MORPHOLOGICAL ASPECTS OF THE EMBRYONIC DEVELOPMENT OF 
AEGLA PLATENSIS (DECAPODA, AEGLIDAE)

BY

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ABSTRACT

The present study characterizes the embryonic development of Aegla platensis Schmitt, 1942 by means of optical microscopy and electron microscopy. The sequence of morphological changes observed in the egg allowed to describe ten embryonic stages: the unsegmented egg, cleavage, blastula, gastrula, nauplius, metanauplius, early zoea, late zoea, decapodid, and juvenile. Cleavage was centrolecithal and the blastula was regular. The nauplius stage was transient. The development of the gut could be observed during the zoea stages. At the late zoea stage the posterior midgut was well developed showing chromophobous cells. The dark eye pigmentation started to develop at the early zoea stage and was completed at the decapodid stage. Newly hatched juveniles exhibit some yolk incorporation in the cephalothorax and do not need food during the first days after hatching. The morphological changes observed during the development of this anomuran are discussed in relation to patterns of development reported for other allegedly allied decapods.

ZUSAMMENFASSUNG

INTRODUCTION

The Aeglidae constitute the only family of the Anomura that lives in freshwater, and its recent distribution is restricted to South America. It plays an important role in the food-webs of numerous biocoenoses, and its predators include fishes, birds, frogs, and alligators (Bond-Buckup & Buckup, 1994). Magni & Py-Daniel (1989) observed that *Aegla platensis* Schmitt, 1942 is also a predator on the larval stages of Simuliidae (Diptera, Culicomorpha). *A. platensis* is very common in southern Brazil, and lives in lotic or lentic waters (Bond-Buckup & Buckup, 1994). Its biology, reproduction, and ecology have been studied, mainly as a result of its abundance and ecological plasticity (Bond-Buckup et al., 1999; Bueno & Bond-Buckup, 2000; Bueno et al., 2000). However, its embryonic development has as yet remained undescribed.

The embryonic development of the Aeglidae shows secondary embryonation, i.e., the larval stages are passed through inside the egg. The newly hatched animals are similar to the adult, but with juvenile morphology and benthic habits. The female exhibits offspring care and maintains the juveniles attached to her pleopods for over 10 days (Bond-Buckup et al., 1999). In some other crustaceans, the females of which also carry the eggs attached to the pleopods, certain larval phases also occur inside the egg and these changes are characterized by specific morphogenetic and organogenetic processes (Bressan & Muller, 1999). This is the case, e.g., in species of the genus *Macrobrachium* Bate, 1868 (cf. Jalihal et al., 1993). The completion of larval stages in the egg in Crustacea occurs with a simultaneous increase in the amount of yolk in the egg. In fact, the eggs of the Aeglidae are very large and they contain a large quantity of yolk.

The present study characterizes the embryonic development of *A. platensis* by means of optical microscopy using both the stereomicroscope and the compound microscope, as well as by electron microscopy.

MATERIAL AND METHODS

Females, with mature ovaries or eggs attached to their pleopods, and males were collected from streams around Porto Alegre, Rio Grande do Sul, Brazil, during spring and summer. The animals were kept in large glass aquaria, containing sand, stones, and vegetation under natural photoperiod and temperature (23°C in spring and 30°C in summer). The water was continuously aerated. Animals were kept on a special diet including worms, carrots, fish, and crustacean food mixed with plain flour, rye flour, and fish flour (Bond-Buckup et al., 1996). These conditions were similar to those in the natural environment from which they were collected.
Sampling started one day after the arrival of the specimens in the laboratory. Two eggs were removed from females daily during the first week, using a slender brush. Then, afterwards, we removed two eggs every two days, until hatching occurred. The total number of eggs examined was 97, of which 42 were sampled from one single female and the remaining ones were collected from another seven females (CL measurements between 9.87 and 18 mm). External changes were studied under the stereomicroscope, including measurements (length × width) of the eggs in the early stages (unsegmented eggs until gastrula), before fixation. Eggs studied under optical microscopy were fixed in Bouin’s fixative, stored in 70% alcohol, and embedded in paraffin according to Lizardo-Daudt et al. (2000). Serial sections were stained with haematoxylin-eosin (HE) and periodic acid Schiff (PAS).

For electron microscopy, eggs from the early period of development were fixed in 2% glutaraldehyde in 0.23 M Millonig’s buffer for 1 h at room temperature and then postfixed in 1% OsO₄ for 90 minutes at 4°C. After that, the eggs were dehydrated in an ethanol series finishing in acetone and embedded in Epon. Semithin sections (1 μm) were obtained with a Leica Ultratome UCT and stained with Toluidine blue. Ultrathin sections (90-150 nm) were obtained with the same ultramicrotome. These sections were contrasted with lead citrate and uranyl acetate and examined in a Philips EM 208S electron microscope, at 70 KV, and at magnifications ranging from 4,000 × to 20,000 ×. Some eggs were punctured after fixation in glutaraldehyde in order to obtain the best possible postfixation.

A classification of the stages of embryonic development was determined by noting the quantity of the yolk, the space occupied by the embryo, and the appearance and subsequent growth of the eyes, appendages, and other structures. These morphological criteria and the nomenclature used are based on the standardization of postlarval nomenclature as proposed by Felder et al. (1985).

RESULTS

Development of the egg

Mating was not observed in the field but was noted in one case in the laboratory. Unfortunately, the female died and hatching time was not observed. The longest continuous period of development observed in the laboratory from the collection of an ovigerous female to hatching, was 35 days. The main characteristics in a series of embryonic developmental stages are summarized in table I.

Stage 1. — Unsegmented egg

The eggs were attached to the female’s pleopods by a small peduncle as in Decapoda Brachyura. They were coloured orange, were elliptical in shape
Table I

Main characteristics in a series of embryonic developmental stages of *Aegla platensis* Schmitt

<table>
<thead>
<tr>
<th>Stages</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Stage 1: unsegmented egg</td>
<td>High amount of yolk. Chorion clearly discernible.</td>
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<tr>
<td>Stage 2: cleavage</td>
<td>Nucleic division deep in the egg, followed by migration of the resulting nuclei to the surface.</td>
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<tr>
<td>Stage 3: blastula</td>
<td>Regular blastula. Yolk in the center. Nuclei situated in the periphery of the cytoplasm.</td>
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<tr>
<td>Stage 4: gastrula</td>
<td>Blastopore marking the posterior side of the embryo. Three different types of cells.</td>
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<tr>
<td>Stage 5: nauplius</td>
<td>Transient and condensed. Presence of optic lobes, three pairs of naupliar appendages and caudal papilla.</td>
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<tr>
<td>Stage 6: metanauplius</td>
<td>Great development of optic lobes, antennules, antennae, and mandibles. Labrum and cerebral ganglia well developed. Buds of other cephalothoracic appendages.</td>
</tr>
<tr>
<td>Stage 7: early zoea</td>
<td>Nervous system differentiated in brain and ganglia. Midgut starts development. Start of dark pigmentation of eyes.</td>
</tr>
<tr>
<td>Stage 8: late zoea</td>
<td>Posterior appendages with segmentation. Prominent eyes.</td>
</tr>
<tr>
<td>Stage 9: decapodid</td>
<td>Eyes completely pigmented. Appendages completely developed with prominent muscular tissue.</td>
</tr>
<tr>
<td>Stage 10: juvenile</td>
<td>Miniature of the adult without abdominal appendages.</td>
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(figs. 1, 15A) and measured around 0.98 × 0.93 mm (length × width). They contained a high amount of yolk. Each ovigerous female bore approximately 60 eggs, depending on her own size. Different-sized yolk granules could be observed, with some of the larger ones having small vesicles inside them. This granular material exhibited different grades of eosinophilia and electron density. The yolk granules were PAS positive. Surrounding the yolk mass, the chorion was clearly discernible. The vitelline membrane attached to the chorion could hardly be seen.

Stage 2. — Cleavage

The first divisions took place deep within the yolky egg. There were many small and picnotic nuclei inside the yolk. In addition to these nuclei there were a few big nuclei with poorly condensed chromatin. The yolk was formed by many spherical granules, some of them surrounded by a 7 to 8.5 nm thick membrane. These granules showed three different levels of electron density as observed by electron microscopy. Besides the yolk granules, there were scattered droplets of lipids. The nucleic division occurred deep in the egg, followed by a migration of the resulting nuclei to the surface. The nuclei located on the periphery of the egg were larger and less picnotic than the small nuclei found inside the yolk. During intralecithal
Figs. 1-5. Embryonic development of *Aegla platensis* Schmitt, 1942, stages 1-4. 1, stage 1 (unsegmented egg; p, peduncle; c, chorion; y, yolk; scale bar, 0.5 mm); 2, stage 2 (cleavage; c, cleavage furrow; cross section, stained with Toluidine blue; scale bar, 10 μm); 3, stage 3 (blastula; b, blastula nuclei; cross section, stained with HE; scale bar, 20 μm); 4-5, stage 4 (gastrula; electron micrograph; +, structure with fibrillar elements; v, vesicular elements; c, concentric structures; h, homogeneous cell; □, granular cells; m, mitochondria; g, Golgi bodies; scale bar, 3 μm).

nuclear divisions, superficial segmentation could externally be observed, but with a non-pronounced penetration of the cleavage furrows into the cytoplasm (figs. 2, 15B).

Stage 3. — Blastula

The chorion was composed of two layers with different electron density and thickness. The external layer (around 520 nm thick) was more electron-dense than
the inner one (around 680 nm). The blastula nuclei were situated in the periphery of the cytoplasm (figs. 3, 15C), and appeared elongated. The cells exhibited many small (approximately 10 nm diameter) and electron dense granules dispersed into their cytoplasm. Also, they had many granular endoplasmic reticula (GER). The yolk occupied the central position in the blastula. Some nuclei could also be observed deep within the yolk egg.

Stage 4. — Gastrula

Gastrulation took place on the animal pole where the concentration of nuclei increased. The cells migrated into the egg through the blastopore, which marked the posterior side of the embryo (fig. 15D). Three types of cells with prominent nucleoli could be distinguished. The first and more abundant type exhibited homogeneous cytoplasm and was stained blue by Toluidine blue. Electron microscopy showed that this type had many well developed mitochondria and Golgi bodies. These cells were elongated in the opening of the blastopore (figs. 4, 5). The second type of cells had large nuclei located eccentrically and exhibited many yolk granules in the cytoplasm and some dispersed glycogen. There were prominent Golgi bodies, agranular endoplasmic reticula (AER) and some mitochondria. Most of these cells were located in direct contact with the yolk mass. The third type of cells stained purple by Toluidine blue and had granular cytoplasm (glycogen granules). These cells exhibited well developed Golgi bodies and abundant GER and AER. Their cytoplasm was more electron dense than the cytoplasm of the other cells. Inside the opening of the blastopore there were some structures with fibrillar elements of approximately 26 nm in diameter. Near these structures, there were some vesicular elements with small vesicles inside them. These vesicular elements were associated with concentric structures which exhibited large electron dense membranous elements (25 nm) separated by large gaps (approximately 65 nm; fig. 4).

Stage 5. — Nauplius

This embryo form was very transient, condensed, and it was observed only once. It was characterized by the presence of optic lobes, three pairs of naupliar appendages (which are to become later antennules, antennae, and mandibles), and the caudal papilla. The embryo occupied about one-sixteenth of the entire egg. There were many cells undergoing mitosis at this stage, and the cells were arranged in lines, forming small buds demarcating each appendage. The antennae showed a more compact arrangement than the other appendages (figs. 6, 15E).

Stage 6. — Metanauplius

This form was characterized by a great development of the optic lobes, antennules, antennae, and mandibles. The anterior appendages exhibited their characteristic morphology, particularly the antennae and antennules, by being distinctly
Figs. 6-10. Embryonic development of *Aegla platensis* Schmitt, 1942, stages 5-7. 6, stage 5 (nauplius; at, antennae; sagittal section, stained with HE; scale bar, 20 μm); 7, stage 6 (metanauplius; ▼, optic lobe; ○, proximal segment of antennule; ■, second segment; sagittal section, stained with Toluidine blue; scale bar, 40 μm); 8-10, stage 7 (early zoea; a, abdominal ganglia; e, eyes; m, midgut; p, peduncle; pr, proctodaeum; t, thoracic ganglia; y, yolk; fig. 8, scale bar, 0.4 mm; figs. 9 and 10, sagittal section, stained with HE, scale bar, 40 μm).

segmented (figs. 7, 15F). The proximal antennular segment was elongated and demarcated a cavity. The second segment was larger than the proximal one, its shape was square and exhibited spherical cells inside. The distal segment was similar to the second one but it was more elongated and smaller. Antennae were more slender than antennules and exhibited the same type of cells. The labrum was well differentiated. The cerebral ganglia were well developed. Some condensed lobes could be seen in the cerebral ganglia. Buds of maxillae, maxillipeds, and pereiopods could also be seen. The embryo occupied about one-eighth of the total egg volume.

Stage 7. — Early zoea

In this stage, the appendages were larger than in the previous stage. The nervous system had developed into a well-formed brain centrally, and into ganglia in the thoracic somites. Abdominal ganglia could be observed, too, although no appendages were noted. In addition, posterior and anterior midgut could be observed. The proctodaeum was continuous with the posterior midgut, which was in direct contact with the yolk mass. The stomodaeum and the small anterior
midgut were also in contact with the yolk mass. The yolk was very abundant and there were little picnotic nuclei dispersed among the granules. The dark pigmentation of the eye was first visible at this stage and appeared as little rods (figs. 8-10, 15G).

Stage 8. — Late zoea

At the late zoea stage, all appendages were well visible, and the posterior appendages were larger than in the previous stage and exhibited segmentation. The eyes were prominent, pigmented, and looked crescent-shaped (figs. 11, 12, 15H). The yolk occupied about two-thirds of the egg. The posterior midgut was well developed, showing chromophobous cells by HE. These cells were cylindrical with basal nuclei.

Stage 9. — Decapodid

The embryo occupied about two-thirds of the egg. The eyes were pigmented, well developed, and oval in shape. The appendages were completely developed and exhibited prominent muscular tissue (figs. 13, 15I). There were yolk granules associated with the midgut and inside it.
Stage 10. — Juvenile

The animal was a miniature adult at the time of hatching but exhibited no abdominal appendages. There was some yolk incorporated in the cephalothorax. The carapace was transparent and chelae were fully developed. Such juveniles were observed hatching from each female over a period of approximately two days (fig. 14), after which no more eggs or hatching juveniles could be seen attached to the female.

The main morphological changes are summarized in fig. 15.
DISCUSSION

According to Rodrigues & Hebling (1978), the eggs of *Aegla perobae* Hebling & Rodrigues, 1977, are spherical in shape, red, and measure an average 1.3 mm in diameter. The eggs of *A. paulensis* Schmitt, 1942, are also spherical and measure 1.1 to 1.5 mm in diameter (Lopez, 1965). *A. platensis*, however, exhibits an elliptical egg shape, and its egg is the smallest among those of the three species mentioned. The increase in egg size with embryonic development, apparently a pattern in decapods, was not observed in *A. platensis* during the first stages of development. Lardies & Wehrtmann (1996) described the egg volume to increase an average of 40.5% during embryogenesis of the anomuran *Petrolisthes laevigatus* (Guérin, 1875). In addition, egg size of *Erimacrus isenbeckii* (Brandt, 1848), *Palaemonetes argentinus* Nobili, 1901, and *Spongicola japonica* Kubo, 1942, also increased during development until hatching (Nagao et al., 1999; Nazari et al., 2000; Saito & Koya, 2001). Future studies including the measurement of eggs throughout the entire development will clarify this point.

*Aegla platensis* exhibits direct development (sensu Gore, 1985). This type of development, according to Kaestner (1980), is an adaptation to freshwater, in that it protects the animal from the riverine environment. Direct development appears to be the rule among the freshwater and terrestrial brachyuran crabs of the family Potamonidae. Direct development also occurs in the freshwater crayfishes (astacids and parastacids), and in some marine shrimps and some marine brachyuran crabs (Gore, 1985). As can be concluded, the incidence of this type of development within the Decapoda is frequent, and widespread across phylogenetic lines.

The embryonic development of the crayfish, *Samastacus spinifrons* (Philippi, 1882), is also direct. The sequence of morphological changes observed on the egg surface allowed the description of five embryonic forms (Rudolphi & Iracabal, 1994). The unsegmented egg, cleavage, blastula, and gastrula are similar to those described for *Aegla platensis*. The last embryonic form is similar to the juvenile stage of *A. platensis*. There are no corresponding nauplius, metanauplius, zoea (early or late), or decapodid stages in *S. spinifrons*, as described by Rudolphi & Iracabal (1994). These stages were perhaps missed because of the method of analysis of the eggs (their study focused on morphological changes on the egg surface). In addition, the time interval between removal of the eggs from the female in their study might form an obstacle to the observation of these stages as well.

The pattern of embryonic development observed in *A. platensis* is also similar to that of *Potamon fluviatile* (Herbst, 1785). *P. fluviatile* represents the most advanced stage of the trend towards secondary embryonization in the Brachyura, because the stage which corresponds to the megalopa is kept imprisoned in the egg (Pace et al., 1976). *A. platensis* also shows this form (described here as a decapodid) in the
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egg, although some differences can be recognized. The blastula of *P. fluviatilis* is eccentric, different from the regular blastula shown by *A. platensis*. In the metanaupliar stage, abdominal appendages are visible in *P. fluviatilis*, which are not found in hatched *A. platensis*. The newly hatched juveniles of *P. fluviatilis* have no trace of yolk in the alimentary canal, an indication that they need an external food source earlier, while *A. platensis* possesses yolk in the alimentary canal and thus appears not to need food immediately after hatching.

In general, the morphological characteristics of each different stage observed in *A. platensis* are similar to the corresponding stages of *Macrobrachium carcinus* (Linnaeus, 1758) (cf. Muller, 1984), *Palaemon pandaliformis* (Stimpson, 1871) (cf. Muller et al., 1999), *Palaemonetes argentinus* Nobili, 1901 (cf. Nazari et al., 2000) and *Macrobrachium acanthurus* (Wiegmann, 1836) (cf. Bressan & Muller, 1997, 1999), despite the differences shown at hatching. However, the total cleavage observed in *M. carcinus*, *P. pandaliformes*, and *P. argentinus* was not observed in *A. platensis* by the method used. The egg was shown to be centrolecithal without total cleavage. According to Anderson (1982), total cleavage is an ancestral mode of crustacean cleavage. This point may be studied in-depth by using a shorter time interval between the removal of the eggs from the female, particularly for the first period of development.

On the other hand, *Pagurus prideaux* Leach, 1815, an anomuran, hatches as a zoea larva (Scheidegger, 1976). Despite this different pattern of abbreviated development, its embryonic development is similar to that of *A. platensis* until this stage. Cleavage is superficial and the blastula is regular, as in *A. platensis*. The endoderm development of *A. platensis* and *P. prideaux* shows a preponderant caudo-cephalic gradient of differentiation. The nervous system shows a cephalo-caudal gradient, similar to that of *P. prideaux*, and a general feature in crustaceans.

Gastrulation in *A. platensis* shows three types of cells. The first and more abundant type is similar to the mesodermal cells described by Scheidegger (1976). The second type of cell, which exhibits numerous granules of yolk in the cytoplasm, is similar to vitellophages, as described by Sollaud (1923). The third type of cell shows characteristics of a cell that synthesizes something, and is probably a specialized mesodermal cell. The origin and function of the structures with fibrillar elements, vesicular elements with small vesicles inside them, and concentrical structures, need to be investigated further.

The sequence of morphological changes observed in the egg allowed the description of ten embryonic stages: unsegmented egg, cleavage, blastula, gastrula, nauplius, metanauplius, early zoea, late zoea, decapodid, and juvenile. It can be seen from the present study that *A. platensis* exemplifies an extreme form of direct development and secondary embryonization among Crustacea, in that even the decapodid is imprisoned in the egg and the embryo hatches as a miniature adult.
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