Shape and size variations of *Aegla uruguayana* (Anomura, Aeglidae) under laboratory conditions: A geometric morphometric approach to the growth

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**ABSTRACT.** Crustacean growth studies typically use modal analysis rather than focusing on the growth of individuals. In the present work, we use geometric morphometrics to determine how organism shape and size varies during the life of the freshwater crab, *Aegla uruguayana* Schmitt, 1942. A total of 66 individuals from diverse life cycle stages were examined daily and each exuvia was recorded. Digital images of the dorsal region of the cephalothorax were obtained for each exuvia and were subsequently used to record landmark configurations. Moul increment and intermoult period were estimated for each crab. Differences in shape between crabs of different sizes (allometry) and sexes (sexual dimorphism; SD) were observed. Allometry was registered among specimens; however, SD was not statistically significant between crabs of a given size. The intermoult period increased as size increased, but the moult frequency was similar between the sexes. Regarding ontogeny, juveniles had short and blunt rostrum, robust forehead region, and narrow cephalothorax. Unlike juveniles crabs, adults presented a well-defined anterior and posterior cephalothorax region. The rostrum was long and stylised and the forehead narrow. Geometric morphometric methods were highly effective for the analysis of aeglid-individual growth and avoided excessive handling of individuals through exuvia analysis.

**KEYWORDS.** Crustacea, cephalothorax, ontogeny, intermoult, morphometrics.

**RESUMEN.** Variaciones de forma y tamaño de *Aegla uruguayana* (Anomura, Aeglidae): Una aproximación desde la morfometría geométrica al crecimiento. Los estudios de crecimiento en crustáceos típicamente utilizan análisis modal en lugar de focalizarse en el crecimiento individual de los organismos. En el presente trabajo, utilizamos morfometría geométrica para determinar cómo varía la forma y el tamaño a lo largo de la vida del cangrejo de agua dulce, *Aegla uruguayana* Schmitt, 1942. Un total de 66 individuos, en diferentes etapas del ciclo de vida, se examinaron diariamente, registrándose la presencia de exuvias. Imágenes digitales de cada muda fueron obtenidas de la región dorsal del cefalótorax y se utilizaron para registrar las configuraciones de landmarks. El incremento por muda y el período de intermuda se estimaron para cada cangrejo. Diferencias de forma entre tamaños (alometría) y sexos (dimorfismo sexual; SD) se observaron. Se registró la presencia de alometría entre los especímenes; sin embargo, el SD no fue estadísticamente significativo respecto del tamaño. El período intermuda aumentó de manera directamente proporcional al tamaño, pero la frecuencia de muda fue similar entre los sexos. Durante la ontogenia, los juveniles presentaron rostro corto y romo, frente robusta, y ancho del cefalótorax estrecho. Los adultos presentaron la región anterior y posterior del cefalótorax bien definido en relación con los juveniles. El rostro fue largo y estilizado y la frente estrecha. Los métodos de morfometría geométrica fueron muy efectivos para el análisis del crecimiento individual en aeglidos y permitieron evitar la manipulación excesiva de los individuos a través del análisis de las mudas.

**PALABRAS-CLAVE.** Crustáceos, cefalótorax, ontogenia, intermuda, morfometría.
The family Aeglidae is one of the six decapod families observed in the continental aquatic environments of South America (Perez-Losada et al., 2004, 2009; Bond-Buckup et al., 2010; Santos et al., 2010). It is the only one Anomura life cycle in freshwater and exhibits an endemic distribution in the tropical, temperate and cold areas of southern South America (Martin & Abele, 1986). Aeglidae are represented by a single living genus, Aegla Leach, 1820, which includes 75 species, each with an endemic distribution (Santos et al., 2013; 2014) in a variety of environments, such as lakes, swamps, caves, rivers, streams and lagoons. Alternatively: a small number of these species, including Aegla uruguayana Schmitt, 1942, have a wider distribution (Schmitt, 1942; Lopez, 1978; Hobbs, 1979; Bond-Buckup & Buckup, 1994; Bond-Buckup, 2003; Giri & Collins, 2004; Almeida et al., 2009).

Although many Aeglidae species have been described, studies on the growth of these organisms are scarce. The majority of these studies used modal analysis rather than focusing on the individual growth of each organism (Vaz-Ferreira et al., 1945; Bahamonde & Lopez, 1961; Lopez, 1965; Bueno et al., 2000; Swiech-Ayoub & Masunari, 2001; Noro & Buckup, 2003; Boss et al., 2006; Silva-Castiglioni et al., 2006; Gonçalves et al., 2009; Trevisan & Santos, 2011). While these studies can provide relative growth rates for portions of the population (by sex or maturity), they cannot provide size increments or the intermoult period for individuals (Stevens, 2012). There are no studies documenting Aeglidae growth under laboratory conditions that focus on moults.

The aim of our work was to identify and characterize the changes in shape and size of the cephalothorax of the freshwater Anomura A. uruguayana during its ontogeny. Therefore, this study was the first to analyse the variation in these animals (Teodósio & Masunari, 2009). Specimens were acclimatised to laboratory conditions for five days under controlled conditions: temperature (25 ± 1°C), light (photoperiod: 12h/12h light-darkness) and constant aeration. After this period, individuals were separated, and the sex of each crab was identified following the morphological criteria (Martin & Abele, 1988). The crabs were maintained in individual aquaria and fed daily with pellet food designed for crustaceans (Collins & Petriella, 1996). Each aquarium was cleaned prior to feeding. The isolated individuals were observed daily and the presence of exuvia was recorded: subsequently, exuvia were carefully removed and maintained in alcohol (96%). This preservation method had no effect on shape (Rufino et al., 2004).

**Image acquisition and landmark definition.** A total of 159 photographs of cephalothorax exuvia were obtained using a SONY Cyber-shot® digital camera and a stereoscopic magnifying glass with a built-in MOTIC® camera. Subsequently, 21 landmarks were recorded on the dorsal cephalothorax. Landmarks (LM), defined as “points of correspondence on each object that match between and within populations,” (Deyden & Mardia, 1998) were identified and digitalised (TpsDig program, Rohlf, 2004) on the exuvia. Cephalothorax size was represented by a calculation using the centroid size (CS): the square root of the sum of the squares of the distances between the centroid and each point of the homologue object (Bookstein, 1991). This was used as a measure of the crab’s size.

Measurement error (photograph and landmark location) and side-individual variation were tested by Procrustes ANOVA, photographed twice and digitized fourfold for 13 specimens. The cephalothorax is a structure with object symmetry; because of this spatial arrangement, the trough symmetric sides are patterned and partially redundant (Klingenberg et al., 2002). This allowed us to perform the analysis using only one-half of the cephalothorax, as defined by the axis of symmetry (landmarks 1 LM, 10 LM, and 11 LM). This reduced the number of variables required to increase the statistical power (i.e., a greater number of landmarks correspond to a greater number of shape variables, and therefore, more specimens would be needed to equilibrate the matrix for the multivariate analysis) and to avoid algebraic problems (Rufino et al., 2006).

Finally, 12 landmarks representing the half of the cephalothorax were included in the analysis. The following step consisted of removing unwanted parameters, such as position and size by General Procrustes Analysis (GPA) (MorphoJ Klingenberg, 2011). The allometry among individuals was analysed using a regression of shape on CS values or each individual (MorphoJ Klingenberg, 2011). An exploratory Relative Warp analysis (RW) was performed as an initial approach to the relationships between shapes. In this way, variations between the moults were identified (Fig. 1, Tab. I).
The size increase was expressed as:

\[
\text{Rate of increase: } \frac{(Cs_2 - Cs_1)}{Cs_1}
\]

where: \(Cs_1\) is the value of the centroid from an individual first moult and \(Cs_2\) corresponds to the centroid of the second moult. This formula was applied to all moults. The intermoult period was assessed during a daily follow-up of the individuals.

**Data analyses.** Statistical analysis of the data was performed using R software version 2.6.2 (R DEVELOPMENT CORE TEAM, 2008). With data from the animals isolation, a Wilcoxon test (W) was used to compare cephalothorax size, increase rates, and intermoult time between juveniles vs. adults and males vs. females because the data were not normally distributed and/or the variances were not homogeneous. A MANCOVA was conducted comparing the shapes of the individual moults and comparing the male and female shapes.

**RESULTS**

**Error measurement: the relationship between photograph and landmark location.** The error of measurement was acceptable; the mean squares for individual variation were greater than the mean squares of other effects (side, individual-side and error). Side variation by specimen was not statistically significant (Appendix I).

**Shape and size variations during ontogeny.** Different size crabs had distinct cephalothorax shapes and displayed ontogenetic, allometric changes. This variation was explained in 4.38% of individuals \((P < 0.0001)\) (Fig. 2). Furthermore, individual changes at each moult (growth) were similar for juveniles and adults, with certain changes in cephalothorax shape that characterised each ontogenetic phase (MANCOVA: Wilks’ \(\lambda = 0.56, F_{GL} = 5.34, P = 9.03e-10\)). Cephalothorax size, identified by \(Cs\), was also statistically significant in these groups \((W = 20.0, P < 2.2e-16)\), establishing a relationship between shape and centroid size (Fig. 2).

Compared to adults, the anterior and posterior (divided by the landmarks L6-L12) regions of the cephalothorax of smaller individuals were less defined. Juveniles had a particularly short and blunt rostrum (L1), and the forehead region (L1-L3) was more robust than in adults. Additionally, cephalothorax width (L7) was narrower in juveniles. Larger individuals presented well-defined anterior and posterior cephalothorax regions. The

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*Fig. 1. Location of the 12 landmarks (LM) left dorsal half cephalothorax of Aegla uruguayana Schmitt, 1942.*

*Tab. I. Description and location of landmarks (LM) in Aegla uruguayana Schmitt, 1942.*

<table>
<thead>
<tr>
<th>Number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1LM</td>
<td>Tip of the rostrum</td>
</tr>
<tr>
<td>2LM</td>
<td>Orbital sine</td>
</tr>
<tr>
<td>3LM</td>
<td>Tip of the anterolateral spine</td>
</tr>
<tr>
<td>4LM</td>
<td>Union between the first and posterior end of the anterolateral lobe</td>
</tr>
<tr>
<td>5LM</td>
<td>Union between the third hepatic lobe and the epibranchial lobe</td>
</tr>
<tr>
<td>6LM</td>
<td>Union between the epibranchial and the <em>linea aeglica lateralis</em></td>
</tr>
<tr>
<td>7LM</td>
<td>Union between the branchial line and the posterior of the <em>linea aeglica lateralis</em></td>
</tr>
<tr>
<td>8LM</td>
<td>Posterior vertices of the cephalothorax</td>
</tr>
<tr>
<td>9LM</td>
<td>Posterior extreme of the longitudinal dorsal line</td>
</tr>
<tr>
<td>10LM</td>
<td>Centre-posterior extremes of the cephalothorax</td>
</tr>
<tr>
<td>11LM</td>
<td>Centre-anterior extremes of the areola</td>
</tr>
<tr>
<td>12LM</td>
<td>Anterior extremes of the bar line</td>
</tr>
</tbody>
</table>

*Fig. 2. Distribution of variations in the shape of the cephalothorax explained by the relative warp (RW) 1 and 2 for juvenile (gray square) and adults (black diamond) of Aegla uruguayana Schmitt, 1942.*
rostrum was longer and stylised (L1) and the forehead (L1-L3) was narrower in adults than in juveniles (Fig. 3).

Regarding sexual dimorphism (SD), differences in shape were observed between the cephalothorax of males and females (MANCOVA: Wilks’ $\lambda = 0.49$, $F_{GL1} = 2.14$, $P = 0.02$). The first Relative Warp (RW1) explained 21.45 % of the variation in shape and the second Relative Warp (RW2) explained 14.08 %. However, the allometry was not statistically significant between the sexes, explaining 2.78 % of the variation ($P = 0.0562$) (Fig. 4). Furthermore, the variation in cephalothorax size between the males and females was not statistically significant ($W = 549.0$, $P = 0.51$). Males possessed a greater maximum width (L7), the longest rostrum (L1) and a narrower front (L3). In general, the rostrum front (L1-L3) was more robust in males (Fig. 5).

**Growth rate in relationship to size and sex.** Individual growth rates decreased as size increased, shifting with an increase in CS. Furthermore, smaller individuals exhibited greater variability in growth (Fig. 6). While the mean growth rate in adults was significantly lower than that of juveniles ($W = 770.0$, $P = 0.04$), the difference in growth rate of males and females was not statistically significant ($W = 131.0$, $P = 0.48$).

**Intermoult period.** For all groups, intermoult time increased with size (Fig. 7). Beginning in the postmoult stage, juveniles of $1.11 \pm 0.39$ CS passed an average of $32.00 \pm 14.45$ days between ecdyses at $25 \pm 1^\circ$C, whereas adults, with a centroid size of $2.45 \pm 0.56$, had an average intermoult time of $52 \pm 14.05$ days. The mean intermoult

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**Fig. 3.** Deformation grids adult (left black), juvenile (right black) and consensus configuration (grey) of *Aegla uruguayana* Schmitt, 1942 (scale factor 35).

**Fig. 4.** Spatial variations in the shape of the cephalothorax explained by relative warp (RW) 1 and 2 for males (up gray triangle) and female (down black triangle) of *Aegla uruguayana* Schmitt, 1942.

**Fig. 5.** Deformation grids males (left black), and females (right black) and consensus configuration (grey) of *Aegla uruguayana* Schmitt, 1942 (scale factor 0.05).

**Fig. 6.** Growth rate of *Aegla uruguayana* Schmitt, 1942 juveniles (gray square), males (up gray triangle) and females (down black triangle) of different sizes (CS), $r = 0.031$. 

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values were $46 \pm 3.56$ days for males and $56 \pm 17.44$ days for females (average size (CS) 2.49 $\pm$ 0.62 and 2.38 $\pm$ 0.49, respectively). The difference in intermoult time for juveniles and adults was statistically significant ($W = 38.5$, $P = 0.0004$). However, the variation between the sexes was not statistically significant ($W = 13$, $P = 0.52$).

**DISCUSSION**

We observed that during ontogeny the cephalothorax undergoes changes in size and shape. The changes cephalothorax shape are related to the different stages of development (juveniles and adults) and to sexual dimorphism in adults. Sexual dimorphism manifests though variations cephalothorax shape but not size.

The present study observed variations in the size and shape of individuals through multiple moult; this design permitted an original perspective and the recognition of different aspects of growth than are commonly reported. Our growth study during the molt and intermolt periods differ from traditional methods both in controlled conditions and the natural environment (Renzulli & Collins, 2000; Stevens, 2012; Gonçalves et al., 2009; Trevisan & Santos, 2011), which have allowed an integral identification of growth.

The relationship between the one-dimensional measurement, as size represented by the centroid size and the entire shape of the cephalothorax, represented by landmarks, allows to study growth as an integral approximation. In this context, we could identify the degree of the shape change during growth in different regions of the cephalothorax. These differences could reflect internal growth (e.g., gonad and muscle) or hierarchy and agonistic behaviour in the population (e.g different dimensions in cephalothorax, armament and chelae) (Willner & Collins, 2000; Colpo et al., 2005; Giri & Collins, 2004; Viana et al., 2006; Ayres-Peres et al., 2011). Furthermore, differences in the growth of shape across several species could provide information regarding group evolution or interaction with the environment (Hartnoll, 1982; Collins et al., 2007).

Differences in the size and shape of the rostrum and the posterior area of the aeglid cephalothorax were observed between juveniles and adults. These observations are consistent with the location in which the puberty moult occurs. Teodóso & Masunari (2009) observed changes in the size and shape of the rostrum of *A. schmitti* Hobbs III, 1979. Working with juveniles, these authors found that larger individuals had proportionally longer rostrums. Therefore, according to the authors, variation in body proportion is related to the ontogenetic development of the species, which is consistent with variations observed in this analysis. Bond-Buckup & Buckup (1994) describe variations in the anterior region of the cephalothorax (pre-cervical width/forehead width). In our analysis, allometric differences were observed throughout the cephalothorax when juvenile and adult data were analysed through geometric morphometric methods. Regarding sexual dimorphism, differences in shape were observed in the rostrum and more clearly in the posterior region of the cephalothorax. Martin & Abee (1988) characterised the aeglid anterior region as narrow, and posterior region as wide. These features are associated with reproduction because these decapods have large eggs with direct development and the females keep early juveniles in the abdomen (Bond-Buckup et al., 1996; Bueno & Bond-Buckup, 1996). Giri & Collins (2004) observed differences in cephalothorax shape between the sexes in some populations of *A. uruguayana*. Similar to this study, the authors reported that this distinction is most obvious at the posterior vertex of the cephalothorax. Sexual dimorphism was observed throughout the entire cephalothorax in other species, specifically that the posterior lateral region is wider in females than in males (López, 1965; Lopretto, 1978; Bond-Buckup & Buckup, 1994; Jara, 1994; Bond-Buckup et al., 2008; Giri & Loy, 2008; Trevisan et al., 2012; Trevisan & Santos, 2012). These findings will allow us to identify the moment of transition between juveniles and reproductive adults in future studies.

Regarding the relative size of males and females, similar values were recorded for *A. leptodactyla* (Noro & Buckup, 2003) and *A. marginata* Bond-Buckup & Buckup, 1994 (Trevisan et al., 2012). However, in the biometric analysis of *A. uruguayana* individuals, Vaz-Ferreira et al. (1945) observed that males were wider and longer than females in the area of the junction between third hepatic lobe and the epibranchial area (landmark 5 in this study). However, variations in this region were not evident in this study. Other authors (Schmitt, 1942; Ringuelet, 1948; Bahemonde & López, 1961; Rodrigues & Hebling, 1978; Jara, 1980; Bond-Buckup & Buckup, 1994; Sweich-Ayoub & Masunari, 2001; Giri & Collins, 2004; Boss et al., 2006; Silva-Castiglioni et al., 2006; Giri & Loy, 2008; Gonçalves et al., 2009; Barria et al., 2011) have
agreed that males are larger than females. According to Silva-Castiglioni et al. (2006), the larger size of males is most likely because they invest their energy primarily in somatic growth. Females are smaller than males because they invest most of their energy in reproduction (gonad maturation and egg production) at the expense of body growth. Corroborating the findings of the present study, F. Giri (unpublished data) did not find differences in the sizes of males and females of *A. uruguayana* but observed larger sizes in female *A. platensis* and *A. scamosa* Ringuelet, 1948. Bueno et al. (2000) recorded larger *A. platensis* females than males. The authors attributed this difference to the fact that the largest males of the population were rare during sampling. The results obtained here may indicate that male and female of *A. uruguayana* present differences in cephalothorax shape but not size.

Regarding the growth rates found for males and females, there was similarity and consistency with observations of other crustaceans (e.g., crab *A. leptodactyla*; prawn *Macrobrachium borelli* Nobili, 1896 and crayfish *Parastacus pugnax* Poepping, 1835) (Collins, 1996; Noro & Buckup, 2003; Ibarrá & Araña, 2011). However, the growth rate for females was slightly higher in other species, such as *A. paulensis* (Cohen et al., 2011). Conversely, in other species, including *A. platensis*, *A. jaraí, A. longirostri*, and *A. itacolomiensis*, growth was more intense in males than in females (Bueno et al., 2000; Boss et al., 2006; Silva-Castiglioni et al., 2006; Gonçalves et al., 2009). All of these observations were obtained using a classical methodology, and it is possible that morphometric geometry may cause us to consider growth as a more integral process and not as a one-dimensional event. Furthermore, this information may allow new interpretations of the groups’ phylogeny or the effects of environmental forces upon each species.

Individual tracing allowed us to determine the intermoult period and its variability as a component of growth. The similarity in the intermoult periods observed in males and females was consistent with the previous reports in the prawn *M. borelli* (Collins, 1996). By contrast, *Palaeomonetes argentinus* Nobili, 1901 females had longer intermoult periods than males (Schildt & Damborenea, 1989), which is similar to other decapod species (Hartnoll, 1982). The differences in intermoult period between sexes were not observed for *A. uruguayana* in this study, but this may be related to the study seasonality, period between sexes were not observed for *A. uruguayana* species (Collins, 1996; Vega-Villasante et al., 2006), in which the size increase was substantial for juveniles and decreased linearly with age.

Finally, we consider the techniques and procedures used in this study, which allowed separate analysis of morphological aspects of growth, such as the shape and size, enabling a thorough study of the growth process and broadening the scope of traditional methods. Additionally, the methodology of the analysis (with the exuvia of cephalothorax being removed during moultting) reduced handling of the individuals, which decreased the likelihood of inducing stress and allowed the individual to be released after the study was completed. This was a key factor for our analysis. Thus, this new approach, in combination with traditional methods offers an integral approach to the study of decapod growth.

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REFERENCES


Appendix 1. Error measurement of the photos and landmarks in *Aegla uruguayana* Schmitt, 1942.

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