# CONTRIBUTIONS TO THE BIOLOGY OF MOLTING AND GROWTH OF THE LONGARM RIVER PRAWN MACROBRACHIUM TENELLUM (DECAPODA: PALEAMONIDAE) IN MEXICO

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Abstract - Studies on the molting cycle of Macrobrachium tenellum are not available, which limits the scope of studies of growth and reproduction. The duration of the molt cycle was determined under controlled experimental conditions. The prawns were divided into four groups according to weight: Group 1 (1.0–1.9 g), Group 2 (2.0–2.9 g), Group 3 (3.0–3.9 g), and Group 4 (4.0–9.0 g). The increase in total weight was higher in groups 2 (23.4%) and 3 (20.3%) than in group 1 ( $\sim$ 18%) and 4 ( $\sim$ 18%). The increase in size after molting among all groups was statistically different: in group 1 it increased 6.1% (highest) and in group 2 it increased 2.2% (lowest). The duration of a complete cycle was 8.9, 9.4, 10.4, and 15.1 days for groups 1, 2, 3, and 4, respectively. The lengths of the molting cycles were not significantly different between groups 1 and 2, but group 4 was different from group 1.

Key words: Prawn, ecdysis, development, exoskeleton, growth, molting cycle, Macrobrachium tenellum, Mexico

## INTRODUCTION

The life of crustaceans, including feeding, reproduction, mobilization of reserves, etc., is organized around and based on the molt cycle (Vega-Villasante et al., 2007; Vega-Villasante et al., 2000). According to Drach (1939, 1944), molting is not a physiological process of limited effects, but profoundly affects the life of decapods. This phenomenon is cyclical, alternating phases of relative external rest with others of intense activity (Petriella and Boschi, 1997; Ismael and New, 2000). Observed morphologic changes in the soft parts of the crustaceans allow characterization of the states of molting in diverse species. The study of the molt cy-

cle has focused mainly on biological aspects and the particular characteristics of each species during molting. However, it has also been widely studied from the standpoint of endocrinal basic and applied studies of digestive physiology and the assessment of toxicity of certain compounds (Vega-Villasante et al., 2000; Gaxiola et al., 2005). It has also been used to establish the stages of the cycle in bioassays as a physiological parameter of equality between individuals (Rosas et al., 1998). In *Palaemon serratus*, Drach (1939) defines four main stages, which are also found in other crustaceans: molt or ecdysis (E), postmolt (AB), intermolt (C1–C4), and premolt (D0–D4). With the profound metabolic changes associated with each stage, Petriella

and Boschi (1997) consider the morphological characterization of the molt cycle essential as a prelude to the study of growth.

The genus Macrobrachium of the Palaemonidae family has been of great research interest, having nearly 200 species. It occurs over a wide geographic range, and is cultivated in many countries. Fresh water shrimps or prawns of the Macrobrachium genus are distributed in the tropical and subtropical zones, with 26 species in the Americas (Holthuis, 1980). All are adapted to freshwater habitats and some occupy brackish water. Most of the early stages of development take place in the neritic zone (Román-Contreras, 1979; Holthuis, 1980; Hendrickx, 1995; New, 2000). M. tenellum (Fig. 1) has been recommended for cultivation because, unlike the majority of species, it is not aggressive, does not display much cannibalism, and lives at high densities (Ponce-Palafox et al., 2006). It also tolerates a wide and fluctuating temperature range (16-32°C; Guzmán, 1987), up to 20 psu salinity (Hendrickx, 1995; Arroyo and Magaña, 2001) and oxygen concentrations of 0 to 5.59 mg/L (Schiff and Hendrickx, 1997). Experimental cultures during summer in coastal tropical zones suggest economic advantages for commercial purposes (Vega-Villasante et al., 2011), yet there are no studies on the molting cycle of this species.

Further research, mainly on growth and reproduction, requires detailed understanding of the molt cycle to manage this species efficiently under commercial conditions. This study provides information on the biology of molting and growth in *M. tenellum*.

## MATERIALS AND METHODS

### Experimental site

Observations were carried out at the Experimental Aquaculture Laboratory of the Centro Universitario de la Costa (CUCOSTA) of the Universidad de Guadalajara, located at Puerto Vallarta, Jalisco, Mexico (20°42′19″N, 105°13′16″W), (10 m above mean sea level).

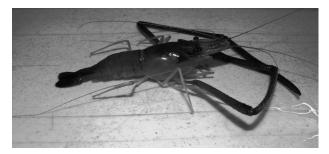


Fig. 1. Adult male specimen Macrobrachium tenellum.

### Animals

Specimens of 50–90 mm of longarm river prawn *M*. tenellum were captured between June and October 2010 with a fishnet in an artificial lake fed by a temporary stream that allows the migration of these organisms from the estuary to this water body (approx. 6 km). The prawns were classified by weight groups and underwent acclimatization for 7 days before the start of individual confinement. The sex of the prawns was determined when possible, but not taken into account in the statistical analysis. The animals were fed extruded shrimp food (Purina Camaronina: 35% protein, 12% moisture, 8% fat, 5% crude fiber, 10% ash, and 30% nitrogen-free extract). The feeding schedule was set at 13:00 h (one feeding each day). The food ration was calculated according to 10% of the initial biomass.

## Experimental design

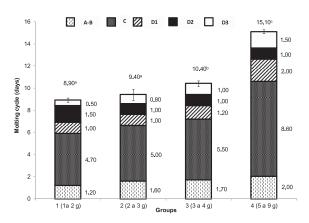
The prawns were kept in 40 L aquaria. Water quality was maintained with filters (Elite 450) which allowed recirculation of at least 400% of the total volume of water a day. The temperature was set at 28°C with a thermostatically-controlled heater (Sunny). Feces, molts, and residual food were removed daily. Measurements (temperature, oxygen, pH, salinity, and total dissolved solids) of the tank water were taken daily at 14:00 h, using a digital thermometer (Hanna), an oxymeter (YSI), a field potentiometer (Hanna), and a conductivity meter (YSI), respectively. Partial water refills (20%) were done every three days. The photoperiod (13 light:11 dark) was the natural photoperiod during the summer and autumn.

The prawns were divided into four weight groups: Group 1 (1.0-2.0 g), Group 2 (2.1-3.0 g), Group 3 (3.1-to 4.0 g), and Group 4 (4.1-9.0 g). To determine the stage of molt, the uropods were studied under a stereoscopic microscope, using the technique described by Oliva et al. (1988) for the classification of the molt stages in Farfantepenaeus notialis. Individuals whose molt cycle was identified as intermolt (stage C) were confined individually and periodically observed to determine progression from one stage to another until molting. The prawns were monitored for three consecutive cycles. We recorded the number of days in each phase of the cycle, as well as size and weight before and after molting. All observations were conducted with five replicates.

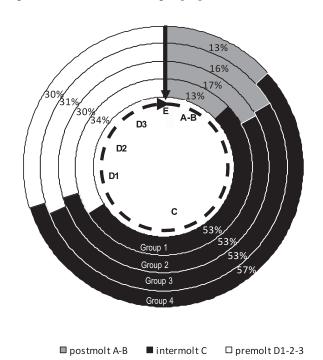
Growth (total wet weight in g; TW) in the postmolt (stages A-B) was expressed as the percentage of increase in weight in relation to the weight in premolt (stages D0-D3). Growth = (TW in postmolt – TW in premolt)/(TW in premolt) × 100. The increase in size (total length in mm; TL) was defined as the difference between length of the postmolt and length recorded in premolt. The weight of prawns was determined using a three-digit laboratory scale (OHAUS) and length was measured with a vernier ruler (from the tip of the telson to the tip of the rostrum).

## Statistical analysis

Growth parameters (wet weight and total length before the molt, wet weight and total length after the molt, wet weight and total length gain between molts, wet weight and total length gain, and specific growth rate) and total days between molts, were compared with one-way ANOVA, previous tests of normality (Kolmogorov-Smirnov;  $\alpha=0.05$ ) and assuming homoscedasticity (Bartlett;  $\alpha=0.05$ ). All statistical analyses were performed using SigmaStat 3.1 (2004). The data on percentage increase in weigth and length were analyzed by arcsine square root transformation (Zar, 1984). Significant differences between treatments were determined by Tukey's multiple comparisons test ( $\alpha=0.05$ ).



**Fig. 2.** Duration (days) of *Macrobrachium tenellum* molting cycle per group. Numbers to the right of each bar indicate the mean days for stage of molting. The values on the bars represent the total cycle time per group. The vertical lines in each bar represent standard deviation. Different superscripts show statistically significant differences between groups (p <0.05).



**Fig. 3.** Duration (percentage) of the molt cycle of *Macrobrachium tenellum*. The rings represent the full cycle per group. The numbers indicate the percentage of the total length of the cycle stage.

## **RESULTS**

The difference in percentage increase in length af-

ter the molt for each weight group was statistically significant. Maximum growth occurred in group 1 (6.1%) and the minimum in group 2 (2.2%). The duration of a complete cycle was 8.9, 9.4, 10.4, and 15.1 days for groups 1, 2, 3, and 4, respectively (Fig. 2). There were no significant differences between groups 1 and 2, with less than a day apart in the duration of a cycle. Between groups 4 and 1 there were statistical differences in the length of molt cycles; group 4 was almost twice as long. Generally, the duration of the stages in the molt cycle increased with the increase in size (Fig. 3). In all cases, stage C was longer (53-57%) and stages A-B the shortest (13–17%). Stage D was 30-34% of the cycle. Group 4 had the longest intermolt and shortest premolt, while the opposite occurred in group 1. The averages of the physical and chemical parameters were: pH (7.8  $\pm$  0.5), temperature (28.1  $\pm$  0.8 °C), oxygen  $(4.0 \pm 1.2 \text{ mg L}^{-1})$ , salinity (0.2-0.4 psu), and total dissolved solids (230.2  $\pm$  7.6 mg L<sup>-1</sup>).

#### DISCUSSION

These results are the first report of the length of stages of the molt cycle of the longarm river prawn M. tenellum under controlled conditions. The molting cycle in the laboratory provides a close approximation, not an accurate one, since experimental conditions and food are different and limited compared to natural environmental conditions (Reyes-Ávalos and Luján-Monja, 2003). Although information on natural conditions of temperature, oxygen, pH, and salinity have been published (Guzmán-Arroyo, 1987; Ruiz-Santos, 1988; Schiff and Hendrickx, 1997; Signoret-Poillon and Soto, 1997; Ponce-Palafox et al., 2002), most of these measurements have been determined in the field in very specific areas (Guzmán-Arroyo, 1987; Ruiz-Santos, 1988; Hernández et al., 2007) and few studies have been conducted in captivity (Aguilar-Juarez, 1995; Hernández et al.,1995; Hernández et al., 1996; Signoret-Poillon and Soto, 1997; Aguilar et al., 1998). In our study, the parameters were approximately the conditions that occur in the central mainland coastal area of Mexico during the summer and early autumn. This set of conditions may not fully assess the maximum

duration of the molt cycle and subsequent growth. Most species of the genus *Macrobrachium* carry out their life cycle moving from brackish water (larva and postlarva) to freshwater (juvenile and adult); however, there are stable populations in coastal brackish water bodies and in freshwater, so it is still unknown if there is an optimal range or comfort zone for the prawns.

Generally, the molt stages in M. tenellum correspond to those described by Oliva et al. (1988) and Promwikorn et al. (2004) in Farfantepenaeus notialis and Penaeus monodon, respectively. Visual inspection of the uropods, using a stereoscopic microscope, allowed for the identification of the main sequences of molting. However, in groups 1 and 2, this inspection was extremely complicated because the prawns were very small and lacked the tegument coloration of prawns (transparent light gray) with slightly pigmented chromatophores. Thus, there was potential bias in the identification of the change from stage C to D and the substages of D. Stage C was, in all groups, the one with the longest duration, over 50% of the cycle. The cumulative percentage of the various substages of D was 30-34% of the cycle, while stages A and B lasted only 13-17% of the cycle. In other crustacean species, similar results occur. In the swimmer crab Ovalipes trimaculatus, Alvarez et al. (2009) found that stages A, B, and early C represent ~19% of the cycle, while later stage C represents ~55%, and the substages of D represent 23-30%. Wickins (1972), in one of the first studies on the molt cycle of Macrobrachium rosenbergii, suggested a cycle of ~50 days, of which the intermolt represents more than 50%. Vega-Villasante et al. (2007) determined the duration of molt in different sizes of the crab Callinectes arcuatus, finding that stage C represented 34-54%, stage D between 35-40%, and stage B between 10.8-26%.

Other studies highlight substantial differences with our study. According to Elorza and Dupré (1996), in the Juan Fernández lobster (*Jasus frontalis*), stage C can last up to 80% of the cycle, while the remaining stages are 5.5% and 14.4% (AB and D, respectively). Hayden et al. (2008) found that in larvae

<b>Table 1.</b> Results of wet weight and	length gain of the longarm river prav	vn Macrobrachium tenellum during t	he molt cycle under labora-
tory conditions.			,

	Group				
	1	2	3	4	
Growth parameters	(1-2 g)	(2.1–3 g)	(3.1–4 g)	(4.1–9 g)	
TW before molting (g)	$1.43 \pm 0.03^a$	$2.39 \pm 0.06^{b}$	3.73±0.02°	$6.78 \pm 0.02^{d}$	
TW after molting (g)	$1.70 \pm 0.02^a$	2.95±0.02 <sup>b</sup>	$4.49\pm0.07^{\circ}$	8.03±0.03 <sup>d</sup>	
Weight gain between molts (g)	$0.03\pm0.01^{a}$	$0.06\pm0.01^{\rm b}$	$0.07\pm0.01^{bc}$	0.08±0.00°	
Weight increase (%)	18.96±3.40 <sup>a</sup>	23.17±3.33ª	20.40±2.57 <sup>a</sup>	18.54±0.02	
Specific growth rate (SGR)	$2.00 \pm 0.33^a$	2.40±0.31ª	2.14±0.25ª	1.96±0.00a	
TL before molting (mm)	$50.70\pm0.20^{a}$	58.83±0.12 <sup>b</sup>	63.08±0.03°	81.25±0.06	
TL alter molting (mm)	53.84±0.07 <sup>a</sup>	60.13±0.06 <sup>b</sup>	65.52±0.06°	85.50±0.20	
Lenght gain between molts (mm)	$0.36 {\pm} 0.02^a$	0.15±0.01 <sup>b</sup>	0.28±0.01°	$0.49\pm0.02^{d}$	
Lenght increase (%)	$6.19\pm0.29^{a}$	2.21±0.17 <sup>b</sup>	3.87±0.15°	5.23±0.18 <sup>d</sup>	

TW = total wet weight; TL = total length.(mean  $\pm$  S.D). Different superscripts in each row show statistically significant differences between groups of prawns (P <0.05).

of *M. amazonicum*, the intermolt is 30-40% of the total cycle (3-4 days at 21°C and 1 to 2 days at 29°C). These results are obviously related to the larval stages in the exponential phase of growth.

In the river shrimp *Cryphiops caementarius*, duration of the molt cycle in adult females is about 30 days, of which 63-73% correspond to stage D, 10-19% to stage C and 16-17% to stages AB (Reyes-Avalos et al., 2002; Reyes-Avalos and Luján-Monja, 2003). Molina et al. (2000) reported that juvenile *Litopenaeus vannamei* have a molt cycle of 11.03 ± 1.13 days, with stage D representing 50%, stage C is 33.33%, and stage AB is 16.67%.

The above results highlight the differences between species and sizes in the duration of the molt cycle. With regard to weight gain and size in our study, all groups had smaller increases. Perhaps environmental conditions and food had an adverse effect on growth parameters. Variables, including density of specimens, type and abundance of food, temperature, and absence of shelter, may have an effect on molting, and therefore, on growth (Peebles, 1977). The water quality can have significant effects

on the molting and growth of crustaceans. In the case of water hardness, caused mostly by CaCO<sub>3</sub>, the total dissolved solids (270  $\pm$  7.6 mg L-1) may by near the maximum permissible limit for cultivating aquatic species. According to Wetzel (2001), the ideal range of water hardness is 50-200 mg L-1 CaCO<sub>3</sub>, and Wickins (1972) suggests a range of 65-200 mg L for suitable cultivation of M. rosenbergii. New and Singholka (1985) recommend lower levels of hardness, 40-100 mg L–1, and Brown et al. (1991) report that the maximum growth of juvenile M. rosenbergii occurs at <53 mg L-1 and conclude that hardness less than the above did not affect growth, while higher concentrations did. Howlader and Turjoman (1984) state that adverse effects of water hardness occur at 688-987 mg L-1 on the growth of M. rosenbergii. In the same species, Adhikari et al. (2007) found that the highest survival rate (100%) occurred at 92 mg L-1 CaCO<sub>3</sub>, whereas the lowest survival rate (60%) was at (384 mg L-1). The maximum growth of juveniles (11.6  $\pm$  2.7 mg / day) occurred at 132 mg L-1, while growth was reduced at >228 mg L-1. These results show a wide range of effects of water hardness on the growth of prawns. For M. rosenbergii, suitable ranges of hardness could not be established despite the number of works that have been conducted. In the case of *M. tenellum*, there are no similar studies, so it is only possible to suggest that increased hardness can lengthen the molt cycle and slow growth after ecdysis. To establish optimal growth conditions for *M. tenellum*, it is necessary to determine the optimal conditions of water quality (salinity, pH, temperature, oxygen, alkalinity, hardness). Determining the physiological comfort zone was the objective of our present studies.

Acknowledgments - We thank Ira Fogel of CIBNOR for editorial services. Funding was provided by the Consejo Estatal de Ciencia y Tecnología de Jalisco (COECYTJAL grant 06-2009-661) to support our current research. Additional funding was provided by Consejo Nacional de Ciencia y Tecnología (CONACYT 2010-156252) and Centro de Investigaciones Biológicas del Noroeste (CIBNOR AC2.5). Stig Yamasaki-Granados is a recipient of a graduate fellowship (grant 77608) from CONACYT.

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