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White shrimp *Penaeus vannamei* culture in freshwater at three densities: Condition state based on length and weight

Marcelo Araneda [a,b,*], Eduardo P. Pérez [c,d], Eucario Gasca-Leyva [b]

[a] Universidad Marista de Mérida, Periférico norte tablaje catastral 13941 Carretera Mérida — Progreso, C.P. 97300 Mérida Yucatán, Mexico
[b] Centro de Investigación y Estudios Avanzados (CINVESTAV) Unidad Mérida, Apdo. Postal 73-CORDEMEX, 97310 Mérida Yucatán, Mexico
[c] Universidad Católica del Norte, Facultad de Ciencias del Mar, Departamento de Biología Marina, Casilla 117. Coquimbo, IV Región, Chile
[d] Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Casilla 599, Benavente 980, La Serena, Chile

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ABSTRACT

The commercial culture of shrimp in low water salinities has been successfully achieved. However, there are no long-term studies on the growth response of shrimp culture in freshwater. In the present study white shrimp *Penaeus vannamei* was cultured in freshwater (0 ppt) at three densities (90, 130 and 180 shrimp m^{-2}) and comparisons made of the resulting growth, length–weight relationship and condition factor data. Gravimetric data were recorded during a 210-day trial to estimate growth rates, the length–weight relationship and the condition factor (according to Fulton's equation). Weight gain results showed the freshwater culture system to be viable, with effects from density ($P<0.05$). Growth rates decreased as density increased, exhibiting an inverse relationship ($P<0.05$, $R^2=0.68$). Survival decreased as density increased with differences between treatments ($P<0.05$). A potential model was used to analyze the length–weight relationship of the treatments. The slopes were different between treatments according to an ANCOVA and a Tukey test ($P<0.05$). The function exponents showed that the 130 shrimp m^{-2} treatment produced organisms with isometric growth ($b=2.99$) in good condition ($t=0.96$; $P>0.05$). The 90 shrimp m^{-2} treatment produced positive allometric growth and the 180 shrimp m^{-2} treatment negative allometric growth.
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1. Introduction

Over 90% of shrimp culture in the Western hemisphere is represented by white shrimp (*Penaeus vannamei*) (Wurmann et al., 2004). This species is generally cultivated in semi-intensive systems located near coasts. Successful culture of white shrimp depends on the quality of seawater used in the system, as well as use of a wastewater treatment plant to prevent pollution of adjacent areas. A number of factors have limited expansion of white shrimp culture, including the high cost of coastal real estate and the constant appearance of viral diseases such as white spot (WSSV), which has brought the industry near collapse (Jory and Dixon, 1999).

One proposed solution to white shrimp production problems is the use of water with salinities lower than seawater. Using water with 0.5 ppt salinity, Van Wyk et al. (1999) reported survival and growth rates below those with seawater. In evaluations of postlarvae survival as a function of age in salinities varying from 16.3 to 0 ppt, both McGraw et al. (2002) and Saoud et al. (2003) reported increased survival as the organisms reached 15 days old at salinities greater than 4 ppt, but only 77% survival at 0 ppt. In studies of postlarvae and juvenile growth and survival in seawater diluted with freshwater and supplemented with a salt mixture, Atwood et al. (2003) and Sowers et al. (2005) reported no osmotic dysfunction although the organisms did not grow as efficiently compared to those in seawater. Samocha et al. (2004) and Sowers and Tomasso (2006) reported growth higher than in seawater using low salinity (2 ppt) water.

No research has yet been done on the long-term growth performance of *P. vannamei* in freshwater (0 ppt), including the effect of stocking density on weight gain and condition factor. The present study aims to compare the length–weight relationship and condition state in growth stage white shrimp cultured in freshwater (0 ppt) at three densities (90, 130 and 180 shrimp m^{-2}). The results provide practical data on growth, survival and organism condition indicators (i.e. length–weight relationship) for these culture conditions.

2. Materials and methods

2.1. Water chemical composition

Water used in the experiment came from the subterranean aquifer in the northern Yucatan Peninsula, Mexico, at 18 m depth. Before the trial began, its chemical composition was analyzed in the Marine Chemistry Laboratory, CINVESTAV-IPN, Mérida. The APHA (1989) titration technique was used to determine total hardness ($CaCO_3$) (400 mg l^{-1}),

* Corresponding author. Tel.: +52 999 9410302; fax: +52 999 9410307.
E-mail addresses: maraneda@marista.edu.mx (M. Araneda), eperez@ucn.cl (E.P. Pérez), eucario@mda.cinvestav.mx (E. Gasca-Leyva).

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alkalinity (325.49 mg l^{-1}) and chloride (Cl^-) (125.69 mg l^{-1}). The atomic absorption method (using a CBC 932 PLUS® spectrophotometer) was used to determine the concentrations of the inorganic ions potassium (K^+; 3.37 mg l^{-1}), calcium (Ca^{2+}; 85.49 mg l^{-1}), sodium (Na^+; 68.64 mg l^{-1}) and magnesium (Mg^{2+}; 27.52 mg l^{-1}). Concentration of the $SO_4^{=}$ ion was done with the turbidometric method using a 21D UV® spectrophotometer (269.50 mg l^{-1}). Salinity (0 ppt) was measured with an induction salinometer (KAHLSICO RS-9®), electrical conductivity (1014 μs cm^{-1}) with a conductimeter (CORNING CHECKMATE®) and pH (7) with a potentiometer (BECKMAN 32®). Colorimetric reactions followed by spectrophotometric analysis (SHIMADUZU UV-1201®) were used to determine ammonium (0.027 mg l^{-1}), nitrites (0 mg l^{-1}) and nitrates (6.55 mg l^{-1}).

2.2. Experimental organisms and acclimation

Postlarvae (PL_8; approx. 0.0025 g individual weight according to manufacturer technical data) were acquired from SYAQUA (Mazatlan, Sinaloa, México) and transported by air cargo in plastic bags containing 38 ppt seawater to the experimental unit at the Universidad Marista de Mérida (México).

The postlarvae were stocked in three, 600-l circular tanks with constant aeration and 38 ppt salinity for six days. Acclimation was carried out over 10 days and begun once the organisms had reached PL_{15}. During this time salinity was lowered from 38 to 0 ppt at an average rate of 4 ppt day^{-1}, and salinity measured every 2 h with a refractometer (CHASEBRAN®). Once acclimated, the organisms were allowed to grow to juvenile stage for 30 days in the 0 ppt water at an average of 26 °C, and fed ad libitum.

2.3. Experimental design

The study was designed to evaluate the effect of stocking density on weight gain and condition factor in white shrimp *P. vannamei* cultured in freshwater. The evaluated densities were 90, 130 and 180 shrimp m^{-2}. Juveniles (0.48 ± 0.03 g) were randomly assigned to one of nine tanks, with three tanks per density treatment. The recirculation system consisted of round, polyethylene, 1200-liter tanks (2 m^2 surface area and 0.60 m high); two, 5 μm-capacity filter cartridges (10 in. Big Blue); a wet–dry (or trickling) biological filter with a 405 m^2 m^{-3} effective fixation surface; a rectangular concrete sedimentation tank (2.7 m^2 surface area) and a 1 hp blower (SWEETWATER®) with air diffusers. To maintain good water quality, the water exchange rate was 0.6 renewals h^{-1}, with a daily replacement of approximately 5% total volume and daily removal of unconsumed feed and feces.

The organisms were fed a commercial balanced feed (Purina™, Camaronina, Agribrands, Mexico) containing 35% protein. Feed ion content was determined with an atomic absorption spectrophotometer (GBC 932 PLUS®): K^+ = 8.44 g K^+ kg^{-1}; Mg^{2+} = 1.76 g Mg^{2+} kg^{-1}. Feed rate was four times a day (09:00, 13:00, 17:00 and 21:00 h), as recommended by Van Wyk et al. (1999). Water temperature and oxygen content were measured three times a day (08:00, 13:00 and 18:00 h) in each tank with an oxymeter (YSI 200®). Weekly measurements were made of nitrogenated compounds such as non-ionized ammonium, nitrite and nitrate with a spectrophotometer (HACH®), and of pH with a potentiometer (BECKMAN 32®). These were taken in one randomly chosen tank per treatment.

2.4. Growth rates, length–weight relationship and condition factor

Gravimetric data were collected weekly throughout the 210-day (30-weeks) experimental period. An ichthyometer (mm) was used to measure total body length, and rostrum-to-telson distance. A digital scale (0.01 g; EXCELL®) was used to record weight after excess moisture was removed from each organism. Sample size (n) for each replicate was estimated following the method of Zar (1999). Growth rate was estimated for each density treatment as a function of weight and time with the formula:

$$GR_i = \frac{W_{fi} - W_{oi}}{t} \quad (1)$$

Where GR_i is the growth rate of treatment i; and W_{fi} and W_{oi} are average final and initial weight in time t. The total length–weight relationship for the different ages was adjusted using the potential model:

$$W_i = a \cdot TL_i^b \quad (2)$$

Where W_i is organism weight (g) for treatment i; TL_i is total length (mm) for treatment i; a is the proportionality constant; and b the isometric exponent. Fulton's equation was used to determine condition factor (Ricker, 1975; Chow and Sandifer, 1991), which varies according to organism reproductive stage and general culture conditions:

$$CF_i = \frac{\overline{W}_i}{\overline{TL}^3} \cdot 10^5 \quad (3)$$

Where CF_i is the condition factor for treatment i; $\overline{W}_i$ is average weight (g) and $\overline{TL}_i$ is average total length (mm).

2.5. Statistical analysis

Comparison of growth rates and final survival between treatments was done with a one-way analysis of variance (ANOVA) and a Tukey HSD *a posteriori* test. Data were expressed as the mean ± standard error. The effect of density on growth behavior over the 30-week experimental period was estimated with a single factor repeated-measures variance analysis. A non linear regression analysis was used to determine the relationship between density and growth rates. The resulting potential curves for the three studied densities were compared with an analysis of covariance (ANCOVA; Zar, 1999) and a Tukey *a posteriori* test. When parallelism was present between slopes, an intercept analysis was done with a Tukey test (Zar, 1999). To evaluate the relationship between weight and total length in each treatment, the statistical significance of the isometric exponent (b) was analyzed with a function proposed by Pauly (1984):

$$\hat{t} = \frac{s.d.(x)}{s.d.(y)} \cdot \frac{|b_i - 3|}{\sqrt[2]{1-r^2}} \cdot \sqrt[2]{n_i - 2} \quad (4)$$

Where t is the t-student statistic; s.d.(x) and (y) are the standard deviation of the log TL_i and W_i value of each treatment; n_i is the number of recorded organisms; b_i is the adjusted b value; and r^2 is the potential fit determination coefficient. The significance level was $\alpha \leq 0.05$. The analyses were conducted using the SPSS for Windows v. 11.0.0 program (SPSS Inc., Chicago, IL).

3. Results

The highest growth rate was observed in the 90 shrimp m^{-2} treatment, with an average of 0.38 g $week^{-1}$, and the lowest in the 180

Table 1
Average weekly growth rates and final survival (±standard error) of white shrimp grown in freshwater (0 ppt) at three different stocking densities

Treatment (shrimp m^{-2})	Culture time (weeks)	Growth rate (g $week^{-1}$)	Survival (%)
90	30	$0.38^a \pm 0.011$	$76.1^a \pm 3.16$
130	30	$0.34^{a,b} \pm 0.007$	$68.9^{a,b} \pm 0.78$
180	30	$0.33^b \pm 0.009$	$65.9^b \pm 0.91$

Different letter superscripts in the same column indicate statistically significant differences ($P<0.05$).

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