EMBRYOLOGY OF DECAPOD CRUSTACEANS, II: GROSS EMBRYONIC DEVELOPMENT OF *PETROLISTHES ROBSONAE* GLASSELL, 1945 AND *PETROLISTHES ARMATUS* (GIBBES, 1850) (DECAPODA, ANOMURA, PORCELLANIDAE)

BY

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ABSTRACT

The gross morphology of the embryonic development of two porcelain crabs from distinct habitats, *Petrolisthes robsonae* (coastal lagoon) and *P. armatus* (sea shore intertidal) is described and illustrated at 48 hour intervals from oviposition to hatching. The development of the two species is compared with each other, since no information is available on the embryology of other porcelain crabs. The development of the two species is highly similar and the whole process lasts about 19 days at an ambient temperature of 25-26°C.

RÉSUMÉ

La morphologie de l’embryon de deux espèces de crabes porcelaines provenant de deux habitats différents, *Petrolisthes robsonae* (lagune côtière) et *P. armatus* (intertidal marin) est décrite et illustrée pour chaque intervalle de 48 heures à partir de l’oviposition et jusqu’à l’éclosion. Les deux développements sont comparés entre-eux du fait qu’il n’existe pas de développement connu pour ce groupe. Les deux développements sont extrêmement similaires et durent environ 19 jours à une température de 25-26°C.

INTRODUCTION

Most of our present knowledge on anomuran crabs of the eastern tropical Pacific is related to their taxonomy, geographic distribution, and habitat. A few species of Porcellanidae and hermit crabs inhabiting the intertidal and shallow waters have been more thoroughly studied (see Haig, 1960; Haig et al., 1970; Ball & Haig, 1974; Brusca, 1980) but from a general point of view their biology and

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autecology are poorly documented. A checklist of all anomuran crabs occurring in the tropical eastern Pacific was recently compiled by Hendrickx & Harvey (1999), and additional records for Porcellanidae have been provided by Hiller et al. (2004). Accordingly, there are currently 68 species of Porcellanidae known from the eastern tropical Pacific, of which 30 belong in the genus *Petrolisthes*. Topics related to the ontogeny of these species have received little attention (García-Guerrero et al., 2005).

Anomuran crabs show a great diversity in reproductive strategies, especially in number and size of eggs (Anderson, 1982; Hines, 1982). As in other Pleocyemata, females incubate their eggs, which are partly or completely covered by the abdomen, from spawning to hatching. The incubation period depends primarily on species (Nagao et al., 1999) and temperature (García-Guerrero et al., 2003). Most studies on the embryology of crabs and crab-like species are recent, and deal with a few brachyuran families only, in particular those that are of commercial interest (Nagao et al., 1999; Bas & Spivak, 2000; Yamaguchi, 2001; Pinheiro & Hattori, 2003). Only a few studies on anomuran embryology are available, including a study of the hatching process in *Petrolisthes armatus* (Gibbes, 1850) (cf. Davis, 1966), and another one focused on the embryology of *Aegla platensis* Schmitt, 1942 (cf. Lizardo-Daudt & Bond-Buckup, 2003).

The eastern tropical Pacific is rich in a wide variety of habitats for permanent populations of porcelain crabs. Species of the genus *Petrolisthes* are very abundant among the invertebrate communities associated with prop roots of mangroves in the coastal lagoons near Mazatlán, Sinaloa, Mexico. This ecological niche features a high diversity of species, in part due to the presence of abundant colonies of mussels (*Mytella* spp.) and barnacles that provide shelter for small, cryptic species (Salgado-Barragán & Hendrickx, 2002a, b; García Guerrero & Hendrickx, 2004a). The embryonic development of species of Porcellanidae associated with shallow, coastal ecosystems is considered fundamental, as these ecosystems are often under strong ecological stress due to coastal development. This is especially so, because embryonic development represents the first step in the life cycle and is probably very sensitive to alterations in the natural environment. *Petrolisthes robsonae* Glassell, 1945 is typically associated with mangrove prop roots and other habitats (e.g., submerged rocks, intertidal debris) in coastal lagoons, thus occasionally enduring adverse conditions like high turbidity and highly variable salinity and temperature. It is usually very abundant and one of the dominant species in this habitat. *Petrolisthes armatus* is another species of Porcellanidae commonly found in the rocky intertidal throughout the area.
METHODS

Males and females of *Petrolisthes robsonae* were captured from prop roots of *Rhizophora mangle* L. in the Estero de Urías, SE Gulf of California, Mexico, about 3 km from the laboratory facilities. Specimens were transported to the laboratory and placed in plastic aquaria. Aquaria were filled with filtered sea water, maintained with constant aeration, and provided with empty sea shells as shelter. The photoperiod was kept at 12 : 12 h (light : dark). Water was maintained at room temperature (25 to 26°C) and at a salinity of 35-36‰. Minced shrimp pellets were offered as food to the crabs. Females were checked for eggs daily, and three females laid eggs during the first two weeks. Two females of *Petrolisthes armatus* with recently-laid eggs were separated from a series of adults collected from the rocky intertidal community in Topolobampo, Sinaloa, north of Mazatlán, transported immediately to the laboratory in Mazatlán and underwent the same treatment. A subsample of eggs was taken from each female, and examined under a dissecting microscope in order to ensure that embryological development was in its earlier phase. Every ovigerous female (both species) was placed in an individual three-liter glass jar with sea water (36‰) and continuous aeration. No food was offered to the females during incubation. A sample of at least 3 eggs was removed from each female every 48 hours, beginning on the day they were first detected as ovigerous.

Eggs were placed in depression slides with sea water and examined in vivo for gross morphology with a compound microscope (25 and 40x magnification). Embryonic morphology, growth, and yolk-tissue proportion were observed for each sample, in lateral and frontal views. The pattern of coloration was also noted. Development was divided into periods of 48 hours, from oviposition to hatching, in agreement with García-Guerrero & Hendrickx (2004b) who recommended the use of the term “periods” instead of “stages” or “steps” in order to reflect the nature of the embryonic development, which is continuous. Illustrations were made with the aid of a camera lucida, as a composite of observations made on every egg in each sample. In all illustrations, structures were drawn separated, to provide clear images, although they are compactly packed in the egg. Smallest and largest widths of the eggs were measured to ±0.01 mm with an ocular micrometer. Egg volume was calculated as an ellipsoid. The diameter was considered as the average between the largest and the smallest width of all eggs measured in a particular period. The accumulative increase in egg volume was calculated by using average volumes observed at the end of each period.
RESULTS

No significant differences in development were observed among embryos of either species for any given period. Average diameter, volume, and accumulative increase of volume of the eggs are presented in table I. The complete embryonic development of both species lasted 19 ± 1 days and included eight embryonic periods from egg extrusion to hatching. Egg volume increase from one period to another was reduced in *P. armatus*, except towards the end of the development process; in *P. robsonae*, volume increase was irregular during the process but eventually reached a cumulative increase of ca. 31%, similar to what was observed in *P. armatus* (table I), except towards the end of the process. Descriptions and illustrations are presented for each 48-hour period (figs. 1-2).

Embryonic development

Period 1, day 1. *Petrolisthes armatus* (fig. 1A) and *P. robsonae* (fig. 2A). — Recently laid eggs. The eggs are macrolecithal-centrolecithal and ellipsoidal, filled with dark brown yolk droplets. No tissue observed.

Period 2, day 4. *Petrolisthes armatus* (fig. 1B) and *P. robsonae* (fig. 2B). — A presumptive patch of primordial cells is located in ventrolateral position.

Period 3, day 6. *Petrolisthes armatus* (fig. 1C) and *P. robsonae* (fig. 2C). — The cluster of primordial cells has differentiated into major embryonic structures, placed in ventral position (ocular, cephalic, and thoracic-abdominal). Some depletion of yolk is noticed.

Period 4, day 8. *Petrolisthes armatus* (fig. 1D) and *P. robsonae* (fig. 2D). — All primordia have increased in size and are more defined. Antennule-antenna,

<table>
<thead>
<tr>
<th>Period</th>
<th><em>Petrolisthes armatus</em></th>
<th><em>Petrolisthes robsonae</em></th>
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<tr>
<td></td>
<td>Diameter (µm)</td>
<td>Volume (µm³ x 100)</td>
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<tr>
<td>1</td>
<td>448 ± 18.1</td>
<td>19.48</td>
</tr>
<tr>
<td>2</td>
<td>451 ± 11.4</td>
<td>19.61</td>
</tr>
<tr>
<td>3</td>
<td>453 ± 14.9</td>
<td>19.69</td>
</tr>
<tr>
<td>4</td>
<td>466 ± 11.7</td>
<td>20.26</td>
</tr>
<tr>
<td>5</td>
<td>488 ± 07.1</td>
<td>21.22</td>
</tr>
<tr>
<td>6</td>
<td>521 ± 18.4</td>
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</tr>
<tr>
<td>7</td>
<td>548 ± 14.0</td>
<td>23.83</td>
</tr>
<tr>
<td>8</td>
<td>589 ± 21.2</td>
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</tbody>
</table>

TABLE I
Average volume, diameter (± std), and cumulative volume increases in percent, of the eggs of *Petrolisthes armatus* (Gibbes, 1850) and *P. robsonae* Glassell, 1945 during periods of embryonic development.
Fig. 1. Periods of embryonic development of *Petrolisthes armatus* (Gibbes, 1850). A, period 1: yolk divided in droplets; B, period 2: primordial cells (PC) visible; C, period 3: primordial structures, optical (OP), cephalic (CA), and thoracic-abdominal (TA) buds; D, period 4: antennule-antenna (AA) and maxilliped (MX) buds; E, period 5: eye ovoid, abdomen enlarged; F, period 6: eye differentiated, appendages segmenting; G, period 7: all appendages have grown, abdomen segmentation completed, digestive gland (DG) and heart (H) visible; H, period 8: few traces of yolk remain, embryo about to hatch. Scale bar = 200 µm.
Fig. 2. Periods of embryonic development of *Petrolisthes robsonae* Glassell, 1945. A, period 1: yolk divided in droplets; B, period 2: primordial cells (PC); C, period 3: primordial structures, cephalic (CA), and thoraco-abdominal (TA) buds; D, period 4: differentiation into antennule-antenna (AA), maxillule-maxilla, and maxillipeds (MX); E, period 5: differentiation into antennule-antenna (AA), maxillule-maxilla, and maxillipeds (MX); F, period 6: differentiation into antennule-antenna (AA), maxillule-maxilla, and maxillipeds (MX); G, period 7: differentiation into antennule-antenna (AA), maxillule-maxilla, and maxillipeds (MX); H, period 8: differentiation into antennule-antenna (AA), maxillule-maxilla, and maxillipeds (MX). Scale bar = 200 µm.
maxillule-maxilla, and maxillipeds are now tiny buds, arising below and behind the primordial optical structures. Tissues appear as transparent.

Period 5, day 10. *Petrolisthes armatus* (fig. 1E) and *P. robsonae* (fig. 2E). — Ocular processes are ovoid and have moved to the rostral part of the embryo. Antennule-antenna, maxillule-maxilla, and maxilliped primordia have enlarged. In *P. robsonae* there is a noticeable depletion of yolk and the abdomen is divided into somites (metameres).

Period 6, day 13. *Petrolisthes armatus* (fig. 1F) and *P. robsonae* (fig. 2F). — The abdomen is enlarged and its segmentation is more advanced. The eyes are differentiated into cornea and retina. All appendages are segmented. Contractions of thoracic and abdominal processes are perceived. In *P. armatus*, the eyes are densely pigmented. In *P. robsonae* the depletion of yolk is substantial. Cephalothorax and digestive gland are now visible.

Period 7, day 16. *Petrolisthes armatus* (fig. 1G) and *P. robsonae* (fig. 2G). — The embryo occupies the entire space of the egg. Yolk droplets are still stored in the cephalothorax, which is now completely visible. The abdomen is fully segmented. The telson has formed. The heart is visible and beats vigorously. In *P. armatus*, the depletion of yolk is substantial. The digestive gland is more obvious.

Period 8, day 19 ± 1. *Petrolisthes armatus* (fig. 1H) and *P. robsonae* (fig. 2H). — A few small droplets of oil remain. The maxillipeds are complete and bear large setae. The embryo is about to hatch.

**DISCUSSION**

To our knowledge, there is no previous description of the embryonic development in any other species of *Petrolisthes*. The 19 ± 1 days needed from spawning to hatching in *P. robsonae* are considerably longer than for species of Brachyura inhabiting the same coastal lagoon (i.e., 14 days for *Aratus pisonii* (H. Milne Edwards, 1837); 15 days for *Goniopsis pulchra* (Lockington, 1877); 13 ± 1 days for both *Panopeus chilensis* H. Milne Edwards & Lucas, 1844, and *Eurypanopeus canalensis* Abele & Kim, 1989) (cf. García-Guerrero & Hendrickx, 2004b, unpubl. data) and is in agreement with observations made on specimens of that species kept in captivity for larval description. The duration of embryonic development proved to be similar in *P. armatus*, from the open shore, and in *P. robsonae*, from coastal lagoons and estuaries, indicating that, in this case at least, the habitat has no influence on the duration of the embryonic stage.

Observations made during this study indicate that there are no major morphological or size differences between eggs from the estuarine species, *P. robsonae* and the open-sea species, *P. armatus*. Both exhibit similarities related to their close
phylogenetic position. During embryogenesis, the chronogram of emergence and differentiation of primordial structures shows the same trend. Slight differences related to the arrangement of primordial cells and (later) of primordial structures, were nonetheless observed. Yolk depletion also occurs earlier in *P. robsonae* (compare the 6th period in both species).

A cumulative increase in the size of the eggs, throughout development, is noticeable only after the 4th (*P. robsonae*) or the 5th (*P. armatus*) period of development (>8%) and reached similar value (ca. 31%) at the end of the 8th period (table I). The increase in egg-volume in the later periods of development period is a regular phenomenon in all crustaceans. First observed by Pandian (1970), this swelling is attributed to the necessity for the embryo to force and break the chorion by the end of the developmental period, and it has been reported for many species of crabs, mostly brachyurans (see Nagao et al., 1999; Pinheiro & Hattori, 2003).

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**REFERENCES**


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