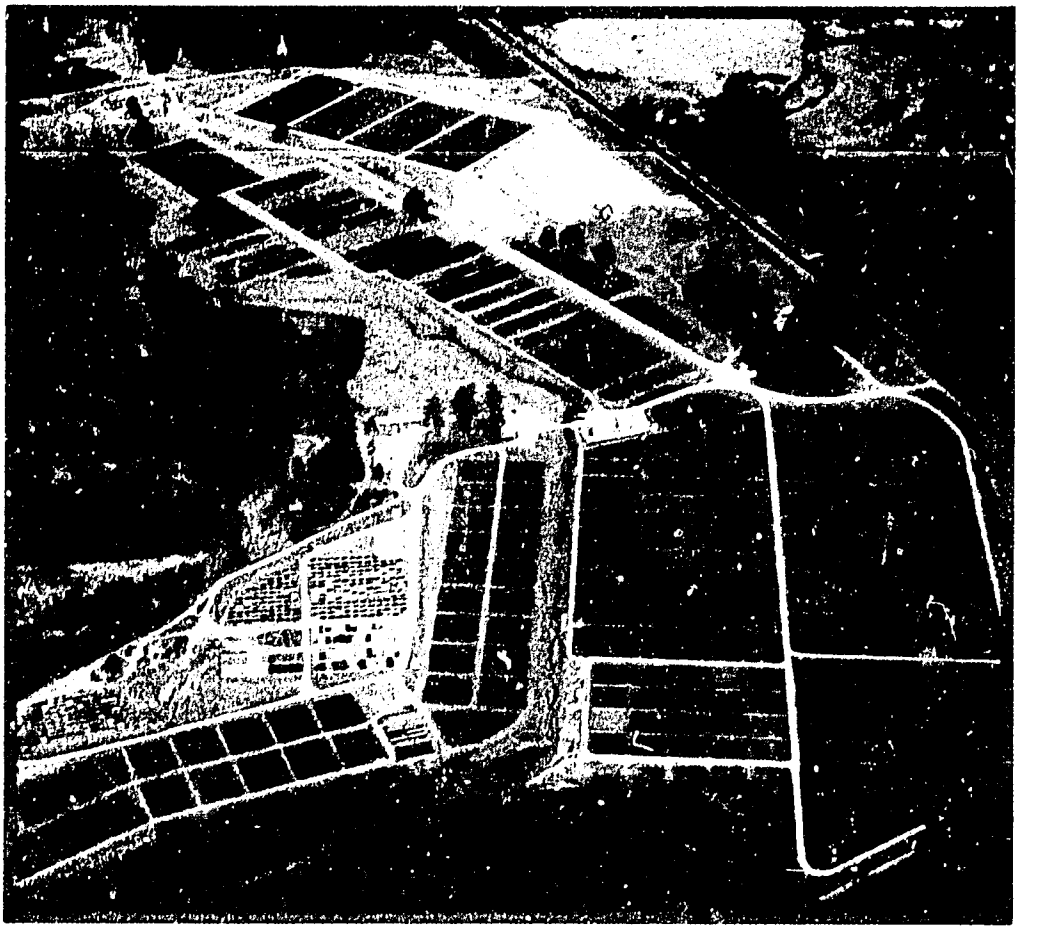
PN-AAQ-547



Fish Farming Research

E.W. Shell, Head

Department of Fisheries & Allied Aquacultures

Alabama Agricultural Experiment Station Auburn University
Gale A. Buchanan, Director Auburn University, Alabama

Fish Farming Research

E.W. Shell, Head
Department of Fisheries & Allied Aquacultures

Alabama Agricultural Experiment Station Auburn University
Gale A. Buchanan, Director Auburn University, Alabama

ACKNOWLEDGMENTS

This book had its beginning when the late Dr. H. S. Swingle introduced me to experimentation in pond aquaculture in the early fifties. With his genius for the use of inductive and deductive logic and the scientific method in solving problems in applied aquatic ecology, he was the father of fish farming research. I also want to acknowledge the contributions of the Auburn staff and graduate students who have labored long and productively in and around Swingle's "outdoor test tubes." They provided most of the material for the book.

A portion of the cost of preparing this publication was defrayed by Contract AID/DSAN-G-0039

CONTENTS

Page

ACKNOWLEDGMENTS ii
LIST OF FIGURES vi
LIST OF TABLES ix
INTRODUCTION 1
THE ROLE OF RESEARCH 1
ADMINISTRATION OF RESEARCH 4
The Scientist as an Administrator 5
Responsibilities of the Aquacultural Scientist 7
Research and Extension 9
Obtaining and Allocating Resources 10
Staff Motivation 11
Staff Required for Research 13
Facilities and Equipment Required for Research 14

PLANNING EFFECTIVE RESEARCH 31
On the Nature of Experimentation and the Scientific Method 31
The Nature of Fish Production Experiments 34
What Research to Do 35
Identifying Specific Research Needs 38
Factors to Consider in Designing Experiments 46
What is the Question? 46
Accuracy Required 47
Significant Differences and Measuring Systems 47
Experimental Variation 51
Number of Replications 53
Controls 54
Using Prepared Feed, Inorganic Fertilizer, or Manure 59
What Species? 59
Monoculture or Polyculture? 62
Size of Test Environment 67
Duration of the Experiment 68
Carry-over Effect 70
Maximum Growth or Maximum Yield? 71
Weight, Size, and Number to Stock 72
Genetic Variation 75
Differential Growth of the Sexes 77
Growth and Nutritional History 78
The Research Plan 79

PROBLEMS ENCOUNTERED IN FISH PRODUCTION EXPERIMENTS 81
Mortality of Stocked Fish 81
Reproduction of Stocked Fish 83
Presence of Wild Fish 85
Feed Poorly Utilized 85
Adjusting Feeding Rates 86
Changes in the Test Environment with Time 87
Infestations with Aquatic Weeds 88

EVALUATING EXPERIMENTS 88
Methods of Expressing Data 88
Standing Crop 89
Net Production 90
Relative Gain 91
Gain Per Day 93
Growth Curves 94
Transformation of Data 96
The Role of Statistical Analysis 101
REPORTING THE RESULTS 103
REFERENCES 105

Information contained herein is available to all without regard to race, color, sex, or national origin.

VI

LIST OF FIGURES

Page

Figure 1. Map of Alabama showing the major soil associations and the location of agricultural experiment stations 16

Figure 2. The large (8.5-hectare) pond in the aerial view is the water supply for a series of smaller ponds used in research at Auburn. The large pond is supplied with water from rainfall on a forested watershed. Seepage and overflow from smaller storage ponds in the watershed help to maintain the water level in the supply pond. Water is supplied by gravity to the smaller ponds in the valley below. 22

Figure 3. The aquacultural research facility at Central Luzon State University in the Philippines 23

Figure 4. A series of research ponds at Auburn. These ponds are similar to those that fish farmers use in the surrounding area. The largest pond is approximately 10.3 hectares. Water for these ponds is provided by harvesting rainfall. 23

Figure 5. A series of experimental ponds at Pentecoste, Ceara State, Brazil 24

Figure 6. Circular, fiberglass tanks used in fish nutrition research at Auburn. Water is supplied by gravity flow from a water storage lake (Figure 2) 24

Figure 7. Rectangular troughs used in research 25

Figure 8. Concrete holding tanks 25

Figure 9. A series of aquaria (40 liters) used in fish nutrition research. 26

Figure 10. Ponds constructed with concrete retaining walls to prevent erosion and to maintain exact area 27

Figure 11. Pond with retaining walls of treated wooden boards 27

Figure 12. Water supply on an experimental pond fitted with a saran screen sock to prevent the entry of wild fish and fish eggs. Note the concrete waterway to prevent erosion as the pond is filled. 28

Figure 13. Water supply for a concrete tank that provides maximum aeration and circulation. Note the large water supply that allows the tank to be filled rapidly 29

Figure 14. Outside drain structure on concrete tanks. With this arrangement, the water can be maintained at a full or half-full level 29

Figure 15. Schematic presentation of a systematic procedure for making decisions on research needs 40

Figure 16. Net production (final weight less the stocking weight) of bluegill and largemouth bass in ponds receiving different levels of fertilization and no fertilization 66

Figure 17. Average weights of channel catfish fed a vitamin-complete diet and a vitamin B-12-deficient diet. Data from Dupree (1977). 69

Figure 18. Absolute growth rate (0) and net production (●) of the original stock of Java tilapia recovered per hectare from ponds receiving no fertilization, (0-0-0), 0-8-2 fertilization, or 8-8-2 fertilization and stocked with 4,940, 9,880, 14,820, or 19,760 fish per hectare. Data from Dendy *et al.* (1968) 71

Figure 19. Growth of male and female bluegill stocked in an earthen pond. Data from Schmittou (1968) 77

Figure 20. Production of young Java tilapia in ponds that received different fertilizer treatments and different rates of stocking. Data from Dendy *et al.* (1968) 84

Figure 21. Relationship between the initial weight and net production of channel catfish being fed two diets. Data from Nail (1962) 92

Figure 22. Growth of channel catfish being fed two diets. Each point represents an average of five replications. Data from Nail (1962) 95

Figure 23. Mean standing crops and variances of three experiments in earthen ponds. Data from Swingle *et al.* (1963) 97

Figure 24. Means and variances from a fish production experiment in plastic pools. Data from Greene (1969) 98

Figure 25. Means and variances from an experiment in steel troughs on channel catfish nutrition. Data from Nail (1962) 99

LIST OF TABLES

Page

Table 1. Range of coefficients of variation obtained in a number of production experiments with several species of fish and in several types of experimental units. 51

Table 2. Frequency of occurrence of coefficients of variation from 13 fish production experiments recorded in table 1 52

Table 3. Percentage survival of similar numbers and weights of fathead minnows in containers used in 10 tests over a 6-month period (Data from Pongsuwana 1960) 55

Table 4. Partial results from an experiment to determine the effect of levels of dietary protein and carbohydrates on the growth of channel catfish in troughs (Data from Nail 1962) 90

INTRODUCTION

AQUACULTURE AS AN ART has been known for centuries. Aquaculture as a science is less than 100 years old. Research on pond aquaculture did not begin until approximately 50 years ago and the total effort involved has been limited. Consequently, the information base for fish farming is relatively meager.

Aquacultural production is increasing rapidly worldwide and in many situations is expanding rapidly beyond its information base. While there are numerous situations where an extension of current information can result in significant increases in production, there are others where new information is needed. Research must be increased at a rapid rate if we are to meet the needs of fish farmers and other scientists for information.

While the design, execution, and evaluation of research in agriculture are relatively well understood, experimentation in aquaculture is not. The purpose of this book is to assimilate and present some of the available information on the application of the scientific method to fish farming research. Emphasis is given to conducting research on the production of fish in earthen ponds and to identifying and solving farmers' problems. Finally, I discuss some of the social science aspects of aquacultural research, such as administration, staff motivation, and accountability. While not an intrinsic part of the scientific method, these people-related factors often determine the effectiveness of its application.

THE ROLE OF RESEARCH

Food production is the primary base of the wealth of a nation; it also is the basis of survival. As Dr. Boysie E. Day (personal communication) stated it, "Food production is not just one of the essential industries; it is the only essential industry." Wealth is derived from the exploitation of renewable and non-renewable natural resources by a nation's people and the conversion of those exploited resources into products of commerce. Oil, timber, fish, coal, minerals, water, climate, and soil are exploited by oilmen, loggers, fishermen, miners, and farmers, and these resources are converted to finished products. The only essential part of this process is the exploitation of climate, soil, and water for food production. Food is the "fuel" that drives the system.

Many nations of the world are constantly on the edge of chaos because of the imbalance between food needs and food production (Brady 1981). For these, increasing food production is not a matter of increasing wealth; it is a matter of national survival. Even those nations able to meet their food needs cannot be complacent. Because of the interdependent nature of our world, the spectre of hunger bears an ominous grin for all.

In a major study, the President's Science Advisory Committee (1967) described and discussed the complex phenomenon of world food production and food shortages. The report acknowledged the contribution of research to modern agriculture. Wortman (1980) has written an excellent article on the role of science and technology in food production. He suggests that production of sufficient food can be achieved by the application of a broad spectrum of technologies based on advances in the biological, social, and physical sciences. Evans (1980) seems to be less enthusiastic about the role of research (new technology). He presents evidence that indicates that in some food crops, yields are approaching limits set by biological constraints.

Research plays a significant role in the food production scheme and there are many indications that it should be expanded significantly; yet, it is important that its role be kept in perspective lest the expectations of the public who must pay the cost of scientists, laboratories, and experimental fields be raised beyond the researcher's ability to deliver. There are many positive attributes and constraints to food production that are beyond the realm of research. In the United States, research has played an important role in the development of our agriculture; yet, there is little doubt that given its climate, geological history, favorable ratio of arable land to population, and demographic history, this country would be the major food producing nation in the world, even though at a much lower absolute level of productivity, if there had been little or no research. Similarly, there are areas of the world where food production probably cannot maintain pace with population increase regardless of the amount of research done.

The production of food requires that certain inputs be available to the farmer. A list of these includes:

1. A benevolent physical environment
2. A suitable economic environment
3. An equitable regulatory environment

4. Incentive (usually profit)
5. Land
6. Water
7. Capital
8. Labor
9. Seed
10. Feed and fertilizer
11. Equipment and chemicals
12. Management
13. Markets
14. Information (research, extension, demonstration, training)

The same inputs are required regardless of whether the crop is wheat or fish or whether it is produced in the United States or any other country. The most efficient production results when all of the required inputs are available in adequate quantity at the proper time; conversely, the lack of a single input at a crucial time can result in little or no production.

The input of primary concern to those in fish farming research is information. Information is the cement that holds together the other inputs into an organized system. It is knowing when to stock, what to stock, when to treat for disease, when to harvest, and how to market. It is the organizing of the understanding of the laws of nature into a form that is applied through the farmer's mind and hands to the ponds, water, climate, and seed to produce a crop with a relatively high degree of predictability from year to year.

In the early history of fish farming, information was obtained by farmers through trial and error, and passed from father to son and neighbor to neighbor. This system still produces useful information. Fish farmers are creative people. Given their intense interest in the success of their efforts and constant day-to-day contact with their problems, they are able, through trial and error and intuition, to arrive at solutions. However, this process may be relatively slow. The production of information or the solving of problems can be compared to a chemical reaction that takes place slowly in the absence of a catalyst, but proceeds much more rapidly in its presence. When the trial and error process is organized or systematized, it becomes research. The research acts as a catalyst in the reaction. Given time, farmers would eventually meet much of their own needs for information, but with research the whole process proceeds more rapidly.

ADMINISTRATION OF RESEARCH

The primary purpose of this book is to provide the individual involved with the planning and execution of fish farming research with guidelines for doing meaningful research; however, a few comments are in order for the research administrator.

Administration is defined as the process of managing or directing execution, application, or conduct. In practice, administration is the development and maintenance of relationships between people for the purpose of transferring authority and responsibility. The importance of people cannot be over-emphasized. Rosenthal (1981), in an article summarizing a considerable amount of information on administration, emphasizes the importance of people rather than organization. He notes that administration involves four, people-related components: delegation, responsiveness, responsibility, and motivation. Each of these is vitally important in the administration of research programs; because of the nature of the research process, motivation is probably the most important one. I will have more to say on this subject in a following section.

The first responsibility in administering a research program is to represent a higher level of administrative authority in the use of public resources. The administrator is responsible for observing the rules and regulations and at the same time he is also responsible for solving problems. These two responsibilities are often contradictory. For example, regulations governing the use of public funds for purchasing are often relatively complex and restrictive. The purchasing process often requires an inordinate amount of time. Regardless of the care exercised in advance planning, unexpected needs can develop quickly. It is often difficult to purchase necessary supplies or equipment to solve the unexpected problem. As a result, an important experiment may be ruined. A situation such as this creates the dilemma of dealing with two simultaneous, often conflicting, responsibilities:

1. Following procedures developed to prevent the misuse of public funds.
2. Effective management of research resources to obtain the maximum amount of information.

In many research organizations, there is a strong tendency toward the development of restrictive rules and regulations that diminish productivity. Research administrators must be alert to the development of rules and regulations that increase the difficulty of

managing the resources and must be prepared to protest those that limit the effectiveness of their units.

It is the role of administrators to encourage individual staff members to realize their full potential in the application of the scientific method to produce new and useful information, but their responsibility extends beyond this point. The administrator should encourage the individuals in such a way that their accomplishments as a group are greater than the sum of their individual accomplishments. The contributions of the organization as a whole should be greater than the sum of individual efforts.

The Scientist as an Administrator

Administration of research is complex. The complexity of administrative responsibility often is accentuated because few research administrators are trained to deal with personnel management, budgeting and accounting, allocation of resources, competition for funds, and enforcing regulations mandated by higher levels of administration (Rosenthal 1981). Many research administrators are scientists themselves who accept the responsibilities for a complex program. Usually, they have little idea of the complexities of the responsibility and are forced to learn "on the job," usually without much guidance from higher level administrators. The "on the job" learning experience often is further complicated because newly appointed administrators attempt to cling tenaciously to their scientific careers.

Because of their education and experience, scientists should make good administrators. Their study of the application of the scientific method to develop new information trained them to identify problems and constraints to progress. They have experience in reducing complex problems into simpler components and in selecting, from a series of options, the most likely solutions. Unfortunately, many scientists who become administrators leave this elegantly simple system for the identification and solution of problems behind. Systematic progress in dealing with problems is replaced by "crisis fighting"; non-problems are attacked with fervor; and solutions to problems that are implemented are seldom effectively evaluated. There often is little or no recycling of inquiry to encourage continued progress.

Although it is difficult, a scientist who decides to administer a complex research program should be prepared to give up his career

as an active researcher. Careers in either science or administration are full-time responsibilities. Only especially gifted individuals can simultaneously do both well. The research administrator must be prepared to watch his staff advance as productive scientists while his scientific career stumbles to a halt. He must be prepared to suppress his interests as a productive scientist and to re-direct his creative energy to support the work of others. The administrator must be prepared to see the reputations of his staff surpass his own. By working to advance the reputations and effectiveness of his staff, the administrator will find that there is more than ample recognition for all. An administrator concerned that his scientific reputation and accomplishments will be less than that of his staff almost invariably will reduce the effectiveness of the entire organization.

Providing effective leadership for a research staff is a complex responsibility. Progress in science can be a relatively slow process. For example, most fish production experiments require at least a year to design, conduct, evaluate, and report. Usually much more time is required. Given the nature of the scientific method and the turnaround time involved in going from one experiment to another, a productive scientist will conduct a relatively small number of significant studies in an entire career; consequently, under the best conditions, a researcher may have little effect on the fish farming industry. Because of the slow pace of research, it is essential that the administrator provide the type of leadership that encourages relevant, incisive, definitive experimentation and that the recycling time between experiments is kept to a minimum.

Effective administration of research involves a considerable amount of "art" and a little "science." The "art" is dependent on the administrator's personality and is difficult to change. The "science" is equally difficult to apply. There are few proven rules. Some successful corporate executives have developed general rules that help them in managing complex organizations, and these are probably appropriate for research administrators. One list has been developed by Charles Knight, of Emerson Electric (Loeb 1980):

1. Be able to set priorities.
2. Be able and willing to deal with tough problems.
3. Set and demand standards of excellence.
4. Develop a sense of urgency.
5. Pay attention to details.
6. Develop a sense of commitment.

7. Concentrate on the possibles.
8. Be willing to accept some mistakes.
9. Be tough but fair.
10. Develop a sense of enjoyment in leading a group of talented people.

Responsibilities of the Aquacultural Scientist

With agricultural commodities such as poultry and beef cattle, the private sector spends a considerable amount of money on research and development. Ultimately, much of the information required by fish farmers may be provided by the industry; however, because of its small size, the fish farming industry cannot provide the necessary funds to pay for the research it requires. For many years, most of the effort in aquacultural research will have to be supported by public funds. This is a large investment for the public sector to make. These funds are amassed primarily by taxing individuals and businesses and represent a form of public trust. The aquacultural researcher should be aware of the responsibility for accountability associated with the use of those funds. The opportunity to do aquacultural research should not be accepted without an urgent sense of this responsibility.

The research scientist is trained to understand the laws of nature and to develop information based on those laws that can be utilized by farmers or other scientists. The researcher has the responsibility to interpret the laws as revealed by his experiments without bias or prejudice. If the results of one's experiments are interpreted according to one's political affiliation or business associations, the long term effect will be a loss of confidence and support by the public. Further, biased interpretation leads to information of poor quality. The public has the right to demand that its funds provide unbiased information.

Because of the potential importance of fish farming in the production of food, aquacultural researchers should have adequate facilities and adequate funds to produce the information required by farmers, and they should be held accountable for the productivity of those resources. Most scientists would agree that the wanton misuse of a laboratory balance or a vehicle constitutes a betrayal of public trust; yet misuse of experimental ponds and laboratory equipment in poorly designed or conceived research is equally a betrayal. Similarly, the accumulation of data without converting them into

useful information through interpretation and synthesis also betrays public trust.

Stoltenberg *et al.* (1970) suggested that searching for knowledge of the natural world through research, like art and philosophy, offers a sense of participation in life that transcends normal experience. Goldstein and Goldstein (1978) term it a sense of exhilaration. A great danger is that those doing research will view it as an end in itself, a God-given right to indulge in an ego trip among the laws of nature without a clear vision of their roles or responsibilities in the wealth producing process. In this situation, data or even information may accumulate with little benefit. Aquacultural research scientists are unique people. They are trained to enter undaunted into the very bowels of the created order; yet without the correct relationship with fish farmers, they can spend a lifetime "tilting with windmills." Scientists are like packrats; they are attracted by hard, bright facts, by colorful theories, and strings of correlations. These baubles store easily and accumulate rapidly and can give one a deep sense of satisfaction; yet they can be virtually useless in the process of development of useful information or in contributing to a nation's wealth.

Aquacultural scientists should strive to increase their productivity in developing new and useful information or in maintaining a high level of innovation in their work. Unfortunately, there are few guidelines as to how this might be done. Mosteller (1981) discussed the problem of the lack of research on how to do research. He reported the results of study done in Britain on industrial innovation. That study seemed to indicate the following:

1. Successful innovators better understand user needs.
2. They pay more attention to marketing.
3. They develop more efficiently, but not necessarily faster.
4. They make better use of outside technology and advice.

While the characteristics probably are more appropriate for industrial research, there certainly is some applicability to aquacultural research.

Aquacultural researchers must constantly remind themselves that they meet but one of the needs of fish farmers. They are but a part of the wealth producing system. Their contribution is important, but no more important (and possibly less) than that of other contributors (banks, fingerling producers, fertilizer manufacturers, salesmen). Unless they work effectively and in harmony within the

system supporting the farmer, much of their effort and the funds provided can be wasted.

Research and Extension

Research results in the accumulation of data that may or may not be converted into information. Similarly, it is possible to accumulate information without obtaining additional fish or income. The process is a complex chain that can be easily broken at any of its linkages.

Without the effective extension of information, research is of limited value. It is unfortunate that some researchers and research administrators consider extension of lesser importance than research. They often assume that their responsibility ends when the experiment is completed and the scientific paper is published. This attitude indicates a grave misunderstanding of the role of research in improving the lives of people. Over time, this attitude can be just as damaging to the effectiveness of the scientist as lack of concern for the condition or maintenance of experimental equipment and facilities.

Extension workers also are extremely important to researchers for another reason. They are involved on a daily basis with farmers and are conversant with their problems, and are one of the best sources of information on research needs. This relationship provides the scientist with a "window" to the real world that is invaluable. The relationship also provides research administrators with some of the information on which to base decisions on program development and resource allocation.

Researchers and research administrators should take a strong interest in people and programs that have the responsibility of extending information produced through their research. They should make every effort to encourage the development of strong extension programs and to cooperate in every way practical with their extension counterparts.

Obviously, both research and extension are important in providing information for farmers; however, emphasis required for each does not remain static. In some situations, research may be many years ahead of farm practice and considerable emphasis on extension is needed. Evans (1980) suggested that there are many countries where national crop yields could be improved several fold through the use of improved varieties and agronomic practices. In other situations, general farming practices may be close to practices

being used on research stations. In this case, further advances can be realized only by developing new technology. For example, Thompson (1975) noted that average corn production on Iowa farms is rapidly approaching experiment station yields. Where farm practice approximates research station practice, the allocation of resources to research should be increased.

Obtaining and Allocating Resources

One of the primary responsibilities of the administrator is to obtain funds to support the program. Research programs require relatively large sums of money, and for maximum effectiveness these funds must be provided continuously. Unexpected fluctuation in funding may destroy the effectiveness of a program. Generally, aquacultural research is funded as part of a larger program involving agriculture and/or fisheries; consequently, the administrator must compete with those representing other commodities for funds. The total amount of food produced from aquaculture is low compared to production of traditional crops. Because the allocation of research funds is usually based to some degree on the value of the commodity, aquaculture receives a relatively low level of funding.

The problem of obtaining funds for fish farming research is compounded because agricultural research in general is underfunded (Evenson *et al.* 1975 and Evenson *et al.* 1979). Underfunding results in part because research benefits in agriculture and in aquaculture "spill over" to and from adjacent regions, reducing the incentive for local support. Also, American taxpayers tend to respond to "crises" in funding research. They reacted to the "sputnik," ecology, cancer, and energy crises by literally throwing money at them. Food production in the United States is an "anti-crisis." We only spend approximately 16 percent of our disposable income for food. Our only "crises" is what to do with the surplus. Taxpayers are not likely to invest heavily in that "crisis." Regardless of the fact that rates of return on research expenditures in agriculture are about 50 percent, funding is lagging. Tichenor and Ruttan (1971) note that regardless of our dependence on science and technology (research), society has been slow to critically examine criteria for deciding which research shall be undertaken. It seems that national ability to do research may outrun the capacity to allocate judiciously scarce economic and social resources. To compete effectively for research funds, the administrator must sell his program as a significant investment opportunity for public funds. The fish farmers and the

fish-buying consumers must be convinced that it is in their best interest to purchase research information.

Another major task is the allocation of resources (funds, facilities, labor) within the organization. The impetus and direction of a program is determined by the allocation of these resources. The relative proportions of short term, intermediate term, and long term research in the unit is determined by the allocation of funds; consequently, it is important that the administrator be knowledgeable of the fish farming industry and of the local, national, and even international events that will affect both short and long term needs of the industry for information. A program can be severely damaged if a wrong decision is made.

The administrator should be careful that his personal research interest does not obscure his vision. It is difficult not to be more interested and more supportive of research with which one is more familiar and knowledgeable. It is also important not to unconsciously use reverse bias or to avoid supporting one's area of research interest for fear of being accused of favoritism.

Allocation of research resources cannot be determined effectively by committee. Decision by committee makes it possible to spread the blame for mistakes; however, it is not an effective way to allocate scarce resources. The strongest personalities on committees often determine the course of action which may or may not be the best solution. Further, if difficult decisions must be made, committees usually cannot make them without strong personal conflict. Committees often "divide the pie" or provide something for everyone. A committee might serve to effectively delineate a series of alternatives, but the administrator should make the final decision. In making the allocations, the interests of all parties directly and indirectly involved (research staff, farmers, consumers, taxpayers) should be considered.

Staff Motivation

The research administrator is responsible for the motivation and performance of highly trained scientists. Among their most valuable characteristics are independence of thought and action. Developing solutions to problems requires these characteristics. The administrator should do everything practical to encourage and guide the innovative character of the staff, but within the bounds of the organization's mission.

Motivation of a research staff is generally not a difficult problem, but maintaining a highly motivated staff must concern the administrator (Rosenthal 1981). Usually, research scientists are "self-motivated" and feel that learning new things and developing new knowledge are their own reward. In some situations, however, scientists do lose their motivation. Long periods with little reward or recognition for effort and a lack of resources (facilities and operating funds) can lead to a loss of motivation. At the beginning of this section, I referred to Rosenthal's (1981) paper regarding the importance of motivation in administration. He refers to Cooley's work in noting three desires that play important roles in determining a person's motivation:

1. Recognition and respect
2. Security
3. New experiences

All of these were more important than salary in determining motivation.

Often the loss in motivation and, in turn, reduced productivity develops rather slowly and is not apparent until well advanced. This is a dangerous point in the career of a scientist. Often it will mean the loss of a productive individual for several years, or in the extreme case the scientist may never reestablish an effective, productive career.

When there is a loss of motivation, it is the administrator's responsibility to help the person realize that productivity has diminished and to help restore a high level of accomplishment if possible. This can be a difficult task. It often requires a significant change in personality on the part of the individual, and an awareness of the importance of regaining productivity. Often, the staff member must work hard to master new techniques and learn a new vocabulary. This situation requires considerable effort and may lead to considerable mental anguish.

Given the difficulty of detecting the loss of motivation and the problems associated with remotivating a scientist, it is much better for the administrator to foster a spirit in the organization that will prevent the situation from developing. The administrator must be strongly motivated himself. An effort should be made to encourage the staff to stay abreast of their fields and to remain productive. The administrator should provide opportunities and encourage the establishment of contacts between his staff and staff at other institutions through attendance at meetings and seminars and through

visits to other institutions. He should encourage the staff to accept a major role in working directly with farmers and farmer organizations. Productivity should be rewarded to the degree practical by promotion and increases in pay. In addition, the administrator should seek every opportunity to demonstrate to the staff his appreciation for their efforts, and he should make an effort to inform higher levels of administration and the public of staff accomplishments.

Staff Required for Research

People do research. Good facilities and modern equipment extend the effectiveness of the human intellect to solve problems, but in the final analysis it is the human element that is the determining factor. Facilities and equipment cannot make up for the lack of ability to understand and apply the scientific method to the solution of problems.

Developing and maintaining a staff to conduct research is a responsibility of the administrator. There may be several problems involved. In many countries, aquacultural research is expanding rapidly and there is a shortage of scientists trained to conduct production experiments. The relatively high cost of trained personnel is also a concern. These costs have increased rapidly in the past few years because of worldwide inflation. Personnel costs have increased at a much faster rate than funding for research. At many research stations, personnel account for more than 80-90 percent of all costs, leaving only limited funds for operations and maintenance.

Another concern is that the number and training of the staff available be appropriate for the amount of data to be collected from experiments. The yield of fish from a pond involves the interaction of a number of complex variables. Alikunki (1968) listed a number of observations that might be made in fish farming experiments. Experiments involving a small number of ponds in which the only data desired are the number and weight of fish present at draining (in many experiments no more data are required) can be conducted by one trained research scientist plus a small field crew of two or three persons to assist with stocking and later to assist with removing the fish. Large, complex experiments requiring the simultaneous collection of data on micro-climate, zoo-plankton and phytoplankton production, presence of fish pathogens, and water quality would require a team of several scientists plus a field crew.

Staff members that have the educational background to work

effectively with the problems in aquacultural research usually expect a relatively high standard of living. They want to live and work in an area where they have access to good housing, adequate shopping facilities, good schools for their children, and opportunities for rewarding leisure time activities. Often aquacultural research facilities are located in areas where it is difficult to provide these amenities. This is an especially troublesome problem in developing countries, and is often an underlying reason for low staff productivity even in the developed countries. As a result, it is difficult to attract and hold competent research scientists at stations where they are needed badly. There is relatively little that can be done to solve this problem except to make an effort to locate research stations, when practical, where a satisfactory living environment is available. Another long-term solution, especially in developing countries, is to train more aquacultural scientists with rural backgrounds. They often welcome the opportunity to be involved in productive research in a rural setting.

Staff with a farm background also bring to research efforts a unique understanding of farmers and their problems. Little research has been done on this subject, but I suspect that much of the success of the State Agricultural Experiment Stations in recognizing and solving problems of American farmers can be traced to the high percentage of agricultural scientists with farm backgrounds.

Facilities and Equipment Required for Research

Adequate facilities and equipment are essential for effective aquacultural research. Administrators are responsible for obtaining and managing these resources. They are responsible for making decisions about what facilities are needed and for making requests for funds to provide and maintain them. Facilities for fish farming research are expensive. Because of the high cost, it is important that every effort be made to locate, design, construct, and equip them for maximum contribution to meeting the needs of fish farmers and of other scientists for information.

The key to the development of adequate facilities is to understand the different "levels" of research required. Wortman (1980), in his excellent article on the role of research in food production, described five categories of research:

1. Operational-adaptive research on individual farms
2. Tactical-research of regional importance

3. Strategic-research of national and international importance
4. Supporting-fundamental research that is likely to lead to new advances in crop production
5. Basic-research to expand the frontiers of knowledge

All five categories are required for an effective information generating system, and different facilities (and staff) may be needed for each category.

The State Agricultural Experiment Stations in the United States are a good model for development of aquacultural research facilities. These stations play a major role in generating information and solutions to problems in support of American farmers (Horsfall 1976). Everson *et al.* (1979) attributed some of the success of the agricultural experiment stations to the association of research oriented to science with that oriented to technology and to farming. This articulation is important. It allows scientists advancing knowledge through basic research, scientists developing technology, and farmers producing food to work as a "team."

Although there are some differences, in most states the State Agricultural Experiment Station is really a network of several stations. A central or main station is located on the campus of the agricultural university, usually the land-grant university, and branch stations or substations and experimental fields are located in important agricultural areas across the state. The combination of these three types of facilities makes it possible to combine the research laboratory, the experimental plots, and the working farm into a functioning unit for the benefit of farmers. An outline map, figure 1, shows the location of the major soils associations in Alabama. The location of the various components of the Alabama Agricultural Experiment Station network also is shown. There is an agricultural research unit in every major soil area in the State.

Usually associated with the central or main station are experimental farm facilities plus a variety of laboratories for both applied and basic research in the several areas of agriculture (horticulture, entomology, dairy science, veterinary medicine). Here, senior scientists are involved in research and development, often relatively basic in nature, in a number of areas.

The branch research stations or substations are located where farming is taking place. These are operating farms where "trial and error" can be organized to arrive at predictable solutions to problems, where "trial and error" can be replicated in space rather than time. The branch stations usually do not have sophisticated laboratory equipment. They depend on the central station for special

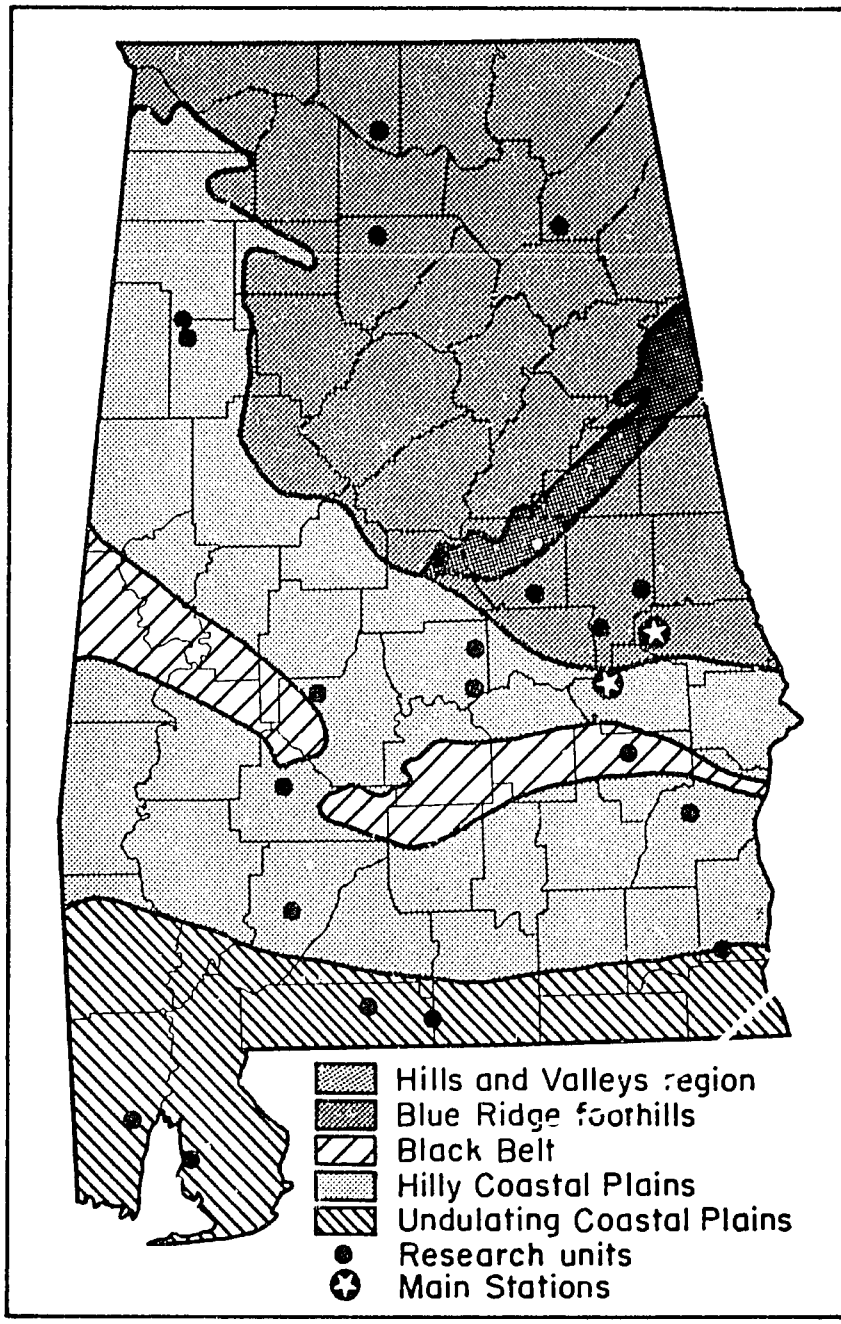


FIG. 1. Map of Alabama showing the major soils associations and the location of agricultural experiment stations.

analyses and support when required. Rather, they reflect the "art of the possible" with respect to research. They have the same types of equipment and facilities as the better than average farms in the area and they grow the same crops.

The branch stations also serve as field research locations for senior scientists from the central or main station. The senior staff may be primarily responsible for the design of the experiments, and the staff at the branch station will be responsible for the day-to-day supervision of the research. Both groups collaborate on data interpretation and on writing reports and scientific papers. The cooperative effort between central and branch stations provides the senior scientists an opportunity to learn firsthand of the problems associated with a commercial farm. This effort also provides the staff of the branch station with the opportunity to improve their knowledge of the scientific method and the basic sciences supporting it. The branch stations also provide an excellent facility to be used in extension programs. Effective result demonstration activities and field days can be sponsored which bring together farmers and senior scientists under optimum conditions.

The number of branch stations required is dependent on the nature of farming in the state. For example, if farming is relatively similar and farmers face the same types of problems throughout the state, then only one branch station would be required. In this case, facilities might be developed at the central station for practical farming research and a separate branch station would not be necessary. However, if there are major differences in soil type, water quality, climate, and fish cultured, and if farmers are faced with different types of problems, more than one branch station would be required.

Some experiment station networks also include experimental fields. These generally are relatively small experimental units. They are usually located in a restricted but important soil type or geographical area where a calibration of research results under specific conditions is required.

The concept of the decentralized experiment station system described above is in contrast to the concept of international agricultural centers (e.g. the International Rice Research Institute), regional centers, or national centers. These centers are supposed to provide opportunities for solving problems relating to agriculture that are of international, regional, and national significance. Facilities of these large centers are impressive, but they are extremely expensive to develop and maintain. They may be ineffectual in the

long term because there are not many international, regional, or national problems. Farmers' problems tend to be relatively site specific, and require relatively site specific research. Usually if a problem is of such nature that it exists worldwide, basic research will be required to solve it. This type of research can be done in many laboratories and does not require the creation of an international center. Most often, international, regional, or national laboratories are, in reality, doing site specific research utilizing a highly trained staff and expensive facilities. No matter how hard a scientist might try, production research in ponds or field plots cannot be divorced from local soils, climates, or pests.

Tichenor and Ruttan (1971) discussed the concept of centralized and decentralized agricultural research facilities. They quoted Schultz, who contended that without a special organization, a national agricultural research center is ineffective. Schultz also felt that there was considerable evidence to support the concept of decentralized stations even in developing countries. In a similar context, Feder (1979) suggested that international research centers tend to discourage local research in agriculture. International or regional centers usually are well funded. They have the best of facilities and equipment. High salaries must be paid to attract and maintain adequate staff with their families, usually from developed countries. Unfortunately, they also attract the best trained local scientists. Also, the enormous investment required probably diminishes the funds available for the development of adequate national, decentralized facilities.

Aquaculture is relatively unimportant compared to agriculture in most countries; consequently, fish farmers may be fortunate to have a single station, much less a network of stations, to help them solve their problems. Often there is only one fish farming research station in an entire country. In this situation, it is virtually impossible to do research on all the site specific problems encountered by fish farmers. As funds become available, it is important that facilities be located, designed, constructed, and equipped for maximum contribution to meeting the needs of fish farmers in different localities.

Experimental ponds are the key to the development of good facilities and, in turn, a good fish farming research program. Because the primary objective of research is to obtain information for the use of fish farmers, adequate experimental ponds are a necessity. Unfortunately, buildings are much more impressive than ponds (holes in the ground), so it is natural to first plan the labor-

atories, the office buildings, and other similar structures. Laboratories do add an extra dimension to research, but the most important element, and often the most neglected one, is the ponds. Satisfactory fish farming research can be carried out with few, if any, buildings but not without ponds. Even if funds are limited, the best investment will be to build ponds. With ponds available and being used effectively to obtain information, additional funds usually will be made available for buildings.

It is possible to conduct research in farmers' ponds, but in many cases the results are poor. Farmers want proven technology used on their ponds, and are reluctant to take the chance of a poor crop by using technology that has not been proven. Even when a farmer agrees to the use of his ponds, he often will not follow the experimental plan when he sees that the fish are not growing well or when other effects of the experimental procedures appear. There is one advantage to utilizing ponds owned by successful farmers for research. Other farmers look to them for leadership and for trend-setting because they are successful. Especially if they are cooperative, successful farmers can be extremely helpful in extension efforts when research and demonstration is located on their land.

Once the need for ponds has been met, then the supporting buildings and laboratories can be developed, but even then, care should be exercised. Buildings and laboratories require that ideas and plans be set in concrete, brick, and mortar. It is not easy to change the structures; consequently, these facilities should be designed and constructed to be as flexible as possible, so that the use might be changed in the future without expensive alteration.

In developing facilities there are three major characteristics to consider:

1. Location
2. Water supply
3. Design

Location is extremely important. Large sums of money have been wasted because of the poor choice of a location. Most problems associated with fish farming are relatively site specific. They result from conditions that are related to local soil, water, climate, market, and economic characteristics. Effective solution of these problems requires that research be done under the same or similar conditions. For example, the farming of channel catfish (*Ictalurus punctatus)* in the Mississippi Delta (Yazoo Basin) results in different types of

problems than in the hill country of east Alabama. In Mississippi, the ponds are large (5-20 hectares) and relatively shallow (1-2 meters). Because of the nature of the soil and the topography, construction is accomplished by pushing up dams surrounding a flat-bottomed basin. Water of uniformly good quality is supplied by pumping from a large aquifer 20-25 meters below the surface. The individual fish farms are generally a part of a larger farming complex. The farmers in the area have a long history of successful farming of cotton and other crops. Credit for production needs is readily available.

In contrast, the ponds used in fish farming in most of Alabama and especially in east Alabama are relatively small (0.5-2 hectares). The ponds are constructed usually by placing an earthen dam across a small valley, creating a V-shaped basin of variable depth. Maximum depth in the ponds is 2-5 meters. Water is often supplied from rain falling on the pond surface and runoff from the surrounding watershed. Water quality depends on the nature of the soil in the watershed, and the intensity and duration of rainfall. The quantity of water available is linked closely with seasonal rainfall patterns. Total fish production from the individual farms, because of the small size of the ponds, is relatively low. Because of the major differences in the two fish farming systems, production problems in the two areas are dissimilar. Research on practical problems conducted for Alabama farmers, especially in east Alabama, has limited application for the farmers in the Mississippi Delta. Similarly, research conducted in Mississippi under specific pond and farming conditions would have limited application in Alabama. Obviously in the example cited above, a single research station located in either Alabama or Mississippi would not adequately serve the farmers in the other area. At least two stations would be required. Even within Alabama, fish farming characteristics are different enough in the Black Belt soil areas in the western part of the State and the Piedmont soils area in the east that two separate stations would be required to fully meet Alabama farmers' needs for information pertaining to their problems.

Several factors should be considered in locating an aquacultural research station. These include:

1. The station should be located where fish farming is, or is likely to be, practiced. Consideration of this factor is essential in locating branch research stations and should be considered strongly when locating a central station. Usually if the area is

already being used for fish farming, soil and water can be found that will be adequate for a station.

2. An area should be chosen where trained scientists will be content to live with their families.
3. Locate the station where future growth of the surrounding area is not likely to create an unsatisfactory environment (pesticide pollution, excessive poaching, vandalism) for research.
4. The area should not be subject to periodic, excessive flooding.
5. The area chosen for development should be large enough to provide for expansion.
6. The station should be located where there is good access for visiting farmers and other scientists.
7. Supporting services, such as telephone, electricity, fuel, spare parts, and maintenance of equipment, should be available. Usually it is necessary to locate the facility near a trade center to obtain these services.

Water supply is equally important as location in the development of an aquacultural research facility. Characteristics that should be considered with respect to water include:

1. Generally, a research facility should have the same type of water as farmers in the local area.
2. There should be an adequate, year-round supply, not subject to periodic flood or drought.
3. The water should have relatively constant quality.
4. Movement of the water to and away from ponds, tanks, and aquaria should be accomplished with as little pumping as practical (figure 2).
5. A water supply that does not contain wild fish is preferred.

Design is the third major factor to be considered in the development of an aquacultural research facility. Design, in reality, includes several different types of factors and will depend on whether the facility will be a central station or a branch station:

1. Layout or location of ponds and buildings
2. Sizes and number of ponds, tanks, troughs, and aquaria
3. Types, design, and location of supporting buildings and laboratories
4. Selection of equipment

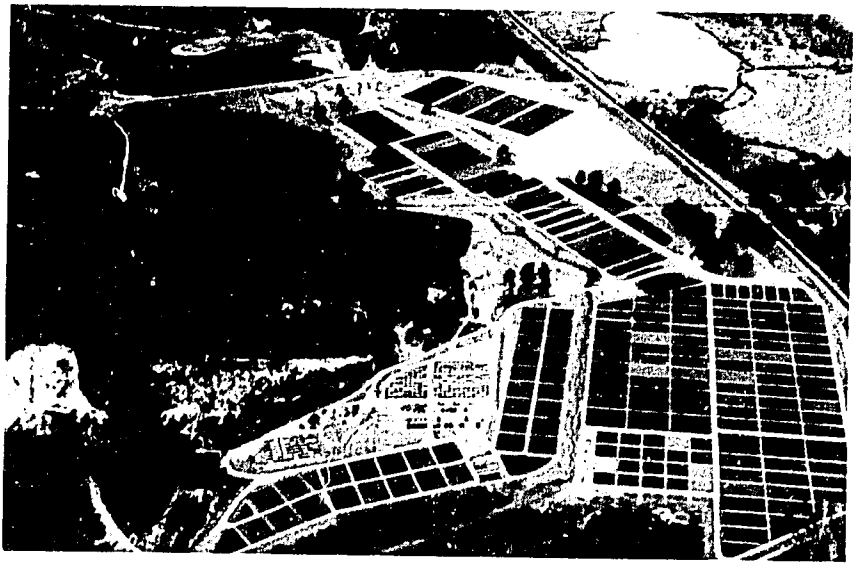


FIG. 2. The large (8.5-hectare) pond in the aerial view is the water supply for a series of smaller ponds used in research at Auburn. The large pond is supplied with water from rainfall on a forested watershed. Seepage and overflow from smaller storage ponds in the watershed help to maintain the water level in the supply pond. Water is supplied by gravity to the smaller ponds in the valley below.

The ponds and buildings should be arranged with the following factors in mind:

1. Arranged for optimum use of available space (figure 3).
2. Ponds and buildings located with concern for possible expansion of facilities.
3. Service buildings located for easy access from ponds and roads (figure 3).
4. Amount of soil to be moved (cut and fill) should be considered in locating ponds.
5. Quality and quantity of soil for building ponds are important.

The sizes and numbers of ponds, tanks, troughs, and aquaria required will depend on whether the facility will be used as a branch station or a central station. The following list includes fish growing, spawning, and holding units that would be needed in a relatively small central station that also serves as a branch station in its area. Obviously this list provides only a guide of facilities required. Depending on the specific mission of the station, the types of problems to be researched, and the type of aquaculture practiced in the surrounding area, the facilities listed might be increased or decreased:

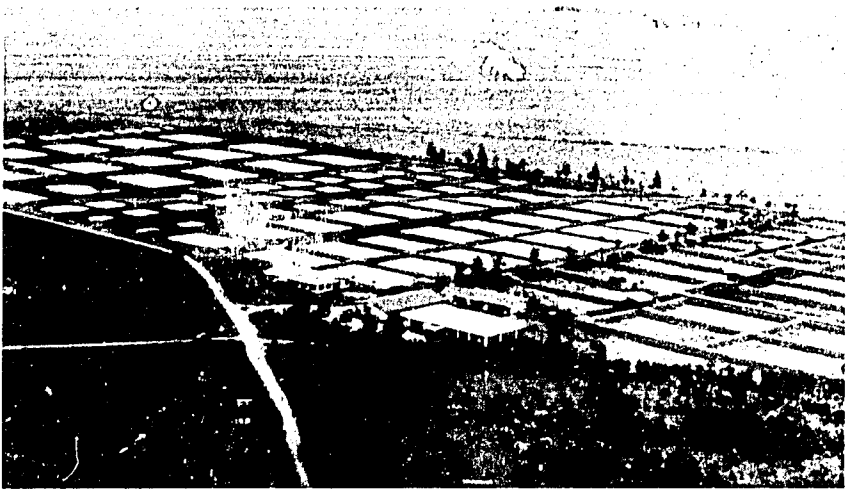


FIG. 3. The aquacultural research facility at Central Luzon State University in the Philippines.

FIG. 4. A series of research ponds at Auburn. These ponds are similar to those that fish farmers use in the surrounding area. The largest pond is approximately 10.3 hectares. The water for these ponds is provided by harvesting rainfall.

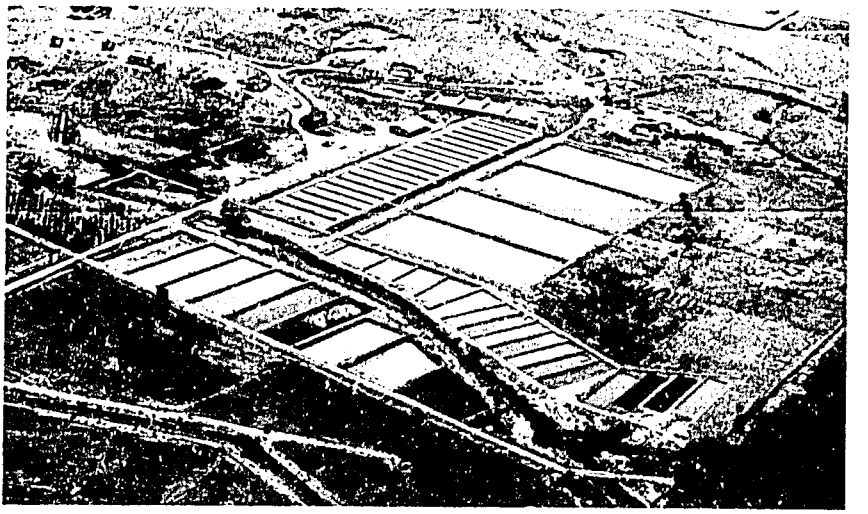


FIG. 5. A series of experimental ponds at Pentecoste, Ceara State, Brazil.

1. A series of 8-12 commercial production ponds, similar in size, water supply, construction, and configuration to those that farmers are utilizing in the area (figure 4).
2. Six to eight ponds, each with an area of 1,000 square meters, for holding brood fish and for preliminary production experiments (figure 5).

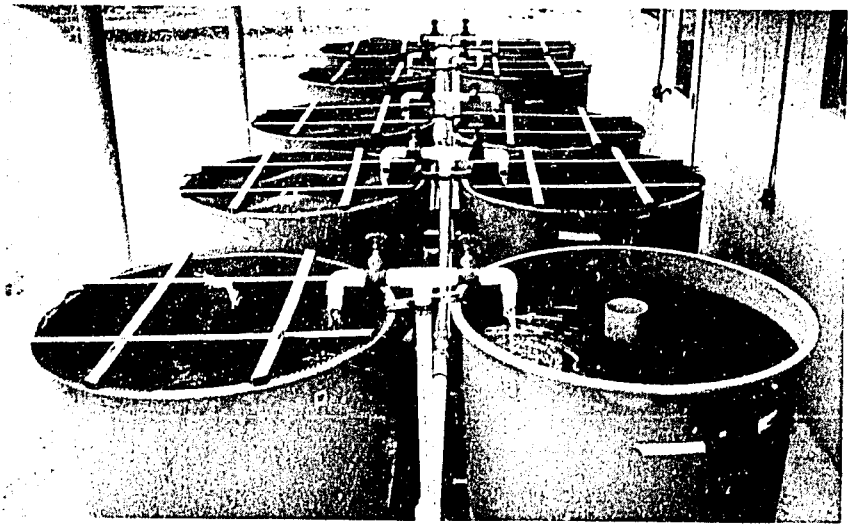


FIG. 6. Circular, fiberglass tanks used in fish nutrition research at Auburn. Water is supplied by gravity flow from a water storage lake (figure 2).

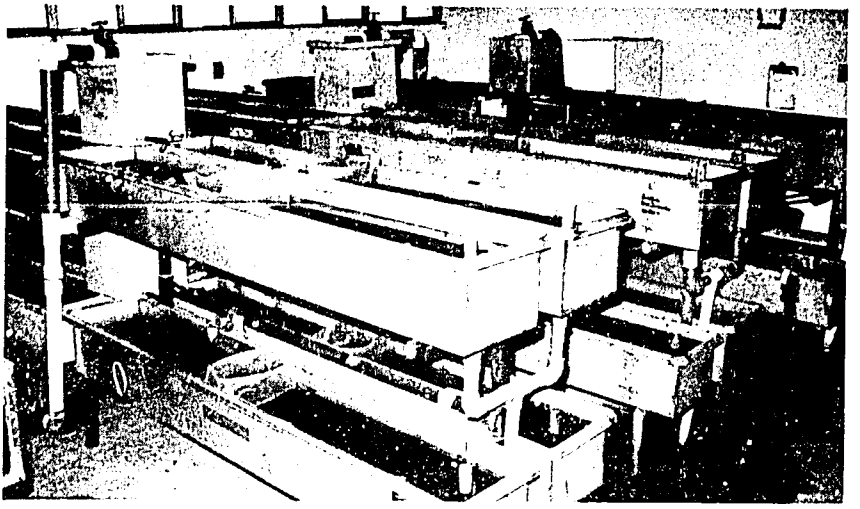


FIG. 7. Rectangular troughs used in research.

3. Ponds for preliminary experiments, 24 to 36 units each with an area of approximately 500 square meters (figure 5).
4. A series of 12 ponds, each with an area of approximately 250 square meters for spawning and for rearing of fingerlings (figure 5).
5. Circular tanks (1.5-meter diameter x 0.75-meter depth) constructed of fiberglass, concrete, or some similar material for holding fish and for miscellaneous research (figure 6). A series of 12-15 of these is required. Preferably, these should be located under a rainproof shelter.

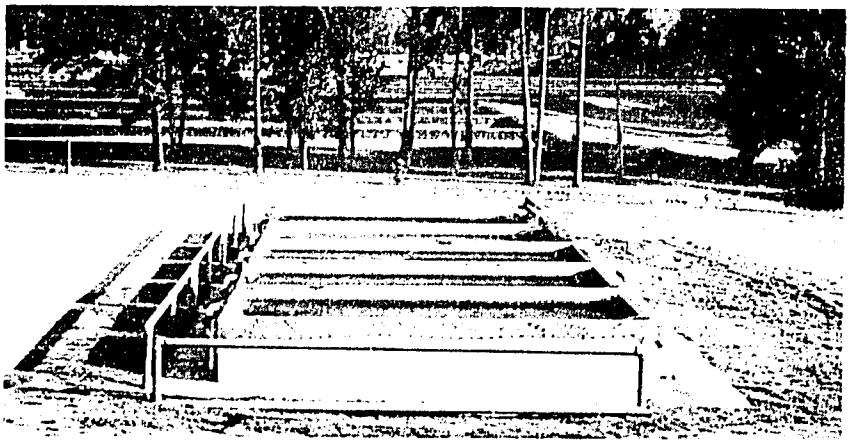


FIG. 8. Concrete holding tanks.

6. Rectangular troughs (60 centimeters wide x 30 centimeters deep x 125 centimeters long) of fiberglass or similar material (figure 7). A series of 36 to 48 of these troughs would be needed for several types of research. These troughs also should be located in a rainproof shelter. In areas with cool or cold winters, the troughs should be located inside a building where the temperature can be controlled to some degree.

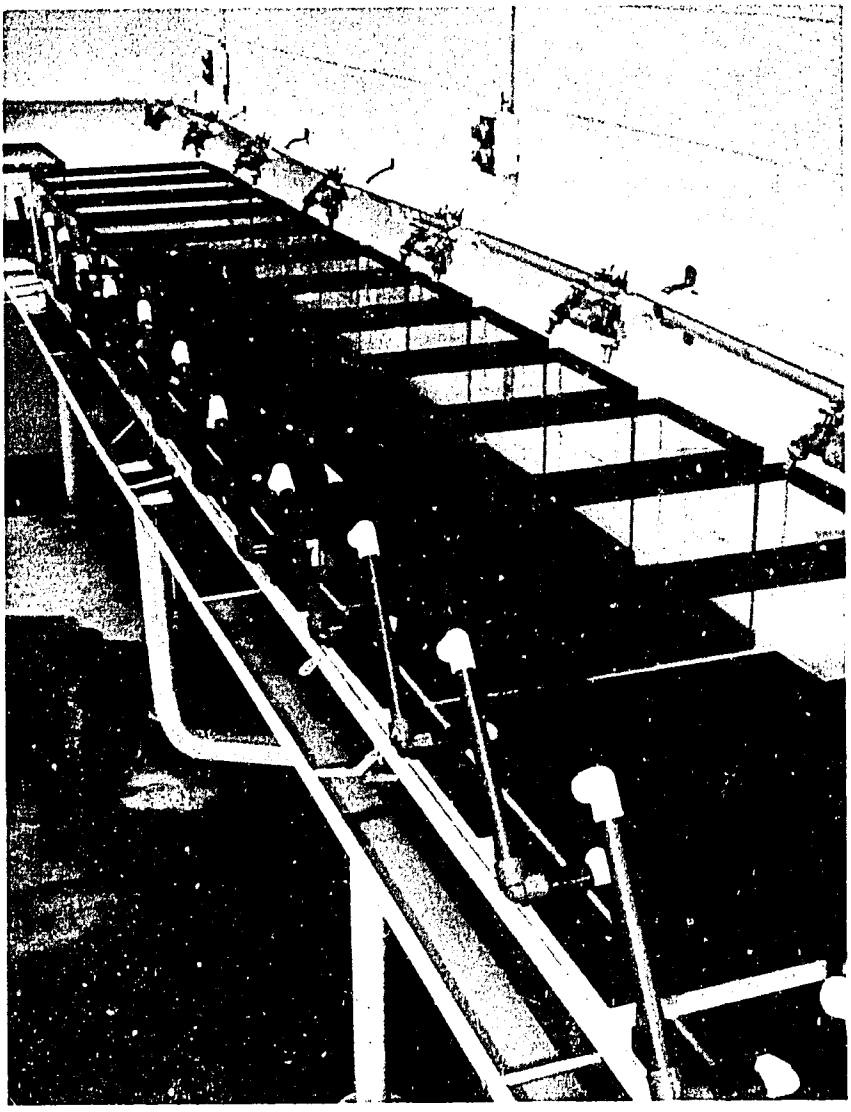


FIG. 9. A series of aquaria (40 liters) used in fish nutrition research.

7. A series of six to eight concrete holding tanks (figure 8) for holding fish prior to stocking into experiments and after removing them. These tanks provide an excellent facility for treating fish for parasites and disease prior to stocking.
8. Approximately 72 aquaria should be available for research (figure 9). They each should have a capacity of approximately 40 liters.

FIG. 10. Pond constructed with concrete retaining walls to prevent erosion and to maintain exact area.

FIG. 11. Pond with retaining walls of treated wooden boards.

Experimental ponds should have the following characteristics:

1. Constructed to minimize seepage and to prevent erosion of the margin (figures 10 and 11).
2. Constructed so that most of the fish can be removed by seining.
3. Arranged so that transportation can be located near the point where the fish will be removed during draining.
4. Each pond should have a water supply and drain control.
5. The water supply for each pond should be fitted with a plastic screen device with sufficiently small mesh to prevent the entry of "wild fish" eggs and fry (figure 12).

Tanks, troughs, and aquaria should have the following characteristics:

1. Each should have its own water supply and drain (figures 8, 13, and 14).
2. The water supply should be adequate to provide two to three complete water changes per hour for the largest tanks.

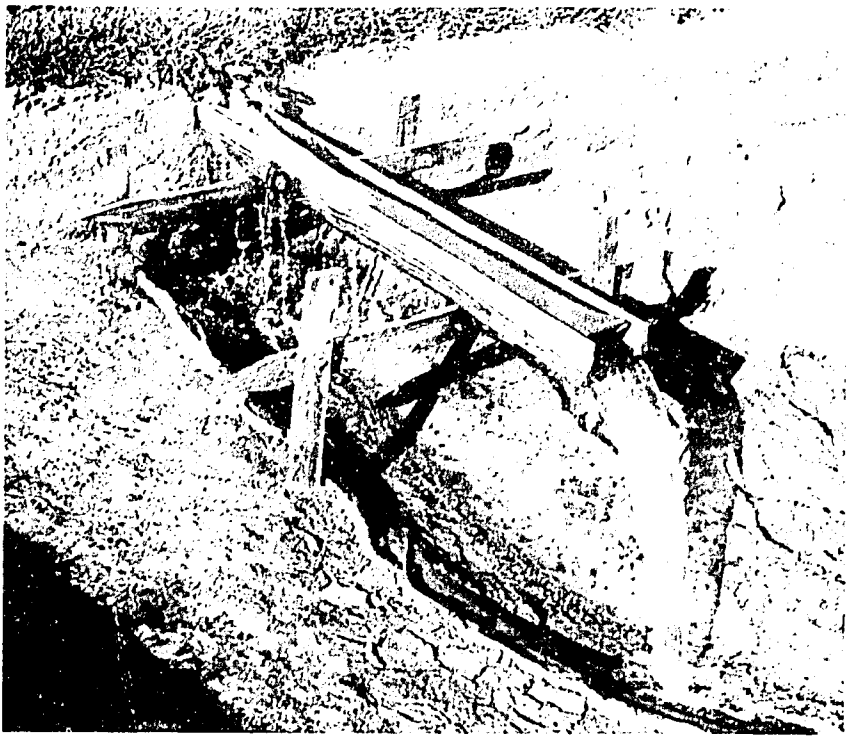


FIG. 12. Water supply on an experimental pond fitted with a saran screen sock to prevent the entry of wild fish and fish eggs. Note the concrete waterway located to prevent erosion as the pond is filled.

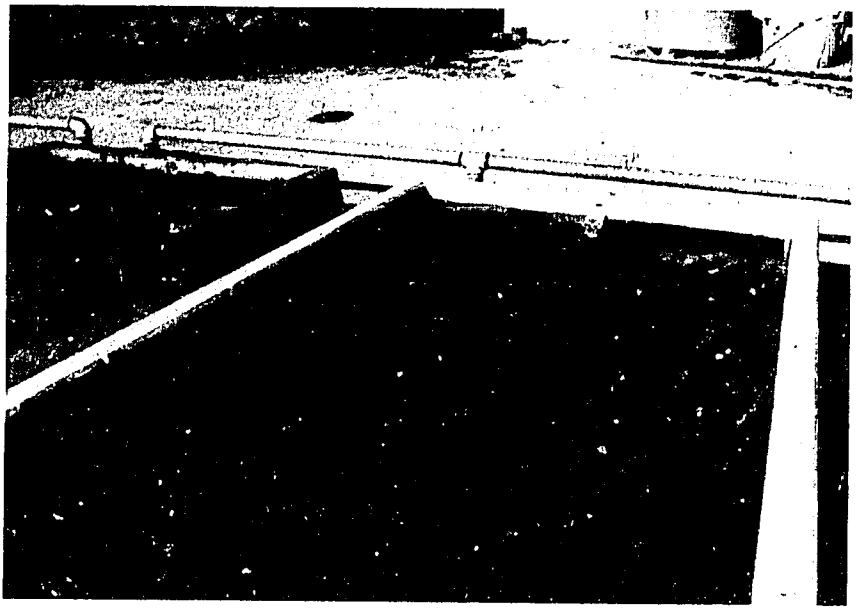


FIG. 13. Water supply for a concrete tank that provides maximum aeration and circulation. Note the large water supply pipe that allows the tank to be filled rapidly.

FIG. 14. Outside drain structure on concrete tanks. With this arrangement, the water can be maintained at a full or a half-full level.

3. If practical, an air supply or aeration device should be available for each of the tanks, aquaria, and troughs. When treating fish with chemicals and in certain other situations, it is necessary to hold them in static water for periods of several hours to several days.
4. The tanks and troughs should be provided with a screen which keeps the fish away from the drain. This screen prevents loss of fish and makes it easier to remove them. It also helps to promote a sort of self-cleaning action. Covers also are needed to keep fish from jumping out.

Support buildings also are an important component of aquacultural research facilities. The nature and extent of buildings needed will depend on whether they will be used on a branch or central station. Fewer and less elaborate buildings are needed for the branch station. A generalized list will be presented for a central station that also serves as a branch station or experimental farm in its particular area. These buildings include:

1. A general purpose building designed with space and equipment for sorting, grading, counting, and treating fish prior to stocking in experiments. The same facilities will also be needed when experimental ponds are drained and data are collected. This building would be the probable location for the concrete holding tanks (figure 8) described in a previous section. Ideally, the building also should contain space for the other tanks, troughs, and aquaria described earlier. This building should be located for easy access by large trucks and tractor-drawn wagons capable of transporting large tanks of water and fish. The large holding tanks in the building should be located so that fish can be unloaded from hauling tanks without requiring excessive handling.
2. A building or space is required for storing inorganic fertilizers.
3. A building or space is required for processing, weighing, and storing feed ingredients and feed. The storage facilities should be constructed to be as pest-free as practical and constructed to be fumigated periodically to eliminate rodents and insects that ultimately gain entrance.
4. A laboratory building is needed with space and equipment for the following purposes:
   a. Routine water analysis
   b. Fish health diagnostic work
   c. Processing and technology research

5. A building or space is needed for staff offices and for maintaining records.
6. A classroom or auditorium is needed to hold extension meetings.
7. General purpose buildings are required for the following uses:
   a. Storing and maintaining seines and nets
   b. Metal working and carpentry shop
   c. Storing tools and equipment
   d. Storing boots, rain gear, and special clothing for the field crews

No effort will be made to provide an exhaustive list of equipment required. The equipment should be selected carefully with the mission of the research facility and the capability of the research staff in mind. There is a strong temptation to over-equip laboratories. Most scientists are impressed by "picture-book" laboratories, but given the ever increasing cost of purchase and maintenance, the acquisition of unneeded or little-used items should be avoided. The availability of service and replacement parts also is of major concern. Purchase of highly specialized equipment and instruments should be avoided. Also, equipment should not be purchased that provides accuracy beyond that required in the research to be undertaken. Such equipment is expensive and difficult to maintain, especially when it is not used regularly.

PLANNING EFFECTIVE RESEARCH

On the Nature of Experimentation and the Scientific Method

Some fish farming problems can be solved by inductive or deductive inference; however, most will require the use of trial and error (experimentation). Sir Frances Bacon emphasized the role of trial and error in problem solving when he said that "truth will sooner come from error than from confusion. The induction which is to be available for the discovery and demonstration of sciences and arts, must analyze nature by proper rejections and exclusions. To man it is granted only to proceed at first by negatives and at last to end in affirmatives after exclusion has been exhausted (Platt 1966)." Rigorous application of "rejections and exclusions" to problem solving has been given the name, scientific method. Little and Hills

(1978) characterize the scientific method as consisting of the following steps:

1. Formulation of a hypothesis—a tentative explanation or solution.
2. Planning an experiment to objectively test the hypothesis.
3. Careful observation and collection of data.
4. Interpretation of experimental results to confirm, reject, or alter the hypothesis.
5. Recycle the procedure until a suitable explanation or solution is achieved.

The scientific method is a systematic approach to problem solving. Used properly it will add significantly to the research process. Used incorrectly, it can lead to confusion. Like any fine tool, its effectiveness largely resides in the hands of the user.

Systematizing inquiry through the use of the scientific method is the heart of science and discovery; yet it also has a soul—a natural skepticism, imagination, the subconcious, chance, and even circumstance. Goldstein and Goldstein (1978) cite the accomplishments of the mathematician Poincaré, the chemist Kekule, and the endocrinologist Nalbandov as examples of the role of "soul" in the discovery process. In these examples, the final "breakthrough" to an important discovery came as a result of occurrences not related (a dream, an accident, a chance observation) to the scientific method; yet even in those cases, systematic inquiry provided the base on which those occurrences manifested themselves.

Effective application of the scientific method begins with the formulation of a hypothesis. Goldstein and Goldstein (1978) define a hypothesis as the perception of some pattern in a phenomena, the establishment of some expectation of what will happen next. These authors note that the development of hypotheses is not an uncommon procedure and is not unique to scientific activity. Almost from birth, we form hypotheses (acceptable explanations) for the things we feel, see, and hear. We are by nature hypothesis formers. By nature we want to explain, to our satisfaction, the world around us. In applying the scientific method, we simply develop an explanation (hypothesis) for an observed occurrence. Then we proceed with the use of an experiment to decide if our explanation is correct. LeClerg *et al.* (1962) suggested that to be relevant a hypothesis should have three essential features:

1. It affords some correlation of facts.

2. It affords a basis for the prediction of other facts.
3. It provides a means for discrimination between valuable and useless information.

The evaluation (rejection or acceptance) of a hypothesis is usually done through the use of an experiment. Experiments play a central role in the process of learning things. Goldstein and Goldstein (1978) suggest that it is the use of the experimental test that distinguishes science from other ways (theology, art, poetry) of reaching understanding. They further suggest that experimentation is the natural outgrowth of the ancient and long-lived capacity to learn from experience.

Confirmation, rejection, or alteration of a hypothesis or proposed solution to a problem requires a carefully planned experiment; an experiment crafted to elicit a specific response appropriate to that hypothesis and no other. Little and Hills (1978) suggested the following characteristics of a well planned experiment:

1. Simplicity
2. Appropriate degree of precision
3. Absence of systematic error
4. Adequate range of validity of conclusions
5. Provision for calculation of the degree of uncertainty

These authors further listed the steps in an experiment that should be considered in planning:

1. Definition of problem
2. Statement of objective
3. Selection of treatments
4. Selection of experimental material
5. Selection of experimental design
6. Selection of the unit for observation and the number of replications
7. Control of the effects of adjacent units on each other
8. Consideration of data to be collected
9. Outlining statistical analyses
10. Conducting the experiment
11. Analyzing data and interpreting results
12. Preparation of the report

The Nature of Fish Production Experiments

Many of the farmers' problems involve some part of the commercial production sequence. Solution of these problems usually requires that the part of the sequence in question be established in a series of experimental plots (ponds), and possible solutions to the problem are applied as treatments. Later, the comparative yield of products from the experimental plots is used as an indication of the effectiveness of the respective proposed solutions (treatments). Production experiments play a central role in agricultural research (LeClerg *et al.* 1962). Without the use of these techniques, agriculture would not have advanced to its present state. Consequently, the philosophy and methodology have been afforded considerable attention. Production experiments are also used extensively in aquacultural research, but relatively less attention has been given to the philosophy and methodology of the technique.

The production experiment in agriculture consists of determining the yield of plants or animals under some predetermined set of standard conditions over a period of time. The methodology varies considerably depending upon whether the production of plants or animals is being measured, but the principle is essentially the same. Either of these components may be varied. An experiment may be designed to determine the yields from several varieties of rice or several breeds of cattle under a single set of standard conditions. Alternatively, an experiment may be designed to determine the yield from a single variety or breed under several sets of standard conditions. For example, yields may be determined from a single breed of cattle on several rations, or from a single variety of rice using several rates of nitrogen fertilizer. In more sophisticated experiments, both components may be varied in the same experiment. The yield could be determined for several varieties of wheat from experimental plots fertilized with different amounts of phosphorus.

Production experiment techniques employed by the agronomist (LeClerg *et al.* 1962) and the animal husbandman have been largely standardized. A considerable amount of information has been accumulated over the past 100 years. The use of the technique in aquaculture is not so well advanced, primarily because relatively less is known about the two components, the fish and the standard conditions (the environment), and their interactions, in aquaculture than is known about the two components in production experiments

in agriculture. For example, the agriculturist generally is using a plant or an animal where the genotype and the phenotype are relatively well known. This is not the case with the aquaculturist. Most of the species of fish that are being cultured are at best only a few generations removed from their wild parents. Genetic variation in these species of fish may be considerable. In most cases, little or nothing is known about this variation, yet it becomes part of the experiment. Further, standard conditions are difficult to develop and maintain in aquacultural research. Experimental ponds may be filled with water from the same source and receive fertilizer of the same type and amount. Yet, in adjacent ponds treated in an identical manner, the type of plankton bloom may be quite different. Obviously, the fish in those two ponds will not be subjected to the same conditions. In one pond, a dense population of *Microcystis* sp. may develop as a surface scum which restricts the penetration of sunlight, resulting in lower oxygen production. In the adjacent pond the water may remain relatively clear. It is questionable whether these two ponds can be considered replications of the same treatment.

Although a production experiment is planned to measure the yield from a particular species of fish under a specific set of standard conditions, a completely different set of standard conditions may develop. For example, rather than measuring the effect of the specified set of conditions on yield, the experiment may be measuring differences in water chemistry from one pond to another, effects of disease in one or more ponds that have gone undetected, or differences in the availability of natural food that are unrelated to the treatments or differences in plankton.

What Research To Do

There is virtually no limit to the number of experiments that minds of scientists can imagine they would like to do, and they are adept at explaining how each is justified. This intuitive, inquisitive spirit is one of the reasons for the success of the scientific method in producing new insight and new knowledge. Unfortunately, because of the high cost of research, it is not possible for scientists to do all the things they would like to do with the hope that some of them will produce beneficial results.

How can scientists be encouraged to be creative and at the same time do "important" things? How does the aquacultural scientist identify those important things that should be done? First, it is

important to realize that the immediate client of research is the fish farmer but that the ultimate client is the consumer. The farmer and his problems are of major importance. He is the one who will use the information; however, he pays only a small portion of the cost of the information he needs. Most of the cost is borne by taxpayers in general, or consumers, who will ultimately benefit from more efficient production techniques or better aquacultural products.

Aquacultural research can provide information that will be used in the production of more fish for food and more income. The immediate goal is to help the farmer. This help can be provided in three ways:

1. Solve immediate problems that are limiting farm production and income.
2. Work on problems expected in 3-5 years.
3. Try to predict the problems and the needs of the farmer 15 to 20 years hence and begin long-term basic research and development.

Most of the research effort (approximately 75 percent) should be devoted to solving immediate, day-to-day problems. The production of a crop of fish is a rather complex sequence of events. Numerous constraints occur or develop. Further, as one constraint is removed another will likely develop. Constant attention of the scientist to the production sequences will indicate problems that should be solved.

Approximately 20 percent of the research should be directed at solving problems expected 3-5 years in the future. The effective researcher should be aware of regional, national, and international trends that will affect the farmer. Of course there is uncertainty in predicting the future, but it is necessary to attempt to guess at changes that will take place. For example, in the United States, the cost of energy will likely have an important impact on both the production and marketing functions of aquaculture. The effective researcher will attempt to visualize what effects this factor will have on the entire production and marketing sequence over the next 3-5 years and will plan appropriate research.

Some 5-10 percent of the research effort should be on basic problems that may have no immediate application, but which will contribute to the understanding of the life processes underlying applied production and marketing procedures. Basic research on physiology, nutrition, breeding, and pathology should be con-

ducted along with applied research; however, emphasis on basic research must be controlled. LeClerg, *et al.* (1962) warned that one of the most difficult problems in the development of a research program is the maintenance of an effective balance between applied and basic research. Basic research is generally easier to do, requires less facilities, is less expensive, and likely to be more professionally rewarding to scientists. Scientists are more likely to earn the acclaim of fellow scientists through basic research than when working on farmers' problems. Publications based on basic research are usually easier to publish than those reporting research on practical, farm problems.

Given the almost infinite number of possibilities for experiments in basic research, relevancy can be ensured by encouraging a close association between basic and applied research. Decisions regarding what basic research to undertake should be based on results of the applied research that is being done. Often necessary research on an applied problem cannot be designed because of the lack of a critical piece of information that can be provided only from basic research. If the two types of research are closely linked they complement each other effectively.

As noted previously, in deciding what research to do, it is important to keep in mind the needs of the consumer as well as the farmer. It is the farmer who has the problems with production and marketing, but it is the consumer who will pay for most of the research. Achieving a balance between the needs of the two is further complicated by the fact that conditions that benefit one group may be detrimental to the other. The consumer is benefitted when food production is efficient and there is a surplus, causing prices to be low. Unfortunately, these same conditions are detrimental to farmers. Farmers are benefitted when food supplies are somewhat restricted commensurate with demand, thus forcing prices up. When food prices rise, the consumer becomes interested in investing more money in research, hoping thereby to bring about increased production and lower prices. At the same time when prices received for their crops are high relative to costs, farmers may lose interest in research.

There are three broad classes of consumers that should be given consideration in research:

1. Low income people—Purchase of food requires most of the money available.
2. The middle class—There is some discretionary income remaining after food needs are met.

3. The wealthy—Food purchases represent a relatively small fraction of family expenditures.

Low income consumers can afford only the less attractive, poorer quality fish. In some countries, these people are able to purchase fish just before they spoil. Fish fresh from the farmer bring high prices shortly after reaching the market. In the absence of adequate refrigeration and storage, price and quality decrease with time. Before the fish must be discarded, low income people are able to purchase them. Further, these consumers cannot afford to pay for special packaging and preparation or any other service that increases the basic cost. This group of consumers presents the researcher with special problems. They pay a relatively small portion of the cost of research and generally have little voice in setting research priorities. Further, farmers have less interest in consumers with little money with which to purchase fish. They are unlikely to grow fish that the poor can afford to purchase. Yet, these poor people must be given some consideration through research. A nation cannot be wealthy where there are many hungry people because they cannot afford to purchase food.

The middle class consumer is extremely important to the fish farmer; most of his sales will be to this group. They can afford better quality fish and some added costs of special processing and handling. This group also pays most of the costs of research.

The wealthy class, although not large, is important to fish farmers. They are willing to pay high prices for certain species. They are also willing to pay for special sizes of fish, fish imported from other countries, and fish that have been given special treatment and handling.

Identifying Specific Research Needs

To identify the specific information needs of farmers, the researcher must identify with the farmer. The classical closed loop system among the farmer, the extension worker, the researcher, and the teacher, described in a previous section, provides a feedback system by which the needs of the farmer can be transmitted back to the scientist. He must be close enough to the whole production process to have an intuitive feeling about the nature of the problems. Researchers with a farm background often have this intuitive feel for problems that scientists without that background do not have. When the researcher does not have this background,

he should be prepared to spend some time becoming familiar with the farm situation.

In many cases, environmental or economic conditions may be such that there is no practical way the fish farmer can be successful regardless of the effort expended by researchers and extension workers. For example, fish farmers in an encroaching urban area will probably be displaced because of increasing land values and zoning restrictions; there is little that aquacultural research can contribute. Farmers must deal with many different types of problems. Only a few of them are amenable to solution through research. To be effective, a researcher must be able to identify those problems that can be solved through research and those that cannot.

Deciding what research to do is extremely important. General David Sarnoff is reported to have said that 40 percent of inventing is knowing what to invent (David 1980). A similar statement could be made regarding research; 50 percent of being successful in research is knowing what research to do. Some scientists decide what research to do almost intuitively. Where the intuitive approach is lacking, the farmer's operation and his production scheme may be analyzed in a systematic fashion and the decision can be made without difficulty.

The number of problems that can be studied by scientists is virtually limitless. Some method must be used to select those for evaluation that will provide the most information on critical fish farming problems. A systematic procedure should be used which leads quickly to the most critical hypotheses or to propose the most penetrating questions for consideration in research. This approach involves systematically breaking down farmers' problems into smaller and smaller pieces or sub-problem units until a simple question can be proposed that can be answered, altered, or revised by an experiment or a series of experiments. This procedure of deciding what experiments to conduct is like climbing a tree (Platt 1966). One begins at the base, then proceeds up the trunk to a fork where a decision is made to go right or left, to a second fork where a decision is required, and so on. When rigorously pursued, the method encourages one to continue to climb rather than stopping at a fork to view the scenery.

A schematic representation of the sytematic, "decision tree" procedure for deciding what research to do is shown in figure 15. For example, at step 1, discussions with farmers might indicate that their major problem is that they simply aren't making any money.

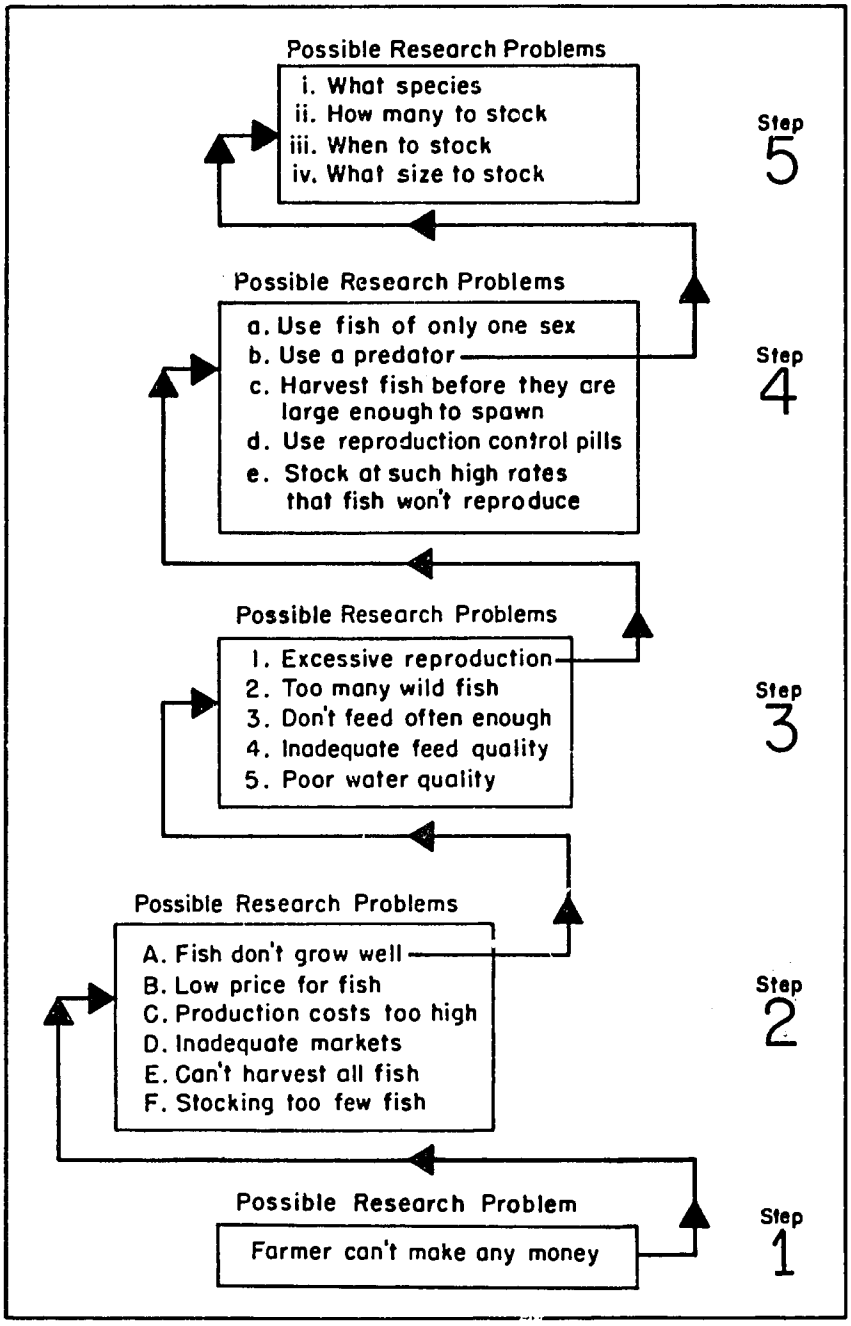


FIG. 15. Schematic presentation of a systematic procedure for making decisions on research needs.

This problem is a broad, general one. Few farmers anywhere in the world would admit that their return on investment is adequate. This problem is too broad and general to use as a basis for deciding what specific research to do to assist them. Obviously, an effective experiment cannot be conducted on why the fish farmer isn't making any money. The next step (step 2) is to break the general problem from step 1 into a series of possible sub-problems or alternative hypotheses. A few examples are listed at step 2. There might be many others. Listing alternative hypotheses in this manner forces the scientist to focus on the farmer's entire operation and aids in the process of deciding the key problems in the production system that might require research. More will be said about the value of utilizing alternative hypotheses later.

Each of the proposed explanations (hypotheses) of why farmers aren't making money should be evaluated using inductive or deductive logic, common sense, observations, literature surveys, or possibly experimentation. For example, it was hypothesized that the farmers are not making any money because their production costs are too high. This hypothesis might be rejected when it was determined from examining farm budgets that the cost of inputs is relatively low. Similarly, the hypothesis that profits are low because of inadequate markets is rejected when it is determined that customers are clamoring for fish. Each of the alternative hypotheses would be evaluated and rejected or accepted.

Assume that after consideration of all the alternative hypotheses in step 2, only the first one is accepted. It might be determined that the most serious problem was that fish grow slowly or not at all and that few reach marketable size. As before, this problem is still general in nature. Because it is impractical to design a definitive experiment at this point, another series of alternative hypotheses should be written as shown in step 3. These hypotheses are evaluated as before. Assume that it is determined through observation and analysis that all of the hypotheses except the first should be rejected. After careful consideration, it is decided that there are too many small fish in the ponds for the food available. Reproduction of fish stocked in the production ponds is excessive. Here is a problem that merits consideration for research. Unfortunately, there are numerous possible solutions. Several possible hypotheses can be formulated. Some further refinement is needed. A list of proposed solutions is shown at step 4.

Remember that the objective of fish farming research is to solve

problems that make it difficult for farmers to increase production and/or to make a profit. We are attempting to determine what experiment(s) can be designed that might lead to solutions to those problems. We are trying to reduce complex, many faceted problems to a manageable dimension through a logical, systematic process. We are attempting to reach a point where a hypothesis can be formulated that can be tested with a specific experiment. The list of possible solutions (hypotheses) includes some fairly obvious ones and others not so obvious. In this situation, it is usually a good idea to "brainstorm." Solutions that on first consideration may seem to offer little promise, may on further study or through the development of new technology be the most successful.

All of the possible solutions (hypotheses) to the problem of excessive reproduction (step 4) may require research. In most cases, only one can be chosen for initial attention. The choice should be based on facilities and resources available and a best guess as to which has the most immediate promise of success given the nature of the production process being used by the farmers. For example, it might be decided, based on available information, that the most practical solution to the problem of too many small fish would be the addition of predatory fish to the production ponds to consume excess reproduction.

The systematic "decision tree" process has led us to the point where it is possible to formulate a hypothesis and to design an experiment to test it. For example, we could formulate a hypothesis that growth of stocked fish in ponds containing no predators is equal to growth of fish in ponds containing 200 predators per hectare. For this experiment, six to eight ponds would be stocked with the species the farmers use. Three or four of the ponds would be stocked with predators at a rate of 200 per hectare. All of the ponds would be treated alike, using the same management procedures (feeding, fertilization, disease treatments) normally used by farmers. At the end of the usual production period, the ponds would be drained and the growth rates of stocked fish in ponds with and without predators compared and the hypothesis of "no difference" accepted or rejected. The same experiment could be used to test the hypothesis that there is no difference in the number of young fish (reproduction) in ponds with and without predators at the time of draining. There are numerous other hypotheses that might be formulated and tested with the same experiment. In fact, scientists representing several disciplines might collect data during the experiment which

would allow them to test hypotheses related to the presence or absence of predators.

The experiment described above is designed to answer a rather specific question. It would allow us to reject or accept the hypothesis that stocking 200 predators per hectare would result in a higher growth rate of commercial species or in smaller numbers of small fish. Unfortunately, before we could go to the farmer with that information, further refinement is required. For example, only one stocking rate was tested. Another rate might be superior.

Refining and focusing the research might take the form of deciding what information farmers will need to utilize predators in their ponds. Four obvious questions that must be answered are shown in step 5 (figure 15). Each of these questions can be answered or be further refined with the use of an experiment. There are still some questions that must be answered before a definitive experiment can be designed. For example, a decision must be made on which species to use in the experiment. There are several hundred or maybe even thousands of species of predators in the world. Obviously all of these cannot be evaluated. The list of possible candidates for use can be determined in part based on reviewing the experiences of others in the literature, personal knowledge of the biology of various species, observations of natural predator-prey populations, intuition, and the practical matter of availability. In the extreme case, a series of tests might have to be conducted to screen a large number of available predators for use in pond fish production. Once the more promising ones are identified, definitive research could be initiated to answer the specific questions posed in step 5. A similar decision would have to be made on the number of predators to stock. The decision could be based on intuition, the work of others, or observations of natural fish populations in rivers or lakes.

The questions of "when to stock" and "what size to stock" also might have to be answered through experimentation; however, it is likely that intuition, observation, and deductive logic would provide sufficient information so that separate experiments would not be required. For example, observations on the spawning habits of the "prey" species in the farmers' ponds would give an indication of when the predators should be introduced. Similarly, observations on the size prey that a given size predator can swallow or studies on the food habits of predators in the wild to see what sizes of prey they prefer could be used as a basis to determine what size of predator to stock in the experiment.

Assuming that experiments would not be required to determine the size to stock and the time to stock, an experiment could be designed to compare the effects of three stocking rates of a predator in reducing the number of small fish in farmers' ponds. Other experiments would be required to determine the best stocking rate for other promising species. A more effective approach, if facilities are available, would be to compare promising species at different stocking rates simultaneously. For this purpose, the following experiment might be conducted:

Number of predators stocked per hectare	*Species of predator stocked*		
	A	*B*	*C*
100	3[1]	3	3
200	3	3	3
400	3	3	3

[1]Replications.

Each of 27 experimental ponds would be stocked with fish using the same species, size, and stocking rate generally used by farmers. The ponds would be treated in exactly the same manner as the farmers treat theirs, except that in this case three species of predators would be stocked according to the schedule in the table. At the end of the culture period, the effect of the predators would be determined by comparing total fish production and the production of young fish in the ponds. If 27 similar ponds were not available, the researcher might choose to determine the value of only one species of predator or possibly only two stocking rates. This would require fewer ponds. Other species and stocking rates might be evaluated in future years.

Note that this experiment would allow us to evaluate a number of hypotheses comparing the three species and the three stocking rates. It would not allow evaluation of the hypothesis that the use of either of the three stocking rates of the three species are better than using no predators at all. This comparison could be made only if some ponds without predators were included in the design.

The procedure outlined for deciding what research to do is based on a rather simplified example. Nevertheless, the systematic, logical methodology is applicable to more complex situations. The procedure of writing down each step is essential. Without going through this exercise, it is too easy to overlook promising solutions to problems. Further, trying to follow the system without listing the steps usually results in undue emphasis on those solutions of most interest to the researcher. By writing down several possible so-

lutions to the problem and different levels of sub-problems, the researcher is forced to think more deeply about the process of problem solving and to do so in a logical manner.

Platt (1966) outlined a similar procedure for choosing research problems and designing experiments which he calls the strong inference method. He traces it to Sir Francis Bacon, whom he quotes as saying, "My way of discovering science goes far to level men's wit and leaves but little to individual excellence because it performs everything by the surest rules and demonstration." The strong inference method involves the systematic application of inductive and deductive reasoning so as to prevent dawdling or wasting precious resources on irrelevancies. It encourages "problem" orientation rather than "method" orientation.

The logical procedure described above will be strengthened considerably if associates are asked to review the scheme leading to a decision on what research to undertake. There is certainly truth in the old adage that "two heads are better than one." Unfortunately, too many scientists are as protective of their ideas as they are of their children. They simply refuse to offer them for constructive criticism, even if it means that their ideas will be improved in the process. The procedure of listing multiple solutions (alternate hypotheses) will alleviate this problem somewhat. A scientist who has proposed only a single possible solution to a problem tends to be overly protective of it. Criticism or suggestions directed against a single solution are viewed as a personal attack. Where several solutions are proposed, suggestions or criticisms are more easily dealt with. A system of proposing multiple solutions also encourages more effective review by other scientists who are reluctant to offer constructive criticism when it must be directed at a single proposed solution.

It is not necessary to repeat this entire sequence of steps each time a new experiment is to be designed or another question is to be asked. In some cases, the specific answer to a problem might require several years of experimentation. For example, in the situation described previously, with less than 27 experimental ponds available for the research, several years might be required to determine the best predator species to use and the optimum stocking rate. In most cases, however, it is a good idea to go through the process of identifying the most promising research relatively often. This procedure prevents spending time on areas of research of interest to the researcher long after the fruitful aspects have been investigated. It

also tends to sharpen ones inductive inferences and the subconscious mental process (Ladd 1979) so that more sharply drawn experiments can be designed.

Factors to Consider in Designing Fish Production Experiments

What is the Question?

Probably the most important aspect of the design of a production experiment is the question that is being asked. Effective solutions cannot be proposed or good hypotheses formulated without a clear understanding of the exact nature of the question involved. In a previous section, considerable emphasis was devoted to identifying farmers' problems and to developing questions and proposed solutions (hypotheses) related to those problems that could be answered or evaluated with an experiment. Unfortunately, in too many cases the experiment that is being designed will provide an answer to a different question than is being asked. For example, an experiment may be designed to answer a question about the effects of various types of inorganic fertilizer on the production of a species of tilapia *(Tilapia sp.)* in ponds. Unfortunately, the relationship between the addition of inorganic salts to a container of water and the yield of fish is complex. The route from inorganic phosphate or nitrogen to tilapia protein, fat, and bone is tenuous. Rather than a single experiment, several are being conducted simultaneously. There is the production of a number of species of phytoplankton, the production of aquatic insects from the phytoplankton and zooplankton, and finally there is the production of tilapia from the phytoplankton, the zooplankton, and the aquatic insects. Rather than a single question, several have been asked. The investigator may simplify the question by relating the type of fertilizer to the production of tilapia; however, the process by which the fertilizer is converted to tilapia is not simple. When ponds are drained, the weight of tilapia produced in each pond is related to the type of fertilizer. In reality, the type of fertilizer was only one factor influencing the yield of tilapia and may have been no more important than a number of other factors. When an experiment is being planned, the aquacultural researcher should be aware of the various questions that the particular experiment is answering. Only in this way can he know if he has any chance of answering the question that is of primary interest.

Accuracy Required

Accuracy of information is an important factor to consider in the design of production experiments. If the information is to be used by fish farmers, the accuracy may have to be of a different magnitude than if the information is to be used as a basis for further research. Fish farmers should not be given unsubstantiated information. They generally do not have sufficient captial to gamble on new methods unless the probability of success is high. In many cases, one poor crop of fish can have a disastrous economic effect. Consequently, production experiments that are to provide information for direct use by fish farmers should be designed so that there will be little doubt of the validity of the conclusions.

Where the information is to be used by fishery scientists or in the design of other experiments, the accuracy of the conclusion may be of less concern. This does not mean that these experiments can be conducted in haphazard fashion. Where the information is to be used only by other aquacultural scientists as a basis for future research, more complicated experimental designs may be utilized. In many cases, the effects of interactions are more important to the scientist than the main effects of the treatments. Learning something of the nature of an interaction between two factors may have greater significance than learning that there is a difference in the effect of the treatments. In the design and analysis of experiments, if extra precaution is taken against drawing an incorrect conclusion, the probability is increased that promising differences in treatments will be overlooked. For a discussion of this subject and its relevance to experimentation in agriculture and biology, consult one of the many excellent biometry and biological statistics textbooks available. A particularly good treatment of this subject can be found in the book by Sokal and Rohlf (1969).

Significant Differences and Measuring Systems

In planning an experiment, the researcher must deal with the question of significant differences. The concept of statistically significant differences plays an important role in problem solving through experimentation. Statistical science and the determination of significant differences provide an objective basis for the analysis of problems in which the data depart from the laws of exact causality (Little and Hills 1978). Unfortunately, too much emphasis can be placed on significant differences. Stoltenberg *et al.* (1970) observed that headlong pursuit of significance may lead researchers to design

larger and more rigidly controlled experiments so that they can detect smaller and smaller differences. This emphasis acknowledges no optimum level of time and money spent.

Research to provide new information for fish farmers is a complex interaction of physical and biological science and of philosophy. The goal of the research is to find new treatments or new ways of doing things that will result in increased production, increased quality, or reduced costs. The decision as to whether two treatments are different or whether a new method is better may be complex. Most often the researcher is concerned only with differences in treatment effects or whether the mean of one treatment is greater or smaller than the mean of another. When two or more treatments are applied in an experiment, they are always different. By definition they are different. Further, their effects, by definition, must be different; however, because of the type of measuring device or system used, differences in effects may not be detected. For example, two pond fertilization treatments differing by only a few grams of phosphorus per application are different treatments. They are different by definition. Further, the effects are different. A suitably sensitive test would demonstrate that different amounts of phosphorus also would have an effect on the biological systems of the pond waters. If the element was labeled with radioactivity, it probably could be shown with a suitably sensitive instrument that there was more phosphorus in the plankton in the ponds that had received the larger amount. It is not likely that the small difference in treatments would be reflected in measurable differences in fish production; however, if an adequate measuring system were available, it probably could be demonstrated that the small amount of phosphorus did have an effect. Unfortunately, the production experiment in aquaculture is not a highly sensitive measuring device. Similarly, there would be little economic value to the farmer in changing to a new fertilization regime which involved adding a few additional grams of phosphorus per application. The two phosphorus treatments probably are different by definition, different by physical effect, but not different by biological or economic effects because of the insensitivity of those measuring systems. Other treatment combinations might differ by definition and by physical and biological effect, but not by economic effect. For example, two fish feeds differing in protein content by 50 percent might be compared in an experiment. Obviously the two treatments would be different by definition, by physical effect, and likely by biological effect (fish production), but if the higher protein

feed cost 50 percent more, there might be little if any economic difference. In fact, the farmer might actually make less profit by using the higher protein feed.

The identification of differences between treatments depends on the sensitivity of the measuring system employed. An important aspect of planning experiments is the choice of a measuring system that is capable of detecting differences appropriate to the researcher's needs. The logical nature of the system of comparing different treatments forces the investigator to face the dilemma of devising or choosing a measuring system that will detect those particular differences that are of interest to him or his client, if they do exist, but not those that are of little interest. Citing a previous example, research probably could demonstrate that there was a difference between the effect of adding two levels of phosphorus fertilizer on the phosphorus content of the water; however, if the primary interest was in recognizing differences in economic effect of the two treatments on fish farming, a completely different type of measuring procedure could be used. In this situation, the investigator is forced to devise a measuring device that is insensitive enough not to recognize those differences that are of no interest.

In experiments in earthen ponds at Auburn, the coefficient of variation (100 x standard deviation/mean) is approximately 20 percent. With this amount of variation in ponds treated alike, it is not practical to detect small differences in treatment effects on fish production if the usual statistical tests are used. For example, in an experiment with four replications per treatment, assuming a coefficient of variation of 20 percent, the usual statistical tests would not detect differences less than approximately 25-30 percent of the mean even if they existed. In an experiment where the mean is 1,000 kilograms per hectare, differences smaller than 250-300 kilograms would not be judged significant. Differences smaller than this have relatively high probability of being the result of chance variation rather than treatment effect. This pond production measuring system is not sensitive enough for detecting small differences that are of interest in certain types of basic research; yet, it is sensitive enough to detect differences important to the farmer.

Measuring systems that are not sufficiently sensitive to detect differences of the desired magnitude should be avoided for obvious reasons. Similarly, there are problems resulting from the use of measuring systems that are too sensitive. Where the system is excessively sensitive, differences may be detected that have little

meaning in a specific situation. There is also another problem. The cost of a measuring system is usually proportional to its sensitivity. A balance that weighs to the nearest milligram is generally more costly than one that measures to the nearest kilogram. Similarly, a pond production measuring system that will detect differences as small as 50 kilograms per hectare would likely be more expensive than one that would detect differences as small as 200 kilograms per hectare. The use of an excessively sensitive (excessively expensive) measuring system not commensurate with the need for the degree of sensitivity of the information required is unwarranted.

When the information is to be used by farmers, the experimental measuring system should closely approximate the farming system (Brady 1981). In this way the researcher can detect those differences that are of importance to the farmer. This is the reason why research to provide information to the farmer is so site specific. Experiments conducted in an area dissimilar (different soil, microclimate, water quality) to the area where the farmer will use the information will involve a different measuring system. In an experiment, the pond soils, water quality, and microclimate and their interactions are part of the measuring system. Where the conditions in the research and the farming area are different, results obtained in the research area may have limited applicability in the farming area.

Because of the wide variety of experiments that might be conducted, it is not practical to pose even general rules for selection of effective measuring systems. The only generalization is that the choice of a measuring system is extremely important in the detection of differences. That choice should be done with care and after considerable study and deliberation.

There is another matter to be considered in selecting measuring systems. The very act of measuring changes the system in unpredictable ways. When an experimental pond is seined to remove a sample of fish to determine growth rate, it is changed. The act of seining renders it so. Abnormal currents may be created as the seine travels along the bottom that will change the benthic environment from what it would have been otherwise. Similarly, the simple act of removing a fish from the water changes it. If we attempt to weigh it with a high degree of accuracy, it must be blotted dry, which could affect the response of the animal to pathogenic bacteria or viruses or affect its growth rate. Goldstein and Goldstein (1978) refer to this as the uncertainty principle. We are unable to measure anything we want, with any accuracy we choose.

Experimental Variation

Variable production in ponds, troughs, or cages treated alike (experimental error) is an important consideration in designing experiments (Wohlfarth and Moav 1968). Prior information on experimental error is especially important in determining the number of replications required to demonstrate a significant difference between two or more treatments if such a difference, in fact, really exists. Also, information on experimental error is helpful in determining if the differences in treatments can be detected using a set of experimental conditions or a specific measuring system. Obtaining this estimate of variation in advance may be difficult, especially when the experiment is being developed in a new or relatively new area of aquacultural research. Generally, however, the experienced aquacultural scientist can make an educated guess of the expected variation from experiments conducted previously.

Through the years a considerable amount of information has been accumulated at Auburn and at other research stations on experimental error in aquaculture (Swingle *et al.* 1963; Buck *et al.* 1970; Annual Reports of Auburn Fisheries Research Unit 1952, 1962, 1969; Shell 1966; Tiemeier *et al.* 1964; Loyacano 1970; Prather 1958; Nail 1962). Although direct use of the information in other parts of the world is questionable, it should serve as a guide to the magnitude of variation

TABLE 1. RANGE OF COEFFICIENTS OF VARIATION OBTAINED IN A NUMBER OF PRODUCTION EXPERIMENTS WITH SEVERAL SPECIES OF FISH AND IN SEVERAL TYPES OF EXPERIMENTAL UNITS

Species of fish	Experimental unit					
	Earthen ponds		Plastic pools		Cages	Troughs
	Feed	Fertilizer	Feed	Fertilizer	Feed	Feed
Channel catfish	15.1-15.7	11.5-23.3 11.4-34.6	--	56.6-81.5	3.2-5.5	1.6-6.8
White catfish (*Ictalurus catus*)	9.4	--	--	--	--	--
Common carp (*Cyprinus carpio*)	--	8.6-18.3 12.3-22.7	--	--	--	--
Largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*)	--	4.2-10.1 10.4-24.8	--	--	--	--
Java tilapia (*Tilapia mossambica*)	--	--	--	11.6-32.3	--	--
Fathead minnow (*Pimephales promelas*)	3.1-11.5	--	--	11.9-51.9	--	--

TABLE 2. FREQUENCY OF OCCURRENCE OF COEFFICIENTS OF VARIATION FROM 13 FISH PRODUCTION EXPERIMENTS RECORDED IN TABLE 1

Class values of coefficients	Frequency
1- 5	8
6-10	13
11-15	10
16-20	7
21-25	6
26-30	4
31-35	3

in response that might be expected. A summary of this information is presented in tables 1 and 2. The variation is reported as coefficients of variation (standard deviation/mean, expressed as a percentage).

Simpson *et al.* (1960) and Snedecor and Cochran (1967) have discussed the value of the use of the coefficient of variation and its utility in planning and evaluating experiments. The data presented in tables 1 and 2 indicate that coefficients of variation in production experiments with fish were variable; however, a majority of the coefficients were in a relatively narrow range. The data presented in table 1 represent 13 different experiments. Included were 56 different treatments and 274 replications. These are distributed as shown in table 2. Five of the coefficients were so far outside (51.9-81.9) the range of the remainder that they were not included in the frequency table. Although the coefficients represent a wide variety of experimental conditions, they appear to be distributed in a somewhat normal fashion with some positive skewness. Thirty-one of 51 of the coefficients are 15 percent or less.

Snedecor and Cochran (1967) noted in corn variety trials that although mean yield and standard deviation vary with location and season, the coefficient of variation is often between 5 and 15 percent. Apparently there is a considerable degree of constancy in coefficients of variation in production experiments involving fish as well as corn.

There is not sufficient information available to analyze the causes of differences in coefficients of variation encountered. Most of the information presented previously is based on experiments in earthen ponds with several different species of fish and on experiments with channel catfish in several types of units. Coefficients obtained from experiments in earthen ponds varied widely within a range of 4.2 to 34.6 percent. Feeding did seem to result in lower

coefficients in earthen ponds as compared to fertilization. The distribution of coefficients was generally similar for experiments in earthen ponds with all species.

Coefficients of variation from experiments with the channel catfish in the several types of experimental units varied more widely (1.6-81.5 percent) than the coefficients from earthen ponds with several species of fish. Both the highest and lowest coefficients obtained were with channel catfish (1.6 percent with catfish in cages receiving feed and 81.5 percent with catfish in plastic pools with fertilization).

It is significant that the lowest coefficients were obtained in rather artificial environments (cages and troughs) where the fish were stocked at high rates and fed a nutritionally complete ration. Coefficients for the experiments with channel catfish stocked in cages ranged from 3.2 to 5.5 percent. Coefficients for experiments with channel catfish stocked in troughs supplied with running water ranged from 1.6 to 6.8 percent. These data indicate that the primary causes of variation are differences in environmental conditions (water quality, food production, etc.). This conclusion is supported by results obtained by Pretto (1976) in an experiment on the production of channel catfish in polyculture research. In the experiment involving 31 earthen ponds, he managed the water quality of each pond individually. He did whatever was necessary (stopped feeding temporarily, aerated, added ground limestone, added inorganic fertilizer) to maintain good water quality. By doing so, he obtained an average coefficient of variation of 2.4 percent.

Number of Replications

One of the important decisions to be made in the design of a production experiment is the number of replications required. An experiment often involves determining whether there is difference in the effect of two or more treatments on production of fish in experimental units (ponds, tanks, cages, troughs). Unfortunately, because of experimental variation (normal differences in experimental units treated alike) it is often difficult to determine whether an observed difference or lack of difference between treatments is real or the result of chance variation. To overcome this problem, each treatment is applied to a number of similar units (replications) to lessen the likelihood of chance having a major effect on the decision. It is assumed that while chance might cause a wrong decision where only a single observation is involved, it would be highly unlikely that it would affect a decision based on several observations.

It is important that an estimate of the number of replications required be determined during planning. Even when there are true differences in treatment effects, they may be masked because not enough replications are used to get a good estimate of the effect of the treatment. In those cases, it is a waste of effort to conduct the experiment; the conclusion is determined before the experiment begins. On the other hand, because of the cost of replicating experiments, the use of too many replications is also a mistake. The proper approach is to use only enough replications, given the amount of experimental variation expected, to show differences in the effect of the treatments assuming that such differences do, in fact, exist. Considerable amounts of research funds and research effort are wasted each year on experiments that were doomed to failure before they are started. Another possible unfortunate result is that promising new treatments or procedures that would be of significant benefit to fish farmers might be discarded because too few replications are used to provide a definitive answer as to their effects on yield.

It is beyond the scope of this book to describe the procedure for determining the number of replications required. Many textbooks of statistics contain explanations of these procedures. One of the better of these books is by Sokal and Rohlf (1969). If those planning experiments are not conversant with statistical procedures and terminology, they should consult a biometrician for assistance.

Controls

Experiments in aquaculture often involve the comparison of a number of treatments, one of which is a control. For example, an experiment might be designed as shown in the following table:

Treatment			
No fertilizer	*0-8-2 fertilizer*	*8-8-0 fertilizer*	*8-8-2*[1] *fertilizer*
4[2]	4	4	4

[1]8N - 8 P_2O_5 - 2 K_2O.
[2]Number of replications.

In this example, the "no-fertilizer" treatment would be the control. Controls are an important element of many aquacultural experiments. In the example, the "no-fertilizer" treatment provides a reference point for the evaluation of the other treatments. Also, this particular control when used in experiments over a long period of

time provides a mechanism for continuous evaluation of the measuring system. For example, research on pond fertilization was begun at Auburn in the mid-1930s. The early experiments always included a "no-fertilizer" control. In later years, other experiments were conducted on pond fertilization. These latter experiments also included "no-fertilizer" controls. It was noted that over a period of time fish production in response to the "no-fertilizer" treatment increased significantly. Apparently, the inorganic nutrient concentrations increased in the muds, changing the ponds as experimental units. The measuring system involving the same ponds was not the same in the mid-1960s as it was in the late 1930s. Without the continuing use of the control, this change would not have been detected.

Another example of the role of controls in research and of changes in a measuring system with time is available in research completed by Pongsuwanna (1960). He ran a series of tests to determine whether certain chemicals would increase the survival of the fathead minnow under conditions simulating those encountered in hauling the fish to market. He compared the survival of fish in jars containing the various chemicals to the survival of fish in containers receiving no chemical (the control). These tests were conducted over a period of several months. In all tests, the containers were stocked with similar sizes and weights. Table 3 includes data on the percentage survival of fish in the control containers in 10 different tests over a 6-month period. These data indicate highly significant differences in the survival of fathead minnows under similar experimental (control) conditions over time. Apparently the fathead minnow changed with time as an experimental animal. The measuring system was not exactly the same at different times of the year. The use of controls in that experiment was essential. The controls

TABLE 3. PERCENTAGE SURVIVAL OF SIMILAR NUMBERS AND WEIGHTS OF FATHEAD MINNOW IN CONTAINERS USED IN 10 TESTS OVER A 6-MONTH PERIOD (DATA FROM PONGSUWANA 1960)

Date of test	Percentage of survival
April 25-26	13.1
May 23-24	3.3
June 30-July 1	1.7
July 12-13	9.4
July 29-30	84.2
July 29-30	88.9
September 10-11	95.7
September 28-29	68.0
September 28-29	59.3
October 3-4	44.6

also provided some valuable information on the relative resistance of these fish to death while being held in crowded conditions.

In some experiments, the control is a standard management technique currently used by farmers. The other treatments may be promising new techniques that might improve fish farm production systems. In this case, the control serves as a reference or a standard with which the new techniques can be compared. In this situation, the control is much like the standard chemical solution that is used to calibrate a measuring instrument. If the production in the control changes appreciably in successive experiments, the researcher will have serious problems evaluating (the potential worth) new techniques for the farmers. The use of the standard technique as a reference will be difficult. Of course the experiment itself might be carried to a successful conclusion and comparisons made between the standard and new techniques; however, because the measuring system has changed, making recommendations to the farmer based on the experiment would be risky.

Hollerman (1980) conducted an experiment on the use of nighttime aeration to increase production of channel catfish. He noted that harvest weights in the unaerated treatment (controls) were much less than those reported by Tucker *et al.* (1979) in an experiment in the same ponds a year earlier. Stocking rates and feeding rates were the same in both years. Hollerman concluded that the differences in the two experiments resulted from differences in the amount of solar radiation received by the ponds in the 2 years. In 1980, radiation per day was 414 langleys, while in 1979 the ponds received an average of 344 langleys per day or 17 percent less. The measuring system obviously was different in the 2 years. Depending on the degree of change, significant differences could be demonstrated between the standard and new techniques in the experiment, when in actual practice the new techniques are no better or possibly poorer than those the farmer is already using.

In some types of aquaculture experiments a simple approach to the use of controls can lead to serious problems in the evaluation of the experiment. For example, in an experiment to compare two devices used to increase the carrying capacity of ponds by aeration, the following scheme might be used:

Treatment		
No aeration device	*Aeriation device No. 1*	*Aeration device No. 2*
3[1]	3	3

[1]Number of replications.

In the experiment the fish are stocked at a relatively high rate because aeration can exert an effect only after the "normal" carrying capacity of the pond is exceeded. The fish are fed daily at 3 percent bodyweight. The "no-aeration" treatment is included as a control or a reference point against which the value of aeration with two different devices can be compared. All experimental characteristics (size of fish stocked, number stocked, weight stocked, size of ponds, amount of feed fed, disease treatments) are the same for all treatments.

Soon after beginning the experiment the researcher is faced with a dilemma. As the "natural" carrying capacity of the ponds is reached, the aeration devices begin to exert an effect; however, in the control, the fish face increased problems with low dissolved oxygen. They may go off feed. If feeding is continued the fish may be killed; however, if the feeding is stopped the control will be compromised and the basic experimental conditions common to all treatments will be altered. Kilgen (1969) encountered a problem of this type in an experiment conducted on the value of polyculture in enhancing water quality and thereby increasing production. In the latter stages of the experiment, fish in the controls died. It was obvious from the experiment that because of polyculture the water quality in those ponds was better; however, because the reference point (the controls) had been lost, it was not possible to determine whether the improved water quality had resulted in increased production. The problem of how to deal with controls in experiments of this type is a complex one. Obviously a reference point is needed; however, if the experimental conditions are altered drastically, for example by stopping all feeding to the fish in the control ponds, the reference point may be compromised. This problem of the management of controls is relatively unique to aquaculture. In experiments involving terrestrial animals, overfeeding is not a major problem. The animals consume all they want and the remainder can be removed and discarded. In aquaculture, if the fish are fed in excess or if for some reason they are not feeding well, the uneaten feed decomposes, leading to a rapid deterioration of the environment and eventually to the death of the fish or at least to reduced growth rate. This unique treatment-environment interaction is the primary reason for the difficulty in managing controls in these experiments.

Pretto (1976) approached the problem of management of controls in a polyculture experiment in a unique manner. His experiment was designed to study the effect of other species of fish on the

production of channel catfish in polyculture. He stocked ponds with the following combinations of fish:

Combination 1 - Channel catfish (the control)
Combination 2 - Channel catfish
Blue tilapia
(Sarotherodon niloticas)
Common carp
Combination 3 - Channel catfish
Blue tilapia
Hybrid buffalo
(Ictiobus cyprinellas x *I. niger)*

He fed all of the fish at the same rate. The amount was based on the weight of fish in the control ponds. By feeding all fish based on the performance of the control ponds, if one of the polyculture combinations was superior in the utilization of food and feed resources and in the maintenance of good water quality, its superiority would be expressed as increased production. He decided that rather than maintain equal inputs to all ponds, including the control, he would vary the inputs to maintain suitable or equal environments. Water quality measurements were made daily. He fertilized, aerated, limed, and interrupted feeding according to the water quality in each pond.

Pretto's approach is an interesting one. In experimentation in agriculture, usually all inputs are the same, except for differences related to treatments. Equal inputs are utilized to establish equal environments in all the replications so that the only differences will be those resulting from the treatments. In aquacultural experiments, however, the use of equal inputs commonly results in unequal environments (for example, ponds treated alike develop different plankton blooms). By varying the inputs according to the water quality, Pretto maintained more or less equal aquatic environments. For example, when the environment in the control ponds began to deteriorate, he changed the inputs to allow those environments to recover. By working to keep all environments equal, he made it possible for any advantage inherent in the treatment (polyculture) combinations to be expressed, yet the control ponds were not eliminated as reference points. Because of the unique interaction between treatments and the aquatic environments, some new approaches to the problem of management of controls must be developed. The proven concepts and principles utilized in terrestrial animal production research are not easily applied in certain

types of aquaculture experiments. The approach used by Pretto represents an attempt to deal with the problem. Other approaches need to be developed. Obviously considerable work remains to be done in refining research techniques.

Using Prepared Feed, Inorganic Fertilizer, or Manure

Usually the question of whether to feed or fertilize in a production experiment is determined by the nature of the problem being studied. For example, in the United States, the major species being cultured (channel catfish and rainbow trout, *Salmo gairdnerii)* and the quantity and price of feed grains available dictate that most research involve the use of high quality, manufactured feeds. These are the feeds that the farmers are using. In developing countries, the choices of which trophic level to use are more restricted. Often plant and animal wastes, or possibly inorganic fertilizers, are the only choices. Prepared feeds are not available. These choices dictate the trophic levels that must be utilized in research. At any time, the trophic level used by a farmer is the result of a complex interplay of several factors. The requirements of the fish are fixed. The fish must have minerals, vitamins, amino acids, fats, and an energy source. These needs may be provided directly to the fish in the form of a prepared feed or by encouraging the production of natural foods in the pond through the use of fertilizer. Which of these trophic levels is actually used depends on the availability of feeds, fertilizers, plant and animal wastes, and their costs.

It is possible that we are on the threshold of some truly exciting developments in providing food for animals in aquaculture. There has been relatively little research on energy flow in aquaculture systems and how it can be optimized. Likely, we will find that by driving the complex metabolic machinery of an aquatic polyculture system with the combination of solar energy (through photosynthesis) and the energy available from the degradation of organic matter (plant and animal waste and feeds), excellent energy efficiencies and high productions of aquatic animal biomass can be realized. Some applied research has demonstrated that this is a promising approach in aquaculture; however, much research remains to be done.

What Species?

In most situations where experiments are designed to provide information to solve farmers' problems, there is no question about

the choice of a species; the species that the farmer uses is used in the research; however, in some cases, it may be necessary to choose a species. In production experiments the fish is often used as an indicator organism in the same way that an indicator is used in a chemical test; consequently, a species should be chosen that is the most responsive to the test being made. For example, to determine the effect of various methods of fertilizing production ponds, a fish should be used that feeds on natural foods (algae) near the base of the food pyramid. A piscivorous fish feeds on organisms so far removed from the point of reaction, the point where fertilization exerts its primary effect, that it would be rather insensitive to all but the largest differences between treatments. An experiment conducted at Auburn (Annual Report of Auburn Fisheries Research Unit 1964) illustrates the point. Twelve, 0.1-hectare ponds were divided into three groups of four ponds each. One group of ponds was not fertilized; the second group received 0-8-2 (N-P_2O_5-K_2O) fertilizer; and the third group received 8-8-2 fertilizer. Ponds were stocked with bluegill sunfish, a forage fish, largemouth bass, a piscivorous fish, and fathead minnow, a forage fish.

The results of the experiment are shown graphically in figure 16 where the average net production (standing crop at the time of draining minus weight stocked) for four replications of each treatment is plotted against type of fertilization. As expected, net production of the forage fish (bluegill) was significantly greater than net production of the piscivore (largemouth bass). The main point, however, is the response of the two species to increasing levels of plant nutrients. In the following table, data are shown as the comparative production of the two species in response to the different fertilizers.

Species	*Percentage increase in net production*	
	0-0-0 compared to 0-8-2	*0-8-2 compared to 8-8-2*
Largemouth bass	85.0	0.8
Bluegill	123.1	30.2

The data on bass production indicated that there was little value in adding nitrogen as a fertilizer to ponds receiving phosphate and potassium; in contrast, the bluegill data indicated that there was considerable benefit in terms of added weight of fish produced from the addition of nitrogen. The results of this experiment demonstrate the importance of choosing the correct species. With one species

one answer may be obtained from a particular experimental design; whereas, if a second species were used, a different answer might be obtained. As a result, it is imperative that the researcher consider all aspects of life history of available species of fish when an experiment is being designed so that a suitable species can be chosen as the indicator.

Swingle (1968) published data that illustrate the importance of the selection of the species of fish to be used in production experiments. Listed in the following table are the maximum productions obtained in the monoculture of four species at Auburn using inorganic fertilization:

Species	*Feeding habit*	*Maximum production, kg/ha*
Largemouth bass	Piscivorous	196
Channel catfish	Insectivorous	370
Bluegill	Insectivorous	560
Java tilapia	Plankton-feeder	1,612

As indicated by these data, the response to a particular question may be largely determined by the species of fish.

In recent years the question of which species to use for research has taken on an added dimension. The question now is whether to conduct research on native or exotic species. In the period 1945 through 1970, there was considerable interest in moving promising aquacultural species from one country to another. Several species of tropical cichlids were spread throughout the world. Similarly, several species of Chinese carps also were introduced in a number of countries. For a time it was extremely popular to import a new species and conduct experiments with it. Several of these species have become valuable aquacultural animals in their new environments; others have become pests.

Beginning in the early 1970s, a worldwide interest in ecology and the value of "native" ecosystems slowed the rapid dispersal of "exotic" species. Most recently, many countries have passed stringent laws governing the importation of exotic species and have decreed that national aquacultural programs will be based solely on native species. Obviously the pendulum has swung widely in both directions regarding the proper role of exotic species. Proponents and opponents of the use of exotics in aquaculture are equally vociferous. The introduction of exotics into fragile ecosystems has led to permanent changes in some cases. In other cases, the introduction of exotic species has had little effect.

If experience in agriculture is an appropriate guide, the correct

use of exotics should be encouraged. Virtually all of the important plants and animals used for food are exotic in many countries. Nature has not uniformly distributed plants and animals with characteristics appropriate to domestication. In the process of evolution in which living things are constantly changing in response to changing environments, characteristics required for domestication may be quite transitory. In this ever changing system, fish that are amenable to effective domestication may be relatively few in number. In aquaculture as in agriculture, useful animals must be distributed widely by man if the farming of water is to fulfill its potential role in meeting the food needs of an ever expanding world population.

There is still much research needed to identify the most promising species for culture. Some of those in use, common carp and rainbow trout, are not necessarily the best. They have simply been the easiest to culture or to domesticate. It is likely that the most important species in the future, especially in the tropics and possibly even the temperate zone, will be the tilapias. Several of these species have considerable promise for domestication. There are probably other valuable species in the large, slow moving rivers of the world (the Amazon and the Mekong for example). Research should be continued and even intensified to identify more promising species, and considerable care should go into the planning and implementation of these studies; however, the development of aquaculture in a country should not be linked irrevocably to the identification of native species for domestication. Many years can be lost searching for a suitable species among the native fish fauna and still the efforts may prove fruitless. There are many countries that do not have native species available for domestication equal to those already in culture in other countries. To deprive one's people of the contributions that aquaculture can make because of a sense of national pride in the "native" ecosystems is a questionable practice. Wholesale introduction of exotic species without some evaluation and testing is unwarranted, but the use of proven exotic species can be used to initiate a program of fish farming while the process of testing native species proceeds.

Monoculture or Polyculture?

Although aquaculture can be carried on in monoculture, the nature of the aquatic culture matrix makes polyculture more advantageous. Ecologists have long known that multiple species animal

and plant communities are more stable and more efficient in the utilization and transfer of energy than single species systems. By combining species of fish with differing feeding habits and spatial preferences with phytoplankton, zooplankton and insect production, a "food web" can be devised that will produce large quantities of high quality protein food.

A majority of the production experiments conducted by aquacultural researchers have involved the use of only a single fish species or monoculture; however, in recent years there has been considerable interest in combinations of species of fish. Polyculture is ancient, as noted by Hepher (1967). It is currently receiving considerable attention throughout the world. If an experiment is to be designed to learn of the maximum amount of fish that can be produced in a hectare of water in a period of time, a combination of species should be used. Generally, combinations of species will result in higher production than a single species. Considerable attention has been given to this subject in Israel and recently at Auburn University. It was shown at Auburn (Kilgen 1969) that the presence of blue tilapia in ponds containing channel catfish receiving feed resulted in significantly increased production without reducing the growth rate of the catfish. Up to 3,395 kilograms per hectare of blue tilapia were produced while production of the channel catfish was being enhanced. The tilapia fed on the wastes and phytoplankton resulting from feeding of the catfish. The work by Kilgen (1969) also demonstrated that the presence of tilapia increased the survival rate of the catfish. By removing wastes as well as phytoplankton from the pond water, tilapia significantly reduced the incidence of fish kills resulting from low oxygen concentrations.

Yashouv (1968) demonstrated that the addition of blue tilapia increased the daily production of carp from 10.01 to 10.23 kilograms per hectare. The daily production of the tilapia was 3.38 kilograms per hectare. Thus, the presence of tilapia increased the daily production of fish in the pond from 10.01 to 13.61 kilograms per hectare.

Dunseth (1977) reported results of combining up to three species in experiments. He stocked 0.04-hectare ponds with the following combinations of species:

Combination 1	300 Channel catfish
Combination 2	300 Channel catfish 80 Blue tilapia

Combination 3	300	Channel catfish
	100	Silver carp *Hypophthalmichthys molitrix*
Combination 4	300	Channel catfish
	80	Blue tilapia
	100	Silver carp

Stocking combinations were replicated in five ponds. The fish were fed throughout the production period with a pelleted catfish feed. Feeding rates were based on the amount of feed consumed in ponds containing only catfish. All ponds received the same amount of feed. After approximately 180 days, the ponds were drained and the following results were obtained:

Stocking combination	*Production (kg/0.04-ha pond)*			
	Channel catfish	*Blue tilapia*	*Silver carp*	*Total*
(1) Catfish	109	-	-	109
(2) Catfish and blue tilapia	100	36	-	136
(3) Catfish and silver carp	113	-	45	158
(4) Catfish, blue tilapia, and silver carp	109	36	50	195

These results indicate that stocking three species with different feeding habits significantly increased total production of fish in the ponds compared to production in ponds containing either one or two of the species. The effect of the different species was additive. Growth of channel catfish was not affected by the presence of the other species.

Pretto (1976) demonstrated that the combination of channel catfish, blue tilapia, and common carp and the combination of channel catfish, blue tilapia, and hybrid buffalo increased total production in ponds compared to production in ponds stocked with channel catfish alone; however, production of channel catfish was lower when included in the combination than when it was stocked alone. In the same experiment, production of channel catfish was essentially the same whether stocked alone or with blue tilapia. These data indicate that there was some interspecific competition for food between channel catfish and common carp or channel catfish and hybrid buffalo, but not between channel catfish and blue tilapia.

Although the value of mixed culture has been established, it is well to remember that the results of an experiment involving more than one species often are more difficult to evaluate than those from

an experiment involving a single species. The presence of a second species may elicit behavior in both species that would not occur if either species was stocked alone. This phenomenon was noted in the experiment reported by Pretto (1976). Further, the degree or extent of behavioral changes is dependent on the relative abundance of the two species. Nilsson (1967) provided an excellent summary on the phenomenon of interactive segregation between species of fish. It is the interaction between species in mixed culture that leads to the difficulty of evaluating the results. For example, in an experiment to determine the response of a combination of species to three types of fertilizer, the response is a measure not only of the effect of the three fertilizers on the production of the combination, but also the effect of the interaction of the different species. The production obtained with one of the fertilizers may be greater because it was more effective in a specific environment in promoting phytoplankton production, or greater production could have been a result of differential response of the two species to the treatments and of the resulting change in interactive segregation.

Data presented in figure 16 and discussed in the preceding section indicated the difficulty of evaluating experiments involving a combination of species. The response of the predatory fish (largemouth bass) to the fertilizer treatments was not the same as the response of the forage fish (bluegill); consequently, the predator-prey relationship was considerably different in ponds with different fertilizer treatments. This fact is demonstrated by the data on the ratio (F/C) of the weight of forage fish (F) to the weight of the piscivore (C) in the following table. Each value is the average of four replications.

Treatment	*F/C*	S_F(%)
No fertilizaton (0-0-0)	3.4	7.8
0-8-2	4.0	30.4
8-8-2	5.7	47.5

The interaction between bluegill and largemouth bass was different in ponds receiving no fertilization and those receiving 8-8-2. The greater part of the response of the bluegill to the different types of fertilization in the experiment was young fish spawned during the experiment. Few, if any, of the young produced during the experiment grew to a total length larger than 8.6 centimeters (3.4 inches). The average percentage, by weight, of young-of-the-year bluegills in four replications of each treatment is presented in the previous

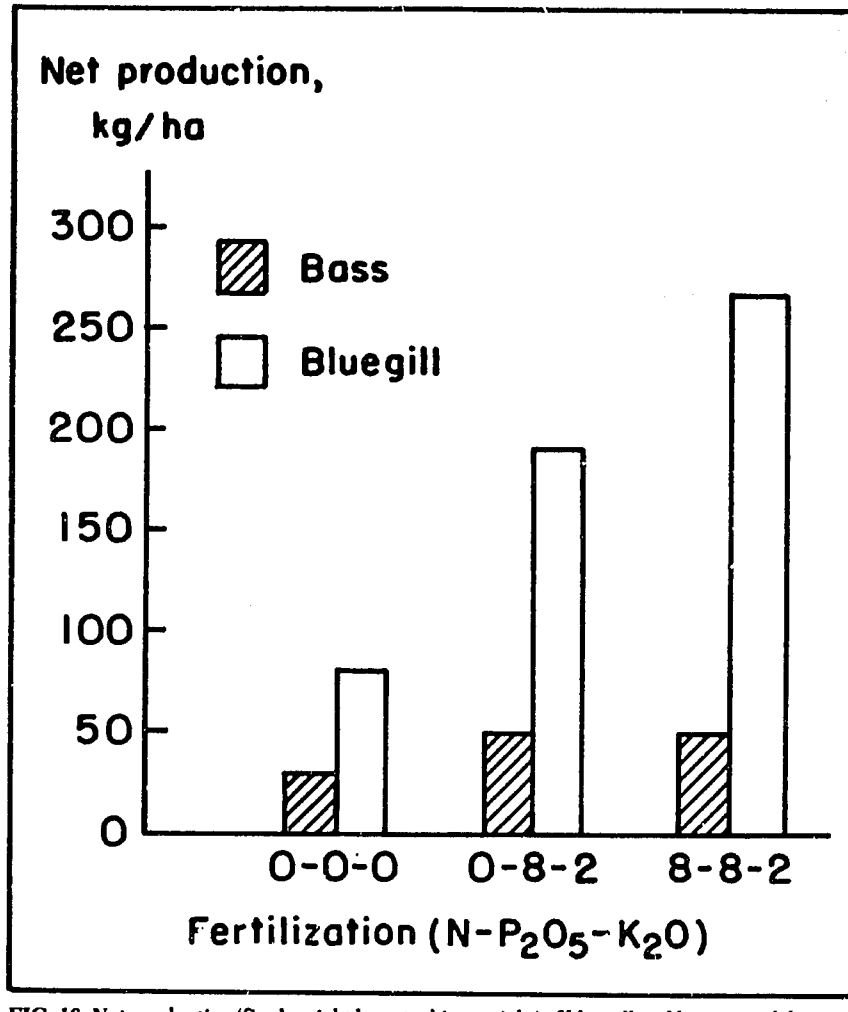


FIG. 16. Net production (final weight less stocking weight) of bluegill and largemouth bass in ponds receiving different levels of fertilization and no fertilization.

table in the column labeled S_F. The S_F values indicate that the percentage of small fish in the ponds increased with the level of nitrogen and phosphorus fertilization. The results of the experiment are relatively clear: total fish production increased as the level of fertilization increased; however, the interpretation of the results is not clear. It is difficult to tell whether the increased weight of bluegill in the ponds receiving 0-8-2 and 8-8-2 fertilization was a result of the response of the forage fish to fertilization or a result of the decreasing severity of the predator-prey relationship or a combination of both.

The predator-prey relationship probably represents the most severe form of interaction between species in mixed culture. Production experiments involving forage fish and piscivorous fish are the most difficult combination to evaluate; however, there likely will be some interaction between any two species of fish in the same pond, especially when they are stocked at the high densities commonly used in aquaculture. Sumawidjaja (1969) obtained experimental data demonstrating the effects of the interaction of two species of forage fish stocked together. He stocked Java tilapia in ponds with blue tilapia. The total number of fish stocked in all ponds was the same; however, he varied the ratio. He noted that as the percentage of blue tilapia was increased in the ponds, the growth rate of both species decreased.

If production experiments involving combinations of species are designed to obtain descriptive information, then the interaction between environment and species is of little consequence. For example, in spite of the problems resulting from the interaction of the fertilizer treatments and the predator-prey relationship described previously, there is little question that complete fertilization (8-8-2) was the best treatment in terms of the weight of fish produced; however, if the experiment had been designed to provide understanding of the biological phenomena concerned, then the question would have been considerably more difficult to answer.

Size of Test Environment

The appropriate size for the test environment may not concern those researchers having ponds or experimental units of only one size, but in some instances research stations may have different sizes. The aquacultural researcher may have available jars, aquaria of various sizes, plastic pools, concrete pools and ponds, and earthen ponds of several sizes. It becomes important then to know something of the appropriate size for the test environment in experiments.

Whenever possible, the size of the test environment should approximate the size of the body of water where the results will be utilized. Experiments conducted in plastic-lined pools should be verified in earthen ponds before recommendations are made that involve earthen ponds. Expected results of fish production in a hectare of water cannot be projected by multiplying the results obtained in a 0.01-hectare pond by 100. Shell (1966) showed in comparing yields of catfish in 0.00059-hectare plastic-lined pools,

0.002-hectare concrete tanks, and 0.10-hectare earthen ponds, all stocked at the same rate per hectare, that there was considerable difference in the relative yields from the three types of experimental units. Fish production was lowest in the plastic-lined pools, followed by the concrete tanks. Yields per hectare from the earthen ponds were many-fold greater than the yield from the plastic-lined pools even though both were stocked at the same rate per hectare and received the same fertilizer treatment.

Production experiments are often conducted in ponds of different size and the results converted to some common denominator, usually a hectare. Little research has been conducted on this problem; however, it is suggested that the practice be avoided if possible. Differences in ponds of the same size often result in considerable variation in experiments. Dissimilarities in ponds of different size doubtlessly would result in even more variation. Prowse (1968) noted that the growth rate of the fish increases as the size of the pond increases. Jeffrey (1969) showed that in shallow, 0.04-hectare ponds at Auburn, the waters cool enough at night in summer to break up thermal stratification. Conversely, shallow, 0.1-hectare ponds at Auburn remain thermally stratified for a considerable period of time in the summer and large ponds, 1 to 10 hectares, are thermally stratified for most of the spring, summer, and early fall.

There is a tendency to conduct experiments in small ponds because more of them can be operated on a smaller land area and require less water, feed, fertilizer, or manure, and fewer fingerlings. However, we should be careful in extrapolating the results obtained in small experimental ponds to the larger ponds that the farmer uses.

Duration of the Experiment

In cases where the research involves studying some phase of the production sequence used by farmers, the length of the experiment should be approximately the same as the length of the farmers' production cycle. When the research is more basic in nature and does not involve a typical commercial production sequence, other factors should be considered. If experimental periods are too short, desired treatment effects may not develop and if the experimental period is too long, the effects of the treatments may often become blurred. When the experimental period is too long, the fish receiving the better treatments may reach a growth plateau while the fish receiving the poorer treatments may continue to grow; after a period

of time there may be little difference in the yield. For example, an experiment may be designed in which the treatments are three types of feed. Type of feed is the limiting factor, but as the fish respond to the different treatments, another limiting factor may appear which will limit the effectiveness of the best feed. When the fish receiving the best feed reach a given biomass, water quality may become the limiting factor. In this situation, the type of feed would no longer make much difference. Fish receiving the poorer feeds would not attain a critical biomass until later. However, if the experiment is continued long enough, fish receiving all feeds might reach the same limiting biomass determined by water quality.

Dupree (1966) did research on the vitamin requirements of channel catfish. He fed groups of fish diets with and without specific vitamins. Figure 17 presents data he obtained from experiments with vitamin B-12. The data indicated that channel catfish required this vitamin in the diet, but apparently this need did not appear until the fish had been without it for approximately 21 weeks. Until then, there was enough vitamin stored or available in the body that it was not required in the diet. If the experiment had been terminated earlier than 21-24 weeks, the need for vitamin B-12 would not have been demonstrated.

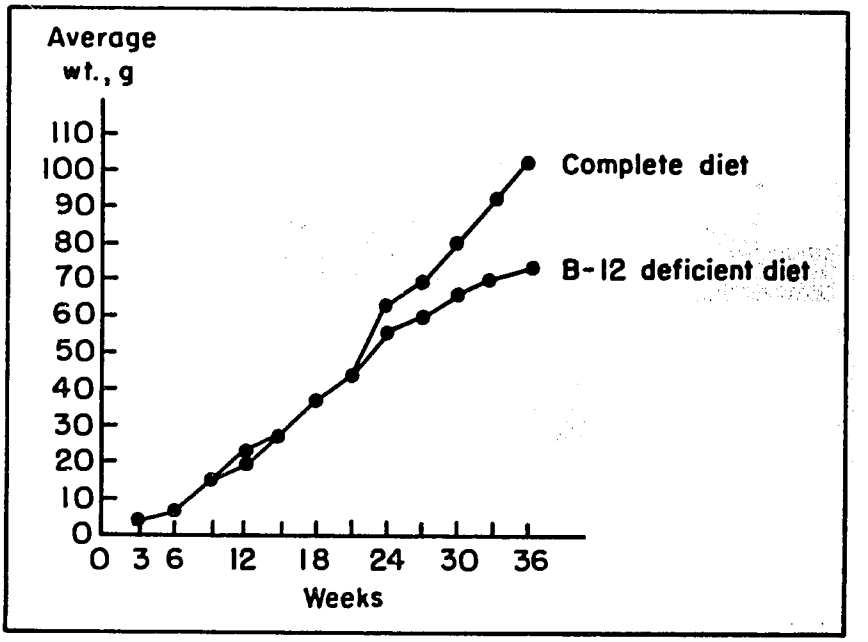


FIG. 17. Average weights of channel catfish fed a vitamin-complete diet and a vitamin B-12-deficient diet. Data from Dupree (1966).

In temperate areas, length of the experiment is quite often taken as the length of the growing season or the season in which the water temperature is high enough for the fish to grow at a maximum rate. In tropical areas, water temperature does not limit the length of experiments; however, lack of seasonal changes in temperature is not the only factor to be considered in determining the length of the experiment. While water temperatures may remain in the range where acceptable growth occurs, they do vary somewhat with time. Even minor changes in temperature probably affect experiments when there is a regular trend upward or downward for a time. Also, there are other climatic changes in the tropics that probably have a significant effect on experiments. Hughes (1977) suggested that production of tilapia in an experiment in El Salvador may have been affected by excessive turbidity in the ponds during the rainy season. Similarly, strong, dry winds in some areas of the tropics may lower the water temperature in ponds at certain times of the year through evaporative cooling. When practical, experiments should be planned so that significant time-related environmental changes are kept at a minimum.

Carry-over Effect

In some cases, an effect of a treatment in a previous experiment may be carried over into a new one. Prowse (1968) has referred to this problem. Though the water of the test environment may be discarded at the end of an experiment, the mud is retained; consequently, the effect of fertilization or feeding may carry over. It was demonstrated by Swingle *et al.* (1963) that previous fertilization affected fish production subsequently for several years. Fish production in ponds that had received 8-8-2 fertilizer for 15 years did not decrease to the unfertilized level until 2 years after fertilization was stopped. Also, sufficient nitrogen had accumulated in the mud so that in subsequent experiments, production in ponds receiving 0-8-2 fertilizer was equivalent to production in ponds receiving the complete (8-8-2) fertilizer. It is not possible to discard ponds after an experiment; consequently, the potential of a carry-over effect exists in most experiments. Unrecognized carry-over effect appears as experimental error in the statistical analysis of the data and affects the precision of the measuring system. If good records are kept on the previous history of experiments in a set of ponds, new experiments can be designed in such a way that some of the carry-over effect can be neutralized. By "blocking" the ponds according to past treatment, it is possible to compensate for it to some extent in the statistical analysis of the data. If carry-over effect is expected to be a

problem and if "blocking" is to be used, a statistican should be consulted during the design of the experiment.

Maximum Growth or Maximum Yield

Yield of fish in a pond is in reality the sum total of the growth of individual fish. In determining the design of a particular production experiment, the aquaculturist must decide whether he wants maximum growth of the individual fish or whether he wants maximum production of fish from the pond. It is virtually impossible to obtain both in the same pond experiment. If maximum yield is obtained—that is, if every morsel of available food is being used—there usually must be a maximum number of fish feeding on that food. This generally will mean a reduced growth rate of the individual fish. On the other hand, if the object is to obtain maximum growth, the fish must be stocked at a rate so that there will be an excess of food available for each.

An example of the relationship between rate of growth and net production in ponds is demonstrated in figure 18. Net production and absolute growth rate (average weight increase of an individual fish) are plotted against the number of adult Java tilapia in each of 12 ponds. Data presented in the figure are re-calculated from the original data of Dendy *et al.* (1968). These data indicate that at three levels of pond fertilization, maximum growth rate and maximum production could not be obtained at the same rate of stocking. As the

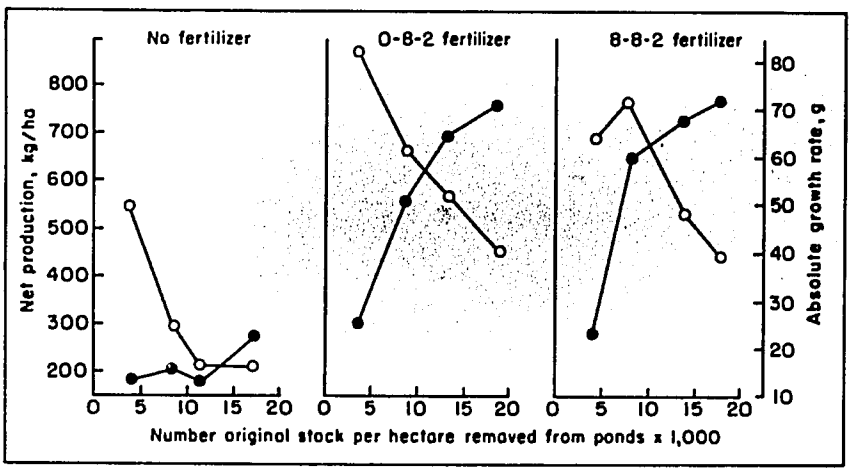


FIG. 18. Absolute growth rate (o) and net production (•) of the original stock of Java tilapia recovered per hectare from ponds receiving no fertilization (0-0-0), 0-8-2 fertilization, or 8-8-2 fertilization and stocked with 4,940, 9,880, 14,820, or 19,760 fish per hectare. Data from Dendy *et al.* (1968).

level of production increased, the growth rate of the individual fish decreased.

Weight, Size, and Number to Stock

In an experiment, generally the objective is to determine the response of a weight of fish to a given treatment. Because of size specific differences in metabolism (Winberg 1960) and food habits (Lagler *et al.* 1962), the specific effect of the treatment will vary for different sizes of fish. The beginning point for determining the weight, size, and number of fish to stock in an experiment is to determine the correct weight.

We are often interested in the yield that may be obtained from a particular set of treatments; yet, if the wrong initial weight of fish is stocked, the carrying capacity of the particular environments may be reached before the treatments exert a definitive effect. For example, two treatments are being compared in an experiment, one inferior to the other. The experimental ponds may be stocked with a relatively high weight of fish so that the carrying capacity of the environment for the superior treatment will be reached approximately halfway through the experimental period. Beyond that point fish biomass may increase little if at all. In the inferior treatment, however, the carrying capacity is not reached until near the end of the experimental period. When the results are compared, the yields might be nearly equal.

An initial stocking weight that is too low can also bias an experiment because treatments affecting density of fish may exert little if any effect during the experimental period. Hepher (1967) suggested that low population density in experimental ponds may explain the lack of effect of fertilization on fish production as compared to its effect on the production of fish food organisms. Where fish are present in such a low density that they are already presented with adequate food, increasing the food supply with fertilization will have little effect on production.

Loyacano (1970) encountered a problem in attempting to determine the effect of mechanical aeration on production of white catfish because the initial stocking weight was too low. He stocked 0.04-hectare ponds with 800 fish weighing a total of 11.1 kilograms. He ran the experiment for approximately 200 days. When he drained his ponds, there was virtually no difference in the yield at the two higher levels of aeration. The first conclusion might be that higher levels of aeration were not needed; yet, this was not the case

at all. In a subsequent experiment in which he stocked larger fish (718 fish weighing 78.6 kilograms), there was a significant increase in the yield of catfish at the higher levels of aeration. In the initial experiment, the fish in both treatments were growing as rapidly as possible, and they did not get large enough during the experimental period to approach carrying capacity or to show the beneficial effect of aeration.

The choice of the weight of fish to be stocked is crucial. Although the success of an experiment is not assured when the correct stocking weight is chosen, choosing an incorrect one makes failure a certainty. The following example describes a procedure for determining the correct stocking weight. The experiment in question is designed to determine the comparative value of two feeds of different quality in the production of 0.4-kilogram channel catfish from 16-gram (12.5 centimeters) fingerlings. A 240-day experimental period (March-October) is chosen because this is the normal production period in Alabama. Both feeds are to be fed at the same rate. A higher feeding rate will be used while the fish are small, but at the end of the experiment, it is expected that the feeding rate will be approximately 2 percent of body weight per day.

It is generally accepted that when the amount of feed added to a catfish production pond exceeds approximately 48 kilograms per hectare per day, water quality may deteriorate. When this rate of feeding is exceeded, the dissolved oxygen budget (the relationship between oxygen production, absorption, and utilization) may become unbalanced and the death of the fish may result. Thus, water quality may become the factor limiting fish production no matter what treatments are being compared.

Because 48 kilograms per hectare per day is the limiting amount of feed that can be added to the ponds and a 2 percent rate of feeding is to be used in the final stages of the experiment, the limiting standing crop will be approximately 48/0.02 = 2,400 kilograms per hectare.

It is expected, based on past experience, that under optimum conditions, the fish will grow from a weight of 16 grams in March to a weight of 400 grams in October. With this information plus the estimate of the limiting standing crop, the weight of fish to be stocked initially can be calculated as follows:

$$\frac{16\text{g}}{400\text{g}} = \frac{(x)\text{ kg}}{2{,}400\text{ kg}}$$

Where "x" is the weight of the initial stock = 96 kilograms. Since the fingerlings to be stocked weigh 16 grams each, approximately 6,000 fingerlings per hectare would be stocked in each of the replications.

If a higher stocking weight were utilized, the limiting rate of feeding and the resulting poor water quality might be reached by mid-summer rather than in October. In fact, because of higher water temperatures, the limiting rate of feeding might even be lower than 48 kilograms per hectare per day in summer. Also, if the experiment had to be terminated by mid-summer, the fish would weigh considerably less than the desired 0.4 kilogram each at that time and one of the original objectives would not be realized; the comparative performance of the fish on two feed formulations growing from 16 grams to 400 grams could not be measured. If a lower stocking weight were utilized, both groups of fish might obtain enough natural foods throughout the test period to mask any differences in feed quality.

Each experiment has some limiting or critical factor that may be used as a basis for determining the initial stocking rate. For example, consider an experiment to compare the growth of fish in earthen ponds on two feeds that are identical except one feed contains added vitamins and the other does not. The critical factor would be the weight of fish required to exhaust the natural food supply so that fish would not obtain sufficient vitamins from natural sources for maximum growth. Until the point is reached that the fish must rely on the vitamins provided by the feed, there would be no test of the effect of vitamins added. If the initial stocking rate were too low, the critical point might not be reached during the entire experiment.

In designing some experiments, there may be insufficient information for determining the optimum stocking weight; in others, the determination of the optimum stocking weight may be the objective of the experiment. In these situations, a series of stocking weights is selected. Ponds are stocked with each weight and the yield determined after a period of time. If the correct series of stocking weights has been chosen, there will be a commensurate increase in yield with each higher stocking weight until the optimum stocking weight is reached. Beyond that point there will be little further increase. In choosing stocking weights, the objective is to select a series with a range broad enough to include the optimum stocking weight yet with the individual stocking weights spaced close enough together so that the optimum rate can be determined fairly accurately. For example, an experiment might be designed to determine the opti-

mum stocking rate of male blue tilapia in monosex culture utilizing 0-8-2 fertilization in ponds. A series of stocking weights, such as the following, might be chosen: 10, 15, 20, or 25 kilograms per hectare. Obviously this range is too narrow and probably would not include the optimum stocking weight. The series 50, 150, 250, or 350 kilograms per hectare would probably include the optimum stocking rate, but the interval between rates is so broad that the optimum weight might not be determined with accuracy.

Although the initial decision should be to determine the weight of fish to be stocked, some decision must be made on the size of fish to stock and a number of factors must be considered. For example, larger fish that are accustomed to feeding on natural food are difficult to train to take artificial feeds. If the researcher wishes to determine the effect of supplemental feed on the growth of the larger-sized fish, generally it will be difficult to train the older or larger specimens to take artificial feed. Fingerlings or smaller-sized fish may be trained more readily. In this case, the lack of the expected result in the experiment involving the larger fish might have been a result of their lack of interest in the artificial feed rather than the treatment (feed).

I have already noted that it may be advisable to use combinations of species in experiments. In some cases it may be advisable to include more than one size of the same species in a yield trial. Hepher (1967) discussed the advantage of stocking several sizes of the same species in a pond to increase production. The optimum size to stock is a complex problem, and it is even more complex if comparisons are to be made between species with different growth rates (Swingle 1968). Certainly more research is needed on this problem. The best general rule is that a size of fish should be chosen for stocking that will be the most responsive to the treatments to be evaluated.

Genetic Variation

In agriculture, experimentation is done with relatively well-defined genetic lines of plants and animals. In aquaculture, with the possible exception of common carp and rainbow trout, most of the fish used for culture are but a few generations removed from the wild state. Little is known of the heritability of the various traits important in the culture of these species.

Generally the genetic component of experimental variation is of relatively little consequence in animal agricultural research. The

fecundity of most farm animals is relatively low; consequently, when experiments are stocked, there is a range of genetic variability included. Conversely, in the case of fish, depending on the species, one female may produce several hundred thousand offspring. Depending on the management of spawning and rearing of fingerlings, an entire experiment can be stocked with the offspring from a single mating. This situation would guarantee genetic uniformity, but it also could mean that the entire group would be uniformly poor. Certainly, there would be a relatively poor representation of the range of genes available in the species present in the experiment. A relatively narrow genetic base in the experiment could mean that the results would not be applicable to a much broader and variable gene pool that might characterize farmers' fish.

There also is another potential problem. Under a given set of circumstances, genetic variability may be distributed in such a manner in the experiment that the evaluation of the data would be biased. If all replications of one treatment were stocked with offspring from one mating and the replications of another treatment stocked with offspring from another mating, in the statistical analysis, genetic variation would be confounded with variation associated with the treatment means. Differences thought to be treatment effects might be a result of differences in the genetic effects of the two different matings. Even when there is less restrictive distribution of genetic material than this, partitioning of variation in an experiment can be affected.

There are few specific rules to follow in averting the problems that can result from poor distribution of genetic material in experiments. It is usually wise to include offspring from a number of matings in an experiment. Further, the different matings should be randomly distributed throughout the experiment. Finally, the matings used as the source of experimental animals should be representative of the gene pool of the fish where the results of the experiment will be applied.

If a serious problem is anticipated, random distribution of genetic material can be obtained by combining the fish from different matings and using a random stocking order. Each pond would first be stocked with a third of its complement of fish, with the order of stocking randomly determined. The same procedure would be followed for stocking the second third of fish in each pond. Finally, the last third would be stocked in the same manner. The stocking of one-third of the complement of fish three times is not required to receive a degree of randomization. Alternately, one-half of the

complement could be stocked two times. The objective is to eliminate, to the degree practical, systematic assignment of genetic material in the experiment unless it is part of the design of the experiment. This procedure virtually guarantees random distribution of genetic material, but it is relatively complicated, especially if there is a large number of ponds to be stocked. Without good record keeping and without close attention to detail by those actually stocking the fish, some ponds will inadvertently receive more or less fish than expected.

Differential Growth of the Sexes

In most species of fish the male grows faster than the female. Animal husbandmen have to deal with this problem, but in aquaculture it is particularly perplexing. A good example of the differential growth of the sexes is shown in figure 19, based on research by Schmittou (1968). In stocking experimental ponds, the sex ratios should be nearly equal. In ponds containing predominately males, it would be unusual if the yield at the end of experiment was not significantly larger than in an adjacent pond where the sex ratio favored females.

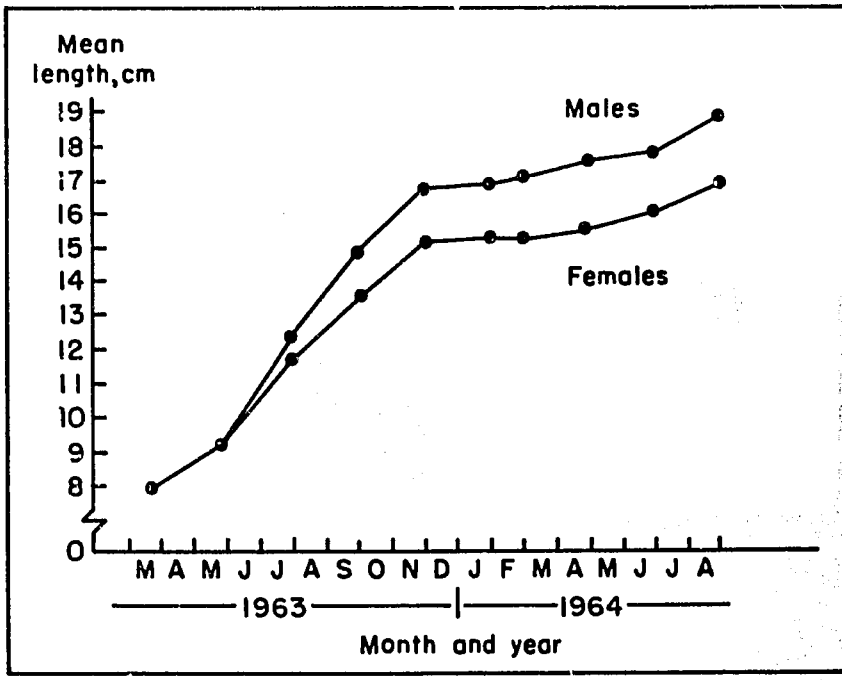


FIG. 19. Growth of male and female bluegill stocked in an earthen pond. Data from Schmittou (1968).

Sex-dependent differences in growth can appear early in the life of some species; consequently, it is easy to inadvertently sex fish during stocking. Pruginin and Shell (1962) showed that blue tilapia can be sexed with a considerable degree of accuracy using size alone when the fish are small (13 to 18 grams). Unless a great deal of care is used when fish are selected for stocking to be certain that neither large fish nor small fish are stocked first, it is likely that there will be a biased sex ratio.

Stocking ponds with fish with unequal sex ratios tends to be a systematic error. The larger fish in a container may be males and are selected without realizing it; consequently, the sex ratios in the first ponds stocked will be in favor of the males; whereas, in the last ponds stocked, the ratio will be reversed. If the ponds are stocked in systematic fashion—that is, if one treatment or all replications of one treatment are stocked first, followed by all replications of the second treatment and so forth—there will be a systematic bias introduced in the treatments that may seriously affect the experiment. This problem can be minimized by following the procedure of random selection and random stocking described in the section on "Genetic Variation."

Growth and Nutritional History

Shell (1963) has shown that there is growth compensation in channel catfish. Two groups of fish were fed experimental diets, one containing a low level of protein and the other a high level of protein. As expected, fish receiving the low protein diet grew much slower than fish receiving the high protein diet; yet, in a subsequent experiment, when both groups were placed on a different, high quality diet, the fish that had grown slower previously grew faster than the group which had received the better diet. These results indicate that growth and nutritional history of the fish can affect the outcome of an experiment. For example, if fish with a poor growth history or nutritional history are stocked into replications of one treatment while fish with a different nutritional or growth history are stocked into replications of another treatment, the results might depend partially on the nutritional history rather than on the treatment alone. Interaction between the previous growth history of a particular group of experimental fish and the treatment might also give an entirely different picture than if the fish with the same nutritional history were used. Whenever possible, fish used in

experiments should be from a stock that has a common nutritional or growth history. When this is not possible, the different groups of fish should be distributed randomly throughout the experiment utilizing the stocking procedure described previously.

The Research Plan

The primary purpose of aquacultural research is to provide solutions for farmers' problems. In a previous section, a procedure was described to reduce a problem into a manageable unit or to a simple question that could be answered by an experiment. The next step is to develop a plan of action for answering that question. Although many senior aquacultural research scientists conduct experiments without a specific plan, their research would be more effective if they prepared one. All younger researchers should prepare plans before they begin. Developing a good plan of action and writing it down require that the researcher think through the entire experiment before it is begun. When writing the whole process, problems in the experiment might be identified and preparations made to avoid them.

Writing, like good research, is a logical process. The exercise of choosing a correct subject and verb to express a thought and the linking together of sentences to form a coherent paragraph is a rigorous logical process. Writing a research plan, or writing to remind oneself (the stimulation of subconscious mental processes) of why and how the research is to be done, often results in the generation of knowledge that the writer does not know he possesses (Ladd 1979). The logical exercise of writing often allows one to discover things about the logical sequence of events in the planned research that were not apparent before.

The research plan consists of the following components:

1. Introduction
2. Review of the literature
3. Materials and methods

This is a relatively standard outline. Most scientists are thoroughly familiar with these elements, but some specific comments may be helpful.

The introduction should answer the following questions:

1. What is the specific problem?

2. Why is it a problem?
3. What is to be done about the problem (objectives)?

Items 1 and 2 are relatively easy to deal with if the suggested procedure of problem identification has been followed. Similarly, the objectives (Item 3) are derived through the same procedure. The value of writing down the objectives is that they are indicators of the effectiveness of the problem identification procedure. If the objectives cannot be easily or clearly written, this is evidence that the problem has not been identified effectively.

A rather exhaustive review of the literature is essential. Because fish farming research is in its infancy, it is not likely that there will be much duplication of research; however, this might occur and should certainly be avoided. The best reason for looking at what others have done is that better methods of carrying out the research might be discovered.

Methods and materials should be chosen that will clearly answer the questions. Each proposed method should be evaluated in relation to the question or the portion of the question it is supposed to answer. Data that are not needed should not be collected on the assumption that they might be useful later. Uncertainty of what data to collect indicates a lack of refinement and understanding of the objectives. Methods should be described in sufficient detail for another scientist to continue the experiment if the one writing the research plan cannot do so. Standard methods need not be described because the description is available elsewhere. All methods and procedures should be time-scaled as part of the planning procedure. For example, diurnal changes drastically affect the aquatic environment. Often data must be collected from a number of experimental units within a short period or diurnal changes will affect the measurements. The researcher might find it physically impossible with the resources available to make all the necessary measurements within the required time interval. Such problems can be avoided by careful planning *before* the experiment begins.

From the methods, lists of materials can be developed. It is important that materials and labor are available when required. Materials and labor should be listed on a time scale showing when a specific item will be needed. In this way, the use of items can be scheduled. This procedure is especially important when new items of equipment or materials must be ordered or when several scientists are utilizing the same equipment.

PROBLEMS ENCOUNTERED IN FISH PRODUCTION EXPERIMENTS

Conducting experiments in aquaculture is somewhat more difficult than in agriculture. This section deals with several specific problems that are particularly troublesome. Many of these problems are difficult to solve, and until solutions are available, the conduct of experiments on fish production will continue to be a relatively imprecise endeavor compared to experiments with plants and animals on land.

Mortality of Stocked Fish

This is the most difficult problem encountered in conducting production experiments. When fish die in an experiment, it is compromised. The degree to which the experiment is changed by the death of fish will determine the value of the resulting information. In some instances, dead fish in the replication of a treatment can be replaced without the loss of information. However, this is true only if the fish being used to replace those dying are of the same stock or have the same history as those that died. A replication of a treatment might be maintained to provide replacements in case some of the experimental fish die. Some workers maintain that when fish die in an experiment the remaining fish make up the difference or utilize the food and space to grow larger and thus replace the fish or weight that was lost. This has not been demonstrated conclusively and cannot be used as a basis for conducting and evaluating experiments.

The major problem is to determine how much mortality can be tolerated and still allow the replication to remain as part of the experiment. Obviously, if only a few fish die, or a relatively small percentage of fish die, the problem will be minimal. Yet, as the mortality rate increases, the value of that replication diminishes.

Another major problem involved when there is mortality of experimental fish is determining the time when mortality takes place. In most cases, only a relatively small percentage of the fish dying in a pond will float to the surface and unless the researcher is diligent in searching for these, fish, birds, or other animals will remove them. In this case, the researcher not only loses the fish but does not know when they were lost. In an experiment at Auburn involving striped bass *(Morone saxatilis)*, an effort was made to

count all the fingerling fish dying in a series of 0.025-hectare earthen ponds. A concerted effort was made to record and remove all dead and dying fish. The ponds were visited several times each day. The dead fish in each pond were replaced; yet, when the experiment was terminated, the ponds contained only about 50 percent of the fish expected.

In many cases when fish die in ponds, the cause of death does not affect all sizes of fish to the same degree. Often when the death of fish is the result of anoxia, it is the larger fish that die first. Thus, a partial kill not only results in fewer fish in the pond but also may leave only the smaller of the fish alive. Prather (unpublished) in research at Auburn noted that following a partial kill of channel catfish in a pond, the average size of remaining fish was smaller than the average size before the kill. A large sample of fish taken before the kill indicated that the mean weight of fish in the pond was 395 grams. Following the loss of approximately 20 percent of the population, the mean weight of a large sample of fish was 335 grams.

As noted above, it is difficult to compensate in a satisfactory way for mortality of fish. The best solution is to prevent it. Precautions should be taken to prevent the stocking of weak, diseased, or unduly stressed fish. In reality, an experiment begins several weeks or even months before the fish are stocked. The process of preparing the fish for stocking should be well planned and executed. Steps should be taken to reduce injury and undue stress, and should include:

1. Feed a good diet which includes a good vitamin package or hold fish to be stocked uncrowded in ponds where there is adequate natural food available.
2. Inspect the fish carefully for parasites, indications of disease, and general condition before removal from the holding pond.
3. Take fish off feed, if possible, 24 hours prior to removal.
4. Remove fish quickly and carefully.
5. Transport to holding tanks quickly, maintaining best possible water quality.
6. Hold fish in tanks for counting and weighing no longer than absolutely necessary.
7. Processing of fish (counting, measuring, and weighing) should be planned so as to stress the fish as little as possible.
8. Transport fish quickly to experimental ponds, again maintaining good water quality.

9. Fish should be stocked in the ponds so as to reduce injury, and care should be exercised to prevent temperature shock if it is a potential problem.

Sampling of fish during the experiment also can result in mortality. Often injury and stress will result in death, but of greater importance is that these weakened animals become the focal point for the development of disease outbreaks. Removal of fish is necessary in many experiments to determine rates of growth and to obtain other information. It is usually necessary to return them after the data are collected. This type of sampling should be kept to an absolute minimum consistent with requirements for data. Where sampling is required, the operation should be well planned and precautions taken to prevent injury and stress. The fish should be removed quickly and transferred into clean, well-oxygenated water. If the weather is hot, cooling of the holding water is helpful, and may be accomplished by using water from a stream or a well. In some situations, ice can be used to cool the water slightly. Another alternative is to do the sampling soon after daylight when the pond water is coolest. In some cases it is effective to add oxygen directly to the holding water, especially when the fish are crowded. At some stations, antibiotics or bactericides are added to the holding water. Also, tranquilizers sometime are added to the water to reduce the stress induced by handling.

When ponds are being stocked with fry or small fish, predatory aquatic insects often will consume large numbers. When insect predation is a problem, the ponds should be kept dry until shortly before the fry are stocked. If it is not practical to fill the pond this late, chemicals can be used to eliminate many of the insects without killing the fry and all the plankton (McGinty 1980).

Reproduction of Stocked Fish

Reproduction of stocked fish is an expected result in some experiments and therefore creates no problems; however, reproduction often adds variables that are uncontrollable and lead to difficulty in evaluation. Reproduction should be avoided whenever possible unless evaluation of the number of young obtained is part of the experiment. Variable numbers of young obtained and variable times of spawning add to the difficulty of evaluating experiments where reproduction occurs. In a study by Greene (Annual Reports of Auburn Fisheries Research Unit 1969) at Auburn of the effects of

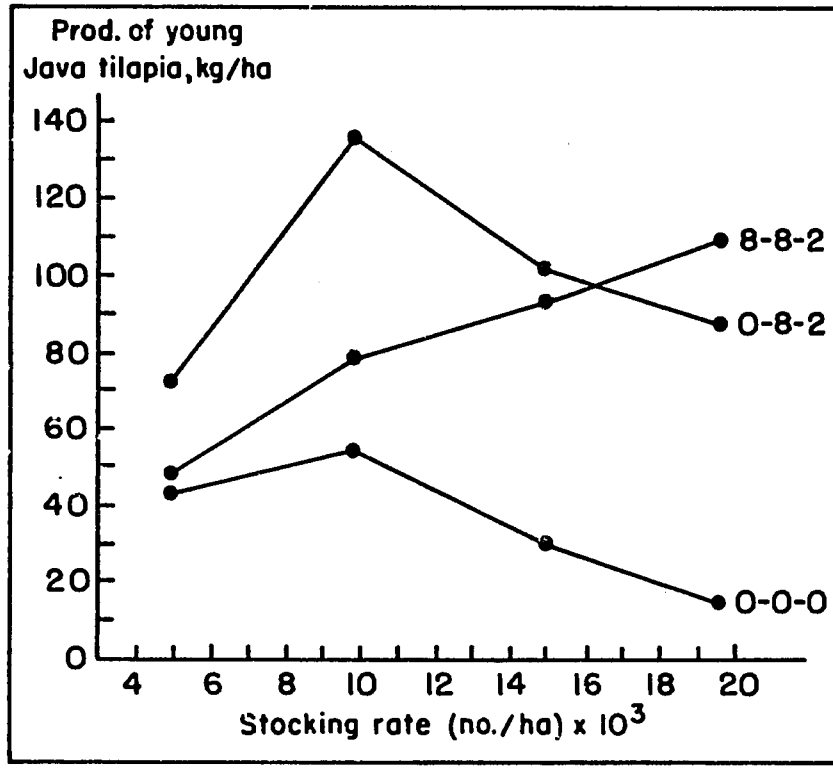


FIG. 20. Production of young Java tilapia in ponds that received different fertilizer treatments and different rates of stocking. Data from Dendy *et al.* (1968).

various forms and amounts of water hardness on production of the fathead minnow, he found an interaction between the treatments and reproductions. In some of the ponds, there were only the stocked adults to utilize the feed. In other ponds there was also reproduction. In this case, the interaction between treatment and reproduction became an unplanned variable in the experiment.

Data from Dendy *et al.* (1968) demonstrate one of the problems resulting from reproduction by stocked fish (figure 20). In that experiment, the weight of young was affected by stocking rate of adults and by types of fertilizer. The data indicated that there was interaction between those two factors. Variable reproduction resulted in data that were difficult to evaluate. Although the three ponds were each stocked with the same number of fish, at the end of the experiment the numbers were significantly different. By the end of the experiment there was little similarity among any of the ponds with respect to the number of fish they contained.

It is not always possible to escape the effects of reproduction, and it is not a serious problem in situations where the experiment is conducted over a period of time so that slight differences or even fairly large differences in reproduction are smoothed out. In short term experiments, differences in reproduction can create havoc. Whenever possible, reproduction of stocked fish should be avoided unless the investigator is relatively certain that it will be similar in all replications.

Presence of Wild Fish

When wild fish get into the experimental ponds, the experiment may well be ruined. Whether or not the results can be used would depend on the degree of contamination. It also depends on the nature of the wild fish themselves. Wild fish that are direct competitors with the experimental fish, or piscivorous fish large enough to consume some of the experimental fish, can create problems in evaluation. Wild fish also can affect experiments by introducing pathogens to a susceptible population. Usually, the major problem is that the number of wild fish may vary widely from replication to replication. It is often difficult to tell when the fish entered the pond. Generally, when wild fish do gain entrance, the researcher attempts to make the best of the situation; yet, because of the possibilities of interaction between the wild and stocked fish, evaluation of the data is difficult.

There are so many uncertainties involved when wild fish gain entrance to experimental ponds that a concerted effort should be made to exclude them. When ponds are drained, the small amount of water remaining should be treated with a fish toxicant to eliminate any fish. Then as the ponds are filled, the water should be passed through a fine mesh screen (figure 12). Of course this latter precaution is not necessary if the source of water is a well or is free of fish. Ponds can be treated with toxicant after filling and before stocking, but it is much more expensive to treat the larger volume of water.

Feed Poorly Utilized

Poor food utilization can be a major problem in experiments where feeding is a part or is one of the treatments. When the feed is uneaten, it usually decays in the water and acts as a direct pollutant. The fish not only fail to benefit from eating the food, but also the

accumulation of wasted food may result in a serious deterioration of water quality that will affect the experiment. Thus, the refusal to take the experimental diet being offered may result in a yield of fish that would be much lower than just the poor quality of the diet itself would indicate. The refusal on the part of the fish to take a particular diet may result in such poor water quality that many of the fish die, thus eliminating some of the replications. In this case, the effect of poor water quality becomes fairly evident. A more serious problem arises when the fish take only a portion of the feed, and the water quality does not become bad enough to cause the death of the fish, but the metabolism of the fish is impaired to the point that the experiment is affected.

Adjusting Feeding Rates

Adjusting feeding rates as the fish grow can cause problems in production experiments. In most experiments involving feeding, the fish are fed an amount each day based on a percentage of bodyweight. Periodically, samples of fish are removed from the ponds and average weights determined. The estimated total weight of fish is obtained by expanding the sample mean weight per fish to the total number in the pond. The amount of feed to be fed daily is then adjusted based on the new estimated total weight. Timing of the adjustment of feeding rates can cause problems in feed utilization. Theoretically, a new feeding rate should be determined each day because the fish are growing each day. If a pond of fish is fed at a rate of 3 percent of bodyweight on 1 day and if they utilize that feed efficiently for growth, on the following day, if they are fed the same amount of food, they will be receiving feed at a rate less than 3 percent. After several weeks, the fish are being fed at considerably less than 3 percent bodyweight rate. If the amount of feed could be adjusted each day, the same percentage rate could be maintained. Unfortunately, it is not practical to readjust the amount fed on a daily basis. The labor required would be prohibitive, and repeated disturbance of the fish by sampling could disrupt the experiment.

When the amount fed is adjusted too infrequently while the fish are growing rapidly, the fish may not receive enough feed for maximum growth. In the extreme case, the feeding rate may finally approach a maintenance level before it is readjusted and the fish may cease growing; then when the amount fed is adjusted, feed may be wasted while the fish are adjusting to the increased level.

Another problem can arise if the amount fed is adjusted too

infrequently. The better treatments in the experiment may be more seriously affected, because those fish are growing more rapidly. In fact, the better treatment may be affected so severely that fish production in a poorer treatment may actually overhaul production in the better one until there is little difference in production associated with the two treatments. Then when the amount of feed is finally readjusted, growth of fish in the better treatment may "spurt" only to slow down as the feeding level approaches maintenance again.

There are no good rules for determining how often the amount fed should be readjusted. The interval is dependent on how fast the fish are growing. In experiments involving fingerlings, the growth rate may be so high that the amount of feed fed should be readjusted on a weekly basis. In adult fish, the weight of an individual fish may change relatively slowly and feeding levels can be adjusted at 2- or 3-week intervals. In large brood fish, the feeding level might be adjusted only once per month or even more infrequently.

Changes in the Test Environment with Time

Changes in the pond environment often are independent of treatment and will occur in replications of the same treatment. Thus, in one pond a dense bloom of algae may develop which will result in poor water quality; whereas, in a replicate pond nearby no such bloom develops. Obviously, the two environments are not the same.

Other changes in the test environments also cause problems. Unequal seepage from the experimental ponds may result in unequal volumes of water in the experimental units. If flow rates are increased to maintain equal water levels, differences may be introduced. In the extreme case, a high seepage rate in a pond may require such a large volume of replacement water that a limited flow-through system is created. Also, when additional water is required for only some of the ponds, unavoidable differences may be introduced when it is added. All ponds might be filled with water from a stream or reservoir at the beginning of the experiment. Later, seepage might result in low water levels so that additional water might be needed in some of the ponds. This "new" water likely would be different from the water used originally. It might be more or less turbid. It might contain recently hatched wild fish fry, an increased number of fish pathogens, or more or less nutrients.

Climatic changes also can cause changes in experimental environments. As noted in a previous section, Hughes (1977) observed a significant increase in turbidity in experimental ponds in El Salvador as a result of the high winds associated with the rainy season. Although it was not recorded in this instance, it is likely that the turbidity varied considerably from pond to pond depending on exposure to the wind.

Infestations with Aquatic Weeds

Infestations with aquatic weeds in ponds can cause serious problems in production experiments. Weeds can interfere with the normal phytoplankton production. Where the fish are being fed with pelleted feeds, the weeds affect feeding behavior. The presence of weeds affects the relationship between predator and prey in ponds where this relationship is part of the expected response. The presence of weeds in ponds usually will make the removal of fish extremely difficult. This difficulty is exaggerated when there are small fish in the pond that must be removed. One of the most troublesome problems with weed infestations is that they usually are not uniformly distributed from pond to pond. One pond will have a heavy infestation; an adjoining pond will have a light infestation. This unequal distribution of plants between ponds adds to the experimental error.

Because of the deleterious effect of weed infestations in production experiments, they should be controlled. Control can be achieved in several ways (Snow 1972, Whitwell and Bayne 1979). At Auburn, Chinese grass carp *(Ctenonpharyngodon idella)* are stocked in most experimental ponds for this purpose.

EVALUATING EXPERIMENTS

Methods of Expressing Data

Several methods of expressing yield in aquacultural research have been suggested. Some have definite advantages, but in most cases they also have disadvantages. The most useful methods are:

1. Standing crop
2. Net production
3. Relative gain
4. Gain per day
5. Growth curves

I will discuss each one, giving the advantages and disadvantages of each. Data presented in table 4 (Nail 1962) will be used in this discussion.

Standing Crop

Standing crop is defined as the "weight of the fish in the experimental unit (pond, tank, or trough) when the experiment is terminated." Standing crop data do not indicate whether there was any increase in the weight of the fish after they were stocked. It is possible to obtain a rather high standing crop without having any increase in weight, or for the fish to actually lose weight. For this reason, standing crop data give a minimum amount of information about the changes that have taken place in biomass during the experiment. Although the disadvantage of using standing crop is obvious, it still is a useful method of expressing the results of experiments. Standing crop data are useful in evaluating experiments for recommendations to fish farmers. They are interested in the total weight of fish that can be harvested. Monetary returns from the fish crop are usually based on the standing crop.

In the following table, standing crop data on five replications of each of two treatments from Nail's (1962) experiment are presented. These standing crops are calculated from data in table 4.

	Standing crop (g)	
	Diet III	*Diet IV*
	2,070	2,374
	1,977	2,339
	2,003	2,226
	2,124	2,301
	2,143	2,190
Mean	2,063.4	2,286.0

At the end of the experiment, the troughs contained between 1,977 and 2,374 grams of fish. Analysis of the data utilizing the "t" test as applied to group comparisons (Snedecor and Cochran 1967) indicates that the hypothesis of "no difference" between the mean standing crops for the two diets should be rejected ("t" = 4.77, 0.005 $P < 0.001$). It is fairly obvious from the data that the standing crop of fish receiving diet IV is greater than for fish receiving diet III. The analysis of the data substantiates this observation.

TABLE 4. PARTIAL RESULTS FROM AN EXPERIMENT TO DETERMINE THE EFFECT OF LEVELS OF DIETARY PROTEIN AND CARBOHYDRATE ON THE GROWTH OF CHANNEL CATFISH IN TROUGHS (DATA FROM NAIL 1962)

Trough	Initial weight, g	Total weight (g) of fish on:			
		July 10	July 31	Aug. 20	Sept. 3[1]
Diet III					
11	846	1,056	1,439	1,656	2,070
12	765	990	1,335	1,546	1,977
27	806	1,015	1,386	1,641	2,003
32	900	1,089	1,470	1,706	2,124
34	833	1,095	1,486	1,719	2,143
Mean	830.0	1,049.0	1,423.2	1,653.6	2,063.4
Diet IV					
4	880	1,138	1,580	1,895	2,374
7	844	1,096	1,537	1,786	2,339
36	797	1,068	1,460	1,722	2,226
37	840	1,095	1,525	1,803	2,301
40	785	1,089	1,449	1,741	2,190
Mean	829.2	1,097.2	1,510.2	1,789.4	2,286.0

[1]Final weight.

Net Production

Net production is defined as "the increase in weight of fish in the experimental unit during the experiment." It is the difference between the stocking weight and the standing crop. The use of net production as a method of expressing yield has the advantage that it indicates how much the biomass changed. Because this method does express something of the dynamics of biomass change, it is generally more meaningful than "standing crop" in expressing results; however, there is one major disadvantage in its use. Net production does not give any idea of how many kilograms of fish were being supported in that body of water at the time of draining.

In the following table, net production data on five replications of each of two treatments from Nail's (1962) yield trial are presented. These net productions are calculated from data in table 4.

	Net production (g)	
	Diet III	*Diet IV*
	1,224	1,494
	1,212	1,495
	1,197	1,429
	1,224	1,461
	1,310	1,405
Mean	1,233.4	1,456.8

These data provide a similar conclusion on the effect on growth of the two diets as with data on standing crop. It is obvious that diet IV is somewhat better than diet III. The hypothesis of "no difference" is

again rejected ("t" = 8.40), but at slightly lower probability level ($P < 0.001$) than was the case with the standing crop data. Thus, the two methods of expressing the results lead to the same conclusion. However, net production data tell considerably more about the experiment. These data indicate the extent of growth in each treatment. Net production data indicate the net change in biomass which is the basis for aquaculture.

Relative Gain

The relative gain method of expressing yield is also called "relative growth rate," or in some cases "percentage growth rate." It is defined as "the difference between the initial stocking weight and the standing crop divided by the initial weight stocked expressed as a percentage." Or it is net production divided by the initial stocking weight expressed as a percentage.

Relative gain indicates the degree to which the initial weight is compounded during the experimental period. For example, for diet III, trough 11 (table 4), the initial weight was 846 grams and the weight gain (net production) was 1,224 grams; thus, the initial weight was compounded by 1,224/846 = 144.7 percent. This expression measures the gain in weight in terms of the initial weight.

Nail (1962) stocked the same number of fish into each replication, but there were small differences in initial weights in the troughs. An examination of data in table 4 indicates a positive correlation between the initial weight and the standing crop in each trough, and that there was a similar correlation between initial weight and gain in weight (figure 21). This is to be expected if the fish in each replication are growing at the same rate. Different amounts of principal invested at a bank at an equal rate of interest return different amounts of interest but always in proportion to the amount invested. Where there is a correlation between initial weight and weight gain, the magnitude of the experimental variation or error is partly dependent on the difference in the initial stocking weight. By expressing Nail's data as relative growth rates, the results are independent of the initial weight.

Relative gain, although correcting to some degree for the mathematical problem of having unequal stocking weights, does not correct for the biological effect. Simply putting weight gain on the same common denominator or on a percentage basis may not correct for the biological effects of differences in weights of fish stocked. Conditions in a pond which contains the higher weight of stocked fish may be different from conditions in the pond containing the

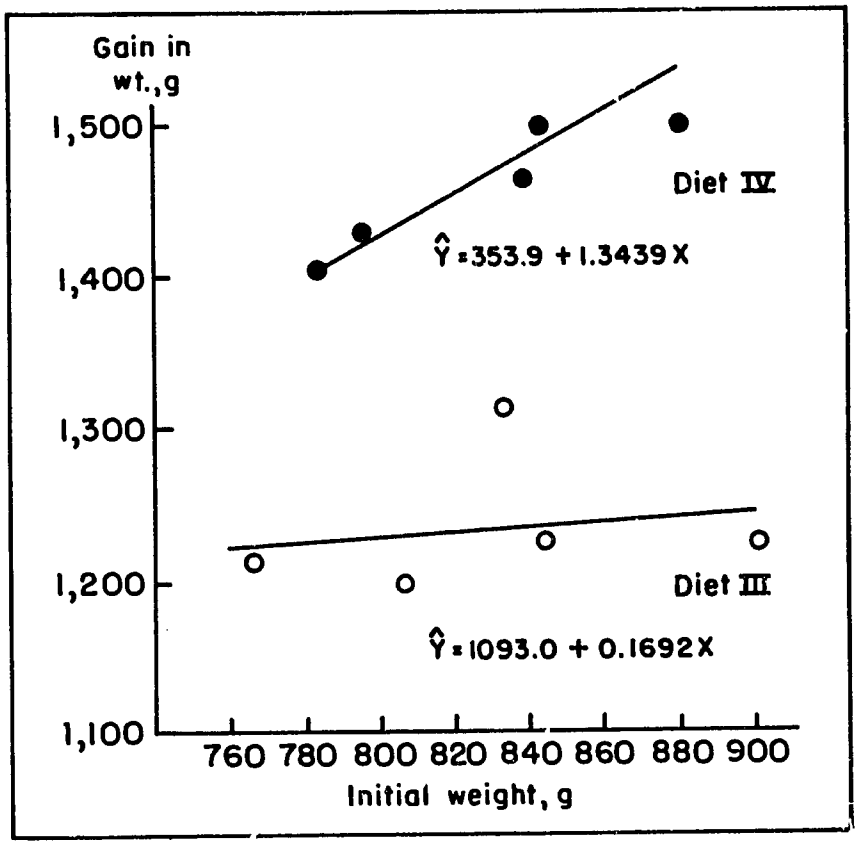


FIG. 21. Relationship between the initial weight and net production of channel catfish being fed two diets. Data from Nail (1962).

lower stocking weight from the beginning. This is especially true if there are considerable differences.

In the following table, relative gain data on five replications each of two treatments from Nail's (1962) experiment are presented. These relative gains are calculated from data in table 4.

	Relative gain (pct.)	
	Diet III	*Diet IV*
	144.7	169.8
	158.4	177.1
	148.5	179.3
	136.0	173.9
	157.3	179.0
Mean	148.98	175.82

Relative gain data also demonstrate the superiority of diet IV. Analysis of the data indicates that the hypothesis of "no difference"

should be rejected ("t" = 5.94, $0.005 < P < 0.001$). Relative gain data tell a little more about the results of the experiment than either standing crop or net gain. With relative gain, we know not only that the fish receiving the diets have increased in weight, but also we know something of the relative magnitude of the increase in weight. For example, in the case of diet III, the mean relative gain for the fish in the five replicate troughs was 149.0 percent. Thus, the mean gain in weight (mean net production) was 1.490 times the initial or stocking weight.

The advantage of the use of relative gain as a method of expressing the results of experiments is that it is a measure of the dynamics of biomass production. Unfortunately, the quantity is without units, which is a disadvantage. The method is of greater utility when the results are to be interpreted by researchers than when reporting results to fish farmers.

Gain Per Day

Gain per day is a method of expressing growth or yield often used by animal husbandry researchers. With this method, the net production over a given period of time is divided by the number of days in the period to obtain an average gain per day. The method is more applicable where the daily weight plotted against time is rectilinear rather than curvilinear. Where the biomass versus time relationship or growth rate is curvilinear, gain per day leaves much to be desired as a method of expressing growth because the value is constantly changing. The method may work for individual animals, but it does not work well when expressing the growth of a large number of fish because the increase in weight of fish in ponds or troughs, plotted against time, is seldom rectilinear.

Gains per day are calculated from data presented in table 4. Gain-per-day data lead to the same conclusion as the other methods. Analysis of the data indicated that the hypothesis of "no difference" between means should be rejected ("t" = 8.14, $0.005 < P < 0.001$).

	Gain per day (g)	
	Diet III	*Diet IV*
	17.0	20.8
	16.8	20.8
	16 6	19.8
	17.0	20.3
	18.2	19.5
Mean	17.12	20.24

From the above table it would be concluded that the fish receiving diet III gained 17.12 grams per day each day for 72 days and that fish receiving diet IV gained 20.24 grams per day; however, neither value represents the actual gain per day. Nail weighed the fish in each trough at intervals in order to adjust the amount of feed. The weights of fish in each trough on each of these dates were presented in table 4. With these data, gains per day were calculated for each time interval and are presented in the following table:

Interval	*Gain per day (g) for the indicated interval*	
	Diet III	*Diet IV*
June 3-July 10	12.9	15.8
July 10-July 31	17.8	19.7
July 31-August 30	11.5	14.0
August 30-September 3	29.3	36.0

The data presented in this table indicate that the gain per day for the different intervals differed considerably from the gains per day (17.12 grams and 20.24 grams) calculated for the entire experimental period of 72 days. Only during the interval July 10 to July 31 were the two estimates relatively close. In the data reported by Nail (1962), the use of a single gain per day value for the entire period would not adequately reflect daily changes in biomass in response to the treatments.

Growth Curves

Another method of expressing the results of production experiments is the growth curve. The growth curve provides an estimate of the weight of fish in the experimental unit at intervals throughout the experiment. These weights are plotted against time to obtain a time-size vector diagram. This method provides a figure or a graph showing the change in biomass with time. Using this method, treatments which result in rapid gains at the beginning of the experiment only to reach some limiting point and resulting in a plateau in the vector diagram are easily observed and can be differentiated from treatments where the increases may be smaller but regular over the entire period.

A primary advantage of using growth curves to express the results of experiments is that it is possible to determine the approximate time in which the treatments begin to exert a significant effect on growth or production. Growth curves of Nail's (1962) data for diets

III and IV are presented in figure 22. The differences in the diets were apparent when the fish were first weighed (on day 20). In other types of experiments, the effects of treatments on growth may not be apparent until much later. Dupree (1966) used growth curves to express data obtained in an experiment to determine the essential vitamins for channel catfish. One of the growth curves was shown in figure 17. It is obvious that there was little effect of the treatment or of the diet deficient in vitamin B-12 until between the 21st and 24th weeks. After that time the difference is dramatic. Data on standing crop obtained at the end of the trial would have demonstrated that channel catfish on vitamin B-12 deficient diets grow much slower than channel catfish on vitamin complete diets; however, with the growth curves it is possible to determine when the effect first

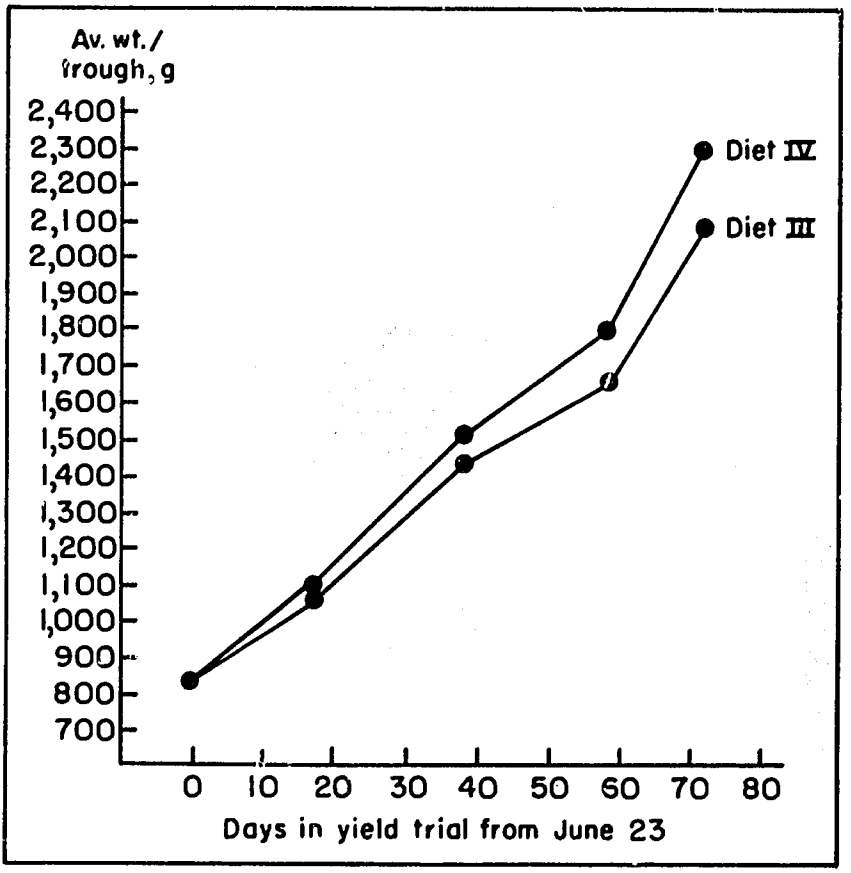


FIG. 22. Growth of channel catfish being fed two diets. Each point represents an average of five replications. Data from Nail (1962).

appeared. This type of data in turn can be used to obtain information on the storage of vitamin B-12 in the channel catfish and the time required to deplete this storage.

There is still another advantage in the use of growth curves. Where the effect of treatments appear early, experiments can be terminated early thus reducing the cost. For example, in Dupree's work there was little need to continue the experiment past the 30th week. An analysis of variance of his data at that time indicated that the variance ratio for treatment effect was significantly greater than could be accounted for by chance alone. The trial could have been terminated at that time, therefore reducing the length of the experiment by 6 weeks.

Growth curves are nothing more than a series of standing crop data obtained over a period of time. When fish are confined to troughs or cages, accurate standing crop measurements are relatively easy to obtain; however, when fish are in ponds, obtaining periodic estimates of standing crop is difficult. Representative samples often are difficult to obtain unless the ponds are small and a large proportion of the fish can be sampled. Also, repeated handling of the fish to obtain data may lead to injury as a result of handling and to lowered resistance to disease.

Transformation of Data

Statistical evaluation of production experiment data generally involves either the "t" test or the analysis of variance ("F" test) (Snedecor and Cochran 1967). With both of these tests, the assumption is made that the variances associated with different treatment means are homogenous. The variances must be independent of the means. When variances are heterogeneous, the tests usually give too many significant results.

Although little attention has been given to this problem, the meager amount of available information suggests that variances of aquacultural production data are generally not homogeneous but rather are directly proportional to the mean. Data from Swingle *et al.* (1963), presented in figure 23, are indicative of the relationship between means and variances in production experiments. Data from Greene (Annual Reports of Auburn Fisheries Research Unit 1969), presented in figure 24, also demonstrate the positive correlation between the mean and the variance. Data from Nail's (1962) experiment, presented in figure 25, give a somewhat different

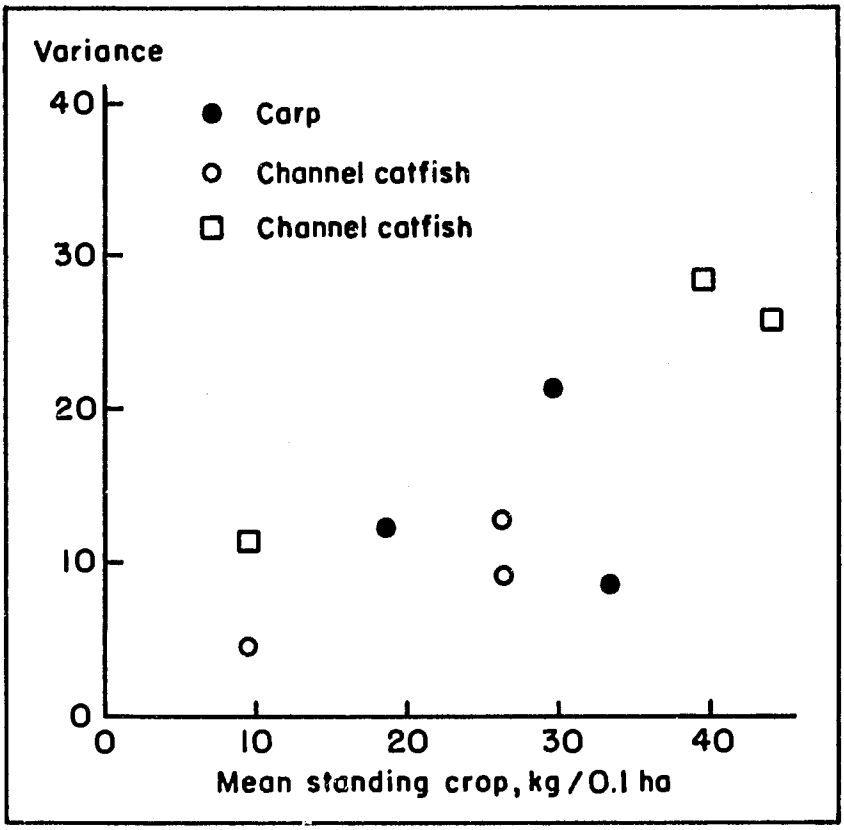


FIG. 23. Mean standing crops and variances of three experiments in earthen ponds. Data from Swingle *et al.* (1963).

picture. Nail's experiment utilized a factorial design in which levels of carbohydrate (9.3 and 18.6 percent) and levels of protein (6.3, 15.8, 25.3, and 34.8 percent) were the two factors. As is shown in figure 25, the relationship between the mean and the variance for fish receiving diets containing 9.3 percent carbohydrate was only slightly curvilinear; however, the relationship between mean and variance for fish fed diets containing 18.6 percent carbohydrate was strongly curvilinear. When the variances cited in the examples above are tested with Bartlett's test for homogeneity (Sokal and Rohlf 1969), in all three cases the tests indicated that the variances were heterogenous.

The examples cited above are not isolated ones. Apparently, heterogeneity of variances is to be expected in production experiment data. The reason for this phenomenon is not known. It appears

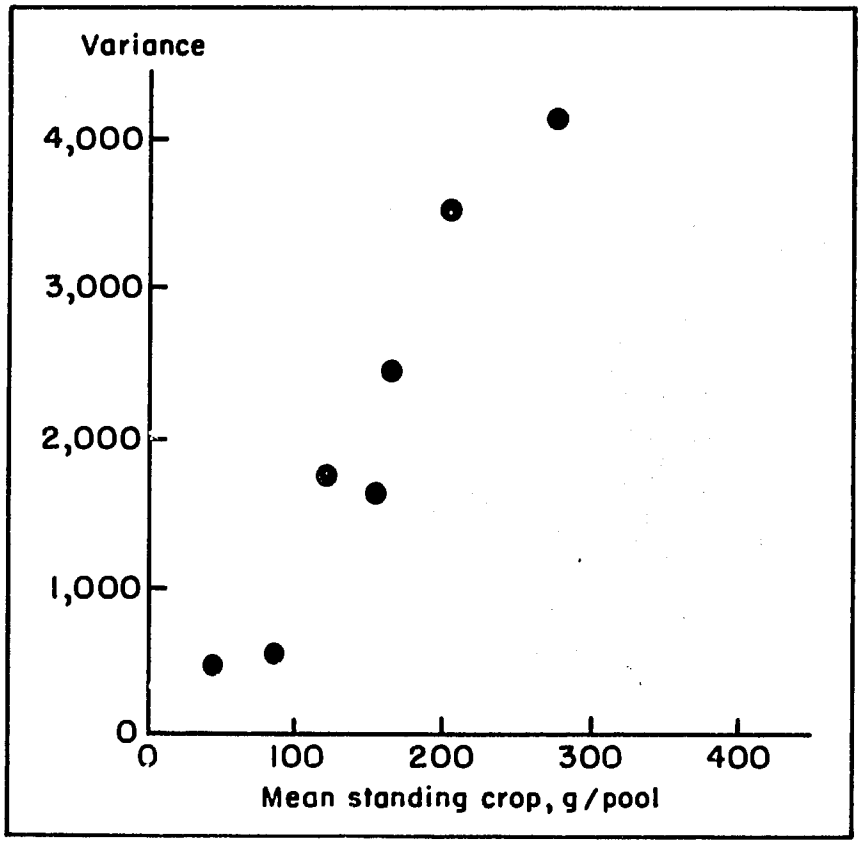


FIG. 24. Means and variances from a fish production experiment in plastic pools. Data from Greene (Annual Reports of Auburn Fisheries Research Unit 1969).

in pond experiments (Swingle *et al.* 1963) where the possibilities for interaction between environment and treatment generally increase with increased production; however, it also appears in flow-through systems (Nail 1962) where there is less chance of treatment-environment interaction. It is possible that the phenomenon is related to some characteristic of these "cold-blooded" animals in a "weightless" environment. Possibly, variation in genetic expression increases as the opportunities for such expression increases. For example, the variation in genetic expression may have increased in Nail's experiment as the quality of the ration improved.

Statistical tests, especially the analysis of variance, can lead to incorrect conclusions when the variances are heterogeneous. When heterogeneity occurs, the researcher has the choice of using an alternative method of analysis in which the assumption of homo-

geneity of variances is not necessary or the data must be transformed to a scale other than the linear scale of numbers. Means calculated from transformed data are generally homogeneous. Sokal and Rohlf (1969) include in their book an excellent discussion on the purpose and results of the transformation of biological data.

When the mean is positively correlated with the variance (greater means are accompanied by greater variances) a common logarithmic transformation of the mean is often appropriate (Sokal and Rohlf 1969). In the transformation, the individual observations are con-

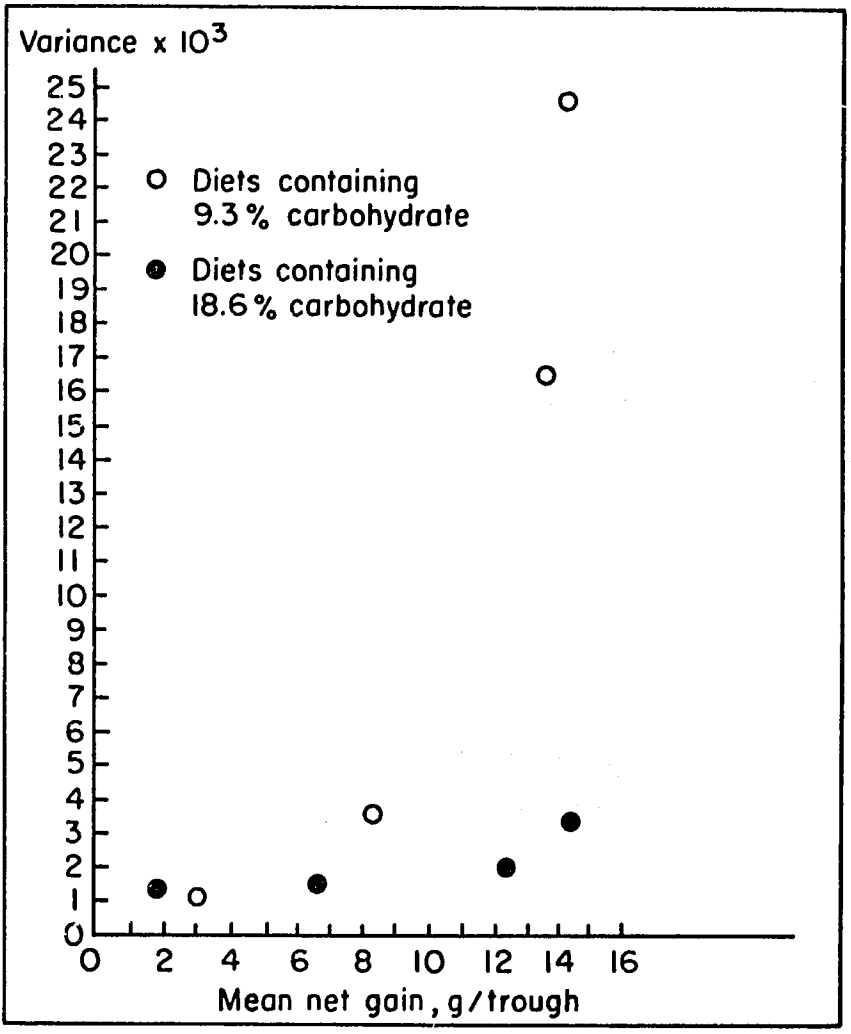


FIG. 25. Means and variances from an experiment in steel troughs on channel catfish nutrition. Data from Nail (1962).

verted to the common logarithmic scale for analysis of the data; however, results of tests are usually given in un-transformed form.

Presented in the following table are means and variances from diets V, VI, VII, and VIII of Nail's data in both the normal or decimal scale (net gain in grams per trough) and in the logarithmic scale.

	Normal		*Logarithmic*	
Diet	*Mean*	*Variance*	*Mean*	*Variance*
V	301	1,205	2.4772	0.00244
VI	844	3,509	2.9254	0.00091
VII	1,354	16,526	3.1300	0.00192
VIII	1,409	24,700	3.1469	0.00242

It is obvious from data in the above table that the variances in the normal scale are heterogeneous. The ratio of the largest to the smallest is 24,700/1,205 = 20.5. As expected, Bartlett's test for the homogeneity of variances indicates that they are highly heterogeneous. On the other hand, the variances on the logarithmic scale are much closer together. The ratio of the largest to the smallest is now 0.00244/0.00091 = 2.7. Bartlett's test indicates that the variances on the transformed scale are not heterogeneous. There still remains an indication of a positive correlation between mean and variance on the tranformed scale, but it no longer is as strong as it was when the data were in the normal scale.

The heterogeneity of variances in the above example can also be corrected by using a cube root transformation. The cube roots of weights of fish are used in the analysis. The growth curve of a fish in terms of length over a short period of time may be generally rectilinear; however, the growth curve in terms of weight is almost never rectilinear. Results of production experiments are generally expressed in some form related to weight, which increases generally as the cube of length. The cube root transformation tends to convert the results from a curvilinear to a rectilinear scale. Haskell (1959) discussed the use of the cube root of weight gained in trout for analyzing the results of experiments.

In the following table, the means and variances obtained from a cube root transformation of Nail's (1962) data on diets V-VIII are shown:

Diet	*Mean*	*Variance*
V	6.6984	0.0651
VI	9.4455	0.0481
VII	11.0535	0.1335
VIII	11.2005	0.1814

The ratio of the largest to the smallest variance with this transformation is 0.1814/0.0481 = 3.8, which is slightly larger than the maximum ratio obtained from the data transformed by logarithms. The effect of logarithmic transformation is much more drastic than the cube root transformation.

The Role of Statistical Analysis

Statistical analysis is often incorrectly considered to be a decision making process when in reality it is but a tool in the process; there are other equally important parts. Design and analysis of experiments provide a systematic procedure for collecting, assimilating, and summarizing data in a standardized format. Data summarized in this manner then can be used in the decision making process. The process, if it is to be effective, must also involve knowledge of the factors and their interactions involved in the phenomena being compared or characterized, through an understanding of the measuring system that has been used to obtain the data, experience with the use of statistics in the decision making process, and an appreciation of the role of decision making in the production of useful information. One of the common problems in the use of statistics in research is the over-emphasis on achieving a result that can be called statistically significant (Stoltenberg *et al.* 1970). Snedecor and Cochran (1967) warned that a biologist seldom if ever rests his decisions wholly on tests of significance of hypotheses.

I can remember my excitement when, as a college senior, I first learned the mechanics of making a "t" test. I could hardly wait to get back to the laboratory to run some tests. I ran them on virtually all of the data that I could find. I was enthralled to see how easy it was to determine if there was a difference in means. It was not until sometime later when I realized how my decisions might affect farmers or other scientists that I decided that there were other aspects to the decision making process than simply a comparison of an observed and tabular "t" value.

Statistical tests are based on precise mathematical relationships. The mathematical theory underlying these relationships is rather well developed. It has been determined through observation that certain quantitative characteristics of biological systems approximate mathematical relationships. For example, it has been observed that frequency distributions of numerous biological phenomena approximate a precise mathematical distribution, the so-called "normal" or "bellshaped" distribution. Because of the

apparent similarity of the distributions, it is assumed that certain characteristics (means, variances) of the distributions also are similar. These characteristics can be used to develop probability statements that can be used in comparing distributions, or samples, from those distributions. Where the degree of approximation between the biological and mathematical or theoretical is rather high, decisions based on the comparisons are dependable; however, as the distributions become more divergent, the decisions become more questionable. Unfortunately, it is often difficult to determine with confidence how much the two systems diverge. This uncertainty is a result of the complexity of biological systems and our lack of knowledge of the multiplicity of factors and interactions of those factors that determine the characteristics of these biological distributions. Comparisons of mathematical distributions may be made with precise accuracy, but because of the uncertainty, comparisons between biological distributions are much less precise; consequently, decisions regarding differences in samples drawn from distributions representing various treatments contain an element of doubt.

Over the years in agricultural research, repeated utilization of statistical tests to evaluate experiments has resulted in the development of a considerable degree of confidence in the value of these tests. Repeatedly, decisions based on statistical analyses of experimental data were validated when results of those experiments were put into practice by farmers. Because of this repeated validation, agricultural scientists consider statistical procedures indispensable in the design and analysis of experiments and have considerable confidence in their use.

The biological and physical processes and their interactions seem to be somewhat more simple in the animal-plant-air-soil environment of agriculture than in the fish-plant-water-dissolved gas-soil environment of aquaculture. Because of the basic differences in the two environments, it is not clear whether the confidence developed in the value of statistical methods in agriculture can be developed in the case of aquaculture. Research in aquaculture is relatively new. Decisions based on statistical analysis of experimental data have not been adequately validated. It is likely that statistical procedures will finally be as important in aquaculture as in agriculture, but until adequate validation is accomplished, aquacultural research should use the decision making or hypothesis testing process with care. In the meantime, considerable effort is needed to validate the use of statistical procedures in aquaculture and to develop the information on application and theory necessary.