

## 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. Moulting 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 moulting 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 cefalotórax 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 cefalotórax estrecho. Los adultos presentaron la región anterior y posterior del cefalotórax 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, cefalotórax, ontogenia, intermuda, morfometría.

Generally, species shape varies from the birth to death of each individual according to the development of the growth that characterises the species. These ontogenetic variations represent different physiological, morphological, ethological, and/or population events or conditions of species (e.g., puberty, adults, hierarchy, kairomone, and reproduction) (KLINGENBERG, 1998). Some impermanent variations are initiated by outside factors and revert over time, while others represent a definitive shift to a new life stage (ADAMS *et al.*, 2004).

Like many other freshwater animals, the growth of crustaceans is a discontinuous process that occurs in cycles due to the shedding of the exoskeleton in each ecdysis event (KURATA, 1962; PETRIELLA & BOSCHI, 1997; LUPPI *et al.*, 2004). There are two basic components to this phenomenon, both of which are regulated by both exogenous and endogenous factors; the increase in size during each moult and the intermoult period. Each of these periods of transformation that occur between the two moults marks a full course of morphological, physiological and biochemical factors that are responsible for the growth

and the shape of the individual (DRACH, 1939; HARTNOLL, 1982; WENNER, 1985). Identification of how these factors interact with moulting increment in individuals is relevant to understanding growth.

Direct or indirect methods must be adapted to gain insight into growth in the absence of permanent structures. Typically, crustacean growth is evaluated by one morphological dimension, such as length or width (HARTNOLL, 1978; PETRIELLA & BOSCHI, 1997). However, such an analysis does not consider variation in shape throughout the life of these species. Geometric morphometrics capture the geometry of structures and maintains this information across analyses, combining geometry, statistics and biology. Furthermore, it promotes a more integral understanding of growth that includes changes in shape through the life of a species (ROHLF & MARCUS, 1993; ADAMS *et al.*, 2004).

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). 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; LOPRETTO, 1978; HOBBS, 1979; BOND-BUCKUP & BUCKUP, 1994; BOND-BUCKUP, 2003; GIRI & COLLINS, 2004; ALMERÃO *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 & LÓPEZ, 1961; LÓPEZ, 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 the size and shape of *A. uruguayana* individuals at different developmental stages by tracking individual moult cycles and considering exuvia as evidence of changes in growth.

## MATERIALS AND METHODS

### Field collection and laboratory maintenance.

Sixty six *A. uruguayana* individuals of both sexes were analysed, including specimen from each size range recorded in their habitat. Specimen were separated into three categories, juveniles (N = 45; LC range: 2.99-10.71 mm), males (N = 13; LC range: 12.21-28.66 mm) and females (N = 8 non-ovigerous; LC range: 12.05-19.02 mm) according to cephalothorax length (LC) following the sexual maturity criteria outlined by VIAU *et al.* (2006).

Specimens were collected at Las Pencas Stream, in Entre Ríos province, Argentina (32°17'23.8"S, 60°26'30.53"W). Individuals were transported to the Instituto Nacional de Limnología (INALI-CONICET-UNL) in plastic containers filled with stream water. Individuals were placed in an aquarium containing small shelters (rocks, vegetation, etc.) that were brought from the sampling site in accordance with the ecological requirements of 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,” (DRYDEN & MARDIA, 1998) were identified and digitalised (TpsDig program, ROHLF, 2004) on the exuvia. Cephalothorax size was represented by a calculation using the centroid (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 preform 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 GPA, General Procrustes Analysis (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:

$$\text{Rate of increase: } (Cs2 - Cs1) / Cs1$$

where:  $Cs1$  is the value of the centroid from an individual first moult and  $Cs2$  corresponds to the centroid of the second moult. This formula was applied to all moults. The

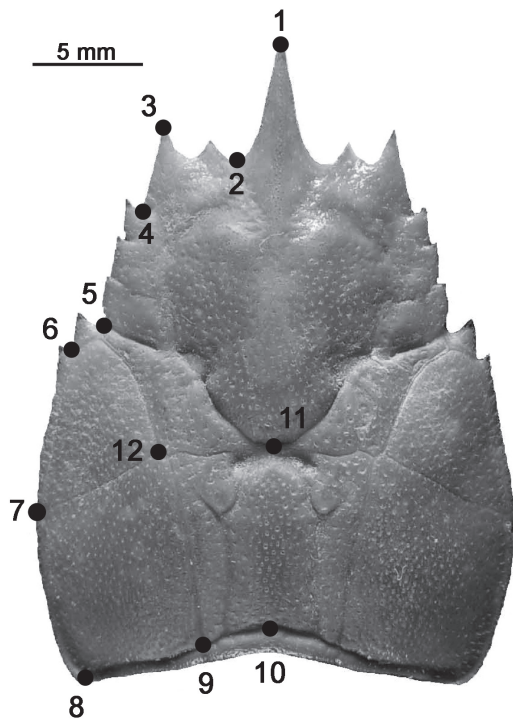


Fig. 1. Location of the 12 landmarks (LM) left dorsal half cephalothorax of *Aegla uruguayana* Schmitt, 1942.

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

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

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'  $\lambda = 0.56$ ,  $FGL_1 = 5.34$ ,  $P = 9.03e-10$ ). 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).

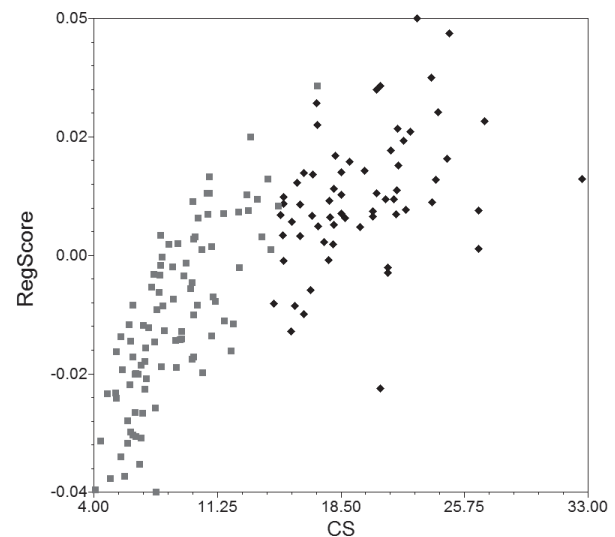


Fig. 2. Distribution of variations in the shape of the cephalothorax explained by the relative warp (RW) 1 and 2 for juvenile (gray square) and adults (black diamond) of *Aegla uruguayana* Schmitt, 1942.

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 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

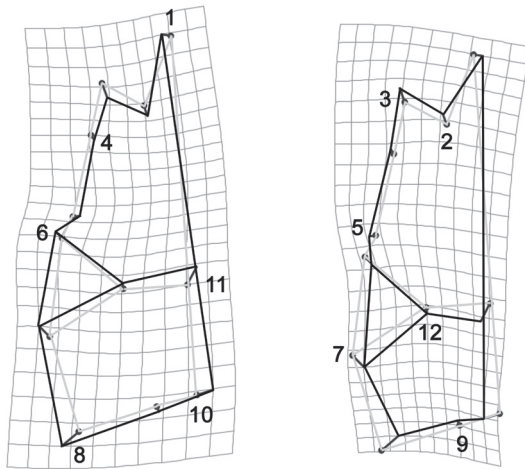


Fig. 3. Deformation grids adult (left black), juvenile (right black) and consensus configuration (grey) of *Aegla uruguayana* Schmitt, 1942 (scale factor 35).

% 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

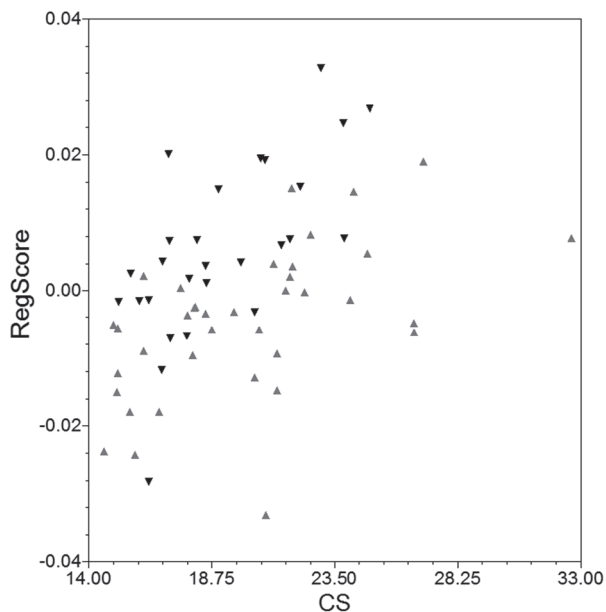


Fig. 4. Spatial variations in the shape of the cephalothorax explained by relative warp (RW) 1 and 2 for males (up gray triangle) and female (down black triangle) of *Aegla uruguayana* Schmitt, 1942.

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  $25 \pm 1^\circ\text{C}$ , 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 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 ( $W = 13$ ,  $P = 0.52$ ).

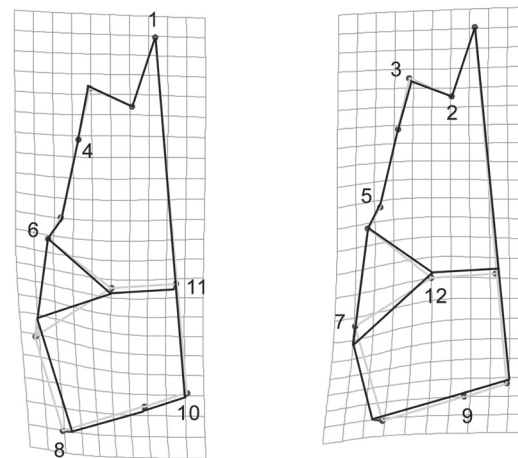


Fig. 5. Deformation grids males (left black), and females (right black) and consensus configuration (grey) of *Aegla uruguayana* Schmitt, 1942 (scale factor 0.05).

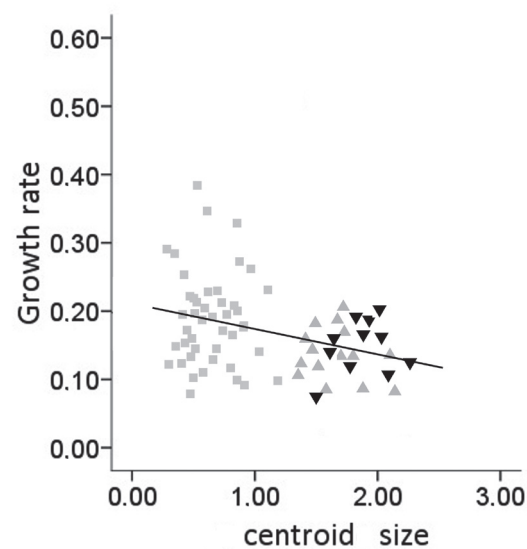


Fig. 6. Growth rate of *Aegla uruguayana* Schmitt, 1942 juveniles (gray square), males (up gray triangle) and females (down black triangle) of different sizes (Cs),  $r = 0.031$ .

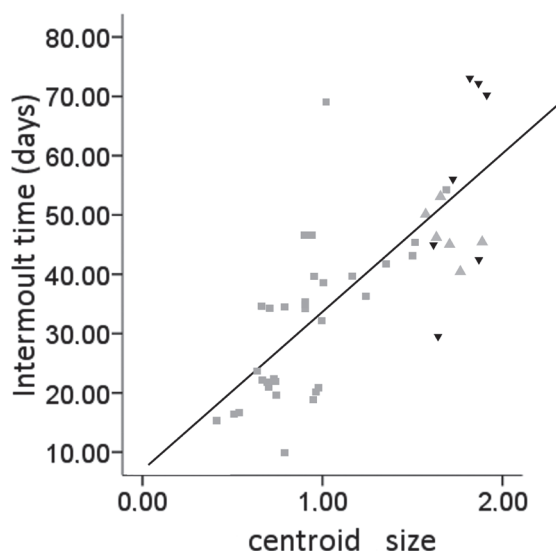


Fig. 7. Relationship between intermolt time (days) and size (Cs) of *Aegla uruguayana* Schmitt, 1942 juveniles (gray square), males (up gray triangle) and females (down black triangle),  $r = 0.552$ .

## 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 through variations cephalothorax shape but not size.

The present study observed variations in the size and shape of individuals through multiple moults; 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) (WILLINER & COLLINS, 2000; COLPO *et al.*, 2005; GIRI & COLLINS, 2004; VIAU *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. TEODÓSIO & 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 & ABELE (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; SWIECHAYOUB & MASUNARI, 2001; GIRI & COLLINS, 2004; BOSS *et al.*, 2006; SILVA-CASTIGLIONI *et al.*, 2006; GIRI & LOY, 2008; GONÇALVES *et al.*, 2009; BARRÍA *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* Ringuélet, 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 borellii* Nobili, 1896 and crayfish *Parastacus pugnax* Poepping, 1835) (COLLINS, 1996; NORO & BUCKUP, 2003; IBARRA & ARANA, 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. jarai*, *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 intermolt period and its variability as a component of growth. The similarity in the intermolt periods observed in males and females was consistent with the previous reports in the prawn *M. borellii* (COLLINS, 1996). By contrast, *Palaemonetes argentines* Nobili, 1901 females had longer intermolt periods than males (SCHULDT & DAMBORENEA, 1989), which is similar to other decapod species (HARTNOLL, 1982). The differences in intermolt period between sexes were not observed for *A. uruguayana* in this study, but this may be related to the study seasonality, or to environmental conditions (e.g., temperature, salinity, and food availability) and the need for gonad maturation for the production of a new generation, as occurs in other species (SARDÁ, 1983; COLLINS, 1996; RENZULLI & COLLINS, 2000; VEGA-VILLASANTE *et al.*, 2006). According to KURATA (1962) and HARTNOLL (1982), feeding is one of the most influential factors of growth. Similarly, VEGA-VILLASANTE *et al.* (2006) describe the relationship between the duration of the moulting cycle and environmental factors in the habitat.

Furthermore, in *A. uruguayana*, as in other crustaceans (HARTNOLL, 1982), growth was slowed as animal size increased. Therefore, as HARTNOLL (1985) explains, growth in Anomura can be considered undetermined, i.e., the animal undergoes continuous ecdysis after puberty, but it does not have unlimited growth. In other decapod's taxa, IBARRA & ARANA (2011) observed that the growth rate for burrowing crayfish *Parastacus pugnax* decreased linearly as the individuals grew and reached zero at their

maximum length. Similar results were obtained for other crustaceans (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 moulting) 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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Appendix 1. Error measurement of the photos and landmarks in *Aegla uruguayana* Schmitt, 1942.

Centroid size	SS	MS	df	F	P (param.)
Effect					
Individual	12560824.14	1046735.34	12	4073.72	<0.0001
Error1	3340.32	256.95	13	10.05	<0.0001
Residual	664.97	25.57	26		
Shape	SS	MS	df	F	P (param.)
Effect					
Individual	0.02009	0.00009	228	3.77	<0.0001
Side	0.00061	0.00003	19	1.38	0.1361
Ind* Side	0.00532	0.00002	228	1.03	0.4002
Error1	0.01123	0.00002	494	3.97	<0.0001
Residual	0.00566	0.00001	988		