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Molting behavior and growth in the giant barnacle
Austromegabalanus psittacus (Molina, 1782)

Muda y crecimiento en el cirripedio gigante Austromegabalanus psittacus (Molina, 1782)

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Abstract.- The effect of temperature and photoperiod on molt production in juvenile specimens of the acorn barnacle Austromegabalanus psittacus (Molina, 1782) was evaluated under controlled conditions. Growth was determined both under controlled and natural conditions. The molt frequency, intermolt period and instantaneous growth rates of the carino rostral lengths were measured in specimens subject to two temperature conditions (10ºC and 16ºC) and photoperiods (8 hours light: 16 hours darkness and 16 hours light: 8 hours darkness). Growth of specimens in natural conditions was evaluated in substrates suspended from floating rafts in Metri Bay (41°36’S; 72°43’W) at two depths: between 1 and 2 m and between 4 and 6 m, during the autumn-winter and spring-summer seasons. Molt frequency was greater at 16ºC than at 10ºC, with no evidence of variations due to photoperiod. The intermolt periods were greater at 10ºC than at 16ºC and during the photoperiods 8:16 than at 16:8. Instantaneous growth rates, on the other hand, were greater at 10ºC than at 16ºC and under photoperiod conditions of 8:16 than at 16:8. No relation was observed between instantaneous growth rate and molt frequency. Under natural conditions, a larger increase in length was observed in individuals growing at greater depths. The results obtained in a controlled environment and in the natural environment were consistent, given that the higher growth reported in suspended systems at greater depths, was associated with lower luminosity conditions and lower temperatures; a similar situation was observed under controlled conditions.

Key words: Balanidae, giant barnacle, temperature, photoperiod, Chile

Introduction
Temperature and photoperiod generate functional and behavioral changes in barnacles that can be expressed not only in the condition of individuals, but also at a population level (Clare 1987, Sarojini & Nagabhushanan 1999). It has been reported that photoperiod and temperature can directly and synergistically affect metabolic activities, and also cause specific endogenous behavior, influencing growth and molt frequency in specimens of various intertidal species, such as Semibalanus balanoides, Balanus amphitrite, Balanus eburneus and Balanus trigonus (Crisp & Patel 1960, El-Komi & Kajihara 1991). Furthermore, their effect and the result of any interaction between them may vary according to the seasonal variations of these factors (Barnes & Stone, 1974). Austromegabalanus psittacus (Molina, 1782) is a species of endemic barnacle
distributed along the entire Chilean coastline, from southern Peru (6°S) to the Straits of Magellan (58°S), including the Juan Fernández archipelago (Nilsson-Cantell 1957, López et al. 2007a) and the southern zone of the Atlantic coast (Young 2000). This species mainly inhabits the subtidal zone, between 5 and 7 m depth (Pilsbry 1916, Santelices 1991), forming dense groups that can reach large dimensions (López et al. 2007b). This species is economically important in Chile, because it is traditionally consumed as human food. At present culture technologies are being developed that require knowledge of the factors that affect biomass production, growth and periodic changes in the exoskeleton or molt. Although it is not possible to directly relate biomass growth to molt production frequency in barnacles (Costlow & Bookhout 1956, Sarojini & Nagabhushanam 1999), ecdysis processes permit an understanding of the functioning of the neuroendocrine system associated with growth. Studying the effects of environmental variables that fluctuate seasonally, such as temperature and photoperiod, can help to determine how these marine organisms adjust functionally and behaviorally to the physical-chemical factors present in the environment. This information could, secondarily, be considered both in the design and development of efficient culture technologies, or in the management of natural populations. In this species, it has been established that molt frequency is affected by fluctuations in salinity and hydrocarbon concentrations in the water. Thus, evidence indicates that molting frequency was greater at 30 psu than at 20 psu, while diesel concentrations of 0.1% v/v, produce an increase in molting production frequency with respect to concentrations of 0.5% v/v and treatments without petroleum (López & López 2005).

The aim of this study was to establish the effects of variations in temperature and photoperiod on the production of molts and the growth of *Austromegabalanus psittacus* juveniles, maintained under controlled conditions, comparing this with observations made on growth in the natural environment.

**Material and methods**

**Experiments in controlled environments**

Sixty *Austromegabalanus psittacus* juveniles were collected from Metri Bay in southern Chile (41°36’S; 72°42’W), during August and October, 2005. Size range fluctuated between 0.7 cm and 1.0 cm carino-rostral length (maximum distance between the carinal plate and the rostral plate), which is a density-independent measurement of age (López et al. 2007b). They were transported to aquaria in the Marine Aquaculture Laboratory of the Universidad de Los Lagos, after eliminating sediments and epibionts. They were acclimatized for a period of 15 days in 15 l trays with sea water and a constant air supply at an environmental temperature (15 ± 2°C), a natural photoperiod and fed *ad libitum* with the microalga *Dunaliella tertiolecta*.

To evaluate molting behavior, a binary factorial experimental design was used, at two photoperiod regimes (8 hours light: 16 hours darkness and 16 hours light, 8 hours darkness) and two temperature conditions (10°C and 16°C). Temperature values were selected according to the range of surface water temperatures present in southern Chile, that fluctuate between an average of 10°C in the winter and 16°C in summer. Similarly, photoperiod regimes were selected based on the seasonal fluctuations of this factor in the same area. For each treatment, juvenile specimens were placed individually in 1 L glass containers, with filtered seawater (0.45 μm) and a constant air supply. Fifteen replicates were undertaken for each treatment, over a total experimental period of 60 days. The individual specimens in each experimental treatment were fed three times weekly with the microalga *Dunaliella tertiolecta*, at average concentrations of 50,000 cells mL⁻¹. The number of light and dark hours in each of the experimental treatments was regulated by using two structures (‘black boxes’) that contained a luminosity system consisting of two 20W fluorescent tubes. Each of the structures was connected to a digital electronic timer, permitting automatic regulation (on/off) of the light source, according to photoperiod. A constant temperature was maintained, to ± 0.1°C accuracy. Daily controls were undertaken to determine molting frequency, quantifying the number of exuviae produced by each of the individuals over the total 60-day period. The intermolt periods per individual (time lapse between one molt and another) were expressed in days. Similarly, specimen growth was evaluated, calculating the instantaneous growth rates (d) of the increase in carino-rostral length, according to the formula: \[ d = \frac{\ln FS - \ln IS}{\Delta t}, \]

where \( FS \) is the final size at the end of the period, \( IS \) is the initial size at the beginning of the period and \( \Delta t \) is the period in days.

Comparisons of accumulated molt frequencies were analysed using the Kruskal-Wallis non-parametric test (Sokal & Rohlf 1979). In the case of the intermolt periods and instantaneous growth rates, comparisons between treatments were undertaken by 2-way variance analysis, after complying with normality and homocedasticity assumptions, using the Shapiro-Wilk and Bartlett tests, respectively. Significant differences were analysed according to an *a posteriori* Tukey test. Furthermore, the relationship between molting frequency and growth was determined by carrying out lineal regression analysis...
and Pearson correlation (Sokal & Rohlf 1979). The STATISTICA 6.0 (StatSoft 2001) computer statistical package was used for statistical analysis.

Experiments in natural systems

During February and September, 2005, artificial substrates, suspended from a floating raft, were installed for cypris larval settlement and subsequent growth of Austromegabalanus psittacus specimens in Metri Bay (41°36'S; 72°42'W), southern Chile. This is a wave-protected location, with average tidal fluctuations of 7 m. Two types of growth systems were designed: - four 33 cm x 33 cm polyethylene plates, 0.6 cm thick, separated by a distance of 30 cm, and joined by a polypropylene cord (n = 5); - three polyethylene tubes, 70 cm long and 10 cm diameter, placed 50 cm apart, and joined by a 6 mm polypropylene cord (n = 5).

These systems were located at two depths: between 1 and 2 m and between 4 and 6 m. Surface temperature fluctuated between 9 and 17°C on average. As depth increased, temperature decreased by between 1°C and 3°C. Average natural photoperiod during this season of the year was 16:8. At depths between 4 and 6 m, the relationship was inverse. Growth was determined bimonthly in samples of 100 specimens, according to depth, and the increase in carino-rostral length was determined with a caliper, to 0.01 accuracy, over a total period of 20 months.

Comparison of the average increases in carino-rostral length according to depth was carried out by one-way variance analysis, with repeated measurements (Zar 1999).

Results

Experiments in controlled environments

a) Molting frequency

Temperature modified molting frequency, which was greater at 16°C than at 10°C (H de Kruskal-Wallis = 25.017; g.l. = 1.41; P<0.05). Nevertheless, there were no statistically significant variations under different photoperiod regimes (H de Kruskal-Wallis = 0.023; g.l. = 1.41; P = 0.877) (Fig. 1).

b) Intermolting periods

Statistically significant differences occurred in intermolting periods according to photoperiod regime. The intermolting periods were longer at photoperiod 8 h light: 16 h darkness than at 16 h light: 8 h darkness. Temperature also significantly modified this variable, which was more prolonged at 10°C than at 16°C. No significant interaction was observed between photoperiod and temperature (Table 1, Fig. 2).
c) Instantaneous growth rate

Significant differences were observed between the two photoperiod regimes, with greater growth at photoperiod 8:16 than at 16:8. Similarly, statistically significant differences in growth occurred between temperatures, being greater at 10°C than at 16°C. Interaction between photoperiod and temperature was not significant (Table 2, Fig. 3).

d) Relation between molting frequency and growth in carino-rostral length

Molting frequency and instantaneous growth rate in carino-rostral length were not related. The Pearson correlation coefficient (r) between these variables was 0.27; the linear regression slope was not statistically significant ($P = 0.086$) (Fig. 4).

Experiments in natural systems

The increase in carino-rostral length differed significantly between specimens growing at different depths ($F = 5.6$; $gl = 1;198$; $P<0.05$). After 20 months, average size of specimens growing at a depth of between 4 and 6 m was $3.4 \pm 0.17$ cm carino-rostral length, while average carino-rostral length of those that grew a depth of between 1 and 2 m was $2.92 \pm 0.14$ cm (Fig. 5).

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Table 1

<table>
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<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
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<tr>
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<td>60.05</td>
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<tr>
<td>Photoperiod*Temperature</td>
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<td>0.007</td>
<td>3.72</td>
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<tr>
<td>Error</td>
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<td>37</td>
<td>0.002</td>
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</table>

Source of variation = sum of squares, df = degree of freedom, MS = mean square, $F = F$-ratio, $P = p$-level

Table 2

<table>
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<th>Source of variation</th>
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<th>MS</th>
<th>F</th>
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<tr>
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<td>Error</td>
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</table>

Source of variation = sum of squares, df = degree of freedom, MS = mean square, $F = F$-ratio, $P = p$-level
Discussion

Molting in barnacles includes discarding the prosoma, cirrus, penis, digestive tract teguments and internal mucous membrane (Darwin 1854). The main factors that affect the molting cycle are temperature, photoperiod and food availability (Clare 1987, Sarojini & Nagabhushanam 1999). Costlow & Bookhout (1953, 1956) reported that Balanus improvisus and Balanus amphitrite niveus juveniles maintained in laboratory under different photoperiod conditions and at a temperature of 20°C, molted during periods of 2-3 days and shell growth was continuous; similarly, they established that individuals subject to total darkness conditions reached a higher growth rate (shell basal area) than those kept under continuous light conditions. In relation to food availability, Crisp & Patel (1960) observed that molting frequency in Semibalanus balanoides individuals starving for a period of 30 to 50 days, declined considerably. On contrast, specimens fed with Artemia sp. larvae, continued molting at the same frequency. These authors also verified that molting frequency in individuals that were fed, linear to temperature increase, from 3°C to 20°C, while in those animals without food, this effect was only produced from 3°C to 12°C, decreasing considerably at higher temperatures. In Austromegabalanus psittacus, molting frequency in juveniles was affected by temperature, with greater molting frequency at 16°C than at 10°C. This could be due to decreasing metabolism at lower temperatures or to effects at the neuroendocrine system level, associated with the molting cycle. These results are congruent with those reported by Crisp & Patel (1960) and El-Komi & Kajihara (1991), who, in their studies of other species of barnacles, established that molting frequency increased as temperature increased up to a certain level, depending on the species; above this temperature level, molting frequency began to decrease drastically. It is probable that thermal regimes in the natural environment are an influencing factor. Average winter temperatures in southern Chile are around 10°C and in summer, 16°C. On the other hand, molting frequency did not differ according to photoperiod regimes (24 hours light; 24 hours darkness; 12 hours light and 12 hours darkness). Nevertheless, constant illumination during the reproductive intermolt led to the rapid reestablishment of molting frequency, thus indicating the importance of an adequate photoperiod during this condition (Barnes & Stone 1974). According to Darwin (1854), molting frequency would be related to
growth rate. Nevertheless, in studies undertaken on *Balanus improvisus* and *Balanus amphitrite niveus*, Costlow & Bookhout (1953, 1956) established that shell growth is not associated with molting frequency. Similarly, in *Austromegabalanus psittacus*, no association was found between these variables, given that molting frequency was not higher in those individuals that grew more. The instantaneous growth rate of *A. psittacus* was affected by variations in temperature and photoperiod. However, no interaction was observed between both variables. Instantaneous growth rates were greater at 10°C than at 16°C. Since individuals kept at temperatures of 10°C have lower molting frequencies than those maintained at 16°C, they also have lower energetic expenditure (Newell 1979).

Among the environmental factors, temperature tends to increase growth in many species of marine invertebrates (Newell 1979). However, the metabolic costs could change this pattern. Growth depends, for the most part, on the way species distribute energy obtained from food towards the different physiological processes (Diaz & Martinez 1992). *Austromegabalanus psittacus* has accelerated growth rates at an early age, with a subsequent and considerable decrease during further age. In the natural environment, growth was higher at greater depths, where temperature and quantity of light hours are lower. If feeding is similar at different depths, a drop in energetic costs could imply increased energy available for growth. Growth in individuals subject to the 8:16 photoperiod was greater than in those maintained at 16:8 photoperiods. Given that the 8:16 photoperiod has a greater number of dark hours (2/3) than the 16:8 photoperiod (1/3), this result could be explained by the fact that availability of carbonates for shell growth is lower at greater luminosity conditions, due to the consumption of carbon dioxide provided by the unicellular algae used as food for specimens, both under laboratory and natural conditions. The opposite occurs when the darkness period is increased. Growth obtained under laboratory conditions cannot be entirely extrapolated to natural conditions, given that the different environmental factors are not fixed and interact with a complex series of variables. Nevertheless, *A. psittacus* growth in suspended systems was higher at greater depths than at surface level, which is congruent with the results obtained under controlled conditions. The information gathered from the results obtained is applicable to the future culture of *A. psittacus*. At present, cultures are being carried out on a semi-industrial scale, taking advantage of the favorable biological characteristics of this species (López et al. 2005). The molting period is considered to be a highly vulnerable stage for crustaceans, due to their greater propensity to infectious diseases that tends to increase mortality rates. The results show that molting frequency is greater at higher temperatures and that the opposite occurs with growth. Thus, lower temperatures would favor the growth and survival of juveniles of *A. psittacus*. On the other hand, greater growth was observed during the photoperiod 8 h light: 16 h darkness than at photoperiod 16 h light: 8 h darkness, indicating that biomass production in cultures would be higher under conditions of longer darkness hours. As a consequence, during the fattening phase, cultures should be undertaken at depths of 4 to 6 m, where temperatures are more stable throughout the year, bordering on 10°C, and, furthermore, luminosity conditions are low.

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