

EFFECTS OF ADAPTATION TO LABORATORY CONDITIONS ON GROWTH, MOLTING, AND FOOD CONSUMPTION OF JUVENILE FARFANTEPENAEUS DUORARUM (DECAPODA: PENAEIDAE)

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ABSTRACT

Growth patterns and biochemical analysis at laboratory conditions (temperature 28°C, salinity 22) were analyzed in juveniles wild pink shrimp, *Farfamepenaeus duorarum* (Burkenroad, 1939), eaught in the Gulf of Mexico compared with cultivated (F2) organisms. Shrimp were maintained for 55 days to monitor the molt frequency and weight gain over time. We determined the frequency of molting and wet weight gain per day over the experiment time. Wild shrimp had an average molting frequency of 10 ± 2.03 days with an increase in wet weight of 0.024 ± 0.001 g day⁻¹; whereas cultivated shrimp had an average frequency of 11 ± 2.15 days and a growth rate of 0.084 ± 0.002 g day⁻¹. Osmotic capacity was determined in wild shrimp at 154.85 ± 73.47 mOsm kg⁻¹ and for eultivated at 128.67 ± 42.24 mOsm kg⁻¹. Total protein concentration was 35.15 mg ml⁻¹ for wild shrimp, while values for cultivated shrimp were significantly different at 112.22 mg ml⁻¹ (ANOVA; p < 0.05). Also, an experiment was conducted to determine the amount of food consumed before and after ecdysis in wild and eultivated shrimp. No significant differences were found in the food consumption (F = 0.220, p = 0.641) between groups before and after ecdysis. We determined that food consumption rate fell by 30% during molt and that feeding did not cease when ecdysis occurs.

KEY WORDS: ecdysis, *F. duorarum*, growth, molting frequency, shrimp food consumption DOI: 10.1163/1937240X-00002125

INTRODUCTION

Growth is one of the most important parameters affecting the dynamics of animal populations under commercial production (Hartnoll, 1982). In crustaceans, the rigid exoskeleton restricts growth, which therefore occurs in spurts related to the molt cycle (Lockwood, 1967; Petriella and Boschi, 1997); before ecdysis, the exoskeleton is split through the absorption of water by the tissue (Stevenson, 1985; Fenucci, 1988), and this allows the internal tissues to increase in size before the new integument hardens. During the life cycle of shrimp the succession of molts is more frequent in the early stages of life, decreasing or ceasing in adults (Petriella and Boschi, 1997).

The pink shrimp, *Farfantepenaeus duorarum* (Burkenroad, 1939), is one of the most valuable species of penaeid from the Gulf of Mexico (Rosas et al., 2007). It is considered as an indicator of health and productivity of the ecosystem in Florida Bay (Browder et al., 2002). However, its relative economic importance has been reduced by a steady decline in annual catch. Gracia (1995) pointed out that the increasing overfishing of juveniles is the main factor in this decline, since it reduces both the potential growth and levels of re-

cruitment in ocean and broodstock populations. This species is highly sensitive to change in the physical-chemical environment, and stress may result in faster growth or death (López-Téllez et al., 2000; Browder et al., 2002; Ramírez-Rodríguez and Arreguín-Sánchez, 2003).

Molting represents a physiological crisis and many events condition its success, adaptation to controlled conditions being one of them (Galindo et al., 2009). Also, osmotic capacity and total protein concentrations reflect the physiological status of wild and cultivated shrimp and, as proposed by Charmantier et al. (1994) and Galindo et al. (2009), can be considered good indicators of adaptation. Metabolism of proteins is an important source of energy (Waterman, 1961) so shrimp must consume food with a balance of proteins in order to optimize physiological efficiency (Dall et al., 1990; Lim et al., 1997). It has been shown that hyperosmoregulatory conditions, at least in cultivated F. duorarum, cause an increase in the energy expenditure required to maintain cell volume (Bonilla-Gómez et al., 2012). Osmorregulatory capacity varies with the molt stages, especially in natural conditions (Charmantier et al., 1994).

The aim of this research was to record the molting frequency, growth and biochemical analysis to access the adap-

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tation of juvenile wild-caught *F. duorarum* under laboratory conditions compared to cultivated; and second to determine the food consumption that precedes molting to test whether shrimp stop to eat when molting. In addition, monitoring of the frequency of molting in juvenile *F. duorarum* may identify fundamental variations in growth.

MATERIALS AND METHODS

Juvenile pink shrimp (mean wet weight of 4 g) were collected at night using casting nets from a coastal lagoon in Sisal, Yucatan $(21^{\circ}09'48''N, 90^{\circ}01'65''W)$, in the Gulf of Mexico. They were transported to a marine laboratory in Yucatan, Mexico in tanks with pumped air. In addition, second-generation juvenile pink shrimp were cultivated from wild broodstock eaptured offshore in the Gulf of Mexico and were sampled (at an approximate weight of 4 g) in the laboratory of shrimp production at UMDI-Sisal. Water salinity was maintained at 22 (726 mOsm kg⁻¹), temperature at 28°C, and shrimp were fed a diet based on 40% animal protein (Table 1) until the start of the experiment.

Experiment 1, Molting Frequency and Growth

Two sets of data were collected, one for wild lagoon-caught shrimp and one for cultivated second-generation shrimp. A reeirculating water system with ten 40 L tanks, each with 9 divisions, was set. One of the divisions was used for aeration while the others were used to isolate individuals, at 80 individuals per treatment. Water salinity of 22 (± 1) and temperature of $28^{\circ}C$ ($\pm 1^{\circ}C$) were controlled in the system to maintain consistency. The daily photoperiod was 12 hours light: 12 hours dark. Shrimp were fed three equal portions daily (at 8:00, 14:00, and 20:00 honrs). These conditions were maintained for 55 days. The molt of each individual was collected and registered daily. The first molt or eedysis event was considered the initial molt, after which the duration of each molting eycle was recorded and molting frequency obtained. Individuals were weighed weekly as well as each exnviae obtained. Special care was taken to avoid excessive handling that might affect survival or growth. To determine the increase in wet weight per day, an average per each individual was calculated weekly and later divided over time (days), while the average weight gain per molt was determined by the difference of individual wet weights before and after each ecdysis event, both in wild and cultured shrimp.

Biochemical Analysis in Hemolymph After Laboratory Conditions

Hemolymph (100 μ l) was sampled in 80 wild and 80 eultivated shrimps with a new syringe previously rinsed with an anticoagulant solution, SIC-EDTA (SIC: 450 mM NaCl, 10 mM CaCl, 10 mM KCl, 10 mM Hepes, 10 mM; EDTA-Na2: 1033 mOsm kg⁻¹, pH 7.3 at 2-8°C). This solution was disearded immediately before the syringe was used to puneture the ventrolateral sinus of the first pleomere to obtain hemolymph (Vargas-Albores et al., 1993). Hemolymph was placed on a piece of Parafilm[®] over cooling gel to keep the sample cold. Subsamples were taken for analysis of total protein. Immediately after hemolymph sampling, osmotic pressure was measured with a micro osmometer (American Advance Instruments) (Pascual et al., 2007). Osmotic capacity (mOsm kg^{-1}) was calculated as the difference betweeu the osmotic pressure in the hemolymph and in the external medium (Charmantier et al., 1989).

To measure the total protein eoneentrations (mg ml⁻¹) in the hemolymph, the extracted plasma was diluted (1:20) in an isotonic solution with a complex added to avoid coagulation (SIC-EDTA, pH 7.3; at 2-8°C) according to Vargas-Albores et al. (1993). It was then centrifuged at 2500 revolutions per minutes for 3 minutes at 4°C and the supernatant was collected in Eppendorf tubes. Aliquots of 20 μ l with 200 μ l of Bradford reactive solution (1976) were placed in microplates and read in an ELISA lector (Bio-Rad Benchmarek Plus) at 595 nm. SIC-EDTA was used as a blank. Biochemical analysis was compared through the molt stages of each individual of shrimp over the course of 55 days.

Experiment 2, Food Consumption Before and After Ecdysis

A second experiment was designed to estimate the mean food consumption before and after ecdysis in both wild and cultivated shrimp, in order to determine if both groups stop feeding when eedysis oceurs. We used 20 wild-caught and 20 cultivated juvenile shrimp of 4 g. Ten 40 l aerated tanks (salinity 22 and temperature 28°C), each with four divisions to isolate individuals, were used. Individuals were fed three times per day (at 8:00, 14:00, and 20:00 hours) with 0.500 g of food (Table 1) during a complete molting cycle (approximately 10 days). Food uneaten after 2 hours was removed with a siphon and placed in a 500 ml plastic bottle, taking care to minimize handling of individuals. Feces were extracted with a Pasteur micropipette. Water samples were filtered through pre-weighed Whatman filters GF/C (1.2 μ m pore, 55 mm diameter). Filters were dried at 60°C for 48 hours then re-weighed to determine the dry weight of the uneaten food. Total food cousnmption per individual was determined from the expression: Ai = (A0 - A1) - L; where, Ai is total food consumption, A0 is the dry weight (g) of food offered, AI is the dry weight (g) of food recovered from filtered water, and L is the lixiviation constant determined as the weight loss of food when is in contact with water. A lixiviation test of 0.500 g of food (form of pellets) was performed obtaining the average values of the difference between the dry weights of 10 filters with food recovered after 6 hours in water and the weight of the pre-weighed filters. Food consumption was eompared before and after each eedysis.

Data Analysis

Statistical differences in molting frequency, weight gaiu, and food consumption were analyzed between wild and cultivated shrimp with oneway ANOVAs. Also, differences in the osmotic eapacity and total protein eoncentrations between wild and eultivated shrimp were analyzed with one-way ANOVAs, having previously verified the assumptions of uormality (Kolmogorov Smirnov) and homogeneity of variance (Bartlett and Cochran) (Zar, 1999). Analyses were performed using STATISTICA 7.0 (StatSoft Ine., 2004).

Table 1. Diet formulation used in the experiment in percentages (%) of dry matteri ^a 65% protein, Apligen S.A. Mexico; ^b 70% protein; ^c DSM Nntritional Products, Mexico; ^d Sodium ascorbate (stay C).

Ingredient	Total (%)	Protein (%)	Lipids (%)	Carbohydrates (%)
Chilean fishmeal ^a	46.0	29.4	3.7	
Squid meal	10.0	7.1	0.5	1.1
Soluble fish proteic concentrate ^b	5.0	3.5	1.0	
Cod liver oil	3.0		2.0	
Wheat starch	20.0			20.0
Soy lecithin	1.0		1.0	
Cholesterol	0.5		0.5	
Vit premix ^c	1.5			
Robimix C ^d	0.5			
Carboxymethyl Cellulose	1.0			
Filler (Talc)	12.0			
Total (%)	100	40	8.7	21

RESULTS

Molting Frequency and Growth

A total of 509 molts were registered throughout the experimental period. Molting occurred mainly at the same time or within the same day. Few shrimp, whether wild or cultivated, molted twice during a period in which others were only able to molt once (Fig. 1). The frequency of the molt cycle in laboratory conditions for wild shrimp was around 10 ± 2.03 days and for cultivated shrimp was 11 ± 2.15 days (Table 2). No significant differences (F = 0.052, p = 0.822) in the molting frequency between wild and cultivated shrimp were obtained. However, there was a significant difference (F = 29.645, p = 0.000) in molting frequency between consecutive molting cycles of the combined data (wild and cultivated pooled) of *F. duorarum* over 5 complete cycles showing a decreasing trend after each molt.

The increase in wet weight in wild *F. duorarum* was 0.024 ± 0.001 g day⁻¹ and 0.245 ± 0.171 g molt⁻¹. On the other hand, the increase in wet weight of cultivated shrimp was 0.084 ± 0.002 g day⁻¹, and 0.343 ± 0.333 g molt⁻¹. No significant differences were found among groups (F =

Table 2. Data of the molt registered over 55-day period in wild and cultivated F duorarum in temperature of 28°C and salinity 22.

Data registered	Wild $(n = 80)$	Cultivated (n = 80)	
Number of total molt occurred	316	274	
Mean of molt cycle (days)	10.11	11.39	
Standard deviation	2.03	2.15	
Shortest time to molt (days)	5	7	
Longest time to molt (days)	17	18	
Total of most frequent molt cycle	72	59	

0.406, p = 0.542) considering 5 complete molt cycles under the experimental conditions (Fig. 2).

Osmotic Capacity and Total Protein Concentration in Hemolymph

After the 55 days of the experiment, wild *F* duorarum showed a mean osmotic capacity around of 154.85 ± 73.47 mOsm kg⁻¹. Cultivated shrimp had a mean of 128.67 ± 42.24 mOsm kg⁻¹. Osmotic capacity differences were significant (ANOVA; F = 5.7002, p = 0.0185) between wild and cultivated shrimp (Table 3, Fig. 3). Total proteins



Fig. 1. Frequency of the molt cycle in juvenile wild and cultivated Farfantepenaeus duorarum during 5 complete molt cycles over a 55-day period.



Fig. 2. Average growth \pm standard deviation during 5 complete molt cycles in juvenile wild and cultivated *Farfantepenaeus duorarum*. First molting event (day 0 = 4.0 g) was considered the initial molt, after the time between consecutive molts was recorded to obtain the molting frequency.

Table 3. Osmotic capacity and concentration of total proteins in the blood. Values are mean \pm standard deviation. Results of the one-way ANOVA to test significant differences (*p < 0.05) between the wild and cultivated shrimp in *F. duorarum*.

Biochemical analysis	Wild $(n = 80)$	Cultivated $(n = 80)$	F	p	
Osmotic capacity (mOsm/kg) Total proteins (mg/ml)	$\frac{154.85 \pm 73.47}{162.94 \pm 35.15}$	$ \begin{array}{r} 128.67 \pm 42.24 \\ 112.22 \pm 44.03 \end{array} $	5.7200 44.5550	0.01846* <0.0001*	

concentration showed a mean concentration of $162.94 \pm 35.15 \text{ mg ml}^{-1}$ in wild shrimp. Cultivated shrimp showed a lower and significant (ANOVA; F = 44.5550, p = 0.0000) mean concentration of $112.22 \pm 44.03 \text{ mg ml}^{-1}$.

Reduction in Food Consumption Before Ecdysis

The amount of lixiviated food was determined as 0.085 ± 0.010 g of loosen material when a 0.500 g pellet was in contact with water for 6 hours. Mean food consumed per each feeding period in both wild and cultivated was 0.278 ± 0.106 g. No significant differences were found in the food consumption (F = 0.220, p = 0.641) between groups measured at each time before and after ecdysis. The mean amount of food consumed per animal at each feeding time declined 20 hours before ecdysis, and maintained low values until about 4 hours before ecdysis (30% less food). Eight hours after ecdysis, consumption began to increase and about 40 hours after ecdysis it had returned to former levels. Although consumption fell during molting, it did not cease in both wild and cultivated (Fig. 4).

DISCUSSION

Determination of growth in crustaceans is complex, especially when dealing with wild animals, since growth is linked to the molting process. In crustaceans, growth pattern has two major components: the increase in growth that can occur at molting; and the interval that occurs between two molt events (Hartnoll, 1982). Growth and molting frequency in crustacean are most commonly analyzed in captive or cultured organisms. Thus, observations in cultivated shrimp can be used to determine differences between weight and size before and after ecdysis (Petriella and Boschi, 1997); whereas these measurements are difficult to obtain in natural environments. Usually, organisms are removed from their natural environment and subjected to an experimental system that allows for individualized monitoring. This enables measurements over several successive molt cycles. In this study, we found that wild shrimp do not performed as well when they are first introduced to laboratory conditions compared to organisms belonging to a second-generation raised in captivity. However, the increase in weight of wild and cultivated shrimp showed a similar pattern mainly due to the 40% animal protein diet used which offers nutrients required for the synthesis of body tissues and exoskeletons (Gaxiola et al., 2004). The transfer of wild shrimp to culture conditions using this diet did not change the molting cycle and wet weight gain rate compared to organisms adapted to laboratory conditions.

The molt cycle in wild-caught juveniles lasted 10 days, whereas the duration in cultivated juveniles was 11 days;



Fig. 4. Food eonsumption (g eonsumed per feeding) in wild (A) and cultivated (B) Farfantepenaeus duorarium (4 g) as a function of time before and after molting or ecdysis. Values are average \pm standard error. Time 0 = ecdysis.

other research results suggest durations of 10-19 days (Eldred et al., 1965) or 4.8-9.6 days (Gracia, 1995). Duration of the molt cycle in other penaeids is closely related to or influenced by temperature, salinity, or size. *Litopenaeus vannamei* (Boone, 1931) at 28°C a molt cycle of 14 days (Charmantier et al., 1994), 11.7 days (Betancourt et al., 1993), or 9.3 days (Galindo, 2007); at 27-29°C, *Litopenaeus setiferus* (Linnaeus, 1767) has a 13.6 days cycle, and *Litopenaeus stylirostris* (Stimpson, 1874) has a cycle of 11.5 days (Robertson et al., 1987; Lemaire et al., 2002).

The variation of metabolite concentrations in hemolymph was the result of the biochemical adaptations displayed by wild shrimp to laboratory conditions, which are necessary to maintain homeostasis (Galindo et al., 2009; Bonilla-Gómez et al., 2012). Due to that the intensity in the osmorregulation mechanisms between hemolymph and environment depends on the concentration of salts in the environment (Lemaire et al., 2002). According to Péqueux (1995) the osmoregulation is an important mechanism of environmental adaptation in aquatic species, particularly in crustaceans. Those were relatively constant in *F. duorarum*, as has been observed in *L. vannamei* (Pascual et al., 2003) and in *L. setiferus* (Rosas et al., 2004). Growth was similar in both wild and cultivated organisms, indicating that *F. duorarum* could be adapted to laboratory conditions, with no difference in weight gain and frequency of molting even if *F. duorarum* have been described like a highly sensitive species to change in the physical-chemical environment that result in death (López-Téllez et al., 2000).

Proteins (amino acids) are a component of the reserves that are accumulated along the intermolt to be used in all the cyclical process of growth in wild and cultivated shrimp. Weight gain was slightly greater in cultivated shrimp, despite having on average a molt cycle one day longer than wild shrimp. This may be attributed to adaptation to food, in this case the artificial diet supplied. This supports the view of Rosas and Carrillo (2006) that cultivated shrimp are well adapted to use protein as an energy source, and as a molecule for growth. Surprisingly, wild shrimp were unable to consume the total amount of food provided, which indicates that growth is controlled hormonally by ecdysone. However, since the hormonal control that produces this effect also affects cultivated shrimp, the difference would be marked by adaptation. Cultivated shrimp are efficient reserving nutrients for the process of calcification and energy expenditure involved in ecdysis. Despite this, the wild and cultivated shrimp did not differ significantly, so from the statistical standpoint, the same amount of biomass is created during intermolt with either treatment, even though the duration of the molt cycle differs.



Fig. 3. Osmotic capacity (mOsm/kg) and total concentration of proteins in hemolymph (mg/ml) over a 55-day period in wild and cultivated juvenile *Forfantepenaeus duorarum* in relation to molt stage. Values are mean \pm standard error (vertical bars denote 0.95 confidence intervals).

Food intake is also linked to the molt cycle and it is clear that nutrition in Decapoda is a broad topic to study, because in the natural environment such organisms have specific diet requirements (Petriella and Boschi, 1997). After ecdysis, ingestion increased providing evidence that a rapid restoration of the digestive structures occurs rapidly in the first molt stages. These results contrast with previous reports mentioning that shrimp stop eating before ecdysis (Chan et al., 1988; Dall et al., 1990; Ceccaldi, 1997; Anger, 2001).

In conclusion, food intake shows a decline in both wild and cultivated organisms, which occurs 20 hours prior to ecdysis. The minimum values were registered 4 hours before molting and the consumption was recovered 8 hours after. Ingestion does not stop before or after ecdysis occurred. Thus, the mouthparts that are synthetized with the new exoskeleton in *E duorarum* calcify rapidly so organisms can take advantage of food resources sooner than other species do. In the wild, competition for the resource may be a limiting factor, and reducing the time required to feed is an advantage (Schwamborn and Criales, 2000) considering that growth depends on the adequate supply of food (García-Galano, 2006).

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