PN-ABN-941
82038

UNITED STATES-ISRAEL COOPERATIVE DEVELOPMENT PROGRAM - CDR

FINAL REPORT

The role of sulfate ions in the potential to replace refined feeds with agricultural residues in mariculture

(C7-042).

Grant number DHR-5544-G-SS-9068-00

Principal Investigator:

Dr. Gerald L. Schroeder

Aquaculture Research Unit

Agricaltural Research Organization

P.O. Box 6, Bet Dagan ISRAEL

Cooperating Investigator:

Dr. Romeo Fortes

University of the Philippines in the Visayas

Iloilo City, The Philippines

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Dr. Gerald L. Schroeder
Aquaculture Research Station, Agricultural Research Organization
P.O. Box 6, Bet Dagan, ISRAEL 50250.

Cooperating Investigator:

Dr. Romeo Fortes
University of the Philippines in the Visayas
Iloilo City, Philippines.

Signature of Principal Investigator:

Gerald Schroeder

OVERALL OBJECTIVES are to evaluate the role of the sulfate-sulfide transition, and specifically the role of sulfide as a toxin that limits the availability of natural foods in brackish and salt water, earthen fish ponds. Utilizing this information, pond management strategies will be evaluated with the intent of improving fish production with reduced reliance on refined feeds.

OBJECTIVES OF FINAL RESEARCH PERIOD were to establish the experimental fish tanks stocked with mullet (*Mugil cephalus*) and complete the sulfate-sulfide experiments using this fish species. Parallel to this, a second out-door tank experiment using a simple carbohydrate as the only organic input was to be performed. We were to complete the evaluation of stable carbon isotope data taken from the initial out-door tank experiment in which a simple carbohydrate plus mineral fertilizers (and sun light) were the only inputs.

ACHIEVEMENTS

The above objectives were accomplished.

Experiment #6: The effect of sulfate-sulfides on mullet (*Mugil cephalus*).

The experimental plan here follows that of the previous indoor experiments. Washed beach sand was spread on the floors of 15 glass aquariums creating a four centimeter layer. These and 15 similar aquariums with no sand bottom were each filled with enough tap water to provide 30 liters of active water volume. On 2 June 1991 ten mullet fingerlings with a mean weight of 2 grams each were stocked in each aquarium. The mean stocking weights were very similar, varying by less than 0.05 grams among the 6 separate treatments. Sodium sulfate was added to produce concentrations that were 0.5%, 50% and 100% of the sulfate found in sea water (2.7 grams sulfate per liter). The aquariums were indoors and the light was dim. No algal growth was evident. Feed was supplied daily to all tanks at 7% of the fish biomass present in the heaviest tank population. Water temperature was 26 to 28° throughout the test. Water pH was approximately 7. The fish were harvested 42 days later, on 7 July.

Continuous aeration maintained oxic conditions in the water column at all times. In the tanks with no sand there was no indication of sulfide accumulation. In the tanks with sand bottom, a black anoxic layer appeared in the sand after about a week of feeding. The interstitial water of this layer had a strong smell of sulfide. The interstitial sulfide concentration in this black layer was measured.

To reduce the accumulation of metabolites in the water column, crushed stone biofilters were installed in each tank. The aeration provided an air lift which continuously circulated the tank water through the 0.5 liter biofilter. These filtered the water column while leaving the interstitial waters in the sand bottoms relatively undisturbed, thus allowing the accumulation of sulfides within the sand intersticies. As demonstrated in our second report, the biofilter did not alter the water's sulfate concentration.

Fish growth, survival and sulfide data are listed in Table 1. Weight gains in all the treatments were low, about 2 to 3 grams per fish over the entire 42 day period. At harvest, there is a trend of increasing weight with increasing water sulfate concentration. This might have been expected since the mullet are a sea fish. A clear correlation between sulfate and growth is confounded by the decrease in rate of fish survival with increasing sulfate concentration. As survival decreased, the population pressure on available resources

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decreased. This in turn may have allowed better fish growth. It should be noted that in the tanks with 90% or greater fish survival there was no trend of increasing fish growth with increasing sulfate concentration.

Within a given sulfate treatment, the fish survival in aquariums with sand bottoms was higher than in aquariums without sand bottoms. The bottom acts as a sink for solid wastes and as a passive biofilter. The transition from oxic to anoxic conditions allows a wide range of microbes to flourish and process potentially toxic metabolites. Parallel to this, in experiment #7 (see further; out-door tanks supplied with simple carbohydrates), only after a porous bottom was provided did fish growth occur.

Although the only place where dissolved sulfides occurred was in the intersticies of the sand bottoms, neither fish growth nor survival was related to the presence of this sulfide. The benefit of the porous bottom out-weighed any negative effects which the sulfide might have produced.

Experiment #7: Tilapia hybrid growth in out-door tanks receiving simple carbohydrates plus chemical fertilizers.

The experimental fish tanks here are those used in experiment #1. They are located out-doors in open sun light, 0.88 sq meters, 65 cm water depth, 0.57 cu meter active water volume with continuous aeration. The bottoms of tanks #3, 4 and 6 had 6 cm of washed beach sand. The bottoms of tanks #1, 5 and 7 had 2 cm of sand. The outlets of the aeration (porous air stones) were suspended at mid water depth to minimize disturbance of the bottom sand by the air flow. On 8 August

1991, six tanks were stocked with 8 tilapia hybrid fingerlings, averaging 30 grams each. The fish were harvested 61 days later, on 10 October.

Three to 5 days each week, tanks #1, 5 and 7 received pelleted trout feed containing 38% protein at a daily ration equivalent to 5% of the fish biomass. At the same time, tanks #3, 4 and 6 received granulated cane sugar at a nominal daily rate of 10% of the fish biomass. The sugar, a simple carbohydrate, was the only organic input to these tanks. These three tanks also received calcium superphosphate and ammonium sulfate at rates to provide a C:N:P ratio of 100:5:1. In total there were 49 feeding days.

To avoid any confusion, let it be noted that we are not suggesting farmers add sugar to their fish ponds. The sugar provides, for experimental purposes, a source of carbohydrate that is pure, of a known composition and relatively cheap. Molasses or properly treated crop wastes would be the logical choice in a commercial fish farm.

Data related to fish growth are listed in Table 2. There was 100% survival in all tanks. Wild spawn was observed in both pellet-fed and sugar-fed tanks. The weight of this spawn was not included in the data. The fry were only a fraction of a gram each. The pellet feed produced good growth, averaging 1.4 g/fish/day. The sugar yield was very much lower, averaging 0.36 g/fish/day. The rates of feed conversion were even less similar than the growth rates. A total of 1487 grams of pellet feed were supplied to each tank, resulting in a feed conversion ratio (FCR) of 2.16 (2.16 kilo of supplied feed per kilogram of fish weight gain). A total of 3470 grams of sugar were supplied to each tank, resulting in a FCR of 19.7. With such disparate results, there can be no justification for use of this simple carbohydrate as a substitute for pelleted feed.

The fish growth did not show any correlation with the Secchi depth, either in the pellet-fed or in the sugar-fed tanks. Seston concentration is related to Secchi depth. The seston in these tanks was almost entirely composed of suspended organic matter. Hence, the concentraions of suspended organic matter was not the limiting factor in the fish growth.

The growth rates here in the sugar-fed tanks are similar to thsoe attained in experiment #1 after cotton cloth, placed on the tank bottoms, provided a porous bottom interface. Here, the sand bottom most likely served the same function. Recall that, in the initial stages of experiment #1 when no porous bottom was present, there was no fish growth in the sugar-fed tanks.

Stable carbon isotope data from Experiment #1 (THE OUTDOOR TANK EXPERIMENT)

The experimental approach was detailed in our December 1990 progress report. In brief, eight tanks, 0.88 sq meters, 65 cm deep water or 0.57 cu meters actual water, out-doors, sunlit were stocked with either 25 or 75 red aurea tilapia hybrid fingerlings (about 6 g/fish). Two tanks with 25 fish received feed pellets (final rate 2.5 to 3% biomass/day, 6 days per week). Three tanks with 25 fish and 3 tanks with 75 fish received sugar at a rate of 5% to 6% fish biomass plus ammonium chloride (7 grams daily) or ammonium sulfate (5 grams daily), calcium superphosphate (2 g daily), potassium chloride (1 g per week). The C:N:P input ratio in these "sugar" tanks was approximately 100:5:1. A ninth tank was stocked with 5 fish and had no inputs otner than the minerals in the tap water. Secchi depth in this tank after a few weeks reached 15 cm. Therefore the minerals of the tap water supported a reasonable algal growth.

All tanks were aerated 24 hours daily. Only make-up water to compensate for evaporation was added.

During the first 30 days, the pellet-fed tanks received chemical fertilization at a rate equal to 20% of that given to the sugar-fed tanks. After this no further chemical fertilization was added to the pellet tanks.

The experiment lasted from 18 July to 25 October. Water temperatures were 28 to 31° C until mid-September and then 25 to 28° C until harvest. pH was 7 to 7.5 throughout the entire time. Survival exceeded 90% in all tanks except one of the 25 fish tanks.

The rate of sugar addition was approximatley twice that of the rate of pellet feeding. It is not certain that this is necessary. The rate was chosen because when sugar is consumed by bacteria, about half is used for the metabolic energy of the bacteria and so is lost as carbon dioxide.

During the first 2 weeks, pellets and sugar were supplied at 1/4 the rates listed above. Although secchi depths were 15 cm (a good value from pond experience) and the color a rich-olive green, fish growth was 0.01 to 0.1 g/fish/day. Based on the water color and secchi depth, the seston had adequately developed but it could not support any significant growth. Note, however, that these same fish when held in clear water lose about 0.1 g per day. The seston maintained their weight but did not permit growth. Therefore the seston contributed to the maintenance of the fish.

After these 2 weeks, on 9 August, feed and sugar rates were raised to the higher values. Growth immediately increased in the two pellet-fed tanks to 0.4 and 0.7g/fish/day. Growth in the sugar tanks did not increase although the effect of the sugar was immediately seen in the seston. Secchi depth fell from 15 cm to 10 cm. Water color changed from deep olive green to brown and few algae were evident although they were clearly abundant in the pellet ponds.

After 12 days with these conditions, on 21 August, unbleached muslin (100% cotton) cloth, with thickness similar to a bed sheet (19 mg/ sq cm), was placed on the bottoms of all sugar tanks and one of the pellet tanks (tank #2). The othe. pellet tank (#8) did not receive the cloth. The cloth was weighted down with bricks.

After the cloth was added, growth in the 25 fish, sugar tanks immediately increased to 0.2 and 0.4 g/fish daily. Growth in the 75 fish sugar tanks remained at less than 0.1 g/fish daily. These tanks were eventually dropped from the experiment. The high density of the fish (75 fish in approximately half a cubic meter of water) exceeded the carrying capacity for the conditions of this experiment.

It is not clear why the cloth on the tank bottom makes a difference in the fish growth. Similar results in smaller indoor experiments were attained about 3 years ago. We had theorized that the cotton was acting as a substrate upon which bacterial slimes could form. Fish could then graze upon this detritus. In the out-door tanks the produciton of algae was intense. Previous measurements in similar conditions showed photosynthetic production of algae at approximately 5 g algal carbon/sq meter/day. The detritus resulting from this photosynthesis produced a detrital mat which might have acted as a sub-trate upon which the bacterial slimes could form and upon which the fish could graze. But the fish did not do well on this. The algal detritus does not seem to be what is needed. It may be that the cotton cloth functions as a porous bottom as well as a substrate for grazing. The water under the cotton sheet was anoxic. The organic matter which accumulated under the cotton sheet was microbially processed. This was evident from the black color of the slimes and the high concentrations of sulfide (at times exceeding 10 ppm) in this region.

Based on delta C data, there is no evidence of cotton assimilation by the fish. Delta C data indicate that the fish muscle in the sugar-fed tanks comes 1/4 directly from sugar carbohydrate and 3/4 from seston. (The tank water had a slight but definite sweet taste.) Comparison of seston delta C in the pellet-fed tanks with the seston delta C in the sugar-fed tanks indicates that the sugar tank seston is based half on carbon originating from the sugar (now incorporated into the microbial slime) and half on algal-based carbon. The delta C of fish muscle taken from pellet-fed tanks shows no evidence of seston. Growth in these tanks appears to be totally based on the supplied feed pellet. This is not surprising since the pellet is of very high quality.

The seston in these sugar-fed tanks was chocolate brown. Not at all like the olive green seston of the pellet tanks. From the almost immediate change in water color from olive green to brown when the input of sugar was increased at 2 weeks into the experiment, it is clear that the bacteria using the sugar for energy compete well against the algae for the added minerals. During the hour following the daily addition of the sugar, the dissolved oxygen of the aerated tank water dropped from 6 or 7 ppm to 1 to 3 ppm even in the presence of the strong continuous aeration. This is the result of the bacterial respiration during the aggressive uptake of the sugar by the microbial population of the tank. There is no such massive drop in DO when the pellets are added to the tanks.

In preliminary experiments, chitin flakes helped the fish growth in sugar tanks (indoor, 50 liters) as much as did cotton cloth. As with the cotton, carbon from the chitin did not appear in the fish muscle. The use of chitin flakes as a recycleable added substrate plus the simple carbohydrate (molasses) might be of practical value. It does require though that there be a cheap source of simple carbohydrate, or a way to stimulate microbial growth which is much cheaper than conventional feeds. One method to achieve this goal might be through the use of a reactor fed with a locally available agri-residue. In intensely stocked fish tanks, where the demand for food per unit volume of water is high, the demand for feed exceeds the capability of the fish tank itself to produce the needed quantity of food.

Regarding microbial processing of added minerals: Although ammoniumn salts were being added to the tanks of this experiment at rates of 7 grams ammonium chloride or 5 grams ammonium sulfate/tank/day, or nitrogen in the form of protein in the pellet-fed tanks at rates reaching 30 grams of pellets containing 15% protein/tank/day, all at 6 days/week, after 70 days the nitrate concentration ranged between 0 and 3 ppm. Nitrite concentration was >>1ppm during the first 40 days and then fell rapidly to close to zero. Ammonia rarely exceeded a few tenths of a ppm and often was not detectable.

Overall Conclusions

The toxic nature of the sulfide ion is well known. In the experiments here, although sulfide concentrations exceeding 2 ppm were present in the intersticies of the sand bottoms of the aquariums, no correlation was found between sulfide and fish growth. This result may be due to the beneficial effects of the porous bottom acting as a passive biofilter as well as a site of microbial processing of organic detritus. If such is the case, then the benefits of the bottom masked the detriment of the sulfide.

To gain insight into marginally effective parameters, it is necessary to have reasonablly high fish growth rates. This requires a directly supplied feed able to produce this growth. The natural foods of the aquarium are insufficient. In out-door, densely stocked tanks, natural food production can maintain a fish yield of about 25% that attained with supplied high (38%) protein pellet. Approximately half the fish growth in the tanks receiving a simple carbohydrate was based on the carbon of this carbohydrate. Of this carbon, half was ingested after it was incorporated into seston. This is in sharp contrast to the fish reared in pellet-fed tanks where the fish growth was entirely based on the supplied pellet.

Although the rates of nitrogen input are very high in the densely stocked fish tanks, the processing of the metabolites by algae and other microbes was sufficiently rapid to keep ammonia levels at a fraction of a ppm. This was not the case in indoor aquariums stocked at similar rates. Here active biofilters were necessary to maintain water quality and fish survival.

TABLE 1
Fish growth, survival, and sulfide concentrations in indoor 30 liter tankks stocked with Mugil cephalus (mullet) fingerlings.

		2/6/91			16/6/91			14/7/91			%	mean sulfide
TREATMENT	TANK	weight	no.	mean	weight	no.	mean	weight	no.	mean	survival	concentration (ppm
	13	20.5	10	2.05	24.2	10	2.42	37.8	9	4.20		
	15	19.2	10	1.92	21.4	10	2.14	35.9	10	3.59		
0%+SAND	17	20.3	10	2.03	25.0	10	2.50	38.7	10	3.87	96	0
	23	20.3	10	2.03	29.0	10	2.90	39.4	9	4.38		
	30	19.4	10	1.94	25.3	10	2.53	42.9	10	4.29		
MEAN TOTAL				1.99			2.50			4.07		
	2	21.5	10	2.15	23.7	9	2.63	32.6	7	4.66		
	6	20.4	10	2.04	24.1	9	2.68	33.9	9	3.77		
0% NO SAND	8	21.2	10	2.12	24.7	10	2.47	36.5	9	4.06	82	0
	18	18.2	10	1.82	21.6	10	2.16	30.3	8	3.79		
	25	19.5	10	1.95	22.0	10	2.20	37.2	8	4.65		
MEAN TOTAL				2.02			2.43			4.18		
	5	21.8	10	2.18	26.0	9	2.89	33.5	6	5.58		
	7	19.9	10	1.99	25.5	9	2.83	37.9	9	4.21		
50%+SAND	9	19.9	10	1.99	22.3	9	2.48	41.1	7	5.87	76	2
	19	18.7	10	1.87	21.4	9	2.38	35.8	8	4.48		
	28	19.3	10	1.93	22.0	9	2.44	36.4	8	4.55		
MEAN TOTAL				1.99			2.60			4.94		
	4	20.1	10	2.01	18.4	9	2.04	---	---	---		
	12	18.8	10	1.88	24.8	10	2.48	33.5	9	3.72		
50% NO SAND	16	20.1	10	2.01	20.6	9	2.29	24.6	6	4.10		
	20	19	10	1.90	22.2	10	2.22	29.2	6	4.87	54	0
	29	19.4	10	1.94	12.8	6	2.13	24.2	6	4.03		
MEAN TOTAL				1.95			2.23			4.18		
	1	20.9	10	2.09	26.6	10	2.66	42.1	7	6.01		
	3	19.8	10	1.98	18.2	6	3.03	---	---	---		
100%+SAND	11	19.1	10	1.91	20.0	9	2.22	36.7	7	5.24		
	21	19.3	10	1.93	21.8	8	2.73	37.6	8	4.70	60	
	26	20.3	10	2.03	25.9	10	2.59	40.9	8	5.11		
MEAN TOTAL				1.99			2.65			5.27		
	10	19.8	10	1.98	22.9	10	2.29	35.7	9	3.97		
	14	19.4	10	1.94	21.6	9	2.40	28.7	5	5.74		
100% NO SAND	22	19.2	10	1.92	21.9	9	2.43	28.6	5	5.72	68	
	24	19.6	10	1.96	20.9	9	2.32	31.5	8	3.94		
	27	18.3	10	1.83	17.5	8	2.19	30.3	7	4.33		
MEAN TOTAL				1.93			2.33			4.74		

weight= total fish biomass/tank (grams)
no.= number fish/tank
mean= mean weight (grams/fish)

Table 2. Data related to tilapia hybrid yields in out-door tanks receiving either pelleted trout feed or a simple carbohydrate plus inorganic fertilizers.

	"Feed"	Number of fish per tank	Average fish weights (g/fish) Stock	Harvest	Fish growth (g/fish/day)	Average Secchi depth (cm)
Tank #1	pellet-fed	10	39.6	129.8	1.5	7.2
Tank #5	"	10	41.2	121.1	1.3	14.2
Tank #7	"	10	40.4	128.1	1.4	7.6
Tank #3	sugar-fed	10	37.9	48.8	0.18	7.8
Tank #4	"	10	34.9	66.3	0.51	19.0
Tank #6	"	10	38.5	62.3	0.39	4.2

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Table 3. Summary of delta C data for samples taken in experiment #1 (out-door fish tanks receiving feed pellets or sugar and inorganic chemical fertilizers).

All values are averages of 3 or more measurements. The spread in any one set of delta C data was less than 1 o/ou.

	"Feed"	Number of fish per tank	Fish growth g/fish/day	Delta C of fish muscle or item noted
Tanks #2, 8	pellet-fed	25	0.4 and 0.7	-21.0; -21.1 muscle
Tanks #4, 7	sugar-fed	25	0.1 and 0.5	-16.8; -16.5 "
Tanks #5, 6	sugar-fed	75	0.07 both	-17.9; -15.5 "
Tank #9	no-inputs	5	0.03	-21.0 "
Tanks #2, 8				-24.0 seston
Tanks #4, 7				-18.7 seston
Tank # 5				-15.2 seston
Tanks #2, 8				-20.5 feed pellets
Tanks #4, 7, 5, 6				-14.0 sugar

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THE ROLE OF SULFATE IONS IN THE POTENTIAL TO REPLACE REFINED FEEDS WITH AGRICLTURAL RESIDUES IN MARICULTURE

A. INTRODUCTION

The commercial operation of aquaculture farms in the Philippines can still be considered in the fertilization level of management although a number of prawn (*Penaeus monodon*) farmers have attempted to use higher levels where stocking density is high, supplemental or intensive feeding is necessary and use of life support systems is required. The trend of pond aquaculture is obviously going into the higher level of management. In the country, at least three levels of management or methods of fish farming are practiced as follows:

1. **Extensive method.** This utilizes relatively bigger areas of ponds and lower stocking densities (about 5,000 to 30,000 post larvae/ha. The stocks are raised primarily on natural food grown in the pond.

2. **Semi-intensive method.** This is characterized by smaller pond compartment and relatively higher stocking densities (>30,000/ha to about 150,000/ha); use of artificial feeds as a regular supplement becomes necessary; pumps become a necessity and aeration is sometimes required to maintain optimum water quality.

3. **Intensive method.** This is likewise characterized by smaller pond compartments and high stocking densities (>150,000/ha); intensive feeding is practiced; use of life support systems such as water pumps, aeration and others are required to maintain an optimum condition for the growth of the species.

It is unfortunate however, that most of the practitioners can not identify one from the other (Fortes, 1989). Using any one of such management scheme and the technology associated with it, many of the users fail to attain their expectations because the cost of production from such system increases every year while the prices of the products decreases. In prawn farming, for example, the cost of feeds alone is about 50-70% of the operating cost which is also true in other forms of animal husbandry. Inasmuch as natural foods account for more than 50% of the growth of fish or prawn (Schroeder, 1983), a significant amount of the operating cost is therefore wasted in terms of feeds. This can be minimized, if not prevented, if the individual niches of natural foods in fish and prawn diets are known thus, it is important that the role of sulfate-sulfide reaction, specifically the role of sulfide as a toxin that limits the availability of natural foods in brackish and salt-water earthen ponds, be evaluated. In freshwater ponds, which is relatively sulfate-free, the anaerobic microbial processes convert the crude organic matter of the added fertilizers into a main source of nutrition for the target animals of these ponds (Schroeder, 1983). In salt water ponds, however, the presence of toxic sulfides which are intimate by-products of these microbial processes and are toxic to most forms of life, may exclude the use of these foods by target animals. The potential of fresh and salt water ponds in terms of fish production appear to be similar, however, experience has been that, for fish farming based on agricultural residues as

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substrates for intense in-pond growth of natural food or based on direct refined feeds, freshwater ponds out perform salt water ponds. In theory, sulfate-sulfide reaction influences microbial activities on the processing of sediment organic matter into useful fish food. It is therefore necessary that a thorough study of the sulfate-sulfide processes be made in order to correlate the role of these ions in the microbial processes/metabolism of natural food production, which would ultimately affect fish yield. If fish and shrimp yields are independent of sulfide and ammonia concentrations in the sediments, then the potential to replace feeds with feed substrates (i.e. agricultural residues) is as great in salt water as it is in freshwater provided a proper management strategy is used. On the other hand, if the fish is found to avoid food niches that are sulfate-rich, this will have a significant implication for mariculture management.

B. OBJECTIVES

On the basis of the above, the project attempted to evaluate the role of sulfate-sulfide reaction, specifically the role of sulfide as a toxin, that limits the availability of natural foods in brackishwater earthen ponds. With this information, pond management strategies will be evaluated with the intent of improving fish production with reduced reliance on refined feeds. Consistent with these overall objectives, the following were the specific objectives:

1. To determine the effect of varying levels of sulfate in sea water on fish yield;

2. To determine the role of sulfate and sulfide ions on natural food production;

3. To determine the effect of sulfate concentration on the nitrogen content of cellulose-rich feed substrates after a period of microbial processing. Part of the results of this project were presented in two (2) international forums as follows:

Fortes, R.D., N.R. Fortes and I. G. Pahila (in press). Effect of varying levels of sulfate concentration in saline waters on fish yield. BOSTID-ICLARM Aquaculture Workshop for PSTC/CDR Scientists, August 6-10, 1991, ICLARM Headquarters, Manila, Philippines.

Fortes, R. D., N. R. Fortes and I. G. Pahila (presented in the scientific session). Agricultural residues as feed substrates for milkfish production in brackishwater ponds. The Third Asian Fisheries Forum, October 26-30, 1992, World Trade Center, Singapore.

(Please see Appendices A and B for the manuscripts)

This report covers the period from September 1, 1990 to August 31, 1992.

C. LOCATION AND DESCRIPTION OF THE STUDY SITE

The site is located in barangay Nabitasan, municipality of Leganes, province of Iloilo in the island of Panay, Philippines (See Figures 1 and 2). The municipality of Leganes is located N 10° 8′ longitude; E 122° 5.4′latitude. The facilities used are part of the Brackishwater Aquaculture Research Station, Institute of Aquaculture, College of Fisheries, University of the Philippines in the Visayas.

D.RESEARCH HIGLIGHTS

1. Preliminary Run

Twelve (12) units of aquariums (90 x 30 x 45 cm) with approximately 10 cm of sand at the bottom and 30 cm of filtered seawater were used in this run. Seawater from four (4) different sources with varying sulfate concentrations were used as treatments which were replicated 3 times each. Air-lift aeration system was provided. No fertilizer was added. On Nov. 2, 1990 sixteen (16) tilapia (Oreochromis mossambicus) fingerlings, approximately 4.0 g.each were stocked in each aquarium. The fish were uniformly fed with rice bran incorporated with 1% cane sugar at 2% - 5% of the fish biomass. However, the experiment was terminated after 24 days because of high mortality. Water parameters such as sulfate, sulfide, DO, pH salinity, and temperature were monitored.

The ranges of sulfate concentration and salinity levels of the water from the 4 sources are given in Table 1. Treatment I, taken from Iloilo Strait between Panay Island and Guimaras Island

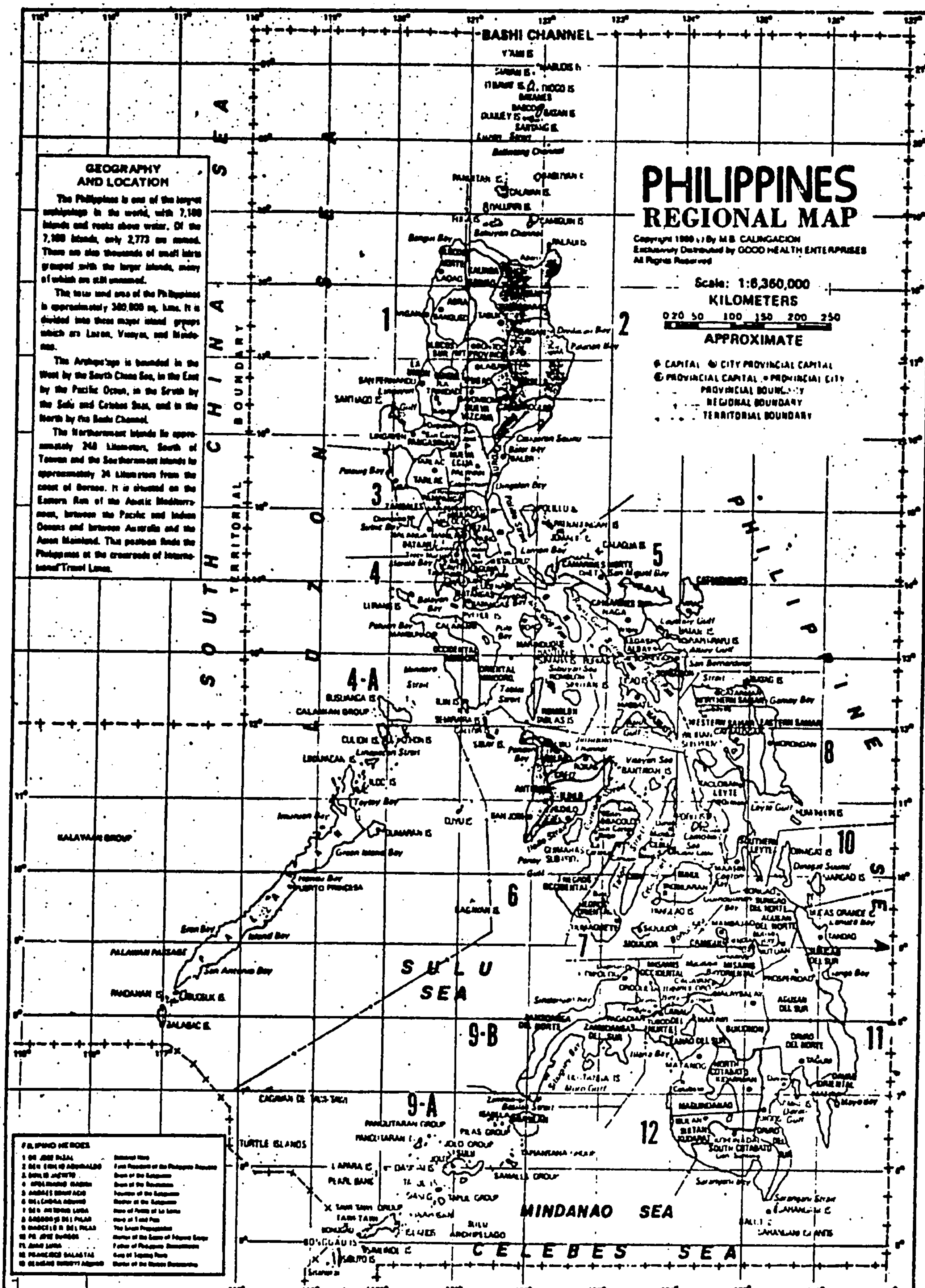

Figure 1. Map of the Philippines showing the location of Iloilo Province in the island of Panay relative to the City of Manila in the island of Luzon.

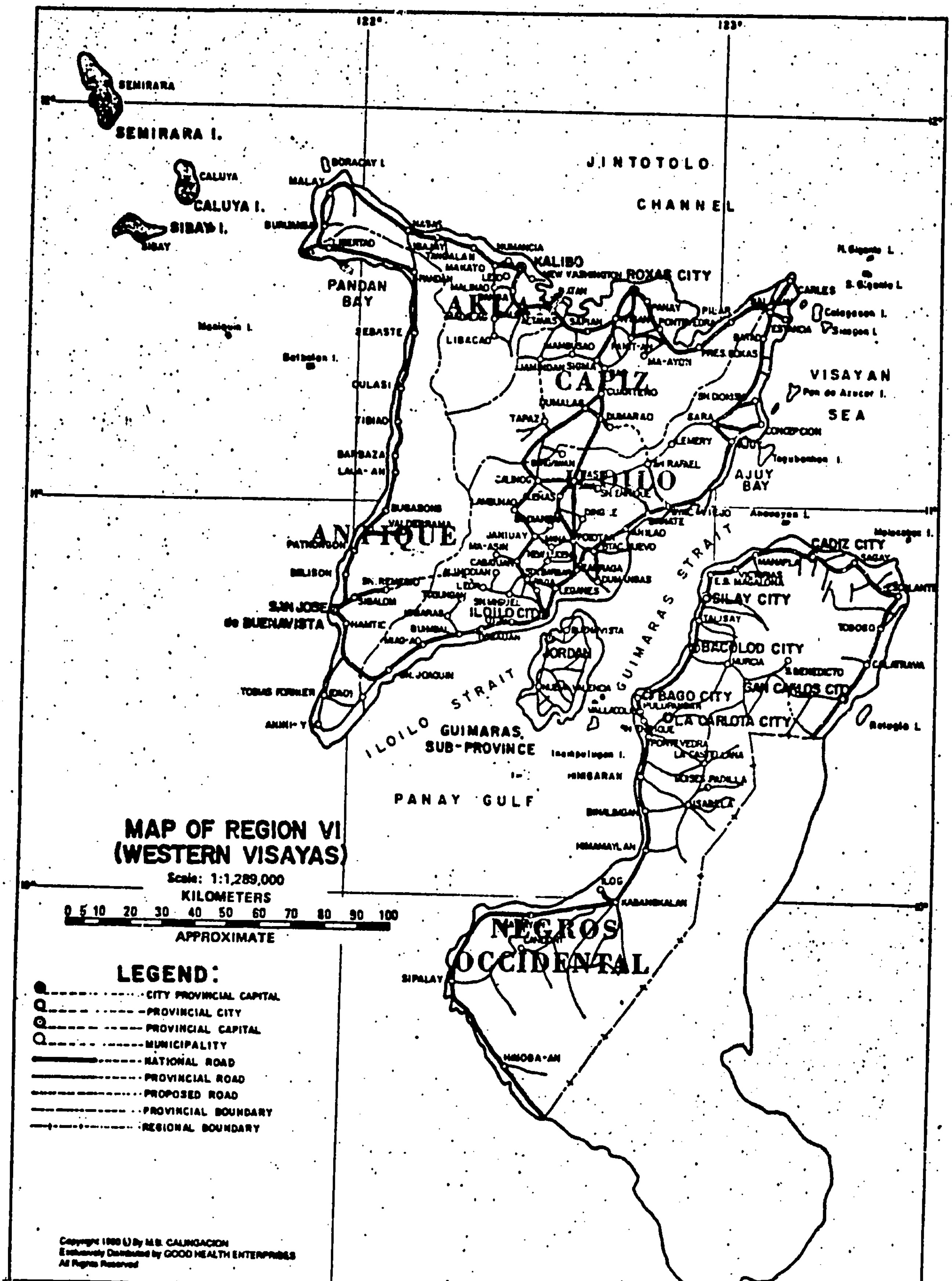

Figure 2. The map of Western Visayas showing the location of the municipality of Leganes in the province of Iloilo where the Brackishwater Aquaculture Center of the Institute of Aquaculture, College of Fisheries, University of the Philippines in the Visayas is situated. This also shows the Iloilo Strait and Guimaras Strait where water samples were collected.

has a mean sulfate concentration of 976.98 mg/l and salinity of 35 ppt. Treatment II, taken from Gui-gui Creek near the Brackishwater Aquaculture Research Station, Leganes, Iloilo, Philippines has a mean sulfate concentration of 840.52 mg/l and salinity of 33 ppt. Treatment III, a mixture of seawater near the shore of Leganes, Iloilo and and brackishwater from Gui-gui Creek has a mean sulfate concentration of 772.56 mg/l and salinity of 35 ppt. Treatment IV, a mixture of seawater from the shore of Leganes, Iloilo and rainwater has a mean sulfate concentration of 441.07 mg/l and salinity of 17 ppt. Church (1975) reported that theoretical sulfate concentration of seawater is 2,657 mg/l at 34.4 ppt salinity which is said to contain higher sulfate concentration than freshwater.

Fish mortality was observed 8 days after stocking and increased day after day . Water was observed to be turbid and highest level of ammonia (>2.0 ppm) was recorded. On the 9th day, Nov. 13, 1990, a super typhoon hit Iloilo and there was power black out for more than a month. A small generator was provided but intermittent power failure was still observed. This caused low DO levels in the water. On the 24th day only few fishes were left and the experiment was terminated. The only data gathered were the initial sulfate concentration of the different water used as treatments and the weight of the fish. Fish mortality could also be attributed to insufficient food supply. No visible growth of green algae was observed. The water had developed a brownish color with suspended brownish material. Whitish particles had settled in some parts of the sand bottom. This white particles could be the unconsumed rice bran used as fish feed. A black slimy coloration also developed on the lower portion of the sand bottom. Rotten-egg odor (sulfide) was observed in almost all aquariums especially when the bottom was disturbed and when the water was drained during the termination of the experiment.

On the basis of these observations, an experiment was conducted to determine the transformation of the organic residue (rice-bran) when added to seawater containing different sulfate concentration. In this run, bottles of 2-liter capacity were used. The nitrogen content in rice bran was determined by Kjeldahl digestion over a period of time. The results are presented in Table 2. Nitrogen declined towards the end probably because of the decline in the organisms (Table 3).

2. MILKFISH EXPERIMENTS

2.1. Aquarium Experiments

2.1.1. Aquarium Experiment No. 1. The same twelve units of aquariums were used with 10 cm washed sand at the bottom. These were filled with filtered sea water to 30-cm of varying sulfate concentration which served as treatments. The water was taken from different sources. Air-lift aeration was also provided. Monoammonium phosphate fertilizer (16-20-0-12; N:P:K:S) was added at a rate of 0.1 g/aquarium initially and every 2 weeks to enhance the growth of natural food. On Jan 4, 1991, fifteen (15) milkfish (<u>Chanos chanos</u>) fingerlings approximately 1.5 g each were stocked into each aquarium. Rice bran-cane sugar (0.1%) mixture was given daily to the fish (5%)

of the fish biomass for the first 2 weeks and later increased to 10% of the fish biomass. Sulfate, sulfide, ammonia and phosphorus, in the water were determined using the methods described by Strickland and Parsons (1972), The digital pH tester, Atago refractometer and YSI DO meter were used to measure pH, salinity temperature and dissolved oxygen, respectively. Sediment samples were taken and analyzed for organic matter content, phosphorus, nitrogen and sulfate. Fish sampling was done every 14 days to monitor growth rate of the fish. The experiment was terminated on Feb. 1, 1991.

Ceramic tiles were placed in each aquarium for analysis of the microorganisms that may serve as fish food . The organisms adhering to the tiles were scraped off and weighed. Also a drop was diluted with water and the organisms were counted in a haemacytometer (Table 4). Apparently higher biomass of food organisms were present in treatment IV where seawater was mixed with freshwater and with relatively low sulfate content. The different soil and water parameters monitored such as sulfate, sulfide, ammonia, pH, DO, temperature and salinity are presented in Table 5.

Sulfate. Analysis of variance on the data showed significant treatment differences ($P<0.01$) in the sulfate concentration of the water taken from different sources. Treatment IV which was a mixture of seawater and rainwater showed significant differece over the other treatments. Treatments I, II and III did not differ much in the sulfate concentration. The sulfate concentration in all treatments were observed to decrease several days after stocking but subsequently increased a few days before termination of the experiment. Significant reduction in the sulfate concentration could be attributed to the anoxic condition in the bottom water as observed in the decreasing dissolved oxygen level of the water. Sulfate was found to be positively correlated with dissolved oxygen and salinity (P< 0.01, P< 0.01). As dissolved oxygen level increased, sulfate concentration subsequently increased in all treatments. Sulfate concentration was relatively higher in the water with higher salinity levels. This observation supported the fact that seawater with higher salinity level actually contain higher sulfate ions than freshwater with lower salinity (Church 1975). Significant correlation was also found between sulfate and ammonia (P< 0.01).

The washed and dried sand bottom, initially contained no sulfate in all the treatments but after harvest analysis showed a significant value of sulfate (1668.51 mg/l to 1991.85mg/l) in the sand bottom. Some of the sulfate in the sediment could be due to the accumulated unconsumed feed (rice bran which contain a small amount of protein). The sulfate concentration of the sediment was observed to be higher than the sulfate concentration found in the water. Statistical analysis showed that sulfate in the sediment was highly correlated (P<0.01) with sediment organic matter.

Sulfide. Analysis of variance on the sulfide showed significant differences between treatments (P<0.01). Hydrogen sulfide was observed to be relatively higher during the later

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part of the experiment maybe because of the unconsumed feed. This phenomenon was especially triggered at times when aeration system malfunctioned. Hydrogen sulfide is formed by heterotrophic bacterial metabolism , thus unionized hydrogen sulfide usually does not occur in well-oxygenated water (Chiu 1988). Sulfide was found to be positively correlated with salinity, pH and temperature ($P<0.01$, $P<0.05$ and $P<0.01$), respectively).

Ammonia. Ammonia concentration was relatively high a few days after stocking due to poor aeration based on records of low dissolved oxygen level. After a week, ammonia concentration in water decreased, especially after water exchange. However a gradual increase was again observed until the later part of the experimental period. This could be due to the accumulated feed that settled on the bottom. Correlation analysis showed that ammonia was negatively correlated with temperature and dissolved oxygen ($P<0.01$) and $P<0.05$) which means that at lower dissolved oxygen and temperature, ammonia accumulates in the water column.

Sediment Organic Matter. Initially the sand bottom was devoid of organic matter. However after 28 culture days, an appreciable amount (in approximately equal amounts, 0.4 g in all the 4 treatments) of organic matter was observed in the sample taken from the sand bottom.

2.1.2. **Aquarium Experiment No. 2.** After the termination of the first experimental run the water was changed and the aquariums prepared for the second experimental run The treatments used in this run were the different kinds of feed as

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follows: (1) rice bran + cane sugar (1%) fed at 10% body weight + 0.98 g diammonium phosphate fertilizer every week; (2) rice bran + cane sugar (1%) (3) Natural food (lumut or flamentous algae) + 0.98 g diammonium phosphate every week; (4) commercial feed at 10% body weight wss given. Fifteen (15) pcs. of milkfish fingerlings at approximately 1.0 g each were stocked on April 10, 1991. Water parameters such as sulfate, sulfide, ammonia, phosphorus, pH, DO, salinity, and temperature were monitored. Sediment samples were also taken and analyzed for organic matter, phosphorus, nitrogen, sulfate and pH. The fish were sampled after 14 days. The experiment was terminated on May 10, 1991.

The different treatments were started on April 10, 1991. Based on data gathered, the fishes on treatment 4 (commercial feed) had a highest growth compared to the other treatments. The water and sediment parameters were determined and summarized in Table 6.

Sulfate. Analysis of variance on the data significantly showed that the treatments contained different amounts of sulfate in the water with treatment 1 having the highest mean sulfate content (919.51 mg/l). The sulfate concentration fluctuated in all the treatments. Based on the correlation analysis, sulfate in water was negatively correlated to phosphorus and temperature ($P<0.01$) but slightly correlated to organic matter content of the soil ($P<0.05$). However, sulfate in soil did not show significant difference among treatments based on data analysis done before stocking and after harvest.

Sulfide. Analysis of variance showed that there was no treatment differences for the sulfide content in the water. Mean sulfide concentration ranges from 0.0225 mg/l to 0.0254 mg/l. It was observed that sulfide concentration in water tended to follow an increasing trend for all the treatments. This could be due to the accumulation of decomposing organic material from the unconsumed feed in the bottom and an increase in the metabolic wastes of the fish on the later part of the experiment which could be justified by the correlation analysis . Sulfide was found to be significantly correlated with ammonia, pH, soil organic matter, soil phosphorus and soil nitrogen .

Ammonia. No difference was found among the treatments. The concentration of ammonia in the water followed a fluctuating trend from one sampling period to another wherein ammonia was controlled by some factors like dissolved oxygen, temperature and organic or nitrogen input. Correlation analysis for this run showed significant correlation of ammonia with dissolved oxygen ($P< 0.05$), soil nitrogen ($P<0.01$) and sulfide ($P< 0.01$).

Sediment Organic Matter. It was observed that organic matter content of the sediment increased after each experimental run, obviously because of the accumulated unconsumed feed which was observed visually on the sand bottom as white precipitate. Mean organic matter of the sediment ranged from 0.6% (treatment III) to 1.035% (treatment I). Treatment I was supplied with rice bran with 1% cane sugar plus fertilizer while treatment III has no feed supplement except fertilizer. It was evident that the unconsumed feed was contributing more to the organic matter in the sediment. Correlation analysis between organic matter and the different parameters showed significant relationship with sulfide, soil and water phosphorus , and soil nitrogen. It is a fact, that upon decomposition of the organic matter it will release sulfide, phosphorus as well as inorganic nitrogen.

Phosphorus. Analysis of variance for water phosphorus showed significant differences among treatments. The highest mean phosphorus concentration in water was found in treatment I with 1.59 ppm P and the lowest mean concentration in treatment IV with 1.19 ppm P which was not significantly different from treatment III . Phosphorus in the water was found to be correlated with sulfate, water pH, sediment organic matter and soil phosphorus. Sulfate has an effect on water pH and pH determines the solubility of phosphorus in water, while organic matter and soil phosphorus is directly related to soluble phosphorus in water and natural food organisms. Data on the plankton count and wet weight of organisms adhering on tiles are shown in Table 7. Highest amount of natural food was observed in treatment III, apparently however, the volume was inadequate to contribute significantly to fish growth.

2.1.3. Aquarium Experiment No. 3.

This run was aborted after a few days due to very high mortality resulting from poor water quality.

2.1.4. Aquarium Experiment No. 4

This experiment was started on May 28, 1991 and completed on July 4, 1991. The different treatments used in this experiment were I. rice bran + cane sugar; II. rice bran + cane sugar + fertilizer; III. Natural food + fertilizer; and IV.

commercial feed. Sixteen (16) milkfish fingerlings at approximately 2.5 g each were stocked in each aquarium. The fish were sampled after 14 days. Stocking, sampling and harvest data of the milkfish are summarized in Table 8. Obviusly, the fish that received commercial feed (Treatment IV) had the highest biomass (40.60 g). This was followed Treatment I - rice bran + cane sugar + fertilizer (29.6 g); then followed by Treatment II-natural food + fertilizer (23.94 g); then Treatment III-rice bran + cane sugar (19.84 g). It appears that the presence of fertilizer in treatments I and II improved fish growth. Perhaps the fertilizer enhanced the growth of natural food organisms or contributed to the degradation of rice bran making it more easily digestible. Also, perhaps the fish had better appetite. Water parameters were also monitored regularly (Table 9). It can be noted that ammonia levels in treatment IV where fish biomass was high were considerably lower than in treatments I and III.

2.1.5. **Aquarium Experiment No. 5)..**

On July 6, 1991, ten (10) pcs milkfish fry were stocked in each of the twelve (12) aquariums. The stocking density was reduced because of poor water quality (due to high feed input) observed in the previous aquarium experiment. This time water quality was better (Table 10). On September 12, 1991, the experiment was terminated after 68 days with higher survival and growth rate. Results of experiment are shown in Table 11. The results obtained in experiment 5 was consistent with experiment 4. Commercial feed gave the highest fish biomass; while natural food/fertilizer gave the lowest. This was probably due to insufficient growth of natural food since there was limited light in the wet laboratory. It can be noted (Tables 12 and 13) that before the stocking of milkfish there was high count of organisms composed mainly of bacteria. However lowering of count was observed after the fish had been stocked and apparently growth was not sustained.

2.2. Milkfish Pond Experiments

2.2.1. Pond Experiment No. 1.

The pond experiment was conducted from March 23 to August 30, 1991. Twelve (12) units of 500 m^2 earthen ponds were used. The ponds were dried, levelled and repaired of leaks. Natural food was allowed to grow in all ponds with the addition of 157.5 g urea and 87.5 g diammonium phosphate fertilizer. On March 23, 1991, 205 pcs milkfish fry approximately 0.7 g each were stocked in each pond. This is equivalent to 4,000 fish/ha stocking density. Different inputs in the ponds were supplied starting April 10, 1991 with the following treatments: (1) 500 g rice bran + 1% cane sugar + 87.5 g diammonium phosphate fertilizer; (2) 500 g rice bran + 1% cane sugar without fertilizer input; (3) natural food + diammonium phosphate fertilizer only; and (4) 50 g commercial feed and later adjusted to 10% body weight after the first sampling.

The mean weight gain (g) expxressed in average body weight of milkfish during the first, second and third sampling was very encouraging but a decline was observed at harvest (Table 14).

The fish were harvested on August 30, 1991 (161 days of culture) and the results are shown in Table 14. Milkfish production of 11.3, 53.4, 23.1 and 65.02 kg/pond were attained in treatments I, II, III and IV which were equivalent to 226, 1,068, 462 and 1,300 kg/ha, respectively. These fish production figures demonstrated the potential of rice bran + cane sugar as feed substrate for milkfish production. In Table 15 are shown the survival and mortality of milkfish from the different treatments.

Soil and water parameters were monitored regularly and are summarized in Tables 16 and 17, respectively. The means and ranges of pH, temperature, salinity and dissolved oxygen are given in Tables 18, 19, 20 and 21, respectively.

Sulfate Data gathered showed that sulfate concentration of the pond water has relatively higher sulfate concentration than the aquarium water even though the water came from the same source. The higher sulfate content of the pond water could be attributed to a quite significant amount of sulfate in the pond soil which was an acid sulfate soil years back. The brackishwater used in the aquarium was filtered using a sand/gravel filter system. Filtration reduced the sulfate concentration (1,188.76 mg/l) observed for the unfiltered seawater (2,351.75 mg/l). It is likely that sulfate ions are trapped in the sand during the filtration process.

Sulfide The sulfide concentration of the pond water was relatively higher than in the aquarium water. It was observed that the sulfide concentration increased with decreasing DO level and sulfate concentration. The relationship was observed in both ponds and aquariums.

Ammonia The ammonia level in the pond was relatively lower than in the aquarium water which could be due to higher oxygen concentration. Besides, the ponds were bigger and volatilization of ammonia could occur faster in bigger surface area. Ammonia concentration fluctuated as affected by some other factors in the pond such as utilization by algae, excretion by zooplankton, and precipitation may have a significant influence.

Phosphorus The phosphorus in water was relatively lower than that observed in the aquarium water. Higher mean phosphorus concentration was observed in Treatment I (rice bran with cane sugar plus fertilizer) than the other treatments.

The potential food organisms collected by sampling the pond water are listed in Table 22. Those from the sediments that attached on ceramic tiles (lab-lab collectors) are given in Table 23. The quantity of these organisms in terms of population count and weights are given in Tables 24, 25 and 26.

2.2.2. **Pond Experiment No.2**

The second milkfish pond experiment was implemented from Nov. 26, 1991 to April 7, 1992. The pond was prepared in the same manner as in the first run. The treatments were as follows: I- rice bran + cane sugar + fertilizer; II- natural food + fertilizer; and III - commercial feed.

Milkfish was stocked at 150 fingerlings per pond (equivaent to 3,000 fingerlings/ha) and harvested after 133 days. The same water parameters were measured using the methods as those used in the first run. The data on sulfide, sulfate, nitrite and ammonia are in Table 26. It can be noted that ammonia was high during the beginning but then decreased towards the end. Ammonia could have been removed by the phytoplankton and lab-lab growing in the ponds. Sulfate and sulfide increased towards the middle of the culture season and decreased towards the end.

The harvest data for milkfish are shown in Table 27. The highest weight gain was observed in milkfish that was fed with commercial feed (Treatment III) followed by treatment I (rice bran + cane sugar + fertilizer) then followed by Treatment II (fertilizer only). In terms of survival highest survival was observed in treatment II followed by treatment I. Highest mortality was in treatment III. Survival was relatively low probably because of the parasites (Ergasilus sp. and Argulus) that infested the fish. Highest weight gain was observed in Treatmnent I. It was shown in this experiment that milkfish production in the treatment with rice bran + cane sugar + fertilizer was comparable to the treatment that used commercial feed. These results were obtained despite the observation that lab-lab biomass was highest in treatment with commercial feed (Tables 28 and 29). However, zooplankton volume was observed in moderate abundance in Treatment II (Table 30) but decreased towards the end of March probably because they were consumed by the fish. It was apparent however, that at such volume, the available food was not adequate to sustain the the growth of fish in the pond. To determine the effect of the different food sources on the muscle growth of the fish, samples of fish flesh and the different possible food sources were sent to 2 laboratories in Israel for delta C analysis. The results of these analyses are shown in Table 31. On the basis of this information, the importance of natural foods that are present in the pond for fish growth was demonstrated.

The selected water parameters that were monitored in the ponds used in the second run of the pond experiment that were stocked with milkfish are given in Table 32. Ammonia concentration was initially high but decreased towards the end of the experiment, indicating the uptake of ammonia as natural food developed in the pond. The levels of sulfate, sulfide and nitrite were fluctuating and there was no significant trend.

3. Tilapia Experiments

3.1. Aquarium Experiment

3.1.1. First Aquarium Experimental Run

Sixteen pieces of tilapia (Oreochromis mossambicus) were stocked into each of the twelve 90-liter aquariums that were set indoor. The fish were fed with rice bran mixed with 1% cane sugar and given at 2% of fish biomass. Results was not very encouraging. A range of survival from 25 to 100% were recorded. The ABW of the fish at the end of the experiment was lower than when they were stocked. Because of this condition, the run was terminated (See Table 33).

3.1.2. Second Aquarium Experimental Run

Twelve units of the aquariums from the previous run were used in this run. The various treatments that were tested in this particular run were: I-rice bran + cane sugar + fertilizer; II-rice bran + cane sugar; III-natural food + fertilizer; IV-commercial feeds. Fifteen pieces of tilapia were each stocked in the aquariums. This run however, was terminated after 17 days due to the increasing mortality observed (Table 34).

3.1.3. Third Aquarium Experimental Run

After the milkfish aquarium experiment was terminated, the same twelve units of aquaria were washed and prepared for the next experimental run. This time the filtered brackishwater used for all the twelve experimental units came from the same source, at UPV-BAC experimental brackishwater pond . Air-lift aeration was also provided. Urea (46-0-0) and diammonium phosphate fertilizer (18-46-0; N:P:K) were added initially at a rate of

40.15 g and 22.49 g/aquarium, respectively to enhance the growth of natural food. Fifteen (15) tilapia fingerlings approximately 2.5 g each were stocked into each aquarium but in the 3rd week of May but actual observation on treatments started on May 28, 1991. This run was terminated on June 16, 1991. The treatments that were replicated three times each were: (1) rice bran + cane sugar (1%) + 0.98 g diammonium phosphate fertilizer where the feed components are given at 5 - 10%g of fish biomass; (2) rice bran + 1% cane sugar (given also at 5-10% of the fish biomass); (3) no feed was given except for 0.98 g diammonium phosphate fertilizer. Lab-lab from the pond were initially seeded at approximately 20% - 60% (wet weight) of the fish biomass to supplement the fish with natural food; (4) commercial feed were given at 10% of the fish biomass. Water parameters were monitored regularly. However, 11 days later, higher fish mortality was observed. The experiment was terminated due to very poor water quality.

The survival, biomass and average body weights of tilapia are given in Table 35.

In all the attempts above to test the effect of the different food sousrces on tilapia growth, significant information was derived despite the premature termination of the experimental runs. In order to make confirmatory runs, experimental trials in tanks were resorted to which is the object of the next activities.

3.2. Tilapia Tank Experiment

3.2.1. Tank Experiment No. 1.

Twelve (12) units of concrete tanks (1.5 m Deep x 1.2 m Wide x 2 m Long) filled with soil to 10 cm from the bottom were each stocked with 75 pcs tilapia of approximately 1.2 g each on June 21, 1991. These were held in the tanks for about one month until they were conditioned to their new environment. Finally, on July 19, 1991 actual observation on the fish under the different treatments was carried out. The different treatments (different food sources) were randomly distributed among the 12 experimental units. Three days before stocking 3.5 g of diammonium phosphate fertilizer were added to each tank to enhance the growth of natural food. Soil and water parameters that are needed in the study were monitored regularly. Observations on the growth of tilapia ended when the experiment was terminated on October 15, 1991.

The growth of tilapia (*Oreochromis niloticus*) in concrete tanks fed with natural and artificial feeds from various sources (the treatments) based on initial and final average body weights are given in Table 36. There was a positive response of the fish to the feeds given although the growth was slow. Treatment differences were observed in all treatments although at this point in time it can not be said that they are significantly different from each other because the data were not yet analyzed statistically. Suffice it to say however, that tilapia production from treatments I (Rice bran + cane sugar + natural food) and II (Rice bran + cane sugar) were comparable; fish production from treatment IV (commercial feeds) is significantly greater than fish production from all treatments. Fish production from treatments I and II however could be significantly higher than those from treatment III. It would therefore appear that rice bran as a source of feed for tilapia could give better fish production than those fed with natural food in tanks. Furthermore, it was observed that there was an increasing number of potential food organisms in the tank water where tilapia were stocked. Despite however, the greater number of organisms in the water in treatment III (natural food) it appears that these are not enough. On the other hand, the lower number of organisms in water in the other treatments appeared to be supplemented by the artificial feeds (commercial feeds and rice bran) which probably explains the better fish production in the other treatments. The survival and mortality of the tilapia in tanks in the different treatments are given in Table 37. The survival of tilapia was not very good (means per treatment: 59.05%; 56.67%; 65.71% and 60.47% for treatments I, II, III and IV, respectively).

It was also possible that the different water and soil parameters that were monitored may have affected to certain extent the growth and production of tilapia in the tanks. These parameters are: sulfide, sulfate, phosphorus, nitrite, ammonia, pH, dissolved oxygen, salinity and temperature and their values in the course of the experiment are given in Table 38. The levels of nitrogen, pH, organic matter and phosphorus in the soil which were used to fill the tank to about 10 cm are given in Table 39. The potential food organisms that were observed to have developed in the tank water under the conditions of the experiment are given in Table 40. In Table 41 are given the weight of organisms that adhered on the screens installed in the tanks; Table 42 gives the number of organisms counted from the water samples collected from the tanks.

3.3.Tilapia Pond Experiment

Six 500 sq.m. ponds were used for this run. Pond preparation was done prior to stocking of tilapia as follows: flushing of ponds, draining and drying of pond bottom and basal application of organic and inorganic fertilizers at a rate of 50 kg and 100 kg per hectare respectively. Fish were stocked in June 1992 and harvested on August 28, 1992. This run tested three treatments with two replicates each as follows: I-rice bran + cane sugar + fertilizers; II-Natural food + fertilizer; and III. Commercial feeds.

The initial average body weight (abw) of tilapia were as follows: I-2.8 g; II-2.5 g; and III-1.1 g. The final abw ranged from 60.29 g, 80.87 g and 119.53 g for treatments I, II and III, respectively. Percentage recovery was low, 32.8%; 29.6% and 43.8% for treatments I, II and III, respectively. Table 43. Water parameters were also monitored specifically sulfide, sulfate, ammonia, nitrite, which are presented in Table 44. Ammonia was relatively high at the beginning probably because of the products of decomposition after the initial application of chicken manure, however the concentration of ammonia decreased toward the end, probably because of the uptake of ammonia by the microorganisms especially the algae. The biomass of food organisms in the tilapia ponds are shown in Tables 45, 46, and 47. It appears that the amount of food available in the ponds are quite similar with very little variation. Data on fish biomass are shown in Table 43. Obviously, higher biomass was obtained in treatment III or those fed with commercial feeds; similarly the increment in weight was also high. Survival was also highest in treatment III. Survival in treatment II (natural food) was lowest (29.6% compared to 32.8 for treatment I and 43.8 for treatment III. In terms of biomass treatment II ranked second to treatment III which indicated that tilapia could grow with natural food if there is adequate supply to sustain them.

Table 48 shows the **delta** C values of tilapia and the different food sources. Apparently, mixes of lab-lab (benthic algae), zoo- and phytoplankton and filamentous algae are food source that could contribute to the muscle growth of tilapia.

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4. Prawn Experiments

4.1. Pond Experiment 1.

The first pond experiment to determine the effect of the different food sources on the growth of prawn (Penaeus monodon) was initiated on December 10, 1991 and completed on April 15, 1992. Routine pond preparation as was done in the milkfish and tilapia experimental runs was followed. These activities consisted of flushing of ponds, draining and drying of pond bottom and initial application of organic and inorganic fertilizers at a rate of 50 kg and 100 kg per ha, respectively. The ponds were filled with brackishwater and then the prawns were stocked at 1,035/pond (equivalent to 20,700 fry/ha). The different treatments are as follows: I-Rice bran + cane sugar + fertilizer (18-46-0); II-Natural feed/lablab/lumut + regular appplication of fertilizer; and III-Commercial feed. Sulfate, sulfide, ammonia and nitrite were monitored using standard methods.

Stocking and harvest data are shown in Table 49. Apparently the shrimp did not grow. Recovery of prawn was very low: 33.7% for treatmeent I; 5.4% for treatment II; and 44.5% for treatment III. The biomass was similarly very low (Table 49). Several factors could be attributed to this. Overage and weak fry are among them. Water quality could be another reason. Similar trend in the ammonia concentration was observed. Ammonia was high at the beginning and decreased towards the end, indicating the uptake of ammonia as natural food developed in the pond. Sulfide, sulfate, and nitrite was low at the beginning and increased towards the middle and then decreased towards the end of the culture period.

Lab-lab biomass and quantity of zooplankton are given in Tables 50, 51 and 52. Data indicate that the natural food present in treatment II was relatively higher but this may not be sufficiently enough. Food (floating lab-lab) in treatment with commercial feed was also high.

4.2. Pond Experiment 2

A second experimetnal run for prawns in ponds was conducted from June to August, 1992. Pond preparation was done in the same manner as in the first run. The different food sources were used as the different treatments as in the first run. Prawn with an average initial weight of 0.35 g were stocked into each pond on June 5, 1992 at stocking rate of 1,330 fry/pond (equivalent to 26,600 fry/ha. Sampling was done 4 times at approximately one month interval except for the 4th sampling (15 days). The prawns were harvested after 109 days of culture (September 22, 1992). Data of stocking, sampling and harvest are given in Table 53.

The analytical methods for chemical and biological (microorganisms) parameters were the same as those used in the previous run. The concentrations of sulfide, sulfate, nitrite and ammonia in water are presented in Table 54. Ammonia decreased towards the later period of the culture period. This behavior was consistent in all treatments which were also manifested in the previous trials. This decrease may probably be due to the ionization products of the chicken manure and inorganic fertilizer applied during pond preparation. Sulfate levels showed an increase towards the end of the culture period; sulfide and nitrite however, exhibited a decline.

The quantity of natural food organisms present in the ponds with different treatments are given in Tables 55, 56 and 57. It appears that the amount of natural food present in treatment III was also high. Zooplankton population was higher in treatment II especially towards the end of the culture period.

Table 1. Sulfate concentration of the seawater from different sources used in the aquaria experiment.

Treatment	Water Source	Range of Sulfate Conc. (ppm)
I	Guimaras Strait	1,500 - 2,000
II	Modified seawater	700 - 900
III	BAC canals	400 - 650
IV	Artificial seawater	10 - 20

Table 2. Changes in the Nitrogen Content (mg/l) of Seawater of Varying Sulfate Concentration with Organic Residue (rice bran).

Exposure Time	Treatment I	Treatment II	Treatment III	Treatment IV
0 hour	0.064	0.257	0.407	0.500
1 hour	0.311	0.504	0.568	0.611
2 hours	0.203	0.332	0.311	0.268
12 hours	0.825	0.536	0.642	0.852
24 hours	0.986	0.558	0.880	1.308
7 days	12.440	4.647	15.018	3.432
14 days	1.80	2.55	1.80	2.70
30 days	1.17	---	---	0.96

Table 3. Changes in the Total Count of Organisms in Different Seawater Sources Applied with Rice Bran

Treatment	2 days	7 days	14 days
I (Guimaras)	4,210,000	7,109,000	3,468,000
II (Guigui)	1,160,000	3,989,000	920,000
III (SM + Guigui)	6,650,000	13,638,000	655,000
IV (SM + Freshwater)	5,363,000	12,495,000	1,285,000

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Table 4. Population of Organisms (orgs/ml x 10^3) in Seawater with Different Sulfate Concentrations.

Treatments/Replicates	January 17	January 24	January 30
Treatment I (Guimaras)	189.3	839.3	242.6
Treatment II (Guigui)	52	242	358.6
Treatment III (SM + Guigui)	196	334	420.6
Treatment IV (SM + Freshwater)	334.3	756	1209.6

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Table 5. Physicochemical properties of the water and sediment (Milkfish aquarium expt.- first run)

Treatment	Sulfate (mg/l) Dec. 27	Jan. 7	Jan.10	Jan.17	Range
I	976.98	476.98	770.72	824.13	476 - 977
II	840.52	490.79	772.56	813.08	490 - 841
III	772.56	339.78	726.52	811.23	339 - 811
IV	441.07	325.05	536.83	669.43	325 - 670

Treatment	Sulfide (ml/l) Jan. 7	Jan.10	Jan.17	Jan.24	Range
I	0.0187	0.0252	0.0241	0.0248	0.0187 - 0.0252
II	0.0153	0.0271	0.0248	0.0195	0.0153 - 0.0271
III	0.0221	0.0248	0.0240	0.0233	0.0221 - 0.0248
IV	0.0133	0.0236	0.0183	0.0214	0.0133 - 0.0236

Treatment	Ammonia (mg/l) Dec.27*	Jan.7	Jan.10	Jan.17	Jan.24	Range
I	0.0763	0.1398	0.0208	0.0285	0.0308	0.0208 - 0.1398
II	0.0966	0.1295	0.0691	0.0653	0.0383	0.0383 - 0.1295
III	0.0105	0.1468	0.0411	0.0334	0.0464	0.0105 - 0.1468
IV	0.1624	0.1402	0.0289	0.0427	0.0261	0.0261 - 0.1624

Treatment	Dissolved Oxygen (ppm) Jan.7	Jan.10	Jan.16	Jan.24	Range
I	3.63	2.15	4.73	4.73	2.15 - 4.73
II	3.47	2.05	4.77	5.13	2.05 - 5.13
III	3.58	2.07	4.30	4.47	2.07 - 4.47
IV	3.52	2.47	4.73	5.07	2.47 - 5.07

Treatment	Salinity (ppt) Jan.7	Jan.10	Jan.16	Jan.24	Range
I	36.0	35.0	39.3	37.7	35.0 - 39.3
II	35.7	35.0	40.0	37.3	35.0 - 40.0
III	36.3	34.7	39.3	38.0	34.7 - 39.3
IV	19.3	17.7	21.3	19.7	17.7 - 21.3

Treatment	pH Jan.7	Jan.10	Jan.16	Jan.24	Range
I	7.20	7.37	7.17	7.43	7.17 - 7.43
II	7.03	7.17	7.07	7.33	7.03 - 7.33
III	7.27	7.23	7.20	7.50	7.20 - 7.50
IV	7.43	7.53	7.17	7.47	7.17 - 7.53

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Table 5. continued ...

Treatment	Temperature (C) Jan. 7	Jan.10	Jan.16	Jan.24	Range
I	24.1	25.6	25.6	27.0	24.1 - 27.0
II	24.1	25.6	25.7	27.0	24.1 - 27.0
III	24.2	25.8	25.7	27.0	24.2 - 27.0
IV	24.1	25.6	25.6	27.0	24.1 - 27.0

Treatment	Organic Matter (%)			Soil sulfate		
	Dec.27*	Jan.28	Range	Dec.27*	Jan.28	Range
I	0.0	0.81	0.0 - 0.81	0.0	3652.5	0.0 - 3653
II	0.0	0.97	0.0 - 0.97	0.0	3760.2	0.0 - 3760
III	0.0	0.85	0.0 - 0.85	0.0	3983.7	0.0 - 3984
IV	0.0	0.85	0.0 - 0.85	0.0	3337.0	0.0 - 3337

Table 6. Physicochemical properties of the water and sediment (Milkfish aquaria expt. - second run)

Treatment	Sulfide (ml/l) April 10	April 17	April 24	April 29	May 9
Trt I	0.0198	0.0214	0.0275	0.0313	0.0252
Trt II	0.0213	0.0187	0.0264	0.0332	0.0275
Trt III	0.0156	0.0222	0.0187	0.0317	0.0283
Trt IV	0.0168	0.0210	0.0225	0.0248	0.0275

Treatment	Sulfate (mg/l) April 10	April 17	April 24	April 29	May 9
Trt I	1129.70	806.36	973.56	685.96	1001.95
Trt II	1177.02	835.28	1039.80	694.90	1011.41
Trt III	1137.59	835.28	1054.00	761.15	1035.07
Trt IV	1155.99	883.12	1012.46	787.96	1012.99

Treatment	Ammonia (mg/l) April 10	April 17	April 24	April 29	May 9
Trt I	0.1456	0.0560	0.0882	0.1290	0.1066
Trt II	0.1385	0.0357	0.1083	0.5780	0.1246
Trt III	0.1149	0.0658	0.0422	0.0539	0.0679
Trt IV	0.1678	0.0779	0.0443	0.1311	0.5343

Treatment	Phosphorus (mg/l) April 24	April 29	May 9
Trt I	1.28	1.98	1.52
Trt II	1.19	1.54	1.44
Trt III	1.15	1.42	1.15
Trt IV	1.10	1.35	1.11

Table 6. continued...

Treatment	Soil Sulfate (mg/l) Feb. 1	May 10	Soil pH May 10	Organic matter (%) Feb. 1	May 10	P (mg/l) May 10	N (%) May 10
Trt I	3760.2	3662.97	7.57 b	0.98	1.09	90.92 a	0.067 b
Trt II	3347.5	3925.87	7.78 b	0.69	1.31	66.54 b	0.064 b
Trt III	3515.77	3584.1	7.82 a	0.91	0.31	56.43 b	0.012 a
Trt IV	4109.88	3641.97	7.77 b	0.90	0.78	63.14 b	0.053 b

Table 7. Total Population Count (org./li x 10^4) and Weight (g) of Microorganisms on Tiles (Aquaria)

	April 9		April 26	
Treatment	Plankton count	Weight	Plankton count	Weight
Treatment I	18.48	0.075	18.95	0.06
Treatment II	12.90	0.017	13.78	0.02
Treatment III	33.20	0.023	20.03	0.011
Treatment IV	21.35	0.037	16.90	0.011

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Table 8. Stocking, sampling and harvest data of the 5th run of milkfish aquarium experiment

Treatment	No. of fish	Biomass	1st Sampling Abw (grams)	No.of (g)	Biomass sample	2nd Sampling / Harvest Abw (grams)	No. of (g)	Biomass samples	Abw (grams)	(g)
I	16	42.88	3.04	48	43.51	2.72	32	29.60	2.78	
II	16	37.19	2.32	46	34.28	2.23	26	19.85	2.43	
III	16	37.91	2.5	47	37.09	2.36	34	23.94	2.11	
IV	16	39.70	2.5	44	43.85	2.99	33	40.60	3.7	

Date stocked : May 30, 1991
No. of fish stocked / aquarium : 16
Date terminated : July 4, 1991

Treatments

I	Ricebran + cane sugar + fertilizer
II	Ricebran + cane sugar
III	Fertilizer (natural food , lab-lab or lumut ; 250-300 grams wet weight)
IV	Commercial Feeds

Feeding rate : 10% of estimated total biomass.

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Table 9. Selected water parameters in the 4th run of milkfish aquarium experiment (May 30 - July 4, 1991)

Treatment	Sulfide (ml/l) June 6	Sulfide (ml/l) July 1	Sulfate (mg/l) June 5	Sulfate (mg/l) July 1	Ammonia (mg/l) June 6	Ammonia (mg/l) July 1	Nitrite (mg/l) July 1	Phosphorus (mg/l) June 6
I	0.0179	0.1954	766.93	1032.44	2.555	2.228	0.0200	1.369
II	0.0122	0.1187	775.84	1003.52	0.308	1.472	0.0390	0.591
III	0.0134	0.0267	795.84	1053.47	2.753	0.373	0.0570	1.276
IV	0.0145	0.0439	948.32	1029.81	0.225	1.419	0.1300	0.561

Table 10. Selected water parameters in the 5th run of milkfish aquarium experiment (July 6 - Oct 15, 1991)

Treatment	Nitrite (mg/l) July 12	Nitrite (mg/l) July 24	Nitrite (mg/l) Aug 9	Nitrite (mg/l) Sept 2	Ammonia (mg/l) July 12	Ammonia (mg/l) July 24	Ammonia (mg/l) Aug 9	Ammonia (mg/l) Sept 2
I	0.289	0.787	0.028	0.006	0.051	0.056	0.023	0.048
II	0.007	0.003	0.003	0.005	0.063	0.076	0.020	0.038
III	0.698	0.855	0.010	0.004	0.325	0.084	0.021	0.052
IV	0.012	0.005	0.003	0.004	0.056	0.099	0.053	0.047

Treatment	Sulfide (mg/l) July 12	Sulfide (mg/l) July 24	Sulfide (mg/l) Aug 9	Sulfide (mg/l) Sept 2	Sulfate (mg/l) July 12	Sulfate (mg/l) Sept 12
I	0.0198	0.0321	0.0305	0.0275	1008.26	581.33
II	0.0233	0.0336	0.0267	0.0271	1075.03	593.95
III	0.0152	0.0248	0.0244	0.0259	895.20	575.02
IV	0.0217	0.0313	0.0298	0.0248	1011.41	559.25

Table 11. Stocking, sampling and harvest data of the 6th run of milkfish aquarium experiment

Treatment	No. of fish	Biomass (grams)	Abw (g)	1st Sampling No. of samples	Biomass (grams)	Abw (g)	2nd Sampling (terminated) No. of samples	Biomass (grams)	Abw (g)
I	10	4.9	0.5	25	3.3	0.4	16	6.2	1.2
II	10	5.0	0.5	30	4.0	0.4	22	7.5	1.0
III	10	5.7	0.6	27	3.5	0.4	16	3.8	0.7
IV	10	5.0	0.5	26	4.6	0.6	18	12.4	2.3

Date stocked : July 6, 1991
No. of fish stocked / equarium : 10
Date terminated : September 12 , 1991

Treatments

- I Ricebran + cane sugar + fertilizer
- II Ricebran + cana suugar
- III Natural Food + Fertilizer
- IV Commercial Feeds

Table 12. Number of organisms/ml (x 10^3) in water in aquarium stocked with milkfish. (Milkfish Aquarium Expt. 6)

Treatments	July 2	July 12	Aug 2	Aug 27
I	16700	73	232	70
II	12700	49	51	85
III	9300	53	54	57
IV	10700	73	91	58

Table 13. Weight of organisms (g) adhering in tiles in aquarium stocked with milkfish. (Milkfish Aquarium Expt. 6)

Treatments	July 2	July 12	Aug 2	Aug 27
I	0.03	0.12	0.08	0.03
II	0.06	0.09	0.08	0.04
III	0.04	0.08	0.10	0.05
IV	0.03	0.03	0.06	0.05

Table 14. Stocking, sampling and harvest data of the 1st run of milkfish pond experiment.

Treatment	Sample size	Biomass (g)	Abw (g)	Sample size	Biomass (g)	Abw (g)	Sample size	Biomass (g)	Abw (g)	Sample size	Biomass (kg)	Abw (g)
I	71	580	24.5	74	1550	62.3	41	1326.7	97.7	174.7	11.3	64.9
II	76	775	30.5	82	1776.7	65.2	47	1726.7	111.9	160.7	53.4	89.8
III	63	1011.7	48.5	80	3330	124.9	45	2185	142.8	540	23.1	129.6
IV	68	660	29.4	86	1828.3	64.8	44	1286.7	99.4	179.7	65.0	120.4

Date stocked : March 23 , 1991
Date harvested: Aug. 30 , 1991

Treatments
- I Ricebran + cane sugar + fertilizers
- II Ricebran + cane sugar
- III Natural food + fertilizers
- IV Commercial feeds

Table 15. Survival and mortality of milkfish reared in ponds for 160 days in different treatments stocked at a density equivalent to 4,000/ha.

Treatment	No. of fish harvested	% survival	% mortality
I	524	87.33	12.67
II	482	80.33	19.67
III	540	90.13	
IV	539	89.83	10.16

Treatments
- I Ricebran + cane sugar + fertilizers
- II Ricebran + cane sugar
- III Natural food + fertilizers
- IV Commercial feeds

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Table 16. Selected water parameters during the 1st milkfish pond experiment. (April 12 to Aug. 30, 1991)

Trt	Sulfide (mg/l) April 10	April 24	May 9	May 23	June 6	June 25	July 24	Aug. 9
I	0.0416	0.0675	0.0656	0.0462	0.0774	0.0700	0.0828	0.0755
II	0.0725	0.0679	0.0698	0.0572	0.0721	0.0779	0.0790	0.0813
III	0.0198	0.0385	0.0679	0.0557	0.0935	0.0801	0.0889	0.0943
IV	0.0691	0.0557	0.0611	0.0507	0.0607	0.0605	0.0839	0.0736

Trt	Sulfate (mg/l)						Phosphorus (mg/l)				
	April 10	April 24	May 9	May 23	June 6	June 25	April 24	May 9	May 23	June 6	June 25
I	1453.05	1168.61	981.97	1584.49	1823.71	1179.65	0.0671	0.0471	0.0938	0.0589	0.0428
II	1409.94	1246.42	1189.12	1087.64	1342.64	1200.68	0.0745	0.042	0.0416	0.0451	0.0404
III	1468.82	1236.43	1045.06	1142.85	1805.31	1132.34	0.0628	0.0471	0.0989	0.0522	0.0494
IV	1332.12	1242.22	1054.52	945.69	1537.17	1211.20	0.1491	0.0302	0.0310	0.0369	0.0235

Trt	Ammonia (mg/l)								Nitrite (mg/l)	
	April 10	April 24	May 9	May 23	June 6	June 25	July 24	Aug. 9	July 24	Aug. 9
I	0.1276	0.0292	0.0511	0.0217	0.0266	0.0441	0.0786	0.0322	0.0259	0.0208
II	0.1412	0.0271	0.0481	0.0297	0.0285	0.0443	0.0786	0.0415	0.0252	0.0235
III	0.1797	0.0238	0.0534	0.0327	0.0334	0.0460	0.0828	0.0378	0.0322	0.0388
IV	0.1400	0.0231	0.0509	0.0238	0.0271	0.0392	0.0793	0.0315	0.0263	0.0197

Table 17. Selected soil parameters during the 1st run of milkfish pond experiment
(April 12 to Aug 30, 1991)

Trt	Soil Sulfate (mg/l) May 14	June 24	July 2	Aug 8	Soil pH May 14	June 24	July 2	Aug 8	Sept 6	Organic matter (%) May 14	June 24	July 2	Aug
I	4535.8	1186.2	3410.3	2297.7	7.15	6.57	6.38	6.85	7.12	3.16	3.34	2.91	3.1
II	4630.4	1299.8	2320.9	2743.8	7.17	6.37	6.20	6.57	6.92	3.94	3.51	3.43	4.3
III	4530.5	1380.4	3072.2	2217.2	7.22	6.62	6.48	6.83	7.17	3.18	3.45	3.10	3.3
IV	4777.6	1210.4	3033.2	2541.1	7.15	6.47	6.45	6.70	7.10	3.87	3.24	3.19	3.8

Trt	Soil Phosphorus (mg/li) May 14	June 24	July 2	Aug. 8	Soil Nitrogen (%) May 14	June 24	July 2	Aug 8	Sept 6
I	21.57	13.84	17.08	18.35	0.10	0.055	0.067	0.059	0.070
II	21.55	12.69	12.89	14.44	0.135	0.078	0.066	0.060	0.073
III	19.03	12.29	14.98	19.71	0.123	0.071	0.067	0.060	0.087
IV	21.48	11.92	13.95	13.44	0.093	0.058	0.059	0.056	0.046

Table 18. The means and ranges of water pH from ponds in the first run of milkfish pond experiment.

Trt	Apr.9	26	May 3	10	17	24	31	Jun 7	14	28	Jul 5	12	17	Aug.2	9	16	23	Mean	Range
I	8.3	8.4	8.3	8.3	7.8	7.6	7.6	7.9	7.9	8.3	7.6	7.9	8.1	8.8	8.1	8.0	8.1	7.9	7.6-8.4
II	8.2	8.3	8.2	8.1	7.7	7.5	7.5	8.1	7.7	8.3	8.1	8.0	8.0	8.5	8.4	8.0	8.1	8.0	7.5-8.5
III	8.3	8.3	8.3	8.4	7.7	7.6	7.6	7.7	7.8	8.3	7.7	8.2	8.1	8.3	8.9	8.6	8.4	8.0	7.6-8.9
IV	8.2	8.3	8.1	8.1	7.9	7.7	7.5	7.8	7.9	8.1	7.6	7.8	8.0	8.5	8.3	8.0	7.9	8.0	7.5-8.5

Table 19. The means and ranges of temperature (in degrees Celsius) in the first run of milkfish pond experiment.

Trt	Apr.19	26	May 3	10	17	24	31	Jun 7	14	28	Jul 5	12	17	Aug.2	9	16	23	Mean	Range
I	30.6	30.5	32.0	28.8	28.2	27	25.5	29.4	27	26.9	24.0	23.3	24.9	25.0	25.4	26	24.0	27	23-31
II	32.0	30.4	31.9	29.0	28.5	27	25.7	31.3	27	26.9	24.1	22.6	25.0	24.9	24.9	26	24.0	27	23-32
III	31.0	30.0	32.0	29.0	28.0	27	25.0	26.0	28	27.0	24.3	23.3	25.0	24.9	25.8	26	24.3	27	23-32
IV	31.0	30.0	32.0	29.0	29.0	27	26.0	31.0	27	27.0	24.0	23.0	25.0	25.0	25.0	25	24.0	27	23-31

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Table 20. The means and ranges of the salinity (o/oo) of pond water in the first run of milkfish pond experiment.

Trt	Apr 9	26	May 3	10	17	24	31	Jun 7	14	28	Jul 5	12	17	Aug 2	9	16	23	Mean	Range
I	35	35	36	37	36	41	40	38	37	23	26	26	27	17	15	15	10	29	10-41
II	35	36	36	37	36	38	38	38	34	22	26	25	27	17	15	16	10	29	10-38
III	35	35	36	35	36	40	41	38	37	21	24	27	27	17	15	15	11	29	11-41
IV	36	36	36	36	35	39	39	40	38	23	24	26	27	17	15	15	10	30	10-39

Table 21. The means and ranges of dissolved oxygen (mg/l) from ponds during the first run of milkfish pond experiment.

Trt	4/19 T/B	4/26 T/B	5/3 T/B	5/10 T/B	5/17 T/B	5/24 T/B	5/31 T/B	6/7 T/B	6/14 T/B	6/28 T/B	7/5 T/B	7/12 T/B	7/17 T/B	8/2 T	8/9 T	8/16 T	8/23 T
I	4/3	5/4	5/5	6/7	5/5	4/5	4/5	4/5	5/7	6/6	5/5	4/6	4/5	4	6	6	6
II	4/3	5/4	5/5	6/7	5/5	4/5	4/4	4/5	6/7	5/6	5/6	7/4	4/7	4	6	6	6
III	5/4	6/5	6/5	6/8	5/5	4/4	5/5	3/5	6/6	5/5	7/3	6/6	4/5	5	6	8	7
IV	4/4	4/4	6/5	7/8	5/5	4/4	4/5	5/5	6/5	5/6	6/4	4/6	5/5	4	7	6	6

T - top B - bottom

Table 22. List of potential food organisms from pond water with different treatments (Apr10-Aug 14,1991)

Organisms/Treatments	April 10				April 24				May 8				June 6				July 24				Aug 14			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I. Zooplankton																								
Copepod nauplii	*	*		*	*	*	*	*	*	*	*	*	*	*		*	*		*	*	*	*	*	*
Copepodite		*		*	*		*																	
Acartia	*	*		*							*		*	*	*	*					*		*	*
Pseudodiaptomus						*		*	*				*	*	*		*	*	*	*	*	*	*	*
Oithona	*	*		*	*	*	*	*	*	*	*	*	*	*		*	*	*			*	*		*
Harpacticoids	*	*		*	*	*	*		*	*	*	*	*			*				*				
Ostracod					*	*	*		*	*	*		*	*		*				*				
Aquatic insect				*		*																		
Brachionus											*		*	*	*			*						
Mysids														*				*			*	*	*	*
Other Protozoans																	*	*	*	*				
Phacus						*	*			*		*												
Euglenoids																		*						
Ciliates					*			*																
Velliger larvae								*	*		*											*		
Round worm				*	*			*		*	*	*												
Filamentous bacteria												*					*	*	*	*				
Round bacteria									*	*	*	*				*	*	*	*	*				
Cypris							*			*	*			*										
Invertebrate eggs																								
II. Algae																								
Pleurosigma		*		*	*	*	*	*		*	*	*	*		*	*		*		*	*	*		*
Nitzchia	*							*		*					*		*	*	*	*				
Fragillaria				*						*	*	*	*	*	*	*								
Navicula (Diatom)	*			*		*		*	*	*		*		*		*		*		*				
Suririella						*																		
Gomphonema									*	*			*		*									
Anabaena																		*						
Oscillatoria		*			*	*	*		*		*	*	*	*		*								
Lynbya									*		*		*					*						
Protococcus																	*	*	*					
Chlorella				*																				
Nostoc										*														
Chaetomorpha				*			*			*	*		*		*									
Cladophora					*																			
Spirogyra									*	*		*												
III. Fungus																*								

1- Rice bran + cane sugar + fertilizer
2- Rice bran + cane sugar
3- Natural food + fertilizer
4- Commercial feed

Table 23. List of potential food organisms from pond sediments that accumulated in the lab-lab collectors in ponds with different treatments (April 10-Aug 14, 1991)

	April 10				April 24				May 8				June 6				July 24				Aug 14			
Organisms/Treatments	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I. Zooplankton																								
Copepod nauplii																								
Copepodite																								
Acartia																								
Pseudodiaptomus																								
Oithona																								
Harpacticoida																						*	*	*
Ostracod									*													*		*
Aquatic Insect																								
Brachionus										*							*			*	*	*	*	*
Mysids																					*			
Other Protozoans																	*	*	*	*				
Phacus		*		*	*	*		*	*	*	*	*	*	*	*	*								
Euglenoids													*	*		*								
Ciliates																								
Velliger larvae																								
Round worm		*		*				*	*	*	*	*					*	*	*	*	*	*	*	*
Filamentous bacteria																								
Round bacteria								*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*
Cypris														*		*								
Invertebrate eggs																						*	*	
II. Algae																								
Pleurosigma				*					*		*						*	*	*	*	*	*	*	*
Nitzchia															*	*	*	*		*	*			
Fragillaria		*		*	*			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Navicula (Diatom)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Suririella									*												*	*		*
Gomphonema																	*	*	*	*				
Ansbaena						*							*											
Oscillatoria		*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Lyngbya																								
Protococcus	*	*	*	*	*	*	*	*																
Chlorella		*		*																				
Nostoc																								
Chaetomorpha		*		*			*		*		*	*		*		*								
Cladophora	*				*		*																	
Spirogyra									*	*		*		*		*								
III. Fungus																								

Table 24. Plankton count (org/l) x 10^3 in different ponds with various treatments (Milkfish Pond Expt. 1).

Treatments	April 10	April 24	May 8	June 6	July 4	July 24	Aug 14
I	784	91	169	240	360	93	97
II	65	157	128	141	207	111	50
III	95	71	215	239	213	63	76
IV	50	72	73	39	334	97	55

Table 25. Total weight of periphyton on lab-lab collectors in ponds. (Milkfish Pond Expt 1).

Treatments	April 10	April 24	May 8	June 6	July 24	Aug 14
I	2.48	1.90	2.70	6.39	0.57	4.02
II	0.78	1.18	1.89	5.98	2.02	4.86
III	0.86	2.45	19.39	14.47	0.75	2.69
IV	0.05	2.99	3.52	6.57	1.75	3.67

Table 26. Total population of microorganisms (org/ml) x 10^3 on lab-lab collectors in ponds (Milkfish Pond Expt. 1).

Treatments	April 10	April 24	May 8	June 6	July 4	July 24	Aug 14
I	86	178	214	532	203	653	191
II	68	171	244	355	299	618	218
III	94	139	214	669	458	534	179
IV	41	126	302	360	451	554	215

Table 27. Harvest, weight gain, growth rate and survival of milkfish in ponds during the experimental run.

Trt.no.	Pond no.	No. fish harvested	Biomass (g)	Abw (g)	Surv. %	Wt. gain (g)	Growth rate (g/day)
I	4	135	20.385	151.0	90.0	145.0	0.2
	5	137	19.20	140.15	91.33	133.95	0.15
Total/Mean		272	19.79	145.6	90.67	139.48	0.18
II	3	145	19.085	131.62	96.67	125.4	0.14
	6	134	6.650	49.63	89.33	43.33	0.05
Total/Mean		279	12.87	90.63	93.00	84.37	0.08
III	1	107	20.55	192.1	71.33	186.0	0.16
	2	123	13.93	113.21	82.0	107.01	0.1
Total/Mean		230	17.24	152.7	76.7	147.0	0.13

Date stocked: Nov. 26,1991
Application of treatment: Dec. 4/91
Date Terminated: April 7/92

Treatments
I - Rice bran+Cane sugar + fertilizer
II - Natural food + fert.
III - Commercial Feeds

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Table 28. Lab-lab biomass (g/m^2) in ponds with milkfish. Milkfish Pond Expt. No. 2 (January to March 1992)

Treatments	1/20	2/3	2/17	3/2	3/16	3/30
I	12.57	41.70	13.69	12.77	74.27	57.43
II	43.98	23.16	34.13	10.34	75.05	81.60
III	54.76	41.94	30.19	52.38	63.60	61.70

Table 29. Floating algal biomass (ml/l) in milkfish pond (Jan. to March 1992).

Treatments	1/7	1/20	2/3	2/17	3/2	3/16	3/30
I	2.82	2.28	5.05	1.85	2.75	2.80	6.80
II	2.50	2.35	1.56	1.51	1.10	3.05	6.45
III	4.85	2.90	5.00	5.30	8.20	11.90	6.95

Table 30. Sinking volume of zooplankton (ml/m^2) in milkfish ponds (Jan to March 1992)

Treatments	1/7	1/20	2/3	2/17	3/2	3/16	3/30
I	2.15	1.25	0.48	0.03	0.16	0.33	0.25
II	2.35	2.85	1.65	1.58	3.49	1.60	0.16
III	0.65	0.47	0.35	0.09	0.18	0.09	0.10

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Table 31. Delta C Analysis for Different Food Sources and Milkfish. (Milkfish Pond Experiment)

Food Source	Treatment	Code	delta C values	Probable Food source for fish growth
Lumut or filamentous algae		P-L	16.6	
Lablab or benthic algae		P-LL	19.9	
Commercial feed		CF	24.8	
Rice Bran		RB	30.7	
Pond phytoplankton		P-phy	24.4	
Pond zooplankton		P-Z	23.3	
Pond Fish (milkfish)(Sample 1)	II	P-F-1	23.0	Zoo
Pond Fish (Sample 2)	IV	P-F-2	19.2	LL
Pond Fish (Sample 3)	II	P-F-3	19.7	LL
Pond Fish (Sample 4)	IV	P-F-4	20.4	LL
Pond Fish (Sample 5)	I	P-F-5	22.3	Zoo
Pond Fish (Sample 6)	IV	P-F-6	21.1	*
Pond Fish (Sample 7)	III	P-F-7	14.7	
Pond Fish (Sample 10)	II	P-F-10	18.7	**
Pond Fish (Sample 11)	I	P-F-11	20.7	LL
Pond Fish (Sample 12)	III	P-F-12	13.1	
Pond Fish (Sample 13)	III	P-F-13	12.5	
Pond Fish (Sample 14)	I	P-F-14	21.1	*

* probably a composite of benthic algae (LL) and zooplankton (21.6)
** probably a composite of benthic algae (LL) and filamentous algae (L) (18.25)

Assumption: If delta C value of food source is within 1 unit of fish muscle, it indicates that the item is a good food source for fish muscle growth; the result of delta C analysis indicated that benthic algae or lab-lab (LL), zooplankton and combination of benthic algae (LL), filamentous algae (L) and zooplankton could be good sources of muscle growth for milkfish.

Treatments: I - RB + CS + F where, RB - rice bran
II - RB + CS CS - cane sugar
III - NF + F F - fertilizer
IV - CF CF - commercial feed

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Table 32. Water parameters monitored in the pond stocked with milkfish (Milkfish Pond Experiment No. 2, 11/26/91-4/7/92)

Treatments	11/22/91	12/19/91	1/06/92	1/20/92	2/03/92	2/17/92	3/02/92	3/30/92
				Sulfide (ml/li)				
I	.0721	.0859	.0973	.1173	.1242	.0784	.0607	.0515
II	.0572	.1013	.1305	.1660	.1099	.0710	.0676	.0481
III	.0693	.1260	.2118	.1517	.1242	.0658	.0653	.0515

Treatments	11/22/91	12/19/91	1/06/92	1/20/92	2/03/92	2/17/92	3/02/92	3/30/92
				Sulfate (ppm)				
I	627.61	745.90	1053.47	1258.52	1566.09	1573.98	1684.37	730.13
II	635.49	718.30	998.27	1266.41	1396.53	1266.41	1534.55	778.24
III	635.49	761.68	974.61	1132.34	1320.03	1245.12	2220.66	832.65

Treatments	11/22/91	12/19/91	1/06/92	1/20/92	2/03/92	2/17/92	3/02/92	3/30/92
				Nitrite (ppm)				
I	.0238	.0106	.0149	.0140	.0282	.0334	.0194	.0146
II	.0182	.0155	.0254	.0291	.0176	.0286	.0273	.0152
III	.0266	.0266	.0440	.0267	.0188	.0253	.0205	.0144

Treatments	11/22/91	12/19/91	1/06/92	1/20/92	2/03/92	2/17/92	3/02/92	3/30/92
				Ammonia (ppm)				
I	.9100	.1355	.1908	.0490	.0312	.0193	.0298	.0315
II	.7525	.1470	.1995	.0490	.0259	.0168	.0168	.0350
III	.7560	.1348	.1855	.0980	.0231	.0161	.0217	.0378

Table 33. The initial, final biomass and average body weight of tilapia in the preliminary run of the experiment (Novemeber 2-26, 1990)

Aquarium No.	Biomass (g) Initial	Biomass (g) Final	Ave. Weight (g) Initial	Ave. Weight (g) Final
1	78.65	39.24	4.9	3.56
2	71.57	35.71	4.5	3.57
3	76.12	24.38	4.75	2.7
4	74.36	33.64	4.65	3.06
5	70.25	45.67	4.4	3.51
6	60.99	69.82	3.8	4.1
7	54.63	54.4	3.41	3.4
8	48.32	17.0	3.02	4.25
9	55.22	78.92	3.45	4.15
10	50.85	32.3	3.18	2.69
11	61.83	46.9	3.9	3.61
12	50.80	44.03	3.18	4.40

Table 34. Data on the second aquarium experiment of tilapia

		Initial			1st sampling			Inventory		
Treatment	Aquarium No.	Biomass (g)	ABW (g)	No. sampled	Biomass (g)	ABW (g)	No. sampled	Biomass (g)	ABW (g)	Survival (%)
I	1	15.8	1.1	15	16.7	1.1	15	16.8	1.1	100
	11	15.1	1.0	15	17.9	1.2	15	15.0	1.0	100
	12	15.9	1.1	15	17.3	1.2	15	16.3	1.1	100
Mean		15.6	1.1		17.3	1.2		16.0	1.1	100
II	2	20.6	1.4	15	21.2	1.4	15	19.3	1.3	100
	3	16.8	1.1	15	19.3	1.3	15	20.3	1.4	100
	9	15.2	1.0	15	16.3	1.1	13	13.2	1.0	87
Mean		17.5	1.2		18.9	1.3		17.6	1.2	96
III	4	18.0	1.2	15	18.1	1.2	15	17.5	1.2	100
	5	14.9	1.0	15	15.8	1.1	14	14.2	1.0	93
	8	16.0	1.1	15	16.9	1.1	15	15.6	1.0	100
Mean		16.3	1.1		17.0	1.2		15.8	1.1	98
IV	6	14.6	1.0	15	23.2	1.6	15	28.4	1.9	100
	7	17.1	1.1	15	25.8	1.7	15	31.0	2.1	100
	10	17.1	1.1	15	24.1	1.6	15	26.8	1.8	100
Mean		16.3	1.08		24.4	1.6		28.8	1.92	100

Table 35. Data of the 3rd run of tilapia aquarium experiment.

Treatment	Aquarium No.	Fish samples	Biomass (g)	Abw (g)	Survival (%)	Sample size	Biomass (g)	Abw (g)
I	1	15	27.6	1.8	0	-	-	-
	11	15	55.2	3.7	100	6	11.3	1.8
	12	15	60.9	4.1	80	4	9.0	2.3
II	2	15	27.0	1.8	100	5	9.0	1.8
	3	15	24.1	1.6	100	5	7.2	1.4
	9	15	33.5	2.2	100	4	9.0	2.3
III	4	15	30.2	2.0	0			
	5	15	30.5	2.0	27	4	8.2	2.1
	8	15	35.9	2.4	80	3	8.0	2.7
IV	6	15	41.1	2.7	80	3	8.5	2.8
	7	15	39.7	2.6	0	4	11.0	2.8
	10	15	39.9	2.7	100	4	11.0	2.8

Seventeen (17) days after stocking, this run was terminated due to the observed heavy mortality. Samples taken and weights of fish were recorded. Dead fish were removed and their weights were estimated inasmuch decomposition had started.

Table 36. Stocking, sampling and harvest data of the tank experiment of tilapia.

Trt	Tank no.	Biomass (g)	Abw (g)	No. of samples	Biomass (g)	Abw (g)	Feeding rate*	No. of fish harvested	Biomass (g)	Abw (g)
I	2	140	2.1	15	55	3.9	29.4	53	290	5.5
	6	133.5	1.9	15	75	5.0	26.7	43	240	5.6
	8	167.5	2.4	14	60	4.3	33.5	28	175	6.3
	Mean	149.3	2.1	44	63.3	4.4		124	235	5.8
II	3	175	2.5	12	80	6.7	35	36	195	5.4
	4	120	1.7	15	70	4.7	24	51	270	5.3
	10	163.5	2.3	17	90	5.3	32.7	26	175	6.7
	Mean	152.8	2.2	44	80	5.5		113	213.3	5.8
III	5	165	2.4	16	35	2.2	Natural food/ fertilizers	43	135	3.1
	9	172.5	2.5	20	60	3.0		27	105	3.9
	11	173	2.5	14	52	3.7		32	170	5.3
	Mean	170.2	2.4	40	49	3.0		102	136.7	4.1
IV	1	135	1.9	15	65	4.3	27	48	375	7.8
	7	134	1.9	14	85	6.1	26.8	44	380	8.6
	12	181	2.6	21	105	5.0	36.2	58	563	9.7
	Mean	150	2.1	50	85	5.1		150	439.3	8.7

Treatments.

I- Riccbran + cane sugar + fertilizers; III - Natural food + fertilizer
II- Ricebran + cane sugar; IV - Commercial feeds

Table 37. Survival and mortality of tilapia in tanks during the first run of the experiment.

Treatment	Tank no.	No. of fish weighed	Biomass (g)	Abw (g)	Fish left per tank	mortality (%)	survival (%)
I	2	13	50.1	3.9	51	27	73
	6	13	71.3	5.4	42	40	60
	8	31	132.5	4.3	31	55.7	44.3
		19	84.6	4.5		41.0	59.0
II	3	21	102.0	4.9	41	41.4	58.6
	4	22	114.4	5.2	43	38.6	61.4
	10	13	72.4	5.6	35	50.0	50.0
		18.7	96.2	5.2		43.3	56.7
III	5	9	47.8	5.3	53	24.3	75.7
	9	26	104.7	4.0	32	54.3	45.7
	11	10	60.0	6.0	53	24.3	75.7
		15	70.8	5.1		34.3	65.7
IV	1	22	108.8	4.9	40	42.9	57.1
	7	18	80.3	4.5	40	42.9	57.1
	12	8	54.4	6.8	47	32.9	67.1
		16	81.2	5.4		39.5	60.5

Table 38. Selected water parameters during the 1st run of tilapia tank experiment. (July 12 to Oct 15, 1991)

Trt	Sulfide (ml/l) July 12	July 19	July 31	Sept 11	Sept 30	Oct 15	Sulfate (mg/l) July 12	Sept 11	Sept 30	Oct 15	P (mg/l) June 25
I	0.0378	0.0240	0.0343	0.0385	0.0370	0.0870	1072.40	488.28	719.09	730.13	0.298
II	0.0401	0.0283	0.0321	0.0343	0.0357	0.0725	1042.95	437.50	727.50	756.79	0.237
III	0.0378	0.0282	0.0366	0.0279	0.0378	0.1905	1011.94	488.65	709.47	734.86	0.252
IV	0.0347	0.0317	0.0294	0.0301	0.0420	0.1149	966.72	495.11	724.34	725.92	0.259

Treatment	Nitrite (mg/l) July 12	July 19	July 31	Aug 22	Aug 30	Ammonia (mg/l) June 25	July 12	July 19	July 31	Aug 22	Sept 30
I	0.382	0.017	0.075	0.034	0.169	0.079	0.310	0.060	0.429	0.074	0.352
II	0.297	0.018	0.134	0.026	0.084	0.068	0.367	0.054	0.408	0.101	0.369
III	0.751	0.018	0.608	0.026	0.045	0.143	0.265	0.101	0.234	0.157	0.263
IV	0.337	0.009	0.100	0.136	0.233	0.092	0.639	0.072	0.574	0.691	0.334

Treatment	Water pH July 11	July 18	July 25	Aug 22	Sept 12	Sept 26	Oct 15
I	7.17	8.33	7.77	7.40	7.33	7.77	7.77
II	7.33	8.30	7.83	7.40	7.50	7.70	7.70
III	7.23	8.33	7.87	7.60	7.70	7.70	7.77
IV	7.23	8.33	7.77	7.47	7.83	7.77	7.73

Treatment	Dissolved Oxygen (mg/l) July 11	July 18	July 25	Aug 22	Sept 12	Sept 26	Oct 15
I	4.87	4.60	1.73	1.70	2.10	2.37	3.20
II	5.50	4.83	2.70	1.60	1.97	3.20	3.47
III	4.47	4.13	2.93	2.77	2.15	2.37	4.10
IV	4.90	4.57	2.57	1.68	2.17	2.32	4.00

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Table 38 (Continued)

Treatment	Salinity (ppt)						
	July 11	July 18	July 25	Aug 22	Sept 12	Sept 26	Oct 15
I	30.3	23.0	23.7	23.0	18.0	20.0	20.0
II	30.3	23.0	24.0	23.0	18.7	20.0	20.0
III	30.0	23.0	23.7	23.7	18.0	20.0	20.0
IV	30.3	23.0	23.7	23.0	18.0	19.3	20.0

Treatment	Water Temperature (C)						
	July 11	July 18	July 25	Aug 22	Sept 12	Sept 26	Oct 15
I	21.93	24.33	24.17	22.60	22.43	22.70	22.50
II	21.83	24.67	24.17	22.80	22.57	23.70	22.50
III	21.83	24.73	23.67	22.53	22.50	23.60	22.50
IV	21.93	24.47	24.33	22.83	22.43	23.80	22.70

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Table 39. Selected soil parameters monitored during the first tilapia tank experiment (July 12 to Oct 15, 1991)

Treatment	Soil N %	Soil pH	Org. Matter %	Soil P mg/li
I	0.321	6.27	2.68	43.55
II	0.394	6.43	2.91	54.58
III	0.393	6.47	2.63	38.38
IV	0.382	6.55	2.50	34.30

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Table 40. List of potential food organisms from the water column in tanks stocked during the 1st tilapia tank experiment. (July 12 - Oct 15, 1991).

	July 12				Aug 2				Aug 28				Oct 7				Oct 15			
Organisms/Treatments	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I. Zooplankton																				
Copepod nauplii	*	*			*	*	*	*					*	*	*	*				
Copepodite						*	*						*	*		*				
Acertia		*					*													
Pseudodiaptomus		*	*			*	*						*	*	*	*				
Oithona	*	*	*	*	*	*	*	*						*	*					
Brachionus									*					*						
Mysids														*	*					
Protozoans	*	*	*	*	*	*	*	*												
Velliger larvae													*			*				
Round bacteria	*	*	*	*				*	*	*	*	*					*	*	*	*
Jelly fish					*	*	*		*					*						
Invertebrate eggs								*	*	*		*								
Brachyura larvae															*					*
Chlorella					*	*	*	*												
Cypris					*															
Round worm						*	*		*	*		*					*			
Ciliates								*					*							
Harpacticoida								*					*	*	*	*		*	*	*
Dinoflagellates													*			*				
Cyclops														*		*				
Ostracod														*	*					
II. Algae																				
Pleurosigma					*			*	*	*	*	*		*		*		*		
Nitzchia								*	*	*	*	*		*	*			*	*	*
Fragillaria						*			*	*	*									
Navicula	*								*	*	*	*				*	*	*	*	*
Suririella									*	*	*	*								
Anabaena										*										
Oscillatoria								*		*	*				*		*			
Cymbella									*		*	*								
Tabellaria													*	*	*	*	*	*	*	*
Chaetomorpha													*							
Nostoc																	*	*	*	*

1 - Rice bran + cane sugar + fertilizer
2 - Rice bran + cane sugar
3 - Natural food + fertilizer
4 - Commercial feeds

Table 41. Weight of organisms (g) adhering on screens in tanks stocked with tilapia.

Treatments	July 12	Aug 2	Aug 28	Oct 7	Oct 15
I	1.15	1.10	1.09	1.69	0.39
II	2.57	1.65	1.86	2.24	0.87
III	3.69	1.17	1.55	2.69	0.58
IV	1.85	1.14	2.04	1.83	0.55

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Table 42. Number of organisms/ml ($x 10^3$) in tanks stocked with tilapia.

Treatments	July 12	Aug 2	Aug 28	Oct 7	Oct 15
I	32	249	299	80	180
II	47	229	413	134	422
III	37	387	612	47	444
IV	28	224	303	95	391

Table 43. Biomass, Daily Increment and Average Body Weight of Tilapia in Ponds Tilapia Pond Expt. No. 1

					Sampling 1			Sampling 2			Inventory			Survival (%)
Trt. no.	Pond no.	Biomass (g)	Abw (g)	No.of fish	Biomass (g)	Abw (g)	No.of fish	Biomass (g)	Abw (g)	No.of fish	Biomass (g)	Abw (g)	Daily wt. increment	
I	2	60.00	2.4	19	530	27.9	17	840	49.41	61	4,350	71.31	0.53	24.4
	6	80.00	3.2	37	1,100	29.73	29	1,500	51.72	103	5,075	49.27	0.40	41.2
Ave./total		70.00	2.8	56	815	28.82	46	1,170	50.6	164	4,713	60.29	0.47	32.8
II	1	75.00	3.0	69	2,600	38.0	25	2,250	90.00	106	8,715	82.22	0.61	42.4
	3	50.00	2.0	21	780	37.14	20	1,300	65.00	42	3,340	79.52	0.60	16.8
Ave./total		62.50	2.5	90	1,690	37.6	45	1,775	77.50	148	6,023	80.87	0.61	29.6
III	4	25.00	1.0	42	1,915	45.6	35	3,125	89.29	128	15,150	118.36	0.91	51.2
	5	30.00	1.2	25	1,010	40.0	29	2,665	91.9	91	10,980	120.70	0.93	36.4
Ave./total		24.50	1.1	67	1,4025	43.0	64	2,895	90.60	109.5	13,065	119.53	0.92	43.8

Treatments	Initial Weight (g)
I - Rice bran + cane sugar + fertilizer	2.8
II - Natural food + fertilizer	2.5
III - Commercial feed	1.1

Date stocked : June 1, 1992

Date harvested: August 28 , 1992

Table 44. Water parameters monitored in the pond stocked with Tilapia (Tilapia Pond Expt. No. 1, June-Aug '92)

	Nitrite (ppm)					
Treatments	6/11/92	7/1/92	7/15/92	7/28/92	8/11/92	8/25/92
I	.0141	.0094	.0081	.0132	.0184	.0108
II	.0129	.0063	.0146	.0137	.0182	.0117
III	.0107	.0090	.0078	.0144	.0158	.0149

	Ammonia (ppm)					
Treatments	6/11/92	7/1/92	7/15/92	7/28/92	8/11/92	8/25/92
I	.2450	.3325	.1593	.0158	.0322	.0228
II	.2643	.2975	.1621	.0161	.0291	.0238
III	.2275	.2933	.1190	.0161	.0581	.0238

	Sulfate (ppm)			
Treatments	6/11/92	7/15/92	7/28/92	8/11/92
I	720.67	600.00	710.41	698.58
II	704.89	641.80	674.92	698.58
III	738.02	588.96	753.79	694.64

	Sulfide (ml/li)	
Treatments	6/11/92	7/1/92
I	0.0572	0.0509
II	0.0504	0.0481
III	0.0469	0.0521

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Table 45. Lab-lab biomass (g/m^2) Tilapia Pond Experiment (June to August 1992)

Treatments	6/11	7/1	7/15	7/28	8/12	8/26
I	28.74	11.21	10.97	25.78	15.00	20.49
II	49.23	34.81	15.15	51.60	14.95	40.15
III	49.32	15.93	22.04	35.34	27.28	45.97

Table 46. Floating algal biomass (ml/l) in Tilapia ponds (June to August 1992)

Treatments	6/11	7/1	7/15	7/28	8/12	8/26
I	7.65	3.65	5.35	9.55	7.65	5.95
II	6.75	3.33	4.20	9.45	2.40	6.35
III	4.30	1.95	5.05	10.15	4.60	4.95

Table 47. Sinking volume of zooplankton (ml/cu.m.) in Tilapia Ponds (June to August 1992)

Treatments	6/11	7/1	7/15	7/28	8/12	8/26
I	0.30	0.18	0.25	0.24	0.10	1.45
II	2.65	0.14	0.17	0.34	0.75	0.25
III	0.58	0.52	0.30	0.30	0.11	0.45

Table 48. Delta C values of tilapia and the different food sources collected from tanks where they were being reared which possibly contributed to their growth.

Food Source	Treatment	Code	delta C values	Probable Food source for fish growth
Tank treatment 1 fish	I	T-I-F	25.3	Phy, T-L
Tank treatment 2 fish	II	T-II-F	21.9	*
Tank treatment 3 fish	III	T-III-F	22.5	*
Tank treatment 4 fish	IV	T-IV-F	21.9	*
Tank lumut or filamentous algae		T-L	24.4	
Lumut or filamentous algae		P-L	16.6	**
Lablab or benthic algae		P-LL	19.9	**
Commercial feed		CF	24.8	
Rice Bran		RB	30.7	
Pond phytoplankton		P-phy	24.4	
Pond zooplankton		P-Z	23.3	

Note: If delta C value of food source is within 1 unit of fish muscle - indication of a good food source for fish muscle growth.

* probably a composite of benthic algae (LL) and zooplankton (21.6)
** probably a composite of benthic algae (LL) and filamentous algae (L) (18.25)

Table 49. Stocking and harvest data of Prawns stocked in Ponds (Dec - March 1992)
Prawn Pond Expt. No. 1

Initial Data					SAMPLING			HARVEST				
Trt. no.	Pond no.	Days	Amt. of feed per day	TFG	FR mo.	Monthly abw (g)	GR/mo.	Wt. gain	No.of prawns harvested	Biomass (g)	S %	M %
I	11	27	-	Natural feeding -								
		31	25.0	.775	.04	3.4	0.06	2.28				
		27	160.1	4.32	.07	4.83	0.06	1.43				
		42	162.5	6.83	.07	8.0	0.06	3.17	233	1.7	22.5	
Total		127		11.93								
	12	27	-	Natural Feeding -								
		31	25.0	.775	.04	3.13	0.054	2.01				
		27	147.4	3.979	.07	3.5	0.04	0.37				
		42	117.7	4.94	.07	4.0	0.03	0.5	465	1.8	44.93	
Total		127		9.694								
II	7	27	** Natural Feeding and regular application of 18-46-6 **									
		31	**			1.8	0.03	0.68				
		27	**			3.13	0.04	1.33				
		42	**			2.43	0.03	-0.7	37	.09	3.57	
Total		127										
	14	27	** Natural feeding and regular application of 18-46-0 **									
		31	**			1.8	0.03	0.68				
		27	**			1.7	0.02	-0.1				
		42	**			1.15	-0.009	-0.2	74	.085	7.15	
Total		127										
III	10	27		Natural feeding								
		31	43.5	1.35	.07	2.6	0.04	1.48				
		27	122.5	3.31	.07	6.03	0.07	3.43				
		42	112.00	4.7	.03	7.0	0.06	0.97	601	4.150	58.06	
Total		127		9.36								
	13	27		Natural feeding								
		31	43.5	1.35	.07	1.9	0.03	0.78				
		27	89.5	2.42	.07	3.44	0.05	2.1				
		42	74.58	3.13	.03	10.4	0.07	5.0	320	2.82	30.9	
Total		127		6.9								

Treatments

I - Rice bran + cane sugar + Fertilizers (18-46-0)
II - Natural feeds / lab-lab ; lumut , and regular application of fertilizers
III - Commercial Feeds
Date stocked : Dec. 10, 1991; Initial wt. : 0.07 g.
No. of prawns stocked/comp. = 1,035 equiv. to 20,700/ha

Table 50. Lab-lab biomass (g/sqm) from ponds where the 1st prawn pond experiment was undertaken (January to March 1992.

Treatments	1/20	2/3	2/17	3/2	3/16	3/30
I	39.81	12.23	15.83	6.85	77.14	11.75
II	86.31	21.07	24.23	93.26	40.44	145.05
III	51.91	22.33	36.26	86.60	89.91	77.43

Table 51. Floating lalgal biomass (ml/l) in Prawn Ponds (Jan. to March 1992)

Treatments	1/7	1/20	2/3	2/17	3/2	3/16
I	6.05	4.00	2.40	1.95	2.45	8.10
II	3.15	2.88	3.50	1.40	12.30	4.90
III	5.80	7.65	2.95	4.90	5.80	13.25

Table 52. Sinking volume of zooplankton (ml/cu.m.) in prawn ponds. Prawn Pond Expt. 1 (Jan to March 1992)

Treatments	1/7	1/20	2/3	2/17	3/2	3/30
I	0.25	0.38	0.27	0.01	0.17	0.12
II	0.22	0.83	0.50	0.02	0.58	0.05
III	0.28	0.40	0.35	0.02	0.13	0.03

Table 53. Stocking, sampling and harvest data on prawn pond experiment (June to September, 1992)

		1st Sampling			2nd Sampling			3rd Sampling			4th Sampling			Inventory
Trt.	Pond	No.	Biomass (g)	Abw (g)	No.	Biomass (g)	Abw (g)	No.	Biomass (g)	Abw (g)	No.	Biomass (g)	Abw (g)	Biomass (kg)
I	11	12	26.0	2.2	10	23	2.3	13	119.0	9.2	50	500	10.0	4.75
	12	18	33.0	1.8	12	30	2.5	12	49.5	4.13	52	542	10.5	5.37
Mean		15	29.5	2.0	11	26.5	2.4	12.5	84.25	6.7	51	521	10.3	5.06
II	7		not sampled					9	39.0	4.3	8	55	7.0	0.33
	14		- - -					10	39.5	3.95	12	49	4.1	2.1
Mean								9.5	39.25	4.14	10	52	5.5	1.21
III	10	18	40.0	2.2	12	27.0	2.25	8	107.0	13.4	51	1029	20.2	9.0
	13	9	28.0	3.1	7	26.1	3.72	15	196.0	13.1	51	955	18.7	11.2
Mean		13.5	34.0	2.7	9.5	26.7	2.99	11.5	151.5	13.3	51	992	19.45	10.1

Note: 1st and 2nd sampling, done by lifting the feeding trays;
3rd and 4th sampling, by cast net.

1st sampling : July 3 '92
2nd sampling : August 3'92
3rd sampling : September 2'92
4th sampling : September 17'92
Inventory : September 22,'92
Initial wt. : 0.35 g; Date stocked: June 5, 1992

Table 54. Water parameters monitored in the pond stocked with prawn (Prawn Pond Expt. No. 2, June-Aug '92)

	Nitrite (ppm)					
Treatments	6/11/92	7/1/92	7/15/92	7/28/92	8/11/92	8/25/92
I	.0112	.0073	.0056	.0099	.0134	.0096
II	.0126	.0066	.0076	.0131	.0115	.0091
III	.0109	.0044	.0064	.0138	.0132	.0129

	Ammonia (ppm)					
Treatments	6/11/92	7/1/92	7/15/92	7/28/92	8/11/92	8/25/92
I	.2433	.3920	.1120	.0392	.0242	.0315
II	.2485	.3745	.1383	.0473	.0224	.0252
III	.2363	.3255	.1383	.0361	.0217	.0249

	Sulfate (ppm)			
Treatments	6/11/92	7/15/92	7/28/92	8/11/92
I	738.01	618.14	718.30	722.24
II	731.71	532.18	726.19	714.36
III	710.41	633.19	745.90	812.93

	Sulfide (ml/li)	
Treatments	6/11/92	7/1/92
I	0.0498	0.0509
II	0.0521	0.0504
III	0.0515	0.0464

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Table 55. Lab-lab biomass (g/m^2) Prawn Pond Expt.2 (June-Aug '92)

Treatments	6/11	7/1	7/15	7/28	8/12	8/26
I	39.71	13.74	18.26	3.89	-	43.26
II	88.45	124.66	10.23	22.52	13.89	21.07
III	113.11	107.40	11.36	42.87	51.32	92.24

Table 56. Floating algal biomass (ml/l) in prawn ponds.
(June to August 1992)

Treatments	6/11	7/1	7/15	7/28	9/12	9/27
I	3.45	3.80	3.10	1.90	0	15.60
II	7.00	6.40	2.45	4.20	2.70	5.60
III	11.65	9.45	2.40	12.80	9.50	15.95

Table 57. Sinking volume of zooplankton (ml/cu.m.) in Prawn Ponds
(June to Aug, 1992)

Treatments	6/11	7/1	7/15	7/28	9/12	9/27
I	0.73	0.05	0.10	0.07	0.01	0.05
II	0.10	0.07	0.17	0.14	0.05	3.15
III	0.13	0.13	0.05	0.08	0.01	0.32

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A P P E N D I C E S

EFFECT OF VARYING LEVELS OF SULFATE CONCENTRATION IN SALINE WATERS ON FISH YIELD

R.D. FORTES, N.R. FORTES AND I.G. PAHILA

Institute of Aquaculture, College of Fisheries
U.P. in Visayas, Miag-ao, Iloilo 5024, Philippines

ABSTRACT

Milkfish (*Chanos chanos*) were stocked in twelve 90 l aquaria to determine the effect of sulfate on fish yield. Saline waters from various sources with known sulfate levels, and different food sources were used as treatments in 1st and 2nd runs, respectively. The levels of sulfate (1st run) decreased from 1,200, 1,380, 1,700 and 1,100 mg/l to 976.53, 840.53, 772.56, and 441.07 mg/l, respectively, after 24 days; and to 477.00; 490.79; 339.78; and 325.05 mg/l after 28 days. The concentrations of sulfide and ammonia increased (0.0114 to 0.0286 mg/l and 0.0105 to 0.1820 mg/l, respectively). Populations of microorganisms adhering on rice bran particles were highest (450, 570, 850/ml) where sulfate and sulfide concentrations (325.05 mg/l and 0.0192 mg/l) were lowest and ammonia concentration (0.08006 mg/l) was highest.

The levels of sulfate and sulfide were not different among the treatments (range of 919.5 to 970.5 mg sulfate/l) and 0.02252 to 0.02542 mg sulfide/l) 2nd run). The levels of ammonia, however, were highest in the rice bran-sugar (II) and commercial feed (IV) treatments compared to treatments that received fertilizers (I and III). The populations of microorganisms that adhere to substrates (tiles) were fewer in the rice bran-sugar and commercial feed treatments than in the treatments that received fertilizers (I and III).

In both runs, the effect of sulfate concentration on fish yield could not yet be established due to low survival (40 to 67%), poor growth attributable to poor water quality, low feeding rate, and poor food quality (1st run). In the second run, fish survival was very high (100%) except one replicate each in Treatments II (88%) and III (93%). The growth of milkfish in all treatments was low (4% to 6% per day) apparently caused by foul water and acidity which could have resulted from high sulfate concentration in the water. An in-depth study using stable isotope technology is in progress to identify important feeding niches, and to intensify the productivity of these niches. Success in this study could lead to a new pond management strategy.

INTRODUCTION

This is an initial attempt to demonstrate the effect of sulfate concentration in saline water on fish yield with a goal of evaluating the role of sulfate-sulfide reaction, and the role of sulfide as a toxin that limits the availability of natural foods in brackish and saltwater earthen ponds. The role of sulfate as a cause of mineral acidity in ponds through its reaction in water, has been the object of investigations since the 60s. There has been considerable research to help alleviate the negative influence of acid sulfate soils on fish and shrimp yields (Singh 1980); however, the problem of sulfide accumulation in the sediment interstices still exists. The effect of this accumulation on crude organic matter, which is the main source of nutrition for the target animals, needs to be properly understood. In milkfish (*Chanos chanos*) farming, traditional or extensive, modified extensive, and semi-intensive methods that are based on animal density, added food and other inputs, are in use. These provide the necessary organic matter that could be converted into nutritious food for the fish. However, the users have been unable to differentiate these methods (Fortes 1989). Tilapia (*Oreochromis niloticus*) raised in brackish-water ponds that received feed, either alone or in combination with chicken manure and/or fertilizer, exhibited better growth and higher production (Fortes 1986), but the actual sources of growth are yet uncertain. In freshwater, despite the presence of full rations of protein-enriched feed pellets, natural food still accounted for more than half of the growth of the target fish (Schroeder 1983).

The goal of this study is to determine food niches in brackish-water or marine ponds that provide target animals with their nutrition. Knowing this information, a management strategy can be designed that will improve the use of fertilizers as locally available replacements for costly imported feeds. This particular component of the project was implemented to pursue the following objectives, initially using milkfish as the test organism.

1. To determine the effect of sulfate on fish yield.
2. To determine the influence of sulfate and sulfide ions on the production of natural food.
3. To determine the influence of sulfate on the population of microorganisms that adhere to the added organic or inorganic material in saline water.

MATERIALS AND METHODS

Location and Facilities. The site is located in Barangay Nabitasan, municipality of Leganes, Iloilo Province, Philippines (Figures 1 and 2). The municipality of Leganes is located N 10° 8' longitude; E 122° 5.4' latitude. The laboratory facilities included 12 glass aquaria (H= 35 cm; L= 90 cm; W= 35 cm) provided with an airlift system and a bottom filter made of 10 cm sand on perforated marine plywood (0.635 cm thick). Aeration, water delivery and lighting systems were also installed.

FIRST RUN

Collection and preparation of Water. Water used during the first run of the experiment was collected from three points (Guimaras, SM area and Gui-gui Creek) along Guimaras Strait approximately 7 km from the laboratory. This was necessary to insure that pure seawater is collected. A total of 75, 60 l plastic bags filled with seawater to a 40 l line (total of 3 tons of seawater) were transported to the laboratory by means of a 4 ton motor boat. Water from each source was used separately or in combination with water from other sources, including freshwater, and of the 4, each treatment was replicated 3 times, as follows.

Treatment	Initial Sulfate Content (ppm)	Source of Water
I	1,200	Guimaras
II	1,380	Gui-gui Creek
III	1,700	SM + Gui-gui (1:1)
IV	1,100	SM + freshwater (1:1)

Samples of water were also sent to the service laboratory of the Natural Science Research Institute of the University of the Philippines at Diliman, Quezon City, for the analysis for cations.

Monoammonium phosphate fertilizer was added before fish stocking to permit microbial growth. Concentrations of sulfate, sulfide and ammonia in the water were measured before and after stocking and every week thereafter using the methods described by Strickland and Parsons (1972). Salinity, pH, temperature and dissolved oxygen were monitored every other day using an Atago refractometer, digital pH tester and a YSI D.O. meter (model 51B), respectively.

Fish stocking and feeding. Sixteen (16) milkfish fingerlings (average weights: 1.27 g; 1.26 g; 1.23 g; and 1.45 g, for treatments I, II, III, and IV, respectively), were stocked in each of the twelve units of 90 l aquaria (equivalent to 1 fish/6 l). The fish were fed rice bran mixed with 1% refined cane sugar given at 5% of fish biomass daily and adjusted to 10% after the first sampling. The fish were raised in these aquaria for 28 days and sampled at the midpoint and at the end.

Sulfate, ammonia and microbe populations. Finely ground rice bran was mixed with refined sugar at a ratio of 100: 1 (rice bran: refined sugar). Two (2) grams of the mixture were added into a 2 l plastic jar filled with seawater collected from different sources with known levels of sulfate concentration. The mixtures were analyzed for nitrogen after 1, 2, 12, and 24 hours; and after 7, 14 and 30 days of contact with water. A microkjeldahl apparatus was used to determine the nitrogen content of the water. The changes in the nitrogen content in the feed substrate during each time of exposure was taken to represent the microbial processing which could take place if rice bran were not immediately consumed by the fish and remained in the water or sediments to serve as food substrate. The water was observed for visual changes, especially the occurrence of detritus in the container. Water samples and detrital material or organic residues were taken and examined under the microscope. Organisms were counted using a hemacytometer.

SECOND RUN

Due to the difficulty of maintaining the desired level of sulfate concentration in water, the treatments were changed. Instead of using the levels of sulfate concentrations as treatments, different sources of fish food were made the treatments, then the levels of sulfate concentrations in water were monitored. This new experimental design is consistent with the tank experiments of our collaborators in Israel and a Tilapia experiment in aquaria. The new treatments with the initial weights of the fish are as follows:

	Treatment	Initial Weight (g)
I.	Rice bran + cane sugar + fertilizer	1.05
II.	Rice bran + cane sugar	1.17
III.	Natural food + fertilizer	1.09
IV.	Commercial pelleted feed	1.08

Each treatment had three (3) replicates (aquaria) that were stocked with 15 milkfish fingerlings each. The rate of feeding for the three (3) different food sources was 10% of the fish biomass given every day. Natural food in the form of lab-lab and filamentous algae were added to the aquaria. The natural food was given at the rate of 20% of fish biomass, and was later on increased to 40%.

The development of microorganisms was monitored by scattering several ceramic tiles (5.75 cm^2 each) on top of the soil in the bottom of the aquaria. The number of tiles was the same as the number of sampling. It was expected that microorganisms would colonize the tiles quickly. Every sampling, one tile was removed from each replicate then weighed. Then, the brownish-whitish substance adhering on the tiles are scraped off and weighed. The weight of the organisms was estimated using the following formula:

$$OW = WTBS - WTAS$$

where:

OW - weight of organisms (g)
WTBS - weight (g) of tiles before scraping
WTAS - weight (g) of tiles after scraping

The samples were then fixed in formalin and the organisms were enumerated and identified. Population counts of minute organisms were made using a hemacytometer; for larger ones, the Sedgewick rafter counting chamber and cell were used.

RESULTS AND DISCUSSION

FIRST RUN

The initial sulfate concentrations of seawater from the different sources were 1,200 mg/l; 1,380 mg/l; 1,700 mg/l and 1,100 mg/l for treatments I, II, III and IV, respectively. These were significantly lower ($P < 0.05$) than the theoretical concentration of seawater of 2,657 mg/l (Church 1975). These initial concentrations decreased to 976.98 mg/l; 840.53 mg/l; 772.56 mg/l and 441.07 mg/l, after 24 days of storage, and were the initial sulfate concentrations of the various treatments at the start of the experiment (Table 1). They decreased further to 477.00 mg/l; 490.79 mg/l; 339.778 mg/l and 325.05 mg/l, respectively, 11 days after the fish were stocked. However, an increase was observed a few days before the experiment was terminated.

TABLE 1. Sulfate concentration of the different treatment used in aquarium experiment no. 1 and no. 2.

Treatments	Sulfate (ppm)	Salinity (ppt)
I	976.98	36
II	840.52	35
III	772.56	36
IV	441.07	19

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The values of different water parameters that were monitored (sulfate, sulfide, ammonia, pH, DO, temperature and salinity) are discussed as follows and are given in Table 2.

TABLE 2. Physiochemical properties of water and sediment (milkfish aquarium experiment - first run.)

A. Treatment:	Sulfate (mg/l) Dec. 27	Jan. 7	Jan. 10	Jan. 17	Sulfide (ml/l) Jan. 7	Jan. 10	Jan. 17	Jan. 24
I	976.98	476.98	770.72	824.13	0.019	0.025	0.024	0.025
II	840.52	490.79	772.56	813.08	0.015	0.027	0.025	0.020
III	772.56	339.78	726.52	811.23	0.022	0.025	0.024	0.023
IV	441.07	325.05	536.83	669.43	0.013	0.024	0.018	0.021

B. Treatment:	Ammonia (mg/l) Dec. 27	Jan. 7	Jan. 10	Jan. 17	Jan. 24	Dissolved oxygen (mg/l) Jan. 7	Jan. 10	Jan. 16	Jan. 24
I	0.076	0.140	0.021	0.029	0.031	3.63	2.15	4.73	4.73
II	0.097	0.130	0.069	0.065	0.038	3.47	2.05	4.77	5.13
III	0.011	0.147	0.041	0.033	0.046	3.58	2.07	4.30	4.47
IV	0.162	0.140	0.029	0.043	0.026	3.52	2.47	4.73	5.07

C. Treatment:	Salinity (ppt.) Jan. 7	Jan. 10	Jan. 16	Jan. 24	pH Jan. 7	Jan. 10	Jan. 16	Jan. 24
I	36	35	39.3	37.7	7.20	7.37	7.17	7.43
II	35.7	35	40	37.3	7.03	7.17	7.07	7.33
III	36.3	34.7	39.3	38	7.27	7.23	7.20	7.50
IV	19.3	17.7	21.3	19.7	7.43	7.53	7.17	7.47

D. Treatment:	Temperature (C) Jan. 7	Jan. 10	Jan. 16	Jan. 24	Organic matter (%) Dec. 27	Jan. 28	Soil sulfate (mg/l) Dec. 27	Jan. 28
I	24.1	25.6	25.6	27.0	0.0	0.81	0.0	3652.5
II	24.1	25.6	25.7	27.0	0.0	0.97	0.0	3760.2
III	24.2	25.8	25.7	27.0	0.0	0.85	0.0	3983.7
IV	24.1	25.6	25.6	27.0	0.0	0.85	0.0	3337.0

Sulfate. An analysis of variance showed significant differences ($P < 0.01$) in sulfate concentrations in water among the treatments. The sulfate concentration in Treatment IV (mixture of seawater and rain water) was significantly lower ($P < 0.01$) than those of treatments I, II and III. The significant reduction in the sulfate concentration could be attributed to the anoxic condition at the bottom as detected in the decreasing dissolved oxygen level of the water (Table 2). Sulfate was found to be positively correlated with dissolved oxygen and salinity ($P < 0.01$). As the dissolved oxygen level increased, sulfate concentrations subsequently increased in all treatments. Sulfate concentrations were relatively higher in water with higher salinity levels. This observation is corroborated by the fact that seawater with higher salinity levels actually contains higher sulfate ions than freshwater (Church 1975). A significant correlation ($P < 0.01$) was also found between the concentration of sulfate and ammonia. The presence of small amounts of ammonia in the sediment could have been contributed by the accumulated unconsumed feed (rice bran) which contained 5.7% to 10.9% crude protein.

Initially, the washed and dried sand bottom in all the treatments were free of sulfate, but after harvest, significant amounts of sulfate (3,337.0 mg/l to 3,983.71 mg/l) (Table 2) were recorded from the sand bottom. The sulfate concentration of the sediment was higher than the sulfate concentration found in the water. Statistical analysis showed that sulfate in the sediment is highly correlated ($P < .01$) with sediment organic matter. It was also possible that sulfur contained in protein (e.g., methionine) from the accumulated unconsumed food, could have also contributed to some degree to the level of sulfate in the sediment.

Sulfide. Sulfide contents of waters collected from the different treatments were significantly different ($P < .01$) among each other. Hydrogen sulfide was relatively higher during the latter part of the experiment, and may have been contributed by decomposing unconsumed food and enhanced by the breakdown of the aeration system. This phenomenon was highly possible because hydrogen sulfide is formed by heterotrophic bacterial metabolism thus, unionized hydrogen sulfide usually does not occur in well-oxygenated water (Chiu 1988). Sulfide was also found to be positively correlated with salinity, ($P < 0.01$), pH ($P < 0.05$) and temperature ($P < 0.01$).

Ammonia. Ammonia concentrations were relatively high three (3) days after stocking. This could be attributed partly to inadequate aeration as evidenced by the low dissolved oxygen (Table 2). From the 6th day onward, ammonia concentrations decreased, especially after water exchange, but an increase was observed towards the end of the experiment. Again, unconsumed food was noted on the bottom of the aquaria. Correlation analysis showed that ammonia was negatively correlated with temperature ($P < 0.01$) and dissolved oxygen ($P < 0.05$), which indicated that ammonia accumulates in the water column in lower dissolved oxygen and temperature.

Sediment Organic Matter. Initially, the sand bottom had practically no organic matter. After 28 days, an appreciable amount of organic matter was recorded from the samples taken from the sand bottom (0.81%; 0.97%; 0.85% and 0.85% in treatments I, II, III and IV, respectively) (Table 2). This indicated that organic matter was formed from the different inputs, particularly the rice bran which, even when input at a relatively low rate, was not completely utilized by the fish.

Sulfate concentration and microbe populations. A whitish film developed on the water and spread across the surface after 6 days. This film was composed mostly of round and filamentous bacteria and protozoans. The total counts of these microorganisms on the second day were 4,210,000, 1,160,000, 6,650,000 and 5,343,000 for treatments I, II, III and IV, respectively. These populations continued to increase until the 7th day and finally decreased on the 14th day (Table 3). There were indications that the populations of microorganisms were higher in the treatments with lower sulfate concentration (Table 4).

Obviously, the particles of rice bran harbored microorganisms and became food substrates. An organic fraction of the food was apparently converted into an assemblage of microorganisms that could serve as fish food. Schroeder (1978) and Hobbie and Lee (1980) pointed out that the relative contribution of supplied foods and fertilizers to the growth of the fish is attributable to the sunlit pond ecosystem in which minerals and organic fractions of the food and fertilizers are converted into a complex of algae, bacteria, protozoans and their mucopolysaccharide exudates, which can be used as food for fish growth.

TABLE 3. Changes in the total count of organisms in different treatments (water sources) with rice bran.

Treatment	2 days	7 days	14 days
I (Guimaras)	4,210,000	7,109,000	3,468,000
II (Guigui)	1,160,000	3,989,000	920,000
III (SM + guigui)	6,650,000	13,638,000	655,000
IV (SM + freshwater)	5,363,000	12,495,000	1,285,000

TABLE 4. Population of organisms (org/ml x 10^3) in seawater with different sulfate concentrations.

Treatment		January 17	January 24	January 30
		Number of organisms		
I	1	406	1654	224
(Guimaras)	2	120	92	248
	3	150	772	756
	Mean	225.3	839.3	409.3
II	1	4	2	212
(Guigui)	2	112	692	394
	3	30	32	470
	Mean	48.7	242	358.7
III	1	150	200	510
(SM + Guigui)	2	162	158.	358
	3	276	646	394
	Mean	196	334.7	420.7
IV	1	358	108	511
(SM + fresh-	2	800	260	1774
water)	3	2	1600	1344
	Mean	386.7	656	1209.7

Changes in the nitrogen content of water from the first hour to day 30 are shown in Table 5. A build-up of nitrogen in all treatments was observed between day 2 to 7, and then decreased tremendously between day 14 to 30. Fluctuations in the number of microorganisms followed the increase and decrease of the nitrogen content of the water. Increases in the nitrogen content could be due to the organisms that adhere to the food substrate and enrich the protein source of the substrate within the one week period of time (observed in this run). After 14 days, however, the nitrogen content abruptly decreased, probably due to the observed decrease in the microorganisms in the water.

TABLE 5. Changes in the nitrogen content (mg/l) of seawater with varying sulfate concentration with rice bran as the organic residue.

Exposure Time	Treatment I	Treatment II	Treatment III	Treatment IV
0 hour	0.064	0.0257	0.407	0.500
1 hour	0.311	0.504	0.568	0.611
2 hours	0.203	0.332	0.311	0.268
12 hours	0.825	0.536	0.642	0.852
24 hours	0.986	0.558	0.880	1.308
7 days	12.440	4.647	15.018	3.432
14 days	1.80	2.55	1.80	2.70
30 days	1.17	-	-	0.96

<u>Survival and fish yield.</u> The mean survival of milkfish on a per treatment basis ranged from 40% to 67%, although a general decrease in the average body weight was observed in all treatments (Table 6). An inverse relationship was recorded between the average body weight (ABW) of milkfish at harvest and the levels of sulfate concentration in water. The following are the means of sulfate concentrations and the average body weights of milkfish.

Treatment	ABW (g)	Sulfate (mg/l)
I	1.145	477.10
II	1.095	490.79
III	1.195	339.78
IV	1.375	325.05

Although there was a slight negative effect of sulfate on the growth and yield of milkfish, other parameters also could have affected the fish. Salinity, pH and temperature were all within tolerable limits, but the dissolved oxygen contents were all in the lower range of tolerance.

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TABLE 6. The initial, sampling and final weights of milkfish raised in seawater in aquaria for 28 days.

Treatment	Weights in grams				Survival
		Sampling			
	Initial	1st	2nd	Final	(%)
I - 1	1.24	1.26	1.08	1.05	63
2	1.27	1.14	1.08	1.03	66
3	1.30	1.15	1.05	1.11	69
Mean	1.27	1.18	1.07	1.06	67
II - 1	1.30	1.08	1.03	1.13	50
2	1.29	1.06	1.02	0.90	38
3	1.19	1.04	1.03	1.06	31
Mean	1.26	1.06	1.03	1.03	40
III - 1	1.14	1.20	1.13	1.24	38
2	1.30	1.30	1.12	1.20	73
3	1.26	1.31	1.06	1.09	38
Mean	1.23	1.27	1.10	1.18	50
IV - 1	1.54	1.41	1.03	1.23	56
2	1.30	1.71	1.08	1.14	49
3	1.50	1.31	1.26	1.23	62
Mean	1.45	1.50	1.12	1.20	56

Number of milkfish stocked in each aquarium - 16.

SECOND RUN

The different feeding treatments started on April 10, 1991. Based on our data, the fish in Treatment IV (commercial feed) were observed to have a significantly higher growth rate than the fish in other treatments. Statistical analyses were run on the water and sediment parameters to determine treatment differences, and are summarized below.

Sulfate. An analysis of variance showed that the amounts of sulfate in the water of each treatment were significantly different. Treatment IV, which received commercial feed, had the highest range of sulfate concentrations (787.96 to 1155.99 mg/l) (Table 7). The average sulfate concentrations showed lower sulfate, over time, in the treatments that received rice bran (Treatments I and II). Fluctuation in the sulfate concentrations could be due to the water change and other factors that affect the sulfate levels in water. Correlation analysis showed that sulfate in water is negatively correlated to phosphorus and temperature ($P < .01$). Also, a slight correlation was observed with soil organic matter ($P < .10$). However, sulfate levels in soil were not significantly different ($P > 0.05$) among treatments, when based on analyses made before stocking and after harvest.

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TABLE 7. Physiochemical properties of water and sediment (milkfish aquarium experiment - second run).

Treatment	Sulfide (ml/l) April 10	April 17	April 24	April 29	May 9
I	0.020	0.021	0.028	0.031	0.025
II	0.021	0.019	0.026	0.033	0.028
III	0.016	0.022	0.019	0.032	0.028
IV	0.017	0.021	0.023	0.025	0.028

Treatment	Sulfate (mg/l) April 10	April 17	April 24	April 29	May 9
I	1129.70	806.36	973.56	685.96	1001.95
II	1177.02	835.28	1039.80	694.90	1011.41
III	1137.59	835.28	1054.00	761.15	1035.07
IV	1155.99	883.12	1012.46	787.96	1012.99

Treatment	Ammonia (mg/l) April 10	April 17	April 24	April 29	May 9
I	0.146	0.056	0.088	0.129	0.107
II	0.139	0.036	0.108	0.578	0.125
III	0.115	0.066	0.042	0.054	0.068
IV	0.168	0.078	0.044	0.131	0.534

Treatment	Phosphorus (mg/l) April 24	April 29	May 9
I	1.28	1.98	1.52
II	1.19	1.54	1.44
III	1.15	1.42	1.15
IV	1.10	1.35	1.11

Treatment	Soil sulfate (mg/l) Feb. 1	Soil sulfate (mg/l) May 10	Soil pH May 10	Organic matter (%) Feb. 1	Organic matter (%) May 10	P (mg/l) May 10	N (%) May 10
I	3760.20	3662.97	7.57 b	0.98	1.09	90.92 a	0.067 b
II	3347.50	3925.87	7.78 b	0.69	1.31	66.54 b	0.064 b
III	3515.77	3584.10	7.82 a	0.91	0.31	56.43 b	0.012 a
IV	4109.88	3641.97	7.77 b	0.90	0.78	63.14 b	0.053 b

__Sulfide__. An analysis of variance showed no differences among the treatments in terms of sulfide concentrations in water. Mean concentration of sulfides ranged from 0.0225 mg/l to 0.0254 mg/l. Sulfide concentrations, in general, tended to increase in time in all of the treatments (Table 7). This could be due to the accumulation of decomposing organic material from unconsumed food on the bottom and an increase in the metabolic wastes of the fish during the latter part of the experiment. Sulfides were found to be highly correlated ($P < 0.01$) with ammonia, pH, soil organic matter, soil phosphorus and soil nitrogen.

Ammonia. No differences in ammonia concentrations was found among the treatments. The concentration of ammonia in water followed a fluctuating trend from one sampling period to another (Table 7), wherein ammonia was seemingly influenced by certain parameters such as dissolved oxygen, temperature and organic or nitrogen input. An analysis for this run showed significant correlations of ammonia with dissolved oxygen ($P < 0.05$), soil nitrogen ($P < 0.01$) and sulfide ($P < 0.01$).

Sediment Organic Matter. The amounts of organic matter in the sediment increased after each experimental run (Table 7), obviously because of the accumulated unconsumed food which accumulated as a white precipitate on the sand. The organic matter contents of the sediment ranged from 0.69% to 1.31% (Table 7). Treatment I was supplied with rice bran mixed with 1% cane sugar plus fertilizer, while Treatment III had no food supplement except fertilizer. It seems evident that the unconsumed food contributed significantly to the organic matter in the sediment. Correlation analysis between organic matter and the different parameters showed significant relationship ($P < 0.05$) with sulfide, soil and water phosphorus and soil nitrogen. It is a fact that upon decomposition, organic matter will release sulfide and phosphorus as well as inorganic nitrogen.

Phosphorus. An analysis of variance for water phosphorus showed significant differences among treatments. The highest mean phosphorus concentration in the water was found in Treatment I (1.59 mg/l P) and the lowest in Treatment IV (1.19 mg/l P), which was not significantly different from Treatment III (Table 7). Phosphorus in water was found to be correlated with sulfate, water pH, sediment organic matter and soil phosphorus. Sulfates could affect water pH, which in turn determines the solubility of phosphorus in water. Thus, the amounts of organic matter and soil phosphorus are directly related to soluble phosphorus in water.

Sulfate and microbe population. The total population counts for the microorganisms, mostly protozoans and bacteria, are significant. Highest population counts were obtained in Treatment IV (commercial feed) (Table 7) where the sulfate concentration of seawater was recorded as 523.94 mg/l. Total population counts of organisms were significantly different from each other ($P < 0.01$). Based on the mean of sulfate concentration (accumulated values in time), total counts of organisms were greater in the treatments with lower sulfate concentrations. There is an indication that the sulfate-sulfide concentrations in seawater negatively affected the total populations of the microorganisms. This is likely, because of the negative effect of acidity on the organisms resulting from high sulfate concentrations in water. The other measured parameters, ammonia ($P < 0.05$), salinity ($P > 0.10$), pH ($P > 0.10$), dissolved oxygen ($P > 0.10$), and organic matter did not affect the total population.

The density of the microorganisms in terms of the total population counts for the two sampling periods in the aquaria are shown in Table 8. Mean counts show the highest density (26,610,000) in Treatment III (natural food), followed by Treatment IV (commercial feed) and Treatment I (rice bran + refined sugar + fertilizer). In terms of population counts, the treatments were not significantly different from each other, but numerically the treatments that received fertilizers exhibited more microorganisms. Average population counts of 313,000 organisms/l and 266,150 organisms/l were recorded from Treatments I and III, respectively, compared to 133,400 organisms/l and 191,250 organisms/l, respectively, in Treatments II and IV. The positive effect of fertilizer on the development of organisms is consistent with the findings of Hepher (1962) that the primary productivity of chemically fertilized ponds is about 4 to 5 times greater than ponds that do not receive fertilizer. In terms of the weight of microorganisms that adhered to the tiles, Treatment I produced significantly greater biomass than the rest of the treatments, possibly due to microbial organisms associated with the organic matter (rice bran). Schroeder (1978) reported that large increases in sediment-related microbial protein are regularly associated with the deposition of organic matter. An aerobic environment rich in coarse organic matter can produce large communities of bacteria and protozoans in small straw-like particles that serve as substrate for microbial growth (Schroeder 1978).

TABLE 8. Total population count (org./l x 10^4) and weight (g) of microorganisms on tiles (milkfish aquarium experiment second run).

Treatment/Replicate		April 9		April 26	
		Population count	Weight	Population count	Weight
Treatment I -	1	6.15	0.02	9.10	0.00
	2	13.05	0.12	21.40	0.11
	3	36.25	0.09	26.35	0.07
	Mean	18.48	0.075	18.95	0.06
Treatment II -	1	7.20	0.01	17.25	0.02
	2	19.25	0.02	16.40	0.02
	3	12.25	0.02	7.70	0.02
	Mean	12.90	0.017	13.78	0.02
Treatment III -	1	57.35	0.01	10.00	0.01
	2	27.35	0.04	13.95	0.003
	3	14.90	0.02	36.15	0.02
	Mean	33.20	0.023	20.03	0.011
Treatment IV -	1	34.95	0.012	11.70	0.01
	2	6.10	0.009	15.05	0.003
	3	23.00	0.090	23.95	0.02
	Mean	21.35	0.037	16.90	0.011

<u>Fish survival and yield</u>. The survival of milkfish in all treatments was very high (100%) except in one replicate each of Treatments II (83%) and III (93%) (Table 9). Fish in all treatments registered a daily growth rate of 4%, 4%, 4% and 6% for Treatments I, II, III and IV, respectively. Such growth rates were very low, but they provide information about the indirect effect of sulfate on the growth and yield of fish under the conditions of the experiment. The expectation that Treatment IV would have the highest yield was realized. This is mainly because this treatment used commercial formulated food given at 10% of total fish biomass. The highest yield, however, coincided with the

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highest level of sulfate, lowest level of sulfide and a higher level of ammonia (Table 7).

TABLE 9. The initial, sampling and final weights of milkfish raised in seawater in aquaria for 30 days.

Treatments		Weights in grams			Survival
			Sampling		
		Initial	1st	Final	(%)
I	1	1.05	1.11	1.12	100
	2	1.01	1.20	1.00	100
	3	1.10	1.30	1.09	100
	Mean	1.05	1.20	1.07	100
II	1	1.37	1.40	1.30	100
	2	1.12	1.30	1.40	100
	3	1.02	1.10	1.00	88
	Mean	1.17	1.27	1.23	96
III	1	1.20	1.21	1.20	100
	2	0.99	1.10	1.02	93
	3	1.07	1.13	1.04	100
	Mean	1.09	1.15	1.09	98
IV	1	0.97	1.55	1.90	100
	2	1.14	1.72	2.10	100
	3	1.14	1.61	1.80	100
	Mean	1.08	1.63	1.93	100

Treatments: I - Rice bran + cane sugar + fertilizer
II - Rice bran + cane sugar
III - Natural food + fertilizer
IV - Commercial feed

CONCLUSION

A negative influence of sulfate on milkfish yield and on the production of natural food (phytoplankton and other algal forms, bacteria and protozoans) in seawater was found in this preliminary study. The duration of the experiment was not sufficient to draw concrete conclusions; however, a trend can be gleaned from the results. This experiment needs to be replicated several times to ascertain consistent results.

Several experiments addressing the same objectives and the overall goal of the project are in progress. These are being done in glass aquaria, concrete tanks and brackish-water earthen ponds. Identification of important feeding niches with the use of stable isotope technology is also in progress. The results of these new studies should strengthen the findings of this study.

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AGRICULTURAL RESIDUES AS FEED SUBSTRATES FOR MILKFISH PRODUCTION IN BRACKISHWATER PONDS

Romeo D. Fortes, Norma R. Fortes Ida G. Pahila

Institute of Aquaculture
College of Fisheries, University
of the Philippines in the Visayas
Miag-ao, Iloilo 5024, Philippines

Abstract

Two runs were made in 500 sqm ponds to determine the effect of rice bran as substrates for milkfish (Chanos chanos Forsskal) food organisms, on fish yield and compare it with other milkfish production methods. The 1st run tested rice bran + cane sugar + fertilizer (I); rice bran + cane sugar (II); natural food + fertilizer (III); commercial feeds (IV); the 2nd run, rice bran + cane sugar + fertilizer (I); natural food + fertilizer (II); and commercial feeds (III). Stocking densities were 205 (1st run) and 150 fish/pond (2nd run). Fish yields were equivalent to 232.3, 295.6, 478.8 and 443.3 kg/ha for I, II, III, and IV, respectively (1st run); 396.2 (I) 252.8 (II) and 351.4 (III) kg/ha (2nd run). Survival ranged from 80.3 to 90.1 (1st run); and 76.7 to 93.0% (2nd run). Average weights ranged from 64.9-129.6 g (1st run); 90.6-152.7 g (2nd run) The potential of rice bran as substrates for fish food organisms was demonstrated. Mixes of phytoplankton, filamentous algae, lab-lab and zooplankton produced on substrates are food sources that contributed to the muscle growth of fish based on delta C values. More trials are needed to confirm these results.

Introduction

The level of management in the commercial production of milkfish (Chanos chanos Forsskal) in the Philippines is generally in the fertilization level. Few fishfarmers used the semi-intensive method of milkfish farming which requires higher stocking density, feeding, and use of life support systems. These are complemented by feed manufacturers that produce milkfish feeds for grow-out. Unfortunately, many users of the semi-intensive method, can not distinguish it from other methods (Fortes, 1989).

Obviously, the direction of milkfish farming is towards the higher level of management intensity which implies expenditures of foreign currency reserves for importation of refined feeds or ingredients. The Philippines may not be ready to go into this direction if the feeds would require imported ingredients and equipment. The use of artificial feeds in aquaculture should be rationalized too, in the face of global concerns relative to environmental protection. New aquaculture management strategies that address these concerns are needed; and advanced technologies that could result to reduced reliance on refined feeds should be developed. Schroeder (1983a) had shown that even in the presence of full rations of protein enriched feed pellets, natural foods still accounted for half or more of the growth of the target fish and prawns. Furthermore, Schroeder (1983b) showed that in relatively sulfate-free environments, such as fresh water ponds, it is the anaerobic microbial processing that converts the crude organic matter of added fertilizers into main source of nutrition for the target animals of these ponds. It is along this line that this work was conceived with the assumption that milkfish yield from brackishwater ponds is independent of sulfide and ammonia concentrations in the sediments in which case the potential to replace feeds with feed substrates (agricultural residues) is as great in salt water as it is in fresh water provided a proper management strategy is used.

The different treatments used in these experiments were based on the preliminary runs in aquaria where yield of milkfish in saline water with known levels of sulfate and sulfide were determined. Although there appeared to be a negative influence of the levels of sulfides (initial concentrations of 0.0133-0.0187 ml/l and final concentrations of 0.0195-0.0248 ml/l in 1st run; and initial concentrations of 0.0156-0.0213 ml/l and final concentrations of 0.0252-0.0283 ml/l in the 2nd run) on milkfish and natural food production, no concrete evidence is yet available (Fortes et al., In press). Thus, this study initially tested the use of rice bran, as a major input in brackishwater ponds so that its potential to serve as substrates for microbial growth and influence on milkfish yield could be determined. The influence of sulfate-sulfide reactions on the processing of sediment organic matter (in this case the rice bran) into useful fishfoods could be known using the newly developed tool of stable isotope technology (Schroeder, 1983a). The overall goal of this study therefore is to significantly reduce, if not replace, the use of refined feeds in pond aquaculture with inexpensive agricultural residues. The specific objectives are: (1) to determine the effect of rice bran as feed substrates on milkfish yield; and (2) to compare the yield of milkfish from this method with other commercial milkfish production methods.

Methodology

1. Location of the Study Site

The study was conducted at the the Brackishwater Aquaculture Center (BAC), Institute of Aquaculture (IA), College of Fisheries (CF), University of the Philippines in the Visayas (UPV), Leganes, Iloilo, Philippines. Leganes is located N10 8' longitude; and E122 5.4' latitude. Elevation of the pond area is 3.59 meters above sea level and water source is mainly from the sea with seawater salinity; and from Jalaur River with 0 to 35 ppt salinity during the rainy season at low tides and during high tides, respectively.

2. Implementation of the Study

The study carried out two runs. The 1st had a duration of 160 days (March 23 to August 30, 1991; the 2nd, 132 days (November 26, 1991 to April 7, 1992). Both runs utilized 500 sqm earthen ponds.

3. Experimental Design

The 1st run tested the following treatments that were replicated 3 times each: Rice bran + cane sugar + fertilizer (I); rice bran + cane sugar (II); natural food + fertilizer (III); and commercial feeds (IV). The 2nd run, with 2 replicates each tested the rice bran + cane sugar + fertilizer (I); natural food + fertilizer (II); and commercial feed (III). These treatments are described as follows: I-The mixture of rice bran and refined cane sugar was 100:1. The amount added was based on the daily supply of the mixture at 5, 7, 10, 10 and 10% of the fish biomass for the 1st, 2nd, 3rd, 4th and 5th month, respectively. Rice bran served as substrates for microbial growth; sugar provided assimilable carbon for bacteria. Fertilizer (18-46-0) (1.1 kg/pond or 22 kg/ha) was used to cause bloom of natural food; II-Same as treatment I (rice bran + cane sugar) but without fertilizer; III-Natural food (lab-lab or benthic algae, lumut or filamentous algae, etc.) was encouraged to develop using the conventional method of fertilization. (This was II in 2nd run). IV-Commercially-available feeds were used. (Treatment III in the 2nd run). The treatments used in the 2nd run were the same as those described above but without treatment II.

4. Stocking, sampling and harvest

Each pond was stocked with 205 milkfish fingerlings (mean wt.= 0.07g)(1st run) grown from fry (initial wt.= 5mg) in 3m x 2m pond in one corner of pond No. A-11. Stocking rate (2nd run) was 150 per pond (mean wt.=6.2g). The fingerlings were purchased and conditioned in indoor containers for about 2 days before stocking. In both runs, the fish were sampled 3 times at an interval of about 1 month between sampling. About 10% of the fish population was sampled (1st run); 10 to 13% (2nd run). The fish were harvested after 160 (1st run) and 132 days (2nd run). The yield, growth, survival and average body weight at harvest were measured and compared to other treatments.

5. Measurements of physico-chemical and biological parameters

A. Measurements were made of: salinity (ppt) by means of Atago refractometer; temperature(C) and dissolved oxygen (mg/l) by YSI DO meter; pH, by pH tester. Sulfide (ml/l) by phenylenediamine dihydrochloride method; reactive P (mg/l), by colorimetric molybdenum blue method; dissolved ammonia (mg/l) in water by idophenol method; nitrite, by the napthyl ethylene diamine colorimetric method (Strickland and Parsons, 1972) and sulfate (mg/l) by the barium chloride method (APHA, 1971).

B. In the soil, measurements were made of: pH, by pH meter in the laboratory; sulfate (mg/l), by barium chloride method and available P, by molybdenum blue method (Black, 1965); organic matter content (%) by Walkley and Black dichromic acid digestion method and total nitrogen (%) by Kjeldahl extraction and digestion method (Dewis and Freitas, 1970).

C. Populations of natural food organisms were determined in the water column and on the pond bottom. One-liter water samples were passed through a plankton net (100 meshes/inch) then concentrated in 30-ml funnel and collected. The organisms were counted under the microscope using Sedgewick Rafter Counting Chamber and Cell then estimated using the equation: N = C x 1000 - V, where: N - average count/cell; C - count of the organisms; V - volume scanned through the counting cell (Length x Width x Depth)

Zooplankton population in water was estimated using the following: P = N x Vs/Vf, where N - average count per cell; Vs - volume of water sample; Vf - volume of filtered sample. The population of organisms at the pond bottom was determined by placing ceramic tiles (4"x4") at the middle and 4 corners of each pond. These served as substrates. A total of 24 tiles (equivalent to 24 sampling) per pond were used. One tile was randomly picked up every sampling and microorganisms were taken by scraping off the brownish-whitish substance adhering on tiles. The scrapings were weighed and wet weight of the organisms was estimated using the equation: OW = WTBS - WTAS, where: OW - weight of organisms (g); WTBS - weight of tiles (g) before scraping; WTAS - weight of tiles (g) after scraping. Samples were fixed in formalin and the organisms identified and enumerated. Population of minute organisms were counted using haemocytometer; larger ones by Sedgewick rafter counting chamber and cell. Delta C values of different food sources and fish flesh were analyzed at the Dor and Krueger Laboratories in Israel.

Results

Stocking and harvest data from the 2 runs are given in Table 1. The average weight gains of milkfish were low (64.9 to 129.6 g (1st run); 90.6 to 145.6 g (2nd run) giving a gross yield equivalent to 232.3 kg/ha to 478.8 kg/ha and 252.8 kg/ha to 396.2 kg/ha for 1st and 2nd runs, respectively. Survival ranged from 80.3 to 90.1% (1st run); and 76.7 to 93.0% (2nd run). In Table 2 are presented the ranges of selected physical and chemical parameters that were measured. The lower limits of water salinity were 9 to 10 ppt; the upper limit was 41 ppt (1st run). Salinity range was 24 to 25 ppt and 48 to 49 ppt for the lower and upper limits, respectively (2nd run). Generally, pH, was within the normal range except the range in the upper limits (8.7 to 9.3 in the 1st and 9.6 in the 2nd) which were higher than the desirable range (pH 6.5 to 8.5) for aquaculture systems (Stickney, 1979). Dissolved oxygen content of water measured between 0800H and 0900H fluctuated from 1.8 mg/l to 11.5 mg/l and 2.1 to 8.2 mg/l in the 1st and 2nd runs, respectively. Water temperature in the 1st run was generally higher (22.0 to 32.9 C) than in the 2nd run (20.2 to 31.0 C).

Sufate-sulfide levels were measured together with phosphorus, ammonia and nitrite. The levels in all treatments of the 2 runs are given in Table 5. The same trend was observed in the 1st and 2nd runs where soil chemical parameters were measured (Table 6). Where the plankton organisms were counted from the water samples, Treatment III exhibited the highest numerical plankton count. The same trend was observed when the biomass of the organisms was considered (Tables 3 and 4).

Discussion

Yields of milkfish (232.3, 295.6, 478.8 and 443.3 kg/ha for Treatments I, II, III, and IV, respectively) were not significantly different (P>0.05) from each other (Table 1)(1st run). However, in terms of mean individual weight, the superiority of natural food and the commercial feed, over the treatments with rice bran was noted. The mean weignts of milkfish in Treatments III and IV (natural food and commercial feed, respectively) were 31% and 25% bigger than the fish in Treatment II (rice bran + cane sugar). Milkfish in Treatment I (rice bran + cane sugar + fertilizer) however, was even smaller than milkfish in Treatment II. Using the SYSTATS program, analysis of variance did not show treatment differences but the negative effect of high stocking density (4,100/ha compared to 2,000/ha used by milkfish farmers) and limited food on fish growth was demonstrated. This is shown by the mean weight of milkfish at harvest of 64.9, 89.8, 129.6 and 120.4 g for treatments I, II, III and IV, respectively (Table 1) despite a culture period of 160 days (way beyond the usual 120 days). The capacity of the ponds to support the weight of fish at such level and kind of inputs, appeared to have limited fish growth resulting to undesirable size-fish for the market. Furthermore, it was also indicated that under such stocking density and limited food availability, the fish that were fed with commercial feed and those raised on natural food, did not attain sizes acceptable in the market.

In the 2nd run where the stocking density was adjusted equivalent to 3,000/ha, survival in all treatments was higher (90.7, 93.0 and 76.7%) than in the 1st run except in Treatment III which was 76.7%. This however, was not significantly different from the survival of milkfish in the other treatments (P>0.05). Milkfish production in all treatments were not different (P>0.05) from each other. In terms of the mean weight of milkfish at harvest, a significant difference (P<0.05) was noted between Treatments II (natural food) and I (Rice bran + sugar + fertilizer); and between Treatments II and III (commercial feed); but not between Treatments I and III. There was apparently a positive effect on the growth of milkfish when rice bran, upon which various food organisms accumulated on each granule, served as a good source of food for milkfish. These are shown in Tables 3 and 4 where the ranges of plankton count in pond water and the population and biomass of the various microorganisms that attached on the ceramic tiles on the pond bottoms, respectively, were examined. It was observed that the treatment with rice bran + sugar + fertilizer and that with natural food + fertilizer had the highest plankton count (72.2M and 77M, respectively) and biomass (33.01 and 90.22 mg/cm, respectively). Although there was no difference between plankton count in the water between the two treatments, the biomass of the microorganisms that attached on the tiles was significantly different from each other ($P<0.05$). The potential of rice bran as feed substrates for most of the fish food organisms in brackishwater ponds where this study was conducted was clearly indicated. However, it is still necessary to make a more detailed run in order to confirm these results.

The effect of the selected chemical parameters of the water and soil on the microorganisms in the water column and tile substrates may have something to do with the results of the experiment. As shown in Tables 5, the level of sulfides in water increased as the concentration of sulfates decreased. This trend was the same in all treatments in both the 1st and 2nd runs. This observation appears to indicate that sulfide, the most common toxin in mariculture pond has not reached the level by which it could significantly affect the potential food organisms in the ponds despite its presence in water (0.064 to 0.072 ml/l and 0.086 to 0.108 ml/l, for the first and second runs, respectively). In Table 6, the levels of soil sulfate did not vary from each treatment but the levels in the 2nd run were generally lower than those in the 1st run (2414.83 to 2534.55 mg/l in the 1st run; 1224.8 to 1938.7 mg/l in the 2nd run). Based on the sulfide production from sulfate in the water which was about 0.005237 to 0.008547%, it could be expected that sulfide production in the tile substrates could be lesser inasmuch as less bacteria were observed on tiles than in the water. The same was observed by Kirchman (1983; Kirchman and Ducklow, 1987) where organic particles in water was colonized by bacteria while those particles that appear to be inorganic were devoid of bacteria. The importance of bacteria in the production of sulfide from sulfate has been pointed out although sulfide may be reoxidized chemically under aerobic conditions or biologically by sulfur oxidizing bacteria under anaerobic conditions (Fry, 1987). Anaerobic condition was not attained in the ponds inasmuch as dissolved oxygen concentration ranged from a low of 1.8 to a high of 11.5 mg/l in both runs. In view of this, the effect of sulfide as a toxin to potential food organisms was not clearly observed under the conditions of the experiment. However, it was demonstrated that milkfish production from the treatment with rice bran was still higher than the production obtained by most milkfish farmers in the Philippines (350 to 1,200 kg/ha per year) (Philippines Fisheries Profile, 1990). The potential therefore of rice bran as substrates for microorganisms that are potential fish food organisms was demonstrated. Rice bran itself is not a good food source for muscle growth of fish as shown by delta C values. The values of delta C for milkfish obtained from the experimental fish ranged from 12.5 to 22.3 which indicated that mixes of phytoplankton, filamentous algae, lab-lab and zooplankton are good food sources for muscle growth but not rice bran (delta C= 30.7). This experiment demonstrated that rice bran is a potential substrates for fish food organisms in brackishwater ponds which could produce significant amount of food organisms that could reduce the requirements of artificial feeds for milkfish production. Refinement of the methods and techniques is still necessary which shall serve as a basis for the development of a new management strategy for milkfish production.

Acknowledgements

This work was funded under the U.S.- Israel Cooperative Development Research Program (CDR Project No. DHR-5544-G-SS-9068-00) obtained through the initiative of Dr. Gerald L. Schroeder, Aquaculture Research Station, Agricultural Research Organization, Bet-Dagan, Israel. The authors also gratefully acknowledge the support of Dr. Sheenan Harpaz of the same organization. The help of Perfecta J. Carpio, Cecilia J. Jaspe, Josette Bangcaya, and Leopoldo Anas contributed significantly to the success of the project.

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Table 1. Stocking and harvest data of the two milkfish experimental runs which compared the performance of the fish in the different treatments (initial stocking sizes of milkfish were 0.07 g and 6.2 g for the 1st and 2nd runs, respectively).

Treatment*	Ave. weight (g)	Survival (%)	Yield (kg/ha)
First Run (March 23 to August 30, 1991)			
I	64.9	87.3	232.3
II	89.8	80.3	295.6
III	129.6	90.1	478.8
IV	120.4	89.8	443.3
Second Run (November 26, 1991 to April 7, 1992)			
I	145.6	90.7	396.2
II	90.6	93.0	252.8
III	152.7	76.7	351.4

Table 2. The ranges of selected physico-chemical parameters of pond water from the different treatments during the period of study.

Treatment*	Salinity (ppt)	pH	D.O. (mg/l)	Temp. (C)
First Run				
I	10-41	7.2-8.9	2.6-8.2	22.0-32.9
II	9-41	7.0-8.9	1.8-8.3	22.0-32.9
III	10-41	7.5-9.3	3.0-11.5	23.0-32.0
IV	9-41	7.4- 8.7	3.0-8.8	23.5-32
Second Run				
I	25-48	7.9-9.6	2.1-8.1	20.2-31.0
II	24-49	7.5-9.6	2.7-8.1	20.2-30.9
III	25-49	8.0-9.6	2.4-8.2	20.2-31.0

* I-Ricebran + cane sugar + fertilizer (Treatment I for 2nd run)
II-Ricebran + cane sugar
III-Natural food + fertilizer (Treatment II for 2nd run)
IV-Commercial feed (Treatment III for 2nd run)

Table 3. The ranges and averages of plankton count of the pond water from the various treatment in the course of the milkfish experimental runs (April to June 1991)

Treatments	Range (x 100,000)	Average (x 100,000)
I	39.20 - 119.75	72.20
II	32.50 - 70.60	61.36
III	35.60 -119.20	77.00
IV	24.90 - 71.40	42.20

Table 4. Means and ranges of the population of microorganisms that were scraped off from the ceramic tiles which were distributed at the middle and corners of the bottom of the ponds and their biomass.

Treatments	Count (x 100,000)		Biomass (mg/cm)	
	Ranges	Averages	Ranges	Averages
I	8.58 -21.40	15.93	18.45 - 63.30	33.01
II	6.75 - 24.37	16.07	7.57 - 58.06	23.86
III	9.38 - 21.40	14.90	8.35 -188.25	90.22
IV	4.00 - 30.20	15.62	0.49 - 63.79	31.87

Table 5. The ranges of sulfide (A), sulfate (B), phosphorus (C), ammonia (D) andnitrite (E) in pond water of the different treatments during the first and second milkfish experimental runs in earthen ponds.

	Treatments			
First Run	I	II	III	IV
A (ml/l)	0.042-0.083 (0.066)	0.068-0.081 (0.072)	0.020-0.094 (0.067)	0.051-0.084 (0.064)
B (mg/l)	982.0-1179.7 (1365.25)	1087.6-1410.0 (1246.07)	1045.1-1805.3 (1305.13)	945.7-1537.2 (1220.48)
C (mg/l)	0.043-0.094 (0.062)	0.042-0.075 (0.049)	0.094-0.099 (0.062)	0.030-0.149 (0.054)
D (mg/l)	0.128-0.022 (0.051)	0.027-0.141 (0.089)	0.024-0.180 (0.061)	0.023-0.140 (0.052)
E (mg/l)	0.021-0.026 (0.023)	0.024-0.025 (0.024)	0.032-0.039 (0.035)	0.020-0.026 (0.023)
Second Run	I	II	III	
A (ml/l)	0.052-0.117 (0.086)	0.048-0.166 (0.094)	0.052-0.212 (0.108)	
B (mg/l)	627.6-1684.4 (1155.0)	635.5-1534.6 (1074.3)	635.5-2220.7 (1140.3)	
C (mg/l)				
D (mg/l)	0.019-0.910 (0.175)	0.017-0.753 (0.155)	0.016-0.75 (0.159)	
E (mg/l)	0.011-0.028 (0.020)	0.015-0.029 (0.022)	0.014-0.027 (0.025)	

Table 6. Selected chemical soil parameters measured from the ponds used in testing the performance of the different treatments in the milkfish pond experiments. (A-sulfate; B-soil pH; C-phosphorus; D-nitrogen and E-organic matter)

	Treatments			
First Run	I	II	III	IV
A (mg/l)	1242.8-4535.8 (2534.55)	1070.2-4630.4 (2414.83)	1034.5-4530.5 (2446.97)	1076.8-4777.6 (2527.68)
B	6.4 - 7.3 (7.0)	6.2-7.4 (6.8)	6.5-7.4 (7.0)	6.4-7.35 (6.9)
C (mg/l)	13.8-21.6 (17.21)	12.9-21.6 (15.03)	12.3-19.7 (16.28)	11.9-21.5 (15. 18)
D (mg/l)	0.06-0.07 (0.076)	0.06-0.14 0.086)	0.06-0.13 (0.090)	0.10-0.05 (0.069)
E (%)	2.91-4.38 (3.39)	3.43-3.96 (3.76)	3.10-4.85 (3.58)	3.19-4.24 (3.69)
Second Run	I	II	III	
A (mg/l)	1889.9-1559.7 (1224.8)	1505.2-1818.7 (1661.9)	1967.0-1910.4 (1938.7)	
B	6.88-6.98 (6.9)	6.30-6.98 (6.6)	6.12-3.89 (6.6)	
C (mg/l)	15.24-15.77 (16.5)	15.78-16.43 (16.1)	17.26-21.36 (19.3)	
D (mg/1)	0.38-0.40 (0.39)	0.31- 0.36 (0.34)	0.40 0.43 (0.42)	
E (mg/l)	3.04-3.45 (3.25)	2.60- 3.34 (2.97)	3.51- 3.89 (3.70)	

Note: Values in parenthesis are means of the parameter indicated.