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ARTICLE

Effect of Stocking Density on Growth and Survival of the Prawn *Macrobrachium tenellum* Cultured in a Cage-Pond System

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Abstract
Freshwater prawns, *Macrobrachium tenellum*, were reared at four stocking densities (6, 12, 18, and 24 prawns/m^3), with three replicates each, in 12 bottom cages of 3 m^3 capacity that were placed in a 1,422-m^2 earthen pond. The growth, weight gain, production, specific growth rate (SGR), feed conversion ratio (FCR), and survival of the prawns were determined. We stocked 540 juveniles that had an average weight of 1.57 ± 0.09 g (mean ± SE) for 180 d and fed them twice a day with commercial shrimp pellets containing 35% crude protein. Water quality variables were measured during the study. All of the growth and production parameters were affected by stocking density ($P < 0.05$). The mean weight and SGR increased at low densities. The lowest mean weight (17.2 ± 2.0 g) was observed at the higher density (24 prawns/m^3), although production increased at high densities and varied from 1,307.2 kg/ha at a density of 6 prawns/m^3 to 2,013.3 kg/ha at a density of 24 prawns/m^3. Survival varied from 79 ± 1% at a density of 6 prawns/m^3 to 47.5 ± 0.6% at a density of 24 prawns/m^3. The overall results suggested that stocking density affected the growth and survival of *M. tenellum* cultured in the cage-pond system. The initial stocking density represents a very important

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culture variable in terms of marketing for this freshwater prawn because at all tested densities the prawns reached their individual market size.

Freshwater prawn culture on the Mexican Pacific coast is restricted to the exotic species, the giant river prawn *Macrobrachium rosenbergii*. However, certain native species of the same genus, such as the longarm river prawn *M. tenellum* and cauque river prawn *M. americanum*, offer a high potential for use in aquaculture (Ponce-Palafox et al. 2002). The distribution of *M. tenellum* in North and South America ranges from Baja California, Mexico (27°N) to the Chira River, Peru (5°S) (Holthuis 1980). Since the late 1970s, *M. tenellum* has been considered a good candidate for cultivation. It is found at high densities under natural conditions, is not aggressive, and can tolerate a wide range of fluctuating temperatures, salinity, and oxygen concentrations (Ponce-Palafox et al. 2013). In addition, it has weak pincers, is unable to harm organisms that it manipulates, and is evidently unable to leave the water because its pereiopods are not strong enough to lift the weight of its body.

Many studies on the technological aspects of *M. tenellum* culture have recently been conducted and have included research on diseases in the commercial production of prawn in rural farms (Ponce-Palafox et al. 2005), nutrition (García-Ulloa et al. 2008; Espinosa-Chaurand et al. 2012), and grow-out (Vega-Villasante et al. 2011; Ponce-Palafox et al. 2013). Nevertheless, several external culture-related factors, such as density, remain to be studied to facilitate optimal freshwater prawn production. The culture of freshwater prawns has traditionally been performed in ponds (New and Singholka 1985; Correia et al. 2002; Weimin and Xianping 2002; Lam et al. 2006; Murthy et al. 2012) and cage culture (Stanley and Moore 1983; Sagi et al. 1986; Marques et al. 2000; Cuvin-Aralar et al. 2007), primarily with *M. rosenbergii*. The aim of this study was to evaluate the influence of various stocking densities on the growth performance, production, and survival of *M. tenellum* reared in a pond in a system of bottom cages.

METHODS

Locale and experimental structure.—The experiment was performed in a 1,422-m^2 earthen pond located at the San Cayetano Aquaculture Center, the property of the Fisheries Department of the Nayarit State Government, Mexico (21°27′24.23″N, 104°49′29.67″W). Twelve bottom cages (3 × 1 × 1 m), each with a capacity of 3 m^3, were placed in the pond. The cages were constructed from polyethylene mesh (0.7 mm diameter) and iron frames. Circular feeding trays 50 cm in diameter made of polyethylene screening were placed in the cages, and synthetic mesh bags (raffia bags) were provided as refuges for the prawns. The cages were tied to stakes, placed on the pond bottom at a water depth of 1 m, and covered with a polyethylene screen to exclude predators and prevent prawns from escaping. The earthen pond was rectangular, and the cages were placed in two parallel lines, each consisting of six cages occupying one-half of the pond. Water exchange (5% of the pond water) was performed nightly from 1900 to 2100 hours to assure adequate water circulation and prevent stratification of the water column.

Water variables.—Temperature and dissolved oxygen were measured daily with a YSI-85 handheld meter (Yellow Springs Instruments) and pH was measured daily with a digital pH meter (Bernauer F-1002) in the cage area at a depth of 30 cm at 1000 and 1700 hours. Water transparency was measured with a Secchi disk. Samples of water were collected monthly from the subsurface water near the cages to monitor the following variables: alkalinity, ammonia nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), and total phosphorus (total P). All of these analyses were performed with a YSI 9000 photometer (Yellow Springs Instruments).

Experimental procedures.—*Macrobrachium tenellum* juveniles ($n = 540$; weight, 1.57 ± 0.09 g [mean ± SD]) were stocked in 12 cages at densities of 6, 12, 18, and 24 prawns/m^3. Three replicates, randomly distributed in the central area of the pond, were used per treatment. The prawns were fed twice daily with a commercial shrimp diet (35% crude protein, 8% lipid, and 12% moisture; size 2–2.5 × 6–7 mm; Nutripec Camaronina XT, Agribrands, Purina, Mexico). The daily feed ratio was determined based on wet weight and gradually adjusted from 10% at the beginning of the experiment to 5% after 90 d of growth. The 5% ratio continued to be fed until the end of the experiment (180 d). The daily feed ratios were based on feed demand and determined after periodic monitoring of the feed trays. The feeding transitions occurred at the same time for all treatments. All of the prawns were collected and weighed monthly to evaluate their growth and adjust the amount of feed supplied. After 180 d of culture, the prawns were individually counted and weighed to determine mean weight (g), weight gain (g/week), production (kg/ha), specific growth rate (SGR; %/d), feed conversion efficiency (FCE; %) and survival (%). Both SGR and FCE were determined as follows:

$$\text{SGR}\,(\%/\text{d}) = 100 \times [(\ln\, W_2 - \ln\, W_1)/t_2 - t_1)] \quad \text{and}$$
$$\text{FCE}\,(\%) = 100 \times (W_2 - W_1)/C,$$

where W_2 and W_1 are mean body weights (g) at times t_2 and t_1 (d), respectively, and C is the wet weight of food consumed (g).

Statistical analysis.—The prawn performance data were analyzed for normality and homoscedasticity with a Shapiro–Wilk test and a Bartlett test, respectively. As the requirements of

TABLE 1. Pond water quality variables (mean ± SD) in cage-pond system of *M. tenellum* during grow-out (180 d). Within a row, means accompanied by different lowercase letters are significantly different ($P < 0.05$).

Water quality variables	Morning	Afternoon
Temperature (°C)	27.0 ± 1.2 x	28.8 ± 1.2 x
Transparency (Secchi disk depth, cm)	82.5 ± 0.5	
Dissolved oxygen (mg/L)	8.2 ± 1.6 x	8.6 ± 0.6 x
pH	8.5 ± 0.2 x	8.4 ± 0.1 x
Alkalinity (mg $CaCO_3$/L)	110.0 ± 0.90 x	135 ± 1.3 y
NH_3–N (mg/L)	0.19 ± 0.05 x	0.15 ± 0.08 x
NO_2^-–N (mg/L)	0.55 ± 0.07 x	0.42 ± 0.09 x
NO_3^-–N (mg/L)	1.58 ± 0.15 x	1.32 ± 0.35 x
Total P (mg/L)	0.56 ± 0.09 x	0.38 ± 0.10 y

these tests were satisfied by both the prawn performance data and the data on the water quality variables, a paired *t*-test was used to compare the morning and afternoon means of these variables. All data from all treatments were subjected to an ANOVA (Montgomery 1997). Differences among treatments were evaluated with a Tukey multiple comparison test of the means. The results were evaluated at the 5% significance level. Values expressed as percentages were square-root arcsine-transformed prior to analysis, but the nontransformed values are presented for ease of interpretation. The analyses were conducted using Statistica package version10 (StatSoft, Tulsa, Oklahoma). The density–biomass relationship produced a quadratic equation of the form

$$\text{Biomass} = \beta + \beta + \beta_2 d_2, \tag{1}$$

where β_2 is a quadratic coefficient (other than 0), β_1 is the linear coefficient, β_0 is the intercept, and d is the density.

The maximum density ($d_{\max}$) was obtained as follows:

$$d_{\max} = \frac{\beta_1}{2\beta_2}, \tag{2}$$

where $d_{\max}$ = maximum density. The maximum biomass was given by

$$\text{Biomass}_{\max} = \beta_0 + \frac{\beta_1^2}{2\beta_2} - \frac{\beta_1}{2}. \tag{3}$$

RESULTS

In general, the temperature, dissolved oxygen, pH, ammonia, nitrites, and nitrates did not differ significantly ($P > 0.05$) between the morning and afternoon in all cages and thus among treatments (Table 1). Significant differences ($P < 0.05$) were found only in alkalinity and total P concentration. The minimum temperature and oxygen concentration were 25.0°C and 7.0 mg/L, respectively. During the summer, the temperature remained at approximately 27.9°C (1–149 d in culture). From November to December (150–180 d in culture, fall–winter under the temperate climate of the study locale) the mean temperature was 26.4°C (the minimum was 25°C). Dissolved oxygen showed higher values during the grow-out phase (8.2–8.6 mg/L). The pH values varied slightly and remained close to 8.5. The other water quality variables are documented in Table 1.

The growth, production, and survival data showed significant differences ($P < 0.05$) among densities (Table 2). The final weight decreased as prawn density increased, ranging from 27.0 ± 1.9 g in the 6-prawns/m^3 group to 17.2 ± 2.0 g in the 24-prawns/m^3 group (Figure 1). Production varied from 1,307.0 ± 151.3 kg/ha in the 6-prawns/m^3 group to 2,013.0 ± 220.1 kg/ha in the 24-prawns/m^3 group. Survival decreased with density, varying from 79 ± 1% in the 6-prawns/m^3 group to 47.5 ± 0.6% in the 24-prawns/m^3 group. The SGR was significantly higher ($P < 0.05$) in the 6-prawns/m^3 group (1.5 ± 0.1). The FCR differed significantly ($P < 0.05$) among treatments. The

TABLE 2. Data (mean ± SD) for cage-pond system on growth performance and production of *M. tenellum* cultured at different stocking densities. Within a row, means accompanied by different lowercase letters are significantly different ($P < 0.05$).

Parameters	Density (prawns/m^3)			
	6	12	18	24
Initial weight (g)	1.6 ± 0.3 z	1.5 ± 0.5 z	1.6 ± 0.4 z	1.6 ± 0.2 z
Final weight (g)	27.0 ± 1.9 z	21.6 ± 1.2 y	19.0 ± 2.3 yx	17.2 ± 2.0 x
Biomass (g/m^3)	130.7 ± 05.1 z	173.5 ± 10.1 y	192.0 ± 12.7 yx	201.3 ± 14.5 x
Weight gain (kg/week)	1.05 ± 0.20 x	0.84 ± 0.09 y	0.72 ± 0.05 z	0.65 ± 0.02 z
Production (kg/ha)	1,307.2 ± 151.3	1,734.6 ± 163.4	1,920.2 ± 189.0	2,013.3 ± 220.1 z
SGR (%/d)	1.5 ± 0.06 z	1.4 ± 0.05 y	1.3 ± 0.06 x	1.3 ± 0.08 x
FCR	1.2 ± 0.1 w	1.8 ± 0.1 x	2.1 ± 0.1 y	2.4 ± 0.1 z
Survival (%)	79.0 ± 1.0 z	65.5 ± 0.9 y	55.5 ± 0.3 x	47.5 ± 0.6 w

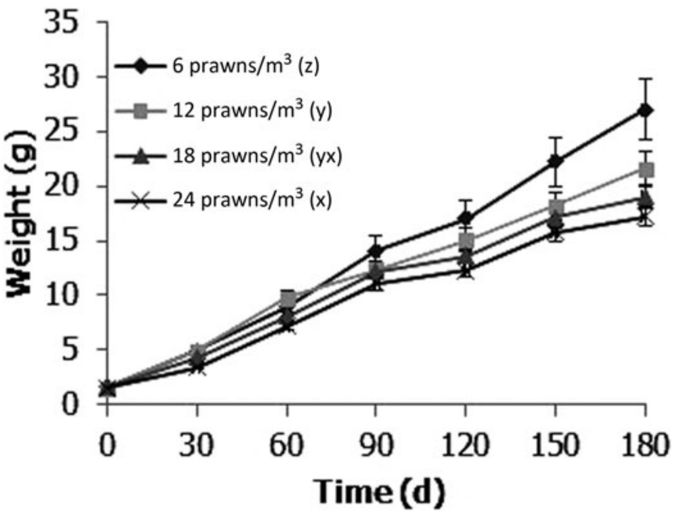

FIGURE 1. Growth of *M. tenellum* at different stocking densities in a cage-pond system. Stocking densities followed by different lowercase letters in the legend are significantly different at $P < 0.05$.

FCR was low at densities of 6 and 12 prawns/m^2, with values of 1.2 ± 0.1 and 1.8 ± 0.1, respectively. Prawn survival differed significantly among treatments ($P < 0.05$). Survival was greater than 50% in the 6- and 18-prawns/m^3 treatments (55.5–79.0%). However, higher levels of production were recorded in the 18- and 24-prawns/m^3 treatments (1,920.0–2,013.0 kg/ha).

Biomass increased up to 201.3 kg/m^3 at the 24-prawns/m^3 density (Figure 2) and then decreased to lower than 190.2 kg/m^3 at the 30-prawns/m^3 density, where growth showed density dependence. Substitution of the values obtained in the field (Figure 2) in the equation for the parabolic curve yielded the following relationship:

$$\text{Biomass} = 74.98 + 10.806\,d - 0.2322\,d^2. \tag{4}$$

Equations (2) and (3) were then applied, and a density of 24 prawns/m^3 was found to produce the maximum biomass.

DISCUSSION

The values of the water quality variables were within the range recommended for the cultivation of freshwater prawns (New 2002). Because *M. tenellum* tolerates a wide range of varying environmental conditions (Ponce-Palafox et al. 2013), this result suggests that the temperature was the only sampled environmental variable that could directly influence prawn performance in this experiment.

The stocking densities used in this study ranged from semi-intensive (6 prawns/m^3) to intensive (24 prawns/m^3) culture systems. These values are higher than those traditionally used for prawn pond culture (Cuvin-Aralar et al. 2007). The mean individual weight of the cultured prawns in this study did not attain the desired market size established for *M. rosenbergii* (35 g) for the principal shellfish markets in Mexico (Ponce-Palafox 1997). Nevertheless, the prawns attained the commercial size (25 g) recognized for this species in local markets in Mexico

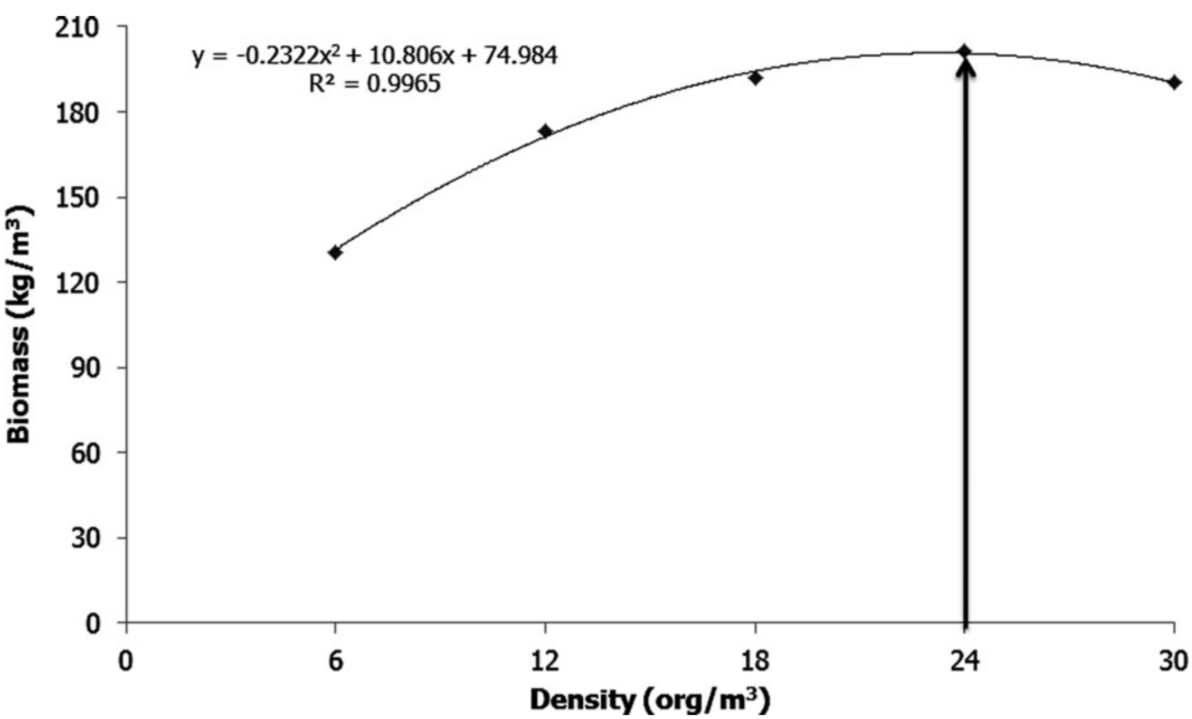

FIGURE 2. Biomass of *M. tenellum* at different stocking densities in a cage-pond system.

(Ponce-Palafox et al. 2002). In fact, the commercial size obtained in this study for *M. tenellum* is comparable with the commercial shrimp size. Compared with the results of a previous experiment (Ponce-Palafox et al. 2013), the greater size of the cage-reared prawn (27.0 g) at harvest in the treatment with 6 prawns/m^3 was presumably due to both the improved water quality and the feeding strategies applied in this study. The mean daily weight gain in this experiment was similar to that achieved in the previous experiment (Ponce-Palafox et al. 2013) at densities of 12–14 prawns/m^3. The most rapid growth and highest survival occurred at 6 prawns/m^3. The decreases in growth found at high density (24 prawns/m^3) appeared to be due to the higher biomass of caged prawns in the ponds and density-dependent effects (Nagarathinam et al. 2000; Cuvin-Aralar et al. 2007).

Macrobrachium tenellum juveniles cultured at an initial stocking size of 0.18–0.35 g in ponds furnished with plastic canvas grew to 9.3 g in 66 d after stocking at 8 prawns/m^3 (Vega-Villasante et al. 2011). Juveniles of the same species reared at an initial stocking size of 1.9–2.5 g in earthen ponds grew to 19.3 g in 144 d after stocking at 14 prawns/m^3 (Ponce-Palafox et al. 2013), results that are similar to those of this study.

Menasveta and Piyatiratitvokul (1982) found that the growth of *M. rosenbergii* in cages was lower than that observed in ponds and ditches. However, survival was significantly better in cages. Marques et al. (2000) and Ang et al. (1992) obtained results similar to those of this study in nursery systems and in the grow-out phase of *M. rosenbergii* in cages, respectively. They reported that a stocking density of 10 postlarvae/m^3 provided better growth than 20 or 50 postlarvae/m^3 although the final biomass was highest at the greatest stocking density. Although the mean final weight obtained in this experiment was comparable with that obtained by Ponce-Palafox et al. (2013) at 14 prawns/m^3 in a semi-intensive system, the yields in the current study were superior for all treatments. A comparison of both studies suggests that this difference is due to differences in water quality and feeding.

An inverse relationship between stocking density and growth has been reported for various species of *Macrobrachium* in different culture systems (Marques et al. 2000, 2010, 2012). Freshwater prawns of the genus *Macrobrachium* show territorial behavior (New 2002). At high densities, even if food is available, access to the food may be negatively affected due to stress caused by aggressive behavior (Wickins and Lee 2002). Comparisons among *Macrobrachium* species show that male *M. rosenbergii* cultured in cages reached 35.6 g in 150 d at densities of 15 prawns/m^3 (Cuvin-Aralar et al. 2007), whereas males of *M. tenellum* reached 27.8 g at 150 d at 12 prawns/m^3. Cuvin-Aralar et al. (2007) reported an FCR of 2.1 for *M. rosenbergii* reared at densities similar to those used in this study, in which the mean final FCR was 1.9. These differences can be partially explained because *M. tenellum* was fed twice daily and *M. rosenbergii* once. Stocking densities of *M. tenellum* up to 6 prawns/m^3 in the experimental cages did not affect survival or performance during the growth phase. However, the results suggest that density-dependent processes regulate the final weight, weight gain, FCR, and survival at densities of 30 prawns/m^3 or more. This pattern has also been found in wild shrimp populations at high densities (Pérez-Castañeda and Defeo 2005)

Farming of *M. tenellum* in a cage-pond system is feasible, especially in terms of survival rates, which were found to be similar to or better than those obtained in conventional earthen ponds (Ponce-Palafox et al. 2013). These data suggest that stocking juveniles in cages at high densities of 30 prawns/m^3 or more for the secondary nursery phase is be a good strategy for reducing the costs of the nursery phase because cages can be placed either within grow-out ponds or in other appropriate water bodies available on the farm. Further research should evaluate the effect of introducing additional surfaces and shelters inside the cages as well as the use of cages for grow-out culture. Cage sizes and other appropriate materials for nets and frames also need to be considered to reduce the costs and allow applications at a commercial scale.

Lastly, we found that density affects the growth and survival of *M. tenellum*. The effect is greater after 150 d of culture for densities greater than 12 prawns/m^3. The choice of a suitable density represents a very important culture strategy for this freshwater prawn in terms of marketing because the animals at all tested densities reached the individual market size.

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