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Short Communication

Effect of stocking density on growth and survival of the prawn *Macrobrachium tenellum,* cultured in a recirculating aquaculture system

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ABSTRACT. The stocking density of the freshwater prawn native to the Mexican Pacific coast, *Macrobrachium tenellum,* has not been evaluated in a recirculating aquaculture system. *M. tenellum* prawns with an average wet weight of 1.71 ± 0.11 g were reared for 60 days at three stocking densities (T1 = 10 ind m^{-2}; T2 = 15 ind m^{-2}; T3 = 20 ind m^{-2}), in nine (1 m^2) tanks connected to a recirculating aquaculture system inside a greenhouse. Average individual weight gain (AIWG), biomass weight gain (BWG), specific growth rate (SGR), food conversion ratio (FCR) and survival rate (SR) were determined. Stocking density affected the BWG and SR. The lowest BWG was observed at the highest density (T1, 20.70 ± 4.75 g m^{-2}; T2, 20.75 ± 4.72 g m^{-2}; T3, 11.31 ± 3.65 g m^{-2}), although weight per area unit increased with densities. SR decreased with increasing density (T1, 77.77 ± 9.62%; T2, 59.25 ± 12.83%; T3, 44.44 ± 4.81%). Other parameters did not show a difference between densities, with a maximum average individual weight at T2 (5.41 ± 1.14 g). The overall results suggested that stocking density affected the productivity and survival but not the individual growth of juvenile *M. tenellum* prawns cultured in a recirculating aquaculture system.

Keywords: *Macrobrachium tenellum*; freshwater prawn; growth performance; survival; aquaculture; Mexican Pacific coast

The stocking density can directly influence the growth and survival of prawns (New *et al*., 2010). A density lower than the optimum could reduce the overall productivity by not using all the available space, while a high density can reduce survival and the individual growth of prawns (D'Abramo *et al*., 2000; Paul *et al*., 2016). These effects are of greater importance in semi-intensive or intensive systems, such as in recirculating aquaculture systems, where available space is restricted (New *et al*., 2010). Available studies, on the stocking density of freshwater prawns, were carried out on the giant river prawn *Macrobrachium rosenbergii* (Sandifer & Smith, 1975; D'Abramo *et al*., 2000; Cuvin-Aralar *et al*., 2007; Ikhsan & Rajaee, 2016; Paul *et al*., 2016). *M. rosenbergii* is the most cultured freshwater prawn on the Mexican Pacific coast (López-Uriostegui *et al*., 2014). Some native species of the same genus such as *M. carcinus*, *M. acanthurus*, *M. tenellum,* and *M. americanum* offer a high potential for use in aquaculture (New *et al*., 2010; García-Guerrero *et al*., 2013). Among these species, *M. tenellum* stands out due to its tolerance to a wide range of fluctuating temperatures, salinity, and oxygen concentrations, it can be found at high densities under natural conditions, it has a low aggressiveness and adults are unable to leave the water (Ponce-Palafox *et al*., 2013; López-Uriostegui *et al*., 2014).

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Research studies of *M. tenellum* stocking density have been carried out (López-Uriostegui *et al*., 2014). However, the reported studies where performed in laboratory conditions or inside grow-out pond systems, and there is no information about the required stocking density to produce *M. tenellum* inside a recirculating aquaculture system (RAS). Available space for RAS is drastically reduced in comparison to traditional pond systems, and these recirculating systems usually present a minimum water exchange with a high flow rate (Ebeling & Timmons, 2012), conditions that could affect the optimum stocking density, since there have been reports that the water replacement rate could affect the growth of freshwater prawns (D'Abramo *et al*., 2000). This study aimed to evaluate the influence of different stocking densities on the growth performance, and survival of *M. tenellum* reared in a recirculating aquaculture system.

Berried females were captured and allowed to hatch in an aquaculture farm (Acuícola S.A. de C.V., Colima, Mexico) to obtain juveniles. Juveniles (0.78 ± 0.31 g) were transported to the experimental facility in a 500 L fiberglass tank with aerators. Upon arrival, individuals were held in a 20 m^3 tank and acclimated for 15 days to recirculating conditions. They were fed with marine shrimp commercial pelletized food (40% crude protein, Camaronina Purina, Mexico) with a 10% daily feed ratio. After acclimating time prawns were transferred to the experimental units in the required densities.

The experiment was performed within a 504 m^2 (18×28 m) polyethylene greenhouse in El Marqués, Querétaro, Mexico (20°42'18"N, 100°15'36"W). The experimental system consisted of nine 500 L High-Density Polyethylene oval tanks (50×120×90 cm) connected to the same recirculating aquaculture system with a 1 m^3 reservoir, and an operating volume of 6 m^3 with a 2% daily water exchange. A canister biofilter with a UV light treatment was used to maintain the water conditions with full water filtration every 25 min. PVC pipes were provided as refuges for the prawns.

Water temperature (°C) was measured every 3 h during the experimental time using a Watchdog 100 datalogger (Spectrum Technologies Inc.). Dissolved oxygen (mg L^{-1}) and pH were monitored daily using the multiparameter probe HQ40D by Brand Hach, USA (LDO101-05 sensor O_2; PHC101-05 sensor pH). Every week analysis of ammonia (mg L^{-1}), nitrates (mg L^{-1}), and nitrites (mg L^{-1}) in water was performed through spectrophotometric techniques using the spectrophotometer Hach DR/6000.

M. tenellum juveniles (n = 81; weight 1.71 ± 0.11 g) were randomly stocked in triplicate experimental tanks at densities of 10 ind m^{-2} (T1), 6 individuals per tank; 15 ind m^{-2} (T2), 9 individuals per tank; and 20 ind m^{-2} (T3), 12 individuals per tank. The stocking densities used in this experiment were inside the range of a semi-intensive (10 ind m^{-2}) to an intensive (20 ind m^{-2}) culture systems (López-Uriostegui *et al.*, 2014). Parameters were obtained per area unit due to the benthic behavior of the species (D'Abramo *et al*., 2000), although the volumetric densities for this study are: T1 (12 ind m^{-3}), T2 (18 ind m^{-3}) and T3 (24 ind m^{-3}).

The prawns were fed daily with a commercial shrimp diet (40% crude protein, 7% lipid, and 12% moisture; Nutripec Camaronina, Purina, Mexico). The daily feed ratio was determined based on 10% of wet weight and adjusted after each measurement (De los Santos-Romero *et al*., 2017). Survival was registered daily. The trial lasted 60 days. At days 0, 20, 40 and 60 all prawns were individually weighed (±0.001 g, Sartorius AY303 Milligram Scale) and total length was measured by linear distance from the tip of the rostrum to the tip of the telson (±0.01 mm, Truper Stainless Steel Vernier).

Average values of each experimental tank were used to determine the following parameters: average individual weight (AIW; g), average individual weight gain (AIWG; g), average individual daily growth rate (AIDGR; g d^{-1}), biomass weight per area unit (W_t; g m^{-2}), biomass weight gain per area unit (BWG; g m^{-2}), specific growth rate (SGR % d^{-1}), food conversion ratio (FCR) and survival rate (SR, %) as stated previously (García-Trejo *et al*., 2016; Peña-Herrejón *et al*., 2016; De los Santos-Romero *et al*., 2017).

The prawn performance parameters and water conditions were analyzed with one-way ANOVA to determine significant differences between treatments. Normality tests were performed (Kolmogorov-Smirnov test, $\alpha = 0.05$). When differences were found, a test of the least significant differences (LSD, $\alpha = 0.05$) was performed. All statistical analyses were performed with 95% reliability using Statgraphics routine Centurion XVI v16.1.11 software.

The water conditions (temperature, dissolved oxygen, pH, NH_4^+, NO_2^- and NO_3^-) during the experimental period were not significantly different between treatments or tanks ($P > 0.05$), as expected since the tanks were connected to the same recirculating aquaculture system. The average values of all tanks are shown in Table 1. The minimum temperature was 26.2°C and the maximum 30.4°C.

Growth performance of *M. tenellum* is presented in Table 2. Initial AIW, initial length, final density, final length, final AIW, AIWG, SGR, and FCR showed no significant differences between treatments ($P > 0.05$). On day 20, individuals density had a significant difference ($P < 0.05$) between T1 and T3, without difference with T2, but on day 40 (T1, 7.77 ± 0.96 ind

Table 1. Average water parameters of the *Macrobrachium tenellum* recirculating aquaculture system during the experimental time. Values are the mean ± standard deviation.

Parameter	Mean ± SD
Water temperature (°C)	27.9 ± 1.1
Dissolved oxygen (mg L^{-1})	6.64 ± 0.67
pH	9.36 ± 0.23
NH_4^+ (mg L^{-1})	0.04 ± 0.05
NO_2^- (mg L^{-1})	0.05 ± 0.01
NO_3^- (mg L^{-1})	1.24 ± 0.69

m^{-2}; T2, 9.44 ± 0.96 ind m^{-2}; T3, 10 ± 1.66 ind m^{-2}) and day 60 (T1, 7.77 ± 0.96 ind m^{-2}; T2, 8.88 ± 1.92 ind m^{-2}; T3, 8.88 ± 0.96 ind m^{-2}); there was no significant difference between densities ($P > 0.05$) (Fig. 1).

Final W_t, BWG; and SR data showed significant differences ($P < 0.05$) among densities. The SR decreased with density, obtaining a significant difference between T1 (77.77 ± 9.62%) and T3 (44.44 ± 4.81%), (T2 59.25 ± 12.83%) had no significant difference with the other densities.

W_t had no significant differences on day 20 (T1 24.58 ± 1.32 g m^{-2}; T2 28.78 ± 6.04 g m^{-2}; T3 32.1 ± 4.7 g m^{-2}). On day 40, T1 (26.78 ± 1.98 g m^{-2}) and T3 (36.30 ± 3.23 g m^{-2}) were significantly different among them, without a significant difference with T2 (31.37 ± 3.04 g m^{-2}) (Fig. 2). On day 60 the significant difference was between T1 (37.84 ± 4.57 g m^{-2}) and T2 (46.77 ± 4.79 g m^{-2}), without a significant difference with T3 (45.39 ± 2.69 g m^{-2}) (Fig. 3). The density-W_t relationship produced a quadratic equation of the form (Fig. 3):

$$W_t = -0.206(\text{density}^2) + 6.936(\text{density}) - 10.93 \quad (1)$$

Equation (1) indicates that 16.83 ind m^{-2} produces the maximum biomass at 60 days.

The maximum BWG of 20.75 ± 4.72 g m^{-2} was obtained at T2 without a significant difference with T1 (20.70 ± 4.75 g m^{-2}) and T3 (11.31 ± 3.65 g m^{-2}), but there was a significant difference between T1 and T3 ($P < 0.05$).

Water temperature, dissolved oxygen, and NH_4^+ were adequate for growing freshwater prawns (New *et al*., 2010; Ponce-Palafox *et al*., 2013); pH 9.36 ± 0.23 was above the maximum recommended value of 8.5 for *M. tenellum* (Ponce-Palafox *et al*., 2002). Therefore, pH could have influenced the prawn performance in this experiment. It has been reported that low and high pH negatively affects growth and survival in *M. rosenbergii* (Cheng *et al*., 2003; Kawamura *et al*., 2015). A high pH (8.81-9.88), as observed in this study, suppress the prawn's immune system (Cheng & Chen, 2000; Cheng *et al*., 2003). As a low concentration of NH_4 was maintained throughout the experimental period, it is considered that the pH effect was not critical (Cheng *et al*., 2003), since growth performance was similar to what was observed in other studies with *M. tenellum* (Vega-Villasante *et al*., 2011).

Densities differences have a tendency to decrease with time, obtaining no significant differences among treatments at the end of experimental time (Fig. 1), this trend is observed in previous stocking densities studies with *M. tenellum* in cage-pond systems (López-Uriostegui *et al*., 2014) and with *M. rosenbergii* (Cuvin-Aralar *et al*., 2007; Sandifer & Smith, 2009). The SR exhibit an inverse relationship with density, as observed in other studies (Cuvin-Aralar *et al*., 2007; Yamasaki-Granados *et al*., 2013; Ponce-Palafox *et al*., 2014). The obtained SR for *M. tenellum* (T1, 77.77 ± 9.62%; T2, 59.25 ± 12.83%; T3, 44.44 ± 4.81%) are similar to the previously observed values for the same densities in cage pond systems (65.5 ± 0.9%; 55.5 ± 0.3%; 47.5 ± 0.6%) (López-Uriostegui *et al*., 2014). Also, previous studies indicate that a survival higher than 50% is acceptable (New *et al*., 2010), what allows us to assume that this recirculating aquaculture system provides adequate conditions for the survival of *M. tenellum*.

Final AIW showed no difference between densities inside the current experimental recirculating aquaculture system in contradistinction to previous reports in pond systems, where a significant difference was observed between densities and their final AIW (López-Uriostegui *et al*., 2014). Though in previous reports in *M. rosenbergii* there was no AIW difference between the higher (40 ind m^{-2}) and lower (20 ind m^{-2}) density, and also there was no difference between the higher and middle (30 ind m^{-2}) density, close to what was observed here (Banu *et al*., 2016). The obtained final AIW in this experiment was lower than the previously observed values for *M. tenellum* at 60 days, which were of about 10 g (Vega-Villasante *et al*., 2011; Ponce-Palafox *et al*., 2013; López-Uriostegui *et al*., 2014). The obtained AIW could have been influenced by the high flow rate of the system (a full water replacement in 25 minutes), since, as previously reported in *M. rosenbergii*, a high water replacement rate can diminish the beneficial effect of increasing the surface area (D'Abramo *et al*., 2000). More studies would be needed to determine if there is an effect on the growth rate of *M. tenellum* or not. Despite the lower final AIW, the AIDGR (T1, 0.054 ± 0.021; T2, 0.061 ± 0.018; T3, 0.057 ± 0.009) was inside the range of previous reports for *M. tenellum*, 0.007-0.135 g for 60 culture days (Vega-Villasante *et al*., 2011; De los Santos-Romero *et al*., 2017), thus, it would be necessa-

Table 2. Growth performance of *Macrobrachium tenellum* cultured at different stocking densities in a recirculating aquaculture system. Within a row, means accompanied by different lowercase letters are significantly different ($P < 0.05$). Values are the mean ± standard deviation. T1: 10 ind m^{-2}, T2: 15 ind m^{-2}, and T3: 20 ind m^{-2}. AIW: average individual weight, AIWG: average individual weight gain, AIDGR: average individual daily growth rate, Wt: biomass weight per area unit, BWG: biomass weight gain per area unit, SGR: specific growth rate, FCR: food conversion ratio, and SR: survival rate.

	Experimental tank		
	T1	T2	T3
Initial density (ind m^{-2})	10.00 ± 0.00	15.00 ± 0.00	20.00 ± 0.00
Final density (ind m^{-2})	7.77 ± 0.96	8.88 ± 1.92	8.88 ± 0.96
Initial AIW (g)	1.71 ± 0.14	1.73 ± 0.10	1.70 ± 0.11
Final AIW (g)	4.97 ± 1.28	5.41 ± 1.13	5.14 ± 0.55
Initial length (mm)	43.98 ± 2.30	45.50 ± 1.06	42.92 ± 1.83
Final length (mm)	74.95 ± 7.77	79.27 ± 10.22	78.71 ± 1.54
AIWG (g)	3.25 ± 1.30	3.67 ± 1.10	3.43 ± 0.56
AIDGR (g day^{-1})	0.054 ± 0.021	0.061 ± 0.018	0.057 ± 0.009
Initial W_t (g m^{-2})	17.13 ± 0.28 z	26.01 ± 0.47 y	34.07 ± 0.97 x
Final W_t (g m^{-2})	37.83 ± 4.57 z	46.76 ± 4.70 y	45.39 ± 2.69 zy
BWG (g m^{-2})	20.70 ± 4.75 z	20.75 ± 4.72 zy	11.31 ± 3.65 y
SGR (% d^{-1})	1.74 ± 0.42	1.87 ± 0.31	1.83 ± 0.19
FCR	5.26 ± 1.81	4.33 ± 0.90	4.71 ± 0.50
SR (%)	77.77 ± 9.62 z	59.25 ± 12.83 zy	44.44 ± 4.81 y

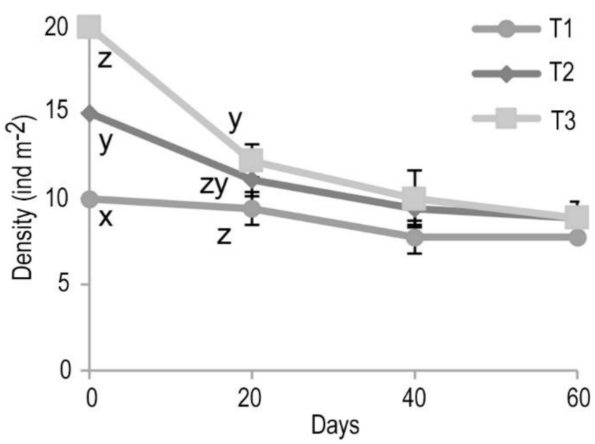

Figure 1. *Macrobrachium tenellum* density changes during the experimental time in a recirculating aquaculture system. T1: 10 ind m^{-2}, T2: 15 ind m^{-2}, and T3: 20 ind m^{-2}. Within a day, different lowercase letters indicate a significant difference between densities ($P < 0.05$).

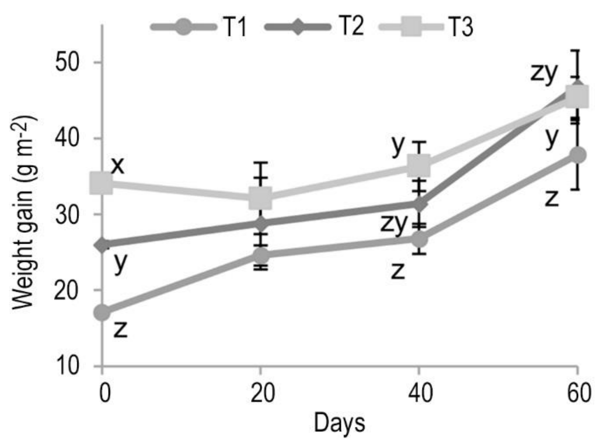

Figure 2. Weight gain per unit area (W_t) of *Macrobrachium tenellum* during the experiment at different stocking densities in a recirculating aquaculture system. T1: 10 ind m^{-2}, T2: 15 ind m^{-2}, and T3: 20 ind m^{-2}. Within a day, different lowercase letters indicate a significant difference between densities ($P < 0.05$).

ry to conduct a study with a longer experimental time to clarity if the recirculating aquaculture system could obtain final AIW similar to traditional systems.

The observed final W_t had no significant difference between T1 and T3, suggesting that the use of a high stocking density of prawns is not justified. T2 final W_t showed a significant difference in comparison to T1 (Fig. 3). Since T2 presented a better W_t than T1, it can be deduced that T2 was the density with the best production for the culture of *M. tenellum* in this experimental RAS. Data that does not correspond with what was observed in previous studies, were the highest densities reported the best W_t for *M. tenellum*, *M. americanum* and *M. rosenbergii* (García-Guerrero & Apún-Molina, 2008; López-Uriostegui *et al*., 2014; Banu *et al*., 2016), possibly due to the difference in experimental times. The maximum estimated W_t from equation (1) was obtained from estimated stocking density of 17 prawns

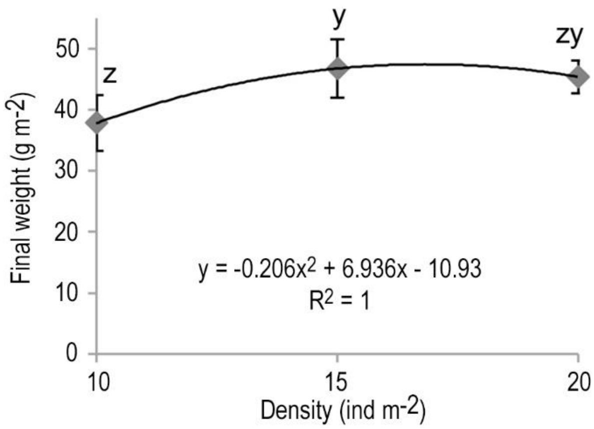

Figure 3. Final weight per unit area (W_t) of *Macrobrachium tenellum* at different stocking densities in a recirculating aquaculture system. T1: 10 ind m^{-2}, T2: 15 ind m^{-2}, and T3: 20 ind m^{-2}. Different lowercase letters indicate a significant difference between densities ($P < 0.05$).

m^{-2} (Fig. 3), a value below what was reported in pond systems (24 ind m^{-3}) (López-Uriostegui *et al*., 2014). BWG at 60 days decreased with the increment of stocking density, due to the mortality of prawns which reduced the number of organisms in the higher initial stocking densities. This result does not correspond to what was observed previously in *M. tenellum*, where the higher densities increased their BWG (López-Uriostegui *et al*., 2014), but in *M. rosenbergii*, it was reported that the net gain in biomass has a negative relation to stocking density (Paul *et al*., 2016), as seen in this study. SGR was inside the reported values for *M. tenellum* of about 1.3-1.5 (Ponce-Palafox *et al*., 2013; López-Uriostegui *et al*., 2014) similar to what was observed in other species such as *M. acanthurus* 1.1-1.4, *M. amazonicum* 0.65-0.90 and *M. rosenbergii* 0.99-1.24 (Marques *et al*., 2012; Banu *et al*., 2016; Hernández-Abad *et al*., 2018), suggesting that the organisms could reach the commercial weight with a longer culture time. The FCR previously reported for *M. tenellum* was of about 1.5-1.9 obtaining the commercial size (25 g) (García-Ulloa *et al*., 2008; Ponce-Palafox *et al*., 2013; López-Uriostegui *et al*., 2014), a lower FCR than the 4.335 ± 0.90 obtained in this study. This high FCR could be because the commercial weight was not allowed to be reached. But, also it need to take into account that the RAS did not allow the natural productivity of microalgae and associated detritus, unlike pond systems where microalgae and bacteria proliferation is allowed, condition that has been reported to impact on the growth of shrimp (Otoshi *et al*., 2003; Wasielesky *et al*., 2006). More tests are required to determine the exact reason for the high FCR; thus, further studies should focus on the effects of the recirculating aquaculture system in the complete culture time required to obtain the commercial weight.

Farming of *M. tenellum* inside a recirculating aquaculture system is feasible; the survival rates are adequate, with similar results to the traditional pond system and the cage-pond system (Ponce-Palafox *et al*., 2013; López-Uriostegui *et al*., 2014). The results suggest that the use of a stocking density close to T2 could be adequate for this recirculating aquaculture system due to the higher W_t and BWG; the traditional culture systems obtain greater productivity but, as stated in studies with shrimp, the RAS can have an effect on growth, through the lack of natural production (Otoshi *et al*., 2003; Wasielesky *et al*., 2006). Further researches should evaluate the correct feeding rate, considering whether the increased flow caused by the recirculating aquaculture system affects the food intake of *M. tenellum*. Future tests should be performed with longer experimental times to allow the organisms to reach the commercial sizes and observe the full effect of the culture conditions.

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