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Effects of stocking density and artificial shelters during the nursery production of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) in net cages

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Abstract: Relative performance of stocking densities and types of artificial shelters in the nursery production of the giant freshwater prawn, *Macrobrachium rosenbergii* in net cages was assessed. For the effects of stocking density, giant freshwater prawn juveniles (mean= 0.2 g ±0.001) were cultured in 12 75-L net cages within a 6 m^3 concrete tank at stocking densities of 8, 15 and 30 individuals/net cage, and each net cage was provided with black polyethylene mesh nets as artificial shelters. For the effects of artificial shelters, the following materials were used: mesh bundles of used black polyethylene nets (Treatment 1), cut pieces of bamboo (Treatment 2) and PVC pipe stacks (Treatment 3), with triplicate for each treatment, was stocked with 15 individuals/net cage. No significant effects of the shelter types and stocking densities on most of the zootechnical parameters were obtained. Feed conversion ratio (FCR) and efficiency (FCE) were significantly better at lower stocking density. There were lesser variations in individual growth of the prawns in net cages that were stocked at a density of 15 individuals/net cage and provided with mesh bundles as artificial shelters, while the net cages with either cut pieces of bamboo or PVC pipe stack had heterogeneous growth during the latter part of the nursery rearing phase.

Keywords: *Macrobrachium rosenbergii*, Bamboo, Growth, Prawns

Introduction

The rapid global expansion of freshwater aquaculture in recent years has resulted in the increase of the Aquaculture industry and number of species that are being cultured (Mather and de Bruyn, 2003). One particular group of freshwater species is composed of members of the genus *Macrobrachium* has increased both in popularity and production (D'Abramo *et al*., 2003). This genus is widely distributed throughout the tropics and subtropics (Holthuis, 1980), but are also found in the North American waters (Murphy and Austin, 2005). The most widely cultured species is the giant freshwater prawn or *M. rosenbergii* (Mather and de Bruyn, 2003), because of their fast growth, good consumer acceptance and high export potential (Whangchai *et al*., 2007).

Giant freshwater prawn is widely distributed in the tropical and subtropical waters and is native to Indo-Pacific region, including the Philippines. They are generally found in freshwater ponds, rivers, lakes, ditches, canals, depressions, low lying flood plains and river mouths. They can be reared in captivity through the introduction of wild-caught juveniles (New *et al*., 2010). The juveniles exhibit nocturnal swimming activity, whereas during the day they settle on the bottom and crawl.

It is evident that in the rearing of crustaceans, it usually implies high stocking densities (Karplus, 2005). In effect, stocking density is one of the major factors that must be taken into consideration when conducting studies in freshwater prawn culture (Garcia-Guerrero and Molina, 2008). Moreover, some form of shelters has to be provided to the cultured stock during the grow-out phase to address the burrowing nature of the animals (Hogger, 1988). Some crustaceans burrow into the soil substrates by forming intricate burrows (Horwitz and Richardson, 1986). Other non-burrowing species utilize rocks, gravel or vegetation as shelters (Hogger, 1988; Eversole and Foltz, 1993; Foster, 1993). It is suggested that these preferences in habitats are able to provide refuge for the crustaceans during periods of vulnerability such as moulting, protect against predation and minimize aggressive interactions and cannibalism (Shivananda Murthy *et al*., 2012).

Several authors have reported on the effects of stocking density in the culture of crustaceans (D'Abramo *et al*., 2000; Gherardi, 2002; Garcia-Guerrero and Molina, 2008). As the stocking density increased, higher mortalities and lower growth rates have been observed in shrimp (Palomino *et al*., 2001; Calado *et al*., 2005), crayfish (Barki and Karplus, 2000; Jones and Ruscoe, 2000), and prawns (Ra'anan and Cohen, 1984; Ranjeet and Kurup, 2002). Moreover, previous studies of freshwater

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crustaceans indicated the importance of the provision of shelters for the early stage juveniles (Du Boulay *et al*., 1993; Jones, 1995; Karplus *et al*., 1995). These studies demonstrated that freshwater crustaceans are able to discriminate between different shelter types and display clear preferences. Shelters play an important role in growth and survival of giant freshwater prawns, because they are aggressive and have territorial and agonistic behavior (Shivananda Murthy *et al*., 2012). According to Smith and Sandifer (2009), the giant freshwater prawns generally prefer a darkened tank and habitat areas. The provision of shelters during the nursery production of the giant freshwater prawns is critical in ensuring good growth and survival of the animals particularly when they are stocked at higher densities (Whangchai *et al*., 2007; Garcia-Guerrero and Molina, 2008; Mamun *et al*., 2010; Shivananda Murthy *et al.*, 2012).

It is common to use bundles of mesh materials as shelters during the juvenile stage of some freshwater crustaceans (Jones, 1990) and these are also used for giant freshwater prawns (Jones and Ruscoe, 2001). For this type of artificial shelter, the mesh materials are bunched together and placed at the bottom of the nursery tanks. Other materials that can be used as artificial shelters include off-cuts of PVC pipes, corrugated plastic sheets, discarded fishing nets, bamboo pieces, or pieces of fallen timber (Jones and Ruscoe, 2001).

As the aquaculture industry for giant freshwater prawns develops, it is necessary to determine the optimum stocking density and the suitable shelter specifications that will result in optimum growth and survival. Ultimately, it is possible to design an artificial shelter based on available materials with characteristics that will maximize its use by the freshwater prawn. In order to determine the ideal stocking density and appropriate artificial shelter for freshwater prawns during the nursery phase in net cages, and with a purpose of providing specifications on the types of materials that could be used for mass production of artificial shelters, an experiment was designed to assess the performance of varying stocking densities and several types of artificial shelters during nursery of freshwater prawns in net cages.

Materials and Methods

Experimental design

The study was conducted in freshwater tanks of the Aquatic Science Laboratory (ASL), College of Fisheries and Aquatic Sciences (CFAS), Western Philippines University (WPU)–Puerto Princesa Campus, Palawan, Philippines. The experiment was conducted for 60 days in nine net cages (0.3 m x 0.3 m x 0.7 m) that were placed inside a concrete tank (3.0 m x 2.0 m x 1.0 m). The concrete tank was used to hold water for the duration of the study. Prior to the experiment, the tank was thoroughly cleaned with freshwater and sundried for a few days. After completely dried, freshwater from a nearby source (DO >5.0 ppm, pH 7.0-8.0, temperature 26-28 ºC) was pumped into the tank and filled at 80% capacity. The water was allowed to age for 2 days followed by the installation of the net cages. The nets were submerged at the tank at a 0.1 m distance from the bottom and tied at four corners at the surface. Each net was supported by a PVC pipe frame base to maintain its square shape while being submerged in water. The water level inside the net cages was 0.7 m.

Three stocking densities were tested, namely: Treatment 1: 8 individuals/net cage; Treatment 2: 15 individuals/net cage and Treatment 3: 30 individuals/net cage. Each net cage was provided with an artificial shelter consisting of black polyethylene mesh nets.

Different shelters represented the various experimental treatments that consisted of the following: Treatment 1: black polyethylene mesh nets, which served as control; Treatment 2: cut bamboo pieces; and Treatment 3: PVC pipes. Figure 1 shows the different shelters used in the study. B-net served as the positive control for this study because this shelter is used at the source of the freshwater shrimp juveniles. For Treatment 2, fifteen pieces of cut bamboo pieces (5.0 cm in length and 2.54 cm in diameter each) were tied together and submerged at the bottom in each of the three net cages. The shelter to shrimp juvenile ratio was at 1:1. For Treatment 3, the same number and size of the PVC pipes were used. A No-shelter control was not included in the study because it was already shown in previous studies that ponds or tanks without shelters had significantly lower production output than those with artificial shelters.

All treatments in the stocking density and artificial shelter studies were done in triplicate and the net cages were assigned using a completely randomized design.

Source of freshwater shrimp juveniles, acclimation and stocking

Freshwater shrimp juveniles (PL20) were transported

Fig. 1: Different artificial shelters that were placed inside the net cage for the nursery production of giant freshwater prawns. (A) Black polyethylene net mesh bundles, (B) cut bamboo pieces and (C) cut PVC pipes.

by air from Southeast Asian Fisheries Development Center - Aquaculture Department (SEAFDEC/AQD) in Binangonan, Rizal, Philippines. Upon arrival at the experimental site, the transport bags were allowed to float in the acclimation tanks for 2-3 h to attain similar water temperatures. This was followed by opening of the bags and the water in the transport bags were gradually replaced with water from the acclimation tanks. Once the dissolved oxygen and temperature were similar in both the transport bags and the acclimation tanks, the freshwater shrimp juveniles were slowly released into the tank by gradually submerging the transport bags. No feeding was done for 24 h and ample aeration was provided. Acclimation was done for at least 2 weeks before stocking.

Stocking of individual net cages was done when the shrimp reached PL35. Each net cage was stocked at a density of 15 juveniles per net cage. Initial length and weight of the juveniles were measured using a plastic caliper and bulk weighed using a digital weighing scale (MH-500 with 500 g capacity and 0.1g sensitivity), respectively.

Feeding and water management

The juvenile freshwater shrimp were fed with formulated shrimp diet (SEAFDEC/AQD) containing 40% crude protein. The daily feeding ratio was computed at 10% of the biomass. Uneaten feeds were collected, dried and weighed. Recorded data were used in the analysis of the Feed Conversion Ratio (FCR). Feeding was done twice daily at 8AM and 4PM, with higher amount of feeds given in the afternoon.

Water exchange was done every 2 days by replacing 20% of the total water volume in the concrete tank. Water temperature, pH and dissolved oxygen were monitored daily prior to the morning feeding to make sure that these levels were at their optimum levels required for the culture of freshwater shrimp juveniles. During water change, uneaten feeds and fecal matter were siphoned out carefully from the bottom of the tank.

Sampling

The freshwater prawn juveniles were sampled of their weight and length every 15 days following the protocol of Shivananda Murthy *et al.* (2012). Only 30% of the total stock in each replicate were collected during sampling to measure the individual length (in cm; from orbit of the eye to tip of telson) and weight (g) using a Vernier caliper and a digital weighing scale (sensitivity 0.1g), respectively.

Data analyses

Weight and length gain, survival rate, specific growth rate (SGR), condition factor (k), Feed conversion Ratio (FCR) and Feed Conversion Efficiency (FCE) were obtained at the end of the experiment following standard computations. Means and standard error (SE) of each zootechnical parameter were obtained. All statistical computations were performed at the 0.05 probability level. Data for all zootechnical parameters for the treatments were analyzed with One-Way Analysis of Variance (ANOVA). If significant, it was followed by pairwise comparisons of means with the Least Significant Difference test. Analyses were performed using Microsoft Excel 2010.

Results

All juvenile freshwater prawns were able to adapt easily to the culture conditions, and feed pellets were readily accepted. The various zootechnical parameters of the various groups at different stocking densities and types of artificial shelters are summarized in Tables 1 and 2, respectively. Highest survival, growth increments and SGR were in freshwater prawn juveniles that were stocked at 8 individuals/net cage but were not significantly different from the other treatment groups (Tab. 1). However, both FCR and FCE were significantly better in the low stocking density group than with the high stocking density groups. In Table 2, net cages provided with both the black polyethylene mesh nets and cut PVC pipes as shelters had similar survival rates of the juveniles, while net cages with cut bamboo nodes as shelters had the lowest survival of the juvenile prawns, although the results were not significantly different among the different groups. Highest increments in both weight and length as well as SGR and condition factor were observed in juveniles reared in net cages with cut bamboo pieces as shelters, but were not significantly different from the other treatment groups. In addition, lowest FCR and FCE were recorded in juvenile prawns provided with bamboo nodes as shelters.

Tab. 1: Means (±SE) of the giant freshwater prawn juveniles reared in netcages at various stocking densities.

zootechnical parameters	8 ind/net cage	15 ind/net cage	30 ind/net cage
Survival Rate (%)	45.83 (±7.22)	37.78 (1±3.88)	26.67 (±0.00)
Initial Weight (g)	0.21 (±0.12)	0.26 (±0.08)	0.38 (±0.06)
Final Weight (g)	1.55 (±0.27)	1.22 (±0.32)	1.55 (±0.03)
Weight gain (g)	1.34 (±0.39)	0.96 (±0.31)	1.17 (±0.09)
Initial Length (mm)	21.20 (±0.35)	20.1 (±0.05)	22.2 (±0.04)
Final Length (mm)	41.10 (±0.09)	38.5 (±0.35)	40.9 (±0.16)
Length gain (mm)	19.90 (±0.26)	18.4 (±0.34)	18.8 (±0.14)
Specific Growth rate (% day^{-1})	1.40 (±0.48)	1.09 (±0.25)	0.89 (±0.12)
FCR	7.60^{a} (±1.15)	17.46^{b} (±3.39)	32.5^{c} (±6.27)
FCE	13.35^{a} (±1.91)	5.88^{b} (±1.22)	3.15^{b} (±0.59)

Row values with different superscripts are significantly different at $p<0.05$. N=3

Tab. 2: Means (±SE) of the different zootechnical parameters of the giant freshwater prawn juveniles reared in net cages with different shelters.

Zootechnical Parameters	Polyethylene nets	Bamboo	PVC Pipes
Survival Rate (%)	55.56 (±21.43)	40.00 (±11.55)	55.56 (±10.18)
Initial Weight (g)	0.20 (±0.01)	0.20 (±0.01)	0.20 (±0.01)
Final Weight (g)	2.17 (±0.19)	2.62 (±0.59)	2.24 (±0.44)
Weight gain (g)	1.97 (±0.90)	2.42 (±0.59)	2.04 (±0.44)
Initial Length (mm)	23.00 (±0.01)	23.00 (±0.01)	23.00 (±0.01)
Final Length (mm)	47.70 (±0.18)	49.60 (±0.32)	47.40 (±0.46)
Length gain (mm)	24.75 (±1.84)	26.58 (±3.22)	24.35 (±4.61)
Condition Factor (k)	0.49 (±0.03)	0.58 (±0.10)	0.48 (±0.05)
Specific Growth rate (% day^{-1})	3.97 (±0.14)	4.26 (±0.38)	4.00 (±0.35)
FCR	5.28 (±1.96)	4.84 (±2.70)	5.75 (±1.03)
FCE	20.96 (±8.36)	24.75 (±11.18)	17.72 (±2.89)

In Table 3, juvenile freshwater shrimps stocked at a stocking density of 15 individuals/net cage exhibited homogeneous individual growth throughout the duration of the study period as shown by the low deviations in both weight and length. Moreover, stocking of juvenile prawns at either 8 or 30 individuals/net cage resulted in homogeneous individual growth at the 30th day of sampling followed by heterogeneous individual growth until the last day of sampling (60th day). On the other hand, homogeneous individual growth was observed in juvenile freshwater prawns when provided with the tree types of artificial shelters up to the 30th day of sampling as shown by the low of standard deviations in their weight and length (Tab. 4). However, at the 45th day of sampling until the last day of sampling (60th day), heterogeneous individual growth was observed in juvenile prawns reared in net cages provided with either cut bamboo pieces or PVC pipes. Only the group provided with B-net as shelter exhibited homogeneous individual growth.

The ranges of selected water quality parameters in all treatment groups are as follows: Water temperature: 25.2-28.0 ºC; pH: 7.24-8.35 and Dissolved oxygen: 6-9 ppm.

Discussion

Stocking density and the availability of shelters have been shown to be a fundamental factor in the culture

Tab. 3: Standard deviations of (A) weight and (B) length of giant freshwater prawn juveniles reared in ne cages at various stocking densities. N=15-20

		D 15	D 30	D 45	D 60
A	8 ind/net cage	0.05	0.48	0.77	1.22
	15 ind/net cage	0.19	0.19	0.43	0.65
	30 ind/net cage	0.14	0.17	0.77	1.21
B	8 ind/net cage	0.24	0.57	1.24	0.96
	15 ind/net cage	0.31	0.39	0.52	0.74
	30 ind/net cage	0.40	0.542	0.76	0.87

Table 4. Standard deviations of (A) weight and (B) length of giant freshwater prawn juveniles reared in net cages with different shelters. N = 15-20

		D 15	D 30	D 45	D 60
A	Polyethylene net	0.21	0.48	0.43	0.61
	Bamboo	0.25	0.57	0.84	1.81
	PVC pipes	0.15	0.43	0.93	1.21
B	Polyethylene net	0.51	0.67	0.59	0.46
	Bamboo	0.61	0.70	0.84	1.11
	PVC pipes	0.38	0.64	0.92	0.84

of burrowing crustaceans (Capelli and Hamilton, 1984; Gherardi, 2002; Garcia-Guerrero and Molina, 2008), such as *Macrobrachium* spp. (New, 2002). Shelters provide protection for recently molted prawns. Previous experiments demonstrated the beneficial effects of using shelters during the nursery and grow-out production of freshwater prawns in concrete tanks and ponds (Jones and Ruscoe, 2001; Whangchai *et al*., 2007; Garcia-Guerrero and Molina, 2008; Shivananda Murthy *et al*., 2012). For example, highest growth and survival was observed at a stocking density of 96 juveniles per square meter of area in concrete tanks (Garcia-Guerrero and Molina, 2008). Shivananda Murthy *et al*. (2012) obtained highest survival and yield of freshwater prawns in concrete tanks that were provided with PVC pipes as shelters. In pond culture, shelter types provided during culture had a significant effect on the biomass upon harvest due to its effect on higher survival (Jones and Ruscoe, 2001). Growth rates and feed conversion ratio of freshwater prawns stocked at a density of 100 juveniles per m^2 were significantly better in ponds provided with plastic sheets as shelters than with the non-sheltered ponds (Whangchai *et al*., 2007). In the present study, no significant differences in the zootechnical parameters of the freshwater prawn during the nursery rearing phase were observed using these three stocking densities and three types of artificial shelters. However, the juvenile freshwater prawns that were reared at low stocking density or provided with cut pieces of bamboo as shelters had the highest growth and best FCR.

The water quality parameters that were tested in the treatment groups during the experiment were within the optimum levels that are required for the culture of freshwater prawns (Nandlal and Pickering, 2006); hence, these were not the critical factors that affected growth and survival of the prawns during the nursery production phase.

Although the juvenile prawns within each treatment were of similar sizes at the beginning of the experiment, variation in size developed subsequently. Growth within a cohort is often heterogeneous, and this is expected among juveniles from multiple spawnings (Ra'anan and Cohen, 1984; Ra'anan *et al*., 1991; Ranjeet and Kurup, 2002) and was observed in this work. Because body size affects survival, variation in growth is an important component in aquaculture studies (Ra'anan *et al*., 1991). In the experiments performed by Ra'anan and Cohen (1984) and Ra'anan *et al.* (1991), about half of the population grew rapidly with variation among specimens. Whangchai *et al.* (2007) obtained greater size variations in pond-reared freshwater prawns when no shelters were provided. Garcia-Guerrero and Molina (2008) also observed heterogeneous individual growth of juvenile freshwater prawns over time regardless of the presence or absence of shelters and stocking density. The causes of this wide variation in individual growth could be related to differences in genetics and physiological condition (Gu *et al*., 1995) or behavior of the animal (Ranjeet and Kurup, 2002). In this study, stocking of juvenile freshwater prawns at a density of 15 individuals/net cage and the provision of mesh net bundles resulted in a homogeneous individual growth of freshwater prawns during nursery production, and it is believed that the nets folded inside the tanks provided some additional shelter for molting prawns (Garcia-Guerrero and Molina, 2008). The efficacy of the mesh bundle shelters may be attributable to the variability in the size of the spaces provided, and the ability of the mesh to separate many individuals relative to the overall volume of the shelter (Jones and Ruscoe, 2001).

In assessing shelters from a commercial perspective, consideration must be given to the cost of manufacture (Jones and Ruscoe, 2001). From a purely economic standpoint, the use of cut bamboo pieces is likely to be the most cost-effective because they are locally available and has a cheap market price. Material costs for mesh bundles are not so expensive; however, they require considerable labor to fabricate and the nature of their design may be an issue during automated production (Jones and

Ruscoe, 2001). On the basis of manual construction, the PVC pipe stack artificial shelters will have the highest material and labor costs.

In conclusion, the stocking densities and the different types of artificial shelters that were used in the present study had no significant effect on the various zootechnical parameters during the nursery production of freshwater prawns in net cages. This study further demonstrated that the levels of the stocking density and the types of artificial shelters had an effect in the variations in individual growth of the freshwater prawn juveniles during the nursery production phase. Lesser variations in individual growth of the prawns in net cages that were stocked at 15 individuals/net cage and those provided with polyethylene net mesh bundles were observed, whereas prawn juveniles that were provided with either cut pieces of bamboo or PVC pipe stack as artificial shelters had heterogeneous growth during the latter part of the nursery rearing phase. The stocking density of 15 individuals/net cage or roughly 150-175 individuals/m^2 during the nursery production phase could be considered as an optimum density that will likely result in uniform growth of the cultured stock. The efficacy of the mesh bundles as artificial shelters in having a homogeneous growth of the juveniles could be attributed to increased variability in the size of the spaces provided by the mesh and the ability of the mesh to separate many prawn juveniles relative to the overall volume of the shelter. However, the use of cut pieces of bamboo as artificial shelter is the most cost-efficient because these are locally available and free of cost.

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