

PRELIMINARY STUDIES OF SELECTED ENVIRONMENTAL AND NUTRITIONAL REQUIREMENTS FOR THE CULTURE OF PENAEID SHRIMP¹

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ABSTRACT

Types of substrate, type of aeration, and stocking density were compared as prerequisites for high-density culture studies with penaeid shrimps. Neither sand-shell substrate nor brick subdivisions of culture tank bottoms produced significantly higher survival rates than bare fiber glass tanks. Forced air supplied via airstones proved to be a more suitable form of aeration than did physical agitation of the water column in culture tanks by high-pressure nozzles. Survival rates of 80 to 90% were achieved when biomass densities did not exceed 40 g/m².

Semipurified pelleted diets (i.e., containing defined chemical ingredients plus one or more natural products) having a complement of nutrients including minerals and vitamins, various ratios of shrimp to fish meal, protein hydrolysates, and such diets fed at three percentages of total biomass daily were compared for their ability to produce increases in growth. Diets without fish or shrimp meal sustained biomass while those diets having the highest proportion of shrimp to fish meal in addition to added vitamins produced over 60% increase in total biomass over a 3-month period. Animals fed a combination of yeast, soy, and casein hydrolysates increased 39% in biomass over the same period of time while those fed each of the above hydrolysates during the 3-month period separately showed only an average of 18% increase in weight. Feeding shrimp with a fish-shrimp base with added vitamins at a rate of 15% daily of the total biomass produced a 164% increase in weight with 95 to 100% survival during the 3-month period. Using semipurified pelleted diets, a food conversion ratio of 5.5 was obtained.

Establishing selected preliminary environmental and nutritional requirements for penaeid shrimp resulted in the successful and reproducible production of major biomass increases with relatively high survival rates and low food conversion ratios.

The harvest of commercial shrimp suffers great seasonal variability and has failed to keep pace with ever-increasing domestic and export demands (Surdi and Whitaker, 1971). In order to supplement the natural harvest and provide a year-round supply of shrimp, several attempts have been made to culture shrimp in natural ponds, restricted portions of bays and estuaries, and laboratory tanks. In general, these efforts have had limited success and have explicitly illustrated the need for more accurately defining the nutritional and environmental requirements

necessary for culturing these species. Although pond culture has produced annual crops of shrimps (Villadolid and Villaluz, 1951; Lunz, 1967; Wheeler, 1967; Broom, 1969; Moore and Elan, 1970³), production has been minimal and highly variable. Attempts to obtain commercial quantities of shrimp by stocking enclosed portions of estuaries have to date not yielded production results (American Fish Farmer & World Aquaculture News, 1970). During recent laboratory studies, Subrahmanyam and Oppenheimer (1969) were able to maintain shrimp in laboratory tanks using a pelleted diet consisting of fish meal, stickwater, and vitamins. However, the

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³ Moore, W. R., and L. L. Elan. 1970. Salt water pond research. Tex. Parks Wildl. Dep., Austin. (Processed.)

total biomass increase of shrimp fed their best diet for 6 weeks was only approximately 50% higher than initial biomass. Better results (on an individual weight basis) using *Penaeus duorarum* were obtained from animals grown on a sand substrate than those grown in bare tanks.

The greatest promise for economical shrimp production lies in determining the exact nutritional requirements and developing an inexpensive artificial diet from feedstuffs for these species. Current commercial practices in Japan employ chopped clam (predominantly *Tapes semidecussata*, Reeve) as a diet for rearing shrimp. Despite the high market price for cultured shrimp in Japan (the retail price of cultured shrimp ranged from \$4 to \$10 per pound in 1970), shrimp farming there tends to be a marginal enterprise because of the high cost of a clam diet. However, in other parts of the world where shrimp does not command such a luxury price, the use of a high-value product such as clam for shrimp feed is prohibited.

Pelleted diets (i.e., pellets containing all the chemical ingredients thought to be important to animal growth) have been designed consisting of purified soybean meal, glucose, sucrose, starch, glucosamine, chitin, cellulose, soybean oil, citric acid, succinic acid, amino acid, minerals, vitamins, and cholesterol (Kanazawa et al., 1970). After growing penaeids on such diets, the animals were in excellent physiological condition, but in the best group, total biomass increase was only 72% of the control group fed chopped clam. Thus, little progress has been made toward establishing nutritional and environmental requirements that will yield optimum growth (total biomass increase) and survival of penaeid shrimp.

In the present study, an attempt was made to develop a suitable experimental culture system which could serve as a model for future nutritional and environmental studies. Several environmental factors were examined, and, as a result, environmental conditions were created which would allow acceptable growth and survival. Having first established suitable culture conditions, several diets were evaluated in preliminary studies of the nutritional requirements of shrimp.

MATERIALS AND METHODS

Both environmental and nutritional studies were conducted in round fiber glass culture tanks measuring approximately 1 m deep by 1 m in diameter and equipped with a venturi type center drain which maintained a water depth of 0.75 m. Three replicates were maintained for all treatments.

Water (ranging in salinity from 26.8 to 29.3‰) from the Skidaway River was filtered through an oyster shell and sand filter to remove major food particles. Filtered water was heated to 30° C in a stainless steel heat exchanger and jetted into each tank at a rate of 1.9 liters/min through flow-control nozzles which were aimed so that the agitation of the water column in each tank was minimal. Temperature ranged from 25° to 28° C in each tank throughout the experimental period.

White shrimp (*P. setiferus*) obtained from the Savannah, Ga., river and tributary systems were used in all environmental experiments, and brown shrimp (*P. aztecus*) obtained from the Tampa Bay, Fla., area were used in the nutritional studies. Shrimp weighing 4 ± 0.8 g (mean and standard deviation based on 480 weighed shrimp) were selected from the above stock and used in all environmental studies (10 animals per tank) and fed pelleted diets (Table 1, Diet 1) at a rate of 5% of their biomass daily, on a dry weight basis. Shrimp were weighed each week and the percent increase or loss recorded on a wet weight basis.

SUBSTRATE STUDY

Sand-shell substrates suitable for burrowing, subdivisions of tank bottoms, and bare fiber glass tank bottoms were provided for replicate groups of shrimp, and relative survival rates among the treatment groups were compared over a 5-week period. Sand-shell substrates were placed directly onto the tank bottom in one group, and in another group, the same substrate was placed on a perforated platform 10 cm above the tank bottom, allowing a flow of water through the drain below the sand surface. Such an arrangement was designed to test the effect of decreasing the

TABLE 1.—Experimental variables and results from four nutritional studies with penaeid shrimp.

	Group I, Diets				Group II, Diets					Group III, Diets				Group IV, Diets				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Casein (%)	50.2	50.2	50.2	--	28.5	8.0	28.5	23.5	--	--	--	--	--	--	8.0	8.0	8.0	--
Methionine (%)	1.0	1.0	1.0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Glycine (%)	0.1	0.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Sodium glutamate (%)	0.2	0.2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Citric acid (%)	0.3	0.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Succinic acid (%)	0.3	0.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Mineral mix ¹ (%)	5.0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fat ² (%)	8.0	8.0	8.0	--	8.0	8.0	--	8.0	--	8.0	8.0	8.0	8.0	--	8.0	8.0	8.0	--
Carbohydrate premix ³ (%)	20.5	20.5	20.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Shrimp meal (%)	--	--	--	--	28.5	69.5	36.5	23.5	--	28.5	28.5	28.5	28.5	--	69.5	69.5	69.5	--
Anchovy meal (%)	--	--	--	--	28.5	8.0	28.5	23.5	--	28.5	28.5	28.5	28.5	--	8.0	8.0	8.0	--
Shrimp muscle (%)	--	--	--	--	--	--	--	15.0	--	--	--	--	--	--	--	--	--	--
Yeast protein hydrolysate ⁴ (%)	--	--	--	--	--	--	--	--	--	24.5	--	--	8.1	--	--	--	--	--
Casein protein hydrolysate ⁴ (%)	--	--	--	--	--	--	--	--	--	--	24.5	--	8.1	--	--	--	--	--
Soy protein hydrolysate ⁴ (%)	--	--	--	--	--	--	--	--	--	--	--	24.5	8.1	--	--	--	--	--
Collagen (%)	4.0	4.0	4.0	--	4.0	4.0	4.0	10.0	--	8.0	8.0	2.0	8.0	--	4.0	4.0	4.0	--
Vitamin mix ⁵ (%)	2.5	2.5	2.5	--	2.5	2.5	2.5	2.5	--	2.5	2.5	2.5	2.5	--	2.5	2.5	2.5	--
Cellulose (%)	7.9	12.9	13.8	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Daily feeding rate (% of biomass)	5	5	5	0	5	5	5	5	0	5	5	5	5	0	5	10	15	0
Duration (weeks)	5	11	11	11	11	11	11	11	11	6	6	6	6	6	6	6	6	6
Results:																		
% increase in biomass	18	0	0	0	44	63	50	37	--	19	18	18	39	--	58	109	164	--
Survival rate (%)	80	80	80	0	90	100	90	80	0	90	80	80	90	27	80	90	90	26

¹ Contains 30.0% K_2HPO_4 , 9.4% KCl, 14.8% MgSO_4 , 27.4% $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 1.4% FeCl_3 , 0.2% $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, and 16.8% CaCO_3 .

² Contains 33 $\frac{1}{3}$ % corn oil, 33 $\frac{1}{3}$ % hydrolyzed vegetable and animal fat, and 33 $\frac{1}{3}$ % menhaden oil.

³ Contains 24.3% corn starch, 24.3% sucrose, 24.3% glucose, 19.5% chitin, and 7.6% glucosamine.

⁴ Nutritional Biochemical Co. enzymatic hydrolysates.

⁵ Nutritional Biochemical Co., Cleveland, Ohio, standard vitamin diet fortification mixture in dextrose.

buildup of anaerobic conditions. Division of tank bottoms into sundry tunnels and levels was created by specific placement of bricks and clay drain tiles.

AERATION STUDY

Aeration provided by jetting streams of filtered seawater into respective tanks was compared to aeration supplied by bubbling air through airstones into tanks in which water was continuously added with no agitation of the water column for an 8-week period. Two airstones were placed in each tank and valve-regulated air lines controlled the pressure at approximately 4 psi. Oxygen levels were monitored periodically and used along with survival rates as a basis for evaluation of replicate groups aerated by each method.

STOCKING DENSITY STUDY

Survival data were compared among triplicate tanks stocked at 10, 20, and 40 shrimp per m² for an 8-week period. These densities of approximately 40, 80, and 160 g/m² were chosen on the basis of data provided in pond and laboratory culture of penaeid shrimp (Broom, 1969; Subrahmanyam and Oppenheimer, 1969).

PRELIMINARY NUTRITIONAL STUDY

Triplicate groups of ten 4 g brown shrimp (*P. aztecus*) were fed a series of pelleted diets. Growth data (biomass increase) was used as a means of evaluation. Diets examined consisted of those patterned after Japanese purified diets (i.e., diets containing only chemical ingredients) (Table 1, Group I) (Diet 1 was conducted for 5 weeks and Diets 2, 3, and 4 for 11 weeks each); a second group of semipurified diets (i.e., containing defined chemical ingredients but containing one or more natural products) providing four combinations of levels of protein, fat, shrimp, and fish meal (Group II) (conducted for 11 weeks); and a third group designed to compare the nutritional value of casein, yeast, and soy hydrolysates (Group III) (conducted for 6 weeks). All of these groups were fed at 5%

TABLE 2.—Percent of pellet dissolved over time and at three concentrations of binder. (Values are means and standard deviation on two replicates with Diet 1.)

Percent binder (collagen) added	Hours		
	6	12	24
1			
3	13 ± 1.2	14 ± 0.9	18 ± 1.7
5	11 ± 0.8	10 ± 0.6	10 ± 0.6
10	10 ± 0.6	10 ± 1.1	10 ± 1.0

of their respective biomass daily. In addition, Diet 6 was fed at 5, 10, and 15% of biomass (Group IV) (conducted for 6 weeks).

Combined environmental factors which produced best survival in each of the environmental experiments (i.e., culture conditions consisting of bare fiber glass tank bottoms, supplied aeration, and a stocking density of approximately 40 g/m²) were used in all nutritional studies. This combination offered a maximum potential for an increase in biomass and therefore allowed accurate evaluation of differences among diets tested. Although survival in bare fiber glass tanks was not significantly different from sand substrates, the fact that bare tanks were simpler to maintain dictated that they be used for the nutritional studies.

Prior to starting nutritional studies, the physical properties of pelleted diets were evaluated for acceptability as shrimp food. Pellet consistency was determined according to its ability to resist dissolution over a given period of time, and texture and size were chosen according to animal performance when presented several choices. Collagen⁴ proved to be a suitable binding agent. Using an experimental design with time and collagen levels as variables, a pellet with 5% collagen added as a binder was found to offer optimum consistency over a 24-hr immersion in salt water (Table 2). Percent dissolution was measured by taking dry weights after 6, 12, and 24 hr of immersion (no shattering of pellets was observed, and all loss of weight was therefore assumed to be from dissolution). Animals were observed to feed most readily on

⁴ Supplied on an experimental basis by the Hides and Leather Division of the U.S. Department of Agriculture Eastern Utilization Laboratory in Philadelphia, Pa.

pellets 0.3 cm in diameter by approximately 1.5 cm in length and which sink in water, and hence, pellets having these characteristics were used in both environmental and nutritional experiments.

RESULTS AND DISCUSSION

SUBSTRATE STUDY

A survival rate of 80% was obtained after 5 weeks in tanks without substrate, 80 to 90% survival was maintained over much of the duration of the experiment among both treatments having sand-shell substrates, and less than 60% survival occurred among tanks having brick subdivisions (Figure 1). Although *P. setiferus* is reported to burrow less than either *P. duorarum* or *P. aztecus* (Anderson, 1966; Pérez Farfante, 1969), it apparently was able to avoid predation, especially during the highly vulnerable moulting period, quite successfully with or without a sand substrate, since 5-week survival data among the two sand-shell treatments and the bare tank bottom treatment were not significantly different ($P < 0.05$) (Duncan, 1955). If the type of shelter is a factor in increased survival for penaeids maintained under culture conditions, the brick subdivisions should have enhanced survival. However, the markedly high mortality

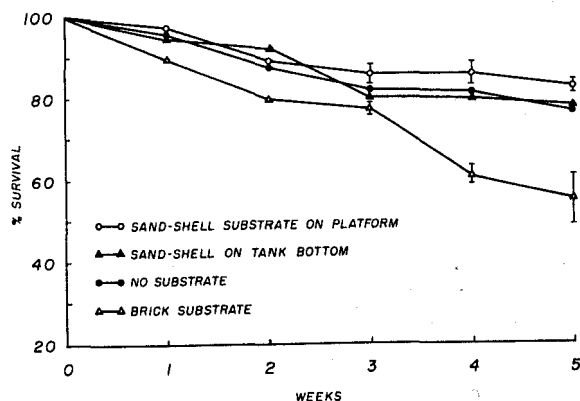


FIGURE 1.—Mean and standard error for percentage of animals surviving after 5 weeks of growth on four different substrates.

rate among this group, significantly different from the other three treatments ($P < 0.05$), may have resulted from either failure of the shrimp to behaviorally segregate and thus fully utilize this protection or from physical abrasion against the sharp and coarse brick surface. Although there may have been toxic substances in the brick materials, the bricks were carefully washed and assumed to be otherwise inert in any chemical effect they may have had on the animals. Although differences in volume of water caused by placing various substrates in their respective treatments was not controlled for, it was felt that these differences in a running water system were not critical to the survival of shrimp. Differences in bottom area among the treatments caused by placement of different types of substrate were neither controlled for nor measured but were also thought to be negligible compared to differences found among treatment groups. The high degree of cannibalism noted by Subrahmanyam and Oppenheimer (1969) in tanks without substrate was not observed in any groups.

AERATION STUDY

The group having oxygen supplied by injecting air through airstones had significantly higher survival rates ($P < 0.05$) when compared with a treatment aerated by agitation of the water column (Figure 2). Although the average oxygen levels were similar between the two treatments (3.4-6.8 ppm), such levels in tanks aerated by high-pressure nozzles often dropped for short intervals due to clogging of the nozzles with silt and biological debris. Electrical power failures which affected water flow but not the compressed air supply (equipped with stand-by DC power) also caused intermittent drops in oxygen levels. Such short-term irregularities may have been more critical to shrimp tolerances than is indicated from reference to average oxygen level values, per se. Also, at the relatively high temperatures maintained throughout the study, short drops in oxygen levels could have been very critical. Decreased survival in tanks with agitation of the 0.75-m water column may also have resulted from physical agitation of the animals.

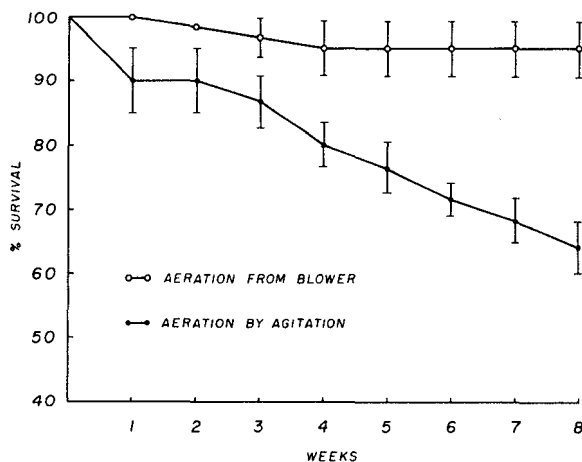


FIGURE 2.—Mean and standard error for percentage of animals surviving after 8 weeks of growth with two types of aeration.

STOCKING DENSITY STUDY

Stocking densities higher than 40 g/m² produced proportionally higher mortalities indicating an approximate carrying capacity for this particular culture system (Figure 3). If shrimp were stocked at 40 g/m², a population of 32 g/m² remained after 8 weeks. Similarly, when shrimp

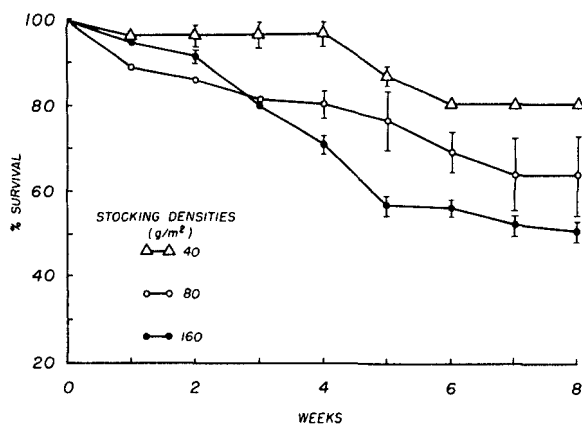


FIGURE 3.—Mean and standard error for percentage of animals surviving after 8 weeks of growth at three stocking densities.

were stocked at 80 g/m², a relatively stable population of approximately 52 g/m² existed during the final 2 weeks of study. Similarly, mortality during the first 8 weeks among a population originally stocked at 160 g/m² created a population of approximately 80 g/m², but in this case the survival rate was still declining after 8 weeks of growth. Therefore, a carrying capacity (maximum biomass obtainable) for this culture system may have been somewhere between 32 and 80 g/m².

Although such a carrying capacity would depend on the particular culture system, applicable calculations, utilizing data from a laboratory culture study (Subrahmanyam and Oppenheimer, 1969) and a pond culture experiment (Broom, 1969), indicate a similar carrying capacity for populations of other systems (ponds, embayments, and laboratory tanks). In the case of the laboratory study, best survival was obtained when shrimp were stocked initially at 34 g/m², yielding a biomass of 27 g/m² at the termination of the experiment. Likewise, best survival and an increase in biomass occurred in the pond culture study when initial stocking densities were below 20 to 30 g/m². Recent data from a commercial operator in Central America indicates that, regardless of stocked biomass, the carrying capacity ranged from 5.5 to 7.3 g/m² (Smitherman and Moss, 1970). Such evidence suggested that final production expectations should be considered in choosing initial stocking densities.

PRELIMINARY NUTRITIONAL EXPERIMENTS

A comparison of several groups of diets (Table 1) revealed that semipurified diets with casein as the major source of protein (Group I), only produced an average of 18% increase in biomass above stocked biomass levels. Group II, having fish and shrimp meal as additional sources of protein, produced approximately 63% growth on the best diet. Group III diets comparing hydrolyzed proteins yielded only 39% growth on the best diet, and animals fed at a rate of 15% of their total biomass (Group IV) increased their initial biomass 164%. In addition to increased

growth above that obtained in the environmental studies, survival was increased from 80 to 90% to 90 to 100% through the information acquired from the above nutritional comparisons.

Shrimp obtained little if any sustenance from organic or settled detritus since the continuous flow of filtered seawater through the tanks kept the system relatively free of siltation and extraneous growth. Furthermore, starved animals were not able to sustain their initial biomass level beyond 2 weeks, and the populations in all three replicate tanks had died after 7 weeks. Cannibalism appeared to be prevalent among starved organisms, and the decline in weight was undoubtedly moderated due to growth of animals preying upon dead shrimp.

Diets in Group I with casein as the major protein source produced little growth above sustenance. Diet 1 with an added mineral mix yielded a significantly higher biomass increase above initial weights at the 5% feeding level than either Diets 2 or 3 which lacked the mix. In addition to the mineral mix, Diet 3 lacked sodium glutamate, glycine, citric acid, and succinic acid and correspondingly caused a loss in biomass over the 3-month growth period. Although the above results showed Diet 1 to be significantly different ($P < 0.05$) from the other diets after the first month of study, results are somewhat confounded with initial differences in stocked biomasses and poor response in general.

These sustenance biomass levels represented far less growth increase than the 72% of control obtained by Kanazawa et al. (1970) and may be due to a lack of cholesterol in our study. Since many crustaceans are not able to synthesize cholesterol (Van Den Oord, 1964; Dall, 1965; Zandee, 1967), including recent evidence for shrimp (Kanazawa et al., 1971), the lack of this entity undoubtedly was attributed to the poor performance of these diets.

Shrimp fed on Group II diets averaged a 37 to 63% increase in growth. Although results from Diets 5, 6, and 7 were not significantly different ($P < 0.05$), Diet 6, which consisted of a high ratio of shrimp to fish meal and a low level of casein, yielded greatest biomass increases. Total biomass decreased in diets having a decrease in percentage of shrimp meal. Growth

from Diet 8, which contained blended shrimp muscle and lower levels of shrimp and fish meal, was statistically less ($P < 0.05$) than the other three diets. Again, the control group of starved shrimp was not able to sustain its initial weight and declined in biomass after the first 2 weeks.

Group III, consisting of yeast, casein, and soy protein hydrolysate diets, produced an average biomass increase of 18 to 39% (Table 1). The combination of diets containing casein, soy, and yeast hydrolysates produced significantly better ($P < 0.05$) growth than individual hydrolysates. Since results from this group were not better than results after 6 weeks from Diet 5 which was similar except it contained only intact protein, these data indicate that hydrolyzed proteins are not utilized more efficiently than intact proteins.

Comparing food supplied at 0, 5, 10, and 15% of total biomass using Diet 6 illustrated that growth was directly proportional to an increase in feeding rate (Group IV), and may reflect the natural feeding habit of the species. While the population of starved animals disappeared after 8 weeks, the treatments fed at 5% of their biomass increased 58% over their initial weight; those fed at 10% of their biomass increased 109%; and those fed at 15% biomass gained 164%. The above results indicate that penaeids are capable of consuming large amounts of food. This may be a reflection of their natural tendency to continuously graze upon large quantities of benthic material rather than feed periodically as would a strict carnivore. Although pellets used in all experiments were textured to maintain consistency in solution for 24 hr, some shattering may occur as shrimp gnaw at them and thus some food may be lost through flushing, thus decreasing the efficiency of ingestion as feed levels are increased.

Although growth was directly proportional to an increase in feeding rate, feeding at low levels was still justified in attempting to determine nutritional requirements of shrimp. The benthic material normally grazed upon is low in energy content and is often of relatively poor nutritional content. Feeding at lower fed levels but with food of proper nutritional value could conceivably produce growth comparable to higher fed

levels of natural or formula diets presently known.

Food conversion ratios (FCR) (weight of food fed for 6 weeks/weight increase) were calculated from results in Group IV (calculated on a dry weight basis). Feeding at 10% biomass yielded an FCR of 6.7 and growth increase of 109%. On the other hand, feeding at the 15% level produced a 164% growth increase and an FCR of 5.5. Such FCR, although not comparable to those obtained for vertebrates such as the 1.6 or less for catfish (Andrews, in press), nonetheless represent a significant decrease over the FCR of 10 or greater reported for shrimp fed on natural foods (Fujinaga, 1963). Further refinement of the FCR for penaeids can undoubtedly be obtained through procurement of a more suitable pellet, better understanding of exact nutritional requirements of specific nutrients, and more information on ingestion and assimilation phenomena.

SUMMARY

1. Environmental conditions yielding 80 to 90% survival in the intensive tank culture of penaeid shrimp encompassed a combination of either no substrate or sand substrate on elevated platforms, air supplied externally by an aeration system, and population density of 40 g/m².

2. Diets having balanced complements of proteins, lipids, carbohydrates, amino acids, fatty acids, minerals, and vitamins produced only sustained biomass levels.

3. Diets having 69.5% of the total diet as shrimp meal produced growth increases of 63%.

4. Examination of soy, casein, and yeast hydrolysates revealed that a combination of each produced 39% growth increase while an average of 18% resulted from feeding each hydrolysate separately. Hydrolyzed proteins did not yield better growth than intact proteins.

5. Feeding at 5, 10, and 15% of the animals' biomass daily yielded directly proportional growth. A growth increase of 164% was achieved with a fish meal and shrimp meal diet fed at 15% of biomass daily.

6. Using semipurified pelleted diets, food conversion ratios were reduced by nearly half of that reported for penaeids feeding on clam and other natural foods.

7. Establishing selected preliminary environmental and nutritional requirements for penaeid shrimp resulted in reproducible production of major biomass increase with relatively high survival and low food conversion ratios.

8. Results from these studies have allowed us to design facilities and experiments for future work with environmental and nutritional factors. Development of an inexpensive diet which will yield rapid and maximum growth will be an essential requirement for economical production of penaeid shrimp.

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