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Effect of stocking density, water exchange rate and tank substrate on growth and survival of post-larvae of *Penaeus monodon* (Fabricius, 1798)

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ABSTRACT

An experiment was conducted with *Penaeus monodon* post-larvae to evaluate the effect of stocking density, water exchange rate and provision of tank substrate on the wet weight of shrimp. Three stocking densities *viz*., 200, 400 and 600 of post-larvae per 500 l tank (corresponding to 0.4, 0.8 and 1.3 animals per litre respectively) under two water exchange systems (10% or 100% seawater exchanged daily) were considered, along with the provision or absence of a substrate in the form of a plastic fiber mat. The wet weight of shrimp was analyzed using a nested generalized linear model, with water exchange and mat provision and their interactions treated as fixed effects, and the effect of tank nested within the combination of the three main effects treated as random. The results demonstrated significant effect of tank substrate, stocking density and rearing tank, while the water exchange rate did not influence wet weight. As expected, the growth was highest with the lowest stocking density and lowest with the highest stocking density. When a tank substrate was provided, survival decreased with an increase in the individual weight of shrimp.

Keywords: Growth, *Penaeus monodon*, Stocking density, Tank substrate, Water exchange, Wet weight

Introduction

The demand for shrimp is increasing rapidly across the countries and shrimp culture is expanding at a great pace to meet this demand. Optimising the stocking density and water exchange rate for shrimp culture will be an important contribution towards meeting this demand. A number of reports in *Penaeus monodon* (Abdussamad and Thampy, 1994), *Penaeus esculentus* (Burford *et al.*, 2004; Arnold *et al.*, 2006a), *Litopenaeus vannamei* (Sandifer *et al.*, 1987; Peterson and Griffith, 1999; Bratvold and Browdy, 2001; Moss and Moss, 2004) *Penaeus setiferus* (Williams *et al.*, 1996); *Penaeus japonicus* (Lanari *et al.*, 1989; Coman *et al.*, 2004), deal with stocking density and artificial substrates on the growth and survival of post-larvae/juveniles. Generally it is observed that the transitional period from post-larvae to juveniles in *viz*., *L. vannamei,* is marked by low survival rates (Samocha *et al.,* 2002). When the post-larvae are grown in the hatchery till the juvenile stage and thereafter stocked in ponds, the survival rates could be expected to increase compared to a system where the post-larvae are directly stocked in ponds (AQUACOP, 1984).

With a view to optimizing the rearing system for *P. monodon*, we have investigated the effect of three stocking densities, two levels of water exchange rates and provision of a substrate in the tank, on thewet weight of post-larvae of *P. monodon*.

Materials and methods

Experimental design

Post-larvae (PL-15) of tiger shrimp from a single female were collected from M/S Best Hatchery, Marakkanam (situated approximately 100 km south of Chennai) and used for this experiment. The experimental design consisted of three stocking densities *viz*., 200, 400 and 600 post-larvae per 500 l fibre re-inforced plastic (FRP) circular tanks having 90 cm inner diameter, holding seawater at a depth of 80 cm (corresponding to 0.4, 0.8 and 1.3 animals per litre respectively); two levels of water exchange (10 or 100% of seawater exchanged daily) and presence or absence of a plastic fibre mat of size 2.5 square feet as tank substrate. Each mat measured 0.762 x 0.762 m and the four sides of the mat were held by a square PVC frame. The frame was anchored inside the FRP tank using two stones at the bottom which held the mat in place, and at the top, the PVC frame was extended to either sides to fit snugly into the tank. A total of 12 (3 x 2 x 2) treatment combinations were tested. Each treatment combination was replicated in three FRP tanks. In the tank with 10% water exchange, 10% of the water in each tank was replaced with fresh UV-treated seawater daily in the morning. In the 100% exchange group, complete water was exchanged and filled with fresh UV-treated seawater.

Management

The tanks were placed in the wet laboratory at the Muttukadu Experimental Station of Central Institute of Brackishwater Aquaculture, Chennai, India. Fresh UV-treated seawater was used in all the 36 FRP tanks and the water was continuously aerated using an air blower. The tanks were carefully cleaned daily in the early hours of the day using a hose and an appropriate net. Commercial post-larval feed (Higashimaru Feeds™) was provided daily @10% of the biomass. Feeding was carried out once in every four hours, *i.e.*, six times in a day.

Data recording

Salinity and pH were recorded daily. In order to ascertain whether the ammonia concentration was well below threshold levels, it was monitored on a weekly basis. On termination of the experiment, *i.e.*, on the 48th day, PL from each tank were collected and weighed individually. The weights were recorded using an electronic balance with an accuracy of 0.001 g. Before weighing, each post-larva was placed on a tissue paper for absorbing the extra moisture. A total of 9,154 PLs were weighed. The count of PL in each combination was used for calculating the survival values.

Statistical analyses

The wet weight was analysed using a nested generalised linear model. The statistical model fitted was as follows:

$$Y_{ijklm} = \mu + S_i + W_j + M_k + T_{l(kji)} + (SW)_{ij} + (SM)_{ik} + (WM)_{jk} + (SWM)_{ijk} + e_{ijklm},$$

where,

Y_{ijklm} is wet weight of the mth juvenile reared in the lth tank with kth substrate management under the jth water exchange and ith stocking density,

μ is the overall mean,

S_i is the fixed effect of the ith stocking density (I = 1 to 3),

W_j is the fixed effect of the jth water exchange (j = 1,2),

M_k is the fixed effect of the kth substrate management (k = 1,2),

$T_{l(kji)}$ is the random effect of the lth tank nested within the kth substrate management under the jth water exchange and ith stocking density (l = 1,2 and 3),

$(SW)_{ij}$ is the interaction effect between stocking density and water exchange,

$(SM)_{ik}$ is the interaction effect between stocking density and substrate management,

$(WM)_{jk}$ is the interaction effect between water exchange and substrate management,

$(SWM)_{ijk}$ is the three way interaction effect between stocking density, water exchange and substrate management, and

e_{ijklm} is the random error assumed to be normally and independently distributed with mean 0 and variance ó^2 e.

The data were analyzed using the SAS Version 9.0 software (SAS, 2002).

Table 1. ANOVA for 48 day wet weight (main effects tested against mean squares of tank effect instead of error mean squares)

Source	df	MSS[a]
Between water exchanges (W)	1	0.019059^{NS}
Between substrate managements (M)	1	0.092251*
Between stocking densities (D)	2	0.348586*
Tanks in treatment combination	24	0.005709*
Water exchange x substrate management (W x M)	1	0.011033*
Water exchange x stocking densities (W x D)	2	0.013189*
Substrate management x stocking densities (M x D)	2	0.010796*
W x M x D	2	0.011057*
Error	9142	0.000444

* p < 0.0001, a = Type III mean squares, NS= non-significant

Table 2. Least-squares means of wet weight (g) with standard errors. Means followed by different superscripts are statistically different (for main effects p<0.0001, for interactions p<0.05)

Effects	LS Mean ± SE (n)
Water exchange	
10	0.04517^{a} ± 0.00121 (4690)
100	0.04196^{a} ± 0.00127 (4464)
Substrate management	
With substrate	0.04709^{a} ± 0.00131 (4079)
Without substrate	0.04003^{b} ± 0.00116 (5075)
Stocking densities	
200	0.05417^{a} ± 0.00186 (1692)
400	0.04476^{b} ± 0.00148 (2809)
600	0.03176^{c} ± 0.00112 (4653)
Water exchange x substrate management	
10 x With substrate	0.04991^{a} ± 0.00180 (2097)
10 x Without substrate	0.04042^{b} ± 0.00162 (2593)
100 x With substrate	0.04427^{b} ± 0.00193 (1983)
100 x Without substrate	0.03965^{b} ± 0.00170 (2482)
Water exchange x stocking density	
10 x 200	0.05865^{a} ± 0.00259 (870)
10 x 400	0.04416^{b} ± 0.00199 (1517)
10 x 600	0.03268^{c} ± 0.00159 (2303)
100 x 200	0.04969^{b} ± 0.00268 (822)
100 x 400	0.04535^{b} ± 0.00220 (1292)
100 x 600	0.03083^{c} ± 0.00158 (2350)
Substrate management x stocking density	
With substrate x 200	0.05934^{a} ± 0.00275 (783)
With substrate x 400	0.04875^{b} ± 0.00234 (1081)
With substrate x 600	0.03317^{c} ± 0.00162 (2215)
Without substrate x 200	0.04900^{b} ± 0.00252 (909)
Without substrate x 400	0.04076^{d} ± 0.00183 (1728)
Without substrate x 600	0.03034^{c} ± 0.00155 (2438)
Water exchange x substrate management x stocking density	
10 x With substrate x 200	0.06418^{a} ± 0.00375 (419)
10 x With substrate x 400	0.05160^{b} ± 0.00308 (618)
10 x With substrate x 600	0.03396^{cd} ± 0.00234 (1060)
10 x Without substrate x 200	0.05313^{b} ± 0.00356 (451)
10 x Without substrate x 400	0.03672^{ce} ± 0.00253 (899)
10 x Without substrate x 600	0.03141^{cd} ± 0.00216 (1243)
100 x With substrate x 200	0.05451^{ab} ± 0.00401 (364)
100 x With substrate x 400	0.04590^{b} ± 0.00352 (463)
100 x With substrate x 600	0.03239^{cd} ± 0.00224 (1155)
100 x Without substrate x 200	0.04487^{be} ± 0.00356 (458)
100 x Without substrate x 400	0.04481^{b} ± 0.00265 (829)
100 x Without substrate x 600	0.02927^{d} ± 0.00222 (1195)

Results and discussion

During the course of the experiment, the average values (with standard deviations in parentheses) of pH, salinity and water temperature were 7.94 (0.33), 28 ‰ (2.14) and 29 °C (1.14), respectively. The levels of ammonia were consistently <0.03 ppm. The coefficient of variation for wet weight at 48 days of age was 53.7%, indicating substantial variation for the trait. The coefficient of determination (R^2) for the model described above was 19.5%, implying that substantial variation was unaccounted for, by the model.

The results of analysis of variance (Table 1) revealed that substrate management and stocking density significantly (p<0.0001) affected the wet weight at 48 days of age, whereas water exchange did not. All interaction effects investigated were statistically highly significant.

The least-squares means with standard errors are depicted in Table 2. Although the mean weight was slightly higher with 10% water exchange as compared to 100% water exchange, this effect was not statistically significant. Provision of substrate increased the wet weight significantly, as evidenced by the lower body weight without substrate. Not surprisingly, the results showed that final weight decreased with increasing stocking densities. A consistent decreasing trend in wet weight was discernible with the increase in stocking density.

Our results are in agreement with those available in literature. A negative correlation between growth rate and density has been documented for *L. vannamei* (Sandifer *et al.,* 1987; Sturmer and Lawrence, 1987; Wyban *et al.,* 1987; Williams *et al.,* 1996; Pérez-Rostro and Ibarra, 2003; Moss and Moss, 2004), *Penaeus japonicus* (Lanari *et al.,* 1989; Coman *et al.,* 2004), *L. setiferus* (Williams *et al.,* 1996), *L. stylirostris* (Martin *et al.,* 1998), *Fenneropenaeus indicus* (Emmerson and Andrews, 1981), *Metapenaeus macleayi* (Maguire and Leedow, 1983) and *P. monodon* (Ray and Chien, 1992; Jackson and Wang, 1998). Forster and Beard (1974) compared the growth of nine species of prawns reared under two densities and in all cases there was an adverse effect of stocking density on growth. Wyban *et al.* (1988) concluded that growth of *L. vannamei* was unaffected by stocking densities ranging from 45-100 shrimps m^{-2}. Lower wet weights observed at high stocking densities could be attributed to reduced space and natural food source availability (Arnold *et al.,* 2006), degradation of water quality (Nga *et al.,* 2005) and accumulation of undesirable sediment (Arnold *et al.,* 2005; 2006b). Moss and Moss (2004) reported that the use of artificial substrates mitigates the potential negative effects of high stocking density on growth of *L. vannamei* in nursery systems, which was not observed in our study. Even with the provision of a substrate, the wet weight of shrimp was significantly lower under higher stocking densities.

The interaction effect of water exchange and substrate management indicated that the provision of substrate resulted in increased wet weights only under the 10% water exchange regime. It is interesting to note here that absence of substrate in both the water exchange groups resulted in the same wet weights. This indicates that in the absence of a substrate, the wet weights were not dependent on the amount of water exchanged (0.04042 g in 10% water exchange compared to 0.03965 g in 100% water exchange). At a stocking density of 200, the water exchange had a very significant effect on wet weight, the weight being comparatively higher with 10% water exchange (0.05865 g) than with 100% water exchange (0.04969 g). When the stocking density increased to 400 (0.04416 g in 10% compared to 0.04535 g in 100% water exchange) and 600 (0.03268 g in 10% compared to 0.03083 g in 100% water exchange), the wet weights were not affected by the water exchange.

The survival values are depicted in Table 3. As the stocking density increased, survival decreased, though this trend was less pronounced when a substrate was provided. The water exchange rate did not affect the survival values. Interestingly, when a substrate was provided, the survival values were consistently lower.

in shelter that enabled newly molted animals to escape from cannibalism. The survival values in the present study exhibited a consistent trend when viewed across the substrate management. The survival values of the combinations without substrate were comparatively higher than those with substrates. In our study, the plastic fibre substrates that were provided, were woven tightly without any perforations, and were black in colour. It was observed that the post-larvae were found clinging onto the mats after feeding. It is quite possible that the post-larvae while clinging onto these mats were exposed to a higher risk of cannibalism. This could have resulted in low survival in the treatment combinations for which substrate was provided.

It could therefore be concluded from the results of the present study that in a rearing system where post-larvae are first grown in tanks and then transferred to ponds, the optimum protocol to maximize wet weight of shrimp (*P. monodon)* from the tank stage is to use low water exchange (*eg*. 10%) and low stocking rates (200 post-larvae per 500 l seawater). Given the discrepancy between our results and those of Abdussamad and Thampy (1994), further investigations are needed in order to evaluate the effect of provision of substrate, including effect of colour and type of substrate.

Table 3. Survival (%) across different water exchange rates, stocking densities and substrate management classes.

Water exchange	Substrate management	Stocking density	Initial number*	No. harvested*	Survival (%)
10	With substrate	200	600	419	69.8
10	With substrate	400	1200	618	51.5
10	With substrate	600	1800	1060	58.9
10	No substrate	200	600	451	75.2
10	No substrate	400	1200	899	74.9
10	No substrate	600	1800	1243	69.1
100	With substrate	200	600	364	60.7
100	With substrate	400	1200	463	38.6
100	With substrate	600	1800	1155	64.2
100	No substrate	200	600	458	76.3
100	No substrate	400	1200	829	69.1
100	No substrate	600	1800	1195	66.4

* pooled over replicates

In the present study, it was observed that wet weight at 48 days of age was significantly affected by stocking density and the presence of a tank substrate. However, this trait was not affected by the levels of water exchange. Given that water exchange rate was not significant, lower exchange rates are advisable in order to reduce disturbance to the shrimp.

The survival values in the present study indicated that the provision of substrate decreased survival rates. These results are in stark contrast to those previously reported by Arnold *et al.* (2006) and Abdussamad and Thampy (1994) in the same species. The higher survival observed in their study when substrate was used was attributed to an increase

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