

**Research Article****The effect of alpha males and shelter type on growth and survival of the longarm prawn *Macrobrachium tenellum* (Smith, 1871)****Rodolfo Benigno De los Santos-Romero<sup>1</sup>, Marcelo Ulises García-Guerrero<sup>2</sup>****Juan José Alpuche-Osorno<sup>3</sup> & Edilmar Cortes-Jacinto<sup>4</sup>**<sup>1</sup>Tecnológico Nacional de México, Instituto Tecnológico del Valle de Oaxaca, Oaxaca, Mexico<sup>2</sup>Laboratorio Experimental de Acuicultura, CIIDIR, Oaxaca

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**ABSTRACT.** The longarm river prawn *Macrobrachium tenellum* is extensively exposed to artisanal fisheries in its distribution range and have culture potential. However, its aggressiveness and territorial behavior limits its management in captivity. Because of this, it is necessary to evaluate the effect of alpha males on regular males, as well as different kinds of shelters, to find how this affects growth and survival. For this, an experimental design was established in order to determine the effect of those issues on growth of cultured juvenile prawns. Growth of prawns with no alpha males had no significant differences ( $P > 0.05$ ). However, the interaction between shelter type and the hierarchical males revealed statistical differences ( $P < 0.05$ ) in comparison with the rest in terms of survival. It is suggested that the effects on growth and survival caused by the social hierarchy of prawns and the use of shelters are part of a wider range of interactions that may be related ecologically, ethologically, and physiologically.

**Keywords:** *Macrobrachium tenellum*, freshwater shrimp, growth, survival, intra-specific relation, morphotypes.

**INTRODUCTION**

The genus *Macrobrachium* of the Palaemonidae family is very diverse and is found in marine, freshwater or brackish environments (De Grave *et al.*, 2008). They are distributed worldwide mostly along the tropics and subtropic riverine basins that flow into the oceans (Vega-Villasante *et al.*, 2011; García-Guerrero *et al.*, 2013). One of the most common species of this genus in Mexico is *Macrobrachium tenellum* (Smith, 1871). This species has potential for aquaculture and it is an important component for the sustainment of food webs in its distribution areas, so its conservation is required. It is still common in freshwater ecosystems and grows fast, tolerates high densities, and has good adaptation to captivity (Ponce-Palafox *et al.*, 2002; Espinosa-Chaurand *et al.*, 2011; García-Guerrero *et al.*, 2013).

However, to achieve the best growth in captivity, it is necessary to know the basic requirements and limitations. Román-Contreras (1991); Signoret & Bralovsky (2002); Espinosa-Chaurand *et al.* (2011);

Vega-Villasante *et al.* (2011) have performed several studies both in culture and in the wild. However, some behavioral issues of the prawns in captivity and their response to the stressful conditions caused by hierarchical differences in captivity are not understood. Vega-Villasante *et al.* (2011) and Yamasaki-Granados *et al.* (2012) have reported the role of intra and interspecific relationships of this species among individuals of different types, determining that this species may have great morphological and size variations among specimens, even those of the same age. The body parts that might vary in size and shape among specimens are mainly the rostrum, the second pair of pereopods and the body size as a whole both in male and females (Hernández *et al.*, 2007; Valencia & Campos, 2007; Espinosa-Chaurand *et al.*, 2011). Particularly among the *M. tenellum* males, there are at least three different morphological kinds produced by these variations. They are recognized as alpha, regular, and dwarf types (Barki *et al.*, 1992).

This is important to be studied and recognized because it is among the main causes of high mortality and low growth rate of freshwater prawns during their culture since the aggressiveness of alpha specimens make them territorial and control shelter and food availability in the pond or tank (García-Guerrero & Apún-Molina, 2008). In *M. tenellum* this may cause a negative effect on the growth and survival of non-alpha specimens because they cannot escape from the area. This effect is known as bull effect (New, 2002) or bull-runt interactions (Kutty & Bhaskara Pillai Kurup, 2016) and is a cause of irregular growth or growth suppression of the rest of the population (Jayachandran, 2001). Implications of this on growth rate and survival should be considered in order to obtain best possible results in culture and management (Harán *et al.*, 2004).

The purpose of this study is to determine the intensity of the effect of males with alpha features, as well as different kind of shelters, over the development of the rest of the prawns during culture.

## MATERIALS AND METHODS

*Macrobrachium tenellum* prawns were collected in the wild with hand net or cast net in the Colotepec River (15°48'19"N, 97°01'09"W) in Oaxaca, Mexico. They were transported in 250 L containers with freshwater and continuous aeration and, then, acclimatized during 15 days to the CIIDIR-Oaxaca-IPN laboratory in a 1000 L tank. During this time, the prawns were fed with 40% protein shrimp pellet (Camaronina®). After 15 days of acclimatization, prawns average weight was  $0.03 \pm 0.01$  g and total length of  $16 \pm 2$  mm. They were stocked in 50 L experimental tanks and tank was covered with a mesh to avoid escaping. Ten juveniles of  $16 \pm 2$  mm were stocked in each replica. Alpha males had a size of  $65 \pm 6$  mm and well developed second pereopods pair. To evaluate the effect of *M. tenellum* alpha males over other males maintained with different types of shelters, a completely randomized experimental design with a 3×3 factorial arrangement was performed with nine treatments (Table 1). Each treatment had four tanks as replicas. Shelters were placed as following: PVC tubes, 3.1 cm of diameter and 5 cm of length, 10 pieces per tank; green plastic folded net mesh 0.5 m<sup>2</sup> per tank; pieces of clay tiles in the bottom of the tank, 10 pieces per tank.

A water recirculation system in cascade was used to maintain high [O<sub>2</sub>] levels and to eliminate dissolved wastes. The lowest container in each treatment had no organisms but a submersible water pump (Atlas World Stadar®, 1200 L/h) that flows water into the upper tray. This system recirculated the water among all the units

**Table 1.** Experimental design with a 3x3 factorial performed on *Macrobrachium tenellum* prawns in captivity.

Treatment	Alpha male /shelter combination
1	2 alpha male / plastic mesh
2	2 alpha male / PVC tube
3	2 alpha male / clay tile
4	1 alpha male / plastic mesh
5	1 alpha male / PVC tube
6	1 alpha male / clay tile
7	0 alpha male / plastic mesh
8	0 alpha male / PVC tube
9	0 alpha male / clay tile

of the same treatment and produced the aeration required to maintain oxygen always above 5 mg O<sub>2</sub> L<sup>-1</sup>. Temperature was maintained at 28°C by placing a 300 W thermostatic heaters (Elite®) inside the lowest unit.

Animals were feed once a day at 6:00 PM with shrimp pellets (Camaronina® 35% protein). Feces, uneaten food, dead animals, and carcasses were discarded every other day by siphoning while replacing 50% of the water in the tank. Individual total weight was registered every 15 days during all the experimental period with a digital scale (0.001 g precision; Denver Instrument).

Those parameters were calculated: Survival index (%):  $S = 100 \times (\text{initial number} - \text{final number} / \text{initial number})$ ; average individual weight increase AIWI (g) =  $\text{final weight} - \text{initial weight} / \text{initial weight}$ ; weight increase per day WIPD (g/day) =  $(\text{final weight} - \text{initial weight}) / \text{time}$ ; weight increase in percentage WIP (%) =  $100 \times (\text{final weight} - \text{initial weight}) / \text{initial weight}$ ; and the specific growth rate SGR (%) =  $100 \times [(\ln \text{final weight} - \ln \text{initial weight}) / t]$ . SRG was calculated as in Cortés-Jacinto *et al.* (2003), WIP and WIPD were calculated as in Gitte & Indulkar (2005); S and AIWI were calculated as in Vega-Villasante *et al.* (2011). For growth estimations in length, same formulas were used but using weight data.

To calculate significant differences among treatments over growth, survival and weight increase (average individual weight increase, weight increase per day, weight increase in percentage and specific growth rate) Tukey tests ( $P < 0.05$ ) were applied. Previously, normality test was executed (Kolmogorov-Smirnov,  $\alpha = 0.05$ ). Analysis were made with the SAS 8.2® statistical software.

## RESULTS

Growth increases in different treatments are presented (Table 2). Increase in weight was statistically significant

**Table 2.** Growth variables for *Macrobrachium tenellum* in different combinations of alpha male presence and shelter type. Initial, final and average increase of weight  $\pm$  standard deviation.

Parameters	Two alpha males			One alpha male			No alpha male		
	Plastic mesh	PVC tube	Clay tile	Plastic mesh	PVC tube	Clay tile	Plastic mesh	PVC tube	Clay tile
Initial weight (g)	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01	0.03 $\pm$ 01
Final weight (g)	0.27 $\pm$ 11	0.29 $\pm$ 13	0.42 $\pm$ 24	0.45 $\pm$ 21	0.40 $\pm$ 18	0.19 $\pm$ 08	0.36 $\pm$ 15	0.68 $\pm$ 45	0.78 $\pm$ 33
Average individual weight increase (g)	0.24 $\pm$ 10	0.26 $\pm$ 12	0.39 $\pm$ 22	0.42 $\pm$ 20	0.37 $\pm$ 19	0.167 $\pm$ 08	0.33 $\pm$ 15	0.656 $\pm$ 42	0.75 $\pm$ 32
Weight increase per day (g d <sup>-1</sup> )	0.003	0.003	0.005	0.006	0.005	0.002	0.004	0.009	0.009
Weight increase in percentage (%)	807	870	1307	1400	1240	557	1103	2187	2300
Specific growth rate (%/day)	2.94	3.03	3.53	3.61	3.46	2.51	3.32	4.17	4.24

different in treatments with no alpha males in comparison with any other ( $P < 0.05$ ). Treatments without alpha male and clay tile or PVC tubes as shelter, produced a daily increase in weight of 0.008 g d<sup>-1</sup>, whereas those with mesh were lower (0.004 g d<sup>-1</sup>). Specimens in the treatment with two alpha males had a daily average weight increase of 0.005 g d<sup>-1</sup> with clay tile shelter, whereas the lowest weight increase (0.003 g d<sup>-1</sup>) occurred with those with mesh. Growth with one alpha male was from 0.002 g d<sup>-1</sup> to 0.004 g d<sup>-1</sup>.

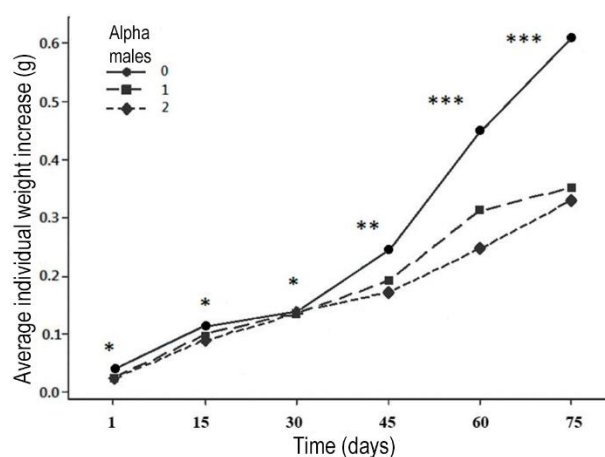
The total individual weight increase without alpha males was statistically different from those with clay tiles and PVC tubes ( $P = 0.046$ ) at day 45 and highly significant ( $P = 0.0002$ ) after day 75 (Fig. 1).

Growth speed in weight increase per day was highest at day 15 for all treatments. Later, growth rate lowered with significant differences among treatments until day 75, at which the treatment with no males and tile shelter had the highest growth rate with significant differences in comparison with the others ( $P < 0.05$ ).

No significant differences in survival among treatments were observed for treatments with no alpha males. However, significant differences in survival among all treatments with one or two males were observed ( $P < 0.05$ ). Initial density was 65 ind m<sup>-2</sup> whereas the mean final density was 14 ind m<sup>-2</sup>, resulting in survivals from 17 to 30% in all the treatments. In relation with asymmetry coefficient, treatments with no males, independently on shelter type, had a regression coefficient  $b = 3,245$ . The  $R^2$  value for the treatment with one male was 2.8348 and 2.8318 for those with two males. Table 3 shows the effect of the interaction between the alpha males and tiles as shelter on weight gain.

## DISCUSSION

The effect of dominant or alpha males on growth of regular males but independently of the shelter type has been studied before in species such as *Macrobrachium rosenbergii*. In *M. tenellum*, however, there is no pre-

**Figure 1.** Average individual weight increase in relation to alpha males for *Macrobrachium tenellum* cultured in a combination of alpha male presence and different shelter types. \* $P > 0.05$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .**Table 3.** Average weight of *Macrobrachium tenellum* specimens in treatment with different combinations of alpha males and shelter type. Values with the same letter (a, b, c) did not reveal statistically significant differences at ( $P < 0.05$ ) Tukey test ( $\alpha = 0.05$ )

Alpha male /shelter	Average weight (g)	Tukey test
0 alpha male / clay tile	0.780 $\pm$ 0.33	a
0 alpha male / PVC tube	0.687 $\pm$ 0.45	a b
1 alpha male / plastic mesh	0.450 $\pm$ 0.21	a b
2 alpha male / clay tile	0.422 $\pm$ 0.24	a b
1 alpha male / PVC tube	0.402 $\pm$ 0.18	a b c
0 alpha male / plastic mesh	0.361 $\pm$ 0.15	b c
2 alpha male / PVC tube	0.291 $\pm$ 0.13	c
2 alpha male / plastic mesh	0.272 $\pm$ 0.11	c
1 alpha male / clay tile	0.197 $\pm$ 0.08	c

vious information regarding this topic. But, present results suggest that there is in fact a negative effect produced by alpha male over growth of regular males. However, this study also suggests that this effect can be

counteracted by shelter type with significant differences ( $P < 0.01$ ).

Jayachandran (2001) and Moraes-Riodades & Valenti (2004) have stated that in addition to genetic and environmental factors, freshwater prawns from the same tank or pond can differ in growth rate among them because of the "bull effect" (Vega-Villasante *et al.*, 2011). This behavioral phenomenon may cause variations in sizes among specimens of the same stock because of the pressure that alpha males put over the rest as stated previously by Casas-Sánchez *et al.* (1995). In fact, Karpus (2005) stated that growth rate differences and, consequently, size variations in males of *M. rosenbergii* populations are due to the apparition of three clearly different morphotypes: small males, orange arms males, and blue arms males, all of them different in size, morphology, and behavior. In the present study, only two morphotypes were recognized, the alpha male and the small male. However, the pressure of dominant males over small males were evident since all those stocked with no alpha males grew faster, some of them becoming alpha males. This phenomenon may start when prawns are juveniles and seems to be enhanced as they grow. With no alpha males, a more regular and faster growth is observed until some of them become dominant. Fernandes-Silva & Arruda (2014) report that *M. rosenbergii* juveniles can grow differently depending on this hierarchy relationship that affects behavior. Previous studies suggest that there is a genetic factor that triggers this unpaired growth so eventually alpha males will appear in the tank. Barki *et al.* (1992), Karpus (2005), and Harrington & Hovel (2015) established that social interactions between large males affect the growth of the smaller, suggesting that social mechanisms such as direct competition for food and food conversion efficiency could increase under such pressure.

In fact in the present work smaller prawns from day 15 grew faster and Barki *et al.* (1991, 1992) stated that dominant alpha males require some time to become such. In present work and from day 15 the hierarchies became evident, producing aggressive interactions. The faster growth of juveniles at the beginning of the trial suggests that some with faster growth rate since starting may have advantages in the access to food once their status as alpha is established, making them able to grow faster almost from the beginning as previously observed in *M. rosenbergii* (Fernandes-Silva & Arruda, 2014).

In addition to this kind of interactions, the structure of a group in the same tank should be considered as a limiting factor for growth. At smaller stages, all prawns seem to interact and grow similarly. However, after maturity, growth rate of smaller males

tend to be slower in comparison to alpha or dominant males, so the chances of being cannibalized increase (Ranjeet & Kurup, 2011).

Maintaining high densities regularly produces low survival in *M. rosenbergii* cultures mainly because of the competition for food and space caused by the larger adult males. It is suggested that the amount of small males is high in comparison with large ones, as in present work, this trend would be observed after certain culture time as observed previously (Ranjeet & Kurup, 2011; Vega-Villasante *et al.*, 2011). Factors such as density, food and shelter might determine the apparition and intensity of this phenomenon. In present work, it is suggested that the low survival (17 to 30%) with a density of 64 ind m<sup>-2</sup> was due to the pressure of dominant males. López-Uriostegui *et al.* (2014) obtained a survival of 47% for *M. tenellum* with a density of 24 ind m<sup>-2</sup> and suggests that survival can be improved if only prawns similar in size and features are cultured as also stated by (Malecha *et al.*, 1981; Ranjeet & Kurup, 2002).

To achieve the best results in the cultivation of native prawns it is important to minimize the aggressive negative interactions because this will reduce the stress that restricts growth and affects survival. Culture density and shelter availability are among the factors required for this (Soares *et al.*, 2015). Since prawns are omnivorous with carnivorous trends, they tend to be cannibalistic (García-Guerrero & Apún-Molina, 2008) but to provide shelters may improve survival (García-Guerrero *et al.*, 2015). In the present work, plastic mesh as shelter combined with the presence or absence of alpha males were not significantly different in survival. With no alpha males and clay tile as shelter, the best growth was observed. No alpha males and PVC tubes always gave good results. However, the use of plastic net did not offer good results, even with no alpha males. This last material seems not to be suitable in terms of the shelter it may provide was not suitable whereas clay tiles might provide better opportunities to hide perhaps since this offers a more similar shelter in comparison with those in the wild. For example, Vásquez *et al.* (2000) mention that *M. brasiliense* prefers roots, leaf litter, and sticks, and bury themselves if shelter is not available. *M. rosenbergii* prefers coarse materials as stones in comparison to sand or mud (Murthy *et al.*, 2012) and better growth is obtained with that option in comparison with PVC pipes or plastic mesh as seen for *M. rosenbergii* (Tidwell *et al.*, 1998, 2000). In present work, weight gain and specific growth rate (SGR) are comparable to those previous findings in where clay tiles as shelter produced better growth in comparison with other materials so it is suggested that this material is more similar to those found in the wild,

cobblestones for example, as observed with *Cherax quadricarinatus* juveniles (Jones & Ruscou, 2001; Viau & Rodríguez, 2010).

Shelter material can be also important as observed in the present work. The use of materials similar to those in the wild seems to be suitable. Tayasu *et al.* (1996) indicate that in the wild, aquatic vegetation and crevices could produce a complex and dense network of hiding places that act as good shelter, helping to avoid negative interactions. In freshwater prawns, suitable shelter would minimize unwanted encounters, reducing stress and cannibalistic behavior (Marshall *et al.*, 2005). Clay tiles produced best results since they are similar in material to natural shelters while plastic mesh material was not recognized as shelter perhaps because of its artificial texture. However, meshes could be good for very small juveniles (García-Guerrero & Apún-Molina, 2008) so size has to be considered when providing shelters. Lammers *et al.* (2009) mention that alpha and subordinate prawns respond similarly when the highest growth rate is achieved, so at higher sizes they might need more shelters or different kind of shelters.

As for the combined interaction between shelters and hierarchies with *M. tenellum*, Vega-Villasante *et al.* (2011) studied this phenomenon associated with a particular type of shelter, stating that this may cause differences in growth, but with no information about interactions among specimens. The growth and survival trends obtained in present work are common because of interactions between alpha males and subordinate ones but a proper shelter might minimize it. In present work, two alpha males might compete ignoring smaller specimens so they may have more chance to grow. Alpha males are more active outside shelters when there are more than one since they need to have more need of controlling its space. That may or may not cause interactions with the lower ones, so this issue needs further investigation.

Physiological functions, particularly those in relation with hormonal balance are another issue that might influence behavior and thus growth through the hierarchies. About this, Vásquez-Acevedo *et al.* (2009) mentioned that *M. rosenbergii* has certain neuropeptides that stimulate aggressiveness and other neuropeptides that control behavior. Therefore, the level of certain peptides in most prawns may determine aggressiveness and, consequently, have an influence on hierarchies in addition to size.

The results of this work suggest that *M. tenellum* that alpha males in the culture can negatively affect growth and survival of the rest of the stock. Since this species prefers shelters made of natural materials as the clay tiles, these are recommended to improve growth

by minimizing undesired encounters with alpha males and giving them places to hide. However, more studies on ecological, ethological or physiological aspects are required for a better understanding of the mechanisms that control domination and aggressiveness among different prawn types. A more detailed study on the relationship among alphas and the influence of this interaction on the rest of the population is also required.

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