections are made for empirically determined extraction efficiencies and generous allowances made for the variation in ATP:cell mass ratio with growth rate, cell type and photoperiod. Thus, estimates based on ATP content provide an imperfect yet useful measure against which we can compare other estimates.

MORIARTY: No. I disagree. I agree that with chlorophyll as well there is huge range, so that estimates of biomass based on chlorophyll determinations can be out by plus or minus an order of magnitude. The same applies with ATP-based estimations. The most you can get is a very rough idea. For your investigations in which you are trying to be fairly precise—saying whether you have 5, 10 or 20% of detrital-aggregates microorganisms and is determining their importance for consumers—ATP determinations are not appropriate. You said that 10% of your material is amino acids and that these are important for the consumers. Now, if the total bacterial biomass is 1 or 5%, then obviously there must be other amino acids present than those present in the microorganisms. We are running here into the problems of defining 'detritus'. Detritus is so variable in composition. We need to define the source of any detritus and its composition in a given environment and the associated microorganisms rather than trying to generalize about the relative importance of living and nonliving components. So your comments need to be qualified as applying to the type of detritus which you studied, *Potamogeton* for example: its source and composition.

BOWEN: I have listed the source of the detritus in each case.

MORIARTY: On coral reefs, bacteria comprise about 5% of the organic matter present, algae perhaps another 10%. The maximum contribution of microorganisms here is say 20-25% which is up to 5 times higher than the system you have described.

BILIO: It is correct to emphasize this diversity. Dr. Bowen has done this in his table.

MORIARTY: I think your Table 1 is biased towards low values for bacterial or microbial biomass. There are problems with all methods for measuring biomass. For example, ATP is difficult to extract completely and is susceptible to decomposition during extraction. With direct counts, cell sizes must also be measured and carbon content estimated; recently some workers have presented evidence to show that previously accepted values for biomass may be too low (e.g., Bratbak and Dundas 1984)*. Other workers have published data showing bacterial biomass to be around 3 to 5% of organic matter, as for example, I have found for coral reef sediments and aquaculture ponds. Would you agree that in some environments or types of detritus, the proportion of bacteria to organic matter could be higher than you indicate?

BOWEN: I agree that there are many problems with our current methods for estimation of microbial biomass, and expect the depth of our understanding will increase as methods become more accurate. With methodological difficulties in mind, I was somewhat selective in compiling Table 1. I included estimates from ATP only when the efficiency of ATP recovery was determined directly by the investigator. I also declined to include estimates for polluted waters (where anthropogenic microbial biomass is high) and microsites such as the surface of a decaying algal cell or microbial films on the surfaces of mineral substrates in the presence of abundant dissolved organic matter. Such microsites may be important food resources for invertebrate "sediment grazers", but they are unlikely to represent a food source for fishes that lack the ability to isolate efficiently the microbes from indigestible substrates. Nonetheless, the figures you quote of 3 to 5% lie within the range of values cited in Table 1. Thus, I believe that Table 1 offers a realistic summary of our current knowledge, and is not biased toward low values for microbial biomass.

PULLIN: I was intrigued by the fact that you have not only studied what you called 'amorphous detritus' but you have also 'made' it. Could this be synthesized for use in aquaculture as 'artificial detritus'. I am thinking of controlled synthesis rather than the processing of organic materials by composting, etc. which we have in present use. This would be a new direction.

BOWEN: I thought for a while that this could be done. My graduate student, Molly Ahlgren and I spent a great deal of effort working with organic compounds leached from leaves and grasses. We worked a lot with lawn grass because of its availability and lack of toxic compounds. We tried a variety of techniques for making nutritious precipitates. We got positive growth feeding precipitates which had been made simply by autoclaving the material. In other words, we had sterile precipitates produced when the tertiary structure of the macromolecules was wrecked by the autoclaving

process. We grew tadpoles on these precipitates in paired treatments: precipitates held under sterile conditions and precipitates colonized by bacteria. In all such paired treatments, the microbially-colonized material produced much better growth. Our conclusion is that in an aquaculture situation, such precipitates are probably more useful as substrates for bacterial growth. In this way, you can feed microbes to the fish, rather than detritus *per se*.

ANDERSON: I broadly agree with your conclusions. However, we must distinguish between availability and quality. For example, if you consider changes in the quality of leaf litter inputs to streams (and I am extrapolating here from data for a terrestrial situation), you can study the N-content of the leaves and the availability of this N (through treatment with protease). With time, the N-content rises but the N-availability declines quite dramatically. So if shredding organisms attack such materials early, they can produce a high quality particulate detritus. The materials which come from precipitation may not be of such high quality. They may involve a whole lot of recalcitrant complexes. These precipitates of fine particulate organic matter will, therefore, contain some material which is highly available and some which is not. The key thing is the feeding strategy of the consumers. For example, the microzooplankton feeding among the sediment grains can select bacteria. These are not really detritivores. They are better regarded as predators. They are picking out a high quality resource. Conversely, other animals which are less selective are consuming a diluted, lower quality resource. So, there are two strategies here with a very narrow corridor. For a plant-feeding detritivore *sensu strictu*, there is a very short period at an early stage within the timeframe of decomposition of that resource when it can be said to be feeding on original material. This period is before the soluble organic compounds and soluble N have been lost and the microbial activity has conditioned the material to be highly assimilable. If this period is missed, the subsequent materials coming from the dissolved organic matter will be very diluted in quality by comparison with the original material. Thus, there is a big difference between the strategies employed by different organisms: fish or fine grain feeding invertebrates.

In summary, we have three variables: availability, the size of the resource and its quality which we must consider. The options for utilizing detritus may be fairly limited.

BILIO: So it follows that the stage at which a detrital resource is used is very important for different types of cultured animals. We should follow a strategy which brings together the cultured animal and the resource when the latter is at the right stage.

BOWEN: Yes. The cultured organism may require the original material or the opportunity to use a pathway associated with some later stage.

BILIO: And the longer you wait, the more you lose with respect to utilization for aquaculture.

FRY: The dissolved organic matter may be important for the production of the detritus which we find in natural ecosystems, but it is probably not important in the total processing of organic matter through an ecosystem. Bacteria are very efficient at using low molecular weight carbon compounds at very high rates. The flux of these compounds through bacteria is enormous, so most of the carbon is processed by this route and not through synthesis of particulate organic matter.

BOWEN: I know that's the dogma, but some work in the past few years (not my own) has shown some different evidence. Certainly if you use specific substances like acetate or glucose in a controlled environment, you find that many of these substances are lost from cells and then react very rapidly in the surrounding medium. Observations by Jensen and Sondergaard** showed that compounds lost from actively photosynthesizing algae resulted in the formation of fine abiotic particles so rapidly that there was no chance of bacteria taking up these compounds. In other words, this fine particulate organic matter was formed after a very short dissolved organic matter phase.

GRAY: What you mean by short?

BOWEN: The experiments, if I recall correctly, were of the order of minutes. One further comment here is that we should perhaps be culturing herbivores rather than true detritivores. The latter seek

out sites at which relatively new detritus is formed from recently precipitated organic matter. The tilapia which we studied were, I think, taking in detritus produced on plant surfaces, the source of which was the tremendous primary productivity in the overlying water. Benthic detritivores also feed at sites where detrital precipitation has occurred. Strict detritivores in the natural environment are thus highly evolved and specialized. Of course, some fish are facultative herbivores as well. *Oreochromis mossambicus* and *O. niloticus* have tremendous trophic plasticity and could grow well in a heterotrophic or an autotrophic based system.

BILIO: When we consider the alternatives of plant feeding and detritus feeding, let us not forget the basic philosophy of this conference which is that detritus *is there*. We are trying to optimize utilization of a resource which would not otherwise be fully utilized. So, these are not really alternatives in the context of this conference.

BOWEN: My suggestion may not have been clear. I feel that herbivorous fishes might be better adapted to utilize the microorganisms that grow on organic matter.

WOHLFARTH: With regard to the choice of target animal and the dynamic conditions in the pond, the choice is fairly obvious to me from a fishfarmer's point of view. The answer is not to stock this fish or that, but to stock both or to use many species in polyculture. The Chinese have been doing this for the last 3,000 years. Of course, you could manage such systems in many ways—for example, stock one species in a polyculture and then harvest it when its resource is no longer there; or leave it in and add some more of the required resource.

BOWEN: That was the idea behind my suggestion: that we look for animals to match available resources.

PRUDER: Are the organisms associated with detritus considered part of the detritus? It seems that we must clarify our concepts of grazing and detrital foodwebs. One further point is that we can input very high quality materials to ponds, so detritus need not be derived from, say, ground-up leaves. We don't have to input low quality residues or wastes.

BILIO: Your second point was made in my overview (Table 1).

EDWARDS: On Dr. Wohlfarth's comment, the Chinese do tend to use a large number of species in polyculture, but I doubt that they understand well the pond dynamics.

WOHLFARTH: I bet they do.

EDWARDS: There are management problems in using large numbers of species. You have to produce all the fry. This may be worth it if the markets are there. However, consider the case of Taiwan. The Taiwanese started with a traditional Chinese freshwater polyculture system but have now moved more and more towards a system in which the main cultured species is tilapia. I agree that we need polyculture systems, for example, we must include a good herbivore to eat aquatic macrophytes and microphagous species to use plankton and detritus but we should aim for a more rational system, not just a traditional mixture of lots of species.

PULLIN: I agree.

WOHLFARTH: Regarding the production of detritus in the pond, an obvious example is the grass carp *(Ctenopharyngodon idella)*. It is an inefficient converter of plant material. Its feces sink and become detritus. Hence, the Chinese claim, which no one has ever investigated, that if you feed one grass carp you are feeding three other fish.

SRINIVASAN: The same thing happens in ruminants. You feed the treated straw and it has to be processed, then the organism gains weight.

SCHROEDER: Taiwanese ponds are rather a special case. They tend to be intensive and heavily dependent on supplied feed. About 10% of the water is drained every day to try to get rid of some of the detritus. Hence, their tendency to monoculture and intensive feeding.

EDWARDS: Such very intensive systems of tilapia culture in Taiwan are not common at all. Most freshwater polyculture systems in Taiwan are *integrated with livestock*. Less vegetation is added to the ponds compared to mainland China. The major inputs are detrital organic matter, principally animal manure.

SCHROEDER: I think at Dor, Israel, we have tried 70-80% tilapia and 30% other species and vice-versa.

WOHLFARTH: We never used 80% tilapia.

EDWARDS: Tilapias are generally more tolerant of low dissolved oxygen than other species. Therefore, if you increase the proportion of tilapia, you should be able to add more organic matter and get higher yields.

SCHROEDER: Yes, provided that there are sufficient other species present to balance the eco-system.

PULLIN: Many of the species used in polyculture are probably far more versatile feeders than they have ever been given credit for. There are tremendous overlaps between species with respect to feeding niches. Tilapia is the best example. It essentially eats what's there! It feeds in the water column; it eats plankton; it feeds on the bottom; it eats detritus; it eats supplemental feed; it has even been entrained in some systems to eat fresh vegetation (I am referring here to *O. niloticus*, not to any more specialized macrophyte feeding species). There are advantages in polyculture, but when it gets to the point of using six or seven, or even up to nine species (in Indian composite culture), this has little rational basis from critical experiments. Perhaps, we should try taking out some of the less marketable species from such polycultures to see what happens. Polyculture needs a reappraisal—on biological and economic grounds.

WOHLFARTH: Well, monoculture vs. polyculture is not really our topic. However, we did perform one test comparing tilapia monoculture with what we think at Dor is a balanced polyculture. The tilapia monoculture gave a lower yield and the monoculture pond had a bad smell. The net used for seining smelled like a cowshed.

BILIO: Only the pond, or also the fish?

WOHLFARTH: No, the fish were fine. This was an *O. niloticus* x *O. aureus* hybrid: better than *O. niloticus*. They were obviously not using everything in the pond. However, this was only one experiment. More work needs to be done.

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**Jensen, L.M. and M. Sondergaard. 1982. Abiotic formation of particles from extracellular organic carbon released by phytoplankton. Microb. Ecol. 8: 47-54.

Carbon Pathways in Aquatic Detrital Systems

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Abstract

Organic matter enters detritus as added manures or feeds, as precipitated algal cells and as dissolved organic matter formed when cell solubles leach from dead cells or are exuded by living algae. The products or microbial processing of detritus, and hence the paths of carbon flow within detritus, are largely determined by the local oxygen regime. Theoretical calculations of the flux of oxygen into detrital mass and *in situ* measurements of oxygen uptake by fresh and saltwater sediments both indicate that half or more of the carbon that enters sediment detritus is fermented anaerobically. Detritus typically has low concentrations of bacterial cells and relatively high concentrations of amorphous extracellular compounds. These extracellular products are held in a web of polymeric fibers produced by sessile bacteria and algae.

The ratio of $^{13}C:^{12}C$ (reported as δC) of zooplankton in manured ponds indicates that they take their nutrients from microalgae, with only small input from detritus. The flow of carbon in the aquatic detritus systems of fishponds appears to go from deposited organic matter to microbial slimes and then to harvesting by deposit feeding fish. This carbon flow, in combination with directly harvested autotrophic growth, is adequate to sustain fish yields of 25 to 35 kg/ha/day. Based on relative delta C values, 60 to 70% of this yield may originate with detrital feeding.

Introduction

The detrital system consistently appears as a major (and possibly the major) contributor to the total target animal yield in conventional (nonraceway) fresh and saltwater aquaculture. This is true for earthen environments with or without supplied feeds provided there has been adequate fertilization. This conclusion may be based on fish gut analyses (c.f. Spataru et al. 1983 and references cited therein; Day et al. 1973), on *in-situ* observations of fish feeding habits (Odum 1970; Day et al. 1973;

Bowen 1981; Bowen et al. 1984), on relative yields of fish from ponds receiving chemical fertilization vs. yields from parallel ponds receiving organic manures (Hopkins and Cruz 1982) and on comparisons of isotopic compositions of available foods with target animal flesh (Fry and Parker 1979; Schroeder 1983 and unpublished data related to milkfish; Shan et al. 1985), for work in saltwater and freshwater with animals as diverse as carps, milkfish, mullet, tilapia, penaeids and *Macrobrachium*. What makes the detrital system a vigorous producer of target animals appears to be its channelling of initially unavailable energy sources and chemicals into a harvestable and assimilable complex community of autotrophic and heterotrophic microorganisms, their associated exudates or slimes, and the host of dissolved organic compounds trapped in their extracellular fibers.

The fact that manured, nonfed fishponds have approximately the same maximum fish yields, 25 to 35 kg fish growth/ha/day averaged over the growth season from fingerling to market size, in China (Shan et al. 1985), Israel (Moav et al. 1977; Schroeder 1978), the Philippines (Hopkins and Cruz 1982), and the USA (Buck et al. 1978) implies that there is some maximum rate at which a pond ecosystem is able to convert metabolites into matter that does not inhibit growth and/or to convert coarse organic matter into useful detritus-based foods. The importance of the manure organic matter to the detrital system, as opposed to the chemical components of the manure alone, has been demonstrated by the twofold increase in fish yield for ponds receiving pig or chicken manures as compared with parallel ponds receiving only inorganic chemical fertilization (Hopkins and Cruz 1982). It is instructive that adding grain to manured ponds increased total fish yield only by approximately 30% (Moav et al. 1977). In these ponds there was no evidence that there was a concurrent increase in available natural foods when the grain was added. The limit of fish yield may still have been the limit of natural food availability.

Detrital communities are often considered to be a complex community of heterotrophs feeding on dead organic matter. These communities may contain autotrophs, especially algae and diatoms. Both produce extensive networks of extracellular fibers. These fibers are a means of attachment to host surfaces and may trap dissolved organic carbon compounds for subsequent assimilation (Costerton et al. 1978; Costerton and Geesey 1979). Measurements of bacterial biomass indicate that bacterial cells contribute a small (≤5%) fraction of total organic weight in a variety of freshwater and saltwater detrital slimes (Odum 1970; Day et al. 1973; Paerl 1978; Schroeder 1978; Bowen 1981; Moriarty 1982).

Even though bacterial densities may be high (up to 10^{10}/ml) in sediments, cellular carbon probably contributes <5% of the mass of organic matter in sediments of typical fishponds (Schroeder 1978). Most of the organic matter is usually associated with an amorphous slime and with debris of algae and macrophytes. Epilithic slimes in the littoral, sunlit zone may contain 1,000 times more algal carbon than bacterial carbon simply because the mass of a single algal cell is orders of magnitude greater than the mass of a bacterial cell (Geesey et al. 1978).

Organic matter enters the benthic detrital system of a pond as sedimenting algae from the overlying water column, as unused portions of supplied feeds and manures,

and as organic matter washed or blown into the pond. With both liquid cow manure and field-dried chicken manure, approximately 90% of the organic matter settles to the pond bottom within two hours after application to the pond. Dissolved organic matter in the pond water may be exuded from growing algae or may be leached from lysed algae, bacteria and other organisms.

In the manure-loaded ponds at Dor, on the coastal plain of Israel, manure carbon is added at rates similar to rates of carbon fixation by net primary production (Schroeder 1978). Comparison of the ratios of the two stable isotopes of carbon, $^{13}C:^{12}C$ (reported as δC; see Schroeder (1983) for a detailed discussion of the use of δC in tracing food webs) as found in the manures and algae with the δC of the pond sediment organic matter (work in progress) indicates that approximately half of the carbon originates with the manure and half with the originally pelagic algae. Particles of detritus, i.e., non-living organic matter, are rapidly colonized and solubilized by bacteria and Protozoa. This is evidenced by the high rate of weight loss of cotton cloth strips placed in fed and/or manured fresh and salt water ponds. These strips consistently lose 30 to 50% of their original weight of cotton in the first five days (Fig. 1). This weight loss was measured after the microbial slime that coats the strip was washed off. Cotton is 99% crystalline, lignin-free cellulose and so is accessible to microbial action in oxic and anoxic environments, i.e., above or within the sediments. The partially decomposed cotton containing the microbial slime possesses 10 to 20% crude protein (calculated as 6.25 x Kjeldahl N; Schroeder 1975). The colonization of insoluble cell wall material and subsequent leaching of soluble cell carbon by heterotrophic microorganisms is the initial pathway by which carbon enters the detrital food web.

Aerobic vs. Anaerobic Microbial Activities

The characteristics of the carbon flow are strongly affected by redox potential at the site of decomposition. When oxygen is the electron acceptor in the microbial processing of organic matter, the main products are water, respired carbon dioxide (accounting for half or more of the original organic carbon) and bacterial and fungal biomass (Tusneem and Patrick, Jr. 1971). A common example of the phenomenon of weight loss in aerobic microbial processing of organic matter is the large reduction in organic matter during (aerobic) composting.

When the environment is anoxic, fermentation of detrital organic matter results in much of the original carbon being converted into extracellular, low molecular weight organic compounds (Van Soest 1980). A common example of this is the production of extracellular organic acids in (anaerobic) silage. These acids reduce the pH of the silage to levels of five or lower. Whether the detrital system is a pond or the rumen of an animal, an anaerobic system of fermentation produces organic matter in the form of low molecular weight, organic carbon compounds. Anaerobic processing retains more of the original carbon in the form of organic compounds than does aerobic processing, provided that the energy-rich extracellular byproducts are not lost (Van Soest 1980). Had the rumen been aerobic, the host animal's survival would

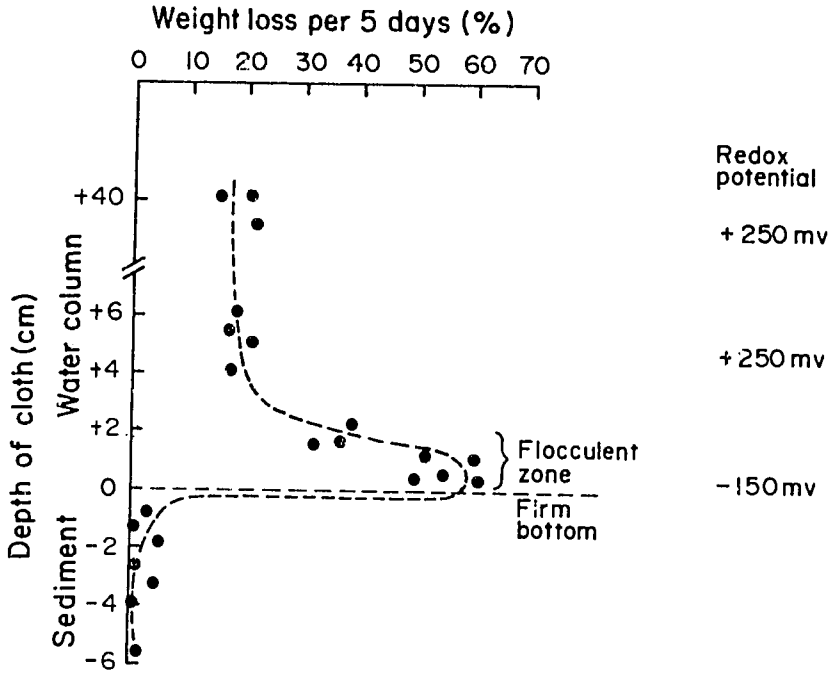

Fig. 1. Rate of weight loss of cotton cloth placed in fertilized, earthen fish pond vs. position of the cloth in the pond. Redox potential refers to redox at the indicated depth. Initial cloth density was 15 mg/cm^2. The data were taken 30 days after filling and stocking the pond. Water temperature was 27 to 30°C. Cotton cloth is approximately 100% crystalline, lignin-free cellulose. After 5 days residence in the pond flocculent zone, the Kjeldahl nitrogen content of the residual cloth plus microbial slimes on the cloth was 2 to 3%. This represents a crude protein content of 10 to 20%. The data presented in this figure were taken in a freshwater pond. Similar rates and patterns of cellulose microbial processing are observed in marine ponds (Schroeder, in Colwell et al. 1984).

have been dependent upon bacterial cell production with all the carbon losses inherent in maintenance of the cells. In the rumen, the extracellular compounds may be absorbed through the rumen wall. In the detrital zone of a fishpond, these compounds may be harvested by target animals. Alternatively, if not harvested rapidly, they may be converted to inorganic nitrogen and carbon compounds by anaerobic bacteria.

Many bacteria, especially those associated with particulate matter, produce extracellular polymers in the form of slime or capsules. Factors affecting the amounts and composition of slime, and the role of the slime in aquaculture food webs require study.

As mentioned previously, only a few per cent of detrital dry weight is composed of bacterial cells. Based on redox potential profiles, fresh- and saltwater sediment becomes anoxic in the first few millimeters of depth (Schroeder 1978; Howarth 1984; Blackburn, this vol.). In the organically rich silt of carp and tilapia pond bottoms on the coastal plain of Israel, the sediments become anoxic within 0.5 mm of the sediment-water interface. The redox potential drops from +250 mv in the water column a few cm above the bottom to −150 mv, 0.5 mm into the sediment (Fig. 1). Within slime layers on detritus, the transition from oxic to anoxic is even more rapid. Approximately 70 microns within a slime layer diffusion of oxygen is sufficiently retarded to cause anoxia (Geesey 1982). Because much of the volume of the zone where detritus settles is anoxic, fermentation and other anaerobic processes predominate in the decomposition of the detritus. Low molecular weight products of decomposition may be adsorbed to the extracellular polymers or to inorganic sediment particles and thus become available to large deposit feeders (e.g., tilapia).

In nature, where nutrients are sufficiently scarce to warrant scavenging, bacteria produce masses of fibers, extending 50 microns or more from the cell. The fibers attach to organic films that coat most submerged surfaces (Costerton and Geesey 1979; Geesey 1982). In addition to securely fastening the bacteria, these fibers act as miniature ion exchange systems, removing organic molecules from the surrounding water and concentrating this dissolved organic carbon in the fiber matrix. Costerton and Geesey (1979) have noted in controlled experiments that the rate of slime formation is proportional to the concentration of dissolved nutrients in the water. Algal exudates may be a significant stimulant to slime formation in the eutrophic conditions of a fishpond. Although polymer fibers may not be easily digestible, the entrapped microbial cells and soluble and insoluble organic compounds may represent a form of concentrated food for deposit feeders.

The abundance of this extracellular food may have been what caused Odum (1970) and Bowen et al. (1984) to conclude that the concentration of microorganisms in detritus was insufficient to account for the nutrition derived by mullet and tilapia from the detritus. Geesey (1982; pers. comm.) noted that the extracellular polymers consisted mainly of polysaccharides and protein. In one instance, Geesey determined the composition ratio to be protein: carbohydrate : DNA = 1.5 : 1 : 0.1. This high protein concentration of the polymer mass is consistent with Bowen's (1980) observation of an amino acid concentration of 7 to 24% in the total detritus weight. Tusneem and Patrick, Jr. (1971) noted in freshwater detrital systems that anaerobic fermentation of straw produced a higher rate of accumulation of amines than did aerobic decay. More nitrogen was associated with amino acids than amino sugars.

Nutrient Uptake by Attached and Unattached Microbes

Several investigators have noted that both for ponds and rivers the number of bacteria/cm^2 of sediment is 100 to 1,000 times greater than the number of bacteria/

cm^3 in the overlying water (Schroeder 1978; Costerton and Geesey 1979; Ladd et al. 1979). In a typical pond of 1 to 2 m depth, these data imply that in absolute terms, there are more bacteria associated with the sediment than in the total water column. Lock and Haynes (1976) observed that dissolved organic carbon was removed from natural waters four to five times more rapidly when over sediments than when isolated from the sediments. We regularly observe in aerated, manured, sunlit aquaria, a succession during the first week of dominance from pelagic to sessile autotrophs and heterotrophs. The fixed detrital layer appears to have an advantage over unattached organisms in nutrient capture. The advantage may be related to nutrient trapping by the fibers used for attachment. When it is considered that between 10 and 60% of total photosynthate may be released as dissolved organic carbon by phytoplankton (Paerl 1978; Carlson and Carlson 1984), the adsorption of these soluble compounds by microbial fibers could be a rich source of organic carbon flow into the detritus web.

Evidence for the influx of organic matter to sediment detritus from pelagic algae, whether settlement of dead cells or as capture of dissolved compounds, is seen in the δC of the sediment organic matter. The δC values of the sediment are similar to the average for the δC of the algae and the added organic fertilizer in the intensely manured ponds at Dor (Table 1). Net primary production and added manure supply carbon to the ponds at similar rates in the pond management strategy used at Dor, each nominally at 3 to 5 $g/m^2/day$.

The Oxygen Regimen in Aquatic Detrital Systems

Steep concentration gradients across the sediment/water interface of oxygen, ammonia and phosphate imply nutrient uptake by a community active in the interface region. Concentrations of ammonia and phosphate drop from >50 ppm 1 or 2 cm within the interstitial water of manured fishpond sediment to <1 ppm in the water 1 cm above the bottom. Oxygen concentration drops from the pond water daytime value of >5 ppm to zero 100 to 500 microns within the interstices of the pond sediment.

The mass transfer of oxygen across the sediment-water or detrital slime-water interface has an important effect on pathways of carbon flow within detrital systems. Assume a uniform one-dimensional flux of oxygen, based on Fick's first law of diffusion:

$$J = -D \frac{\delta C}{\delta X} \qquad \ldots 1)$$

where J is mass transfer (flux) by diffusion (in this case oxygen diffusing from the water into the sediment or detritus where it is consumed by aerobic bacteria) units are g oxygen/m^2/day; D is the oxygen diffusion coefficient, approximately 2 x 10^{-5} cm^2/sec for oxygen in water (Kanwisher 1963; van der Laeff et al. 1984); C is the oxygen concentration; and X is the distance into the sediment of detritus.

Table 1. Delta C ($\delta^{13}C$) values observed in manure-loaded fishponds.

	Manure type	
	Liquid goose manure (δC)	Dry chicken manure (δC)
Muscle of target animal		
Common carp (*Cyprinus carpio*)	−18	−22
Tilapia (*Oreochromis niloticus* x *O. aureus* hybrid)	−20	−25
Silver carp (*Hypophthalmichthys molitrix*)	−22	−29
Natural foods and added manure		
Zooplankton >150 microns	−28	−34
Algae <37 microns	−28	−31
Seston <37 microns	−25 to −28	−27 to −31
Chironomids	−21	−29
Littoral organic matter	−23	−26
Sediment organic matter	−23	−25
Added manure	−13	−17

Notes:
1. δC values are relative to the conventional PDB standard (Schroeder 1983).
2. Chironomids were found only in regions protected from fish grazing such as under rocks.
3. More than 90% of planktonic primary production was by algae <37 microns in size.
4. Each datum represents an average of values for 3 or more individual samples taken from each of 2 or more replicate ponds. For each average, the range of values was <1.2 δC units and the standard deviation was <0.8 δC units.
5. Portions of these data are adapted from Schroeder (1983).

Assume that the oxygen concentration decreases from 5 ppm in the water above the pond bottom to zero over a diffusion distance of 200 microns: 100 microns of stagnant water above the sediment (Kanwisher 1963) plus 100 microns within the sediment or detritus (Geesey 1982).

Substituting these values into Equation (1), after correcting for consistent units, gives a flux (J) of 4 g oxygen/m^2/day. Blackburn (this vol.) reported that the dissolved oxygen decreased to zero over a depth of 2 mm into a marine sediment layer he was studying. When this value is substituted into equation 1, the predicted flux is 0.4 g/m^2/day. Values of 1 to 4 g oxygen uptake by sediments/m^2/day have been measured in fishponds, lakes and salt marshes (Teal and Kanwisher 1961; Day et al. 1973; Schroeder 1975; Hopkinson et al. 1978; Revsbech et al. 1980; van der Laeff et al. 1984). The values for all the environments tested peak at 3 to 4 g oxygen uptake/m^2/day provided that the measurements are made *in situ*, with undisturbed, submerged sediments. If the surface of the sediments was exposed to air and the interstices not flooded, the flux of oxygen was greater because the value of D in air is about 1,000 times greater than in water (Schroeder et al. 1965; Schroeder 1975).

The measured values of J, so similar to the predicted maximum value obtained in Equation (1), reveal an important fact: the aerobic pathways of carbon flow in detritus are diffusion limited. All the oxygen that can diffuse into the detritus is used. Beyond this, anaerobic processes dominate and alternative electron acceptors must be used.

Sources and Sinks for Detrital Carbon

In salt marshes, sulfate is the main anaerobic electron acceptor, accounting for >50% of the total decay of organic matter (Howarth and Teal 1979; Hargrave and Phillips 1981; Howes et al. 1984). Estimates of total organic carbon fermentation in salt marshes range from 2 to 5 g C/m^2/day. Based on a simplified statement of carbohydrate oxidation;

$$CH_2O + O_2 = CO_2 + H_2O,$$

3 g of oxygen (the maximum amount of oxygen that can diffuse into the sediments/m^2/day) can account for the aerobic respiration of only about 1 g carbon. The remaining 1 to 4 g carbon fermented must be consumed anaerobically.

In the freshwater fishponds at Dor, net primary production is 3 to 5 g C fixed in algae growth/m^2/day (Noriega-Curtis 1979). Feed or manure, supplied at a seasonal average of about 100 kg dry weight/ha/day, adds about 3 g C/m^2/day. Fish growth (0.3 g C/m^2/day) plus respiration account for approximately 1.5 g C uptake/m^2/day. A total of 5 to 6 g C/m^2/day settles to the sediments. In the 200-day growing season, the influx of carbon would add about 1 kg organic matter/m^2 annually were it not for the decomposition.

There is strong empirical evidence that the main flow of carbon in pond detrital systems takes place anaerobically. Fig. 2 shows a piece of cotton cloth after five days in a manured, freshwater fishpond. The cloth extended from the water column into the sediment. Note the nearly complete decay of the cloth a few millimeters below the surface of the sediments. In the region of decomposition, the redox potential was −150 mv, i.e., anoxic and highly reduced. Similar patterns of decay have been observed in saltwater marshes (Howarth and Hobbie 1982) and saltwater fishponds (Schroeder, unpublished data). Based on measurements of the decay of cotton strips placed horizontally just within the pond sediments (as well as in the water column and 2 to 3 cm within the sediments where decay was 2 to 10 times slower; Schroeder 1978), cotton weight loss averaged 7 g C/m^2/day.

Extended microbial processing of the detritus can result in conversion of the organic matter to the end-products of carbon dioxide, water and soluble organic compounds. This is indicated in Fig. 2 by the total disappearance of part of the cloth. However, slime accumulation is an intermediate phase in this total digestion. This process is evident from the highly decayed, slime-coated portion of the cloth adjacent to the region of total decay. It is this slime that may be harvested by fish.

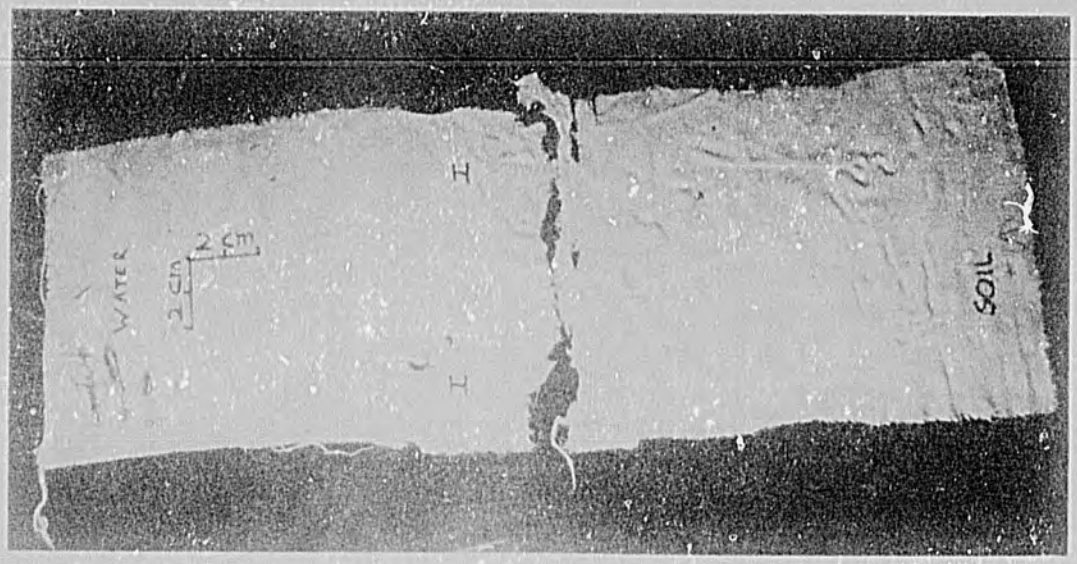

Fig. 2. Cotton cloth after being suspended vertically for 5 days in a fishpond receiving feed pellets and chicken manure. The cloth extended from the water into the bottom sediments. The bottom was flocculent and not clearly defined. "I" marks the approximate location of the soil/water interface. Note the intense digestion of the cloth just below the interface.

Pond conditions: Water column: temperature 27° to 31°C, dissolved oxygen early morning >1 ppm, early afternoon >7 ppm; redox potential +250 mv; phosphate <2 ppm; ammonia <3 ppm; pH 8 to 9; crude protein (as Kjeldahl N x 6.25) of cloth plus slime <2%.

Within the interface interstices: anoxic; redox −150 mv; phosphate about 100 ppm; ammonia 10 to 100 ppm; pH 6.5; crude protein of cloth plus slime 10 to 20%.

Density of cloth prior immersion was 15 mg/cm^2. (Cloth is turned sideways here)

Although the cellulose of cotton decays more rapidly in the anoxic detritus zone than does fiber containing lignin, such as found in manures, the high rate of cotton decay indicates the potential for anaerobic microbial activity on crude fiber. The nature of the electron acceptors is not obvious in the freshwater ponds. In one experiment (Schroeder, unpublished data) where superphosphate and ammonium sulfate were added daily to a manured freshwater pond 60 days after the start of manuring, the rate of cotton weight loss increased approximately 50%. This increase may have been attributable to the N and P supplying essential chemicals for microbial growth consuming the cotton. However, the sulfate may have served as an electron acceptor. The pH of the sediment interstitial water of the freshwater ponds was 6.5. This is favorable for bacterial fermentation.

Carbon Flow in the Trophic Levels Above Bacteria

The trophic level above bacteria is usually considered to be protozoans (Fenchel and Jørgensen 1977). That ciliates abound in anoxic media is clear from a casual survey of rumen fluids and from study of organic deposits in ponds (Schroeder

1980). In oxic media, larger zooplankton and protozoans eat bacteria or algae. The carbon isotopic composition (δC) of zooplankton taken from pond water is consistently more similar to the δC of microalgae than to the δC of the pond detritus (Table 1). This implies that their feeding is based primarily on grazing of the algae.

In manured ponds stocked with one or more fish/2 m^2 of pond area, the intense grazing on the natural foods lowers the standing stock of zooplankton to less than a few tenths of a milligram dry weight/liter. As the generation time of most zooplankton is two or more days (see references cited in Schroeder 1978) and their δC value is similar more to microalgae than to detritus δC values, the growth of zooplankton cannot account for a significant flow of carbon out of the detritus.

Chironomids ingest particles in the size range of bacteria and microalgae. For all the pond treatments studied to date where the organic input was dry manure or feed pellets, the chironomid δC matched that of the microalgae δC. For ponds receiving liquid manures, the δC of the chironomids showed the influence of the manure carbon in their diet. These data indicate the origin of their food web, but not whether they subsist on the food before or after bacterial processing. As with the zooplankton, the quantity of detrital foods harvested by these zoobenthos is small because in these fishponds stocked with one or more fish 2 m^2, the number of chironomids is grazed to only a few/m^2.

Because the consumption of detrital carbon by zooplankton and zoobenthos is slight in fresh- and saltwater aquaculture ponds, it appears that the flow of carbon within the detrital systems of these ponds, prior to harvesting by deposit feeding fish and crustaceans, stops at the microbial stage.

Microbial Paradox

Bowen et al. (1984) and Odum (1970) state that microorganisms account for a small per cent ($<5\%$) of detritus organic weight. Yet detritus is consistently the main component in the guts of deposit feeding fish and crustaceans in manured ponds (where fish yields reach 30 kg/ha/day) and salt marshes where crustacean yields reach 10 kg/ha/day (Day et al. 1973). A food in which $<5\%$ was assimilable by the consumer would not seem able to support the high growth observed in these detrital feeding systems. As Bowen et al. (1984) discussed, the rate of passage of ingested material through the fish intestines is not sufficiently rapid to enable the fish to consume adequate quantities of the detritus to sustain the measured animal growth if this growth is based on the 1 to 5% that is composed of bacteria.

Although the standing stock of bacteria accounts for 1 to 5% of the detrital mass, estimates of bacterial production based on oxygen demand in the detritus (see discussion above) indicate a significant production rate: 1 to 4 g C fixed in bacteria/m^2/day. To take advantage of this rate of production in view of the low standing stock of bacteria, the fish would have to select for bacteria among the total detrital mass. Observations by Bowen et al. (1984) of concentrations of bacteria in tilapia gut contents indicate that tilapia do not select bacteria specifically.

The δC of the tilapia grown in manure-loaded ponds indicates that ≥70% of their growth was associated with algal carbon webs (Table 1). Inspection of tilapia intestines from manure-loaded ponds revealed the main component to be unidentifiable organic matter, visually quite similar to the organic matter of the pond bottom. Algal cells were rarely observed. The implication is that the algal carbon assimilated by the tilapia had been processed in the detrital system of the pond prior to ingestion by the tilapia.

Reviews of the use of manures in fish farming (Wohlfarth and Schroeder 1979; Schroeder 1980) revealed that when the manure was presented as a direct feed (i.e., included in a pellet) and not allowed to enter the detritus system, such as in the case in a cement tank that was cleaned daily, the yield of fish decreased with increasing proportion of manure in the pellet. When the manure was allowed to enter the detrital web, as when the unused portions of the feed or manure remained on an earthen pond bottom, the yield of fish based on manure was quite similar to the yield based on standard feed pellets. Some processing of the detritus, but not necessarily a high standing crop of bacteria, is apparently essential for its assimilation by fish.

Conclusions

From the observations stated above, one may derive the following: manure and other forms of fiber-rich organic matter are poor fish foods at the time they enter fresh- or saltwater detrital systems. Microbial processing upgrades the quality to a good food, yet the concentration (i.e., the standing stock) of the bacteria is not sufficient to account for this quality. The *rate* of bacteria production in the detrital mass can account for significant quality improvement. Because much of the microbial processing takes place anaerobically and because anaerobic fermentation produces considerable amounts of extracellular, low molecular weight products, it is possible that organic matter resulting from bacterial processing, although not comprising bacterial biomass, is nutritionally valuable for detrital feeding animals.

In addition to the actual products of fermentation within the detritus, the ion scavenging characteristics of the fiber mass produced by sessile microbes and the adsorptive potential of sediment solids have the potential to concentrate within the detrital layer the soluble exudates of the pelagic algae and bacteria.

The contributions of algae and manure to the detrital food webs in manure-loaded ponds are evident from the δC of deposit feeding fish grown in these ponds. For ponds receiving daily inputs of field-dried poultry manures, ≥70% of the tilapia growth is based on food webs originating with algal organic matter.

The intensely manured fishpond is a system to which mineral and fiber-rich organic matter is added frequently (often daily). In this system, when managed properly, grazing by the target animals is sufficiently frequent to harvest the web of natural foods generated from the added matter prior to its loss by mineralization to carbon dioxide and methane.

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Discussion

MORIARTY: Regarding your comments on aerobic and anaerobic production, particularly your analogy of the pond as a 'rumen' in which anaerobic processes produce extracellular products rather than microbial biomass, in aquatic ecosystems slime is usually produced when the C:N balance is wrong. That usually occurs when there is not enough N. Anaerobic activity can be very high with little slime production. You said that carbon was not lost in anaerobic processes. Carbon is not lost in anaerobic processes as long as the products are small organic molecules like organic acids, but as soon as processes like nitrate or sulfate reduction are involved, then of course carbon is lost. I feel you have oversimplified matters.

On your cotton cloth method, your cloth did also lose weight in the water column as well as at the pond bottom. If you were to quantify this, perhaps more cellulose digestion in the water column volume is taking place than in the area of the thin upper layer at the water-mud interface?

BILIO: Let us consider the cotton cloth method first.

SCHROEDER: It is true that the cotton cloth has significant weight loss in the water column and none deep into soil. But when you add a dried or aged manure to the pond, over 90% of the organic matter settles out in less than two hours. So while there may be significant digestion of

cellulose (perhaps algal cellulose) in the water column, the bulk of the added cellulose lies on the pond bottom.

BILIO: Were your ponds and those mentioned by Dr. Moriarty comparable, particularly in depth?

MORIARTY: Both are around 70 cm to 1 m deep. My main point is that just because you get a higher weight loss in your cloth at the water-sediment interface does not mean that most of the processing of cellulose in the pond is happening there.

SCHROEDER: But much of the *added* cellulosic material will be processed there, even if you do have carp disturbing the bottom.

MORIARTY: If you quantify this for the sediment surface layer and the whole water column, how does it add up?

SCHROEDER: To do this, I would have to quantify the cellulose present in the water column.

KIRCHMAN: The water column is important, but there are roughly 10^6 bacteria per ml of water compared to 10^9 per gram of sediment, so perhaps the processing at or in the sediment is more important. It really depends what your fish is going to do. If it can feed more effectively on the bottom, obviously pond bottom products are more important and vice versa.

SCHROEDER: I think that our isotope data define pretty well where the fish are feeding and our results are usually confirmed by gut analyses. In our ponds, silver carp is a filter-feeder. Tilapia and common carp are bottom feeders. Their delta-carbon values match those of the bottom feeds almost exactly and are most unlike the values for water column feeds. The bottom feeds are the settled added manure and the algal 'rain'. The common carp appear to chew on the settled manure fragments whereas the tilapia vacuum the pond and thus their delta-carbon matches that of the whole substrate. Tilapia have been observed skimming the pond bottom and even chopping chironomids in half. We don't see this in common carp. We can surely all agree on where the available foods are. For example, the values for the filter-feeding silver carp match those of the microalgae almost perfectly. They will filter almost anything, but their delta-carbon values are most unlike those of the pond bottom. Now, when you add a fresh, highly flocculent manure like fresh pig or goose manure rather than a dried manure like dried chicken manure (or an aged manure like fragmented cow manure) then the seston changes and there is more of the added material in the water column. It would be nice if more of the added manure remained in the water column, but most settles to the bottom.

BOWEN: The cotton cloth method is a nice *in situ* technique, but all it gives us is a rate of activity. We would have to multiply this by the concentration of substrates available in the different parts of the pond to work out actual contributions to total production. It is a good approach to looking for sites of cellulose breakdown. The rate is clearly greatest at the sediment-water interface.

EDWARDS: A lot of organic matter does settle, but a pond is not such a continually static system. Settled material is being resuspended not only by the activities of fish but also by diurnal changes (stratification and destratification). There is a lot of mixing.

SCHROEDER: I don't agree. If this were so, we would expect to see it reflected in our delta-carbon values, but we don't.

COLMAN: You could probably check whether you are in fact getting as much microbial processing at the water-sediment interface as you propose, by measuring oxygen balances. Granted the anaerobic processes will not use oxygen, but the processing of their products will, once these move to the water column. In this way, if we assume a relatively steady state, we can regard the anaerobic processes as using oxygen indirectly. Now, if you work this out for your ponds, I don't think you will find enough oxygen demand in your system to account for the bacterial processing which you are proposing.

SCHROEDER: I know that there is a controversy at present, for between Howarth at the Marine Biological Labs at Woods Hole and Howes at Boston University. Howes' paper said what you are saying, that the products of anaerobic process should move up and be oxidized. But consider ammonium ion NH_4 and H_2S as typical products. These do not oxidize in the water column despite all the activity around, neither does methane.

COLMAN: Methane would form very low in the sediments.

SCHROEDER: I am just giving examples which run counter to the hypothesis that these highly reduced endproducts are re-oxidized in the water column. I suspect the truth is somewhere in the middle between some reduced endproducts remaining in the sediments and others coming up with oxidization of some products following.

COLMAN: I agree that NH_3 and H_2S would not oxidize exactly at the sediment-water interface. I don't think methane would be found very often.

SCHROEDER: What about free fatty acids? These are the most important endproducts of anaerobic processes in terms of their food value, just as in ruminant animals. Would these get oxidized? It is not clear to me that they would.

MORIARTY: Well certainly there is a balance between all these processes; free fatty acids are used by the bacteria that produce methane and sulfide. If NH_3 does come up into the water column, it will be oxidized by nitrifying bacteria. It's what they are waiting for!

SCHROEDER: But it may be used up first.

MORIARTY: Well, it may be taken up by phytoplankton or by bacteria that are degrading cellulose, but it *is* either oxidized or used up. Sulfide is oxidized by thiobacilli, etc., *Beggiatoa* and similar organisms. Therefore, we must distinguish here between measuring short-term and long-term processes. John Colman used the term 'steady state', but we are really faced with a dynamic equilibrium of interacting processes. It is difficult, therefore, to make deductions concerning oxygen demand. We need to understand the processes. Perhaps the balance is such that the system can support the level of production that Dr. Schroeder is proposing. This is in fact what we need to take from this conference. What levels of production can we get from these processes?

BILIO: Then let us now leave this topic for a later general discussion. Are there more specific questions for Dr. Schroeder?

PRUDER: In anaerobic processes, about 70% of the substrate conversion becomes usable biomass. However, to be used it must be oxidized by the users. Therefore, you cannot say that anaerobic production is advantageous because it does not consume oxygen.

SCHROEDER: Not entirely. For example, if the target animal takes in a volatile fatty acid, it can convert it directly into a lipid or a carbohydrate. It is not necessarily oxidized. There is an oxidation step in such a conversion and some CO_2 is released, but most of the carbon stays in the animal.

BLACKBURN: I agree that you do not often get a good balance between oxygen consumption and carbon dioxide production. In general, you get much more CO_2 production than oxygen consumption, the difference being due to anoxic processes that accumulate reduced products like ferrous sulfide or pyrite which are non-diffusible. These simply do not come up again.

SCHROEDER: But you are talking about products at the far end of these processes. What about the beginning? What about free fatty acids?

BLACKBURN: There is no buildup of free fatty acids!

SCHROEDER: I meant to say oligosaccharides.

BLACKBURN: I don't think your analogy of the pond as rumen is a good one.

SCHROEDER: Why?

BLACKBURN: The main problem is the time factor. The rumen turns over once every 24-36 hours whereas the pond turns over every month (or year) and is nonmixed. By this I mean there is no output. Therefore, the rumen has a fast fermentation which only goes partially to completion, that is to the volatile fatty acids. These are then absorbed and metabolized in the ruminant's liver. They are removed from the system. By comparison, the pond is a closed system. In the rumen, because of the short turnover time, bacteria with long generation times, like the methanogenic bacteria which utilize acetate, do not have time to grow. So acetate cannot be broken down into methane in the rumen. It can in a pond. Moreover in a pond, substrates like propionate and butyrate would be fermented further to acetate with the generation of hydrogen, which could then be removed by methanogens and finally the acetate itself would be decomposed to methane and CO_2. Therefore in a pond, you would get a terminal fermentation to methane and CO_2 compared to a partial fermentation to volatile fatty acids in a rumen.

SCHROEDER: You are not the first to disagree with my analogy. I accept what you are saying but I don't think you can take such a static view of a pond. While it is true that we drain ponds say, once every 120 days, we feed or fertilize daily or once every few days. If we input much less frequently, say every few weeks (as used to be the practice in Israel), the productivity goes right down. This says to me that something is happening in the pond that has a time cycle of the order of days. Whether it is two or four days is probably not critical. Also, you mentioned removal of fermentation products from the rumen. In the pond, there is also removal by the fish. The fermentation is not terminal in the pond (to CO_2 or methane) because it doesn't have time to get there.

BLACKBURN: That is supposition.

SCHROEDER: Except for the fact that it 'works'.

BLACKBURN: It would still 'work' with the process going to terminal fermentation.

SCHROEDER: No, if all the carbon went to CO_2 and methane, we wouldn't get the production that we do get.

BLACKBURN: But I am not saying that all the carbon goes to methane and CO_2. What I am saying is that what is *fermented* goes to these endproducts.

SCHROEDER: So you are saying this would leave the 'chopped up' polysaccharides, etc. for the animals to eat? The animals do not grow on manure. Remember if you only put chemicals in once a week and just grow algae, you get about half the fish production. If you only put manure into a concrete-lined pond and clean the bottom every day, you get low production. If you feed manure pellets, the fish don't grow. The organic matter input to the pond is important. Something is *happening* to it in the pond. I have demonstrated with isotope work that the carbon is used to produce fish, but indirectly—not by direct feeding. So there is 'conditioning' of the organic matter in the pond. This might be just microbial growth, although from Bowen's work the numbers look too low.

BOWEN: The detritus that I work with is not comparable.

SRINIVASAN: Even if you add manure daily, your pond is a batch culture whereas a rumen is a continuous culture. The pond is analogous to a mixed batch culture. You are adding nutrients at intervals over a 120-day cycle. You can have aerobic and anaerobic processing of accumulated or recycled material. It doesn't really matter which. It is probably mostly the bacteria upon which

the fish graze. This may account for your increased production with manure as opposed to other inputs.

BOWEN: It is clear to me that fish in ponds can derive a lot of their nutrients from direct digestion of microorganisms. It is quite conceivable that 10 or 20% of the organic matter in your ponds could be microorganisms, which we know can be digested with near—100% efficiency.

FRY: It serves no useful purpose to call the sediment a rumen. The contents of a rumen are completely mixed by muscular action. The pond sediment is always layered and has a complex and important spatial organization of microorganisms. By calling a pond sediment a rumen, you will encourage people to read up on ruminant processes and apply their principles to ponds. This will create all sorts of misconceptions.

ANDERSON: That's right, but we are getting caught in semantics. The original use of the analogy was to indicate increased availability of C and N through microbial activity. We need not throw away this analogy. In the rumen, of course, it is mostly microbial protein and Protozoa which go through to benefit the N requirements of the animal. This is a different pathway to the intake of fatty acids, but perhaps not unlike the pathways in the pond.

BILIO: I would like to suggest we leave this topic. All such analogies have their limitations.

SRINIVASAN: But we must be careful with the terms we use otherwise this will mislead microbiologists and aquaculturists.

ANDERSON: This is teleological.

BILIO: If you specify the limitations to the analogy, I think it is all right.

SRINIVASAN: Even then, I do not think you can say the pond can be compared to a rumen. All that one can say is that there may be anaerobiosis in the sediments and what this may lead to. I think this is a very bad comparison.

WOHLFARTH: Perhaps I can make the closing remarks on this rumen story. If Dr. Schroeder wishes to view the pond as a 'sunlit rumen' why should we spoil his fun? This is obviously semantics. A rumen is different to a fishpond. What Dr. Schroeder is really saying is that the fishpond is an extremely efficient converter of substances which are bad fish foods to substances which are good fish foods. The conversion efficiency of manure to fish is not as high as that for a high protein feed, but it is considerably better than that for a grain feed.

SCHROEDER: I have used the term 'sunlit rumen' not just 'rumen' in this context.

BLACKBURN: I think there is a serious misconception here about the role of anaerobic bacteria. There seems to be an assumption that they are going to create all sorts of useful compounds which will be available to other organisms. These are very 'hungry' bacteria! which can and do consume almost everything in reach. There is not going to be an accumulation of oligosaccharides or any other tasty compounds for fish to come along and eat. Anything that can be hydrolysed and fermented is going to be used up.

ANDERSON: That is a function of time. In the fishpond, you have serial inputs, therefore you must get partially degraded products appearing.

SRINIVASAN: Dr. Wohlfarth referred to conversion of materials like cellulose in the pond. The horse does the same thing. It does not have a rumen.

MORIARTY: Let's close this topic. Dr. Schroeder has drawn attention to the large amount of microbial activity at the sediment-water interface. What we now need is for microbial ecologists to investigate this in fishponds to quantify the various processes, both aerobic and anaerobic. I hope that we can formulate some proposals for such experiments before the end of this conference.

BILIO: Because it is a controversial subject, let us consider further the production and nutritional value of extracellular products. Would anyone like to comment further on Dr. Schroeder's statement that only 10% of the anaerobic microbial production is living cells, the rest is extracellular products. Is this acceptable?

MORIARTY: You cannot easily generalize on this. It depends upon the conditions, the nature of the substrates and the microorganisms present. It needs much more experimentation. From the literature, you can make some estimates of conversion efficiencies. I would like to discuss this further in more detail. It involves the conversion efficiencies of the bacteria into cellular and extracellular products, their respiration, etc. This varies with the type of organism and the C:N ratio of substrates. If there is a lot of N, they will put more into biomass than into extracellular products. If, however, there is very little N, they do tend to produce a lot of slime or internal storage compounds like poly-β-hydroxybutyrate which is a C-H-O compound. The short answer is, we cannot generalize.

BILIO: This is a useful statement nevertheless.

BLACKBURN: I would anticipate that the pond bottom material derived from manure or feed inputs will have a reasonably high N:C ratio. Therefore, the anaerobic bacteria will probably not be starved for N. Rather they may be starved for C. Bacteria which are starved for C generally do not put out a lot of compounds; they use what C they have for energy.

KIRCHMAN: Even if such bacteria did put out a large amount of polysaccharides, these would probably not be very digestible.

BILIO: Let us now discuss the degree of confidence we can place in the carbon isotope method.

PULLIN: For those of us who are not experienced in using the carbon isotope method, could Dr. Schroeder please comment on the confidence limits about his data points and the significance of differences between the values for different fish and feeds? How certain can he be that a fish is or is not ingesting (and of course utilizing) a given feed? This is crucial to our discussions, especially with respect to the whole rationale for polyculture. How different do delta-carbon values need to be before we can make significant conclusions?

BLACKBURN: It is a very reliable method and very useful in this context. It has been used very successfully in following the food webs of seagrass communities.

SCHROEDER: We have very good data showing the basic reliability of the method, including analysis of muscle biopsies from the same fish fed on different foods. The delta-carbon values of the fish match those of the foods very closely. For such work one should have at least a 10-fold weight gain in the fish and/or a 2-3 month residence time (considering the biological half-life of carbon) so that the carbon in the fish's body is representative of that of the food it is eating. Only one set of data has consistently been difficult to interpret: for prawns grown on soybean meal. This is toasted soybean meal, so there is no inhibition involved. The data for prawns and soybean meal lie well off the curve, which is a 45° line for all our other data covering a large range of foods and terrestrial and aquatic organisms. Perhaps the reason is that soybean meal is about half protein and half carbohydrate, so the delta-carbon values of these fractions are different. The rest of the data for the organisms we have looked at all fall within about ±1 or 1/mil. Their greatest deviation from the foods is about −1 or −2/mil. This means that the animal's carbon is slightly heavier than that of the food, i.e., heavier means less negative: it has more ^{13}C.

You just have to present a range of options. For example, two sources like a C3 and a C4 plant source are easily to interpret. However, a fish meal input would lie between C3 and C4. For an animal offered all three sources, we would take its delta-carbon value and work out the worst possible combinations of inputs, then give a range of possible utilization of each.

COLMAN: But what if one or more of your three sources has different delta-carbon values within it? For example, some work with kelp has shown differences as high as 15 or 10/mil in different parts of the same kelp frond.

SCHROEDER: We find this in alfalfa and corn, etc. also.

COLMAN: It has been suggested that this is because different biochemical components have different delta-carbon values.

SCHROEDER: Yes, like muscle and fat. Lignin is very negative. Cell solubles are very negative.

COLMAN: So, instead of three points, you may have, say, six.

SCHROEDER: Yes. It may be more. It may be ten. Alfalfa has been fractionated into cellulose, hemicellulose, lignin, total cell wall, total cell solubles and say into total protein and total carbohydrates. However, the delta-carbon values of animals *do* match those of the total foods they eat. For a corn eater, the value matches the total corn not the cell solubles or the cell wall. When you consider digestion, you can understand why, because cell wall material is digested and utilized just about as efficiently as cell solubles—contrary to the view that prevailed for a long time.

BOWEN: The significance of the differences you have presented between the delta-carbon values of fish flesh and feeds could probably be investigated by a simple analysis of variance of the data behind your summary graphs. Have you done this?

SCHROEDER: I have just calculated the standard deviations about the means of fish samples taken from the ponds.

BOWEN: A simple analysis of variance would give you an answer to the questions whether there are real differences between different treatments. You can define 'treatments' in various ways to test your hypotheses. Until you make such comparisons, we can't distinguish between your data and randomly distributed observations.

SCHROEDER: Oh. If you look at the spread of the data, the significance is obvious.

BOWEN: No, you cannot say that at all. We have no basis for concluding that any of your treatments are different until such comparisons have been made.

BILIO: Well, you have an idea if you have very small standard deviations.

BOWEN: It can give you an intuitive feel, but I would feel much more comfortable with a standard comparison.

PULLIN: Only if the data do not overlap can you avoid doing significance tests.

SCHROEDER: Let's pretend that there are no differences in the data. Then the animals don't look very different whether you put chicken manure or pelleted feed into the pond. Chicken manure is working just about as well as pelleted feed to grow common carp, tilapia and silver carp and prawns; as far as the origin of growth is concerned.

EDWARDS: As I recall, didn't you show by using this technique that grass carp do not derive any nutritional benefit from grass?

SCHROEDER: No, if you read our publications closely, grass carp in ponds where there is no grass present derive no nutritional benefits from grass. This applies to our ponds. Grass carp in China derive considerable nutritional benefits from grass.

EDWARDS: I must have missed this point.

WOHLFARTH: Concerning statistical tests, could this not be approached equally well by an analysis of regression as by analysis of variance? Wouldn't proving that there is a significant regression mean the same thing? Has it been done?

BOWEN: This would seem appropriate to me—if we are looking for significance of cause and effect.

FRY: Analysis of regression is completely different from analysis of variance. To check whether two means are significantly different you *must* do some sort of analysis of variance. If you have a complex data table, such as we have seen, and want to pick out different values and test for differences between them, you *must* use analysis of variance. All tests for differences between means are constructed in a similar way. You use tabulated values of functions which are multiplied by some function of the variance. I don't have such tables with me, but the values of such functions are often in the range 2 to 4. Now from the tables presented, the standard deviations of the delta-carbon values were usually about ±1, your variances are also about 1 (i.e., 1 x 1) and your functions will be (2 to 4) x 1 which = 2 to 4. Thus, two delta-carbon mean values will only be significantly different if the means are different by more than 2 to 4. So I would say you could be in a marginal area with respect to the significances of some of the differences between your means. It is, therefore, vital that you do an analysis of variance.

BILIO: Well, then let us recommend that analysis of variance be done on these data. We should also note Dr. Fry's caution of the dangers of making inferences from single measurements. Dr. Fry has also stressed the complexity of microbial interactions. We must discuss this further when time allows. We have now concluded our first round of discussions on basic aspects and can move towards hearing about aquaculture in the papers which follow. I have several points to make here. First, costs have been mentioned in passing. These are very important. When considering biology and technology, we must not forget economics. These are very important for application and extension of new production procedures (cf. Table 4 of my introductory overview). First, there are the economics of procedures—the costs of materials, transport, storage, pretreatment, application, removal of residues, etc. These should be monitored at an early stage in research and development and tested in a pilot phase. Second, there are various economic preferences in the context of rural development. Aquaculture may be the best option for use of certain detrital resources but this is not necessarily so. Third, there is the question of acceptability of the procedures and their products by the target groups in the farm operators and consumers. Fourth, there is the question of the different economies of operations of different scale.

Potential hazards are also very important (see Table 5 of my overview). On what we might call Level 1, these may be a threat to the microbial populations themselves from chemical (such as heavy metals) or microbial contaminants in pond inputs. Next, on Level 2, there may be a threat to the growth and survival of the cultured organisms from changing environmental conditions; for example, lack of oxygen due to decomposition processes. On Level 3, there may be health concerns for the consumers of aquaculture products from microbial and chemical contamination.

May I suggest the following question as an item for our later discussions on aquaculture: What to do with excess detritus that accumulates in ponds?—This is a common situation. There are at least three possible solutions. 1. In traditional Italian valliculture in brackish coastal lagoons, the pond bottom is sometimes plowed and harrowed in an inundated condition using a 'hydro-cultivator'. I have seen similar devices used in Taiwan on a smaller scale. 2. Draining and drying ponds is a well-known procedure in freshwater. However, in some brackishwater situations, like Italian lagoons, drying would seriously disturb the life cycles of some of the principal food organisms of important cultured fishes; for example, bivalves and polychaetes, which are important foods for *Sparus aurata*. Such ponds should not be drained and dried in an annual culture cycle. This option is only applicable for a cycle of more than one year, if ever. 3. The pond bottom deposits can be removed and used as a fertilizer in agriculture, but does this practice really produce benefits? We have one experience from a GTZ-project in which even darkly colored pond bottom sediments seemed to have almost no benefits as a land fertilizer. Or perhaps, the problem is the way that such sediments are applied. Perhaps direct application in horticulture and agriculture is too crude an operation. We can consider all these questions after the aquaculture papers.

Session on Productivity and Detrital Food Chains

CHAIRMAN'S OVERVIEW

J.S. Gray

Coming from Norway where aquaculture is concerned almost solely with the raising of salmon and trout, detritus has a rather different role than that in tropical systems. The Norwegian aquaculture industry is in a phase of rapid expansion from a present production of 30,000 t/year of salmon to over 120,000 t projected for 1990. Salmon are raised largely in nets in the sea and fed on pelleted food made from trash fish. Yet only 50% of the food is calculated as being eaten directly by the fish, the remainder goes through the net meshes and causes eutrophication problems in the surrounding areas. Thus efficient ways of utilizing this high quality food would be of great benefit to the economics of Norwegian aquaculture. Yet the pathways of detritus through the marine ecosystem are not well known and recent findings have radically altered our views on the structure of the system.

Steele's 1974 model of the structure of marine ecosystems assumed that phosphorus and nitrogen in the presence of sunlight allowed production of phytoplankton which was all consumed by zooplankton. Zooplankton feces sank to the seabed and was the principal food resource for the benthic system. Yet based on known standing crop biomasses of fish populations and benthic fauna the system was not in balance as the food resources were in short supply. The model served to focus attention on poorly understood parts of the system such as nutrient regeneration within the sediment and water column and measurement of sedimentation rates. In general, there was a lack of knowledge of the dynamics and fluxes of nutrients and energy through the system.

New techniques have greatly advanced our knowledge in the last few years. It was generally assumed that phosphorus recycled rapidly within the surface layers of the sea whereas the usually limiting nutrient nitrogen was recycled slowly. But studies of concentrations of nutrients alone cannot give information on dynamic aspects. With the application of ^{15}N techniques it was shown that nitrogen in fact could be mineralized very rapidly so that even if concentrations of zero nitrogen were found plants could still be capable of growing due to the almost instantaneous uptake of nitrogen produced for example by zooplankton excretion. Similarly although phosphorus concentrations in the water may be zero, by mobilizing phosphorus

stores plants may still be able to grow. Thus studies of limiting nutrients for phytoplankton growth are concentrating on fluxes of nutrients.

Much debate centers now on whether it is phosphorus or nitrogen that limits primary production in the sea. Freshwater systems are in general phosphorus limited whereas marine are nitrogen limited. Where the transition occurs from a freshwater phosphorus to a seawater nitrogen controlled system has not been adequately defined nor have the circumstances been defined under which marine systems at times are phosphorus and not nitrogen limited. This debate is far from academic as sewage treatment plants have traditionally been based on phosphorus removal which is much cheaper than nitrogen removal. If nitrogen is generally limiting phytoplankton growth then, where eutrophication is a problem in the marine environment (enclosed sea areas such as the Baltic and Mediterranean Seas and fjords), great expenses is likely to be incurred in controlling pollution.

New findings on the role of bacteria and bacteria-eating colorless flagellates have completely changed our views on the structure of the surface water systems. Rather than being the most important mineralizers of organic matter it is now felt that bacteria may be important competitors with phytoplankton for available nutrients. The bacteria are consumed by colorless microflagellates between 3 and 10 μm in size. The microflagellates in turn are consumed by microzooplankton where the primary mineralization is now thought to occur. This has been termed the "Microbial Loop" (Azam et al. 1983). Fig. 1 summarizes a generalized model of recent ideas on the feeding structure of the water column system. Zooplankton feeding on phytoplankton is known not to be very efficient and results in rupture of cells and leakage of dissolved organic matter into the water, termed "sloppy feeding". Measurement of sedimentation rates show that at most 50% of annual primary production is in fact eaten in the water column by zooplankton, the rest sedimenting to the seabed. Yet the process is not continuous and nearly all sedimentation in temperate areas occurs in a limited period of time in spring and autumn In polar regions, the coupling of primary and secondary production in time is less tight and a higher percentage sediments to the seabed in polar compared with temperate regions. In tropical open ocean systems, phytoplankton and zooplankton productions are probably tightly coupled throughout the year so that little material sediments to the benthos.

Whilst our knowledge of the water column system has altered radically, little attention has been given to the sediment. It seems unlikely, from the admittedly few studies that have been done, that colorless flagellates play the same role in sediments that they do in the water column. It is likely that in sediments ciliates and specialized bacterial feeding meiofauna are the important consumers of bacteria. The paper by Dr. Warwick addresses this problem.

Similarly, does mineralization of detritus by bacteria and bacterial consumers give a potential food resource for fish feeding in aquaculture ponds or is the energy conversion efficiency so wasteful that ciliates and meiofauna are undesirable competitors within the system?

Are there organisms that by special culturing methods can be used selectively to increase the efficiency of detrital systems? Such aspects have been little studied and undoubtedly require more research, but will be considered in this session.

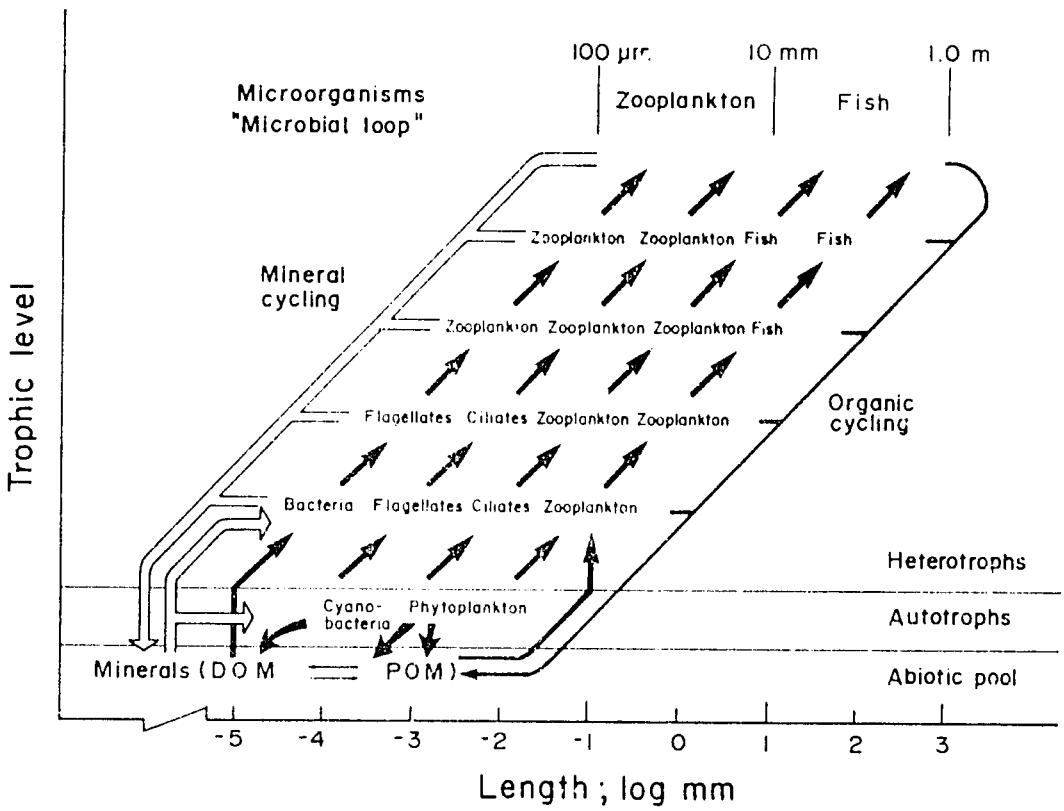

Fig. 1. Semi-quantitative model of planktonic food chains. Solid arrows represent flow of energy and materials; open arrows, flow of materials alone. It is assumed that 25% of the net primary production is channelled through dissolved organic matter (DOM) and the "microbial loop", bacteria, flagellates and other microzooplankton (e.g., ciliates); POM = particulate organic matter. It is further assumed that the most efficient predator:prey size ratio is 10:1, hence the slope of the lines relating trophic status to log body lengths is 1:1. The food chain base represents a size range over 3 orders of magnitude (smallest bacteria 0.2 µm, largest diatoms 200 µm; therefore, any trophic level will have a size-range factor of 10^3 and conversely each size class of organisms (100 µm) will represent at least 3 trophic levels. The thickness of open arrows (left) represents the approximate relative magnitude of minerals released in excretion at each trophic level; corresponding organic losses (feces, mucus, etc.) are shown on the right hand side (from Azam et al. 1983).

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Feeding Pathways and Environmental Constraints in Waste-fed Aquaculture: Balance and Optimization

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Abstract

The fertilization of fishponds with organic matter is a traditional method of raising fish in Asia but it produces a large percentage of the yield of the world's farmed fish. Methods of waste-fed aquaculture were developed empirically by farmers and have yet to be subjected to the close scientific scrutiny which could provide an impetus for their wider dissemination in developing countries. There is considerable controversy concerning the relative importance of the various feeding pathways in waste-fed ponds. The autotrophic pathway and the interdependence of the autotrophic and heterotrophic pathways are considered here. Various aspects concerning the autotrophic pathway are reviewed: a historical perspective of algae in waste-fed aquaculture in both East and West; gross and net primary production; pond fertilization; feeds of filter-feeding fish, with emphasis on phytoplankton; algal periodicity; fish kills; and pond balance and optimization. Filter-feeding fish are probably omnivorous but tilapia and silver carp are able to filter and assimilate planktonic algae. Digestion in tilapia is especially adapted for blue-green algal assimilation. Since blue-green algae comprise a better source of nutrition than other algae, it is suggested that studies be undertaken to stimulate the development of the blue-green alga *Microcystis* in waste-fed ponds stocked with tilapia. Grazed or harvested algal communities exhibit positive net photosynthesis, are net contributers to pond oxygen levels on a 24-hour basis and could be used as biological aerators in ponds used to grow high market value species of finfish, giant freshwater prawn *(Macrobrachium)* and penaeid shrimp.

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It is the custom of those who write concerning the food of fishes, to confine themselves solely to a discussion of animal food . . . . . it is time that the primary links of the food chain receive due consideration. The algae, and the animals which feed directly upon the algae, should be studied with all possible care, both as a scientific problem and as a basis for practice.

J.E. Tilden (1929)

Introduction

Traditional methods of raising finfish in Asia employ a wide variety of organic matter inputs as pond fertilizers (Ling 1967; Prowse 1967) and such methods produce a large percentage of the world's farmed fish yield (Wohlfarth 1978).

The practices of waste-fed aquaculture have been developed over a period of centuries by fish farmers by trial and error; although there is an impressive body of accumulated experience, particularly in China, the underlying mechanisms of operation are still poorly understood. Detailed scientific study would explain not only how such systems function, but in addition, should enable substantial increases in yield to be made through their optimization.

It is widely accepted that there are three basic feeding pathways by which pond organic matter inputs provide nutrition to fish: direct feeding of fish on the organic matter input and feeding along heterotrophic and autotrophic pathways which develop as a result of the fertilization of the pond by the organic matter input. There is a general consensus that all three feeding pathways are operative in a given aquaculture system although their relative importance has been the subject of intense debate. The heterotrophic pathway in particular has received considerable attention and it has been stated that it is of greater importance in waste-fed aquaculture than the autotrophic pathway (Schroeder 1977, 1978, 1980a; Wohlfarth and Schroeder 1979). To redress the imbalance in the literature, the autotrophic pathway is reviewed here in depth together with the interdependence of the autotrophic and heterotrophic pathways and an assessment of how they can best be coupled under the environmental constraints of the fishpond environment.

A Historical Perspective on the Importance of Algae in Waste-fed Aquaculture

There is a considerable dichotomy in both philosophy and pragmatism concerning the nutrient enrichment of waters in the East and West. There is a voluminous literature in the West on the eutrophication of water bodies (Anon. 1969; Likens 1972a). The word literally means "becoming well fed" and eutrophication implies an enrichment with nutrients and a consequent increase in biological productivity, particularly involving the development of large standing crops of phytoplankton, which are considered to be a nuisance. The literature is replete with references to undesirable phytoplankton blooms which may have several adverse effects on the

water: increased treatment costs to provide domestic water; decomposition of algae leading to unpleasant smells and occasional mass mortality of fish; the occasional production of toxins which kill birds and cattle, and which may cause gastroenteritis in man; and decreased recreational value for swimming and boating (Fogg 1969; Vallentyne 1974; Reynolds 1984a). At first sight it seems ironic that increased primary productivity of a water body would not be viewed as beneficial to fish, but eutrophication is associated with species change: in northerly latitudes more commercially valuable trout (salmonids) and whitefish (coregonids) are gradually replaced by perch and carps of a lower market value, although fish productivity does increase in response to eutrophication (Hasler 1947; Opuszynski 1978; Willemsen 1980).

Eutrophication is regarded as a natural succession of a water body in geological time due to weathering and nutrient buildup, but it is now recognized that the process has been accelerated considerably by man's influence, for which the terms artificial (Hasler 1947), or, more recently, cultural eutrophication have been coined (Likens 1972b; Reynolds 1984a). The ultimate cause of the present problem of eutrophication in the West was the decision in the mid-nineteenth century to adopt a water transport system for toilet waste, which broke the cycle of nutrients between man and the soil and led to a unidirectional flow of nutrients to receiving water bodies (Vallentyne 1974). The process has been exacerbated by the urbanization of the last 100 years and the recent development of intensive livestock rearing in feedlots, with associated substitution of inorganic fertilizers for organic manures in agriculture. Borgstrom (1973) has referred to these processes as "the breach in the flow of mineral nutrients", and has emphasized the need to channel organic wastes back into human food, with fish or livestock as an ultimate step; possible strategies to achieve this have recently been reviewed by Edwards (1980a).

In parallel with the increasing concern over eutrophication in the West, there has been the development of the mass cultivation of algae, which it was hoped would become an important human supply of protein (Spoehr 1951; Milner 1953). Yields of 20-25 g of dry algal biomass/m^2/day (73-91 t/ha/year) have been consistently attained over short periods of time, but because production costs are high, due mainly to harvesting and processing the algal cells, the product cannot compete with soybean meal; to date it is commercially profitable only as a "health" food and as an ingredient for pet fish food (Goldman 1978; Soeder 1980). Oswald began the "green trend" in sanitary engineering in the 1950s in which waste water replaced the "clean" culture media in the mass cultivation of algae, but despite the simultaneous treatment of waste water, reclamation of water, and production of a high-protein animal feed, harvesting and processing the algae in an economically viable way remains a major bottleneck (Shelef and Soeder 1980).

Ryther (1971) proposed to harvest the sewage-grown algae with a herbivore which itself could be used directly as food for man and/or animals, because harvesting algae was expensive by centrifugation or flocculation from the Oswald high-rate sewage stabilization pond. Rather surprisingly he wrote that "no such commonly used food

species exists in the freshwater environment", perhaps referring exclusively to the USA, but he considered bivalve molluscs as suitable herbivores for the marine habitat. Ryther and colleagues subsequently outlined the concept of "controlled eutrophication", an essential feature of which was the physical separation and compartmentalization of the producer and consumer levels (Ryther et al. 1972). They believed the separation was necessary because both primary and secondary production must proceed at constant and controlled rates, an impossibility for combined systems in temperate latitudes, where daily incident radiation varies by almost two orders of magnitude annually, and even daily by almost one order of magnitude, depending on the degree of cloud cover. Such perturbations would lead to wide variations in photosynthesis, upset the delicate balance between primary productivity and respiration, and even result in anoxia and mortality.

A two-stage pilot-project was built in the tropics where fluctuations in primary productivity are less than in temperate latitudes; the algae-laden effluent from a sewage-fed high rate stabilization pond was pumped through tanks containing the herbivorous fish *Oreochromis niloticus* (Edwards 1980b; Edwards and Sinchumpasak 1981). Although a linear relationship was established between fish yield and mean phytoplankton biomass up to 70 mg dry weight/l, the production of algae in the high-rate pond and its subsequent utilization by the herbivores could not be optimized simultaneously and a significant phytoplankton biomass passed through the system in the effluent (Edwards et al. 1981a). Subsequent studies (Asian Institute of Technology, unpublished data) have attempted to optimize fish production in a single, combined system consisting of an effluent-free pond modified for fish culture as well as for waste treatment, as recommended by Edwards et al. (1981b).

Eutrophication and aquaculture are intimately linked in many aquaculture systems in Asia; manures (livestock and/or human depending on the culture) comprise basic organic matter inputs in most traditional systems (Prowse 1967; Wohlfarth and Schroeder 1979; Edwards 1980a) and fish may be the cheapest form of animal protein when grown in such a way (Wohlfarth 1978). The best-known Asian system is the Chinese system of polyculture (Hoffmann 1934), the evolution of which was facilitated by the zoogeographic advantage of China: an outstanding diversification of the carp family including two efficient filter feeders, the silver carp *(Hypophthalmichthys molitrix)* and the bighead carp *(Aristichthys nobilis)* (Kafuku 1966). These fish were introduced to Southeast Asia at least 50 years ago, particularly Malaysia, Singapore and Thailand, and more recently have been widely disseminated (Welcomme 1981). Traditional fish culture in India is based on at least three species of fish that are to a large extent filter feeders: catla *(Catla catla)*, mrigal *(Cirrhinus mrigala)* and rohu *(Labeo rohita)*. Ponds with a pronounced development of plankton in Java are stocked with the filter-feeding tambakan *(Helostoma temmincki)* as the principal species, but polyculture is usually practiced with the inclusion of one or more of the following species which also have some ability as filter feeders: common carp *(Cyprinus carpio)*; tilapia *(Oreochromis mossambicus)*; nilem *(Osteochilus hasseltii)*; and tawes *(Puntius gonionotus)* (Vaas 1954). The planktivorous tilapias

from Africa, particularly *Oreochromis aureus* and *O. niloticus*, are most promising fish for the development of detrital fed systems and are being introduced throughout the tropics (Bowen 1982).

Aquaculture systems in the developed countries—in Europe, Japan and the USA—have evolved along different lines. No European fish possesses the ability to filter phytoplankton like the Chinese silver carp and only a few species exist in the world fish fauna (Opuszynski 1978). According to Huet (1972), cultured herbivorous fish of European origin are practically nonexistent. The common carp, a major cultured fish in Europe (and in some parts of Asia), is basically a bottom feeder, although some varieties are reported to feed on zooplankton (Kafuku 1966). The food and game fish of North America do not generally live directly upon algae (Tilden 1929) and there are no large plankton-feeding carps (Kafuku 1966). In fact, phytoplankton blooms are considered to have an adverse effect on cultured fish production in North America (Boyd 1979; Tucker and Lloyd 1984). There has been a developing tendency in recent years for dry concentrated feed, particularly in pelleted form, to replace fresh feed, and for an intensification of fish culture (Hickling 1971; Huet 1972). Methods now employed in developed countries resemble "feedlot" methods of raising livestock (Wohlfarth and Schroeder 1979). Fish culture in much of continental Europe and Israel, however, has changed over the past two decades from a monoculture of common carp to a polyculture of common carp and filter-feeding Chinese carps, and tilapia in warmer areas. With intensification of fish culture, filter-feeding fish have been introduced to utilize the large amounts of phytoplankton that develop which are not directly utilized by common carp, and this has led to increased fish yields (Reich 1975; Spataru 1977; Opuszynski 1978; Dimitrov 1984).

Primary Productivity

The fish production potential of plant-based feeding pathways depends firstly on the rate of plant productivity possible in a pond environment and secondly on the efficiency of capture and conversion of the plant material produced into fish flesh. Assessment of plant productivity rates in fishponds entails measurement of photosynthesis; simultaneous production of plant material and loss (through cropping, settling and cell death) rule out productivity rate measurement by direct observation of plant biomass change (Ryther 1956; Hepher 1975; Lund and Reynolds 1982).

Unfortunately, quantification of aquatic plant production through photosynthesis measurements is not straightforward. Both of the commonly used aquatic photosynthetic measurement techniques, ^{14}C and oxygen, are difficult to conduct correctly (especially when spatial and temporal integration is required, as for their use in productivity assessment) and are complicated by errors whose significance is still debated (Ryther and Vaccaro 1954; Oglesby 1977; Marra and Heinemann 1984).

Besides having methodological complexities, photosynthetic rate measurements are not easily converted to algal productivity values. In fishponds, photosynthesis measurements have been made almost exclusively by the oxygen light and dark

bottle method or some variation thereof. As was outlined when initially described (Gaarder and Gran 1927), measurements using the oxygen method lead to three productivity parameters, related as follows:

$$GP_a = NP_{wc} + R_{wc} , \qquad \ldots 1)$$

where GP_a is the gross productivity of the algae, NP_{wc} is the net productivity of the water column and R_{wc} is the respiration of the water column. However, investigators of algal use in fish feeding pathways agree that the most appropriate measure of algae productivity is net algal productivity (McConnell 1963),

$$NP_a = GP_a - R_a , \qquad \ldots 2)$$

which is greater than NP_{wc} by the amount of nonalgal water column respiration that occurs. Unfortunately, NP_a cannot be directly measured using the oxygen method but in any case is not much different from NP_{wc} when nonalgal respiration is small, as may be the case in some lakes, for instance. On the other hand, a large difference between NP_a and NP_{wc} may be expected in fishponds receiving large loadings of organic fertilizer, which would increase R_{wc}. As seen from Equation 1, an increase in R_{wc} for a given level of GP_a would cause NP_{wc} to decrease.

Gross Productivity

In part to avoid ambiguities associated with net productivity values, many results from fish-producing environments have been reported and compared simply on a gross productivity basis. Strong correlations have been found between fish production and gross primary productivity over a wide range of values (Melack 1976; McConnell et al. 1977; Liang et al. 1981), giving credence to the use of gross primary productivity as a measure of algal-derived fish food.

The large range of gross productivities cited in these correlation investigations and from other fish-producing environments confirm observations from algal biomass culture regarding light and nutrient requirements for maximum productivity (Goldman 1979) and from freshwater bodies in general regarding the importance of areal loading rates (Schindler 1978). Thus, Hepher (1962) found gross productivity in common carp ponds increased four to five times when fertilizer was added as compared to ponds with no addition. Hepher also reported that doubling the dose of fertilization did not increase gross productivity, presumably because of light limitation. Prowse (1972) found that gross productivity in five ponds stocked with tilapia and carp fertilized with phosphate (but not nitrogen) averaged 2.9 g C/m^2/day. In these inorganically fertilized ponds GP_a is generally greater than in lakes, even especially productive ones such as tropical Lake Lanao in the Philippines, where gross productivity was reported to average 2.4 g C/m^2/day (Lewis 1974).

The highest gross productivity levels have been reported from tropical and subtropical fishponds with organic fertilizer inputs. These include a temple pond, GP_a =

6-11 g C/m^2/day, and a fort moat, GP_a = 6.1-10.0 g C/m^2/day, in Madras State, India (Srinivasan 1964a and 1964b, respectively); septage-fed experimental tilapia ponds in Thailand, average GP_a = 6-7 g C/m^2/day (Colman and Edwards, unpublished data) and investigations in Israel: in one, tilapia were grown with manure and inorganic fertilizer, GP_a = 6.4 g C/m^2/day (Noriega-Curtis 1979); in a second, with manure, inorganic fertilizer and pelleted feed, GP_a = 5.5-11.1 mg C/m^2/day (Zur 1981).

On the basis of the deviation of his primary productivity data from previous regressions (McConnell et al. 1977), Noriega-Curtis (1979) concluded that feeding pathways based on manure carbon were used in addition to fish feeding on algal carbon. However, the difference in algae to fish conversion efficiency between managed ponds stocked with herbivores as compared to less managed ponds with natural fry recruitment could also be important. Hecky et al. (1981) also reported efficiencies comparable to those reported by Noriega-Curtis, but in which organic fertilizer was not a factor.

It is possible, of course, for ponds with large standing crops of algae to have large gross productivity (GP_a) and small net productivity (NP_a or NP_{wc}) if the algal biomass were static and algal respiration were high. The true measure of algal productivity available for fish consumption is net algal rather than gross algal productivity (McConnell 1963).

Net Productivity

Although net algal productivity cannot be directly measured using light and dark bottles, it is possible to estimate net algal productivity by subtracting from gross productivity values the 24-hour algal respiration, evaluated by the product of algal standing crop and respiration rate constant. In a series of 4-5 m^3 concrete tanks loaded with septage between 6 and 9 g COD/m^2/day, GP_a was 7.1, NP_a was 2.2 and NP_{wc} was 1.2 g C/m^2/day. Thus, tank values of NP_{wc} underestimated net algal productivity by a factor of 1.8. Net productivity values were measured over a 24-hour period rather than only over the light period so that even NP_a was only 30% of GP_a (Colman and Edwards, unpublished data).

As appears to be the case for GP_a, NP_a measured by the above method was found to be greater in tanks with both organic and inorganic inputs than in tanks with only inorganic fertilizer (Asian Institute of Technology, unpublished data). Schroeder (1978) compared NP_{wc} values in inorganically and organically fertilized ponds stocked with tilapia with ponds which received only inorganic inputs. Daily oxygen increases during morning hours, a measure of NP_{wc}, between the two systems were similar, which he felt indicated that the net photosynthetic productivities of the systems were equal. He concluded that increased fish yield in the organic-fed system was supported by manure rather than algal carbon. However, the equal rates of oxygen increase imply that NP_a of the manured pond was greater than the NP_a of the pond without manure because one would expect the respiratory oxygen demand to be higher in the manured pond with more heterotrophic organisms.

Where organic fertilizers have not been used, NP_{wc} may approximate NP_a. Hepher (1962) and Prowse (1972) found NP_a values of 2.9 and 2.5 g C/m^2/day for inorganically fertilized ponds on a light-period basis. These values are considerably lower than the highest sustained NP_a level found in a septage loaded tank, 4.0 g C/m^2/day, on a 24-hour basis (Asian Institute of Technology, unpublished data). Although this value was by no means the theoretical maximum for net photosynthesis, which is 15-20 g C/m^2/day according to Goldman (1979), it may have approached the practical upper limit. Indeed sustained yields from algal biomass culture systems are only marginally higher, falling in the range of 5-10 g C/m^2/day (Goldman 1978).

Pond Fertilization

There is a voluminous literature on the fertilization of fishponds with myriad conflicting viewpoints concerning the type of nutrients required, the amount of fertilizer, the relative merits of inorganic and organic fertilizers, and the method and frequency of application. This is reflected by the review of North American pond fertilization by Gooch (1967), who concluded that "the art of pond fertilization is in such a primitive state . . . we are essentially no closer now to predicting results of fertilizer practices than we were 37 years ago". It is beyond the scope of this review to consider the various aspects of pond fertilization in detail, but a brief overview is germane to the subject of this paper.

It is now appreciated that both N and P need to be added to fishponds to stimulate primary productivity for more intensive fish production in the tropics based on natural food (Woynarovich 1975). The N:P ratio in phytoplankton may vary from $< 1.5 : 1$ in nitrogen deficient cells to $> 15 : 1$ in phosphorus-deficient cells by weight, according to nutrient availability and species (Ryther and Dunstan 1971). However, phytoplankton have a remarkable consistency in chemical composition, measured as % dry weight, if nutrients are provided in excess and light is the growth-limiting factor: approximately 45-50% C, 8-10% N and 1% P, i.e., a C:N:P ratio of about 50:10:1 by weight (Goldman 1980). Many earlier studies in Europe and Asia led to the conclusion that nitrogen was not necessary even though ten times more of it by weight than phosphorus is needed for phytoplankton growth. Only phosphorus was considered to be needed (Hickling 1971; Lin 1968), probably because the stocking density of fish in the pond was low and so little P was added that nitrogen fixation by pond biota was sufficient to provide the required need for nitrogen. Lin (1970) even went as far as to caution against the use of nitrogenous fertilizers because with their addition to the pond "blue-green algae will become lazy" and not fix nitrogen, and nitrogen added to the water would be lost through reduction by bacteria. However, even in eutrophic lakes with an excess of phosphorus, annual nitrogen fixation rates extrapolated from relatively short periods of blooms of nitrogen-fixing algae were 44 kg/ha/year (Horne and Viner 1971), 34 kg/ha/year (Ashton 1979) and 5.9-130.5 kg/ha/year (Ashton 1981), an insufficient amount to maintain a relatively intensive plankton-feeding fish system. El Samra and Olah

(1979) did not consider nitrogen fixation to be significant in fishponds because they recorded a rate of only 5.7 kg N/ha/growing season.

Relatively little attention has been given to carbon in fishpond fertilization studies even though it is the most important nutrient by weight in phytoplankton. This is due to an overemphasis on NPK, caused by comparison with agriculture (in which CO_2 is derived from the air) without consideration of the special conditions in the aquatic ecosystem (Woynarovich 1980). In view of the importance of carbon in fishpond fertilization, Woynarovich (1956) referred to the addition of manure and the subsequent bacterial degradation with release of CO_2 for phytoplankton growth as the "carbon manuring method".

There is controversy in the literature concerning the relative merits of inorganic and organic fertilization. During the 1960s there was a campaign to promote the use of inorganic fertilizers in Taiwan because they are easy to manage and hygienic (Lin 1968; Lin 1970) but organic fertilizers are still used almost exclusively in fish culture on the island today, due to the relatively high cost of inorganic fertilizers following the increase in the price of oil, and a continuation of integrated livestock/fish farming. Although both inorganic and organic fertilizers are used in Israeli fish culture, it has been reported that yields with inorganic fertilizers rarely exceed 10 kg/ha/day compared to 30 kg/ha/day with intensive organic fertilization because, the author believed, there is light limitation of phytoplankton that limits fish yields with the former, whereas with the latter filter-feeding fish can rely to a greater extent on heterotrophic organisms which are not light dependent (Schroeder 1977; Schroeder 1978). Hepher (1962) demonstrated the dependence of primary productivity on both nutrients and light; in a "standard" fertilized fishpond primary productivity was higher than in a "double" fertilized pond because in the latter regime higher concentrations of algae in the upper layers of water shaded the lower water layers, which led to a lower total water column primary productivity in the latter compared to the former regime. However, as pointed out by Reich (1975), a fertilized fishpond stocked with filter-feeding fish is a dynamic system; silver carp reduce the self-shading of phytoplankton by continuous filtration of algae, which allows light to penetrate to the deeper layers of the pond so that fertilizer can be utilized throughout the water column and in quantities which would not otherwise be utilized. Yashouv and Halevy (1972) obtained only small differences in the yield of common carp *(Cyprinus carpio)* and tilapia *(Oreochromis aureus)* between weekly and fortnightly applications of the "standard" fertilizer dosage but the yield of the phytoplankton filter-feeding silver carp was increased by 27% with the higher dosage of inorganic fertilizer. Filter-feeding fish are able to feed on dense blooms of phytoplankton in surface waters in heavily fertilized ponds, increase the light penetration into the water and thus stimulate both net primary productivity and fish growth.

Much of the apparently contradictory advice regarding fertilization can be attributed to the variety of culture systems to which fertilizer inputs have been added. Fish not feeding directly on phytoplankton may require different fertilizer rates and element ratios than those which do. If it could be agreed that maximum algal produc-

tivity were the goal, then fertilization schemes already worked out in algal biomass culture could be implemented; some have already been adapted to utilize organic wastes commonly used in aquaculture, with inorganic supplements of nutrients where necessary (Becker and Venkataraman 1982). Primary production rates measured in many fishponds fall somewhat below those routinely achieved in algal biomass sytems, so that implementing the techniques of the latter could be expected to improve production rates in fishponds. However, some of the differences between algal biomass and fish culture systems must doubtless be retained. Fishponds must be deeper than those for algal biomass culture. Amounts and ratios of fertilizers may differ because in algal biomass culture the algae and their nutrient contents are entirely removed from the system at harvest; in contrast, nutrients in algae harvested by fish are partly recycled back to the pond through fish excretion.

The problems that muds in fishponds are said to cause for phosphorus fertilization may be overstated. Earthen ponds with a history of phosphorus fertilization, given initial K_2HPO_4 doses of 12 g P/m^2/day and subsequent additions of 0.14 g P/m^2/day, maintained water column concentrations of 5-10 mg/l of soluble reactive phosphorus (Colman and Edwards, unpublished data). Investigators have reported variously that muds strongly absorb phosphorus (Boyd and Musig 1981) or that muds can be a source of phosphorus for pond algae (Hepher 1966). In fact, whether pond muds are sources or sinks probably depends primarily on whether they are aerobic or anaerobic as well as upon their past history of exposure to phosphorus. Holdren (1974) described the annual rise and fall of phosphorus concentrations in interstitial sediment waters of lakes according to whether phosphorus-absorbing metal oxides were present, or whether these were dissolved under anaerobic conditions. Syers et al. (1973) showed that the type of absorption of phosphorus that occurs on muds is reversible, even at high interstitial phosphorus concentrations, i.e., it is not a chemical precipitation by which phosphorus concentrations are lowered. Although movement of phosphorus to pond sediments inevitably occurs, it is probably caused by settling of phosphorus-containing organic material rather than direct absorption of phosphorus onto the pond mud, at least in cases of ponds with histories of phosphorus fertilization. Furthermore, as the organic material is remineralized at the pond bottom, the contained phosphorus is released back to the water column. The remineralization and diffusion from interstitial waters and thence to the water column is incomplete. This explains, together with phosphorus being exported from ponds in fish, why it has been found that highly productive fishponds require phosphorus addition even after many years of use (Metzger and Boyd 1980).

Feed of Filter-Feeding Fish

There is considerable confusion in the literature concerning most aspects relating to the feeding of filter-feeding fish: the type of organisms consumed, the mechanisms of consumption of plankton and the digestibility and nutritive value of plankton.

Types of Organisms Consumed

It has been the usual practice to divide fish into different types according to their natural nutritional habits, i.e., fish that feed on phytoplankton, or zooplankton, or benthic animals, or macrophytes, or detritus, but this distinction between the different feeding habits may not be absolute. A certain species of fish may prefer a given type of feed if it is abundant, but be able to adjust to food from other sources if the amount of preferred food diminishes (Reich 1975; Schroeder 1980a). A study by Spataru et al. (1983) elegantly demonstrated an inverse relationship between the type of plankton preferentially consumed and the predominant plankton type present in ponds stocked with silver carp (preferential phytoplankton feeder), bighead carp (preferential zooplankton feeder), or their interspecific hybrid; the amounts of phytoplankton and zooplankton found in the guts were 88-95% and 4-7%, 0-2% and 75-95%, and 28-55% and 32-63%, for silver carp, bighead carp and their hybrid, respectively; the proportional amounts of phytoplankton in pond water stocked with silver carp, bighead carp and their hybrid were 12-33%, 54-99%, and 17-77%, respectively.

There are conflicting reports in the literature concerning the selectivity of specific genera of phytoplankton by silver carp. Chiang (1971) reported that silver carp were selective about their food, favoring blue-green algae over green algae and showing an aversion to diatoms. However, Lin (1969) stated that while silver carp ingested principally phytoplankton and detritus particles but very little zooplankton, they did not appear to have a mechanism to select food but ingested whatever was available in the water, provided that the sizes of the food particles were in the correct range; a bloom of three genera of blue-green algae developed in one pond and this was reflected in a higher percentage of these algae in the digestive tract at that time. This conclusion was supported by a study by Cremer and Smitherman (1980), who reported that although silver carp consumed primarily phytoplankton (range of particle size 8-100 μm, majority 17-50 μm), the genera in the water samples were found in proportionate amounts to samples from the guts, indicating no selectivity for specific types of phytoplankton; seasonal variations in the genera were reflected in the gut contents, with the exception of the green alga *Selenastrum*, but its absence could have been due to its small size, usually less than 10 μm.

However, the potential diversity of the feeding habit of silver carp in the absence of the preferred phytoplankton diet has been reported by several workers. According to Borucki (cited by Opuszynski 1978), detritus carried by the waters of the River Amur, the natural habitat of silver carp, in spring and early summer constitutes more than 90% of the feed of the fish; in summer during intensive blooms of algae the amount of detritus declines and phytoplankton constitutes the basic feed component; but towards the end of September when algal blooms disappear, detritus again constitutes 60-100% of the feed of the fish. There are also several reports in the literature of the gut contents of silver carp from ponds consisting mainly of detritus (Opuszynski 1981; Bitterlich and Gnaiger 1984) and of containing signifi-

cant numbers of zooplankton (Lin 1969; Opuszynski 1972, 1978; Bitterlich and Gnaiger 1984).

There are also reports of considerable latitude in the feeding habits of the herbivorous tilapias (Bowen 1982). *Oreochromis aureus* had guts full of the dinoflagellate *Peridinium cinctum* during February to June when the phytoplankton was dominant in the water of the Tsalmon Reservoir in Israel, but from July to January, it fed on the bottom covered with inorganic sediments, organic matter, as well as diatoms and green algae (Leventer 1973). Spataru (1978) reported that the absence of a dense growth of aquatic macrophytes in Lake Kinneret, Israel, led to a change in the typical macrophyte feeding habit of *Tilapia zillii;* gut analyses revealed a great variation in food, both animal and plant, from the plankton and the benthos. A recent study by Maitipe and De Silva (1984) demonstrated considerable trophic plasticity in *Oreochromis mossambicus* populations in twelve manmade lakes in Sri Lanka, involving feeding on phytoplankton, zooplankton and detritus.

Detritus originates directly from plant biomass, or secondarily from animal feces which may comprise a large amount of undigested plant material, and is colonized by a community of bacteria and fungi as primary degraders, and also Protozoa which consume the bacteria (Pomeroy 1980). Fish which feed on detritus presumably derive nutrition from the detrital community, including the bacteria (Bowen 1976; Kuznetsov 1977; Opuszynski 1981).

Mechanisms of Consumption of Plankton

There is considerable confusion in the literature concerning the size range of plankton that can be consumed by filter-feeding fish, due in large part to our incomplete knowledge concerning filter-feeding mechanisms. This has had important repercussions on attempts to optimize fish production in waste-fed systems. Schroeder (1978, 1980b) and Wohlfarth and Schroeder (1979) stated that silver carp were unable to harvest 90% of the primary productivity in Israeli fishponds because the phytoplankton were too small to be harvested by the gill rakers of the fish. They hypothesized that the direct consumption of phytoplankton by fish can account for only a small fraction of the fish production in a manured pond and that most phytoplankton must be "recycled to increase its size to a harvestable dimension" (Wohlfarth and Schroeder 1979). They reported various minimum particle sizes as filterable by silver carp (20-50 μm, Schroeder 1978; 30-40 μm, Schroeder 1980a; 20 μm, Schroeder 1980b; and 40 μm, Wohlfarth and Schroeder 1979): all at odds with the conclusion of Spataru in a paper cited by the above writers, who wrote that "the special pattern of its gills, which are adapted to retain suspended organisms and particles of sizes less than 20 μm, enables silver carp to filter enormous quantities of phytoplankton and organic particles" (Spataru 1977). However, in a later study of a fish-prawn polyculture system receiving manure as the only pond input, Schroeder (1983a) attributed 60-80% of the growth of tilapia and 100% of silver carp to the photosynthetic food web.

There are various references in the literature that silver carp filter nanoplankton. Lin (1969) reported that silver carp is most likely to be capable of filtering particles less than 10 μm with its broad, sponge-like gill plates. Yashouv (1971) demonstrated in aquaria that silver carp fingerlings were able to filter nanoplankton very effectively. Reich (1975) also reported that the anatomical structure of the gills enables silver carp to filter nanoplankton. However, Januszko (1974) and Spataru et al. (1983) both commented that it was difficult to appreciate how silver carp could filter *Chlorella minutissima* with a cell diameter of 3 μm and *Chlamydomonas* sp. with a cell diameter of about 10 μm, respectively.

Despite several studies on the structure of the filtering apparatus of the silver carp (Boulenger 1901; Fang 1928; Verigin 1957; Zambriborshch 1957; Wilamovski 1972) our knowledge of the functioning of the filtering apparatus is still incomplete (Cremer and Smitherman 1980; Jirasek et al. 1981/82). Kuznetsov (1977) has suggested that the labyrinthiform organ (suprabranchial organ) of the fish produces mucus to which small particles, even isolated bacteria and small bacterial aggregates adhere, and that these are then aggregated into larger masses, and then swallowed; microscopic examination of the silver carp guts revealed large quantities of bacterial aggregates, and those in the foresection of the guts were almost invariably surrounded by slime. Plankton-feeding tilapias appear to filter particles by a combination of physical filtration involving gill rakers and fine teeth on the pharyngeal bones (Bowen 1982) and entrapment of particles in mucus (Greenwood 1953). According to Greenwood (1953), suspended phytoplankton are drawn into the buccal cavity where they become entangled in mucus secreted by cells in all areas of the oral and pharyngeal epithelium, the dorsal bucco-pharyngeal cavity being thrown into low folds to increase the surface area; mucus and food aggregates are carried posteriorly but the gill rakers prevent them from escaping with the outgoing current.

The milkfish, *Chanos chanos*, is another species of fish in which a lack of appreciation of its ability to filter plankton may have limited its exploitation as a food fish. Traditionally it has been cultured in shallow brackishwater ponds and encouraged to browse on benthic algal pastures (Chen 1976). Chacko (1945) reported 40 years ago that milkfish fingerlings collected from estuarine waters in India and stocked in freshwater and brackishwater ponds and reservoirs, fed on plankton (based on gut content analyses), but only during the past decade has a significant method of culturing the fish based on its plankton feeding mode been developed, the milkfish pen system in the shallow waters of Laguna de Bay, Philippines (Pullin 1981; Delmendo 1982). Early studies on the mechanism of feeding in milkfish revealed that it has gill rakers and an epibranchial (suprabranchial) organ lined with an epithelium containing mucus cells (Chacko 1945; Kapoor 1954). The function of the epibranchial organ in digestion has been confirmed by more recent studies (Bertmar et al. 1969; Kapoor et al. 1975). A system of milkfish culture in "deep" freshwater ponds has recently been developed in Taiwan but is based on pelleted feed (Su and Ting 1980; Horng 1981); neither farmers nor fish biologists in Taiwan apparently believe that milkfish are able to filter phytoplankton, although it is likely that the fish do derive some

nutrition from the phytoplankton in the "deep" water ponds because the water color is indicative of significant phytoplankton biomass (P. Edwards, pers. obs.).

Nutritive Value and Digestibility of Plankton

Although fish may directly consume some of the manure added to ponds, it has been shown that it is a poor feed due to relatively low contents of metabolizable energy and true protein, but the nutrients and organic carbon contained in the manure lead to the production of natural food (autotrophic and heterotrophic organisms) of high nutritional value (Schroeder 1977; Wohlfarth and Schroeder 1979; Schroeder 1980a). The relative nutritive values of the various types of plankton, particularly phytoplankton, have given rise to a great deal of controversy, and several major questions still remain to be resolved. Initially it was believed that certain species of phytoplankton were indigestible to fish and frequently emerged from the alimentary canal alive and moving or that they could be cultured after passing through the fish (Fish 1951; Prowse 1961). The cell contents of diatoms were reported to be digested by nearly all plankton-feeding fish, including silver carp and *Oreochromis mossambicus*, because the diatoms have cell walls with a series of perforations through which enzymes can penetrate easily, but planktonic green algae, euglenoids, and most blue-green algae including *Anabaena*, *Microcystis* and *Oscillatoria* were reported to be indigestible (Prowse 1961, 1966a, 1966b). Prowse (1969) believed that besides diatoms, plankton-feeding fish could freely digest any cells which break open easily, such as the dinoflagellate *Ceratium*, but not phytoplankton with copious mucilage (blue-green algae), cellulose walls (green algae), or firm periplasts (euglenoids). Prowse (1966a) did not recommend the traditional use of sewage or manure in fishponds because he believed it led to blooms of indigestible algae and considered that the development of such algae made it necessary to provide supplementary feed in Chinese carp polyculture (Prowse 1966b).

The myth that blue-green algae cannot be digested by filter-feeding fish was finally laid to rest by an elegant study using ^{14}C on the assimilation of carbon from phytoplankton by herbivorous fish (Moriarty and Moriarty 1973); during a study of the productivity of Lake George, Uganda it was found difficult to reconcile the high productivity of herbivorous fish with the dominance of blue-green algae in the lake. It was shown that tilapia were able to lyse the cell walls of blue-green algae by relatively low gastric pH values of less than pH 2 (Moriarty 1973; Moriarty and Moriarty 1973). However, good fish growth in waters dominated by blue-green algae had been recorded previously in India (Ganapati 1940; Srinivasan 1964a, 1964b), Sri Lanka (Mendis 1964) and more recently in Laguna de Bay, Philippines (Delmendo 1974; Lee et al. 1983). Analyses of successive portions of the guts of *Tilapia zillii* in Israel revealed that it had the capacity to disintegrate gelatinous colonies of blue-green algae, especially *Microcystis*, and thus feed on them (Spataru 1976). Recent work in China has indicated that tilapia can digest both green and blue-green algae (Zhang Zong-She, pers. comm.).

The literature on the digestibility of phytoplankton by silver carp is somewhat confused. In Russia, diatoms were found to be the most preferred and easily digested algae, followed by the euglenoid *Euglena* and the green algae *Coelastrum* and *Pediastrum*, but blue-green algae such as *Anabaena*, *Coelosphaerium*, *Merismopedia* and *Oscillatoria* were considered to be indigestible; the fish were reported to tend to spit out filtered material consisting mainly of blue-green algae (Savin, cited by Lin 1969). Silver carp were reported to grow more slowly in a pond with abundant blue-green algae than in one with more abundant green algae and diatoms (Lin 1969). Sparatu (1977) observed that only organic particles and the green alga *Scenedesmus quadricauda* were food for silver carp and that ingested euglenoids *Euglena* and *Phacus* remained whole and retained their pigmentation throughout the gut. According to traditional Chinese experience, the suitability of ponds for raising fish is based in part on the color of the water: the best is brown due to the cryptomonad *Cryptomonas*, followed by green water caused by green algae such as *Scenedesmus* and the euglenoid *Euglena;* diatoms are also considered to be good feed for silver carp but are not abundant in fertilized ponds. Blue-green water due to the growth of blue-green algae is regarded as too fertile, possibly because of the danger of low dissolved oxygen concentrations at night, rather than a belief that blue-green algae are indigestible (Zhang Zong-She, pers. comm.).

However, there are reports in the literature that silver carp consume blue-green as well as green algae. Chiang (1971) reported that silver carp digested *Microcystis* and green algae more easily than diatoms, based on an analysis of gut contents. Ghosh et al. (1973) analyzed the guts of silver carp raised in a sewage-fed pond in India and reported that the gut contents were dominated by blue-green algae (61%) and organic detritus (15%); the cell contents of the blue-green alga *Oscillatoria*, which was predominant in both the water and the guts, were observed to be completely absorbed in the posterior gut. *Microcystis* was also dominant in plastic enclosures in a eutrophic pond and in the gut contents of silver carp stocked in the enclosures (Kajak et al. 1975). Recent data from China indicate that silver carp can digest and absorb *Scenedesmus*, *Euglena* and *Microcystis* (Zhu 1982; Zhu and Deng 1983).

There is a general consensus of opinion that fish can digest zooplankton but our knowledge is far from complete concerning the digestibility of bacteria and phytoplankton. Both Kuznetsov (1977) and Opuszynski (1981) reported that silver carp consume bacterioplankton, but the mechanism of digestion remains obscure because vertebrates lack gastric enzymes capable of attacking the prokaryotic cell wall (Bowen 1982). Moriarty (1973) first described the role of gastric acid in the lysis of blue-green algae (cyanobacteria) in *Oreochromis niloticus* and Bowen (1976) demonstrated *in vitro* that the same mechanism allows *Oreochromis mossambicus* to digest bacteria associated with detritus. However, it is not yet known how silver carp can digest bacteria and blue-green algae, nor how silver carp and tilapia can digest green algae with cellulosic walls (Fish 1960; Stickney and Shumway 1974).

There are relatively few quantitative data concerning digestibility of algae by fish, and most relate to feeding dried algal meal. Nose (1960) fed goldfish *(Carassius*

auratus) diets containing various ratios of the green alga *Chlorella* and potato starch and estimated that about 48% of the protein of the alga was digested. Channel catfish *(Ictalurus punctatus)* fingerlings fed on a diet containing 25-75% of five algal genera (green algae *Chlamydomonas, Chlorella, Chlorococcum* and *Chlorogonium* and the euglenoid *Euglena*) gained as much weight as control fish fed a 100% commercial feed, although larger fish of 20-25 cm fed the algae-supplemented diet gained less than control fish in 10 weeks (Reed et al. 1974). Diets enriched with the green alga *Scenedesmus* led to better growth than conventional fish diets at levels up to 50% and 80% for fingerlings of common carp *(Cyprinus carpio)* and grass carp *(Ctenopharyngodon idella)*, respectively (Meske and Pfeffer 1978). Apparent protein digestibilities of algal meal (green algae *Ankistrodesmus, Oocystis* and *Scenedesmus* and the euglenoid alga *Euglena*) by common carp, *Cyprinus carpio* ranged from 69 to 85%, lower than for fish meal but similar to that of oil meals (Sandbank and Hepher 1978; Hepher et al. 1979). According to Hepher et al. (1979), the use of algal meals in fish diets will depend only on its cost of production, harvesting and processing.

Since the mass cultivation of algae as feed has yet to be demonstrated as economically viable, data concerning feeding fresh algae to fish are of greater interest. Stanley and Jones (1976) fed wet concentrates of the blue-green alga *Spirulina* containing 96% water to bigmouth buffalo *(Ictiobus cyprinellus)* and tilapia *(Oreochromis aureus)* in aquaria and obtained a relatively low food conversion ratio of 2.0 (dry concentrate:wet fish weight gain). However, the ingestion and assimilation efficiencies of algae fed to fish as concentrates may differ from those for algae filtered from the water naturally by the fish. Moriarty and Moriarty (1973), using ^{14}C, determined the assimilation (apparent digestibility) of carbon by *Oreochromis niloticus* and reported maximum values ranging from 67 to 82% for the blue-green algae *Anabaena* and *Microcystis* and 78 to 81% for the diatom *Nitzschia* but lower values ranging from 45 to 52% for the green alga *Chlorella*. Mean daily assimilation efficiencies for blue-green algae and diatoms in the lake water were in the range of 45 to 60% (Moriarty and Moriarty 1973). Edwards et al. (1981a) reported food conversion ratios (dry weight algae:fresh weight fish basis) ranging from 1.3 to 2.5 at maximum fish yields for *Oreochromis niloticus* raised in outdoor concrete tanks through which the algae-laden effluent of a sewage-fed high rate stabilization pond was pumped. The ratios were computed using the phytoplankton biomass retained in the tanks (the difference between the biomasses in the influent and effluent) and were relatively crude estimates, because algal productivity, predation by zooplankton and sedimentation rate were not determined. Furthermore, the fish may have derived nutrition from detritus remaining in the high rate stabilization pond effluent.

These experimental constraints have been overcome to a large extent by the simultaneous culture of phytoplankton and tilapia in water enriched with inorganic fertilizers in outdoor concrete tanks. It was necessary to introduce organic carbon initially to induce the blue-green alga *Microcystis* to grow, but it was calculated that the organic carbon was not very significant in fish growth (Colman et al., unpub-

lished data). Feeding rate was estimated from the sum of algal disappearance during the fish growth period (57 days) and the computed net algal productivity, NP_a. Cultures in which either *Microcystis* or *Scenedesmus* predominated were established, three tanks for each algal genus with adequate water quality for fish growth. Fish biomass increase was 3.7 g/m^2/day (wet weight) in the *Microcystis*-dominated tanks, more than three times the rate of increase of only 1.1 g/m^2/day in the *Scenedesmus*-dominated tanks. Feed conversion ratios for the *Microcystis*-dominated tanks were a low mean of 2.0 (dry weight algae:fresh weight fish basis); the feed conversion ratio for the *Scenedesmus*-dominated tanks could not be calculated because the formation of oxygen bubbles in the water precluded the measurement of primary productivity (Colman et al., unpublished data).

Algal Periodicity

Genera Involved

An appreciation of which phytoplankton genera are likely to predominate in waste-fed aquaculture systems is a prerequisite to the optimization of fish production because of evidence that the digestibility and nutritional value of algae may vary. In contrast to higher plants, freshwater phytoplankton generally have a cosmopolitan or ubiquitous distribution with many species found in all parts of the world in a variety of habitats from polar to tropical regions (Smith 1950; Whitford 1960; Reynolds 1984a, 1984b). No correlation has been reported between which species became dominant in sewage oxidation ponds and their geographical location (Fitzgerald and Rohlich 1958).

The composition of phytoplankton communities often undergoes continuous changes, which operate over much shorter time scales than in terrestrial environments. The term "periodicity" is preferable to "succession" when applied to seasonal phytoplankton community changes because in plant ecology the concept of community succession (which may include an aquatic phase) embodies a more or less unidirectional change from a "pioneer" through a distinct series of successional stages to a "climax" community (Reynolds 1984a). The terrestrial stages of succession are characterized by increasing species diversity and biomass but declining productivity (Odum 1969; Reynolds 1984a), whereas a change from an oligotrophic to a eutrophic water body is more likely to involve decreasing species diversity associated with both increasing biomass and productivity of phytoplankton.

The seasonal changes in abundance and composition of phytoplankton in temperate lakes, during which the biomass may change through six to nine orders of magnitude as a response to seasonal changes in daylength, light intensity and thermal structure of the water column, as well as nutrient availability and the abundance of herbivores, are well known. Although there are numerous variations in phytoplankton periodicity, in more productive temperate lakes the spring and early summer diatom populations are progressively followed by maxima of green algae (*Eudorina*, *Pan-*

dorina, Volvox), filamentous blue-green algae *(Anabaena, Aphanizomenon)*, and ultimately by large standing crops of the dinoflagellate *Ceratium* and/or the blue-green alga *Microcystis* (Reynolds 1984a). In shallow water bodies in temperate latitudes that receive high and continuous nutrient loads, such as sewage oxidation ponds, phytoplankton growth continues throughout the year and the community is most likely to be dominated by green algae *(Ankistrodesmus, Chlamydomonas, Chlorella, Micractinium, Pyrobotrys, Scenedesmus)* and the euglenoid, *Euglena* (Silva and Papenfuss 1953; Allen 1955; Fitzgerald and Rohlich 1958; Raschke 1970). However, in warmer latitudes during the summer, blue-green algae may be dominant. In Texas, USA, in a warm temperate latitude, green algae dominated a sewage oxidation pond during most of the year but during the summer, when temperatures were elevated, the blue-green algae *Oscillatoria* and *Microcystis* were dominant (Wiedeman 1965). In warm temperate Florida, the unicellular blue-green alga *Synechocystis* was reported to have a competitive advantage in summer, under unmixed or intermittently mixed conditions, over the green alga *Chlorella* and the yellow-brown alga *Monodus* which were dominant at other seasons (Lincoln et al. 1984). Eutrophication has led to major changes in the structure of the phytoplankton community in the shallow Lake Dong Hu, Wuhan, China, with an increase in the abundance of phytoplankton by an order of magnitude and the previously dominant diatoms and cryptomonads gradually replaced by green and blue-green algae; the summer maximum of blue-green algae was also more predominant and lasted longer than before (Jao and Zhang 1980).

Some of the large and nutrient-deficient lakes in the tropics maintain relatively small algal communities, including diatoms, but the shallow and more productive lakes where the physical constraints of temperate lakes are absent may support persistent blooms of blue-green algae, particularly *Microcystis* (Ganf 1974a; Reynolds and Walsby 1975; Reynolds 1984a, 1984b). There are several references in the tropics, particularly from India, of small water bodies such as temple tanks, fish tanks, fort moats, and sewage stabilization ponds, that contain more or less permanent blooms of blue-green algae (Ganapati 1940; Singh 1955; George 1962; Srinivasan 1964a, 1964b). *Microcystis* is the most common bloom-forming genus in India, but in addition the following genera of blue-green algae have been reported to form persistent blooms: *Anabaena, Anabaenopsis, Aulosira, Cylindrospermum, Gleotrichia, Oscillatoria, Raphidiopsis, Spirulina* and *Wollea* (Singh 1955; Jayangoudar and Ganapati 1965). The eutrophic Beira Lake situated in the city of Colombo, Sri Lanka, has dense year-round standing crops of phytoplankton usually blue-green algae (Mendis 1964; Costa and de Silva 1978). However, it should be noted that other algae may predominate in shallow eutrophic ponds in the tropics. Rao (1980) listed common phytoplankton genera recorded from stabilization ponds in India; in addition to the blue-green algae *Merismopedia, Microcystis, Oscillatoria, Spirulina* and *Synechococcus*, he reported the green algae *Chlamydomonas, Chlorella, Pandorina* and *Scenedesmus*, and the euglenoids *Euglena* and *Phacus*. Green algae were most frequently the dominant algae in an experimental sewage-fed high rate stabiliza-

tion pond in Thailand although blue-green algae, particularly *Microcystis*, were dominant during about one-third of the observations made (Edwards and Sinchumpasak 1981).

It may be desirable to cultivate a certain species of herbivorous fish in a pond with a given genus of nutritious phytoplankton, but despite an enormous accumulation of knowledge concerning phytoplankton periodicity over the past 50 years, our ability to control phytoplankton periodicity in nature is rudimentary (Reynolds 1984b).

An understanding of r- and K-selected algal species may lead to the development of technically feasible strategies to select for either green or blue-green species in waste-fed ponds. According to Reynolds and Walsby (1975), the dominant algae at high nutrient loadings are likely to be those with the fastest rates of growth, such as the small green algae *Chlorella* and *Scenedesmus*, with a higher productivity than *Microcystis*. Uhlman (1971) described models of phytoplankton growth which indicated that small green algae are able to dominate at high nutrient loadings and short retention times due to their rapid growth rates. Blue-green algae have been observed to dominate eventually in experimental static water ponds loaded with organic matter at the Asian Institute of Technology, but months may elapse before the blue-green bloom occurs in cases in which greens have become dominant first (Colman and Edwards, unpublished data).

It is possible to overcome the advantage of r-selected species in newly established culture systems by seeding with *Microcystis*. Attempts to seed *Microcystis* at the Asian Institute of Technology in sixteen 4-5 m^2 concrete tanks and twelve 200 m^2 earthen ponds in which organic fertilizer had been applied resulted consistently in massive *Microcystis* blooms in two to three days. However, the blue-green algal inoculum died when seeding was attempted with only inorganic fertilizers added to tanks.

Since phytoplankton are normally heavier than water, bloom-forming genera have evolved mechanisms to reduce their density and avoid sinking out of the euphotic zone, e.g., small size, cellular appendages, ability to swim, or provision of gas vacuoles (Reynolds 1984a). Shallow tropical water bodies, both lakes and fishponds, have been reported to be thermally stratified during the middle of the day under still, windless conditions and to "overturn" due to wind action and/or cooling during the night (Ganapati 1940; Singh 1955; Ganf 1974b). *Microcystis* in tropical Lake George, Uganda, were reported to be usually evenly distributed during the night and early morning and then to sink during mid-day to avoid prolonged exposure to potentially damaging high light intensity (Ganf 1974b). There is evidence that light intensity provides the basis for the control of the gas vacuoles in blue-green algae and their ability to migrate (Ganf 1974b; Reynolds 1984b). Such diurnal migrations enable *Microcystis* to obtain optimal light intensity for photosynthesis and may also be a mechanism by which it competes with other potentially faster-growing species (Reynolds 1984a). The dominance of the unicellular blue-green alga *Synechocystis* sp. in summer in a high-rate stabilization pond in Florida occurred only under un-

mixed or intermittently mixed conditions; continuous flow-mixing at 20-30 cm/sec was effective in suppressing the alga and led to dominance of green and/or yellow-brown algae (Lincoln et al. 1984). A list of the six major dominant algal genera in sewage-fed high rate stabilization ponds in Israel did not include a single blue-green algal genus (Azov et al. 1980), possibly because the contents of the ponds were usually mixed. However, as noted earlier, blue-green algae, mainly *Microcystis*, were dominant during about one-third of the observations made of an experimental sewage-fed high-rate stabilization pond in Thailand (Edwards and Sinchumpasak 1981).

Factors Involved

Diatom biomass usually declines following silica limitation in lakes, and waters that are so shallow and turbid that diatoms rapidly sediment into aphotic sediments are inimical to diatom growth (Reynolds 1984a). Stratified water columns may be unfavorable for diatoms. Major shifts in dominance from diatoms to green algae and to the blue-green alga *Microcystis* occurred in experimental enclosures in which approximately constant concentrations of dissolved nutrients were maintained by fertilization (atomic ratios 26Si : 33N : 2P), whereas mixing of the water column led to a renewed growth of diatoms (Reynolds 1984b). In a Polish fishpond stocked with silver carp, the sudden change in dominance of diatoms after spring and early summer to green algae did not take place and diatoms were numerous to the end of the growing season, although the euglenoid *Euglena* and the cryptophyte *Cryptomonas* were also abundant (Januszko 1974); a possible reason for the maintenance of the phytoplankton in an earlier stage of periodicity was considered to be the considerable acceleration in the circulation of matter in the water due to the presence of the silver carp (Opuszynski 1978).

Dense water blooms of phytoplankton are dependent on light and nutrients. The relative dominance of green algae and blue-green algae may be related to different evolutionary adaptations to a planktonic existence: "r-selected species" versus "K-selected species", a concept which is well established in theoretical ecology. Green algae and euglenoids are probably "r-selected species", i.e., pioneer or colonizing, opportunistic species with rapid growth that may later be displaced by blue-green algae, "K-selected species" which grow more slowly but which can make better use of available resources and operate more closely to the environmental carrying capacity of the system. *Microcystis*, which has the ability to form permanent blooms in shallow, eutrophic water bodies, may be an extreme example of a "K-selected species" (Reynolds 1984a). Although the shortest doubling time recorded to date for any alga is about 2 hours for the blue-green *Synechococcus (Anacystis nidulans)*, the mean doubling time for *Microcystis* is about 2.1 days at 20°C under optimal conditions, much more than that of several small green algae which often dominate eutrophic ponds *(Chlamydomonas, Chlorella, Scenedesmus)*, the optimum doubling rate of which may be less than 9 hours (Reynolds and Walsby 1975; Reynolds 1984a). Allen (1955) reported that the green algae *Chlorella* and *Scenedesmus* were dominant

due to their high growth rate in sewage oxidation ponds in California where active oxidation of the sewage was taking place. Rao (1980) was able to demonstrate a definite pattern of periodicity in the growth of algae on sewage in India; the lower the BOD (biochemical oxygen demand) of sewage, the faster was the change from green algae to blue-green algae as final dominants. The nature of *Microcystis* as a "K-selected species" may explain what is regarded as a paradox: blue-green algae are characteristic of highly eutrophic waters, but these algae develop in temperate lakes after the initial spring bloom when concentrations of inorganic nutrients such as nitrates and phosphates are relatively low (Fogg 1969; Reynolds and Walsby 1975).

It is often stated that blue-green algal blooms are stimulated by organic matter (Ganapati 1940; Reynolds 1984a), but no specific requirement for an organic substrate has been demonstrated for freshwater blue-green algae (Reynolds 1984a). The role of organic matter in promoting blue-green algal growth remains unclear but may be nothing more than the well-known symbiotic relationship between bacteria and algae in stabilizing organic matter in which organically bound carbon, nitrogen and phosphorous and perhaps other elements are metabolized by bacteria and made available for algal growth in exchange for oxygen (Reynolds and Walsby 1975). *Microcystis* also has a requirement for a high pH, greater than pH 9 (Gerloff and Skoog 1954; Shapiro 1973). Shapiro (1973) reported that both green algae and blue-green algae responded to high nutrient levels but that at high pH, blue-green algae predominated because they were more efficient than green algae in obtaining CO_2 from low ambient concentrations. However, this hypothesis was discounted by Goldman (1973), who pointed out that only the total inorganic carbon concentration in water is important for the growth of algae and that blue-green algae thrive at high pH due to factors other than low CO_2 concentrations. *Microcystis* has also been reported to tolerate low concentrations of dissolved oxygen and be able to withstand periods of deoxygenation (Reynolds and Walsby 1975) which may help it to compete in waste-fed fishponds.

Fish Kills

Water Quality

Fish selected for waste-fed systems must be able to withstand wide fluctuations in dissolved oxygen. Chervinski (1982) described tilapias as very tolerant to low dissolved oxygen concentrations, even to early morning concentrations below 1 mg/l, when they may use atmospheric oxygen. The Chinese carps require higher concentrations of oxygen; bighead *(Aristichthys nobilis)*, grass *(Ctenopharyngodon idella)*, mud *(Cirrhinus molitorella)*, and silver carp *(Hypophthalmichthys molitrix)* grow and develop normally when the dissolved oxygen concentration is above 2 mg/l and the higher the oxygen concentration of the water, the more food is taken. When the dissolved oxygen concentration falls below 2 mg/l, the fish have a poor appetite, below 1 mg/l they stop feeding, and if the concentration drops below 0.2-0.5 mg/l the fish asphyxiate and die (Anon. 1980).

Large-scale fish mortality or "fish kills" have been reported in lakes, ponds and in waste-fed aquatic systems, and have been attributed to various causes. Bhaskaran and Hora (1952) reported a large-scale mortality of fish in the Hanakhali sewage fishery near Calcutta which he attributed to overapplication of wastewater, which presumably led to a decrease in water quality. Krishnamoorthi (1976) reported acute mortalities of fish, common carp *(Cyprinus carpio)* and rohu *(Labeo rohita)* in fishponds fertilized with stabilization pond effluent at Nagpur, India; blue-green algae, particularly *Microcystis*, were dominant and dissolved oxygen concentrations were reported to drop to critical concentrations at night. It has been known for many years that phytoplankton blooms can cause sudden fish kills and these have generally been attributed to the oxygen demand of decaying algae causing the depletion of dissolved oxygen (Tilden 1929; Olson 1932; Smith and Swingle 1939; Boyd et al. 1978). The cause for the sudden death of most or all the phytoplankton, which typically involve blue-green algae such as *Microcystis* or *Anabaena*, but sometimes the green alga *Trachelomonas* and the dinoflagellate *Gymnodinium*, is not known (Swingle 1968; Boyd 1979). The collapse of blue-green algal blooms may be associated with large increases in buoyancy due to inability of the alga to control its gas vacuoles, resulting from deficiencies in N, P or CO_2 (Reynolds and Walsby 1975; Healey and Hendzel 1976). At Auburn, Alabama, die-offs of blue-green surface blooms usually occur during calm, clear, warm weather in late April to early May (Boyd 1979). Excessive radiation may damage the algae and may be the ultimate cause of death (Reynolds and Walsby 1975; Reynolds 1984a). According to Swingle (1968), the presence of a surface bloom may lead to total depletion of dissolved oxygen in the lower water layers because there the lack of light prevents photosynthesis. However, there may still be adequate dissolved oxygen for fish in the upper 0.3-1.2 m. The problem for fish arises when there is an overturn or upwelling of deoxygenated water to the surface caused by a sharp drop in air temperature (for example in late summer or early autumn) by heavy winds blowing the surface water to an opposite bank, or by heavy cold rains falling on the water. Mendis (1964) reported that occasional mass mortalities of tilapia in a highly eutrophic *Microcystis*-dominated lake in Sri Lanka nearly always occurred after heavy overnight showers. Asphyxia of Chinese carp in South China usually occurs between summer and autumn due to heavy downpours of rain which cause the humus on the pond bottom to be stirred up by the sudden fall in temperature and sinking of the surface layers of water, with an increased consumption of dissolved oxygen in the water (Anon. 1980). Sudden mortality of mullet and carp in fishponds in Hong Kong was correlated with the collapse of algal blooms, resulting in oxygen depletion and an increase in ammonia (Sin and Chiu 1982). The collapse of a bloom of the blue-green alga *Aphanizomenon* in southern France caused mortality of carp due to the disease known as infectious dropsy of carp (IDC); the pathological symptoms of the fish carp following the collapse of the bloom were caused by NH_3 poisoning, with concentrations of unionized ammonia estimated to have reached a maximum of 0.30 mg NH_3/l (Seymour 1980).

Toxic Blue-green Algal Blooms

There is a considerable body of literature on the production of toxins by bloom-forming blue-green algae (see Carmichael and Gorham 1980; Codd 1984; Skulberg et al. 1984 for recent reviews), a topic of some concern in view of their potential adverse effect not only on fish cultured in waste-fed systems but also on humans who may consume such fish. Of the three common bloom-forming species of blue-green algae, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, the last is frequently dominant in waste-fed fishponds.

Most reported cases of poisoning have involved livestock that have drunk from bloom-infested waters. Livestock poisonings have been reported with the greatest frequency from western Canada and the midwestern USA but also from similar geographic regions in Asia, Australia, Europe, South Africa and South America. Most of the blooms of the potentially toxic bloom-forming species are nontoxic but the environmental conditions leading to toxicity are not known. Toxic and nontoxic strains of particular species exist but there may be complex mosaics of varying degrees of toxicity within a single bloom. The wide range of toxicological signs reported for affected animals—poisoning and tissue damage to cardiovascular, gastro-intestinal, hepatic, neuromuscular and respiratory systems—implicate different types of toxins. Some toxins are fast acting and are or may be alkaloids whereas others act less rapidly and are or may be peptides.

Despite the significance of the presence of potentially toxic blue-green algae in fishponds, there is a dearth of critical literature on the subject; most of the reports relating fish mortality to blue-green algal blooms may be explained, with a lack of evidence to the contrary, by anoxia or by algal decomposition products (Prescott 1948; Ingram and Prescott 1954; Schwimmer and Schwimmer 1968). However, there are reports that implicate blue-green algal toxin in fish mortality. In a study reported by Kun, Teplyi and Astakhova in 1961 (cited by Schwimmer and Schwimmer 1968), carp (species not given but probably common carp, *Cyprinus carpio*) injected intraperitoneally with a sterile infusion of *Anabaena*, *Aphanizomenon* and *Microcystis* exhibited erratic behavior, labored respiration, convulsions and finally mortality; similar results were reported from feeding a mixture of live *Aphanizomenon* and *Microcystis* to the fish. Gentile and Maloney (1969) reported that intraperitoneal injections of extracts of *Aphanizomenon flos-aquae* lead to mortality of *Cyprinodon variegatus* and *Fundulus heteroclitus;* and in the same paper, the authors reported mortality of the golden shiner, *Notemigonus crysoleucas*, when the extracted toxin was added to the water. A pure strain of *Microcystis aeruginosa* (toxic to mice) was nontoxic to rainbow trout immersed in a culture for 10 days, although an intraperitoneal injection of *Microcystis aeruginosa* caused an acute toxic response with 100% mortality within 36 hours (Phillips et al. 1985).

There are no published data on *Microcystis* toxicity to filter-feeding tilapias, but in a recent series of experiments with Nile tilapia, *Oreochromis niloticus*, there were no significant mortalities in a tank containing *Microcystis* (toxic to mice) compared

to a control tank containing a nontoxic strain of *Microcystis* over a period of 10 days; the fish filtered the algae out of the water in both tanks (M.J. Phillips and G.A. Codd, pers. comm.). The absence of reports of tilapia mortality in many lakes, e.g., Lake George in Uganda and in waste-fed fishponds dominated by *Microcystis* in the tropics, is encouraging but the potential danger should not be ignored. The *Microcystis* toxin is heat labile so proper cooking of tilapia raised in *Microcystis*-dominated ponds should render any toxin accumulated by the fish harmless to humans (B. Hepher, pers. comm.).

Pond Balance and Optimization

There are two types of balances of concern in waste-fed fish culture—organism balance and chemical balance—and the two are closely connected. The metabolic products of respiration, some of which are toxic to fish at elevated concentration, are produced on a net basis by the nonphotosynthetic biota, such as bacteria, zooplankton and fish. These materials provide nutrients for algal growth. The algae in turn produce oxygen required for respiration. A number of models of fishpond dynamics have been developed to attempt to simulate conditions in pond culture (Romaire and Boyd 1979; Talpaz and Tsur 1982; Svirezhev et al. 1983/1984; Wolfe et al., in press) but none has yet been used to determine optimal management practices for commercial operations, and probably for good reason. Even if all possible means and costs of manipulating pond biology and chemistry could be coupled to a pond dynamics computer model to optimize fish production on biological and economic grounds, the effort involved in computing would be very large and the rewards in terms of fish culture possibly too small. Because the conditions for optimal balance may change continually, it is not certain that rule-of-thumb guidelines for pond management practice would be forthcoming from such an analysis. An alternative approach, shown below, is based on the observation that several potentially production-limiting factors (including rates of natural food production and oxygen generation) can reach their optimal values for fish production simultaneously. The conditions necessary for simultaneous optimization, which concern primarily rates of productivity and destruction of pond algae, determine the limits to the output that can be expected from waste-fed aquaculture, as well as limiting values for system segments such as primary productivity, heterotrophic productivity and fish stocking density. The case for simultaneous optimization of productivity-limiting factors is based on the literature on organism and chemical balance reviewed below.

Organism Balance

Optimal population densities of phytoplankton, heterotrophic bacteria and fish in pond culture remain to be specified; there are merely suggestions in the literature that these various groups should be in balance (Hoffmann 1934; Tang 1970).

Balance of the herbivorous fish-algae interaction should be relatively simple as this follows the dynamics of a two-level food chain (Fretwell 1977), in which greater grazing pressure reduces plant biomass and lesser pressure allows more growth. The implication is that the cultured fish alone are responsible for cropping the algae. Indeed, in several investigations of planktivorous fish in culture tanks, the zooplankton were quickly cropped off when fish were added so that the subsequent effect of zooplankton on the phytoplankton was greatly reduced (Wolfe 1981; Costa-Pierce 1983; Elliott et al. 1983; Smith 1985).

Investigators of algal growth in light-, as opposed to nutrient-limited, systems have presented models (Goldman 1979; Laws and Malecha 1981) and experimental data (Goldman et al. 1975; D'Elia et al. 1977), which show that net primary productivity has a maximum value, as a function of algal standing crop, at an intermediate standing crop density (Fig. 1). Standing crop is in turn regulated by "harvesting rate"—the rate of flow-through in algal biomass culture (Goldman 1979) or, in static aquaculture systems, by the combined grazing and algal death rates. Thus, standing crop is at a maximum, determined by a balance between available light for growth and respiration and death of the algae, when the harvesting rate of the algae is zero. As harvesting rate is increased from zero, the standing crop decreases and net productivity increases. The maximum net productivity occurs when the harvesting rate (algal capture) equals the maximum possible rate of algal productivity. This system is stable as long as phytoplankton biomass is larger than the value, X', corresponding to maximum net productivity. There the effect of increased cropping rate caused by an expanding biomass of planktivorous fish would cause a drop in algal standing crop and an increase in net productivity (Fig. 1). Thus, the increased demand for

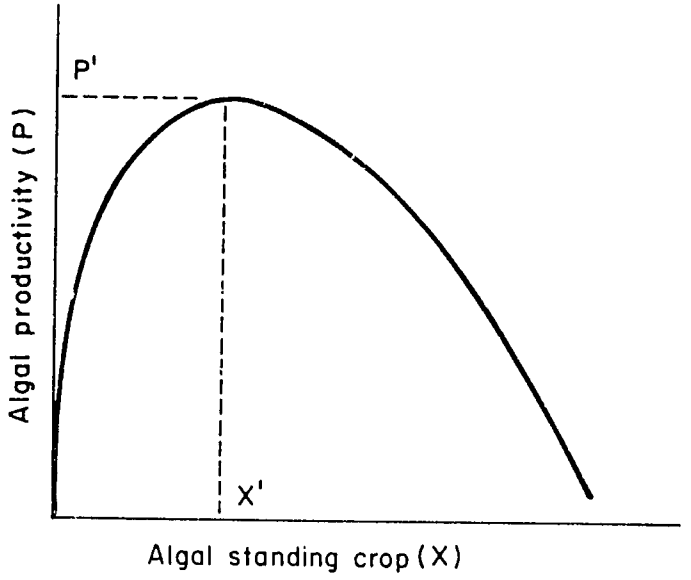

Fig. 1. Generalized relationship between algal productivity and algal standing crop. P', maximum algal productivity; X', maximum algal standing crop.

algae would be met without a major change in the system, such as an algal population crash. However, if the harvesting rate increased enough to push the standing crop below X′, stability would be lost because continued decrease of standing crop from increased harvesting would result in lower net productivity. The lower productivity would increase the rate of algal biomass decline, resulting in still lower productivity, and so on, causing a precipitous drop in algal standing crop.

In waste-fed systems in which phytoplankton are food for filter-feeding fish, it would be desirable to adjust the fish grazing pressure to maintain the phytoplankton standing crop at density X′, corresponding to maximum net productivity of algae, to exploit the algal food source to the fullest possible extent. Actually, a slight compromise of net productivity using phytoplankton standing crop somewhat above X′ would be best, in view of the system stability. Although the relationship described above has been shown to be valid for algal biomass culture, in which mechanical harvesting is employed, it has yet to be adequately demonstrated whether fish cropping of algae can be substituted for mechanical harvesting in optimizing algal productivity. Evidence for equivalence of these processes would be a decrease in algal standing crop as herbivorous fish stocking density increased, an increase in algal net productivity with an increase in fish stocking density and a precipitous decline in both algal biomass and productivity at very high stocking densities.

Reich (1975) may have been the first to appreciate these relationships between algal biomass and productivity and fish stocking density, although he did not present any supporting data. According to him, intensive grazing of algae by silver carp leads to a decrease in algal biomass but an increase in primary productivity. He also believed it possible to increase the number of fish in the pond to such an extent that the rate of grazing exceeds the reproductive potential of the algae with concomitant decreases in both primary productivity and fish yield.

In recent work at the Asian Institute of Technology, four 4-5 m^3 concrete tanks were stocked at 4 fish/m^2 with 70 g Nile tilapia, *Oreochromis niloticus*, and fertilized with septage and inorganic fertilizer and fish biomass increased at rates of up to 4.6 g/m^2/day over a 62-day period. Algal biomass was lowest and net primary productivity levels were highest in the tank with the fastest-growing fish. Nitrite concentrations in the tanks were inversely correlated with fish growth. If we assume the slower growing fish were cropping less algal biomass, these results demonstrate that fish can increase net primary productivity through decreasing algal standing crop (Colman et al., unpublished data). In a second tank experiment in which *Microcystis* dominated all concrete tanks, fish stocking density with 44 g Nile tilapia varied from 0.5 to 10 fish/m^2. Initially fish grew best (biomass increased up to 6 g/m^2/day, average of three replicates) at the higher stocking densities but by the third measured growth interval (day 27-41), treatments with lower stocking density showed better fish growth. No correlation was noted between fish stocking density and chlorophyll *a* concentration, but a change in algal dominance from *Microcystis* to *Scenedesmus* was found at the highest fish stocking rate. Rather than cause a precipitous decline in algal standing crop, high harvesting pressure by fish may simply cause a shift in dominance to those algae that are too small to be filtered or too indigestible to be assimilated.

Several investigations that have indicated a shift to small, relatively indigestible (compared to blue-green algae) green algae in the presence of herbivorous fish have been undertaken in translucent tanks using inorganic fertilizers and high fish stocking rates (Wolfe 1981; Pierce 1983; Elliott et al. 1983; Smith 1985). These systems may in fact favor faster growing green algae over slower growing blue-green by a mechanism that would not occur in earthen ponds. Light penetration through the sides of translucent-sided tanks might remove the competitive advantage of blue-green algae, of positioning themselves through buoyancy regulation in optimal light intensities at or near the surface and thus shading and outcompeting green algae (Reynolds 1984a). If this factor does operate and leads to the development of less digestible algae in fish tanks compared to ponds, it could help to reconcile results from food pathway investigations of these systems using the delta carbon (δC) technique: *Oreochromis aureus* which were raised in tanks and given complete pelleted feed, but which were found also to graze algae, had apparently derived their food entirely from pellets (Schroeder 1983b); but in earth ponds stocked with tilapia hybrids at a density of $0.75/m^2$ with organic manure, algae and pelleted feeds available, the ratio of carbon isotopes in the fish bodies suggested strong reliance on "natural foods of photosynthetic origin" (Schroeder 1983a).

Attempts to control algal blooms by adding herbivorous fish to culture systems have sometimes led to dramatic increases in algal standing crop; for example, when silver carp were added to ponds stocked with common carp (Opuszynski 1972). Addition of herbivorous fish may have the unintended consequence of removing zooplankton predators of phytoplankton, thereby reducing the predation on phytoplankton (Kajak et al. 1975; Opuszynski 1978; Elliott et al. 1983).

The relationship between fish stocking density and cropping of algae may depend on the species of fish cultured. In ponds stocked with silver carp, zooplankton were apparently more efficient croppers of the algae than the fish because the algal biomass increased following stocking of fish that also consumed zooplankton (Opuszynski 1978). However, the increase in algae may also have been facilitated by increased nutrient recycling caused by the stocked fish, i.e., the ponds stocked with silver carp may have been nutrient-limited rather than light-limited before the addition of the silver carp. Perhaps algal dominance in carp ponds shifts to less easily grazed species as the silver carp stocking density is increased. Whether and at what fish stocking density earthen ponds dominated with *Microcystis* might shift to other species because of tilapia grazing, have yet to be shown. However, in earthen ponds seeded with *Microcystis* and stocked with tilapia at 1 fish/m^2, *Microcystis* remained dominant for over three months (Colman and Edwards, unpublished data).

There is little literature regarding organism balance where the heterotrophic bacteria in culture ponds are concerned. Productivity measurements have been carried out by Schroeder (1978) and Rimon and Shilo (1982) but the results were preliminary. The mathematics of bacterial doubling times and removal rates have been carefully considered in waste water biological reactors (see Benefield and Randall 1980), but because detrital-bacterial interactions are complex and rates

and processes of bacterial removal by fish are uncertain, more research is needed before bacterial growth and grazing can be successfully quantified or modelled (see Moriarty, this vol.). For the purpose of optimizing heterotrophic productivity as a natural food source in aquaculture, it may be sufficient simply to optimize the supply of oxygen to the system.

Chemical Balance

The goal of chemical balance in aquaculture should be to generate a sufficient supply of oxygen for growth of the cultured species and their natural food organisms while minimizing the buildup of toxic metabolic products. Whereas other chemical requirements of pond culture such as sufficient supply of nutrients to provide natural food production in the ponds and maintenance of pH at appropriate levels are normally met by pond inputs, the provision of oxygen and removal of metabolites are not. Except on an emergency basis, direct aeration or pumping oxygenated water from outside the pond to boost pond oxygen concentrations is normally too expensive (G.R. Pruder, pers. comm.) as are most water treatment procedures for removing toxic metabolites such as ammonia.

Chemical balance in ponds is generally regulated by adjusting pond organism balance because most of the important chemical transformations that occur are biologically mediated. In the case of oxygen, the biological regulation is between the photosynthetic producers and the respiratory consumers and the goal of management strategy is to produce as much oxygen as possible in the pond so that the consumers, including the cultured organisms, have an adequate supply. Any oxygen not used directly by the fish and other macro- and meiofauna is available for heterotrophic productivity, a potential feeding pathway in a waste-fed system. Indeed, the amount of oxygen available determines to what extent the heterotrophic system can operate.

The phytoplankton-cropping feeding pathway may be the most efficient way to raise fish because net photosynthetic oxygen evolution is proportional to net algal productivity, and thus generation of both algal feed and oxygen reach a maximum value at the same time. Since the limiting factor in heterotrophic productivity is oxygen availability, the implication is that both heterotrophic and autotrophic pathways could be maximized at the same time. Thus, even in aquaculture systems with target species feeding in the heterotrophic food chain, maximum net algal growth to supply oxygen to the heterotrophic productivity should be sought. Harvest of the algae, necessary for the maximum net oxygen production in a pond, is not of course dependent only upon fish; any algal harvesting technique from zooplankton grazing to flushing algae out of the pond by water exchange would suffice to bring the algal crop to the level of maximum net productivity. Oxygen production by algae in a shrimp pond, for example, could be maximized by harvesting algae through herbivores in polyculture, by water flow-through at an appropriate rate or both.

Because there is a close association in the aquaculture literature between algal productivity and oxygen depletion (Smith and Swingle 1939; Boyd et al. 1978; Romaire et al. 1978; Hopkins et al. 1983), a recommendation to run ponds at maximum net algal productivity may seem at odds with currently accepted knowledge of aquaculture systems. However, this can be resolved by maintaining a distinction between harvested and unharvested algal biomass. Maximum net algal productivity occurs only when the algal standing crop is reduced from maximum possible densities by harvesting (Fig. 1).

More factors need to be considered than simply the net oxygen production occurring at various phytoplankton cropping intensities. The dawn oxygen concentration of the diurnal oxygen regime is the critical parameter for many cultured species. This parameter is influenced by consumption of oxygen by algae as well as nonalgal biota and exchanges of oxygen between the air and water. Computer simulations are most useful to realize the summed effect. A computer study indicated that at a net algal productivity of 4 g $C/m^2/day$, perhaps approaching the practical upper limit for fishponds, the maximum oxygen available for heterotrophic productivity would be 4-5 g $O_2/m^2/day$ assuming that the average dawn oxygen concentration did not fall below 2 mg O_2/l (Colman and Edwards, unpublished data). This reveals the basic constraints to the food chains: the autotrophic food chain is limited by light and nutrient availability, the heterotrophic chain by oxygen and organic substrate availability. Nutrient and organic substrate requirements can be met by pond inputs and oxygen can be supplied as a byproduct of the autotrophic productivity, but only to the degree allowed by available sunlight and harvesting regulation of the algal biomass. Schroeder (1978) attributed an increased production of 15 kg/ha/day of tilapia in ponds that received organic (and some inorganic) fertilizer inputs, compared to ponds with only inorganic input, to feeding on heterotrophic bacteria that developed in the organically fertilized ponds; he believed that the photosynthetic feeding pathway was light-saturated. His estimate of the required 10 kg/ha/d bacterial carbon productivity at 35% conversion efficiency from manure would require 5 g $O_2/m^2/day$ to produce. If this estimate were correct, heterotrophic productivity would already be at the limit that could be supported by photosynthetically produced oxygen. In fact, the measured uptake of oxygen in ponds with similar organic manure application was calculated to be about 0.4 g $O_2/m^2/night$ or 1.2 g $O_2/m^2/day$ (Schroeder 1975), which indicates that there was less feeding through the heterotrophic route than originally calculated.

Discussion and Recommendations

With the emphasis in developed countries on raising fish on pelleted diets in a similar way to "feedlot" methods for raising livestock, it is not generally appreciated that most of the world's yield of farmed fish is in fact raised in manured ponds (Wohlfarth 1978). The lack of appreciation in the West of the positive benefits of eutrophication in culture ponds probably results from the lack of economically

important fish that can filter phytoplankton in Europe and North America (Tilden 1929; Opuszynski 1978). Instead, attempts to recycle nutrients contained in organic wastes in the West have included the mass culture of algae as animal feed, although there are problems in harvesting and processing the phytoplankton in an economically viable way (Shelef and Soeder 1980). Ryther (1971) suggested that a herbivore could be used to harvest waste-grown phytoplankton and proposed the concept of "controlled eutrophication" with physical separation of primary and secondary productivity levels (Ryther et al. 1972). However, the most widely used technique to recycle organic wastes, the traditional waste-fed fishpond, is still poorly understood and considerable benefits to aquaculture would accrue from the optimization of a single stage, effluent-free pond modified for both fish culture and waste-treatment (Edwards et al. 1981b).

The data in the literature concerning the feeding of filter-feeding tilapias and silver carp are highly contradictory. This is hardly surprising considering the variability in the type and relative amounts of suspended particles present in the pond when the studies were conducted (phytoplankton, zooplankton, detritus) and the various methods used to study the feeding habit (analysis of gut contents; assimilation; fish growth). However, there is a considerable body of data that suggests that filter-feeding tilapias and silver carp are not strictly herbivores but feed on whatever particles are suspended in the water. Since such "phytoplanktivorous" fish appear to be opportunistic microphagous, or even omnivorous species, it may be more appropriate to distinguish only between fish species that have a strictly carnivorous diet and those that feed on less valuable feed (Bitterlich and Gnaiger 1984; Bitterlich 1985). According to Hickling (1971), fish are more or less omnivorous and consume a wide range of supplementary feed, so there is no reason why the feeding latitude of filter-feeding fish should not be rationally exploited in detrital-fed aquaculture systems. Schroeder (1977, 1980b) stressed the importance of using the pond not only as a medium in which to grow the fish but also as an "external rumen" in which nutrients bound in a relatively indigestible form could be released by microbial activity and provide substrates for both heterotrophic and autotrophic production that could subsequently serve as high protein natural food for the fish.

There is also considerable confusion in the literature regarding the size of particles that filter feeding fish can remove from the water and their digestibility, but it does appear that milkfish, silver carp and microphagous tilapias do in fact remove nanoplankton from the water, possibly involving entrapment by mucus. Our knowledge is incomplete concerning the digestion of phytoplankton by fish but the acid hydrolysis of the blue-green algal cell wall by gastric acid in tilapia has been well documented (Moriarty 1973; Bowen 1982). Despite the fact that Reynolds (1984a) concluded his scholarly treatise on the ecology of freshwater phytoplankton with the sobering statement, "it is humbling to realize that all our acquired knowledge scarcely allows us to make valid predictions about when and what species will be abundant in given waters", it is fortuitous that shallow eutrophic water bodies in the tropics, including fishponds, are likely to be dominated eventually by more or less permanent blooms

of the blue-green alga *Microcystis*. With increasing eutrophication, diatoms tend to be replaced by green and euglenoid algae, which in turn are replaced eventually by blue-green algae. The dominance of blue-green algae in waste-fed ponds stocked with tilapia in the tropics is highly beneficial, in stark contrast to the low regard in which they are held in the West, as eloquently expressed by Goldman and Horne (1983): "Like thistles left alone by cows in a grassy field, blue-green algae are weeds among the more nutritious populations of diatoms or green algae". Ryther and Officer (1981) ranked major taxonomic groups of phytoplankton as food organisms for herbivores according to their decreasing value to man and blue-green algae were ranked bottom of the league of seven groups. However, the ability of tilapia to exploit blue-green algae grown directly in fishponds may lead to the development of badly needed, more cost effective aquaculture in developing countries (Bowen 1982).

The relationship between algae and dissolved oxygen concentrations in fishponds is poorly understood because no distinction is made between harvested and unharvested algal biomass. If maximum net algal productivity were achieved by the addition of sufficient nutrient inputs to make the algae light-limited rather than nutrient-limited, and by sufficient grazing of algae by filter feeding fish to reduce the algal standing crop to the optimal density for net productivity, there would be maximum net generation of oxygen by the algae. In fact, algal populations in any ponds exhibiting positive net productivity values on a 24-hour basis are net contributors to oxygen in the water. In aquaculture systems in which phytoplankton are a feed for fish, maximum net algal productivity would also lead to maximum exploitation of the algal food source. In contrast, unharvested algal biomass may lead to the development of the maximum algal standing crop at which net productivity approaches zero, with concomitant adverse effects on the oxygen regime of the system.

The supply of oxygen determines how much heterotrophic productivity can occur because the most efficient heterotrophic productivity uses oxygen. At the maximum net primary productivity rates that have been achieved in aquaculture (4 g $C/m^2/day$), the amount of oxygen available for heterotrophic productivity, after subtraction for oxygen lost to the atmosphere and lost to fish respiration, would be 4-5 g $O_2/m^2/day$. To avoid oxygen depletion in ponds, the rate of oxygen consumption by the heterotrophic system would have to be limited to a value lower than this. This is true unless artificial oxygenation of the water were economical, for example, by mechanical aerators or by water exchange as occurs in raceway culture, in ponds with tidal exchange and in cage culture.

Considerable increases in the yield of tilapia should be possible in waste-fed ponds by promotion of the growth of the readily digestible blue-green alga *Microcystis*. Studies should be conducted on the feasibility of seeding *Microcystis* into ponds to increase the degree and frequency of dominance. To obtain maximum fish yield, maximum net algal productivity needs to be maintained to provide maximum supply of feed and adequate oxygen for the fish by an optimal rate of cropping algae by tilapia. The optimal biomass of tilapia to maintain maximum net algal productivity would be difficult to achieve, let alone maintain, in a freely breeding population of tilapia with continual recruitment, but the balance between fish biomass and algal

biomass would be easier to maintain with a monosex culture of male fish. Studies are required on the loading rates of various types of organic manures, possibly with supplementation by inorganic fertilizers in the case of nutrient-poor organic matter, to produce the requisite nutrient-saturated and light-limited algal population. The technique of algal tissue analysis, similar to plant analyses employed in agronomy, may be used to determine whether the algae are nitrogen- or phosphorus-limited at any stage during the culture period (Gerloff and Skoog 1954; Colman and Santha, in press).

Therefore, phytoplankton have the potential to be managed as highly cost-effective "biological aerators" in fishponds. *Microcystis* could also be tried in this role in culture systems in which it is not a direct food organism of the principal target species; for example the culture of freshwater prawn *Macrobrachium* and freshwater fish in which nutrition is derived from either the consumption of pelleted feed or cropping from the heterotrophic food web or both. Blue-green algae do develop in such systems and may build up to dangerously high biomass densities because they are not cropped by the target species; the algal standing crop may reach the maximum light-limited density at which net algal productivity is low or even zero, with potentially disastrous effects on the oxygen budget of the pond. However, the algae could be used to generate oxygen for pellet- and detritus-fed systems if an algal-cropping fish species were introduced to reduce the algal biomass to the density at which maximum net productivity and maximum net generation of oxygen occur. Such fish may need to be confined to pens or cages to prevent consumption of pelleted feed meant for the target species.

Since all the above-mentioned systems develop phytoplankton naturally, it would be worthwhile to consider seeding with *Microcystis*, a species that is known to be readily cropped by filter-feeding fish. A second beneficial attribute of *Microcystis* is its tendency to float on the surface and be blown by the wind to the down-wind side of the pond where it could be cropped by fish in pens or cages.

The same principles using brackishwater phytoplankton could be applied to penaeid shrimp culture, using either a herbivore to harvest the phytoplankton, or water exchange. Although water exchange is an inefficient means to transport oxygen to a pond, the stimulation of net algal productivity and oxygen production caused by washing out part of the algal standing crop could be substantial. Either a once-through or a recirculating system could be employed; the latter would draw water from a location in the pond with the highest algal biomass and remove the algae by passing the algae-laden water through a fast-flow sand filter before its return to the pond. Ten per cent water exchange removing 10% of a phytoplankton crop of 25 g C/m^2 would generate 6.67 g O_2/m^2 as the crop grew back to 25 g C/m^2. If the removal and grow-back occurred on a daily basis, the algae would supply 6.7 g O_2/m^2/day, enough to eliminate the oxygen problems of most shrimp culture systems.

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Discussion

SCHROEDER: Have you tested *Spirulina* or *Oscillatoria* as far as feeding is concerned?

COLMAN: It's very hard to run the experiments. You need species which you know will dominate. For example, we have got *Microcystis* and *Scenedesmus* to dominate; not much else.

SCHROEDER: Not *Oscillatoria?*

COLMAN: No, not for long enough to make any conclusions about feed conversion.

SCHROEDER: We have had some blue-green dominated ponds in which there is a shift from blue-greens to greens as the fish biomass grows. We assume that this is because the fish are over-cropping the blue-greens. The greens then get an advantage. The different generation times are also important. With our delta-carbon work, tilapia, silver carp and common carp values do not match those of the blue-greens. The silver carp gut contents had a delta-carbon value which matched that of a mixture of blue-greens taken from the water. The mixture included *Oscillatoria, Spirulina* and *Microcystis* (I cannot say which was dominant). The delta-carbon value of the silver carp flesh was very different..

BILIO: How do you interpret this?

SCHROEDER: I cannot make conclusions at this stage. The silver carp is obviously ingesting these algae, removing them from the water column, but perhaps not digesting them. The greens later took over. As I have said the delta-carbon value of the silver carp gut contents matched exactly that of the algae in the water, but the delta carbon value of its flesh was very different: a 5/ml difference. The gut contents of the tilapia and common carp had flesh delta carbon values very similar to those of their gut contents and the pond bottom. Has anyone here observed silver carp to ingest blue-greens but not digest them?

EDWARDS: There is literature cited in the paper presented here by John Colman and myself (Chiang 1971; Ghosh et al. 1973; Zhu 1982; Zhu and Deng 1983) which shows that silver carp can digest blue-greens. Empty cells were found in the hind-gut. There may be other contrary evidence.

SCHROEDER: Were the types of blue-greens described in this work?

EDWARDS: Yes.

COLMAN: Well, we have pretty good evidence for the utilization of *Microcystis* by tilapia—in one case a conversion ratio of 2; that is dry weight *Microcystis* production: wet weight fish flesh production. Surely this is clear evidence?

SCHROEDER: Certainly. I would not dispute this. Was this in tanks or ponds?

COLMAN: Tanks.

SCHROEDER: Then the available food was almost all *Microcystis.*

MORIARTY: Tilapias have no problem digesting any blue-greens which they can ingest, irrespective of species. They cannot easily digest green algae. I have shown this with *Chlorella.* They can easily digest diatoms. Therefore, if you have significant quantities of blue-greens in a tilapia pond, the fish will surely use them. If the delta-carbon data suggest otherwise, then I would like to look at how this can be. But let us consider the broader question of how to manipulate the species composition of the plankton in a fish pond; for example, encouraging blue-green algae for tilapia, zooplankton for a zooplankton feeder, etc. Dr. Colman mentioned some problems in sustaining *Microcystis* dominance. Perhaps pH is a factor here. Blue-greens prefer a high pH.

COLMAN: I only had trouble in tanks when there were no organic inputs. I never had any difficulty in sustaining *Microcystis* dominance in ponds or in tanks with organic material inputs.

MORIARTY: I can't think why you had this problem in the tanks. *Microcystis* does not need such organic material or the presence of bacteria.

COLMAN: I don't understand why. Anyway organic inputs are simple and convenient.

MORIARTY: Still this problem should be investigated further.

COLMAN: In algal succession patterns, the r and k characteristics of the different species are probably very important. If you wish to sustain a fast-growing species, it might be desirable to have a fairly fast water exchange. For example, in sewage oxidation ponds that have a flow-through of water, fast growing green algae tend to dominate. In our more stationary ponds, *Microcystis* might take a long time to become dominant (two months in some cases) but thereafter it stays. Therefore, I have suggested an inoculum of *Microcystis* be added at the beginning of a tilapia culture cycle. However, we don't yet have enough data to know if this method would result in early *Microcystis* dominance.

WOHLFARTH: Regarding your comments on fertilizer inputs, Hepher (1962)* showed that increasing fertilizer inputs beyond a certain point ceases to yield any benefits. This was done using a monoculture of common carp. I do not know of any other similar investigations.

EDWARDS: There is Israeli work (Yashouv and Halevy 1972)** which has shown that by stocking a filter-feeding fish like the silver carp, the rate of fertilization can be increased and that consequent yield of fish is higher. This work was discussed by Reich (1975)*** and is further reviewed in my paper with John Colman.

PULLIN: Dr. Colman's work has shown that phytoplankton are very efficient biological aerators, but they can let you down. Let us not lose sight of the fact that mechanical aeration is in current use in some highly viable aquaculture production systems.

COLMAN: This probably applies only to intensive aquaculture, not to semi-intensive systems.

PULLIN: Well, many ponds have aerators which are used for some of the time. We can argue about definitions of intensive vs. semi-intensive, but at the end of the day many culturists require safeguards against their fish dying through lack of oxygen and also need to avoid sublethal effects such as growth depression.

EDWARDS: Such farmers give large quantities of supplementary feed, either agricultural byproducts or feed pellets. Waste-fed ponds may not need these safeguards because of the oxygen supply from the phytoplankton.

COLMAN: The more intensive systems have different economics. Waste-fed pond culturists may not be able to afford aerators.

PULLIN: Well, if you want to push a waste-fed pond to maximum productivity, it could be good to have emergency aeration kept handy.

GRAY: We must discuss this further in our general discussion.

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*Hepher, B. 1962. Primary production in fish ponds and its application to fertilization experiments. Limnol. Oceanogr. 7: 131-136.

**Yashouv, A. and A. Halevy. 1972. Experimental studies of polyculture in 1971. Bamidgeh 24(2): 31-39.

***Reich, K. 1975. Multispecies fish culture (polyculture) in Israel. Bamidgeh 27: 85-99.

Meiofauna: Their Role in Marine Detrital Systems

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Abstract

Detritally enriched marine habitats are characterized by high densities of meiofauna which are generally confined to a few taxa: nematodes of the genera *Rhabditis*, *Diplolaimella* and *Diplolaimelloides* and copepods of the genus *Tisbe*, the latter often in multispecies guilds. All species are opportunistic with the ability to utilize a wide variety of food sources, a high reproductive potential and rapid rates of population growth. The nematodes are bacterial feeders and convert detrital to nematode biomass with an efficiency of about 1% in a simple two-step food chain: their presence enhances the rate of bacterial decomposition of the detritus. The feeding behavior of copepods of the genus *Tisbe* is more complex and they may occupy several trophic levels. Meiofauna, particularly copepods, are the main dietary item for the juvenile stages of many commercially important marine food species including fish and crustaceans, and they therefore play a crucial role in the rearing of such species in aquaculture systems.

Introduction

The meiofauna can be defined as an assemblage of small benthic metazoans, ranging in dry adult body mass from about 0.01 to 50 μg and having a coherent set of life-history and feeding characteristics which sets them apart as a separate evolutionary unit from larger macrofauna (Warwick 1984). Arranged roughly in order of numerical abundance, the meiofauna consists of nematodes, copepods (principally harpacticoids), turbellarians, gastrotrichs, tardigrades, kinorhynchs, gnathostomulids, small annelids, hydrozoans and in addition some aberrant members of other phyla which mainly comprise larger organisms.

When considering the role of meiofauna in detrital systems, in the particular context of aquaculture, three main questions seem important: what kinds of meiofaunal organisms are generally associated with detritus both in natural and culture systems; what is the role of the meiofauna in the breakdown and mineralization of the detritus and is the presence of meiofauna detrimental or beneficial in aquaculture systems which use plant detritus as the primary food source? In attempting this review it has become abundantly clear that there is a dearth of published information on these topics and it is obvious that much more research needs to be done.

Meiofauna Associated with Detritus

The meiofaunal taxa which favor habitats which are highly enriched with organic detritus are remarkable in two respects, first they are virtually confined to a few restricted groups of nematodes and copepods, and second these same groups are of ubiquitous occurrence in such situations, at least in temperate latitudes where they have been most studied.

Nematodes are found in very high densities in decaying plant material derived from both macroalgae and terrestrial macrophytes. For example, Odum and Heald (1972) found nematodes in "extremely high numbers" in decaying mangrove leaves and suggested that they "play an important role in the decomposition process". Koop et al. (1982) similarly reported "large populations of nematodes" associated with beds of decomposing kelp. I have examined nematodes collected from South African kelp beds described in this last paper, and found that the community is dominated by a single species, *Rhabditis marina*. This species has a cosmopolitan distribution (Inglis 1966) and is commonly associated with stranded decomposing algae. Inglis and Coles (1961) consider that it is a typical member of the beach fauna and is not a truly marine species: indeed it belongs to a large class of nematodes, the Secernentea, only two species of which have been found as free living organisms in the marine environment.

Detrital systems where marsh vegetation or mangrove leaves predominate are usually dominated by two closely related genera of the nematode family Monhysteridae, namely *Diplolaimella* and *Diplolaimelloides* (Lorenzen 1969; Hopper 1970; Hopper et al. 1973). These two genera are also associated with high-shore and brackishwater environments and may not be truly marine. A particular feature of these three genera of nematodes which are prevalent in detrital systems is their ease of laboratory culture in comparison with truly marine forms, and they have been used extensively in experimental studies (Tietjen et al. 1970; Tietjen and Lee 1975; Tenore et al. 1977; Milton 1981; Warwick 1981; Findlay 1982; Findlay and Tenore 1982; Pamatmat and Findlay 1983; Alongi and Tenore 1985).

A wide variety of environments enriched by particulate organic material are also characterized by the predominance of a limited number of copepod species, notably members of the genus *Tisbe* (Fava and Volkmann 1975). This genus comprises a number of very closely related and morphologically similar species (Volkmann

1979) which are often found in the field in multispecies guilds (Bergmans 1979): the maintenance of this diversity probably results from behavioral adaptations for spatial resource partitioning (Marcotte 1984), or it may be that a temporary superabundance of food obviates interspecific competition for resources. *Tisbe* species are again noted for their ease of culture and many species, particularly the scavenging *T. gracilis* group, are frequently found in neglected aquaria in large numbers (Volkmann 1979). In controlled experiments in which sublittoral muddy sediments were enriched with powdered *Ascophyllum*, Gee et al. (1985) found that the enriched sediments became dominated by a guild of five *Tisbe* species. There is no doubt that these copepods are fully marine, and they appear to be favored in relation to nematodes in sublittoral habitats which are detritally enriched.

Common features of both the nematode and copepod components of the meiofauna of detrital systems are their opportunistic characteristics. Both the nematodes (this paper) and the *Tisbe* species (Berghe and Bergmans 1981) have the capacity to utilize a wide variety of food sources. They also both have a high reproductive potential and rapid rates of population growth as indicated by Warwick (1981) for the nematodes and by Battaglia (1970) and Bergmans (1981) for *Tisbe*. These characteristics enable them to exploit the highly unpredictable environment that natural detrital systems offer, depending as they do on the occurrence of storms or other climatic events.

Role of Meiofauna in Detrital Systems

It can be deduced from the structure of their buccal cavity (Wieser 1953) that the main nematode taxa associated with detritus are selective bacterial feeders. They all have simple unarmed buccal cavities capable of processing bacterial sized particles: for example in *Rhabditis marina* the buccal cavity is about 1.8 μm wide (Inglis and Coles 1961) and in *Diplolaimelloides bruciei* about 1.5 μm wide (Hopper 1970), whilst the larger benthic intertidal bacteria are usually 1-1.5 μm in diameter (I.R. Joint, pers. comm.).

Findlay (1982) showed that the nitrogen content of plant detritus was the best measure of nutritional quality for *Diplolaimella chitwoodi*. Population growth rate (r) and carrying capacity (K) were measured in laboratory culture using different types of plant detritus. Population growth was scarcely affected by the nature or quantity of the detritus except in a few isolated cases, but K was strongly affected by both the food quality and ration. I have done some similar experiments with *Diplolaimelloides bruciei* which largely confirm these conclusions. Freeze-dried and ground material of three brown algae (*Fucus*, *Pelvetia* and *Ascophyllum*) and two salt-marsh phanerogams (*Halimione* and *Spartina*) from the nematode's estuarine habitat were added to 50 ml of 26 ppt autoclaved seawater in 100 ml conical flasks. Three ration levels, 25, 50 and 100 mg per culture, for each food source and five replicates for each treatment were used. The cultures were innoculated with 1 ml of a suspension of *D. bruciei* containing a mean of 16.6 nematodes ml^{-1}. The flasks were stoppered with cotton wool and incubated in the dark at 20°C. After various

intervals of time the population density was estimated by withdrawing 1 ml aliquots and counting the nematodes on a Hawksley eelworm slide. Population growth rate and maximum population density were compared with control cultures containing 50 mg of ground baby food (Gerber mixed cereal) (Fig. 1) on which the stock cultures had been maintained.

The initial exponential increase is represented by a straight line on the graph of log abundance against time, with slope r. It can be seen that this slope is very similar for all food sources except that it is rather greater for *Spartina*. Final population density varies both with the ration and with the food type, being lowest for *Spartina*.

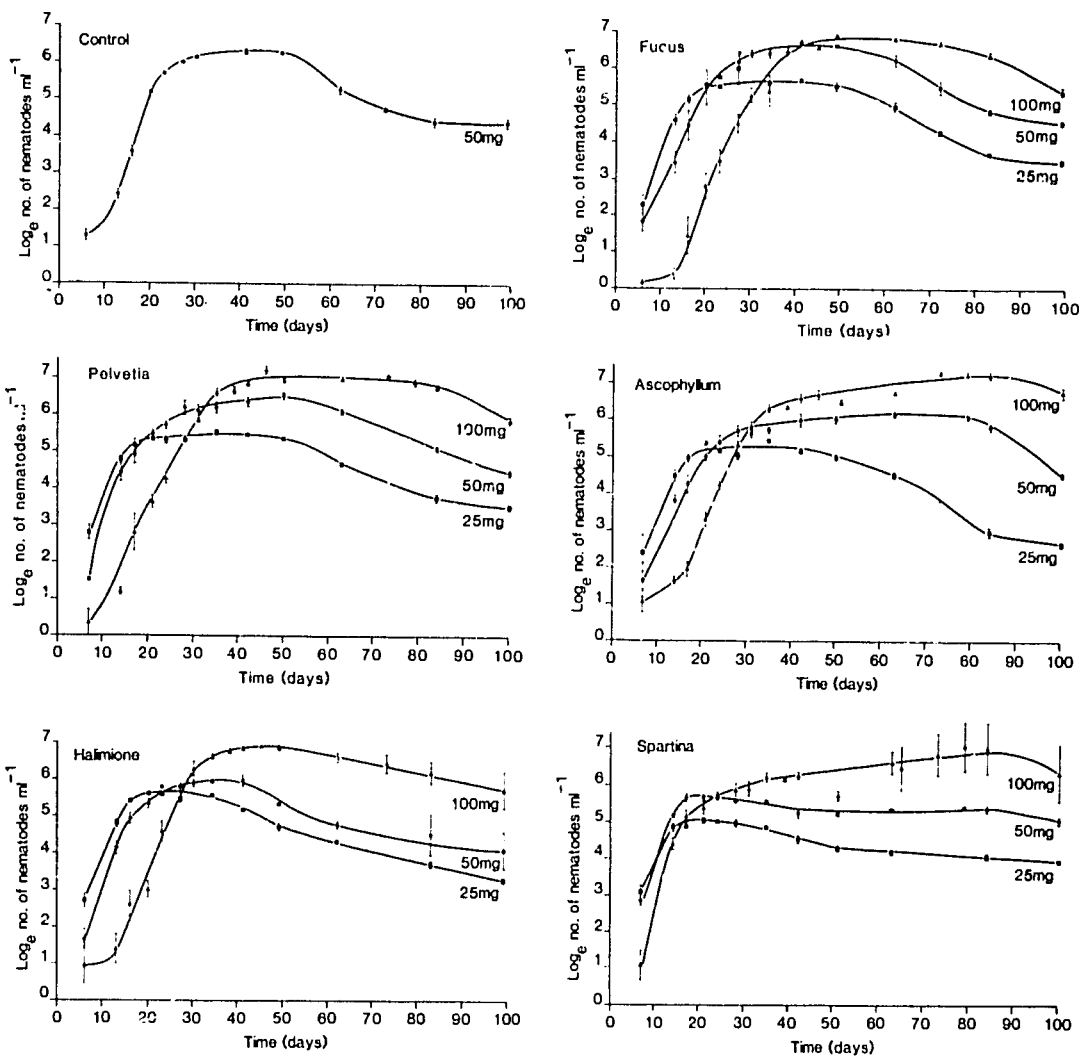

Fig. 1. Population growth of the marine nematode *Diplolaimelloides bruciei* cultured on different detrital sources at three ration levels.

The carbon and nitrogen content of the detrital sources, and the values of maximum population density achieved, are given in Table 1. Linear regression of the maximum population density attained (numbers per ml) against the carbon ration (Fig. 2a) and nitrogen ration (Fig. 2b) indicate a much closer correlation with the nitrogen,

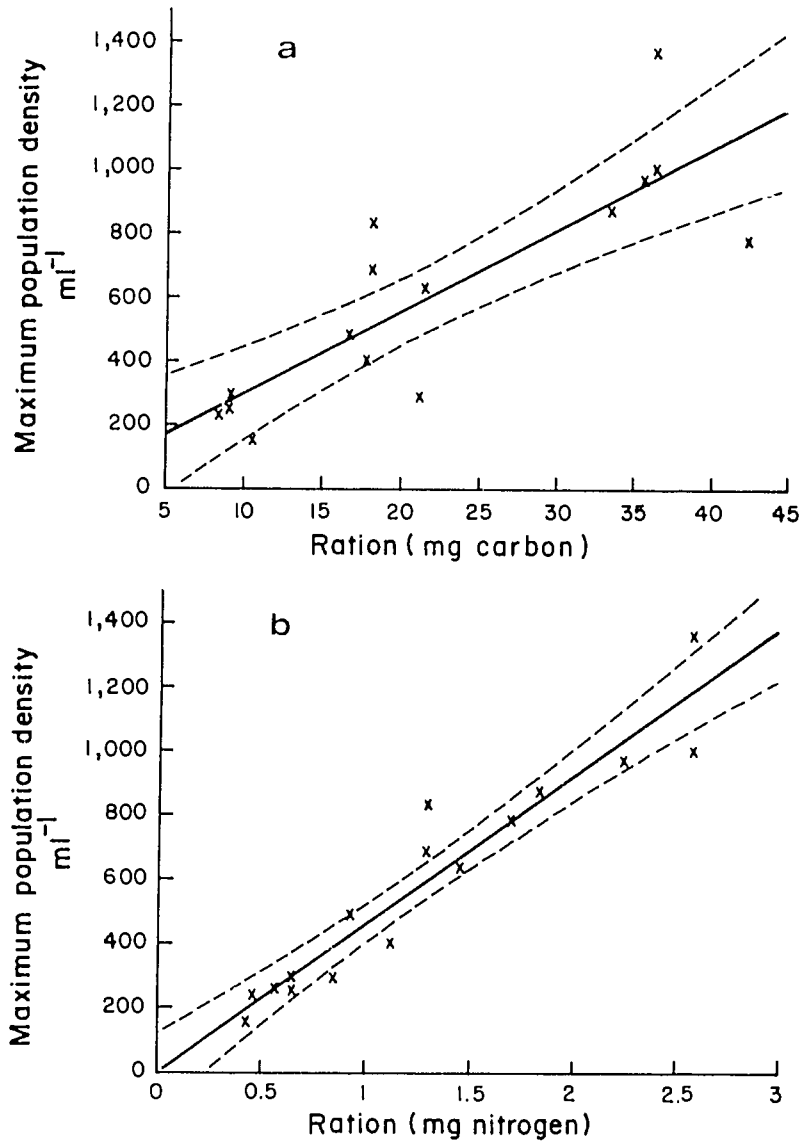

Fig. 2. Maximum population density of the marine nematode *Diplolaimelloides bruciei* attained on different detrital sources and rations (from Fig. 1 and Table 1) regressed by the method of least squares against the carbon ration (a) and nitrogen ration (b). Broken lines are 95% confidence limits of the regression lines.

confirming Findlay's (1982) observation that the nitrogen content of the detrital source is the best measure of its nutritional quality for nematodes.

By converting maximum population density in Table 1 to biomass, it is possible to calculate rough conversion efficiencies (nematode weight/food weight). Warwick (1981) found that these cultures comprise 8.91% adult males (body volume = 0.319 nl), 4.69% adult females (b.v. = 0.411 nl) and 86.4% juveniles (b.v. = 0.0217 nl): an average nematode thus has a body volume of 0.06645 nl, and assuming a specific gravity of 1.13 and dry/wet weight of 0.25 (Wieser 1960) this gives a dry weight of 0.0188 μg. Conversion efficiencies of about 1% are normal (Table 1), but mean values are slightly better on algae (*Fucus* 1.20%, *Pelvetia* 1.17%) than on phanerogams (*Spartina* 0.62%, *Halimione* 0.88%), although the conversion efficiency on the alga *Ascophyllum* (0.87%) is comparable with that of *Halimione*. Interestingly, conversion efficiencies on baby cereal (1.19%), which has a high C and N content and is balanced with vitamins, are no better than on brown algae.

Newell (1982) has reviewed evidence that the conversion of both algal debris and of detritus derived from salt-marsh plants to bacterial biomass has an efficiency of approximately 10%. In laboratory microcosms, in the absence of meiofauna, colonization of such debris follows a characteristic succession of bacteria, followed by flagellates and ciliates which utilize the bacteria with an efficiency also around 10%, so that the overall incorporation of detritus into Protozoa is about 1%. This figure is

Table 1. Conversion efficiency (C.E. = dry weight of nematodes/dry weight of food) for *Diplolaimelloides bruciei* fed on different types of detritus at three ration levels.

Detrital type	Ration mg D.W.	Ration mg C	mg N	Max. nematode density (ml^{-1})	C.E.(%)	Mean C.E.
Ascophyllum	25	8.34	0.455	232	0.87	
	50	16.68	0.910	488	0.92	0.87
	100	33.36	1.821	874	0.82	
Fucus	25	9.06	0.643	292.8	1.10	
	50	18.13	1.285	832	1.56	1.20
	100	36.26	2.571	1,006	0.94	
Pelvetia	25	9.04	0.641	251.4	0.94	
	50	18.07	1.283	686	1.28	1.17
	100	36.15	2.565	1,370	1.29	
Halimione	25	8.86	0.558	258.4	0.97	
	50	17.73	1.116	402	0.75	0.88
	100	35.46	2.231	972	0.91	
Spartina	25	10.56	0.421	153.8	0.58	
	50	21.12	0.842	292	0.55	0.62
	100	42.23	1.683	785	0.74	
Mixed cereal	50	21.47	1.443	634	1.19	

the same for meiofaunal nematodes, suggesting that they are indeed primary consumers of bacteria: if they incorporated plant debris directly, conversion efficiencies an order of magnitude higher would be expected, as are found in the macro-consumers in such systems (Cammen 1980; Newell 1982), and if they were secondary consumers of Protozoa the conversion efficiency would be an order of magnitude lower. The relative development of meiofauna and Protozoa in the potentially competitive situation of natural detrital systems has not been investigated.

As bacterial consumers, nematodes may play an important role in the decomposition of plant detritus. Gerlach (1978) postulated that a large part of the bacterial population is in the stationary phase: grazing by meiofauna stimulates active metabolism and rapid duplications, and this enhanced bacterial productivity is important for the breakdown of organic matter. In sediments, this was shown to be the case by Milton (1981), who found that *Diplolaimella shiewoodi* enhanced bacterial numbers (determined from direct acridine orange counts) in freeze-thawed sediments. However, the evidence is still equivocal as Pamatmat and Findlay (1983) found that *Diplolaimella chitwoodi* inhibits bacterial metabolism as measured by heat production in sand samples. In detrital systems, however, the effect of nematodes on the mineralization rate is significant. Findlay and Tenore (1982) labelled *Gracilaria* (an alga) and *Spartina* by growing them in the presence of $^{14}CO_2$ and measured the mineralization rates (organic ^{14}C mineralized to $^{14}CO_2$) with and without *Diplolaimella chitwoodi*. They found that, at natural nematode densities, mineralization rates of *Gracilaria* were enhanced by 300%, but of *Spartina* by only 50%. This increase in mineralization by nematodes would reduce the overall standing stock of detrital carbon for macro-consumers, but concurrent changes in microbial biomass and/or production might increase the nutritional quality of the detritus.

The trophic position and role of the copepods in detrital systems is more problematical and probably more complex. Harpacticoids may graze bacterial cells off detrital particles or ingest some detrital fragments whole. The mucilage released from macroalgal detritus, with its associated microbiota, may also be an important resource (Hicks and Coull 1983). Members of the Tisbidae may be cultured in the laboratory using a wide variety of plant and animal food sources (Hicks and Coull 1983), but the exact method of utilization of the resource is often unknown. *Tisbe holothuriae* ingests ciliates of the genus *Uronema* (Rieper and Flotow 1981), but the extent to which predation on ciliates occurs in nature is also unknown. *Tisbe furcata* has been known to attack both larval fish and nematodes (Garstang 1900), and there is indirect evidence that its predation on nematodes might be quite significant: Marcotte (1977) noted an inverse density relationship between *T. furcata* and nematodes, and it certainly appears to be the case that detrital systems either become dominated by nematodes or *Tisbe* spp., but rarely by both. Ciliates and flagellates may be important in this relationship: nematodes may outcompete Protozoa for the bacterial food supply in some situations so that if *Tisbe* fed primarily on Protozoa this inverse relationship would become apparent. Alternatively, the inverse relationship may simply be due to conditions of the physicochemical environment, nematodes being favored by brackish intertidal situations and copepods by marine sub-

tidal ones. Such mechanisms are, of course, pure speculation. Much more work needs to be done on the succession of microbial and animal communities on fresh plant detritus of different kinds under differing environmental conditions.

Meiofauna in Aquaculture Systems

Dense populations of meiofaunal nematodes and/or copepods are likely to develop naturally in aquaculture systems which utilize plant detritus as the primary food source, but the question arises as to whether such development is beneficial or detrimental to the production of the target species. Do meiofauna compete with the cultured organisms for the detrital food source and its associated bacterial flora, constituting an energy sink, or are they a crucial step in the transfer of energy up the food chain to the target species?

If the cultured species does not utilize meiofauna as a food source at any stage of its life-history, then the presence of meiofauna could be detrimental to its growth rate. Alongi and Tenore (1985) have shown that, in the presence of both *Diplolaimella chitwoodi* and *Tisbe holothuriae*, the weight specific growth rate of the deposit-feeding polychaete *Capitella capitata* was reduced when mixed cereal and red seaweed detritus were used as food sources. On the other hand, Tenore et al. (1977) showed that the presence of meiofauna *enhanced* the rate of incorporation of aged *Zostera* detritus in another polychaete *Nepthys incisa*. The influence of meiofauna in such cases is unpredictable, and clearly not well understood. Of course, neither *Capitella* nor *Nepthys* are likely candidates for aquaculture, but such experiments serve to illustrate the regulatory role of meiofauna in detrital food chains.

However, many food species reared under aquaculture feed on meiofauna, especially in their juvenile stages. Gut content analysis of the larvae and juveniles of many commercially important species of bottom feeding fish have shown the dominance of harpacticoid copepods in their diet (review by Hicks and Coull 1983), including various species of flatfish (Castel and Lasserre 1982; de Morais and Boudiou 1984) and salmon (Kaczynski et al. 1973; Feller and Kaczynski 1975; Sibert et al. 1977; Sibert 1979). It is probably true to say that harpacticoids are a crucial dietry item in the early life stages of these species. Gut content analyses reveal that nematodes are much less important, although they do not have such permanent exoskeletons and are likely to be more rapidly digested than copepods, so that their importance may have been underestimated. Hofsten et al. (1983) fed four species of nematodes to the fish *Danio* sp. and no identifiable nematodes were found in the gut when the digestion period was 3 hours or more. However, copepods in detrital systems are usually larger and more active than nematodes, and are often brightly colored, and so would be much more attractive to predators foraging visually. With regard to crustaceans, J.M. Gee (pers. comm.) found that harpacticoids comprised the major item in the diet of shrimps *(Crangon)* foraging over sand, and in young individuals (15-19 mm in length) they represented virtually the entire gut content. Bell and Coull (1978) showed experimentally that shrimp populations regulated

meiofaunal densities, and Gerlach and Schrage (1969) showed that shrimps consumed nematodes under laboratory conditions (when given no other choice). Juvenile crabs are also significant meiofaunal predators (Scherer and Reise 1981).

The evidence therefore suggests that the presence of meiofauna is essential in bringing many commercially important marine food species through their early developmental stages. In general, harpacticoid copepods seem to be the favored resource. In pilot studies, *Tisbe* species and several other harpacticoid copepods have been reared in mass culture on a variety of vegetable and other food sources (Kahan 1979 and references therein) with a view to using them a food source in marine fish hatcheries. Kahan et al. (1982) devised a method of presenting the naupliar larvae of harpacticoids to very young hatchlings by culturing them in floating net-bottomed trays with a mesh size of 80-100 μm, through which the older copepods could not pass. Whether nematodes might outcompete the copepods in such systems in the longer term is not known. I have alluded earlier to our lack of knowledge about what conditions favor the relative development of copepods or nematodes in detrital systems, and research into this field is required urgently in order that the conditions favoring harpacticoids can be reproduced. Such research may be crucial in the development of many detrital systems for aquaculture.

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Discussion

ANDERSON: In Dr. Gray's opening comments, there was reference to a 'microbial loop'. If you use Steele's figures for organic carbon transfer, i.e., with 10% conversion efficiency from one level to another, you find that the transfer of energy to zooplankton is negligible. They appear as a very small energy compartment. Dr. Warwick has shown us that the biomass of the nematodes is clearly related to the N-content of their food resources. I would say that when we consider transfers, as energy or carbon transfer, we are missing the point of transfer of *protein* across trophic levels. We saw from previous papers that the food quality (protein quality) of detritus is very important. Have you looked at N transfer efficiencies? Perhaps many of the organisms that we classify as detritivores or even some herbivores and omnivores are using a lot of this animal protein. Many herbivorous or omnivorous animals can cope with a large intake of animal protein if such is available, for example, consumption of the placenta after parturition by ruminants. The capacity to cope with digestion of animal protein is there. Some of our definitions may be somewhat artificial.

WARWICK: Well, the meiofauna are really best considered as predators on bacteria. I have not looked at N transfer efficiencies. I have only measured transfer efficiencies in terms of biomass—a very crude measure.

ANDERSON: But an animal feeding on detritus could be taking in lots of valuable protein from bacteria and meiofauna.

WARWICK: Yes. I am sure it could.

ANDERSON: In that case, an N-transfer budget would show much higher efficiencies than those which you have measured.

MORIARTY: This is very important. If the conversion efficiency of available food to meiofauna really is say 0.5 or 1.0%, then they are not beneficial to aquaculture systems. However, it is the

step between the detritus and the meiofauna which is important, namely the bacteria. We must find out what they are doing in the detritus and their conversion efficiency not only in terms of C but also in terms of N. If the bacteria are converting detrital N into protein which is then available for use by the meiofauna and higher organisms, then this is a key point for our discussions. Only then can we work out how to utilize detritus to maximum benefit. We need to get the best possible conversion efficiencies from the bacteria to meiofauna or high organisms.

WARWICK: Then perhaps we should avoid the meiofaunal step in aquaculture. They may compete with fish for the detrital resource and the bacteria.

MORIARTY: But the meiofauna can be important mineralizers of N and P. In a fertilized pond in which there is a rich layer of organic material on the bottom, perhaps a rich meiofaunal population could help to mobilize dissolved nutrients for algal production.

WARWICK: Meiofauna also excrete lots of ammonia.

MORIARTY: We need to look at all these options and then build systems for specific purposes.

SCHROEDER: Let us reconsider the question of transfer and losses: the idea of a 1% efficiency with respect to carbon fixation in meiofauna. If this is so, the system could not supply enough carbon to produce meiofauna and still support fish production as well. However, for every 100 g food consumed, perhaps about 90 g is released to the system by excretion and respiration. All of this is not lost. If say 20-40 g go off as CO_2, still say 40-50 g are recycled to the system in other compounds. So, the real efficiency may be much higher.

ANDERSON: This is why decomposers are so effective. There is continuous recycling. There are feedback systems.

GRAY: I did some measurements on ammonia excretion by meiofauna in Australia. Their input is very significant and could be taken up by bacteria.

WARWICK: We must not think only in terms of conversion efficiencies. The target species must be able to grow and complete their life cycles.

BILIO: What evidence is there that nematodes are consumed by fish, young fish for instance?

WARWICK: They are consumed by fish and shrimp. Work done by Gerlach and Schrage (1969)* has shown that laboratory cultures of shrimps can be sustained by nematodes alone. However, this was in the absence of other food items. Copepods are usually the preferred item for fish. Many *Tisbe* spp. in detritus are brightly colored—reds and blues—and are easy to locate.

BILIO: What about the digestibility of nematodes? They have a strong cuticle. Wouldn't they be as resistant or more resistant to digestion than copepods?

WARWICK: Maybe. I don't know of any work on this.

BILIO: I suspect this from my observations on nematodes ingested by Turbellaria.

WARWICK: The copulatory apparatus of nematodes, particularly spicules, should be good indicators of their digestion. These should remain very prominent in the gut of consumers.

BILIO: Yes. There was once a new tubellarian species described in error because of the presence of a nematode spicule.

SRINIVASAN: When considering digestibility, we should not forget that some bacteria are also chitinoplastic.

PULLIN: You did not mention rotifers. These are important in aquaculture, particularly in hatchery and nursery systems. Perhaps they are best defined as zooplankton, not meiofauna. In carp hatchery ponds, one technique used is to develop a rich mixed zooplankton and then to apply an insecticide which selectively kills the arthropods. A 'bloom' of rotifers then follows to provide excellent food for carp larvae. Are any rotifers important components of the meiofaunal systems which you have discussed?

WARWICK: Certainly not in marine benthic situations. Rotifers are present but they are very uncommon.

BILIO: They can become abundant in some special situations, for example, in littoral pools.

PULLIN: What about freshwater ecosystems?

WARWICK: I really do not know. Probably they are more important. I would not like to speculate on their importance in systems with rich detritus. I doubt that meiofauna could be important foods, say for fattening up tilapia. However, they may be useful foods during the early life history stages of some fish.

SCHROEDER: Yes. The early life history stages of many fish require high animal protein food—like zooplankton.

WOHLFARTH: Larval feeding may indeed be the most critical part of the growth cycle for fish. David Kahan in Israel has mass-cultured nematodes and copepods for feeding fish larvae and fry and is now at the stage of setting up a pilot plant for harpacticoid copepod culture.

WARWICK: Where is this work in progress?

WOHLFARTH: The Hebrew University of Jerusalem.

MORIARTY: Regarding the need for a link to the heterotrophic food web to generate nutrients, what about the Protozoa? Would they be the main regulators of bacterial production, by grazing on bacteria in the sediments. Certainly, as Dr. Gray has pointed out in the water column, the microflagellate Protozoa have an important role in limiting bacterial production and biomass. Fenchel and Jørgensen (1977) in their review suggested that Protozoa were the main controllers of bacterial production in the sediments, but I know of no clear evidence for this. What do you think are the relative roles of Protozoa and meiofauna?

GRAY: My feeling is that in most sediments the Protozoa play a minor role and the meiofauna are the major bacteria grazers. However, it may be different in highly flocculent material. Here, there may be large ciliate populations. These are very difficult to study.

WARWICK: I agree. I don't think the Protozoa are as important as the meiofauna. Fenchel (1969)** found that they were important in so-called capillary sediments, that is, sediments like muddy sand with a definite pore structure. In most muds and detritus, they are not important bacteria grazers compared to meiofauna.

MORIARTY: Which meiofauna?

WARWICK: Nematodes, which are overwhelmingly abundant.

BILIO: On part of the seashore, where plant detritus accumulates and there may be anaerobic conditions, you can also find large populations of ciliates.

GRAY: Yes, where you get a mass macroalgal debris you can find this, but not say, on the floor of a fjord. So, it depends upon conditions. However, what we can say is that microflagellates are not

important in the sediments. Their role, as performed in the water column, is taken over by meiofauna and sometimes perhaps by ciliates.

BILIO: There is obviously a need for more work on the nematode meiofauna and the ciliates in fishponds and natural systems.

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Detrital and Algal Based Food Chains in Aquaculture: A Perspective

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Abstract

A review is given of the energy flow in semi-intensive aquaculture systems. Such systems require external sources of organic carbon, nitrogen and phosphorus. The roles of photosynthesis and the detrital food chain in semi-intensive aquaculture are discussed with reference to water quality and pond management.

Introduction

Most aquaculture systems throughout the world produce fish and are characterized by annual yields of approximately a few hundred kg/ha. These "extensive" systems are almost wholly dependent upon natural production and receive little, if any, feed supplements. At the other extreme are "intensive" systems yielding tens of thousands of kg/ha annually. Such systems, raceway trout and shrimp production for example, are dependent upon high-quality, nutritionally complete, manufactured feeds. Intensive systems require very high water turnover rates, frequent cleaning and auxillary mixing and aeration in order to maintain acceptable water quality conditions.

Currently receiving worldwide attention are "semi-intensive" systems, which yield from several hundred to several thousand kg/ha. Semi-intensive systems are dependent upon the addition of organic materials, such as feed or waste products, which are seldom nutritionally complete. Here, the target animals are partially dependent upon "natural" feeds (microorganisms and/or detritus) to satisfy their nutritional needs. Natural food chains and microbiological processes could be enhanced to

increase yields and lower costs of aquaculture products. Yet, little is known concerning the productivity and relative importance of various food chains. The proceedings of the Second International Conference on Aquaculture Nutrition: "Biochemical and Physiological Approaches to Shellfish Nutrition" (Pruder et al. 1983) did not include a single paper acknowledging the potential importance of detritus and associated microbes and food webs based upon this energy source.

According to Slobodkin (1970) and Rheinheimer (1980), 80-95% of the chemical energy bound in living creatures may be lost in the transfer from one trophic level to another. If this be the entire issue, it is difficult to envisage large gains in yields being derived from lengthening food chains in aquaculture ponds. Certainly, there are benefits to be derived or the whole notion would have been abandoned. But surely the potential benefits are not fully appreciated. Research to control and manipulate food webs (algal and detrital) is underway in the United States at the Oceanic Institute, the University of Hawaii, the University of South Carolina, Texas A & M University and Auburn University. This research is as much motivated by needs to minimize microbially-induced variation in water quality as to improve nutrition. As will be shown, water quality and nutritive quality are interdependent in semi-intensive aquaculture systems.

There is no shrimp farming technology practiced anywhere in the world that would be commercially viable in the United States, competing in the world shrimp market. The development of a viable US marine shrimp farming industry is currently constrained by low profitability and high risk (Pruder et al., in press). To date, we have been unable to develop acceptable combinations of yield, reliability and cost. However, innovative methods, including some to exploit natural food webs, are under development. If successful, the resulting technology will be available for application to a wide range of production systems throughout the world.

Aquaculture development in the United States has been slow and high expectations remain largely unrealized. Contributing in part to this state of affairs is terrestrial bias: bias towards the application of agricultural principles and methods. This bias has impaired our ability to recognize inherent differences in chemistry and physics between terrestrial and aquatic culture processes (Pruder 1983). Schroeder (1978), describing the autotrophic and heterotrophic production of microorganisms in intensely-manured fishponds, triggered interest in natural food webs relevant to aquaculture. Progress in unraveling the mechanics of differences between "good" and "bad" ponds has been slow. At the Oceanic Institute, research is under way in eight replicate ponds to study pond-to-pond variation. We are monitoring physical, chemical and biological factors, seeking to identify combinations of factors that support good growth, conversion efficiency and survival.

It is important to recognize that we know so very little of the workings of semi-intensive production systems. Can we answer the following questions? What, specifically, is offered by the detrital based food web, or the algal based food web? Is there any part of what is offered that may be of significant value to aquaculture? If so, how do we develop the technology and/or management strategies to exploit potential benefits?

Energy Flow in Aquatic Systems

This analysis is developed from the perspective of semi-intensive aquaculture production systems which yield thousands of kg/ha. What follows is a review of some work on algal and detrital food webs. A diagram showing the heterotrophic conversion of carbon from primary production was selected from Newell (1984), where he considers algal and detrital food chains, points out their inherent interactions in shallow water and makes reference to aquaculture.

In this herbivore dominated pelagic system, 62% of the carbon from primary production, directly or indirectly, enters the detrital food chain (Fig. 1). This may represent a minimum contribution because under other conditions, a greater portion

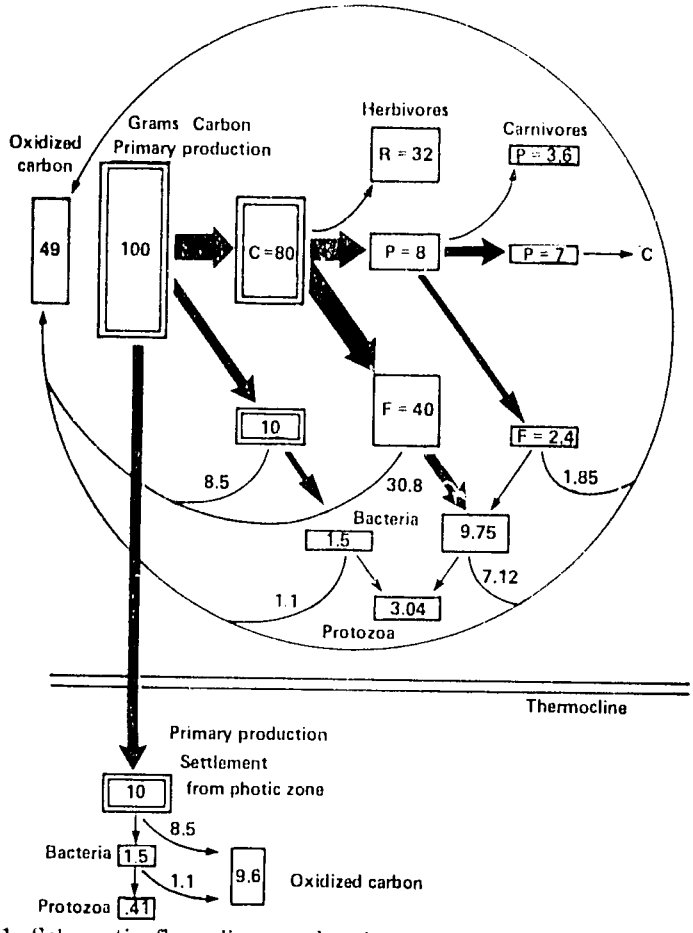

Fig. 1. Schematic flow diagram showing the heterotrophic conversion of carbon from primary production in a herbivore dominated pelagic system. Of the 100 g carbon from primary production, 80 g is assumed to be consumed directly by herbivores, 10 g of the nongrazed particulate matter sinks below the thermocline and 10 g is decomposed in the surface layers. Absorption efficiency of the herbivores is assumed to be 50%, and that of the carnivores 70%. Bacterial carbon conversion of plant detrital material is 23% whilst the efficiency of transfer from bacteria to flagellates is 27%. It will be noted that the feces have been assumed to decompose in the surface waters, but it is recognized that a variable proportion of the fecal material may sediment below the thermocline. C in the figure = consumption; R = respiratory losses; P = production; and F = feces.

(more than 90%) of the primary production could flow directly to the detrital food web (Mann 1972). Note that the biomass production of Protozoa through the detrital food web (3.45% of primary production) is greater than the biomass production of first-order carnivores in the algal food web (2.0% of primary productivity). In both food webs, the bulk of the carbon is oxidized and recycled in subsequent photosynthesis. Preference for one food web over another must be based upon the relative nutritive quality of various carbon forms to the feeding organisms of interest.

The Aquaculture Case

Perhaps the simplest example of high-yield aquaculture is the raceway production of trout. Here, the nutritive needs of the fish are met through nutritionally complete, manufactured feeds. Environmental water quality is maintained by high water turnover rates which remove fish waste and uneaten food, dilute waste metabolites and supply the necessary oxygen. Photoautotrophic and heterotrophic microorganisms and microbial food webs do not play significant roles in this process. This production technique is site specific, requiring dependable, large supplies of high quality and low cost water.

Shigueno (1975) developed an intensive system for the production of marine shrimp in Japan. His system also was dependent upon high quality feeds, frequent cleaning and high water turnover rates. Although the system was technically feasible, it provided economically marginal returns, even when shrimp were sold for over US$30/kg. Shigueno has recently turned his attention to earthen pond marine shrimp production using high quality feeds, but giving special attention to maintenance of desirable algal blooms through bottom cultivation and pond mixing (Shigueno 1984).

A raceway system for producing marine shrimp is operated for economic evaluation by Marine Culture Enterprises, Kahuku, Hawaii. It is somewhat dependent upon *in situ* microalgal photosynthesis. It is not clear whether this dependence upon primary production is related to improved water quality or the production of vital nutrients.

Aquaculture in the foreseeable future is likely to remain largely dependent upon semi-intensive systems in earthen ponds. It is unlikely that water turnover rates will exceed 10% of pond volume due to pumping costs and/or insufficient water quality or availability. Neither is it expected that nutritionally complete feeds will be available or economically justifiable. Other means than those discussed above are required to deal with animal waste, uneaten food, water quality and vital nutrients. Interactions between photosynthetic and heterotrophic microorganisms, including algal and detrital based food webs, can supply vital nutrients and help maintain acceptable water quality.

Simplified diagrams of probable material flow and interactions between microbes and target animals for various hypothetical, semi-intensive aquaculture systems are presented in Figs. 2 to 6. These systems depict the polyculture of marine shrimp and bivalve molluscs. Possible similarities and differences between these hypothetical,

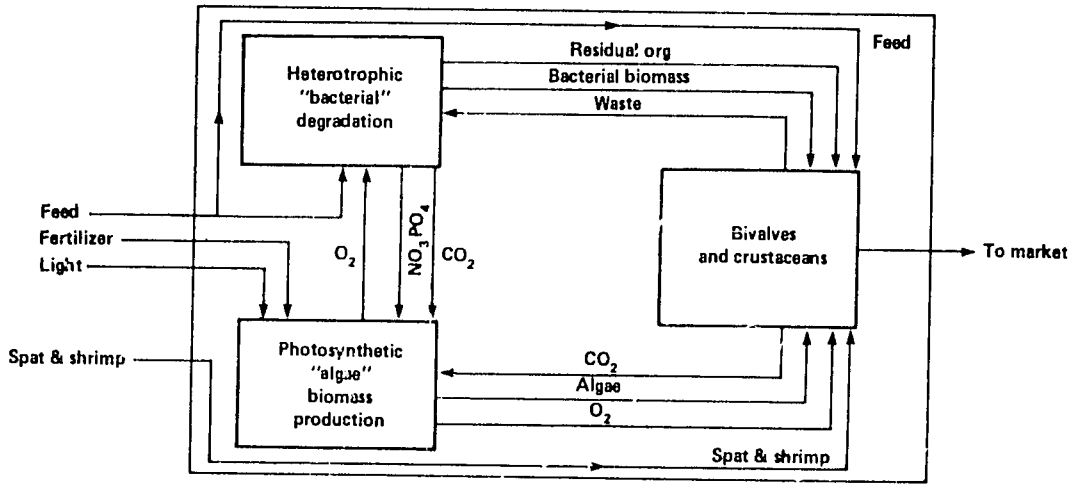

Fig. 2. Diagram of material and gas flow in a semi-intensive aquaculture system.

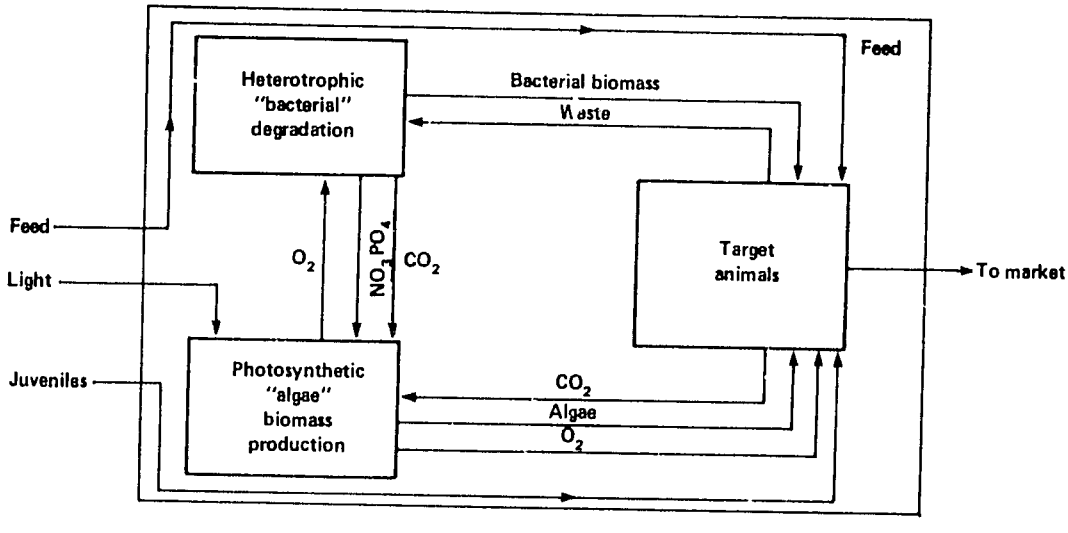

Fig. 3. Diagram of material and gas flow in a semi-intensive aquaculture system, assuming high-quality feed and animals feeding on organics, algae and bacteria.

semi-intensive aquaculture systems and a typical herbivore dominated pelagic system (Fig. 1) are now discussed.

The target animals in the aquaculture model, shrimp and bivalves, feed on algae, detritus and associated microorganisms. All diagrams depict algae and consumer feces entering the detrital food chain marked heterotrophic bacterial degradation in

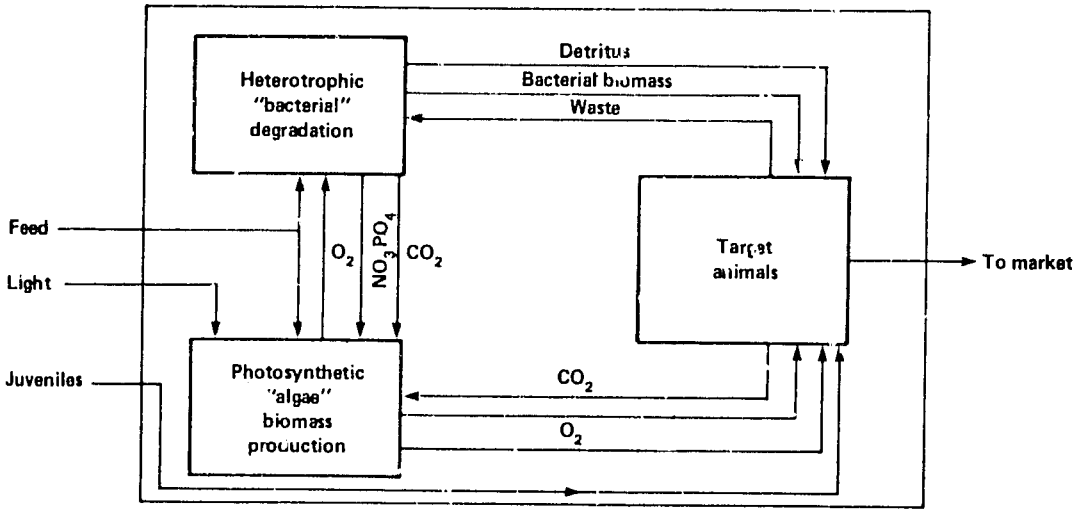

Fig. 4. Diagram of material and gas flow in a semi-intensive aquaculture system, assuming low-quality feed and animals feeding on algae and bacteria, etc.

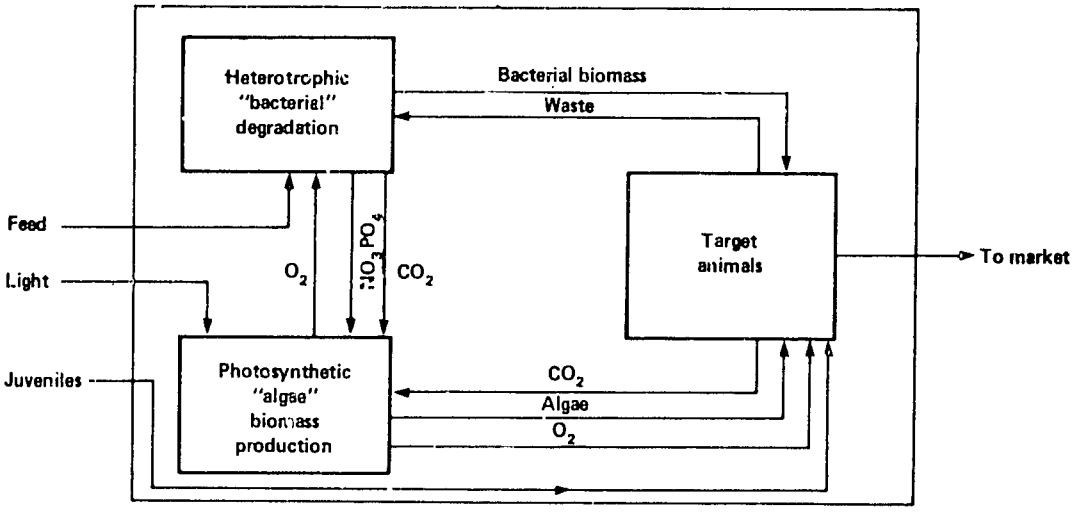

Fig. 5. Diagram of material and gas flow in a semi-intensive aquaculture system.

the aquaculture diagram. All diagrams reflect output from the detrital chain flowing both to consumers and to support further photosynthetic biomass production.

Newell's diagram shows primary production as the base of both algal and detrital food webs. In contrast, semi-intensive aquaculture systems are dependent upon organic material (feed or waste) input that serves as the base of both food webs. These organic materials are either consumed directly by the target animals or enter

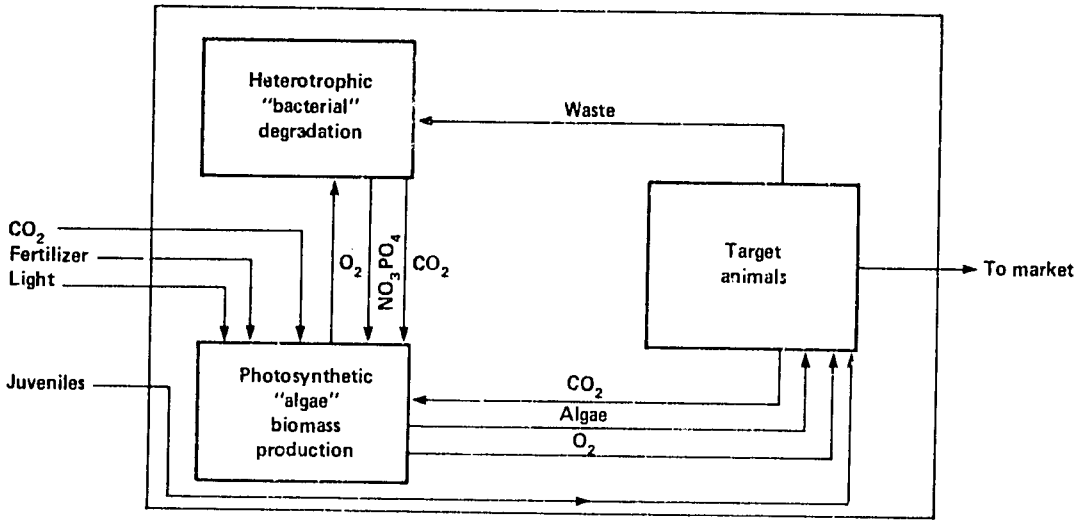

Fig. 6. Diagram of material and gas flow in a semi-intensive aquaculture system, assuming CO_2 and fertilizer input (inorganic) and target animals feeding on algae.

the detrital food web or both. Oxygen is consumed and carbon dioxide and inorganic nutrients are produced. The carbon dioxide and inorganic nutrients are then recycled through algal photosynthesis, which produces dissolved oxygen and algal biomass. The algae produced serve as feed for the target animals or enter the detrital food web. Photosynthesis, although not the base of the food web, is nonetheless of critical importance in semi-intensive aquaculture systems where algal and detrital food webs are interdependent.

Aquaculture ponds have characteristics similar to eutrophic waters where productivity is often light-limited and oxygen depletion is a threat. The system depicted by Newell is more often nutrient-limited and dissolved oxygen and dissolved carbon dioxide seldom vary outside acceptable limits and are seldom considered limiting factors.

As most aquaculturists appreciate, oxygen necessary for aerobic metabolism should not be taken for granted. Cassinelli et al. (1979) have shown that net photosynthesis supplies the bulk of the oxygen to semi-intensive pond systems. Algae, although not the base of the food web, are nonetheless essential for providing oxygen (Pruder and Bolton 1980; Pruder 1981, 1983, in press)..The oxygen supply role leads into suggestions concerning exploitation of the detrital food chain, which will be discussed below.

The rate of oxygen transfer through the air-water interface is often insufficient to meet the pond's oxygen demands, making *in situ* photosynthesis essential. Net primary production becomes dependent upon *in situ* production of nutrients released during aerobic respiration of organic matter. In semi-intensive aquaculture, primary production and respiration are coupled. Photoautotrophs and heterotrophs are interdependent.

An average net photosynthetic rate of 2 g C/m^2/day, would require a surface area of 50 m^2 to produce 100 g C. At a nominal animal loading of 1,500 kg/ha, a 3% by body weight feeding regime and a 50% carbon content in the feed, the feed carbon required per day for an area of 50 m^2, is 112 g. The natural phytoplankton production, if directly consumed by the animal, could satisfy the animal's carbon needs.

If the input feed itself is nutritious and acceptable to the target animal and is cost effective, food web decomposition and conversion would be wasteful and should be repressed. If, however, inexpensive and nutritively poor materials can be converted to nutritious feed by microbial processes and/or provide nutrients for algal photosynthesis, the food web should be exploited.

It is necessary to realize that nutritive quality and photosynthetic enhancement are of equal importance to a consideration of carbon and energy flow. Phillips (1984) discusses the role of different microbes and substrates as suppliers of specific essential nutrients to marine detritivores. I am pleased to second his recommendation that, "studies of nutritional values of detritus and factors affecting trophic transfer consider the importance of animal requirements for specific essential nutrients, as well as energy and protein".

Summary

For all practical purposes, semi-intensive aquaculture technology must include, at least, but is not limited to:

1. An external source of organic carbon, most likely of terrestrial origin
2. An external source of nitrogen, phosphorus, etc.
3. A system of decomposers to make the nutrients available for primary production, maintain water quality and provide nutritive feed materials
4. A system of primary producers to provide oxygen, maintain water quality and provide nutritive feed materials
5. A population of desirable target animals.

The relative importance of algal versus detrital food chains depends upon the nature of the input and the requirements of the target animal. Food web interactions should be considered and exploited.

It is the aquaculturist's responsibility to identify the nutritional and water quality requirements of the target animal and the relative cost and availability of input materials; then, working with microbiologists, ecologists and nutritionists, to devise the most cost-effective pathways or food webs and finally, working with food technologists and engineers, to devise the delivery and control technology.

Lastly, we must not underestimate the importance of timing in management strategy. A pond's capacity to produce oxygen is fixed by the day-night cycle. Maximum biological oxygen demand should occur concurrently with the maximum photosynthetic activity using as much of the water column as possible. Correspondingly, minimum biological oxygen demand should occur during the dark hours especially just before dawn.

Acknowledgements

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Discussion

EDWARDS: Please do not forget in your definition of aquaculture that not all aquaculture products are "for sale". In developing countries, family consumption is extremely important.

PRUDER: I agree. However, the products still have 'value'. They must have to be worth the effort of production.

SCHROEDER: Your discussion on the integration of marine bivalve and crustacean production points up a problem of marine aquaculture as opposed to say a balanced pond culture in freshwater. Your bivalves can be the equivalent of a filter-feeding fish in freshwater polyculture. Therefore, you can have a better balanced system than with crustaceans alone.

SRINIVASAN: You have made a valuable contribution by drawing our attention to the inorganic cycles in aquaculture and in particular to carbon, the importance of which has often been overlooked. Carbon availability for plankton growth, etc. may be a major limiting factor.

One further general point is that we must consider water 'quality', that is how the water chemistry affects the animals, before getting into the more detailed biology and biochemistry of production processes. We must consider buffering capacity, alkalinity, etc.—the total water chemistry.

FRY: When photosynthesis ceases at night, your plankton will change from important producers of oxygen into consumers. In marine systems, high in sulfate, if oxygen is no longer available, H_2S will be produced and will come out from the sediment into the water column.

PRUDER: Ryther's group, which I joined for a while, used secondarily-treated effluent. In other words, it was rich in N and P but had already been stripped of a lot of carbon. The results would probably have been better using the effluent just after primary settlement. However, even in the summer months, the group was able to sustain a diatom bloom. The species was *Phaeodactylum tricornutum*. This is a 'weed' diatom. It never seems to dominate in nature—only in manmade algal ponds! It is no good as a feed for bivalves. We looked at possible limiting factors to phytoplankton succession, such as silicate. When we grew *Phaeodactylum* in comparison with *Thalassiosira* and *Skeletonema* in laboratory culture, it gave no clues as to why it should outrun these species in the ponds until the pH went to 9.3 and the oxygen to about 200% saturation. The work of Ryther's group was fine, given the state of knowledge at the time. In retrospect, we could probably do much better now by not going for a tertiary level of production. Remember as well that whereas mussels eat almost anything and clams are intermediate, among the bivalve filter feeders the oysters require algae almost exclusively.

In summary, oysters could be a useful component of a tropical marine polyculture. We grow then in our shrimp pond outfalls in Hawaii. We do not know what the effect would be of putting them in the ponds. Hard clams *(Mercenaria mercenaria)* will grow in shrimp ponds.

EDWARDS: Are diatoms always present in the water, as well as greens?

PRUDER: Usually when the ponds are productive, diatoms are present. We don't always have that. have that.

BLACKBURN: The balance of CO_2 fixed to O_2 produced is a 1:1 ratio in a coupled system. So I fail to see how a large phytoplankton biomass in pond can do anything but cause you trouble by producing large swings in oxygen concentration. Why not have dark ponds?

PRUDER: I have considered this. The phytoplankton do have a beneficial effect on water quality. For example, they take up ammonia. Light appears to be essential.

BLACKBURN: But they in turn are broken down and produce more such endproducts.

PRUDER: True, but the system is not entirely based on primary production. We are adding other materials which are also being processed.

COLMAN: The phytoplankton effectively store organic matter so that it does not become an immediate oxygen demand to threaten the fish.

BLACKBURN: Yes, I can see that this aspect of oxygen demand will be out of phase, but ultimately you will reach some sort of balance.

PRUDER: If your target animals consume and oxidize the algae, this is a short, efficient food chain.

BLACKBURN: But, as I understood it, your first objective was to use the phytoplankton as incidental oxygen producers in a high energy-input system.

PRUDER: Well, suppose I have no phytoplankton and I use Dr. Schroeder's feeding rate of 5 $g/m^2/day$, the system will not run for lack of oxygen.

BLACKBURN: If you are producing 2 to 3 times oxygen saturation during the day, this is very high production. You must lose a lot of oxygen.

PRUDER: Yes, and we must find out how to improve matters. Most ponds are managed so that maximum oxygen concentrations are around 12-13 mg/l. I have never seen ponds go to 30 mg/l as mentioned by Dr. Schroeder.

BLACKBURN: Well, you could limit this by encouraging heterotrophic production. This would create an oxygen demand. Is this what you want?

PRUDER: It could be if the quality of the material produced is higher than that of the added materials.

BLACKBURN: Has anyone ever tried a dark fishpond?

WOHLFARTH: Yes, by overproduction of duckweeds (*Lemna*, etc.). There was close to total fish mortality. It stopped oxygen from being produced in the water column. It also of course stops diffusion of oxygen.

SCHROEDER: On dark systems, when Coca-Cola and W.R. Grace were developing highly intensive shrimp raceway culture, they got vastly increased survival and production in outdoor as opposed to indoor systems with all other factors kept constant.

SRINIVASAN: The phasing of all this is important. By using the phytoplankton you are 'buying on credit', removing some of the BOD, but the reckoning will come later. One solution is to remove some of the phytoplankton. It can then be regenerated.

BLACKBURN: I don't think the fish biomass is significant in terms of the overall carbon and oxygen budgets.

ANDERSON: Your models showed some interesting feedback effects. The higher the quality of a feed that you give to an animal, the higher the BOD that it will create through its feces, etc. I like the concept of producing oxygen by phytoplankton. However, your system also has a spatial problem because your target organism is a bottom feeder. You need to maintain acceptable oxygen concentrations at the bottom, which is difficult. If you add a spatial dimension to your models, on the one hand, settled organic material can create anaerobic bottom conditions whereas dissolved organic matter can give rise to oxygen deficits in the upper water column. Could you grow your shrimps on a false bottom—a mesh which would keep them off the anoxic sediments and would allow for the diurnal swings in oxygen concentration in the water column while still keeping them in tolerable conditions? In other words, could you take advantage of a stratified system?

PRUDER: It may be possible, I'm not sure it would be desirable. Shrimp do not just crawl around the bottom. Also they are not simply carnivores. The problem with our current strategy is that most of the oxygen is in the upper third of the water column and much is lost to the atmosphere.

It would be good if we could devise a stable lower density phytoplankton-based system with light penetration to greater depths. We could then produce oxygen by day throughout the water column. Thereby we could avoid putting any energy into mixing.

WOHLFARTH: A mesh above the bottom would not be a good idea to keep *Macrobrachium* off the bottom in this way. This is a bottom-living detritivore.

ANDERSON: Could you not arrange a mesh size that would allow waste materials to fall through the mesh to the bottom—there to be processed and to enhance production through the dissolved organic carbon route—while the target animal feeds on the mesh in better quality water conditions?

WOHLFARTH: In our polycultures, *Macrobrachium* eats detritus on the bottom, rather than supplemental feed.

PRUDER: It may be different for penaeid shrimps. We don't know very much about their feeding habits and preferences. We are trying to analyze these at present. Dr. Warwick's idea is just an indication of how we sometimes wish we could take the upper waters of a pond down to the bottom and vice-versa. Although maybe Dr. Schroeder would not agree.

SCHROEDER: I can agree that there are aerobic and anaerobic bacterial processes which are both important.

PULLIN: Let's get back to oxygen. Low early morning dissolved oxygen is common in tropical waste fed ponds, but some of the cultured fish, particularly tilapia, are very tolerant to this. In our manured pond experiments with Central Luzon State University, we had early morning DO's down to zero, but the tilapia survived. However, sublethal effects are important. It is particularly important to have enough oxygen for growth.

Perhaps some of the variability in yields between ponds is caused by variability in available oxygen for finfish, if not for shrimp as well.

EDWARDS: At AIT, we have collected some data on the sublethal effects of low oxygen on growth. We used increasing loadings of septage (cesspool slurry) to tanks. The algal populations built up but they were stable and there was a corresponding relationship between dawn dissolved oxygen and septage loading. We did get down to zero oxygen at dawn for some tanks. The fish grew very fast at first and then growth fell off and we got some mortality. This may have been due to low oxygen and/or increased concentrations of nitrite (and possibly ammonia). I must emphasize that this was under conditions of *stable* not collapsing algal populations.

PRUDER: It is possible to have a healthy algal population of density such that you get just enough light penetration for the algae to produce enough oxygen and use enough CO_2 to stay in balance with the bottom. You can have an algal population which is producing essentially nothing and is stable; just recycling CO_2. Stability is not the important point. It is the net production of oxygen that is important.

COLMAN: In terms of using the algae to get net production of oxygen, some cropping of the algae by fish is probably a good thing even if the amount of production of these fish is small. The standing algal crop is reduced by such fish and the diurnal oxygen swings are smaller. If this is not sufficient, then some of the algal-rich water can be removed and replaced with new water. I have always been struck by how great the algal production of oxygen is when a pond is first set up and the algal population is growing rapidly. Perhaps we could envisage systems of removing algae-rich water for stocking new ponds to be topped up with new water.

BILIO: That is what the Japanese do in eel farms. They have a well-developed system of water exchange and dilution.

COLMAN: You need not throw away the water removed. It can go to other ponds, for example.

WARWICK: You recommend having food chains as short as possible. I am sure that this is right for an aquaculture system in which you are adding food to fatten animals. However, for a self-sustaining aquaculture system in which the animals are reproducing, you would have to consider also the requirements of the early life history stages. We discussed this after my paper. In nature, fish nursery grounds are very different habitats from adult feeding grounds. Can you envisage a compromise system in which fish can be bred and fattened?

PRUDER: Well, I did say the shortest food chain *possible*. For most aquaculture operations, it has been found most efficient to separate hatchery, nursery and growout procedures.

WARWICK: Isn't that very labor-intensive?

PRUDER: I wouldn't like to exclude any possibilities. If a long food chain appeared a viable proposition, we could use it.

PULLIN: Hatchery, nursery and grow-out procedures are usually separated in developing and developed countries.

Session on Manipulation of Detrital Systems for Aquaculture

CHAIRMAN'S OVERVIEW

R.S.V. Pullin

The title of this session is optimistic. Although many aquaculture researchers and producers use detrital food chains to produce fish, their understanding of the flow of nutrients in their systems is very limited. Culture installations and cultured fish are often regarded as 'black boxes': the inputs being water, feeds, fertilizer and solar energy and the outputs fish, sediments and effluent water. A greater understanding of the chemical and biological basis for fish production in such systems is an essential prerequisite to manipulation of detrital food chains.

It is clear from the papers to be presented here and from our discussions so far in this conference that the most interesting and productive culture systems that involve detrital food chains are fishponds for which the major or the sole inputs are organic wastes. The more intensive tank or cage systems in which large amounts of supplemental feed are given are also interesting because fish feces and unconsumed food particles enrich detrital feeding niches. These contribute significantly to production, especially in systems in which detrital substrates are resuspended by aeration, agitation and fish movements. However, it is the waste-fed pond that contributors to this conference have found the most appropriate system for study and discussion. Here, as in the well-established principle of recycling organic wastes in agriculture by land application, the term 'waste' has no derogatory connotations.

Admitting that our knowledge of how waste-fed pond systems work is poor, let us first consider how good existing practices are in terms of fish production. I would say that they are not bad. We can produce about 30 kg/ha/day over say a 300 day growing season in the tropics, i.e., about 9 t/ha/year. Yields of five to eight t/ha/year are more usual in production ponds, but yields of 10 t/ha/year and more have been achieved experimentally, even without inorganic fertilizers and feeds to supplement the organic waste inputs. This is impressive animal protein production by any terrestrial comparisons. However, one must consider the resources (energy, land area, labor, water, etc.) necessary to produce the organic wastes inputted to the pond, not just the pond area, when comparing terrestrial and aquatic food producing systems. This requires a resource economics approach which is outside the scope of this conference.

In this session, we will consider how to increase yields beyond those presently achieved. The carrying capacity of a pond is a useful concept here. It can be increased by using expensive inputs such as high quality feeds and aeration, but can we find other more cost-effective routes? We know how to 'green up' ponds for herbivorous fishes, but not how to manipulate the pond's autotrophic and heterotrophic food webs to channel more nutrients into fish flesh. Our knowledge of how to manipulate the detrital food web is almost zero. It is to be hoped that this session will frame the questions which must be answered by future research and will also consider the practical options which could be tested in the near future, such as improvements in waste handling, waste loading techniques, water management and quality control and associated fish husbandry practices.

The papers to be presented are wideranging and interesting. Dr. Peter Edward's contribution emphasizes the great potential of herbivorous fish as producers of animal protein from low grade vegetable inputs. The grass carp *Ctenopharyngodon idella* is by far the most important species, although some other cyprinids and *Tilapia rendalli* merit much greater study. From the work done by Dr. Edwards and his colleagues at the Asian Institute of Technology, Bangkok, the production of 5-6 t of tilapia/ha/year on nightsoil–water hyacinth compost is particularly impressive.

The paper by Drs. Piedrahita and Tchobanoglous offers a new perspective on health risk assessments for sewage/wastewater fish culture. This remains a controversial topic. The key concept here is 'reasonableness of risk balanced with demonstable benefits.' Public health is the most important issue in this sector of waste-fed aquaculture and this paper is most welcome. It will help those who wish to establish practically attainable standards of safety, similar to those applied to other food producing systems.

The paper by Drs. Wohlfarth and Hulata reminds us again of the high yields attainable from waste-fed systems: up to 50 kg fish/ha/day from static water ponds, if supplemental feed is given as well as inorganic and organic fertilizers. These authors point out that we do not understand how to increase these yields either in absolute terms or in terms of economic efficiency. They emphasize the lack of critical experimentation on the chemical and biological bases of fish production in ponds. Very little critical work has been done apart from the programs of AIT, ICLARM and Israeli groups. We must therefore define research needs for the future with a view to improving the biological and economic efficiency of fish production, keeping the best aspects of traditional and current practices and testing new ideas. The suggestions for practical manipulation of pond systems will likely be the most important output of our conference.

Use of Terrestrial Vegetation and Aquatic Macrophytes in Aquaculture

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Abstract

There is a need to promote fish culture for small-scale farmers in developing countries to augment decreasing supplies of wild fish, the traditional source of animal protein in many Asian countries. The use of terrestrial vegetation and aquatic macrophytes by herbivorous and detritivorous fish that feed low down on the food chain may provide the requisite low cost, low energy technology. Various aspects concerning the use of both terrestrial and aquatic macrophytes in aquaculture are reviewed: characteristics of herbivorous fish; traditional macrophyte-fed aquaculture systems; cultivation of aquatic macrophytes as fishpond inputs; simultaneous pond culture of fish and macrophytes; use of green manure and compost, essentially manmade sources of detritus; and rotation of fish and plant crops. It is recommended that the principles of Chinese integrated aquaculture be more widely applied in developing countries and quantified under tropical conditions. Herbivorous fish are relatively efficient grazers of macrophytes but are inefficient assimilators and accelerate the recirculation of nutrients which stimulate the development of plankton; thus to optimize a macrophyte-fed aquaculture system a polyculture of macro- and microphagous fish is required. Because there are constraints to the use of grass carp in the tropics, the "central" species in Chinese carp polyculture, other macrophagous fish such as the tilapia, *Tilapia rendalli*, and the tropical carp, *Puntius gonionotus*, require evaluation in polyculture with microphagous species such as Nile tilapia, *Oreochromis niloticus*.

Introduction

A recent FAO study has predicted that by the year 2000 a world population of more than six billion will need an agricultural output of 50-60% greater than 1980

and that the demand for food and agricultural production in developing countries will double (FAO 1979). Fish protein forms only about 5% of the total protein available to man (Pimentel et al. 1975), but in much of Asia fish have a much more important dietetic role and comprise more than 50% of the total animal protein supply in many countries in East, South and Southeast Asia (Josupeit 1981).

Although aquaculture has a history of several thousand years in Asia (Tubb 1967) and Asia is still by far the major producer of farmed aquatic products (Pillay 1979), it is only within the last two to three decades that aquaculture has gained momentum in tropical Asia. Most operations are conducted by entrepreneurs to supply urban markets. In contrast to the generally held supposition that, "in general, all fish culturists are farmers and all farmers cultivate fish" (Huet 1972), small-scale farmers, with few exceptions, have yet to become widely involved in aquaculture (Edwards et al. 1983a). The traditional source of fish in most of Asia is "wild" fish caught from natural water bodies, but recent shortages, due mainly to rapid population growth and concomitant overfishing, have led to widespread protein-energy malnutrition in rural areas, particularly among infants and preschool children who derive insufficient protein from the rice based diet (Edwards 1983).

In developed countries, fish farming methods used that are based to a large extent on high protein pelleted feeds are energy intensive (Purdom and Preston 1977; Weatherley and Cogger 1977) and are not suitable for small-scale farmers in the tropics (Edwards 1980a). Hickling (1971) listed several advantages of the increasing tendency to feed fish dry pelleted feed: the formula can be varied as required; the feed can be blended in bulk and therefore provided cheaply and in reliable supply; and the dry feed is compact and easy to transport, store and dispense. However, small-scale farmers with a mean farm size in Asia of only 0.67 ha in 1970 (FAO 1979) and limited purchasing power, are hardly likely to be able to benefit from the convenience of commercially produced pelleted feed and alternative strategies are required for the single most populous group in the world. It has long been appreciated that fish production is highest for ponds with short food chains i.e., those with herbivores, omnivores, plankton and detritus feeders (Huet 1972); thus it is logical to focus attention on aquaculture systems involving such fish.

The various ways in which terrestrial and aquatic macrophyte vegetation may be used in fish culture are reviewed in this paper. Vegetation may be consumed directly by herbivores, but the role of detritus in fish production, a topic that has received scant attention to date, is considered also. In many natural ecosystems, up to 90% of the primary plant production is not directly consumed by herbivores and enters the detrital food web (Pomeroy 1980). Because most detritus originates from plant biomass in natural ecosystems, vegetation and the detrital food web hold promise as the requisite inputs for low cost, low energy aquaculture systems appropriate for the small-scale farmer. The integration of fish with rice cultivation is not discussed but it has been reviewed elsewhere (Ardiwinata 1957; Coche 1967; Huat and Tan 1980; Ruddle 1980).

Herbivorous Fish

World Distribution

Cultured herbivorous fish of European origin that feed on macrophytes are practically nonexistent (Huet 1972) but in Asia and Africa there are many commercially important herbivorous fish (Hickling 1971). According to Nikolsky (1963), there are no herbivorous fish in high latitudes because the growing period for vegetation is short. At lower latitudes, there are facultative herbivores such as the silver crucian carp *(Carassius carassius)* and roach *(Rutilus rutilus)* which do feed on vegetation, although animals comprise their main food source. Plants come to play an increasingly important role as fish feed in lower latitudes with more continuous vegetation growth throughout the year and here there are fish that feed entirely on plants. However, despite the presence of a wide range of herbivorous species in the tropics (Hickling 1971; Edwards 1980b), their potential remains to be fully exploited.

Herbivorous Species

Asia has the widest range of commercially exploited herbivorous species. The best known aquaculture system is the Chinese carp polyculture, the development of which was made possible by an outstanding diversification of carps in China (Kafuku 1966). The system has been described in detail by various authors (Hoffmann 1934; Anon. 1973, 1980; Edwards 1982). The grass carp *(Ctenopharyngodon idella)* is generally the dominant species in areas with abundant vegetation (Lin 1954), but in some areas the herbivorous Wuchang fish *(Megalobrama amblycephala)* (Anon. 1980; Edwards 1982) or the Chinese bream *(Parabramis pekinensis)* (Anon. 1973) may also be included in the polyculture. The mud carp *(Cirrhinus molitorella)*, which is usually a major species in the polyculture in South China, feeds on higher plant detritus (Anon. 1980). The Chinese carps are now cultured outside China, starting about 60 years ago in Malaysia, Singapore and Thailand (Lin 1954). More recently, they have been widely disseminated, for example, to India (Chaudhuri et al. 1975) where the indigenous Indian major carps catla *(Catla catla)*, rohu *(Labeo rohita)* and mrigal *(Cirrhinus mrigala)* may feed to a certain extent on either fresh or decomposing higher plants (Hora and Pillay 1962; Huet 1972), although much less voraciously than grass carp. Common carp *(Cyprinus carpio)* has been reported to occasionally feed on macrophytes (Huet 1972), but normally it is not a herbivore and consumes vegetable food only when very hungry (Chen 1934; Lin 1935). The inhibitory effect of common carp on the development of aquatic macrophytes in fishponds has been attributed to its feeding habit of digging into the bottom mud and increasing the turbidity of the water, thereby impeding the penetration of light and the growth of aquatic macrophytes (Huet 1972; Crivelli 1983).

The culture of herbivorous macrophyte feeding fish is also a traditional practice in West Java, Indonesia, in which the following fish are usually raised in polyculture: gourami *(Osphronemus gouramy)*, nilem *(Osteochilus hasseltii)* and tawes *(Puntius*

ganionotus) (Vaas 1954; Hora and Pillay 1962; Ling 1967; Huet 1972). Gourami and tawes may also be raised on a small scale in other countries in Southeast Asia (Kong 1966; Huet 1972). *Pangasius* spp. (catfish) are mainly carnivorous in open waters but can be fed soft vegetation when cultured in Kampuchea, Thailand and Vietnam (Thiemmedh 1961; Hora and Pillay 1962; Huet 1972). Grey mullet *(Mugil cephalus)* raised in freshwater as well as brackishwater ponds in China, Hong Kong, India and Israel feeds on decaying macrophytes (Hora and Pillay 1962; Huet 1972). Snakeskin gouramy *(Trichogaster pectoralis)* has been fed soft aquatic plants and detritus in ponds in several countries in Asia (Hora and Pillay 1962; Huet 1972), but is now a major cultured fish in a recently developed detrital system in Thailand (see section on Simultaneous Pond Culture of Fish and Macrophytes). The pearl spot *(Etroplus suratensis)*, indigenous to southern India and Sri Lanka, feeds on aquatic macrophytes and detritus (Hora and Pillay 1962; Huet 1972), and is considered to have potential as a farmed species in Sri Lanka (De Silva and Perera 1983; De Silva et al. 1984).

The tilapias from Africa and the Levant are mainly herbivorous and members of the genus *Tilapia; T. rendalli, T. sparrmanii* and *T. zillii* feed on macrophytes (Meschkat 1967; Semakula and Makoro 1967; Huet 1972; Bowen 1982; Trewavas 1982). In contrast to the microphagous tilapias belonging to the genus *Oreochromis*, relatively few attempts have been made to culture the macrophagous tilapias outside their native range; recent attempts have been made in Central and South America (Hepher and Pruginin 1982; Ramos-Henao and Corredor 1980). Culture of herbivorous tilapias has been reviewed by Pullin (1983a).

There are additional fish species that may have potential as cultured herbivores, but these remain to be adequately assessed; for example, certain species from the Amazon River basin: silver dollar fish *(Metynnis roosevelti, Mylossoma argenteum)*, pirapitinga *(Mylossoma bidens)*, and tambaqui *(Colossoma bidens)* (Edwards 1980b).

Structural Adaptations to Diet

Herbivorous fish that feed on macrophytes have anatomical adaptations for their diet. The grass carp, like other cyprinids, has a toothless mouth but has strong and specialized pharyngeal teeth arranged in two rows, those on the lower row with doubled and flattened serrated cutting and rasping surfaces. The grass carp has no stomach and the gut is only two to three times the length of the fish, exceedingly short for a herbivore (Hickling 1966). The macrophyte-feeding tilapias have bicuspid and tricuspid teeth on the jaws and small, sharp pharyngeal teeth that shred vegetation and rupture some of the cells (Trewavas 1982). The Nile tilapia, *Oreochromis niloticus*, which is usually regarded as only a microphagous species, has pharyngeal teeth almost as stout as some macrophyte feeders as well as a relatively high count of fine gill rakers (Pullin 1985). However, in contrast to the grass carp, the tilapias have long coiled intestines up to 14 times the body length (Trewavas 1982; Bowen 1982). There appears to be a correlation between the length of the intestine and the feeding habit of fish with the relative gut length increasing gradually from about 1.6

for strict carnivores to up to 18 for typical herbivores with omnivores in an intermediate range; however, extremely long digestive tracts may be restricted to microphagous fish because grass carp has a relative gut length of only 2 to 2.5 (Bitterlich 1985).

Type of Macrophytes Consumed by Herbivorous Fish

Herbivorous fish do not consume all species of terrestrial and aquatic macrophytes, and even plant species that are eaten are not taken with equal relish because the fish have distinct preferences.

There is a voluminous literature on the grass carp because it is the most voracious feeder among herbivorous fish and there is worldwide interest in its use as a biological means of aquatic weed control (Anon. 1979; Edwards 1980b; Pierce 1983; Shireman and Smith 1983; Zonneveld and van Zon 1985). Like most cultured fish, the grass carp is omnivorous (Hickling 1971), and will feed on almost anything of animal or plant origin when adult: macrophytes, insects, earthworms, small fish, silkworm pupae and even decayed cloth and old shoes thrown into the pond (Chen 1934; Ling 1967). However, there are certain broad feeding preferences concerning the consumption of macrophytes by grass carp (Edwards 1980b). The most favored macrophytes are soft plants such as filamentous algae, submerged aquatic macrophytes, duckweed, and soft leaves of terrestrial plants such as herbaceous plants and vegetables, and grasses (Lin 1954; Anon. 1980). Among the least favored are fibrous and woody plants such as rushes and sedges and also larger floating aquatic macrophytes such as water lettuce *(Pistia statiotes)* and water hyacinth *(Eichhornia crassipes)* (Singh et al. 1967; Alabaster and Stott 1967). Although grass carp do prefer the more soft and succulent macrophytes, taste may be also involved because in a list of 16 plants eaten by grass carp in approximate order of preference, the fairly succulent water cress *(Rorippa nasturtium-aquaticum)* was the 14th species listed (Cross 1969).

Other herbivorous fish species do not seem able to consume such a wide range of plants as the grass carp. Macrophyte-feeding tilapias have been reported in Africa to feed mainly on the leaves of cultivated plants such as banana, cassava, papaya and sweet potato and on grasses and herbaceous plants (Meschkat 1967; Semakula and Makoro 1967; Huet 1972).

Feeding Efficiency of Herbivores

Herbivorous fish consume relatively large amounts of macrophytes, due in part to the high water content of the ingested material (Edwards 1980b). Bhatia (1970) reported that grass carp of 1.00 to 1.25 kg in weight consumed 100 to 174% of their body weight/day of certain species of aquatic macrophytes. Venkatesh and Shetty (1978) reported the consumption by grass carp of 100 and 125% of their body weight/day of the submerged aquatic macrophytes *Hydrilla* and *Ceratophyllum*,

respectively. A 1-kg grass carp was reported to eat its body weight of grass/day (Anon. 1980).

There is considerable variation in the literature concerning the feeding efficiency of herbivorous fish, which is hardly surprising when it is realized that feeding trials were conducted in containers of various sizes from aquaria to ponds, under varying environmental conditions, with different species and sizes of fish, fed macrophytes of various species, which themselves varied in water and nutrient content, the latter ranging widely in a given species depending on the fertility of the environment (Edwards 1980b). Grass carp is notable for its low conversion rate of food into fish biomass which may be due to the inefficient trituration of plant material and the exceedingly short gut for a herbivore (Hickling 1966). Estimates of digestion efficiencies (apparent assimilation efficiency) of plants by grass carp ranged from 50 to 70% (Hickling 1966; Shireman and Smith 1983) although Law (1978) reported a digestibility of napier grass *(Pennisetum purpureum)* by grass carp of only 19.7% on a dry weight basis; however, 65.5% of the protein was extracted by the fish. Law (1978) attributed the low digestibility of napier grass to its more fibrous nature than the guinea grass *(Panicum maximum)* used by Hickling (1966). Van Dyke and Sutton (1977) reported relatively high apparent digestibilities of 53 and 80% for the dry matter and crude protein of a mixture of the duckweeds *Lemna minor* and *L. gibba* by grass carp, respectively. The macrophyte-feeding *Tilapia rendalli* was estimated to have an assimilation efficiency of 47.8 to 58.7% when fed the submerged macrophyte *Ceratophyllum demersum*, which was considered to be very good for a macrophagous herbivore, due to successful trituration and efficient pre-assimilatory processing of food (presumably by low gastric pH) (Caulton 1982). The utilization of crude protein in the duckweed, *Spirodela polyrrhiza*, and the submerged macrophyte, *Elodea canadensis*, by the macrophyte-feeding fish *'Tilapia melanopleura'* (probably *T. rendalli*, see Trewavas 1982, for discussion on nomenclature) was between 47 to 57% for both species (Mann 1967) but was 86% in a hybrid of the normally microphagous hybrid *Oreochromis niloticus* x *O. aureus* fed the duckweed *Lemna gibba* (Gaigher et al. 1984).

Food conversion ratios for macrophytes in the literature expressed on a wet weight basis normally range from about 30 to over 200 (Edwards 1980b; Shireman and Smith 1983), but much of the variation is due to the higher water content of aquatic compared to terrestrial macrophytes, e.g., Venkatesh and Shetty (1978) reported food conversion ratios of 27.0 for hybrid napier grass and 128.4 for *Ceratophyllum*, but the food conversion ratios expressed on a plant dry matter basis are narrowed to only 4.6 and 10.3, respectively. Very low food conversion ratios of less than 2 on a plant dry matter basis have been reported for duckweeds, which have a relatively low fiber and high protein content (Edwards 1980b; Shireman and Smith 1983). These low food conversion ratios are comparable to grass carp fed commercial pelleted feed (Huisman and Valentijn 1981). Gaigher et al. (1984) reported a food conversion ratio of 1 for the hybrid *Oreochromis niloticus* x *O. aureus* cultured at high density in an experimental recirculating unit even though natural food due to

fertilization by uneaten feed and fish feces was not allowed to develop due to possible adverse effects on water quality. Although the growth rate of the fish was relatively slow, the duckweed was readily ingested and efficiently utilized by the tilapia hybrid, which indicates the potential of duckweed in feeding tilapia.

A major reason why the feeding efficiency of herbivorous fish is usually low is that fish do not produce cellulases directly, nor do they have a significant symbiotic gut flora capable of hydrolyzing cellulose (Fish 1960; Hickling 1966; Stickney and Shumway 1974; Buddington 1980). Cellulolytic activity determined in the guts of fish is positively correlated with the amount of processed plant detritus in the guts and is presumably attributable to the bacteria and fungi colonizing the detritus (Prejs and Blaszczyk 1977; Buddington 1980). Microscopic examination of gut contents in grass carp showed that only those cells that were rasped away or ruptured and exposed by cutting during mastication appeared to be digested and large areas of leaf passed through the gut intact; the digestive enzymes were reported to have little lytic effects (Hickling 1966). In contrast, tilapias have both morphological and physiological adaptations to a plant diet; like grass carp they have pharyngeal teeth to triturate plant matter but also gastric acid secreted to an unusually low pH, frequently less than pH 1.5 which can lyse the prokaryotic cell walls of bacteria and blue-green algae (Moriarty 1973; Moriarty and Moriarty 1973; Bowen 1976) and which may aid in the denaturation of eurkaryotic cell membranes and thus expose the cytoplasm to intestinal enzymes (Bowen 1982). In the grass carp, the gut contents were reported to be nearly alkaline to alkaline throughout, with the pH highest at 7.4-8.5 in the anterior part, 7.2-7.6 in the middle part and 6.8 in the posterior part (Hickling 1966).

Traditional Macrophyte-fed Systems

The traditional Chinese system of polyculture comprises a variety of carps with different feeding niches and receives a variety of organic inputs: macrophytes, manure (livestock and human) and agricultural byproducts (oil cakes, rice bran, silkworm pupae). The grass carp is often a major component species and in most cases grass is cut on the pond dikes and in nearby areas as feed. Aquatic macrophytes are also collected from adjacent canals and lakes and in vegetable growing areas waste vegetables are purchased from vegetable gardeners (Hoffman 1934; Lin 1940; Ruddle et al. 1983). When the pond becomes shallow through silting, the soft bottom mud is removed and placed on the pond dikes where mulberry trees or vegetables are grown (Lin 1954).

The main reason that the grass carp is a major species is according to Chinese tradition "by taking care of the *Ctenopharyngodon idella*, the rest of the species will take care of themselves" (Chen 1934); there is a Chinese proverb, "one grass carp supports 3 silver carps" (Lin 1982). Due to the inefficient feeding efficiency of the grass carp, about half of the plant material which is broken into fragments less than 3 mm^2 in area "passes out as feces which may support, directly or indirectly,

an equal biomass of other species of fish including plankton-eating cyprinids" (Hickling 1966). In fact, the grass carp has been referred to as a "living manuring machine" (Hickling 1971). The grass carp is rather susceptible to disease and if it were not raised in polyculture with filter-feeding fish, the water would become too fertile for the grass carp to thrive (Anon. 1973, 1980). According to Chen (1934), such traditional beliefs are not supported by scientific data, but are the results of thousands of years of experience. The statement is still valid today because there have been relatively few studies on aspects of the dynamics of Chinese carp polyculture. Fischer (1970) reported that the assimilation efficiency of grass carp fed only plant food was low, an average of 13%, and that it played an important role in fertilizing water bodies. Shireman et al. (1977) were forced to use a rapid water turnover in fiberglass tanks for grass carp fed the duckweed *Lemna minima* to satiation, due to high fish excretory rates. Stanley (1974) reported that the principal impact of grass carp fed the submerged macrophyte *Egeria densa* in tanks was to increase the rate of recycling of nitrogen and phosphorus. Rather surprisingly, Terrell (1982) did not obtain the expected shift from "desirable to undesirable plankton" species as a result of eutrophication in acidic ponds stocked with grass carp to control aquatic macrophytes; macrophyte growth was controlled but ponds stocked with fish contained fewer individuals, genera and orders of plankton than control ponds, presumably because vegetation was not added to the ponds, which remained relatively infertile.

There are few data from China on the efficacy of grass carp. Liu (1955) reported a net yield of 3,000 kg of fish/ha/year in a pond stocked with a polyculture of Chinese carps fed exclusively with aquatic plants. A second Chinese study obtained a yield of 6,112 kg fish/ha/year with green fodder as the main pond input (99.3% green fodder and 0.7% animal manure) with a food conversion ratio of 26.3 (Anon. 1980).

The principle of polyculture of macrophyte and filter-feeding fish is also utilized outside China. According to Lin (1954), Chinese carps used to be among the principal fish reared in Malaya (Malaysia and Singapore) and Thailand with the grass carp the most important species due to the abundance of grass in the humid tropics. Meschkat (1967) reported that a polyculture of macrophagous and microphagous tilapia had become popular in many areas of Africa for better utilization of natural food in the pond. A study by Sen et al. (1978) in India demonstrated that the fertilization of the water by grass carp excreta may enhance the growth of native Indian major carps to about the same extent as fertilization by the addition of livestock manure.

The traditional Chinese polyculture may still be observed in Asia but the more progressive farms have intensified fish production or have changed to other aquaculture systems that are not dependent on macrophytes as pond inputs. In mainland China more and more supplementary feeds are being used in fish culture, including a wide range of both terrestrial and aquatic macrophytes that are cultivated on or near the farm and which may be added directly to the pond, fresh as green fodder or green manure, or after mechanical or biological processing (Anon. 1973, 1981). Various types of pasture grasses, a member of the Borraginaceae family, sweet

potato and various types of cabbage are cultivated as fish feed (Anon. 1973, 1980, 1981; Ruddle et al. 1983) (Plates 1 and 2). Although Chinese cabbage forms a "quality fresh feed for herbivorous fish" (Anon. 1981), it is likely that only waste leaves are fed to fish (Ruddle et al. 1983). The cultivation of aquatic macrophytes is treated separately below. Mainland China appears to be the only region that has recently promoted the cultivation of macrophytes as pond inputs. In other areas with traditional Chinese carp polyculture systems such as Taiwan, Thailand, and Malaysia, there has been a tendency to decrease macrophyte inputs to the pond and increase the amount of livestock manure, waste food, agro-industrial byproducts and pelleted feed, although these are all used in mainland China. However, the potential importance of macrophytes as a potentially low cost, low energy input to fishponds as demonstrated by the Chinese experience should be fully explored, particularly in the humid tropics with such luxuriant vegetation growth.

Plate 1. Cultivation of elephant or napier grass, *Pennisetum* sp. as green fodder for fish on pond dikes in Guangdong Province, China.

Cultivation of Aquatic Macrophytes

Aquatic macrophytes are cultivated in mainland China as fishpond inputs. The duckweeds *Wolffia arrhiza*, *Lemna minor* and *Spirodela polyrrhiza* are cultured in

Plate 2. Cultivation of a member of the Borraginaceae family (foreground) and rye grass, *Lolium perenne* as green fodder for fish on pond dikes in Jiangsu Province, China.

small shallow ponds fertilized with manure (livestock or human) as feed for grass carp fry and fingerlings in nursery areas. Initially the fry are fed the smaller *Wolffia*, the smallest flowering plant in the world, but when they reach 6 to 7 cm in length they are fed the larger *Lemna minor* and *Spirodela polyrrhiza* (Anon. 1973, 1980). *Wolffia* was also reported by Hoffmann (1934) to be cultivated by Chinese carp fry dealers in Singapore based on a paper by Corner (1930), but rather surprisingly he did not observe it in Kwangtung (Guangdong) Province. The cultivation may be localized in mainland China since according to Anon. (1973) it is carried out particularly in Chekiang (Zhejiang) Province. *Lemna* and *Wolffia* are also cultivated together in Taiwan (Chen 1976; Edwards, pers. obs.) (Plate 3). In mainland China, *Wolffia* was reported to be harvested for five months from April to September in Kiangsi (Jiangxi) and Chekiang (Zhejiang) Provinces with an extrapolated annual yield of 14 to 17 t dry wt/ha/year, assuming year-round growth (Anon. 1973). This corresponds to the extrapolated annual yield for the mixed cultivation of *Wolffia* and *Lemna* reported by a farmer in Taiwan (Edwards, pers. obs.). Extrapolated yields of about 20 t dry wt/ha/year of *Spirodela polyrrhiza* were obtained in a series of 200-m^2 earth ponds on the campus of the Asian Institute of Technology using septage (cesspool slurry), but in a longer experiment of six months duration the mean extrapolated yield was reduced to about 9 t/ha/year due to insect infestation (Edwards et

Plate 3. Cultivation of the duckweeds *Lemna* and *Wolffia* as green fodder for fish in Taiwan, China.

al. 1984). Trials are currently under way at the family level in villages in Thailand in which duckweed raised in a septage fed pond is harvested and fed to the tilapia *Oreochromis niloticus* in an adjacent pond.

The water hyacinth *(Eichhornia crassipes)*, a serious pest in water bodies throughout much of the tropics due to its prolific growth (Edwards 1980b), is cultivated in mainland China, as are two other aquatic macrophytes, water lettuce *(Pistia stratiotes)*, and alligator weed *(Alternanthera philoxeroides)*, which are known as the "three aquatic weeds" (Anon. 1973, 1981). The plants are usually cultivated in rivulets, small bays, or swamps to avoid taking up land and are usually fed to pigs, but can be fed to fish after processing because water hyacinth in particular is not readily eaten by fish (Edwards 1980b).

Simultaneous Pond Culture of Fish and Macrophytes

At first sight it would seem logical to attempt to develop an integrated aquatic macrophyte-herbivorous fish system in which the aquatic macrophytes, the fish feed, were actually cultivated simultaneously with the herbivorous fish in the same pond. However, in practice it would be difficult to optimize such a system to obtain significant fish yields. To provide an adequate supply of plant food for the fish using submerged aquatic macrophytes, it would be necessary to fertilize the pond to

stimulate macrophyte growth but there would be competition with phytoplankton for nutrients and light. Phytoplankton are normally able to outcompete submerged macrophytes, mainly due to shading effects, and eliminate them (Hasler and Jones 1949; Vaas 1954; McNabb 1976; Edwards 1980a). The addition of fertilizers to fishponds has actually been recommended in the USA as a means to control submerged weeds by the stimulation of filamentous floating algae and phytoplankton (Swingle 1967; Blackburn 1968; Lawrence 1968). A report from Michigan where secondary sewage effluent pumped to artificial lakes led to significant crops of the filamentous green alga *Cladophora fracta* and the submerged macrophyte *Elodea canadensis*, which were harvested, would seem to be an exceptional case (Bahr et al. 1977).

The addition of fertilizer to an integrated system involving a floating aquatic macrophyte such as duckweed would lead to the desired increase in growth of the macrophyte and competing phytoplankton would be reduced through shading. Hickling (1971) reported that the growth of duckweed may keep pace with the rate of removal of duckweed by young grass carp stocked in well-manured duckweed production ponds if the pond were not stocked too heavily with fish. However, such a system would be difficult to optimize in practice for good fish production because normally, fish growth would soon outstrip the growth of duckweed and eliminate the production of feed in the system. If the cover of duckweed became complete it would lead to a lower concentration of dissolved oxygen and an increase in the free CO_2 concentration in the water column with a concomitant fish kill (Edwards 1980b).

In actual practice fish and aquatic macrophytes are rarely raised together in the same system. Hoffmann (1934) reported that a farmer reared fish in the same pond as the lotus *(Nelumbo nucifera)* in China, but with only 50% of the usual number of fish because they grew more slowly than when raised alone. Djajadiredja and Jangkaru (1978) described the cultivation of two aquatic macrophytes as vegetables for human consumption (*Ipomoea aquatica* and *Limnocharis flava*) in the same pond with common carp *(Cyprinus carpio)*. However, in both the above examples the space in the pond was effectively the main shared resource, and the fish probably derived little or no direct feed value from the macrophytes.

There is a remarkable system, the culture of the snakeskin gourami *(Trichogaster pectoralis)* in converted paddy fields in Thailand, in which the only nutrition provided to the fish is derived from aquatic macrophytes grown in the same system as the fish (Boonsom 1981; Indrambarya 1981). The Department of Fisheries in Thailand attempted to promote rice/fish culture in the 1950s and was not successful, but its attempts led to the development of this unique aquaculture system in an area of about 20,000 ha of relatively saline soils, which formerly gave poor rice yields (Edwards et al. 1983a). The dikes of the former rice fields have been raised and a peripheral ditch 3-4 m wide and 0.75 m deep constructed. A wide variety of emergent aquatic macrophytes (major species: *Eleocharis equisetides*, *Hymenachne myurus* and *Paspalum conjugatum*) grows naturally on the shallow central platform and is cut at biweekly intervals, manually or by mechanical cutters on boats, and scattered or piled to decompose to provide food for the fish. The potential of the

system for other tropical countries may be limited due to the relatively large size of the farms (range 3 to 20 ha) and fairly low yields of 450-1,700 kg/ha/8-10 month growing period, although yields have recently been doubled in experimental trials on one farm from 1,000 to 2,200 kg/ha/growing season by the application of 156 kg chicken manure/ha/10 days for two months during the fry rearing period which led to greatly increased survival (Boonsom 1981).

Green Manure and Compost

The use of green manure and compost, essentially artificial or "man-made sources of detritus" (Edwards et al. 1983b) are traditional practices in several Asian countries. Because certain species of macrophytes may not be readily consumed even by herbivorous fish, they must be processed in some way before addition to the pond. Furthermore, it may be desired to use macrophytes as green manure to stimulate the production of plankton and other natural food organisms in the pond for filter- and bottom-feeding fish rather than as green fodder for herbivorous fish. However, because there is a plethora of complex interactions in the pond ecosystem, it is frequently difficult to separate the effects of macrophytes, fresh or processed, as supplementary feed and as green manure.

There are sporadic references in the literature to the use in fishponds of green manure and compost, which invariably were regarded as pond fertilizers. Schaeperclaus (1933) reported a 50% increase in fish yield by fertilization of ponds with 30 kg of P_2O_5/ha but a 100% increase in yield when a double quantity (presumably 60 kg/ha of wet material) of decomposed submerged aquatic macrophytes was added. The growth of plankton was stimulated in Israeli fish ponds by the application of farmyard manure and compost (Hickling 1948). Singh (1962) reported that compost was added to fishponds as an organic fertilizer to raise the production of plankton. Hickling (1971) also reported that green manure was placed in heaps on the pond bottom or in wicker cages so that the vegetation would rot slowly, give off nutrients at a slow but steady rate, and prevent widespread deoxygenation of the water. Hickling (1971) also pointed out that the rotting manure was a good substrate for living organisms, which the fish could eat directly. Huet (1972) considered that the best manuring results were obtained with cut submerged aquatic macrophytes, but that young emergent macrophytes could be left where they were cut or heaped up to decompose slowly; manure could also be made with cut cultivated plants.

Much more detail concerning compost and green manuring was given by Martyshev (1983). Compost is prepared by the usual agricultural methods in Russia and well decomposed compost is not considered to be inferior to fresh dung in its action and superior to dung from sanitary and hygienic points of view. The compost is reported to be applied by being placed in small heaps along the pond margin or uniformly throughout the pond, or stored in wattle enclosures on the dike. However, Martyshev (1983) considered that compost will never be widely available for

fish farms because it is labor intensive to prepare. He believed green manure (agricultural crops or grass cultivated on the bottom of the pond or cut terrestrial and aquatic plants added to the pond) to be more economically profitable, although it is difficult to appreciate how green manure could significantly increase the fish yield because most of the plants were reported to have been collected from the pond itself.

There is considerable information regarding green manure and compost from China. There are several references to the use of strongly odorous plants like goat weed (*Ageratum conyzoides*, Compositae) and some mints (Labiatae) and also members of the Gramineae and Leguminosae being cut and stacked fresh in the corner of the pond with the heap being turned every 1-2 days until the plants have decayed, with the roots and stems resistant to decay being later removed from the pond (Lin 1954; Anon. 1973, 1980). Lin (1954) reported that 100 catties (60 kg) of green grass would lead to the production of three 3 catties (1.8 kg) of fish. In Hong Kong, weeds of the Compositae and Labiatae were reported to be mixed with dung before application to the pond (Lin 1940). Tatsao is the Chinese term used to refer to plants of the Compositae family with low fiber and high nutrient content, which readily decompose in water. With the rapid development of fish culture and concomitant increased demand for pond inputs, vegetable waste and other wild plants with soft stems and leaves are also reported to be used to fertilize the water. Tatsao is applied by being heaped in the water along the banks with about 150 kg/pile and after 3-4 days of fine weather it starts to decompose and the water gradually turns greenish-brown. The heaps are repeatedly turned to spread decomposed organic matter throughout the water. Seven to 10 days later, undecayed plant remains may be removed from the pond (Anon. 1980). Tatsao is mainly used to rear fry and fingerlings in China, in addition to inorganic and organic manures, and compost (Anon. 1980).

In recent years in China, several types of compost have been developed for fish culture involving various ratios of green grass, various types of livestock and human manure, and slaked lime, depending on locally available materials. The various ingredients are placed in a fermentation ditch in layers and the ditch then filled with water until the contents are submerged and then sealed with a layer of soil. The mixture is loaded into a boat on completion of fermentation and are best applied by swirling the compost in a strainer in the pond so that only the soluble portion of the fertilizer is dispersed in the water. The insoluble portion is retained and used as a soil fertilizer (Anon. 1973). According to another Chinese source, compost is prepared in a pit for addition to fry rearing ponds using a 1:1 ratio of grass and animal manure with quicklime added at 1% of the total weight, with the grass and manure in layers after which water is added to the pit to soak the manure and then the pit is sealed with mud and decomposition allowed to take place. Compost is added 1-2 times at the rate of 0.5 kg/m^3 of water, 4-5 days before stocking fry and at 0.1-0.2 kg/m^3 of water after stocking 1-2 times/day depending on the water quality. The compost is added directly to the water along the pond margins. The advantage of compost is the decreased oxygen demand compared to the direct addition of the

composted raw materials, but the method requires more labor and there is some volatilization of nitrogen following the opening of the pit (Anon. 1980).

During a study of various strategies to treat and recycle human excreta in fish culture, nightsoil was mixed with chopped water hyacinth and composted above ground by the Chinese method of continuous aerobic composting (Edwards et al. 1983b). The mean extrapolated yield of *Oreochromis niloticus* in ponds fed only with compost was 3.6 t/ha/year, with a maximum yield of 5.6 t/ha/year in two ponds. Much of the nutritional value of the compost was undoubtedly due to its fertilizer effects but fish were observed to consume compost, although much less voraciously than conventional feeds. The mean food conversion ratio was 7.4, only slightly higher than that reported for cereals (4 to 6), assuming a comparable feed moisture content. It suggested that the acid hydrolysis of compost by the low stomach pH reported for tilapia (Moriarty 1973; Bowen 1976), may have enabled the tilapia to digest significant amounts of nonliving amorphous detritus (Bowen 1981, 1982).

Water hyacinth was applied to ponds whole as a feed and fertilizer in China, but the fish were reluctant to take it, it took a long time to decompose, and the rate of utilization was low (Anon. 1980). Ling (1967) reported that water hyacinth and water lettuce were chopped into small pieces and used to feed grass carp and common carp. The so-called "three aquatic weeds" (Anon. 1981) are now more likely to be processed before addition to the fishpond. *Alternanthera* is usually cooked and mixed with rice bran and is reported to be taken by all important cultured carps (Ling 1967; Anon. 1973). Water hyacinth and water lettuce were reported to be processed either mechanically (soaking, mixing, cutting, or grinding) which formerly was labor intensive but which has now been mechanized, or biologically. The latter involved green storage and fermentation in ditches, tubs, or barrels under anaerobic conditions at 65-75% moisture after cutting into 6 cm strips and sealing by a 15 cm layer of dry grass topped by a 15 cm layer of moist soil; if the material was too moist it could be sun-dried or mixed with dry hay before sealing (Anon. 1973). Good results have also been reported by composting water hyacinth with silkworm feces (or animal manure) and quicklime, or composting the chopped water hyacinth with a small amount of salt or saccharified yeast (Anon. 1980).

Experiments have also been carried out at the Asian Institute of Technology with the use of water hyacinth alone as a fishpond input. Good growth and feed utilization efficiencies were obtained with the incorporation of up to 75% composted water hyacinth in a conventional pelleted tilapia feed in a simple displacement (of the pellet ingredients) procedure using *Oreochromis niloticus* (Edwards et al. 1985). Water hyacinth was also added to a series of earth ponds stocked with *Oreochromis niloticus* in three forms: fresh whole plants that decomposed beneath the water *in situ*, freshly chopped water hyacinth spread on the surface, and composted water hyacinth; similar extrapolated yields of 5 to 6 t/ha/year were obtained with all three treatments at the same dry matter loading rate of 200 kg/ha/day, or about 3 kg total Kjeldahl nitrogen/ha/day (Asian Institute of Technology, unpublished data). A wide

range of yields has been reported in the literature (Edwards 1980b; Reddy and Sutton 1984), but if a reasonably conservative potential yield of 100 t dry wt/ha/year were taken for a well managed cultivation system in fertile water in the tropics, it would require 0.73 ha to provide the 200 kg dry matter/ha/day in the experiment described above on an annual basis, a value that indicates that a water hyacinth-fed aquaculture system may be feasible.

Freshwater crawfish (crayfish), the red swamp crawfish *(Procambarus clarkii)* and the white river crawfish *(Procambarus acutus acutus)*, which feed on plant detritus, are cultured in the southeast United States (Huner and Barr 1984; Huner 1985). Sexually mature adults are stocked in ponds in mid- to late spring after which the water is gradually lowered to dry the pond by early summer. Mated crawfish survive in burrows and begin to reproduce. Vegetation is allowed to grow on the pond bottom during the summer, e.g., grasses, sedges and alligator weed *(Alternanthera philoxeroides)*. The annual vegetation starts to die as the pond is refilled in late summer to early autumn and provides not only detritus used by the crawfish as food but also the stalks of plants which enable the crawfish to crawl to the water surface and obtain atmospheric oxygen, which the animal is able to utilize when the dissolved oxygen concentration falls below 2 mg/l due to the oxygen demand of the decaying vegetation. The crawfish are harvested with traps, and when the ponds are drained slowly in late spring, the surviving crawfish burrow to repeat the cycle. Yields in well managed ponds range from 500 to 2,500 kg/ha but introduction of the crawfish to areas outside its range could have a serious ecological impact, possibly including the elimination of native crustacean species (Huner 1981).

Rotation of Fish and Plant Crops

The rotation of fish and plant crops developed out of the need to drain and dry the bottom of fishponds periodically. The reasons for draining the pond as enumerated by Hickling (1971) are: to harvest all the fish; to kill off harmful insects, parasites and pathogenic bacteria; to allow time for routine pond maintenance; to control the density of stocked fish; and chiefly, according to Hickling, to restore the fertility of the pond by aerobic oxidation of organic matter, which accumulates and decays slowly under the anaerobic conditions of the pond bottom. Because the growing period for fish in Central and East Europe is only about 180 days, ponds are normally drained at the end of the growing season and left empty throughout the winter. Because it is not always possible to dry the pond bottom effectively during a single winter, crops are often planted for one or more successive years to improve the fertility of the pond bottom and at the same time provide terrestrial crops (Schaeperclaus 1933; Hickling 1971; Huet 1972; Martyshev 1983). Fish production is stimulated on refilling the pond after the cultivation of the plant crop as a result of the advantages of the dry period listed above, and also because any plant residues left in the bottom rot and provide a source of fertilizer, which may have been augmented further by manure from livestock allowed to graze on the dry pond bottom (Hickling 1971; Martyshev 1983). Such a practice is still conducted in eastern Europe in

large ponds of many hectares, e.g., in Hungary where it may involve a three phase rotation involving integrated-duck fish farming for 4 to 5 years, forage crop production for one year, followed by rice production for 3 years (Müller 1978).

However, as pointed out by Wunder in 1949 (cited by Hickling 1971), the profit from raising fish is usually much higher than that from raising agricultural crops, so rotation can only be justifiable to the extent that it improves the efficiency of the pond for fish culture. Sarig (1956) advocated the culture of carp throughout the winter in Israel because the climate is warmer than that in Central and East Europe and considered that a 10-day exposure of the pond bottom during harvesting was probably a sufficiently long dry period. Hickling (1971) reported that the pond mud cracked open in dry weather in the tropics (Malaysia) after being left dry for several days.

The alternation of fish and plant crops was considered to be a good practice in China with about a 1,000 year history (Hoffmann 1934). The organic matter left on the pond bottom was thought to be an excellent fertilizer for plant crops and after the plant harvest, the crop residues were left in the pond as green manure. Furthermore, rotation of fish and plant crops gave the farmer two chances to speculate on the market rather than one, should a loss be sustained in one of the two ventures. A wide variety of aquatic plant crops was commonly grown: water chestnut *(Eliocharis plantagenea)*, arrowhead *(Sagittaria sagittifolia)*, lotus *(Nelumbo nucifera)*, rice *(Oryza sativa)*, water caltrop *(Trapa natans* or *T. chinensis)*, water cress *(Rorippa nasturtium-aquaticum)*, water spinach *(Ipomoea aquatica)*, wild rice *(Zizania latifolia)*, or various vegetables. The fish used in rotation with plant crops were stocked large and were purchased when fish were cheap. The fish were well fed and sold when the price was at its highest, usually during Chinese New Year in January or February. Sometimes however, the fish gained little to no weight and the pond served primarily as a "storage" place but profit was made on the increase in price (Hoffmann 1934). The practice of rotating fish with plant crops appears to have at least declined considerably in China since it is not mentioned at all in recent texts (Anon. 1973; Anon. 1980). This is supported by the comment made by Hoffmann (1934) that it was more profitable to raise fish year-round, which was possible in deepwater ponds that are typical in China today.

Although rotation of fish and agricultural crops is still practiced in Eastern Europe (Müller 1978; Martyshev 1983), it is done much less frequently now due to the wider use of fertilizers (Huet 1972), and presumably also supplementary feed. Furthermore, the minimum size of a 1 ha pond (optimum pond size 30-50 ha) considered to be necessary for the "aquacultural rotation" in Hungary (Müller 1978) hardly conforms to the typically small farm holding in most developing countries.

Conclusions and Recommendations

The developing world is facing the major challenge of how to increase the productivity and welfare of the single most populous group of people in the world, the

small-scale farmer. Traditional sources of wild fish, the main source of animal protein in much of tropical Asia, have decreased mainly due to overfishing from increased population growth in recent decades. Because small-scale farmers have few available resources, an attempt should be made to apply the major principles of Chinese fish culture to tropical developing countries. The development of integrated farming in China was probably not accidental, but a response to a large population living off a relatively small amount of arable land, a characteristic of most developing countries today. An attempt needs to be made to quantify the wealth of Chinese integrated fish farming methods under tropical conditions.

There is a need to quantify terrestrial and aquatic macrophytes, fresh and processed, as fishpond inputs because the limited data available from China and elsewhere indicate that yields as high as 5 to 6 t/ha/year are possible with macrophytes as the sole pond input (Mathieu 1960, 1961; Anon. 1980; Asian Institute of Technology, unpublished data). Tropical pasture crops, green crop residues and aquatic macrophytes have potential as pond inputs. The Chinese not only harvest macrophytes from lakes but cultivate them for fish culture. In other parts of the world there are innumerable water bodies infested with aquatic macrophytes, which are not utilized for aquaculture (Junk 1977; Fernando 1980) (Plate 4). Duckweed, which is cultivated in China for aquaculture, appears to have great potential throughout the humid tropics (Gaigher et al. 1984). Water hyacinth, the most prolific aquatic macrophyte, needs to be processed prior to its use as a fishpond input

Plate 4. Catching wild fish in Bung Boraped, an aquatic macrophyte infested lake in Nakorn Sawan Province, Thailand.

because it is not readily consumed by herbivorous fish, but the best method to achieve this remains to be ascertained.

Because herbivorous fish are relatively efficient grazers, but inefficient assimilators, they have a limited ability to convert macrophytes into fish tissue, but accelerate the recirculation of nutrients (Prejs 1984). The central species in the Chinese system of carp polyculture is the grass carp, which like most herbivorous fishes, is an inefficient feeder, but due to large amounts of partially and undigested finely divided plant matter is able to also support the growth of filter-feeding planktivorous fish. The grass carp may not be the ideal species for many countries because it is not easy to breed and succumbs easily to disease in fertile water (Lin 1954; Anon. 1980). Furthermore, the Chinese carps are less tolerant to low concentrations of dissolved oxygen that occur in highly fertile ponds than the tilapias, which may have a greater potential in the tropics. Meschkat (1967) reported that macrophagous and microphagous tilapia have been raised in polyculture in Africa, and this system should be studied in more detail (Pullin 1982, 1983b). The microphagous tilapias of the genus *Oreochromis* have been widely disseminated, but much less is known about the performance of the macrophagous *Tilapia rendalli*, alone or in polyculture. An evaluation of a polyculture of the microphagous tilapia *Oreochromis niloticus* and the macrophagous tropical carp *Puntius gonionotus*, which are frequently cultured together in Thailand, should also be conducted.

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Discussion

MORIARTY: Presumably the vegetation which grass carp eat has a high C:N ratio. They then use as much of the N as possible, so their feces have an even higher C:N ratio. Are they then such a good fertilizer source for generating food in the pond for other fish like the filter feeders?

EDWARDS: Well, grass carp have quite a varied diet and a large throughput of material. They eat for long periods. The C:N ratio of the feces may not be so high. It depends upon the C:N ratio of the food and the completeness of digestion and assimilation. The N-content of the feces may be low, but remember the Chinese proverb.

PULLIN: The grass carp is one of the world's fastest growing freshwater fish, but it does have a very low assimilation efficiency relative to the vast amounts of food it consumes.

MORIARTY: So they rely mainly on a triturating process for dealing with vegetation?

PULLIN: Yes, to a large extent.

BILIO: When extrapolating from the results of small ponds to yields/ha, we must be careful. The different economics of different scale operations should also be taken into account. For some operations, a size less than 200 m^2 may not be economically viable.

PULLIN: This is important. In fact, the extrapolation of data from small tanks to yields/ha has a lot of pitfalls. For example, there is the 'edge effect'. The epiphytes growing on the walls of small tanks may be an important food resource. However, a 200 m^2 pond appears to be a useful size for experimentation.

EDWARDS It can also be a reasonable size for a small-scale farmer. With a duck-fish integrated farming technique in Thailand, we obtained an extrapolated annual yield of 176 kg of fish from a 200 m^2 family pond (Edwards 1983).* This is sufficient to supply the animal protein needs of a family of five—assuming that they derive a third of their total protein from animal sources. I regard such ponds as pilot scale production units. I only extrapolate to yields/ha for comparison with other literature.

BILIO: I was just saying we should be careful with extrapolation. It would not even help if we said the yield was per m^2 not per ha. The context is important. For example, GTZ has a research project in Colombia dealing with the economics of fishpond culture in association with agricultural production, e.g., coffee growing. There the minimum economically viable pond size is 200 m^2.

WOHLFARTH: I have several comments. First, I agree with Dr. Edwards on the instability of the duckweed—fish system. Second, on extrapolation, whether we extrapolate yields to per ha per year or intrapolate to per m^2 per day, we are making the same calculation and can easily fool ourselves. Third, on the pond size, we have used mainly 400 m^2 for our experimental work. I am not at all sure that we can make valid extrapolations from data from these ponds of relevance to Israeli production ponds, which are hectares in area. For example, no farmer has yet been able to repeat our experimental production successes with *Macrobrachium*. The problem here is survival: 80-90% in our ponds compared to 0-50% on farms. I don't think we treat our animals any differently. The difference is in the pond conditions among which pond size is definitely a variable.

BILIO: So, in addition to a yield per unit area, one should indicate the surface area from which the data were obtained.

EDWARDS: My main interest is developing systems for the poor small-scale farmers in developing countries. Our production data don't really need extrapolation. I know we all make extrapolations for comparative purposes.

BILIO: As soon as you move from such a small-scale backyard operation to a larger operation, the labor costs (e.g., for putting wastes into the pond) must be accounted for. So the economic situation changes.

BIDDLESTONE: In your compost heaps, did you always have to incorporate a relatively dry carbon source, like leaves or straw? You mentioned night-soil and water hyacinth. Was there a third component?

EDWARDS: In our initial studies, we included rice straw. However in later work, we just used water hyacinth. We dried a certain proportion to ambient (normally about 15% moisture) and then used a formula to mix this with freshly chopped material (about 93% moisture) to arrive at whatever mix we wanted.

In an earlier discussion, Dr. Wohlfarth asked why the Chinese use composting when 50% of the dry matter is lost during the process. The answer, I think, is that most of the loss is carbon. The N is conserved in aerobic composting. In China, they use everything because of pressures on land use. Most of their compost is applied to the land. The loss of carbon is not a serious loss there. I have never seen Chinese use of compost in aquaculture. I don't think it is used in China in growout ponds. The major inputs to growout ponds in China are fresh macrophytes, human excreta, animal manure and now grains and pelleted feeds. According to the literature, the Chinese do apply composts to green-up fry and fingerling production ponds. For this, compost is produced on land or dumped in the pond in piles. The latter method is probably not very efficient because it can easily go partly anaerobic.

PULLIN: I agree, manure used to be applied to fishponds in heaps. A better practice is to broadcast it daily.

SRINIVASAN: Trace elements are also conserved in composting, in addition to N. This could be important. They may be lacking in other inputs.

SCHROEDER: In short-term composting, the cell solubles (or in other words the 'leachate') may be lost. We heard after Dr. Bowen's paper that precipitates, etc. formed from the leachate were not very nutritious (at least for tadpoles). Perhaps the bacterial colonization of compost makes it better nutritionally.

BOWEN: The tadpoles grew better when the material was colonized by bacteria. I thought one rationale for composting was to get over the oxygen demand produced by the buildup of microbial populations *on the land*, before adding the microbially-rich compost to the water. This avoids a reduction in water quality which you would get with decomposition in the pond.

EDWARDS: I don't think this has ever been a problem with small-scale farmers. You are correct theoretically; but I think that deoxygenation problems are more common in intensive aquaculture in which the inputs and feeds are of high energy. Small-scale farmers are usually constrained by both quality and quantity of feed/fertilizer inputs to their ponds so deoxygenation of the water is probably seldom of concern.

PULLIN: These problems of lack of oxygen are likely to be more serious at higher temperatures. Perhaps there is a difference here between tropical and temperate systems.

BIDDLESTONE: Are fish fry particularly sensitive to low oxygen?

PULLIN: Yes. We should return to discuss this further.

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*Edwards, P. 1983. The future potential of integrated farming systems in Asia. Prov. V World Conf. Animal Prod. 1: 273-281.

The Use of Human Wastes and Sewage in Aquaculture

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Abstract

Aquaculture with human wastes has a long history and, currently, is used extensively in China and several other Asian countries. Widespread use of human wastes and sewage in aquaculture is, at present, limited by the real and perceived public health risks associated with this method of fish farming. Although much is known about the health risks associated with pathogenic organisms, the use of human wastes in aquaculture has not been subjected to a rigorous health risk assessment. Because of the significant potential for the production of animal protein using human wastes and sewage, greater emphasis should be devoted to the delineation of critical health risks associated with this practice. Given the existing data limitations, an extrapolation method known as "Probability Matrix Technique" is suggested as a starting point for assessing the risks. The results of individual risk assessment can also be used to rank alternatives and to establish research priorities.

Introduction

The practice of using human wastes in aquaculture is probably as old as aquaculture itself. The principal reason for using human wastes and sewage in aquaculture is to harness the nutrients contained in the wastes for production of a usable protein food. A recent development is the use of aquaculture as a waste treatment method

for the removal of contaminants prior to release into the environment. Although the use of aquaculture for waste treatment is of interest, the primary purpose of this paper is to examine the use of human wastes and sewage for the production of fish and the factors limiting expansion of this practice. The topics to be considered include a brief review of current waste aquaculture practices, characteristics of human wastes, health risks involved in the use of human wastes, a methodology for assessing the risks where human wastes are used, and the opportunities available for risk management. General practices have been reviewed in detail (Allen 1969, 1972; Tsai 1975; Allen and Hepher 1979). In the following discussion, the term human wastes is used to denote wastes collected directly from pit and bucket latrines; pour flush, composting and vault toilets; and septic tanks. Nightsoil is another term commonly used to describe the material. The term sewage is used to denote the mixture of human, industrial and other wastes collected in sewerage systems.

Use of Human Wastes and Sewage for the Production of Fish

Current practices range from the use of subsistence ponds, where toilets are located over the ponds, to managed aquaculture facilities where fish are grown in ponds enriched with human wastes or sewage. Where human wastes are used, they are often brought to the pond site in carts or trucks and subjected to some processing before being added to the ponds (Edwards et al. 1984; Feachem et al. 1977; Polprasert et al. 1982). Where sewage is used, it is usually pretreated and diluted. In all cases, the underlying principle is to recycle and utilize the nutrients and organic matter contained in the wastes for the production of animal protein. Management options typically include the selection of the fish species and density, and control of the waste loading rate. The types of fish cultured include the carps and tilapias (primarily *Oreochromis niloticus*). Reported fish yields from aquaculture ponds fed with human wastes are given in Table 1, Part A.

Based on a review of the available information concerning the use of human wastes and sewage for the production of fish, it can be concluded that this practice is feasible, but largely empirical. This situation is similar to that in the field of commercial aquaculture. A scientific basis for pond management, based on a fundamental understanding of pond dynamics, is needed (Lannan et al. 1986).

Although operationally and technically feasible, the widespread use of human wastes and sewage for aquaculture has been limited 1) by the health risks identified with this practice and 2) by religious and social constraints. Health risks are considered in detail later in this paper. Consideration of religious and social constraints are beyond the scope of this paper.

Use of Aquaculture for Wastewater Treatment

The use of aquaculture for the purpose of treating wastes and for improving the quality of sewage effluent prior to its release to the environment has been practiced

Table 1. Fish yields from aquaculture ponds fed with sewage.

Waste pretreatment	Location	Fish	Yield	Reference
		A. Ponds used primarily for fish production		
Diluted septic tank water	Indonesia	*Cyprinus carpio*	3,000 kg/ha/yr	Vaas (1948)
Primary settling, aerated lagoons, diluted with sea water	USA	*Oncorhynchus* sp.	0-11 kg/ha/d	Allen and Dennis (1974)
Oxidation pond	India	*Cyprinus carpio*	3,200-7,700 kg/ha/yr	Jhingran (1974) Feachem et al. (1977)
Unspecified	India	*Oreochromis* sp. *Cyprinus carpio*	9,500 kg/ha/yr	Srinivasan (1979) Hepher and Pruginin (1981)
Treated sewage (treatment unspecified)	Poland	*Cyprinus carpio* *Ctenopharyngodon idella* *Hypophthalmichthys molitrix*	—	Klekot (1979)
Untreated sewage	Taiwan	*Oreochromis* sp.	6,500-7,800 kg/ha/yr	Chen (1976)
Untreated sewage	Thailand	*Oreochromis niloticus*	6,000 kg/ha/yr	Edwards et al. (1984)
		B. Ponds used primarily for wastewater treatment		
Primary settling	West Germany	*Cyprinus carpio*	400-1,000 kg/ha/yr	Neess (1949) Bardach et al. (1972)
Primary settling	Poland	*Cyprinus carpio*	1,300 kg/ha/yr	Hepher and Pruginin (1981); Wolny (1962)
Secondary (method unspecified)	England	*Cyprinus carpio*	300-500 kg/ha/yr	Noble (1975)
Primary settling and aerated lagoons	USA	*Oreochromis niloticus* *Ictalurus punctatus* *Notemigonus crysoleucas* *Pimphales promelas*	—	Carpenter et al. (1976)
Untreated sewage	India	*Cyprinus carpio*	900 kg/ha/yr	Feachem et al. (1977)
Untreated sewage	Israel	*Cyprinus carpio* *Hypophthalmichthys molitrix* *Oreochromis aureus*	2,800 kg/ha/6 mo.	Schroeder and Hepher (1979)

for a relatively short time. Perhaps the best known example of this practice is the culture of carp *(Cyprinus carpio)* in wastewater ponds in Germany (Metcalf and Eddy 1935; Bardach et al. 1972; Allen and Hepher 1976). In the United States, Carpenter et al. (1976), have reported significant reductions of organic matter, nutrients and suspended solids using a series of waste treatment ponds stocked with fish. Fish yields obtained in ponds designed for the purpose of improving the quality of sewage are reported in Table 1, Part B. Although the reported yields are significant (compare values reported in Table 1, Parts A and B), this method of waste treatment has not gained wide acceptance. Health concerns have been a major impediment in the United States.

Important Considerations in the Use of Human Wastes for Aquaculture

Important considerations in the use of human wastes for aquaculture are related to the chemical and biochemical characteristics of the waste material, the presence of pathogens in human feces, and the presence of anthropogenic compounds (manufactured chemicals) in sewage collected in sewerage systems. The role of pretreatment is also examined.

Characteristics of Human Wastes and Sewage

In comparing aquacultural practices that utilize animal wastes with those that utilize human wastes, it is important to recognize the different characteristics of the wastes. Production rates and composition of human and animal fecal material are highly variable; thus, generalized values such as shown in Table 2 should only be used for comparative and illustrative purposes. Humans produce approximately one-third the amount of waste produced by domestic animals per kg of biomass (see Table 2). In addition, five day carbonaceous biochemical oxygen demand ($CBOD_5$) to chemical oxygen demand (COD) and nutrient ratios are different in human wastes from those in animal wastes. The higher $CBOD_5$ to COD ratio in human wastes as compared to animal wastes can be taken as an indication that human wastes are more readily biodegradable than animal wastes. Nitrogen to phosphorus and nitrogen to potassium ratios are also higher for human wastes than for other animal wastes.

Table 2. Characteristics of human and animal wastes.[a, b, c]

Animal	Waste production g/kg animal/day[d]	Moisture content %	Constituent values, g/kg animal/day[d]						
			$CBOD_5$[e]	COD[f]	Total solids	Volatile solids	Total Kjeldahl nitrogen	Total phosphorus	Total potassium
Dairy cattle	82	87	1.7 (2.1)	9.2 (11)	10.4 (13)	8.6 (10.5)	0.41 (0.5)	0.07 (0.1)	0.27 (0.3)
Beef cattle	60	88	1.6 (2.7)	6.6 (11)	6.9 (12)	5.9 (9.8)	0.34 (0.6)	0.11 (0.2)	0.25 (0.4)
Swine	65	91	2.0 (3.1)	5.7 (8.8)	6.0 (9)	4.8 (7.4)	0.45 (0.7)	0.15 (0.2)	0.29 (0.4)
Poultry	53	75	2.5 (4.7)	8.4 (16)	13.4 (25)	9.4 (18)	0.50 (0.9)	0.20 (0.4)	0.20 (0.4)
Man									
North America, Europe	19	92	0.4 (2.1)	0.7 (3.7)	1.5 (8)		0.2 (1.0)	0.03 (0.2)	0.03 (0.2)
Rural developing country	22	92	0.5 (2.3)	1.0 (4.5)	1.8 (8)		0.2 (0.9)	0.04 (0.2)	0.04 (0.2)

[a] Human wastes after Feachem et al. (1980); Feachem et al. (1983).
[b] Animal wastes after ASAE (1983).
[c] Wastes include feces and urine.
[d] Values in parenthesis represent percent of total production (wet mass).
[e] $CBOD_5$ = carbonaceous 5-day biochemical oxygen demand.
[f] COD = chemical oxygen demand.

Sewage characteristics depend not only on the composition of the waste, but also on the method of collection. In industrialized countries, human and other wastes are collected primarily by central sewerage systems using water as the carriage medium. Typical composition data for sewage from industrialized countries are presented in Table 3. Apart from being quite dilute, sewage often differs from manually collected human wastes, by the presence of industrial wastes. The presence of industrial wastes and anthropogenic compounds may create toxicity problems.

Table 3. Typical composition of dry-weather domestic sewage in industrialized countries.[a]

Characteristics	United States	Japan	United Kingdom	Federal Republic of Germany
Per capita flow, m^3/day	0.35	0.30	0.18	0.16
$CBOD_5$[b], g/m^3	200	140	350	400
COD[c], g/m^3	500	200	500	570
Suspended solids, g/m^3	200	70	350	400
TKN[d], g/m^3	40	21	50	57
P, g/m^3	10	3.5	15	17

[a]From Tchobanoglous and Schroeder (1985).
[b]$CBOD_5$ = 5-day carbonaceous biochemical oxygen demand.
[c]COD = chemical oxygen demand.
[d]TKN (total Kjeldahl nitrogen) = organic nitrogen + ammonia nitrogen.

Pathogens in Human Wastes and Sewage

In addition to the chemical and biochemical characteristics considered above, the presence of pathogenic organisms in feces is of great concern where human wastes are to be used for aquaculture. The level of concern is greatest in those countries where the practice of waste-fed aquaculture might be beneficial, but is not now sanctioned or allowed for public health reasons. In many cases, health risks are more perceived than demonstrated with supporting scientific evidence. In those countries where human wastes are used routinely for the production of fish, public health questions are seldom raised, because most of the diseases associated with this practice are endemic.

Pathogens commonly found in human wastes are reported in Table 4. While fish are not susceptible to infection by most human pathogens, they can serve as carriers and thus transmit many of the diseases reported in Table 4. Fish have been shown to accumulate bacteria and viruses in several body tissues, including muscle and organs such as kidney, pronephros and liver (Buras et al. 1985). The degree of accumulation is dependent on the concentration of the bacterium or virus present in the culture

Table 4. Pathogens found in human excreta.[a]

Biological group	Organism	Disease
Viruses		
	Coxasackievirus	Various
	Echovirus	Various
	Hepatitis A virus	Infectious hepatitis
	Poliovirus	Poliomeyelitis
	Rotavirus	Gastroenteritis in children
Bacteria		
	Campylobacter sp.	Diarrhea in children
	Pathogenic *Escherichia coli*	Gastroenteritis
	Salmonella typhi	Typhoid fever
	Other Salmonellae	Food poisoning
	Shigella species	Bacillary dysentery
	Vibrio cholerae	Cholera
	Other vibrios	Diarrhea
	Yersinia species	Yersiniasis
Protozoa		
	Balantidium coli	Mild diarrhea
	Entamoeba histolytica	Amebic dysentery and liver abscess
	Giardia lamblia	Diarrhea and malabsorption
Helminths		
	Ascaris lumbricoides	Ascariasis
	Clonorchis sinensis	Clonorchiasis
	Diphyllobothrium latum	Diphyllobothriasis
	Faciolopsis buski	Fasciolopsiasis
	Schistosoma sp.	Schistosomiasis; bilharziasis
	Paragonimus westermani	Paragonimiasis

[a]Adapted from Feachem et al. (1983).

water, and is a function of the type of microorganism. In experiments reported by Buras et al. (1985), it was shown that coliform and fecal coliform bacteria "although present in the water in high numbers were not always detected in the muscles of the fish, while other bacteria were recovered". Therefore, particular care needs to be taken in the use of coliforms as indicators of possible bacterial contamination of fish grown in sewage waters, and the risk of disease transmission should always be assumed to be present.

Anthropogenic Compounds in Sewage

Where sewage is used for aquaculture, the presence of anthropogenic compounds is also of concern. The discharge of potentially tainting, toxic, or carcinogenic compounds to sewers will be difficult to eliminate or control even with source control programs. The discharge of such compounds is even more serious in countries with open sewers. The presence of such compounds is of great concern because fish bioaccumulate many anthropogenic compounds in their tissues, especially the fatty tissues (Tinsley 1979). There is little direct information available concerning the bioaccumulation of such compounds in sewage fed ponds.

To illustrate the potential seriousness of the problems that may be caused by the bioaccumulation of various anthropogenic compounds, four Midwestern states and the U.S. Federal government recently issued uniform guidelines on the hazards of eating fish from Lake Michigan. In the new advisory, fish are ranked in three groups: "safe", "moderately safe" and "to be avoided". The rankings are based on tests of fish flesh for levels of contamination with pesticides and polychlorinated biphenyl compounds (PCBs) that surpass pure food limits established by the U.S. Federal Food and Drug Administration (Burko 1985). Because of the potential for bioaccumulation, sewage may require pretreatment to remove various anthropogenic compounds before the nutrients in it can be used for the production of fish. Human wastes directly collected from latrines may be safer with respect to the presence of anthropogenic compounds.

Pretreatment of Sewage for Aquaculture

In addition to differences in sewage characteristics, there are marked differences in the levels of treatment given to sewage before it is made available for aquaculture use. The degree of pretreatment prior to application to the fishponds will affect the nutrient content of the wastes, the initial oxygen demand and the number and type of microorganisms present. A reduction in the initial oxygen demand can be achieved with a variety of pretreatment operations and processes (Metcalf and Eddy, Inc. 1979; Feachem et al. 1980; Tchobanoglous and Schroeder 1985).

Pretreatment can have a marked effect on the survival of viral, bacterial and protozoan pathogens. Without chlorination, conventional primary and secondary treatment systems (primary sedimentation, trickling filters, activated sludge and oxidation ditches) are effective in reducing the number of pathogens, but do not eliminate the danger of disease transmission from wastewater. Low cost treatment methods such as waste stabilization ponds with long detention times and land treatment by rapid infiltration appear to be more effective in decreasing the number of pathogens through natural die away and by filtration/adsorption (Klock 1971; Feachem et al. 1980). In all cases, the effectiveness of removal of pathogenic organisms has been shown to be highly temperature dependent, with increased removal efficiencies at higher temperatures.

In general, the survival of bacterial pathogens is decreased in the presence of large numbers of microorganisms that act as predators or competitors. Viruses, on the other hand, adsorb onto particles, and appear to have longer survival times in the presence of large amounts of suspended matter (Feachem et al. 1980). Moreoever viruses attached to larger particles may settle and remain viable in sediments (Klock 1971). Thus, the danger of transmission of viral diseases to man by fish would presumably be greater for detritivorous fish.

The health benefits of pretreatment must be evaluated for the specific situation and weighed against the costs. Benefits are normally measured in terms of the reduction in pathogenic organisms, the reduction of the nutrient content of the waste and, in some cases, the reduction of the concentration of anthropogenic compounds.

Public Health Risks

The health risks associated with the use of human wastes and sewage in aquaculture are poorly defined. Risk is defined here as "the nature, likelihood and magnitude of an adverse event or effect occurring as a result of the use of a given technology or some other action" (adapted in part from The Conservation Foundation 1985). With respect to the use of human wastes or sewage for aquaculture, different populations are exposed to the risk of disease transmission through aquaculture (adapted in part from Feachem et al. 1983; Shuval et al. 1986):

1. Fish farm workers, exposed to toxicants and pathogens present in the fish culture area;
2. Fish handlers, exposed to toxicants and pathogens on the fish and in the fish guts. Handling of the fish may occur at harvest, during transport, at markets, in processing and in preparation;
3. Consumers exposed to toxicants and pathogens in the fish.

While handlers and farm workers are normally at risk of contracting diseases that are transmitted by routes other than ingestion, the direct pathway for disease transmission from waste-fed aquaculture to fish consumers is normally limited to the ingestion of contaminated fish. The difference in the types of exposure of the different groups to wastes and sewage (or the aquaculture products) means that the groups are at risk of contracting diseases that have different paths of transmission. Some diseases are transmitted with the passive contribution of aquatic organisms, while others involve fish or other pond fauna as intermediate hosts.

Assessment of Health Risks

Risk assessment, as used in this paper, is "the process of determining the adverse consequences that may result from the use of a technology or some other action" (The Conservation Foundation 1985). In the context of the use of human wastes

and sewage in aquaculture, the quantitative assessment of risk involves (1) determination of the presence of pathogens, (2) determination of the manner in which infectious forms of the pathogens may come into contact with susceptible individuals and (3) determination of the number of individuals exposed, the level of exposure and the probability of adverse consequences resulting from exposure.

In assessing risk, extrapolation methods are used most commonly as they are considered to be the most sound scientifically, although revelation and intuition are also used (Kates 1978). Using extrapolation methods, known information about a specific event is extended or projected to predict a future event. Some of the methods that have been used include: standard statistical methodology, causal models based on known phenomenological relationships and formal methods of encoding expert opinion in the form of judgmental probabilities (Moreau 1980). The field data required for the application of quantitative extrapolation methods of risk assessment do not yet exist for the use of human wastes and sewage in aquaculture. Because of these limitations, use of an opinion encoding method known as the "Probability Matrix Technique" is suggested here as a starting point for assessing the risk. In the future, as more information and data are developed, this method can be used in conjunction with one or more of the quantitative extrapolation methods to assess risk. The following discussion is based on the work of Olivieri and Cooper (1981) and Cooper and Olivieri (1982) who have applied this consensus-building technique to assess the health risks associated with wastewater disposal options and onsite systems.

The Probability Matrix Technique Methodology

In waste-fed aquaculture, the probability matrix technique may be used to compare the relative risk associated with different options of treating or using a given water source. To apply the probability matrix technique of risk assessment to waste aquaculture, two groups of health experts are required. The first group is asked to define the public health problems that may be associated with the use of human wastes or sewage in a given aquaculture option. After identifying the problems, members of the first group are asked to make judgments on the probability of problem occurrence and to place a value on the probability using a linear scale of zero to one. Each expert within the first group is also asked to identify what additional information would be needed about each waste-fed aquaculture option to estimate with greater certainty the probability that a specified public health problem would occur. Experts in bacteriology, virology, parasitology, entomology, toxicology, aquaculture and sanitary engineering would be included in the first group.

The role of the second group is to judge the relative severity of the public health problems delineated by the first group. Ranked from zero (least severe) to 100 (most severe), the same severity value can be assigned to more than one health problem as the severity of the problem is compared to the other problems being considered and not to an absolute level of severity. Judgments on severity are

medical opinions based on collective knowledge of the life expectancy, degree of disability and effectiveness of treatment of the public health problem considered. The second group of experts would be composed primarily of physicians and epidemiologists.

The Delphi technique (developed by the Rand Corporation) is commonly used to obtain consensus values for the probability of occurrence of an event and for severity levels (Cooper and Olivieri 1982). The technique is designed to minimize face to face confrontations in arriving at a consensus, by using a series of questionnaires. Experts participating in the exercise are given the opportunity to revise their estimates of the probability of occurrence of an event, after seeing a summary of the values proposed by the group.

Magnitude of the Risk Estimate

Using the judgments made by the two groups of experts, the magnitude of the public health problems that might be expected with a given waste-fed aquaculture option is determined as follows:

$$R_i = \frac{\sum_{j=1}^{n} P_{ij} S_j}{\sum_{j=1}^{n} S_j}$$

where R_i = expected magnitude of risk for aquaculture option i
P_{ij} = subjective probability that aquaculture option i will have public health problem j ($0 < P_{ij} < 1$)
S_j = relative severity of problem j ($0 < S_j < 100$)
n = number of public health problems being evaluated.

The probabilities are subjective, but represent the best estimate that a problem will occur, given the current state of knowledge.

Application of Probability Matrix Technique

The application of the probability matrix technique for a single aquaculture alternative is illustrated in Table 5. As shown, the health problems and probability values are identified by group 1 experts. The severity values are assigned by group 2 experts. The severity values shown in Table 5 would be equal for all options under consideration. Probability values, however, would change between options due to differences in the survival of pathogenic organisms under the conditions created in

Table 5. Example of a probability matrix for risk assessment of the use of human waste in aquaculture option 2 (i = 2).[a]

Health problem, j[b]	Probability[c]	Severity[d]	$P_{2,j} S_j$
1. Infectious hepatitis	0.1	50	5.0
2. Gastroenteritis	0.8	50	40.0
3. Typhoid fever	0.2	20	4.0
4. Dysentery	0.1	80	8.0
5. Cholera	0.1	20	2.0
6. Clonorchiasis	0.5	50	25.0
7. Diphyllobothriasis	0.5	100	50.0
8. Fasciolopsiasis	0.3	80	24.0
9. Schistosomiasis	0.5	100	50.0
10. Paragonimiasis	0.5	80	40.0
		630	248.0

$$R_2 = \sum_{j=1}^{10} P_{2,j} \quad S_j / \sum_{j=1}^{10} S_j = 248/630 = 0.39^e$$

[a]All values shown in table are hypothetical.
[b]Problems identified by group 1 experts.
[c]Probability values assigned by group 1 experts.
[d]Severity values assigned by group 2 experts.
[e]The value of risk computed in this table would be compared to the values computed for the other options under consideration.

the processes being evaluated. The computed R_i value is used to rank each aquaculture option amongst all possible options with respect to public health concerns. It should be noted that in the example cited, only acute health risks associated with pathogenic organisms are considered. In practice, it is anticipated that the list of risks would be expanded to include other acute and chronic health risks. An analysis similar to that shown in Table 5 would be done for all aquaculture options being considered. In addition, other nonaquaculture options being proposed and existing systems of sewage or waste disposal should be analyzed.

Use of the Probability Matrix Results

Results of the probability matrix analysis are used in the decision-making process to compare options and to select those that are to be implemented. The risk estimates generated with the probability matrix technique are indicators of the possible negative public health effects of the various water treatment or aquaculture options analyzed. In deciding which of the proposed alternatives is to be implemented, the negative effects (quantified in the risk analysis), the expected benefits and socioeconomic factors are considered. An alternative that needs to be included in the

analysis, and that is often overlooked, is that of continuing the current waste disposal practices.

A second possible use is in the identification of problem areas. For example, if high risk values are estimated for an aquaculture option that has large potential benefits, attention can be focused on means that can be used to reduce the risks and make implementation of the process possible. Identification of high risks is very important in the evaluation of waste disposal or treatment practices that are in use at the site for which treatment strategy options are being proposed.

Risk Management

Risk management involves the means and methods used to reduce risk. Whenever risk management is considered, what must be determined is the degree of risk that is acceptable. Three categories of risk can be identified (1) zero risk, (2) technology-based risk and (3) reasonableness of risk balanced with benefits. Zero risk is usually unattainable in an economic context. The second two risk categories are where most risk management options are found, especially for the use of human wastes and sewage in aquaculture. Management options for pathogens and anthropogenic chemicals are considered below.

Control of Pathogens

Much has been written on the control of pathogens where human wastes and sewage are used for the production of fish. Feachem et al. (1980), have suggested the following techniques to control pathogens:

- Enriching ponds only with treated sewage, stored nightsoil or sludge
- Allowing fish to reside in clean water for several weeks prior to harvesting (depuration)
- Clearing vegetation from pond banks to discourage the growth of molluscan intermediate hosts of digenean trematodes and the provision of substrates for metacercarial cysts of some species
- Promoting good hygiene in all stages of fish handling and processing
- Discouraging the consumption of unprocessed or undercooked fish

Additional control measures might include:

- Restricting the use of fish grown in sewage fed ponds to processed food applications
- Using the fish grown in sewage fed ponds for nonhuman uses such as in the production of animal feeds or fish silage

While it is known that each of these techniques will help to reduce (or partially manage) the risk, the relative effectiveness of these methods is difficult to quantify.

Control of Anthropogenic Chemicals

Little is known about the control of anthropogenic chemicals in aquaculture facilities where sewage is used. The reason is that the potential problems have only recently been identified. Some management techniques that can be used are as follows:

- Enriching ponds only with treated sewage
- Improved industrial source control and pretreatment programs
- Pretreatment of irrigation return waters before using them

Risk management technologies for the control of anthropogenic chemicals will, unfortunately, be site specific and are likely to be expensive. For example, because conventional treatment methods for agricultural return waters are expensive, it may be necessary to use the soil as a filter or some type of adsorption bed which can be followed by soil filtration.

Summary

Currently, millions of people in China and other parts of Asia rely on waste-fed aquaculture as a source of animal protein. Where human wastes and sewage are now used for fish production it would be difficult to eliminate the practice based solely on public health considerations. However, to understand and reduce the real and perceived health risks of using human wastes and sewage for fish production, it is essential that this practice be subjected to a risk assessment. The risk associated with using agricultural return waters must also be considered.

Given that an extensive database is not available, it is recommended that a probability matrix technique, such as outlined in this paper, be used to assess the risk. Based on the results of the risk assessment, it should be possible to evaluate the impact of several risk management techniques. Ultimately, as the health risks are delineated more completely, the use of human wastes and sewage for the production of fish protein may find a broader application. Because of the potential importance of human waste and sewage-fed aquaculture for the production of fish protein in developing countries, greater emphasis should be devoted to the delineation of the critical health risks associated with this practice and to the opportunities and possibilities for reducing the risks involved.

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Discussion

PULLIN: Considering possible risks, we should not forget the risks to fish farm workers as well as consumers. For example, there have been recent incidents of leptospiral infections among salmonid farm workers. Leptospirosis is transmitted through abrasions being put into contact with water which is contaminated with the urine of infected rats. This has caused some fatalities.

SCHROEDER: Your mathematical technique has one serious flaw. It can camouflage the risk of a fatal, highly contagious infection (say 100% fatal, 100% chance of infection) because your overall figure, once you have made your division, could be, say, 50%.

BOWEN: This technique is used to assess risk from other sources; for example, nuclear power plant accidents. The values given are relative, not absolute. The fact that the probability values are closer to one or to zero has no relevance. These are all relative values.

SCHROEDER: This must be made abundantly clear to anyone who might use these figures to assess risks, say in using sewage-fish culture in a third world country. Someone might take these values as a true indication of risk while they can hide the chance of certain death, as in the example which I have given.

PIEDRAHITA: The probability matrix technique is designed to be used for estimating risks by quantifying opinions and dealing with uncertainty. If a waste is certain to cause a severe public health problem under a given treatment and disposal method, there is no point in including it in the probability matrix analysis. The dangers of using the particular waste should be noted and the recipients of the probability matrix analysis should be informed of the certainty of problem occurrence and of alternative treatments for the waste.

PRUDER: We can build a model to take account of such possibilities.

PULLIN: Is this technique used in risk management; in insurance risk assessment for example?

PIEDRAHITA: Yes, where there is little information available. It is used as a sort of last resort. It has been very useful, however, in small-scale wastewater applications, which are somewhat similar to what we are discussing: you are trying to dispose of a waste with an acceptable level of risk.

MORIARTY: I see no problem. This is a model, which, like all models, is a tool with limitations. It cannot be used in a situation in which one disease gives the prospect of certain infection and certain death. Everyone would know this.

SCHROEDER: But they could not tell it from the numbers.

Editors note: This discussion continued without a clear resolution. However, the authors have addressed in this published version of their paper the use of their suggested technique when highly contagious/fatal diseases are present.

PULLIN: There is another factor here. Where you have sewage to dispose of, particularly in some developing countries, there may be very high risks associated with alternative methods to aquaculture or if nothing is done. We had to face this issue in an appraisal of a GTZ-World Bank sewage-fish culture project in Lima, Peru. Moreover, the public health experts in this case produced very long lists of human pathogens and parasites which they felt might occur in the system, but for many of which there were no data on abundance or even presence or absence. The task of investigating them all looked very daunting. It seems to me, therefore, that this paper has drawn attention to a very cost-effective technique for making a first assessment of the relative risks associated with different sewage disposal methods, when information is limited.

EDWARDS: From a medical point of view, it may also be highly beneficial to recycle human waste into food, perhaps through aquaculture, in areas in which there is protein malnutrition. In other words, the health of the population could suffer if this resource was not recycled in this way. Socioeconomic conditions are of paramount importance here.

BILIO: I agree.

EDWARDS: For example, recycling of human waste used to be widespread through Korea, Japan and China. As affluence has increased in some areas, its use has declined, e.g., in Taiwan (pers. obs.). However, in the poorer countries of Asia, it remains very important as a potential resource for producing high protein food.

BIDDLESTONE: The results of using a model like this are, of course, only as good as the data. There may be a danger in assuming developed-country efficiency in pathogen removal by a given process for parallel situations in developing countries. Perhaps a 90% kill of salmonella in one anaerobic digester is only a 10% kill under less controlled conditions. Have you also looked at aerobic treatments?

PIEDRAHITA: You are right in saying that pathogen removal efficiency is likely to be highly variable and will depend on the waste treatment process being used and on the level of control over the process. There are, however, general trends on the effectiveness of different types of treatment methods for the removal of various pathogenic microorganisms. As an example, Feachem and co-workers (1983)* have presented a table indicating the expected effect of various waste treatment processes on different types of pathogenic microorganisms.

BILIO: But you have not used these in your paper, you have made guesses.

PIEDRAHITA: We have used them in arriving at an overall estimate of the effect of a given treatment method on the probability of problem occurrence. The pathogen reduction efficiency is, however, only one component of the overall probability estimate, which accounts for other factors

such as exposure, form of transmission, etc. The values used in the example are for illustration purposes only and do not necessarily represent accurate probability values for a real situation.

PULLIN: On Dr. Biddlestone's point, Dr. Piedrahita has stressed in his paper that the application of this technique is highly location-specific.

COLMAN: The basic question is, is this a useful technique? I would say it is. When you have assembled your matrix all the information is there—including the 100% probabilities if such exist.

ANDERSON: But the decisionmaking process may be left to say a group of sociologists or laymen, who may not have all the information or your perspectives on that information.

PULLIN: We must close this discussion here. We can continue later if time permits.

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*Feachem, R.G., D.J. Bradley, H. Garelick and D. Mara. 1983. Sanitation and disease, health aspects of excreta and wastewater management. World Bank studies in water supply and sanitation 3. John Wiley and Sons, Chichester. 501 p.

Use of Manures in Aquaculture

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Abstract

The use of manures in aquaculture enables the production of a highly valuable, protein-rich food from inputs of little nutrient value to man or livestock. This practice, used traditionally in China with a polyculture of Chinese carps, has been applied in other areas with different combinations of fishes, including tilapias, a range of stocking rates and different manures. Yields of up to 30-35 kg/ha/day have been obtained in different investigations. Yields as high as 50 kg/ha/day have been reached in standing water ponds using feed pellets with a 25% protein content, but with grains (sorghum) as supplemental feed, yields are lower than the maximum yields from intensive manuring alone. These conclusions are based on a survey of investigations from different parts of the world and from a series of replicated studies in Israel. Yields from manured ponds may be increased by supplemental feeding but the increment is relatively small and is obtained at a low efficiency.

The processes by which manure is converted into fish flesh are either direct consumption of feed remnants in the manure or stimulation of natural food webs to increase autotrophic and heterotrophic production. These processes are poorly understood and have not been quantified. Our lack of understanding may be the main constraint to further increasing fish yields from intensively manured ponds.

Introduction

The traditional management practice in Chinese aquaculture is to use manure as the principal nutrient input to ponds stocked with a variety of Chinese cyprinids. The philosophy of this practice has been described as 'harmony' (Tang 1970), i.e., the fish stocked harmonize with available fish foods. Presumably a major reason for developing this efficient system was the requirement for producing food from inputs which are of no nutritional value for man or livestock. Note that in animal husbandry

this aquaculture management practice is the only method of producing, rather than converting food, other than keeping livestock on pastures. The chronic famines, especially of protein-rich foods, in large parts of the world and rising prices of feedstuffs make this system of particular interest.

Intensive manuring of ponds stimulates natural food webs thereby generating considerable quantities of phytoplankton, zooplankton and benthic organisms. A variety of fishes of different food preferences or a single species which can exploit different feeding niches is required for the effective utilization of this highly heterogenous food web and to prevent the accumulation of nonutilized components. Thus intensive manuring is usually linked with polyculture. With conventional feeds, monoculture is the usual practice.

The use of manures in fish farming has been reviewed by Wohlfarth and Schroeder (1979) and Edwards (1980). The advantages and disadvantages of this practice, and methods used in different production systems are reviewed here, followed by a summary of empirical investigations carried out in Israel.

Use of Manures, Advantages and Disadvantages

The advantages of using manures as the principal nutrient input in aquaculture are:

- The low cost of manure can drastically reduce feed costs, which often account for about half the total production costs.
- In areas where manure is readily available, the need for feedstuffs produced or manufactured elsewhere is avoided.
- Utilization of manures solves problems connected with its disposal and environmental pollution resulting from manure accumulation.
- Because the carbohydrate content of natural foods developing from manure degradation products is lower than that of most conventional feeds, fish particularly common carp *(Cyprinus carpio)* grown on manure tend to have a lower fat content than those grown on conventional feeds (Wohlfarth and Schroeder 1979).
- A variety of fishes can be produced from manured polyculture systems.

However the use of manures, accompanied by polyculture, also suffers from several disadvantages:

- The proper management of these systems is more difficult than feedlot monoculture systems.
- Estimating the appropriate amount of manure to be applied is less standardized than for conventional feed inputs due to differences in the quality of different manures, e.g., contents of moisture, ash and minerals. Such differences exist, particularly when using manure from different animals, but even manures taken from a given production system also differ at various times.
- The processes of manure decomposition and the utilization of its degradation products by the wide variety of organisms in a pond are influenced by factors not under our control, e.g., temperature, intensity of solar radiation and

dissolved oxygen. The amount of manure to be applied depends upon these somewhat unpredictable processes as well as upon the biomass of fish.

- Because the rate of manure decomposition is a function of temperature, its application is more suited to warm climates and warmwater fish (e.g., Chinese carp, common carp, tilapia and catfish) than to salmonids and other coldwater fish.
- Aesthetic objections to manure-fed fish may lead to consumer resistance.
- Manure is continuously available in large amounts only when livestock production is concentrated in feedlot units situated within a reasonable distance from the ponds. When animals are largely raised on pastures, manure availability may be a problem.
- Manure is not a suitable nutrient input for highly intensive aquaculture systems, such as raceways. Even in standing water ponds, maximum yields attained with manure are lower than those attained with nutritionally balanced feeds (Moav et al. 1977).
- The relative economics of manure and feedstuffs in aquaculture depend on the price ratio between fish and feeds. Growing fish on manures is more profitable only when the fish are relatively cheap and feeds are expensive. With fish of luxury prices, using formulated feed pellets is likely to be more profitable due to the higher yields attainable (Wohlfarth and Schroeder 1979).

Methods of Manure Utilization in Aquaculture

Manure may be applied to fishponds by housing livestock over the water or in its vicinity. In such integrated systems, fresh manure is continuously added to the ponds, which avoids losses from degradation of the manure and fodder remnants. Environmental pollution resulting from accumulation of manure is largely avoided. Investigations on the integration of livestock and aquaculture units have been carried out with ducks (Blume 1960; Chislov and Chesnakov 1974; Muller 1978; Cruz et al. 1979; Cruz and Shehadeh 1980; Hopkins and Cruz 1980; Sin 1980; Barash et al. 1982; Plavnik et al. 1983); with pigs (Nugent 1978; Cruz et al. 1979; Burns and Stickney 1980; Cruz and Shehadeh 1980; Delmendo 1980; Hopkins and Cruz 1980); with chickens (Stickney et al. 1977; Hopkins and Cruz 1980, 1982); and with geese (Sin 1980). In most of these investigations the fish were stocked in polyculture, consisting of two or more of the following: Chinese carp, common carp, tilapia (sometimes as the predominant species and accompanied by a predator) or mullets. Monoculture of common carp (Blume 1960; Muller 1978) or tilapia (Stickney et al. 1977; Burns and Stickney 1980) has also been investigated in manured ponds.

Alternatively, manure may be transported to the ponds without integrating the livestock and aquaculture units. This is the prevalent aquaculture system in China; any available manures are utilized, including those from pigs, cattle and horses, as well as from humans ('night soil') (Hoffmann 1934). Manures tested in different investigations include cattle manure (Schroeder 1974, 1975; Moav et al.

1977; Collis and Smitherman 1978; Rappaport and Sarig 1978; Wohlfarth 1978; Schoonbee et al. 1979; Wohlfarth et al. 1980), pig manure (Tang 1970; Stickney et al. 1979; Buck et al. 1979), and chicken manure (Tang 1970; Rappaport et al. 1977; Rappaport and Sarig 1978; Wohlfarth et al. 1980). In most investigations, the fish were stocked in polyculture, although monoculture of common carp, tilapia or silver carp was also tested. The giant Malaysian prawn *(Macrobrachium rosenbergii)* may be added to fish polyculture and grown to market size with chicken manure as the principal nutrient input (Wohlfarth et al. 1985). Yields may be increased by augmenting the intensive manuring management with relatively small amounts of feed pellets, but the marginal feed conversion of these pellets is poor (Moav et al. 1977; Wohlfarth et al. 1980).

The potential importance of manure in aquaculture as a means of preventing pollution is discussed by Edwards (1980), who states that only in China are animal and human wastes fully utilized. Common carp have been used to clean the water of a lagoon receiving the wastes from a poultry processing unit (Anon. 1979) and the pollution of coastal waters by pig dung effluent was reduced by stocking a pond, through which the effluent flowed, with silver carp *(Hypophthalmichthys molitrix)*, bighead carp *(Aristichthys nobilis)* and grass carp *(Ctenopharyngodon idella)* (Seow and Tay 1973).

Yields obtained from manure-fed systems varied from 7 to 36 kg/ha/day among different investigations (Table 1). Fish grown in these systems were mainly different combinations of Chinese carp, common carp or tilapia. Stocking rates also varied considerably. The lowest yields were obtained at relatively low stocking rates, but increasing stocking rates to much above 10,000 fish/ha do not appear to result in further yield increases. Neither is it apparent whether a given type of manure or a particular combination of fish is superior. The highest yields were obtained from integration of fishponds with duck or pig production systems, but yields almost as high were obtained with intensive use of poultry manure.

The main conclusion to be drawn from the results summarized in Table 1 is that high yields may be obtained with manure as the exclusive organic input. Whether this high yield potential is realized apparently depends on management factors not defined in the publications upon which this table is based.

Manuring Tests Carried Out in Israel

Nine tests in which intensive manuring was applied to experimental fishponds are summarized in Table 2. The earlier tests are more fully described by Moav et al. (1977), Wohlfarth (1978) and Wohlfarth et al. (1980). All tests were carried out at Dor in earthen ponds of 400 m^2, between July and November of each year. The ponds were stocked with a mixture of common carp, tilapia (in most cases different interspecific hybrids; Wohlfarth et al. 1983), silver carp and grass carp. In 1979, 1981, 1982 and 1983 freshwater prawns were also included (Wohlfarth et al. 1985). The manuring treatments were integrated into a program of testing different genetic groups of common carp (Moav et al. 1975) or tilapias (Wohlfarth et al. 1983).

Table 1. Fish yields from manured ponds.

Source of nutrients	Fishes stocked	Stocking rate (No./ha)	Daily yield (kg/ha)	References
Pig manure, plants	Chinese and common carps	18,000	40*	Shan et al. (1985)
Duck facility	Common and silver carp, tilapias	10,000 –20,000	36	Barash et al. (1982)
Pig facility	Chinese carps, tilapias	15,500	36**	Behrends et al. (1983)
Cattle manure	Common and Chinese carps, tilapias	9,000 –18,000	32	Moav et al. (1977)
Chicken manure	Common and Chinese carps, tilapias	8,000 –16,000	29-35	Wohlfarth et al. (1980)
Manures, 'nightsoil', rice bran	Chinese and common carps, mullets	23,500	24***	Tang (1970)
Pig facility	Chinese and common carps, buffalo	10,700	17-22	Buck et al. (1979)
Pig, duck or chicken facilities	Tilapias, common carp, snakehead	20,000 10,000	19-20 10-15	Cruz et al. (1979) Cruz and Shehadeh (1980); Hopkins and Cruz (1980, 1982); Hopkins (1982)
Cattle manure	Tilapias	10,000	16	Collis and Smitherman (1978)
Chicken manure	Common carp, *Clarias*	2,100	7	Bok and Jongblood (1984)

*Mean of two ponds, yield computed from seined samples, ignoring mortality.
**Single pond, yield includes 14 kg/ha/day tilapia reproduction.
***Single pond, mean of three repeated investigations.

In the first manuring test (1974) involving two ponds, a constant amount of liquid cattle manure was applied six days per week for the initial 83 days and thereafter the amount was doubled. The decline in growth during the initial period and the immediate response to the increased amount of manure led us to believe that the amount of manure required for sustaining optimal growth needed to be increased with increasing fish biomass, as practiced with supplementary feed. In all subsequent tests, the manure management consisted of applying 50 kg/ha/day dry matter of manure at the beginning of the test and increasing this by 25 kg every two

weeks up to a maximum of 175 to 200 kg (Wohlfarth 1978). Inorganic fertilizers were applied once every two weeks at a rate of 50 kg/ha each of ammonium sulfate and superphosphate. For technical reasons, poultry manure was substituted for cattle manure from 1977 onwards. In 1982, in addition to the 21 ponds with poultry manure, 'goose manure' was used in three additional ponds. This 'manure' resembled a slurry of ground, fermenting corn because it was obtained from production units in which geese were force-fed to enlarge their livers. Total stocking rates varied between 6,000 and 20,000 fish/ha in different treatments and years. The proportions of the different fish also varied, with an emphasis on common carp in the earlier tests and on tilapia in the later ones.

The management variables emphasized in the first test (1974) consisted of two feeds (sorghum or 25% protein pellets), applied at a rate computed as 4% of the biomass of common and grass carp plus 2% of tilapia biomass. These results are presented to serve as nonmanured controls for comparison to the later tests in which intensive manuring was practiced. In most of these manuring tests, different treatments were applied, i.e., varying type of manure and stocking densities, with and without different supplemental feeds; supplemented feeds were presented at one-third of the standard daily rates of nonmanured controls. Fish biomass was estimated from sample weighings every two weeks. Manures and feeds were applied daily (six days per week) except in the low stocking rate treatment of 1981 in which the ponds were manured once a week, but the full standard amount of feed was presented. All treatments in each tests were replicated (Table 2).

Results

Mean survival of fish was high, predominantly between 80 and 90%. The last two columns of Table 2 show the food conversion ratios (FCR) of manure and feed. For tests in which manure plus feed were applied, the FCRs were computed separately, ignoring the effect of the other organic input. Hence, they are partial conversion ratios. With manure as the only organic input, the FCR is remarkably stable at about 3.5 (except in the 1974 test), similar to the FCR of sorghum and only a little higher than that of high protein feed pellets. Supplementing manure with feed resulted in a slight lowering of the partial manure conversion ratio, because the effect of supplementary food on yield is ignored. The partial FCRs in these treatments are very low for similar reasons. The sums of the two partial conversion ratios tend to be a little higher than either feed or manure conversion ratios, indicating that increasing yields by supplementing feed to manure is relatively inefficient. This is also shown by the marginal FCRs in tests in which either manure plus feed or manure alone was applied. Marginal FCRs show the weight of feed required to obtain a further unit weight of fish by supplementing manure with feed. They are computed by dividing the amount of supplemental feed (yield x partial conversion ratio) by the yield difference between manure plus feed and manure only. When cattle manure was supplemented with sorghum (1975) the marginal FCR was about 6; when chicken

Table 2. Managements and results of nine tests in which livestock manures were applied to experimental fishponds in Israel.

Year	No. of replicates	Nutrients Manure	Nutrients Feed	No. of fish stocked/ha CC	T	SC	GC	Total	Survival (%)	Daily yield (kg/ha) CC	T	SC	GC	Total	Conversion ratios Manure***	Feed
1974	4	—	S	11,450	5,000	2,500	750	19,700	82	14	4	11	1	30	—	3.2
	4	—		3,300	1,500	1,250	330	6,380	85	14	1	8	1	24	—	3.8
	4	—	P	11,450	5,000	2,500	750	19,700	77	33	4	10	3	50	—	2.5
	4	—		3,300	1,500	1,250	330	6,380	82	23	2	8	1	34	—	2.9
	2	Cattle		3,050	1,500	1,250	330	6,130	80	11	7	1	1	20	1.8	—
1975	4	Cattle	S	9,050	5,000	3,080	850	17,980	89	21	9	9	2	41	2.7	1.3
	4	Cattle		4,800	2,500	1,520	420	9,240	91	24	6	7	2	39	2.6	1.2
	4	Cattle		9,050	5,000	3,080	850	17,980	91	13	9	9	2	33	3.4	—
	4	Cattle		4,800	2,500	1,520	420	9,240	91	17	5	7	2	32	3.2	—
1977	8	Poultry	—	4,000	3,000	1,000	300	8,300	93	20	5	6	1	32	3.5	—
1978	8	Poultry	—	5,000	9,000	1,500	500	16,000	89	13	12	8	2	35	3.5	—
	8		—	4,000	3,000	1,000	300	8,300	91	17	5	5	1	29	3.5	—
1979	9	Poultry	P	5,200	9,200	1,000	1,000	17,400	86	22	9	8	3	4[illegible]	3.0	0.6
	9			4,000	3,000	1,000	500	8,500	88	24	4	6	2	36	3.0	0.6
1980	11	Poultry	P	5,200	9,000	1,200	350	15,750	78	16	8	7	1	32	3.3	0.8
	11		S	5,200	9,000	1,200	350	15,750	75	15	7	6	1	30	3.8	0.8
1981	6	Poultry	P*	3,000	3,000	1,000	450	7,450	91	18	7	9	2	36	0.6	1.8
	15		S	3,000	7,500	1,000	450	11,950	90	10	11	8	2	31	3.4	0.6
1982	21	Poultry	P	3,550	9,000	1,000	375	13,925	86	13	11	7	3	34	2.9	0.6
	3	'Goose'**	—	3,550	9,000	1,000	375	13,925	92	16	16	6	1	39	3.6	—
1983	24	Poultry	P	3,000	9,000	1,000	300	13,300	91	13	14	6	1	34	3.4	0.5

CC = common carp, T = tilapia, SC = silver carp, GC = grass carp, S = sorghum, P = high protein (25%) pellets.
*Applied at 'full' daily rate (see text): all other entries were at one-third standard daily rate.
**From geese force-fed for liver fattening.
***Computed according to dry matter content.

manure was supplemented with high protein pellets, (1978 versus 1979), the marginal FCR was between 3 and 4.

The effects of different management techniques on total fish yield (Table 3) may be compared from results of different treatments within a given test. Comparisons between tests lead to less reliable conclusions due to differences in total stocking rate, proportions of different fishes and temporal effects which are more difficult to define. Nevertheless both types of comparisons are made here to draw general conclusions from this series of experiments. Total fish yields from different tests are grouped according to management techniques in Table 3. Stocking rates are arbitrarily divided into 'low' (< 9,000/ha) and 'high' (> 12,000/ha). Yields responded to different components of these managements as well as to interactions between them.

Table 3. Mean daily fish yields (kg/ha) from different combinations of feed and manure inputs to fishponds. [Boxes indicate treatments within same test (see Table 2)].

Nutrient management	Type of feed	Fish stocking rate (low, < 9,000/ha; high, > 12,000/ha)	Feed	Organic inputs Cattle manure		Poultry manure			Mean yield (kg/ha/day)
Feed* only	High protein pellets (25%)	High	50						42
		Low	34						
	Sorghum	High	30						27
		Low	24						
Manure + feed*	High protein pellets (25%)	High			42	32	34	34	35
		Low			36		31		
	Sorghum	High		41		30	36		36
		Low		39					
Manure only	None	High		33	35				32
		Low		32	29		32		
				20***					
Mean yield (kg/ha/day)		High	40	37		35			34
		Low	29	36		32			

*Applied at standard rate, i.e., 4% of common carp and grass carp biomass + 2% of tilapia biomass.

**Applied at (1/3) standard rate.

***Not included in mean.

Feed Versus Manure

The highest yields were obtained with high protein feed pellets (at a high stocking rate). Yields attained from intensive manuring, reinforced by feed supplements were

higher than these from manure alone; the lowest yields came from sorghum without manure.

Nutrient Content of Supplemental Feed

When feed is used as a supplement to intensive manuring, similar yields are obtained with high protein feed and sorghum (Table 2, 1980). This is in contrast to the use of feed as the only organic input.

Effect of Stocking Rate

Higher yields were obtained at high stocking rates when feeds were the only organic input, either high protein or sorghum. Only at the high stocking rate did the high protein feed show a better yield than that obtained in manured ponds. Similarly the low yield obtained from sorghum is emphasized at a low stocking rate. The effect of varying stocking rates in manured ponds is more complex. With cattle manure, either alone or supplemented by sorghum, little extra yield was obtained by doubling stocking rates (Table 2, 1975). But with poultry manure, either reinforced by high protein pellets or alone (Table 2, 1979 and 1978), higher yields were obtained at the higher stocking rate. In both these tests the higher stocking rate was largely composed of tilapias. The mean yields over all treatments were similar at high and low stocking rates for both manures (Table 3).

Cattle Versus Poultry Manure

With manures as the only organic input there was little difference in yield between ponds with cattle or poultry manure (Table 2, 1975 versus 1977 and 1978). With a sorghum supplement however the use of cattle manure appears to result in a higher yield than that of poultry manure (1975 versus 1980 and 1981).

Discussion

The traditional method of freshwater fish farming in China and other parts of Asia consists of stocking ponds with different Chinese carps and common carp, and using manures as the principal nutrient inputs. The successful management techniques, practiced for so long by Chinese fishfarmers, are a result of trial and error and tradition handed down from generation to generation. The innovation of the investigations here described is in their use of controls and reasonable numbers of replicates. Furthermore they demonstrate that tilapias and freshwater prawns may be introduced into such systems.

The results presented show that fish yields of up to 35 kg/ha/day may be obtained in polyculture ponds with manure as the only organic input. This was also the yield of our first serious manuring test in which the manuring rate was determined arbitrarily. This apparently maximum yield appears to be stable and hardly influenced by management factors, such as types of fish stocked, total stocking rate (until a certain threshold is reached), proportions of different fish or type of manure used. The rate of manure application was not varied in our tests, but different rates applied in some of the experiments summarized in Table 1 did not produce a large yield response. Moreover their results were not influenced by the use of inorganic fertilizers. Thus, as for stocking rate, a certain threshold of manuring rate appears to be required for obtaining this maximum yield.

The stability of this yield may be due to some limiting factors in the ability of the fishpond to recycle animal manures, themselves a poor direct feed, into different components of 'natural food'. Conversion processes presumably occur at the soil-water interface (Schroeder 1978) or in the water column. These processes may be area limited. The full utilization of this area (or volume) requires adequate distribution of the manure. Most of the investigations described were carried out in small experimental ponds, in which wind and wave action may be sufficient for a reasonable distribution of the manure. This may not be the case in large production ponds. In Israel large-scale application of intensive manuring to production ponds requires satisfactory techniques of manure distribution, e.g., blowing the manure into the pond from one of the longer embankments, in the direction of the prevailing wind.

Little is known of the processes by which manure is converted into food for fish. In most cases all we know is the amount of manure applied and the yield of fish obtained. Possible modes of conversion were defined by Tang (1970) and Wohlfarth and Schroeder (1979) as direct consumption, autotrophic production utilizing the mineral contents, and heterotrophic production based on the organic contents. However, this has not been confirmed empirically or quantified. Manures are considered largely as indirect food, requiring planktonic or microbial activity in order to become available to the fish. Their low value as a direct feed for fish, demonstrated by substituting some of the contents of feed pellets by manures, is explained by their low content of metabolic energy and protein in comparison to conventional feedstuffs (Wohlfarth and Schroeder 1979). Supplemental feeds, with their higher metabolic energy and/or protein contents, are thought of as direct feeds, although clearly fish feces may affect the ecosystem in a way similar to other animal manures. Indirect feeds are expected to be far less effective than direct ones, since at every transition in trophic level in a recycling process, about 90% of the energy and nutrients become unavailable. Therefore, it is surprising that the conversion ratios of supplemental feeds and manures are similar and that manures may even show better conversion ratios than a grain feed (Table 2).

This may be explained by three, not mutually exclusive mechanisms: (1) manure is a better direct feed for fish than is usually assumed; (2) some of the supplemental feed does not act as a direct food; (3) the microbial recycling processes are so efficient that nutrient materials are only temporarily immobilized during the transition

in trophic levels and become available again rapidly. The decreases reported in growth and feed conversion of fish fed on feed pellets, some of whose contents were substituted by manures, may be due to most of these tests having been carried out in tanks or cages where the recycling processes occurring in earthen ponds may not operate.

Yields of intensively manured ponds may be increased by supplemental feeds but the yield increment is obtained at relatively high marginal FCRs. The quality of supplemental feeds, i.e., the presence or absence of a high protein component, appears to have little influence on yields in such systems. This is in contrast to the much higher yields from high protein pellets compared to grains, when these are the only organic inputs.

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Discussion

PRUDER: I am intrigued by your conversion ratios—for example, 3.5:1 and 3.0:1—for manure inputs which go through at least one breakdown or decomposition step. Could you comment on this?

WOHLFARTH: Well, I am equally intrigued by this. I do not understand it. However, these are the conversion ratios as a farmer would work them out. To get a true conversion ratio, you should deduct the natural food production of the pond. However, even if you do this, you can get a conversion ratio of about 5:1 which is still amazingly good.

SCHROEDER: I think if we look at the carbon flow, this tells us that the food chain for the pellet-fed fish is about as short as that for the manure-raised fish. The manure food chain can't go much beyond bacteria (or perhaps ciliates) to fish.

PRUDER: But that is still too many steps. It doesn't explain the comparable conversions for pellets and manure.

SCHROEDER: It does if you consider the conversion ratio is on a dry:wet basis and is about 20:1 for carbon in each case. This would allow a bacterial step.

WOHLFARTH: The situation looks strange when we consider the marginal conversion ratio for supplemental feed added to manured ponds, i.e., the amount of supplemental feed divided by the additional yield obtained. These marginal conversion ratios are quite high: about 6.0:1 for sorghum and 3.5:1 for pelleted feed. This indicates that it is hardly worthwhile giving such supplemental feed and that, if it is given the high protein feeds do better than low protein ones. The only rationale for doing this is to finish your fish to market size rapidly.

PULLIN: I am a little concerned about the comparability of data from different research groups. Does the fact that you use basal and periodic treatments with inorganic fertilizer in your Israeli experiments confuse the situation when you calculate conversion ratios and compare your results with those of other groups? Some other groups avoid inorganic fertilization. Why do you use inorganic fertilizers?

WOHLFARTH: Well, it is really rather silly. The rationale for our experimental work has been to compare the technique of growing fish on manure with the way in which fish are grown in Israel—which was (before we started our work) on supplemental feed. It is also common practice in Israel to fertilize ponds every two weeks with about 50 kg/ha each of superphosphate and ammonium sulfate. We therefore did the same. We wanted to get good results and the fertilizers seemed to us to be relatively cheap (this was probably not true). We decided to give our ponds the benefit of the doubt rather than starve them of N or P. This may have been a silly decision, because there is probably enough N and P in manure anyway.

ANDERSON: You have repeatedly said that your fish do not consume manure directly. Is there any real evidence for this? I have seen tilapias in Africa eating hippopotamus dung almost under the tail of submerged animals—a very short food chain!

WOHLFARTH: I may have expressed myself inaccurately. There are of course three possible pathways for manure utilization: direct consumption; autotrophic and heterotrophic. When you manure a pond, the fish show interest and will burrow into it. We do not know the relative importance of these three pathways. It will obviously depend on the quality of the manure. The fish will feed on food remnants in manure, if livestock feeding has been careless. On the other hand, a cow has very high digestion efficiency.

ANDERSON: Have you considered the effect of manure constituents like blood and mucus? It is possibly a much richer input than you suppose and therefore, your conversion ratios, taking into account direct consumption, autotrophic and heterotrophic pathways are not so amazing.

WOHLFARTH: So, you are suggesting that direct consumption of manure *is* probably significant?

ANDERSON: Yes, but it's pure speculation on my part.

WOHLFARTH: No purer than mine!

EDWARDS: Fish certainly do eat manure.

WOHLFARTH: How could we then investigate this critical question and quantify the proportion of manure consumed directly by the fish?

PULLIN: Let's consider that when we return to discussing methods.

EDWARDS: I have a few comments. First, your Israeli cattle are feedlot cattle which receive high quality feed and therefore produce high quality manure: higher in quality than cows or buffaloes feeding on rough pastures in a developing country. Second, fish culturists in Taiwan claim that tilapia grown in integrated livestock-fish farming systems have a muddy flavor and therefore a lower market value than fish raised on conventional feeds. Could you please comment? You said that your fish raised on livestock manure tasted better.

WOHLFARTH: This is a large question. Muddy flavor in fish has been quite widely studied. It can happen in manured and nonmanured ponds (remembering of course that the fish 'manure' is always present). The flavor is due to pond organisms which can grow in any pond. It has never affected our tests. Our best tasting fish of all were carp grown on manure alone. They were very lean (5 or 6% fat); much less than carp raised on high carbohydrate feeds. Their flesh had a texture like that of tilapia.

EDWARDS: We have started doing palatability tests at AIT. We found muddy flavor in some fish from ponds which had received conventional feed. These were grown in parallel with ponds receiving water hyacinth inputs. The water hyacinth-raised fish were of comparable quality.

PULLIN: This muddy flavor comes from certain bluegreen algae. It is common at certain seasons in fish from Philippine lakes.

SCHROEDER: It has also been reported from the USA.

GRAY: People who work on primary production in the water column now measure and recognize a difference between 'new production' and 'regenerated production'—that is new production from incoming nutrients and regenerated production from nutrients recirculated within the system. They do this by calculating N and P budgets. You could do the same here. You cannot get conversion efficiencies such as you describe on manure alone.

WOHLFARTH: Well, we do and this needs to be explained.

COLMAN: I think we can conclude that not all the conversion is by the bacterial route. To get your yields from bacteria alone would take about 10 g O_2/m^2/day just to produce the bacteria. On top of this, there would be the oxygen required for bacterial respiration and metabolism of the fish. This is far too much of an oxygen demand.

WOHLFARTH. Well, please note that these results are not unique. ICLARM and other groups have comparable data.

SRINIVASAN: We know that fecal material contains a lot of bacteria and therefore a lot of bacterial N; but how it rates as a food, I have no idea.

WOHLFARTH: We have only ever measured percentage dry matter and ash for our manures.

SCHROEDER: But we did measure true protein in our experiments one year. It was less than 1% dry weight.

PRUDER: I am sure that no one is questioning your conversion efficiency figures. However, we must look for an explanation. They cannot be due to a straightforward consumption of the manure. Some other resources created by recycling or other processes in the pond must be involved.

WOHLFARTH: I hoped to find an explanation from our discussions here.

COLMAN: Is there a lot of buildup of organic material in the pond during the growout period?

WOHLFARTH: No, not in our polyculture system. We did get some different results once with a tilapia monoculture at 30,000 fish/ha. When we seined the ponds, the mud and the net smelled like a cowshed. This never happens in our polyculture. Even with this monoculture, there was no significant visible buildup of organic material when we drained the ponds.

PULLIN: In our experiments with Central Luzon State University using an 85% tilapia: 14% common carp: 1% predator *(Channa striata)* polyculture at 10,000 or 20,000 fish/ha, we got no buildup of organic matter. All we could see was a small trail of fibrous material near the pipe which carried the animal house effluent wastes to each pond.

EDWARDS: At AIT, we do get a buildup of organic material. It is not a thick layer but it is significant. In fact between experiments, we always excavate the pond bottom mud to get back to residual basal fertility to avoid complications.

COLMAN: There is also a significant buildup of material in the water, so you do not necessarily see it all on the bottom.

WOHLFARTH: Are these standing water ponds?

EDWARDS: Yes, we are all using essentially the same system.

PRUDER: Dr. Edwards, as you observe this buildup of organic material, are your conversion efficiencies similar to those obtained by Dr. Wohlfarth?

EDWARDS: I have not treated our data in the same way. We get comparable yields. We have not always quantified manure inputs. Instead, we have just run say 27 ducks/200 m^2 pond. This gave an extrapolated yield of almost 9 t/ha/year.

PULLIN: Let us return to these issues in our general discussion.

General Discussion on Detritus and Microbial Ecology in Aquaculture

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Abstract

This discussion considered many of the points raised during the conference and was loosely structured around the following topics: detritus and the microbial ecology of fishponds; culture systems (hatchery/nursery and growout in ponds); suggestions for manipulation of pond food webs; and feeds and other limiting factors in fish production, including a comparison of feed production outside the pond (composting and ensilage) with in-pond decomposition.

Research approaches are also outlined, principally: 1) a major series of factorial experiments to investigate the basis of high fish yields from waste-fed ponds and to suggest how these might be increased by manipulation of the pond food-web; 2) an ecosystems modelling approach to waste-fed aquaculture; 3) socioeconomic investigations. A summary of research needs is given in a closing consensus statement.

Detritus and the Microbial Ecology of Fishponds

There was a discussion on the numbers of bacteria present in materials such as pond bottom detritus. Based on measurements of oxygen uptake of the pond bottom, a typical value for bacterial biomass would be about 1-2% of the organic matter present. However, there is clearly insufficient information available on bacterial production and the flux of nutrients through these materials and their associated microorganisms. There appears to be a paradox here. The pond yields suggest that the pond sediment-water interface, detritus and their associated microorganisms must be important in driving pond production systems and yet we cannot explain the mechanisms for this, based on current estimates of benthic production, such as oxygen uptake. It should be noted that there are serious technical constraints

to measuring true oxygen consumption on a pond bottom. Methods using domes tend to underestimate the true uptake.

In natural ecosystems such as seagrass beds, bacterial production has been estimated using labelled thymidine at up to 4 g C/m^2/day. In mangrove sediments and other systems, values of 500 mg to 1 g C/m^2/day are common. This is high *production*, but the bacterial *biomass* is still only about 2%, up to a maximum of 5%, of the material. Oxygen uptake has not been measured in such systems, but it is important to note that not all the bacteria present are aerobic. It was pointed out that oxygen uptake values for some fjordic sediments, measured at about 100 mmol O_2/m^2/day also approximate to a production of about 4 g C/m^2/day, assuming 50% efficient conversion of carbon.

It was agreed that there is a general lack of knowledge on the bacteriology of waste-fed fishponds. Basic descriptive studies of fishpond bacterial populations and their production are urgently required.

Culture Systems

Hatchery and Nursery Systems

It was recognized that hatchery and nursery operations are usually separated from growout and that certain of the protein-rich live foods essential for many fish species in early life, e.g., rotifers and other zooplankton, can be profitably grown by using organic wastes as inputs. This applies particularly to the carps.

The only suggestion for a new direction was that in marine systems benthic meiofauna, particularly benthic copepods feeding on detritus, could be used as live food organisms. These can be grown in primitive apparatus and low water quality conditions. Some initial work has been done on mass-rearing of nematodes and copepods. The participants felt that copepods—(for example, *Tisbe* which is often seen in large quantities in neglected marine aquaria and is easy to culture) hold more promise than nematodes for use as live food organisms in aquaculture.

For freshwater systems, chironomids are used in some Southeast Asian hatcheries and nurseries. They are often collected from muddy, fecally-polluted water bodies. More research is needed on chironomids as a food resource in aquaculture, their culture and harvesting. It was felt, however, that most freshwater systems will continue to rely on production of zooplankton. The option of raising benthic meiofauna as live food seems unattractive in freshwater.

Growout in Ponds

The microbial food web in growout ponds was felt to be the most important topic of the conference. It was recognized as highly complex, requiring consideration of water column and pond bottom processes and their interactions and the behavior and physiology of the target organisms. Given these complexities, the general point was made that the data available from published work on waste-fed pond production

is largely from scattered observations, using different combinations of target organisms, different inputs and, therefore, different food availabilities. Therefore, any given set of observations should not be taken as representative of a general case. The processes associated with autotrophic and heterotrophic production are broadly similar in different environments (saline and freshwater, temperate and tropical) but the interactions between the target organisms, the available foods and the environment are often site-specific.

It was agreed that the basis of fish production in ponds receiving organic wastes, i,e., the total energy source for fish protein synthesis, is not understood. It is far more complex than production from a simple system such as a herbivore cropping exclusively the phytoplankton produced in the water column through inorganic fertilization. In waste-fed systems, there appear to be three possible sources of food/energy: autotrophic organisms; heterotrophic organisms and nonliving detrital matter (either from the input material or that generated in the pond or both). The growth of target organisms therefore depends on one or more of these.

The microphagous tilapias are known to be able to digest bacterial and blue-green algal (cyanobacterial) cell walls. These are very important food items for tilapias. However, the possible utilization of green algae and green plant material has been little studied. There have been waste-fed pond experiments in which green algae predominated and excellent yields were still obtained, for example in experiments at the Asian Institute of Technology (AIT) in which tilapia were fed with algae produced in high rate sewage oxidation ponds (water residence time about two days). It is difficult to explain such results. Perhaps there is still a significant blue-green algal contribution to the fish's diet in such high exchange-rate systems.

The utilization of various microorganisms by carps is imperfectly known. To what extent and by what digestive mechanisms does the silver carp *(Hypophthalmichthys molitrix)* utilize algae? Ingestion is not the same as utilization. To what extent can the cultured carps utilize bacteria as food? More detailed research on the feeding mechanisms and preferences and digestive physiology of such target organisms is urgently needed, backed up by stable natural isotope analyses to trace the flow of nutrients from pond sources to fish flesh; for example, delta-carbon estimations. Delta-carbon studies have not elucidated complete food chains but the few studies made have already indicated that tilapia and silver carp flesh can be derived from a food chain which *starts* with algae.

Suggestions for Manipulation of Pond Food Webs

Because of the lack of key information, it was agreed that little purpose would be served by discussing the growout of specific target organisms. The discussion turned to what can be produced as foods in a pond and the options for food web manipulation. It was felt that target organisms in mono- or polyculture could then be chosen to match these options.

How then can ponds be treated so as to maximize phytoplankton or bacterial or say, meiofauna production? The view was that there is insufficient knowledge

available. The system as a whole requires much more study, including experiments to study its components in controlled environments before working with culture ponds. The effects of pond ageing and pond drying are complicating factors. However, it is clear that significant information already exists for some aspects, e.g., how to enhance phytoplankton blooms and how to encourage nutrient release from decaying matter as opposed to microbial protein production. Moreover, the growing body of data from fish farms and experimental studies could be used to attempt to model pond systems.

It was suggested that encouragement of microbial production at the pond bottom, which does not use oxygen, was a possible route to improving pond yields. This could be studied in the laboratory using a perfusion system to produce various conditions (anaerobic vs. slightly aerobic). Enhancement of microbial production in anoxic conditions on the pond bottom could release more nutrients into the water column to be taken up in further biomass production in aerobic conditions. Microbial biomass production under such anaerobic conditions is less efficient than in aerobic conditions. Energy conversion efficiency of sulfate-reducing bacteria, for example, is about 15-20%. Nevertheless production of microbial protein without oxygen seems attractive. The problem with trying to manipulate this in a pond is that the fate (partitioning) of added materials and, therefore, the extent to which water-sediment interface biota are involved in their processing are not known. Particle size has a large effect on processing rates and has not been investigated for organic inputs to ponds. Even the rates of sediment accumulation have not been well studied in ponds and a gross balance cannot be drawn between inputs, outputs and residues.

There remains also the question of how to utilize the nutrient-rich sediments which can accumulate in culture systems. This applies not only to ponds but also to the usually anoxic sediments which accumulate beneath floating cages. These have reached 0.5 m depth in Japan.

It is, of course, possible to get some measure of inputs reaching the water-sediment interface by measuring there the rates of various processes of mineralization: methane reduction, sulfate reduction, general fermentation and nitrification/denitrification. Therefore, microbial ecologists tend to focus on these processes and their products—CO_2, NH_3 and bacterial cells. These have yet to be studied to any great extent in fishponds.

The basic processes of mineralization and bacterial production are the same for freshwater, brackishwater and marine environments. However, saline waters have much higher sulfate contents and therefore sulfate reduction is more important in salt- than in freshwater. Marine and brackishwater pond operators tend to renew their pond water every few days or raise oxygen concentrations by other methods so as to cope with the H_2S produced from sulfate reduction. It was suggested that freshwater ponds offer more scope for increased utilization of pond bottom bacterial production than marine or brackishwater ponds because less H_2S would be generated. A further suggestion was to add Fe which complexes with sulfide. If the sulfide became complexed with Fe, then perhaps more sulfate could be added to

increase bacterial production. Sulfide is not usually found free in the water or in pore water but these reactions are pH-dependent. In marine ponds the frequent water exchange employed also counteracts the acidity increase produced by sulfate reduction because the incoming seawater is more alkaline. Freshwater systems are more highly buffered and so again water exchange is not needed for this purpose.

The concept of encouraging anaerobic microbial production in ponds was, therefore, felt worthy of further investigation. One problem will be how to avoid the process depleting the highly aerobic environment required by the target organisms. This sparked a lengthy debate on oxygen in growthout systems. There is widespread utilization of aeration devices in intensive fishponds and tanks and occasional use in semi-intensive ponds, especially at night and early morning when respiratory demand far exceeds the oxygen supply available from prior photosynthesis. Most of these periodic or emergency aeration devices raise oxygen concentrations in only a small portion of the pond, thereby creating an oxygen-rich haven for the fish. This is fundamentally different from the use of continuous aeration as a management tool in intensive culture systems. However, the cost of aeration devices is usually high. For ponds, phytoplankton are virtually cost-free biological aerators by comparison. Oxygen replenishment by water exchange is a routine option only for those systems in which the incoming water brings more food as well as oxygen (for example, flowing water carp culture in Indonesia) and where water improvement can be obtained at little or no cost (for example, tidally-flushed ponds, largely dependent on benthic food production). For most warmwater fertilized fishponds water exchange is to be avoided (except for emergency use) because the discarded water contains valuable foods: plankton and bacteria. However, where algal production is not being cropped by any of the target organisms, water exchange to reduce the algal standing crop can be useful.

A suggestion was made that stirring the pond bottom could be a useful manipulative technique. It may mobilize nutrients for use in the water column. It may also reduce oxygen concentrations to undesirable levels. Mechanical stirring would be similar to the effects of benthic foraging by the common carp *(Cyprinus carpio)* which is encouraged in many polyculture systems.

An interesting observation was that meiofauna will not grow in continually stirred detrital systems. Therefore the meiofaunal 'energy sink' could be eliminated by stirring detrital sediments in ponds, if so desired. In particular, the growth of nematodes is inhibited. These could compete with some target organisms for consumption of detrital bacteria.

Stirring the water column or agitating the water surface layers have already seen considerable use in aquaculture as a means of destratifying ponds and increasing oxygen concentrations. In some Chinese ponds of 2.5-3.0 m depth, paddle wheel aerators are run at full speed in the middle of the day when one would expect that this would be a waste of energy and cause losses of oxygen from the supersaturated water. The explanation given by the operators is that they are mixing the water and thereby storing oxygen in the deep water column for the coming night. Hence these devices are agitators not aerators.

Stirring the bottom sediment by day, when the overlying water is fairly well-oxygenated would increase oxygenation of the sediments and aerobic microbial production (which is more efficient than anaerobic production) but at night or in the early morning stirring could exacerbate oxygen shortages in ponds. Stirring would undoubtedly resuspend formerly settled organic matter, and increase its rate of decay, as is done by the common carp. However excessive turbidity would reduce photosynthesis.

The practical difficulties and costs of stirring were discussed. Sophisticated mechanical devices would obviously be very costly. However a 'hydrocultivator' which turns over the bottom mud of mullet ponds in Italy has given some promising preliminary results. The advantages of hydrocultivated as against undisturbed ponds were clearance of undesirable floating filamentous macroalgae and increased feeding activity of the fish. No problems of oxygen deficiency were found. The disadvantages were disturbance of the bottom fauna and lower populations of algae in the water column. The overall utility of this device has yet to be determined.

It was agreed that even periodic disruption of the sediment-water interface by towing a net or weighted line through the pond was a type of stirring which was practical. The idea was felt to merit investigation.

Feeds as Limiting Factors in Aquaculture Production

It was agreed that it is necessary to understand feed resource partitioning for the target organisms, particularly for polyculture systems and for the tilapias and other species which can exploit a range of feeding niches (benthic detritus, phytoplankton and vascular plants). There was some divergence of opinion on the importance of the species composition of algal and microbial communities in ponds. A minority view was that different ponds with different species assemblages around the world give essentially the same fish yields and that, for example, the relative abundance of green and blue-green algae as food items was not important, the systems being so highly buffered and insensitive to such differences. However, the clear majority view was that the species composition of particularly the algal community is very important—for example, Nile tilapia *(Oreochromis niloticus)* thrives on blue-green algae such as *Microcystis* but not on green algae (see above). Perhaps the explanation for this divergence of opinion is that in the examples published of similar yields from different systems, the production of the target organisms was limited by environmental conditions other than food availability and that all published yields so far are far below those that could be obtained by providing suitable food organisms in all the exploitable feeding niches in a well-balanced pond.

The algal succession in ponds is, therefore, very important since different algae have different food values for different target organisms. The fertilization regimen (i.e., frequency and chemical composition) may select for different algae. One hypothesis is that pulsed inputs of readily available nutrients select for 'r' type

species* whereas inputs of low quality select for 'K' type species* by a slow nutrient release. These processes are buffered by a 'sink' of benthic nutrients. This has not been studied in fish culture systems. It would seem to merit investigation particularly as the appropriate fertilization regimen, perhaps backed up with an inoculum of the desired algal species could be used to control the composition of the algal community, for example, by encouragement of blue-greens rather than greens in tilapia ponds.

Dissolved organic carbon is required for establishment of growth of 'K' algal species. However, 'r' species generate through their high respiration rate an environment which has a positive feedback on microbial and algal activity. Perhaps 'K' species require an available carbon pool (organic or inorganic) and they are best sustained by organic carbon inputs and nutrients recycled from the benthos. There is a whole suite of variables here which needs to be transformed into a set of testable hypotheses before management guidelines can be worked out for ponds. It was agreed that at present it is not possible to predict accurately the algal succession in fishponds. Examples were given of ponds fertilized with human waste (rich in N) which produced initial blooms of green algae before *Microcystis* appeared and also ponds which received only inorganic fertilizers and produced heavy *Microcystis* blooms from the start. As *Microcystis* can be regarded as a 'K' species, these instances contradict some of the ideas suggested above. An opposing view is that all these algal species are 'r' species. Obviously much more work is required on this important topic. The information at present is anecdotal.

Macrophyte feed inputs to ponds were also discussed both from the point of view of direct consumption by the fish and as inputs to food chains as dissolved organic matter and other substrates. Regarding direct consumption, it was agreed that feed digestibility and feed presentation were the paramount considerations. Some feeds must be chopped to an appropriate size for consumption. For fish which cannot forage in oxygen-poor benthic muds, feeds must remain floating or suspended in the water column. The nutrient value of macrophyte feed inputs can be improved by 'microbial preconditioning'; in other words by the onset of decomposition. If this is aerobic, it reduces dissolved organic carbon whereas if it is anaerobic it increases DOC in the form of short-chain fatty acids. So there are options here with regard to the form of carbon produced. Therefore, decomposition of macrophyte feed inputs at the surface or on the bottom are rather different processes with some scope for manipulation.

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*'r' species have a high intrinsic rate of increase, and are typified by 'weedy' opportunistic species that show boom and bust cycles; for example, green algae in sewage oxidation ponds. 'K' species invest in a long-term presence and have a low reproductive output; for example, some blue-green algae.

With regard to feed supply it was recognized that most aquaculture systems use batch culture rather than continuous culture. In waste-fed ponds this means that it is rather difficult to match the fish biomass to the available food. At the Asian Institute of Technology (AIT), Bangkok, a constant rate of organic loading is used throughout each batch culture. This is essentially a compromise. It has been found that plankton biomass tends to increase during the culture period. It appears therefore that the waste loading could be progressively decreased. It also appears that at the start of a culture period when the fish biomass is low, a lower waste loading could also be given. This, however, gives very poor early growth. It seems essential to build up a high concentration of plankton and bacteria at the start and then to maintain or increase these.

At the Dor Fish and Aquaculture Station, Israel, a different approach is used, starting with a minimal manure loading and increasing the rate at biweekly intervals in the same manner as supplemental feeding with pellets is adjusted to an increasing fish biomass in intensive culture systems. The rates start at 50 kg/ha and are increased up to 200 kg/ha. However, basal and periodic additions of inorganic fertilizer are also used.

The point was then made again that maximum yields from warmwater fertilized and fertilized/supplementally fed ponds at different locations are broadly similar (about 30 kg/ha/day). Perhaps this similarity in results can be partially explained if supplemental feeds, like cereals, are to some extent fertilizers (rather than simply direct feeds) and conversely manures are to some extent direct feeds for some target organisms. A further explanation could be that far from obtaining good matches between fish biomass and available feeds, the various experimenters have all arrived at similarly poor mismatches using different inputs. Alternatively there could be some more general limiting factors in the environment. It seems that the fish in these different ponds may be short of food and/or short of oxygen and/or facing some other common barrier(s) to higher yields.

This raises the question of whether fish in the manured pond systems in current use are likely to face food shortages (in quantity and/or quality) during a typical production cycle. There is evidence from AIT that very high production of *O. niloticus* (about 12 g/m^2/day) can be sustained for about four weeks in fertilized concrete tanks with dense blooms of *Microcystis*. Thereafter the production declines suggesting a food shortage.

In all such systems, it should not be forgotten that feeding preferences may change as the fish grow. This is unlikely to be a major complication for carps and tilapias, in which nursery and growout ponds are separate, but it could be important in systems where fish are grown from small fry to adults in the same pond. Broadly speaking, small fry and fingerlings prefer a diverse diet, rich in zooplankton.

It was suggested that the physical dimensions of ponds could influence production; for example, the area of the pond walls and the total surface area of the water-sediment interface could be important factors. There have been some preliminary trials in shallow ponds in the Philippines with flexible plastic mesh walls staked

across the pond bottom to increase the surface area for algal and bacterial growth. No results are available yet.

There is also a general lack of knowledge on the effects of pond area, pond shape, pond depth, stocking density of fish and fish behavioral interactions on fish growth. There is some evidence that fish grow to larger sizes in larger water bodies; which is the opposite one would expect if a high pond edge to area ratio was thought to be beneficial. Spatial and behavioral influences on growth require much more research.

A comparison was made between detritivorous tilapias in natural waters, which are hardly ever confronted with a food shortage (there being abundant high quality detrital aggregate available) and tilapias in culture pond at much higher population densities—a situation in which the adequacy of the detrital food supply is unknown.

Other Limiting Factors

Among the possible physicochemical factors limiting waste-fed pond production, available oxygen and the presence of metabolites were mentioned. The discussion on oxygen is given above. It was felt that ammonia would generally be scrubbed from manured pond systems by the phytoplankton. Nitrite would also be oxidized rapidly by organisms like *Nitrobacter*. However these processes are highly pH-dependent. Nitrite buildup can occur in ponds.

Production of Feeds

General Considerations

It was recognized that there is a major difference between production of feeds and nutrient resources outside a fishpond (e.g., by composting, waste processing, growing excess vegetation, etc.) and production in the pond by aquatic decomposition, etc.

The discussion focused on the relative merits of enriching cellulosic wastes like sugarcane bagasse by composting with an N source outside the pond or adding them directly to the pond, also with an N source. The production of fungal protein on such wastes outside the pond was felt to have advantages because it produced protein-enriched material at no oxygen cost to the pond. Moreover, it was suggested that the production of CO_2 by in-pond decomposition processes—often cited as important in providing CO_2 for photosynthesis—was not very advantageous because the CO_2 requirements of photosynthesizing algae are small and usually easily supplied by the pond ecosystem.

The production of fungal biomass in controlled fermentation systems is expensive, but some of the costs can be lessened by having centralized production units from which the products can be disseminated for farmers (analogous to distribution of fry from large fish hatcheries). However, even this may not be viable in developing-country situations where the numerous small-scale farmers have very limited means.

For production of feeds by decomposition in the pond, the complex nature of algal-detrital-bacterial interactions poses great difficulties for separating the relative importance of different food sources. There is a series of gradients between algal production/algal death/detritus formation/bacterial production/bacterial death/nutrient release/algal production—a dynamic series of cyclical processes, the products of which can be fish foods either singly or in many different combinations. Some target organisms are selective feeders; other are less selective.

Composting

Composting always involves a loss of carbon and N and does not produce an end-product as rich in N as controlled fermentation to produce fungal biomass. It can, however, be a very simple and practicable option for small-scale farmers. The N loss in composting can be minimized, as in Chinese practices.

The best stage in the composting process at which to feed a compost to fish was also considered. Bacterial populations tend to predominate earlier and fungal populations later. It was agreed that N losses can be minimized. For example in 4-6 week aerobic composting experiments at AIT, about 80% of the original N (measured as total Kjeldahl N) was conserved. Experiments at AIT on water hyacinth composting have shown that fish yields were about the same whether the water hyacinth was composted outside or decomposed in the pond. However, when these yields were compared on the basis of total dry matter input, the decomposition in-pond appeared more advantageous because about 40% dry matter is lost from a hyacinth compost heap during the composting process.

The discussion then turned to whether the carbon content of compost was a significant factor, given that it is N (protein) which the fish require to eat. The consensus was that N is more important but that an energy source is also important in all fish diets. The question of whether fish avoid filamentous foods, such as fungal hyphae, was raised but there was no information available.

The main advantages of composting materials were said to be: avoidance of in-pond oxygen demand; removal or reduction of health hazards from pathogens; low bulk of the product and hence ease of storage and transportation. The last two advantages are very significant for developing countries seeking to compost aquatic macrophytes such as water hyacinth. Water hyacinth is about 93% water and transporting large quantities of the fresh product is difficult and costly.

A variation on the theme of composting used in Russia was mentioned. Agricultural byproducts and offal are used as media to mass culture a wingless type of house-fly. The pupae are allowed to fall into fish ponds or animal feed mixing apparatus.**

In striving for a consensus on the pros and cons of composting as a method for producing inputs for aquaculture, it was agreed that local conditions are the most

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**Communicated by Dr. J. Anderson (unpublished).

important factor. Many countries are now installing large (500-700 t/day) urban waste composting plants, therefore the supply of composts, albeit of variable quantity, is likely to increase. These composts must be disposed of. Perhaps application in fish culture offers an attractive alternative to land application. It was agreed that composts can be a useful resource, given appropriate socioeconomic conditions, but that their limitations from a nutritional point of view were clear: principally N and C losses during the process and a limit on the amount of enrichment that can occur. For example, bacteria will not go on increasing in biomass; rather there will be death/turnover resulting in an upper limit of about 10^8 to 10^9 bacteria/g. The biomass of fungi similarly levels off. The upper limit to the microbial biomass of most composts and detritus is about 5%.

There was considerable diversity of opinion on the forms in which N was held in composts—whether as ammonia, as bound nitrogenous compounds or as microbial protein. A minority view was that a large proportion is present in the form of ammonia. However, the basic principle of aerobic composting for land application is that composts allow a slow release of N to the land. This N comes from a mixture of microbial protein and a variety of recalcitrant nitrogenous compounds such as lignoproteins and humic acid compounds. The proportions of such recalcitrant compounds increase with time during the composting process.

Indeed in land application, whereas the P content of applied compost is used up completely and rapidly during a single annual cropping cycle, only about 25% of the N is used up over the same period. This was the majority view. It was pointed out, however, that such a 'slow release' mixture, while ideal for a land-based fertilizer, was not a suitable composition for a direct feed. The use of composting to provide food for fish should, therefore, probably be confined to a short-term process to increase C and N availability, stopping before losses are too great and the N and energy sources remaining become increasingly unavailable. Growing mushrooms on compost cropped at an early phase after the first temperature peak is somewhat analogous. The critical experiments on where to stop composting for aquaculture purposes have yet to be performed.

The final consensus on composting was that aquaculturists should compost only, or use composts only when there is a specific reason for not using the original material available.

Ensilage

The possible use of ensilage of wastes, as opposed to composting, was then discussed. Ensilage is an anaerobic, acidic process that does not allow terminal fermentation to go to completion. The products are a mixture of volatile fatty acids—lactic, butyric, propionic, etc., at high concentrations (about 50 mmol), rather as in a rumen. These products would have an extremely high BOD if put into a pond. The process also makes cellulosic materials much more susceptible to microbial processing. There was no consensus on the potential of silage as a fishpond input because of

a lack of information. No relevant experiments have been performed to date. However, it was agreed that the high BOD of silage should not discourage experimentation. Other pond inputs, including feed pellets have high BODs. In fact, BOD is a measure of nutritive value since it derives from the growth of pond biota.

Research Approaches

Investigating Food Webs

High fish yields and good input conversion efficiencies from warmwater waste-fed ponds cannot be explained by assuming they are based on consumption of primary production. Much material must pass through bacterial food webs. Knowledge of this is very poor and therefore manipulation by aquaculturists is difficult or impossible. Therefore, critical experiments must be designed to increase knowledge of these food webs.

There was a detailed discussion on experimental approaches to this problem. Several approaches were identified. First, a series of factorial experiments was proposed, the main objective of which was to distinguish between the relative importance of new and regenerated primary production and bacterial production in the pond as fish foods.

The basic experimental design proposed was a 3 x 2 factorial experiment to compare three manure loadings (zero, L_1 and L_2; where L_2 is probably 2 x L_1) in two types of pond (clean, C, i.e., concrete lined, and earthen, E, having earthen sides and bottom). The six treatments are therefore: zero, C; zero, E; L_1, C; L_1, E; L_2, C; L_2, E.

All ponds would be stocked with the same density of fish. This outline gives only the basic experimental design. Pond sizes, choice of species, polyculture combinations, fish stocking rates, type of manure (or other organic inputs such as composts or vegetation) input loadings, etc. would all have to be determined according to specific objectives and with reference to published information.

A large number of parameters would be measured during the experiment, (with single, periodic, e.g., daily, diurnal, weekly or continuous measurements as appropriate) including C, N, protein, P of the added material; sedimentation rates; water chemistry (NH_3, NO_2, NO_3, P, pH, oxygen profiles); primary production; temperature and pond bottom respiration. Estimates would also be made of bacterial biomass and production, protozoan, meiofaunal and zooplankton biomass. From these data C, N and P budgets would be constructed to work out the relative importance of new and regenerated primary production and bacterial production as food sources for the fish. The importance of bacteria and the detrital food web would then be clarified.

Such a series of experiments would require international collaboration and major funding. Because of pond to pond variability within treatments (itself an interesting phenomenon for which more key information would emerge from the proposed

work) treatments should be at least triplicated. Five to eight replicates would be better. Thus, a large number of ponds or a large number of units subdivided within the same pond is required. There was no consensus on the number of replicates needed. It was proposed that a preliminary experiment using a standard fish production system be performed first to estimate variability and then define an appropriate number of replicates. Attention was drawn to the paper by Pauly and Hopkins*** and associated correspondence**** which provides an alternative to using many replicates in pond research when a large range of treatment variables is used.

Second, it was agreed that where as such a series of experiments would go a long way towards explaining the basis of fish production, it would not delineate completely the food sources of the fish, i.e., the relative importance of plankton, bacteria, direct manure consumption, etc. It was agreed that there should be parallel studies on the feeding behavior of the fish, supported by stable natural isotope analyses of manure, plankton, detrital sediments, other pond biota, gut contents and fish flesh. If possible and affordable these stable isotope analyses should encompass more than one element; for example C and N. The possibility of compartmentalizing ponds or tanks to isolate fish from certain feeding niches—e.g., grids, cages—could also be useful in determining feeding preferences and feed utilization.

The number of institutions having facilities in suitable climatic conditions for such work is very limited. AIT, the Dor Fish and Aquaculture Station, Israel, and the other institutions cooperating with ICLARM in the tropics, principally Central Luzon State University, were all mentioned. There was a further discussion on increasing the number of experimental units by subdividing ponds with netting. This can reduce variability within treatments but it can be problematical: fish escapes and transfers between compartments can occur and the netting provides a significant additional surface for growth of microorganisms, thereby complicating the results. Whole ponds are preferable.

There was a brief discussion on whether a preliminary series of experiments using only inorganic fertilizers as inputs would be valuable. The majority view was that it would have little predictive value for most real aquaculture situations. An external organic carbon source appears essential for high fish yields. The difficulties of separating the roles of organic inputs as pond fertilizers and potential direct feeds were again stressed.

There was a long and inconclusive discussion on the many variables involved in the proposed series of experiments, particularly the less controllable factors such as pond biota (especially different types of algae) and the difficulties of monitoring direct consumption of organic inputs by fish. It was also agreed that there were important differences between the various types of organic inputs available. Livestock manures differ in N and P content and C:N ratio, according to the animals

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***Pauly, D. and K.D. Hopkins. 1984. A method for the analysis of pond growth experiments ICLARM Newsl. 6(1): 10-12.

****Lovshin, L.L. 1984. Letter to the editor. ICLARM Newsl. 7(2): 30.

used and their feeding regimen. While a lot is known about organic waste composition, it is often not certain which are the key components in terms of fish production—N or P or perhaps C. External C can also be added to ponds as green vegetation. The main complicating factor is the lack of knowledge on the extent to which fish consume organic inputs directly. Tilapias definitely do eat livestock manure and human excreta, but how much of the total input do they consume and how well do they utilize it? What is the composition of their own feces in such pond systems? These are all unanswered questions.

There was, however, a clear consensus that such a series of experiments *should* be performed and that it would generate important new information on the relative roles of different feed sources in waste-fed ponds for fish production, thereby suggesting which food chains should be manipulated and further investigated.

There was a further proposal that ecosystem modelling techniques be applied to existing and future data sets to attempt to identify key factors controlling fish growth and yields. This would assist in the design of experiments such as those proposed above. Modelling techniques are well-known in multicomponent ecosystem studies. It was agreed that this would be a highly productive approach even given the limitations of some data sets.

Social Issues

The prioritization of research on different waste-fed aquaculture systems is to a large extent dependent upon local socioeconomic conditions. For example, research to improve the productivity of sewage-fed fishponds would be of very low priority in the USA (where it is unlikely to become a food producing system) but of very high priority in the People's Republic of China, where it is already a major source of animal protein in human nutrition.

Consensus Statement

The conferees agreed to define detritus as nonliving particulate organic matter. It was further agreed that detritus and its associated biota play a key role in aquatic productivity. For aquaculture, further research into detrital food chains is necessary before these can be manipulated to maximize fish yields. The interrelationships between autotrophic and heterotrophic pathways, environmental constraints to production and the behavior and physiology of cultured organisms are poorly understood.

The complexity of these interrelationships is so great that experimental studies to date have given insufficient understanding of the processes occurring. A major research program is required to analyze these interrelationships. Such a program would need to be multidisciplinary and would require international support since no single agency or institution has all the required capabilities.

The conferees further agreed that research on the following topics should be given high priority for support so that the full potential of detrital food chains in aquaculture can be realized:

Detrital Microbial Ecology

1. The role of bacteria in converting detritus to useable energy and protein for cultured organisms;
2. The role of bacteria in the mineralization of detritus and release of nutrients for algal growth;
3. The role of Protozoa, meiofauna and macrofauna in mineralizing organic detritus together with bacteria;
4. The efficiencies with which different types of detritus are converted into bacterial biomass in the water column and sediment;
5. Tracing food chains from detritus to cultured organisms using stable isotope analysis and detailed biochemical analyses;
6. The nature of detritus and associated biota;
7. The food value of detritus *per se;*
8. Interactions between primary production and fish production;
9. Algal inputs to and effects on the detritus pool and associated processes.

Utilization of Wastes and Green Fodder

1. Assessment of the nutritive value of composts, silages, livestock manures, human wastes and green fodder as pond inputs, either fresh or after various degrees of processing;
2. Production of feeds for cultured organisms from agricultural byproducts, wastes and green vegetation;
3. Toxicity problems—heavy metals and other anthropogenic compounds;
4. Health problems—possible transmission of animal and human pathogens.

Culture Systems

1. Mass culture of meiofauna and other live food organisms on detrital substrates for hatchery and nursery use;
2. Effects of stratification/destratification in waste-fed ponds and evaluation of manipulative techniques (such as stirring) to optimize yields and food conversion of cultured organisms;
3. Integration of aquaculture with agriculture;
4. Size of aquaculture production systems;
5. Choice of cultured organisms;
6. Analysis of existing data and systems modelling;
7. The role of algae in culture systems.

Fish Biology

1. Feeding behavior and feed utilization of fish in detritus-fed systems;
2. Environmental constraints to high fish yields in detritus-fed systems (such as dissolved oxygen and metabolites).

Economics and Social Issues

1. Economics of detritus-based aquaculture;
2. Comparative assessments of waste-recycling through aquaculture and other options;
3. Public health aspects;
4. Public attitudes to waste-recycling through aquaculture, including consumer acceptance of products.

General Index

Water bodies, research institutes and universities are included in the Geographic Index.

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