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RESEARCH ARTICLE

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Influence of density on growth and survival of freshwater prawn *Macrobrachium americanum* (Bate, 1868) (Caridea: Palaemonidae) cultured in a cage-pond system

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Abstract

The domestication and culture of freshwater shrimp native of tropical and subtropical American Pacific zone requires the study and development of technologies in open systems where their response to types of farming, feeding and management strategies generate practical information for the production of this species. The aim of this study was to evaluate the effect of stocking density on growth and survival in *M. americanum* juvenile-adult, cage-cultured and to determine the optimal density for grow-out production. The caridean shrimp *Macrobrachium americanum* was cultured for 152 days in a 16 cage-culture (3 m^3 each) at densities of 1, 3, 6 and 9 org/m^3, respectively, with stocking sizes from 12.1 ± 1.7 to 13.5 ± 2.3 g. The prawns were fed twice daily with Camaronina 35%. Water quality parameters were within standard range for caridean shrimp culture. There was a significant effect of density on final weight, growth rate, specific growth rate, K condition and survival, and an inversely proportional relationship with the feed conversion ratio. The growth was affected by density, resulting to a maximum increase of 6 org/m^3 in the asymmetry of the prawn and also increasing the number of small organisms by 9 org/m^3. However, it is necessary to study such aspects as the rate and frequency of feeding, the initial size-grade and the implementation of shelter, among others.

Additional keywords: biomass; freshwater prawn; optimal growth; stocking size; shrimp.

Abbreviations used: CV (coefficient of variation); DO (dissolved oxygen); FCR (feed conversion ratio); GR (growth rate); HB (harvest biomass); PSI (production-size index); SD (standard deviation); SGR (specific growth rate); TAN (total ammonia nitrogen).

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Introduction

Problems associated with the deterioration of the environmental conditions of the hydrological basins in Latin America have raised concerns to implement systems for aquaculture production of native populations of prawns. Among the species with gradual decline and more aquaculture potential is *Macrobrachium americanum* (Ponce-Palafox *et al.*, 2002; Anger, 2013) among others. These species have the conditions of the key species in the lentic ecosystems, their size, and adaptation to aquaculture production systems, price, and their market demand.

Many studies on biology, reproduction, physiology, biochemistry and growth aspects for caridean shrimp *M. americanum* have been conducted (Ponce-Palafox *et al.*, 2014). However, there are few studies on biotechnological aspects of growth in early juveniles (Mendez-Martinez *et al.*, 2017) and late juvenile-adults (Ponce-Palafox *et al.*, 2014). The generation of technology aspect for the culture of *M. americanum* juveniles, sub-adults and adults is essential for its growth and survival in commercial semi-intensive and intensive farming systems.

The size and survival at stocking density have had a significant impact on production for the culture of crustaceans (Jones & Ruscoe, 2000), so it is necessary to study their behavior in aquaculture systems for different species. Numerous studies on freshwater prawns have shown the effect of density on growth, survival and production in ponds, tanks, cages and pens (Marques *et al.*, 2000, 2012; Lopez-Uriostegui *et al.*, 2014). Density in these species have been compared in mono, polyculture and mixed cultured conditions (Wohlfarth *et al.*, 1985; Alam *et al.*, 2001; Hossain & Islam, 2006; Uddin *et al.*, 2008), suggesting that in the grow-out phase the best densities are between 2 and 6 org/m^2 depending on the species and the culture system. In *M. americanum*, the density effect in juveniles has been studied, obtaining 80% survival (Garcia-Guerrero & Apun-Molina, 2008; Ponce-Palafox *et al.*, 2014). However, little is known as to the effect of density on *M. americanum* in grow-out phase in monoculture systems. The aim of this study was to evaluate the effect of stocking density on growth and survival in *M. americanum* juvenile-adult, cage-cultured and to determine the optimal density for grow-out production.

Material and methods

Site and cage system

The experiment was carried out in a 1,422 m^2 earthen pond located at the Centro de Acuicultura San Cayetano, Tepic, Nayarit Mexico (21°27′24.23″N; 104°49′29.67″W), where 16 bottom cages (3 × 1 × 1 m) were introduced in a semi-rustic rectangular pond. The cage system was used and managed according to what was described by Lopez-Uriostegui *et al.* (2014). 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.

Water quality

Dissolved oxygen (DO), temperature (Yellow Springs Instruments YSI-85), pH (Bernauer F-1002) and water transparency (Secchi disk) were measured daily inside the cage at a depth of 30 cm. Samples of water were collected monthly inside the cages, to determine total ammonia nitrogen (TAN) and alkalinity (YSI 9100 photometer, Yellow Springs Instruments).

Experimental procedures

Shrimps of *M. americanum* (n=228) of 12.8 ± 1.63 g were placed in 16 cages with a stocking density of 1, 3, 6 and 9 org/m^3, respectively, using a completely randomized design with four replicates by treatment. The prawns were fed twice daily with Camaronina 35% (Agribrands, Purina, Mexico, Inc., 35% protein, 8% lipid and 12% moisture), in the same proportion in each cage. The daily feed ratio was adjusted from 5% to 2% at the end of the experiment, based on feed demand and after periodic monitoring of the feed trays. All prawns were collected and weighed monthly to evaluate their growth and adjust the amount of feed supplied. After 152 days, the prawns growth was determined: mean initial weight (W_i) and mean final weight (W_f) in g, growth rate (GR) in g/week, production (P) in g/m^3, harvest biomass (HB) in g/cage, specific growth rate (SGR) in %/day, production-size index (PSI), feeding conversion ratio (FCR) and survival (%). Biological parameters were determined as follows: GR = Final mean weight - Initial mean weight / Time; FCR = Feed fed / Weight gained; SGR = 100 × [(ln W_f – ln W_i) / (t_f - t_i)]; and PSI = (Production × Average weight) / 1000 (Tidwell *et al.*, 1999).

Statistical analysis and model

The growth data were analyzed by Shapiro-Wilks and Bartlett tests, to assess normality and homoscedasticity, respectively. A paired t-test was used to compare the morning and afternoon data of water quality. The growth data were subjected to a one-way ANOVA (Montgomery, 2013). Differences among treatments were measured with Tukey's multiple comparison test of the means. The results were evaluated at 5% significance level. Survival data were square root arcsine transformed prior to analysis. The analyses were conducted using Statistica package v10 (StatSoft, Tulsa, OK, USA). The coefficient of variation was calculated as CV = (Standard deviation / Mean) × 100.

A regression was applied for each stocking density *vs* production relationship of all animals (Bhujel, 2008). The relationship was a quadratic equation of the form:

$$Production = \beta_0 + \beta_1 d + \beta_2 d^2 \tag{1}$$

where β_2 is a quadratic coefficient (other than 0), β_1 is the linear coefficient, β_0 is the intercept and d is density (Bhujel, 2008).

The maximum density was obtained as follows:

$$d_{Max} = \frac{\beta_1}{2\beta_2} \tag{2}$$

where maximum production (*Production* $_{Max}$) is:

$$Production_{Max} = \beta_0 + \frac{\beta_1^{\,2}}{2\beta_2} - \frac{\beta_1}{2} \tag{3}$$

Final optimum production for stocking density was calculated from the first order derivative of the quadratic regressions (*i.e.* when $dP/dS_{density}=0$) (Buhjel, 2008).

Results

The water temperature, DO, pH and TAN concentrations did not show any difference ($p>0.05$) among different stocking densities (Table 1). Alkalinity concentration in the afternoon was significantly higher than in the morning ($p<0.05$).

M. americanum grew from a mean of 12.83 ± 0.64 g to 53.08 ± 29.28 g in 152 days at different stocking densities (Fig. 1; Table 2). After 60 days, differences were found between the densities of 1 to 3 org/m^3 and 6 to 9 org/m^3. Differences were also found between 1 and 3 org/m^3 at 120 days (Fig. 1).

The final weight of the organisms decreased as the density increased from 82.4 to 16.2 g. The SD and CV were the highest in the density of 6 org/m^3 with values of 20.7 and 47.0%, respectively. The organisms with the lowest growth had the lowest weight dispersion in the density of 9 org/m^3 (Table 3).

The mean weight and survival of prawn at harvest (82.4 ± 9.1 g; 100%, respectively) were significantly greater ($p<0.05$) at the lowest stocking density (1 org/m^3), at 152 days (Table 2). Final weight, GR, HB, production, yield, K condition, SGR, and survival were the lowest in the highest density (9 org/m^3). The prawn production and yield in 6 org/m^3 were significantly higher than in other treatments ($p<0.05$) and reached 190.3 g/m^3 and 1,903.0 kg/ha (152 days), respectively. FCR was significantly lower in stocking densities 1 and 3 org/m^3, ranged from 1.4 to 1.6. In general, it was found that there was tendency to present a higher uniformity in the mean weight of the prawn in the treatment with the highest density (9 org/m^3), the highest dispersion of weights of the population in stocking density 6 org/m^3 and the highest weight in the treatment of 1 org/m^3, with ranges from 74.3 to 96.8 g.

Table 1. Variations of water quality variables (mean±SD) in ponds used for rearing of *Macrobrachium americanum* at different stocking densities during a 152 days period.

Variables	Morning	Afternoon
Temperature (°C)	27.0 ± 2.4^a	28.8 ± 2.2^a
Secchi disk (cm)	35.0 ± 0.5	-
Dissolved oxygen (mg/L)	6.2 ± 1.6^a	6.6 ± 0.6^a
pH	8.4 ± 0.2^a	8.5 ± 0.1^a
Alkalinity (mg $CaCO_3$/L)	110.0 ± 0.90^b	135 ± 1.3^a
Total ammonia N (mg/L)	0.19 ± 0.05^a	0.15 ± 0.08^a

Values with the same superscript within the same row are not significantly different at $p<0.05$.

The quadratic regressions= $-8.0222x^2 + 78.629x + 17.54$ ($R^2= 0.9751$; Fig. 2) indicated that the optimum for maximum production increases with stocking density up 3 org/m^3 and was estimated to be 192.1 g/m^3. Since that space does not increase in the cage, prawn production ceases when their standing crop reaches about 6 org/m^3, and survival decreases from 100 to 92%.

Discussion

Water parameters were within range for *Macrobrachium* species (Boyd & Zimmerman, 2000; New, 2002) and those recorded in the culture of

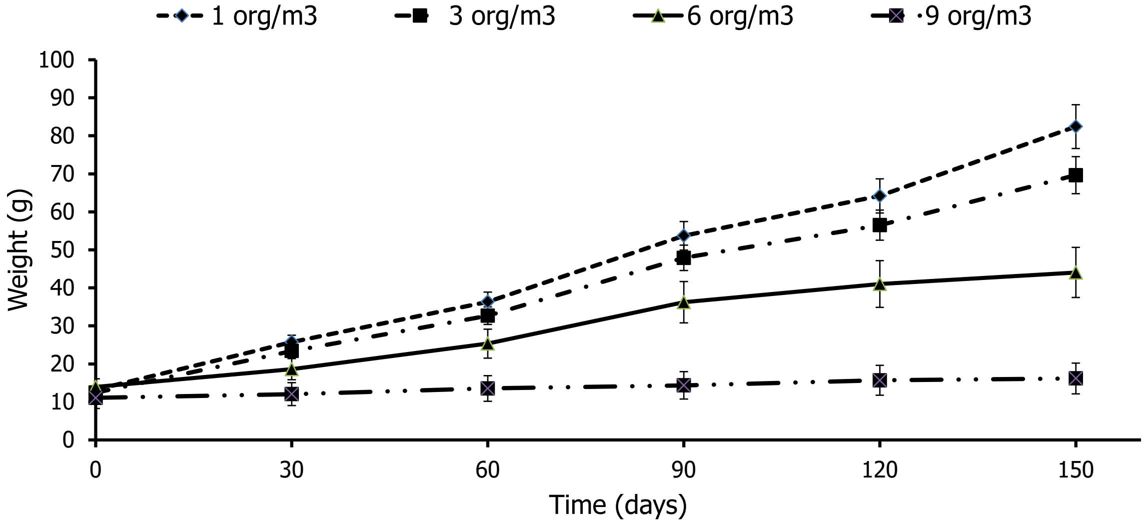

Figure 1. Growth curves for *Macrobrachium americanum* at different stocking densities during a 152 days period. Errors bars: ± SE.

Table 2. Growth performance, production and survival of *Macrobrachium americanum* at different stocking densities during a 152 days period (mean ± SD). T1= 1 org/m^3, T2= 3 org/m^3, T3= 6 org/m^3, T4= 9 org/m^3.

Variables	T1	T2	T3	T4
Initial weight (g)	13.2 ± 2.0^{a}	12.5 ± 1.9^{a}	13.5 ± 2.3^{a}	12.1 ± 1.7^{a}
Final weight (g)	82.4 ± 9.1^{a}	69.6 ± 7.7^{b}	44.1 ± 20.4^{c}	16.2 ± 1.9^{d}
Growth rate (g/week)	3.2 ± 1.2^{a}	2.7 ± 1.1^{b}	1.5 ± 1.0^{c}	0.2 ± 0.1^{d}
Harvest biomass (g/cage)	247.2 ± 9.7^{b}	576.3 ± 11.7^{a}	571.5 ± 11.2^{a}	236.2 ± 8.9^{c}
Production (g/m^3)	82.4 ± 12.7^{b}	192.1 ± 21.9^{a}	190.5 ± 18.5^{a}	78.7 ± 10.6^{b}
Yield (kg/ha (152 days))	824.3 ± 92.4^{b}	$1{,}921.0 \pm 122.2^{a}$	$1{,}905.1 \pm 115.7^{a}$	787.3 ± 63.9^{c}
Specific growth rate (%/day)	1.2 ± 0.1^{a}	1.1 ± 0.2^{a}	0.8 ± 0.1^{b}	0.2 ± 0.1^{c}
Production-size index	67.9 ± 6.9^{c}	133.7 ± 12.1^{a}	84.0 ± 8.6^{b}	12.8 ± 1.6^{d}
Feeding conversion ratio	$1.4 \pm 0.1^{c,b}$	1.6 ± 0.3^{b}	1.9 ± 0.6^{b}	2.6 ± 0.7^{a}
K condition	1.5 ± 0.1^{a}	1.4 ± 0.1^{a}	$1.2 \pm 0.2^{a,b}$	0.9 ± 0.1^{b}
Survival (%)	100 ± 2.1^{a}	92 ± 3.8^{b}	72 ± 2.5^{c}	54 ± 1.2^{d}

Values with the same superscript within the same row are not significantly different at $p<0.05$).

stocking sizes in semi-natural ponds for *M. americanum* (Ponce-Palafox *et al.*, 2014). This study confirms that *M. americanum* can be grown in a grow-out phase in cage-cultured in a pond system at low densities, finding an inverse relationship between size and density, as it has been reported for this species (Ponce-Palafox *et al.*, 2014) and other native species of Latin America (Marques *et al.*, 2010; Lopez-Uriostegui *et al.*, 2014). The high growth rates of the prawn *M. americanum* in the cage-cultured in a pond system was due to the high availability of live food associated with periphyton, zooplankton and benthos (Uddin *et al.*, 2008; Marques *et al.*, 2010; Mohanty, 2010; Marques & Lombardi, 2011), and commercial feed with 35% protein in diet (Ponce-Palafox *et al.*, 2014).

When the density increased from 1 to 6 org/m^3, the weight of the prawn decreased 50%, similar to what happens to *M. rosenbergii* in earthen ponds (Wohlfarth *et al.*, 1985). The positive correlation of FCR and density found showed that as the density increases, the efficiency of the feed decreases (Martin *et al.*, 1998). Stocking size affects the population structure, juvenile grading and selectively harvesting in prawns grown in cages (Karplus *et al.*, 1986a; Lopez-Uriostegi *et al.*, 2014). In this study it was determined that at low density, juveniles play smaller roles in the subsequent morphological development. This showed a structure similar to the ungraded population. This differs from that found in *M. rosenbergii* where juvenile jumpers are transformed into large prawns and laggards into small males (Karplus, 2005). However, this is similar to that found in *M. amazonicus*, where prawn development can show a directional process which produces a stable regulation through environmental factors (Maciel & Valenti, 2009). In 9 org/m^3 there was a partial compensatory growth in this species, which has been reported for *M. rosenbergii* and other species in high densities (Daniels *et al.*, 1995; Preto *et al.*, 2010; Marques & Lombardi, 2011).

Table 3. Characterization of the distribution of weights in *Macrobrachium americanum* at different stocking densities to the final weight (W_f) in a 152 days period. T1= 1 org/m^3, T2= 3 org/m^3, T3= 6 org/m^3, T4= 9 org/m^3.

Parameters	T1	T2	T3	T4
$W_{f\text{-mean}}$ (g)	82.4	69.6	44.1	16.2
SD	9.1	7.7	20.7	1.9
CV	11.0	11.0	47.0	12.0
$W_{f\text{-min}}$ (g)	74.3	63.0	15.5	13.0
$W_{f\text{-max}}$ (g)	96.7	82.0	61.8	18.0

The coefficient of variation of weight was higher in 6 org/m^3 (47.0%) because the larger prawns negatively affect the growth of the smallest ones and the structure of the population was more homogeneous in low densities. The CV (11%) of the densities of 1 to 3 org/m^3 showed that in this species, the population tended to a "steady stable" of the morphotype distribution (D'Abramo *et al.*, 1989; Sampaio & Valenti, 1996). The CV showed that increasing stocking density up to 6 org/m^3 increased production, but decreased commercial production by decrease mean weights.

At the density of 6 org/m^3, strong density dependence was found at a high mortality and low growth rate. Strong density-dependence was established when growth in mean weight was high. The effect of "heterogeneous individual growth" (HIG) was greater at a density of 6 org/m^3. The population was more complex at a density of 9 org/m^3, and smaller sized transformation occurs without much increase to their body size, which

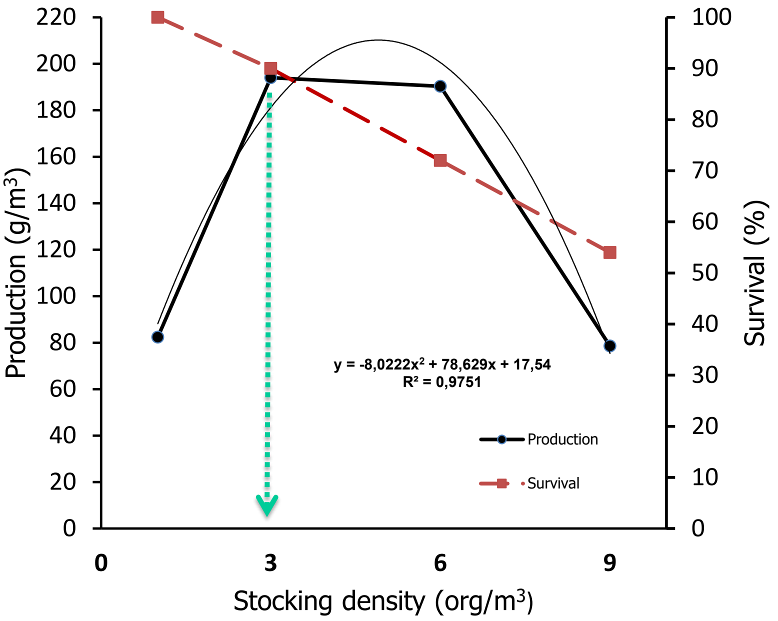

Figure 2. Relationship between production and survival of *Macrobrachium americanum* at different densities in the cage-culture in a pond system.

has been observed in other species (Ranjeet & Kurup, 2002). Space and natural food can be limiting in high densities, but the agonistic and social behavior increases disproportionately (Moraes-Valenti *et al.*, 2010).

In freshwater prawns stocked up to 6 org/m^3, yields were significantly greater (p<0.05) than in 1 org/m^3 (1,905.1 and 824.3 kg/ha (152 days), respectively). Also, weight and SGR were decreased (p<0.05) in prawns stocked at 6 and 9 org/m^3. From 1 to 3 org/m^3, higher values of SGR (1.1 to 1.2) were obtained than those recorded by Ponce-Palafox *et al.* (2014) for this species. However, the SGR was below those recorded for *M. rosenbergii* (3.86 to 4.96) in ponds (Tidwell *et al.*, 2004; Hossain & Islam, 2006).

The results obtained with *M. americanum* were in agreement with those found for *M. rosenbergii*, where the increase in production reduced the sizes at high density (Karplus *et al.*, 1986b). The increase in PSI with increased density up to 3 org/m^3 indicated a positive balance in the production/size ratio. In this density, the production was more important than the low weight (Tidwell *et al.*, 2000). The values obtained in the density of 3 org/m^3 (133.7 ± 12.1) were similar to those reported for *M. rosenbergii* at 133 days (Tidwell *et al.*, 2003), obtaining a high growth.

The quadratic regressions (Fig. 2) showed that the optimum stocking density considering the effect of production and survival (maximum standing crop) in semi-intensive systems was estimated to be 3 org/m^3 or 192.1 g/m^3. This density is within the range reported for high growth in grow-out phase in this species (Ponce-Palafox *et al.*, 2014).

Survival of *M. americanum* was inversely proportional to density, which is in agreement with that found in most of the monocultures of freshwater prawn (Valenti & New, 2000; Uddin *et al.*, 2008). The survival obtained at densities of 1 to 6 org/m^3 was found within the range of 72 to 100%, suitable for commercial freshwater prawn culture (Karplus *et al.*, 1986b; Hosain & Islam, 2006; Ponce-Palafox *et al.*, 2014).

Our results indicate that *M. americanum* shows a density-dependent effect when the density was ≥ 6 org/m^3 in grow-out, and mortality was high from 9 org/m^3. The results of this research utilized bioengineering information on handling *M. americanum* in grow-out cages system. However, further research is required on the uniformity of size, feeding rates, and shelter, among others.

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