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A growth experiment with Penaeus monodon Fab. in a closed system

by

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INTRODUCTION

During the last two years we have been conducting experiments to assess the suitability of a number of prawn species for intensive culture in closed recirculation systems (Forster and Beard, in preparation). With all the species used, growth and survival were adversely affected by a high stocking density (166 animals/m^2) compared with a low density (25/m^2). It was thought unlikely that the adverse effects were due to limitation of food or water quality but rather to a behavioural response, possibly accentuated by the small size of tanks used (0.83 x 0.69 x 0.2 m deep). Accordingly an experiment was made to assess the effect of tank size on growth and survival of the most promising of the species tested, Penaeus monodon.

METHODS

The experiment was made in a laboratory recirculation system consisting of three experimental tanks, 0.84 m^2 (0.915 x 0.915 m), 1.68 m^2 (0.915 x 1.83 m) and 3.36 m^2 (0.915 x 3.66 m), a reservoir (capacity 3200 litres) and a biological filter (capacity 1 m^3). The filter consisted of three (1.22 x 0.61 x 0.61 m deep) mesh-bottomed boxes containing 0.45 m of 10-30 mm gravel topped by a small amount of crushed oyster shell. The experimental tanks were constructed of GRP U-shaped sections, 0.915 x 0.915 x 0.305 m deep, bolted together to produce the required length. Each section had its own inflow of water at 3 litres/min and its own drain. The water depth was approximately 15 cm. Water was circulated from the reservoir to a small header tank and then gravity-fed through the experimental tanks. The outflow discharged on to the surface of the biological filters via a perforated pipe, percolated through the filter and returned to the reservoir. The total water volume was approximately 4000 litres and 25 per cent was changed each week. On weeks 10 and 15, 75 per cent was changed to try and reduce mortalities which were becoming increasingly severe. Aeration was provided at four points in each section at 2 litres/min and in the reservoir through eight points at 3 litres/min. Water temperature was maintained at $28^{\circ} \pm 2^{\circ}C$ and salinity at $28 \pm 3^{\circ}/oo$.

Other aspects of water quality were monitored throughout the experiment at weekly intervals. These included pH determined with a Beckman Zeromatic pH meter, dissolved oxygen determined with a YSI Model 54 oxygen meter, total ammonia estimated by the method of Solórzano (1969), nitrite by the method of Bendschneider and Robinson (1952) and nitrate according to the method of Wood et al. (1967). Total suspended solids were determined by filtering a known volume of water through tared GFC filter papers which were then dried at 100°C for 24 hours and reweighed. In addition, estimates of the total numbers of bacteria were made by counting colonies grown on ZoBell agar plates incubated at 28°C for 48 hours. Total ammonia, nitrite and nitrate were estimated from deep-frozen samples (-20°C) which had been collected during the experiment and filtered through GFC filter papers prior to storage.

P. monodon juveniles were obtained as wild-caught stock from the Philippines (see acknowledgements) in two batches approximately one month apart. Acquisition of the animals in two batches was not desirable but was unavoidable due to shortage of supply. The first batch obtained were weaned on to our standard diet of pieces of shrimp and mussel flesh and then kept cool (approximately 20°C) to slow down their growth. When the second batch arrived they were also weaned on to this diet and held at 28°C until both groups were approximately the same size (290 mg live weight). The first batch were then reacclimated to 28°C and the two groups mixed together and redistributed amongst the experimental tanks at a density of 146 prawns/m^2. This corresponded to 122, 244 and 488 prawns in the 0.84 m^2, 1.68 m^2 and the 3.36 m^2 tanks respectively.

The diet for this experiment consisted of a mixture of mussel flesh (*Mytilus edulis*) and frozen shrimp (*Crangon crangon*) which was provided, in excess, each day. Before feeding, the ingredients were well washed in fresh water, blotted with absorbent tissue and weighed. Any food remaining after 24 hours was recovered from the tanks and similarly treated. An estimate of gross food conversion was therefore obtained.

RESULTS

Growth and survival

The mean live weight and survival for the three tanks during 16 weeks are tabulated in Table 1. Throughout the experiment survival was poorer than expected with this species, and during the last two weeks there were heavy mortalities. These mortalities, the possible causes for which are discussed below, meant that the original objectives of the experiment were not fully achieved, since the stocking density in all tanks was so greatly reduced. However, within this limitation the results suggest that there was little effect of tank size on growth or survival.

Water quality

The changes which were recorded each week in water quality are shown in Table 2. Nitrate accumulated during the experiment and the sudden drops in concentration on weeks 10 and 15 occurred when approximately 75 per cent of the water was changed. Ammonia levels did not exceed 0.61 ppm NH_4-N, and the highest nitrite level recorded was 0.60 ppm NO_2-N. The pH fell rapidly during the first two weeks and then fluctuated between 7.0 and 6.6. These low pH values were due partly to an accumulation of carbon dioxide and partly to acid-producing (oxidative) processes which occur during biological filtration. The precise effect of such low pH values on the prawns is not known, although similar pH values had occurred in previous successful trials with P. monodon (Forster and Beard, in prep.).

Following the high mortalities which occurred, samples of water which had been deep-frozen during the experiment were also analysed for certain metals (see Table 3). The water samples were not prepared or stored specifically for these determinations and the authors are advised that the resulting analytical values are only likely to be accurate to within 20 per cent. Despite this, however, there is no indication of serious accumulation or depletion of any of the metals determined. Compared with values given by Elderfield (1971) for Conwy 'header' tank water, Zn, Cd and Ni levels were in the same range, whilst Ca was nearly twice as high. Cu was 3-4 times higher, which was possibly due to the use of tap water for salinity adjustment. However, such levels are about 10 times lower than that found to be toxic for the prawn Pandalus borealis in 48 hours' acute toxicity tests (Portmann and Wilson 1971).

Food conversion

Attempts to estimate food conversion efficiency (wet weight of food eaten ÷ increase in biomass) were impeded by the high mortalities, but up to week 8 survival was sufficiently good to allow the following estimates to be made:

Experimental period	Tank area (m^2)	Total weight of food eaten (kg)	Biomass increase (kg)	Food conversion ratio
Weeks 2-4	0.84	1.13	0.082	13.7
	1.68	2.19	0.155	14.2
	3.36	4.33	0.326	13.3
Weeks 4-8	0.84	4.38	0.293	14.9
	1.68	8.73	0.508	17.2
	3.36	16.77	1.045	16.1

Conversion ratios in this range are less than hoped for but they do not take into account mortalities or losses of food due to leaching and bacterial action during the 24 hours the food was left in the tanks.

Possible causes of mortality

Results given in Table 4 show that in many cases corpses were not recovered from the tanks and had therefore been victims of cannibalism. In cases where corpses were recovered these were often partly eaten and death could therefore have resulted from cannibalistic attacks. However, a previous experiment with P. monodon (Forster and Beard, in prep.), in which 91 per cent survival during 16 weeks was obtained at a stocking density of 166 prawns per square metre, suggested that this species is not naturally cannibalistic. It is therefore considered that there were other underlying reasons for the mortalities in the present study and that only already weakened or dying prawns were the victims of cannibalism. This suggestion is supported further, since within one week of the end of the experiment the remaining prawns had died and many of the corpses showed no sign of mutilation.

The two most likely causes of mortality were poor water quality or disease. Values recorded for various aspects of water quality (Table 2) show that concentrations of ammonia, nitrite and nitrate were not particularly high and were well below levels likely to be toxic (Wickins 1973). Similarly there was no indication from metal determinations (Table 3) of potentially harmful accumulations or depletions. There is therefore no evidence of inadequate water quality in this experiment, although it is always possible that other factors which were not monitored could have caused trouble. With regard to disease the prawns showed no obvious symptoms of sickness, although some did show a distinct reddening of the gills or erosion of the shell, especially around the pleopods. Microscopic examination of corpses and live specimens revealed no evidence of fungal or protozoan infection, but a Gram Twort stain of the gills showed the presence of high numbers of Gram negative bacteria associated with the periphery of the gill lamellae. Three Vibrios were also isolated, two from the gills and one from a homogenized sample, by plating on agar (Cholera medium).

Attempts to reinfect healthy prawns (*Penaeus setiferus*) were inconclusive. When juvenile *P. setiferus* were placed in one of the tanks in the culture system where they were exposed to the contaminated water but not to the contaminated prawns they all died within 10 days. A control population remained healthy. However, when juvenile *P. setiferus* were exposed to suspensions in clean sea water of bacteria (Vibrios) isolated from the contaminated stock no mortality was observed. Neither did an homogenate of the contaminated animals have any effect in a similar test.

DISCUSSION

The main achievement of the objective of this study was prevented by the heavy mortalities which occurred from the eighth week onwards. Prior to week 8, however, when survival was quite good, there was no effect of tank size on growth or survival. Between weeks 8 and 16 the high mortalities made the results of dubious value. Forster and Beard (in prep.) found that the influence of stocking density on the growth of *P. monodon* in another experiment was apparent after only two weeks and was very marked after four weeks; their results were:

Week	Low density, 25/m^2		High density, 166/m^2	
	Live weight (g)	Survival (%)	Live weight (g)	Survival (%)
0	0.146	100	0.155	100
2	1.27	100	0.86	100
4	3.43	100	1.87	100
8	10.00	100	4.73	94
12	18.45	100	9.00	92
16	25.40	100	13.00	91

It therefore seems reasonable to infer that, under the conditions of the present study, tank size did not affect growth.

For commercial culture the growth of *P. monodon* at the higher stocking density, i.e. from about 0.2 to 13 g live weight in 16 weeks, is quite acceptable, but the species has the potential to grow twice as fast if the effects of stocking density can be understood and circumvented.

One other aspect that this study showed was the severity and rapidity with which mortalities can occur in highly intensive culture systems, and it highlights the lack of knowledge about crustacean diseases and their environmental tolerances.

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Table 1 Mean live-weight (g) and survival (%) of three populations of *P. monodon* grown for 16 weeks in different sized tanks

Time in weeks after the start of the experiment	Tank size		
	91.5 x 91.5 cm 0.84 m^2	91.5 x 183 cm 1.68 m^2	91.5 x 366 cm 3.36 m^2
	(a) Mean live-weight (g)		
0	0.29	0.29	0.29
2	0.95	0.85	0.87
4	1.73	1.61	1.57
8	4.57	4.41	4.36
12	6.84	6.89	7.06
16	10.66	11.46	11.99
	(b) Survival (%)		
0	10.0	10.0	10.0
2	97.5	95.1	96.9
4	92.6	89.8	96.3
8	87.5	79.9	83.8
12	75.4	61.9	59.6
16	33.6	23.4	23.0

Table 2 Weekly determinations of water quality components in a closed seawater system stocked with *P. monodon*

Component determined	Week 1	2	3	4	5	6	7	8
Salinity (°/oo)	29.0	28.4	29.0	25.0	26.0	27.5	28.0	28.0
pH	7.4	7.0	7.1	7.0	7.2	6.9	6.7	6.6
Dissolved oxygen (% saturation)	92.9	89.9	91.4	84.5	88.6	88.4	73.5	80.9
Ammonia ppm (NH_4-N)	0.15	0.28	0.43	0.58	0.36	0.31	0.50	0.61
Nitrite ppm (NH_2-N)	0.20	0.22	0.54	0.60	0.45	0.40	0.45	0.52
Nitrate ppm (NO_3-N)	10.9	16.3	20.4	28.1	40.6	51.6	61.1	67.7
Total suspended solids (mg/l)	5.3	11.1	5.4	7.5	9.7	7.1	6.7	8.8
Bacteria (millions/ml)	0.2	2.2	0.4	1.4	1.9	4.6	18.0	5.3

Component determined	Week 9	10	11	12	13	14	15	16
Salinity (°/oo)	28.5	28.5	28.5	27.0	30.0	31.0	27.5	27.0
pH	6.6	6.6	6.6	6.8	6.9	6.5	7.0	7.0
Dissolved oxygen (% saturation)	90.5	90.0	88.9	80.3	87.1	89.2	85.3	86.1
Ammonia ppm (NH_4-N)	0.55	0.24	0.24	0.47	0.26	0.38	0.37	0.51
Nitrite ppm (NH_2-N)	0.39	0.08	0.10	0.29	0.21	0.20	0.16	0.33
Nitrate ppm (NO_3-N)	88.9	36.4	41.5	76.9	78.7	94.6	79.0	83.4
Total suspended solids (mg/l)	9.1	12.0	7.6	7.1	11.4	12.6	7.2	8.8
Bacteria (millions/ml)	3.0	15.0	22.7	25.0	-	11.8	0.4	1.0

Table 3 Concentration of certain metals in water samples from a closed seawater system stocked with *P. monodon*

Week on which sample taken	Concentration (μg/l)						Conc. (mg/l)
	Zn	Cu	Fe	Mn	Cd	Ni	Ca
2	43	15	2.2	13	2.9	1.6	720
10	51	9.4	1.6	8.5	2.9	1.1	640
15	56	17	2.3	7.3	2.0	2.4	790

Table 4 Mortalities in three populations of *P. monodon* grown for 16 weeks in different sized tanks. A distinction is made between mortalities where corpses were or were not recovered

Period in weeks	Tank size					
	0.84 m^2		1.68 m^2		3.36 m^2	
	Corpse recovered	Corpse not recovered	Corpse recovered	Corpse not recovered	Corpse recovered	Corpse not recovered
0-4th	1	8	0	25	2	16
4-8th	2	4	4	20	20	41
8-12th	5	10	16	28	30	88
12-16th	23	28	60	34	94	85
Totals	31	50	80	107	146	230