Embryology of decapod crustaceans III: Embryonic development of *Eurypanopeus canalensis* Abele & Kim, 1989, and *Panopeus chilensis* H. Milne Edwards & Lucas, 1844 (Decapoda, Brachyura, Panopeidae)

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ABSTRACT. Embryonic development in two coastal lagoon crabs, *Eurypanopeus canalensis* and *Panopeus chilensis*, is described. Embryos were sampled and illustrated at intervals of 48 hours. The complete embryonic development at 26-28°C was similar (13 ± 1 day) for both species. Growth was synchronous and appendages were formed during the same periods for the two species. Differences were observed in eye size and cephalothorax-abdomen proportions. The egg-volume increased significantly, three differences statistically in volume were detected for *E. canalensis* and two for *P. chilensis*, especially at the two last stages of development.

KEY WORDS: Brachyura, Panopeidae, Embryology, Coastal Lagoons.

INTRODUCTION

Most knowledge associated with brachyuran crabs of the west coast of Mexico concerns geographic distribution and habitat. Few autecological studies are available and, although many species are easy to recognize in the field, there has been little study on their biology. A few species of brachyuran crabs (e.g., *Callinectes* spp.; *Cancer jonngarthi* Carvacho, 1989; *Maiopsis panamensis* Faxon, 1893) are important fishery resources in the area, but their biology is poorly documented (Hendrickx, 1984; 1995).

Topics related to the ontogeny of eastern tropical Pacific brachyuran species have received little attention (García-Guerrero & Hendrickx, 2004). Brachyurans show great diversity in reproductive strategies, especially in egg number and size (Anderson, 1982; Hines, 1982). Females incubate eggs attached to the abdomen, from spawning to hatching (Anderson, 1982). The incubation period depends on species (Nagao et al., 1999) and temperature (García-Guerrero et al., 2003a). Most studies of crab embryology are recent and deal with species of only a few families (see Nagao et al., 1999; Bas & Spivak, 2000; Yamaguchi, 2001; Pinheiro & Hattori, 2003).

Coastal lagoons associated with mangrove forests in the eastern tropical Pacific are highly suitable habitats for permanent populations of panopeid crab genera such as *Panopeus*, *Eurypanopeus* and *Hexapanepeus*. Some of these crabs are associated with the red mangrove aerial roots, oyster and mussels banks, and any hard substrate (e.g., pier, ropes) introduced in these ecosystems. There is, however, no information available on the embryology of these crabs even though most species are well known, generally abundant and easy to collect (Hendrickx, 1984; Salgado-Barragán & Hendrickx, 2002). In the Western Atlantic, more attention has been allocated to larval development of panopeid and other crabs and, to some extent, to their embryonic development (Martin et al., 1985; Pinheiro & Hattori, 2003). Because it represents the first step in the life cycle and probably is one of the most sensitive to external factors, study which describes the embryonic development of species associated to critical ecosystems (i.e., ecosystems with strong ecological stress) is considered important to their biology.

Among the crabs associated with mangrove forests of the SE Gulf of California, *Eurypanopeus canalensis* Abele & Kim, 1989 and *Panopeus chilensis* H. Milne Edwards & Lucas, 1844 were studied in order to describe and compare their embryonic events.

MATERIAL AND METHODS

A group of adult males and females of *P. chilensis* and of *E. canalensis* was captured in August 2003 associated with *Rhizophora mangle* prop roots in the Estero de Urias, SE Gulf of California, Mexico. Specimens were transported to the laboratory and placed separately in plastic aquaria (LxWxH= 60x40x40). Aquaria were filled with filtered marine water, maintained with constant aeration and provided with bricks with holes as shelter. Photoperiod was kept 12 :12 h (light :dark). Water was maintained at room temperature (26 to 28°C) and at salinity of 35-36‰. Commercial shrimp pellets were offered every other day as food. During the first two weeks of captivity,
three ovigerous females of *P. chilensis* and five of *E. canalensis* lay eggs. Each female was immediately transferred to an individual three-liter glass jar with continuously aerated sea water. A sample of at least 10 eggs was removed from each female every 48 hours beginning on the day they were first detected as ovigerous. Eggs were placed into depression slides in seawater and examined in vivo to describe the morphology with an optical microscope (25x and 40x magnification). Embryo morphology, growth and yolk-tissues proportion were observed for each sample, in lateral and frontal views. The colouration pattern was also noted. Development was divided into periods of 48 hours from ovoposition to hatching in agreement with GARCÍA-GUERRERO & HENDRICKX (2004) 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 a continuum. Illustrations were made with the aid of a camera lucida, as a composite of observations made on every egg in each sample. Smallest and largest diameters of the eggs were measured to ± 0.01 mm with ocular micrometer. Egg volume was calculated as a perfect sphere. Diameter was considered as the average between the largest and the smallest width of all measured eggs within a particular period. Accumulated increase of egg volume was calculated by using average volumes observed at the end of each period. A one-way ANOVA test (p>0.05) was applied to egg volume to detect possible significant differences over the course of development.

**RESULTS**

Average diameter, volume and accumulated increase of egg volume are presented in Table 1. The complete embryonic development of both species lasted 13±1 days and included eight embryonic periods from egg extrusion to hatching. In *E. canalensis* major volume increase was observed during period 8, while in *P. chilensis* major volume increases were observed in periods 7 and 8; overall, accumulated increase at the end of the development was 101.4% for *E. canalensis* and 45.8% for *P. chilensis*, respectively (Table 1). Although in both species an increase in average egg volume from one period to another is evident, the one-way ANOVA followed by post hoc Tukey test, revealed that egg volume generally increased significantly only when several successive periods are clustered. Three statistically different size increases were detected in *E. canalensis* (i.e., total increase during periods 1-4, 5-7 and during period 8 were different, F= 583.6, p= 0.001) and two in *P. chilensis* (i.e., size increase during periods 1-6 and 7-8 were different, F= 4021.1; p= 0.002) (Table 1).

Descriptions and illustrations are presented for each 48-hour period of development (Figs 1-3).

### TABLE 1

Diameter (mean±σ), average volume and accumulated volume growth in percent in eggs of *Eurypanopeus canalensis* and *Panopeus chilensis* during periods of embryonic development. Periods were arbitrarily designated as 48-hours intervals over the time-course of development. From ANOVA, different letters = statistically significant differences (p<0.05).

<table>
<thead>
<tr>
<th>Period</th>
<th>Eurypanopeus canalensis</th>
<th>Panopeus chilensis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (µm)</td>
<td>Volume [µm³ x 10⁶]</td>
</tr>
<tr>
<td>1</td>
<td>300±11.1</td>
<td>14.14±0.5a</td>
</tr>
<tr>
<td>2</td>
<td>298±21.7</td>
<td>14.09±1.0a</td>
</tr>
<tr>
<td>3</td>
<td>309±14.4</td>
<td>15.46±0.7a</td>
</tr>
<tr>
<td>4</td>
<td>321±17.7</td>
<td>17.32±0.9a</td>
</tr>
<tr>
<td>5</td>
<td>333±09.4</td>
<td>19.31±0.5b</td>
</tr>
<tr>
<td>6</td>
<td>337±14.2</td>
<td>20.04±0.8b</td>
</tr>
<tr>
<td>7</td>
<td>349±11.7</td>
<td>22.21±0.7b</td>
</tr>
<tr>
<td>8</td>
<td>380±12.4</td>
<td>28.70±0.9c</td>
</tr>
</tbody>
</table>

### Eurypanopeus canalensis

Period 1. Recently laid eggs (Fig. 1A). The eggs are macrolecithal-centrolecithal and spherical, filled with a uniform purple yolk mass. No tissue evident.

Period 2. Day 2 (Fig. 1B). The yolk is fragmented into small oily droplets. Yolk colour is lighter than in period 1. A cluster of presumptive primordial cells begins to form as a patch located in ventrolateral position.

Period 3. Day 4 (Fig. 2A). The cluster of primordial cells has differentiated into major embryo structures, placed in ventral position (ocular, antennule-antenna, maxillule-maxilla, maxilliped and thoracic-abdominal).

Period 4. Day 6 (Fig. 2B). Abdominal and cephalothoracic primordia have increased in size and are now separated. Antennule-antenna, maxillule-maxilla and maxillipeds are now tiny buds rising below and back to the optical primordial structures. Tissues and yolk appear transparent.

Period 5. Day 8 (Fig. 2C). Antennule-antenna, maxillule-maxilla and maxilliped primordia have grown. Abdomen is incompletely divided into segments (metameres). Ocular processes are developing a slightly darker oval-shaped core (retina) in the fore portion of the embryo.

Period 6. Day 10 (Fig. 2D). Depletion of yolk is substantial. Cephalothorax is now formed, abdomen is enlarged and its segmentation is almost complete. Eyes are enlarged, differentiated in cornea and retina, and densely pigmented. All appendages are segmented, enlarged. The heart beats slowly. Contractions of thoracic and abdominal processes are observed.

Period 7. Day 12 (Fig. 2E). Embryo occupies all the space within the egg. Yolk droplets are still stored dorsal to cephalothorax, which is entirely visible. Abdomen is
fully segmented. Telson is formed and bears chromatophores, also present on the abdomen. The heart has grown and beats vigorously.

**Period 8. Day 13±1 (Fig. 2F).** A few small yolk droplets remain dorsally on the carapace. The abdomen shows a pair of chromatophores on each segment. Maxillipeds are complete with long setae. The embryo is about to hatch.

**Panopeus chilensis**

**Period 1.** Recently laid eggs (Fig. 1A). The eggs are macrolecithal-centrolecithal and spherical, filled with a uniform brown yolk mass. No tissue evidences.

**Period 2. Day 2 (Fig. 1B).** The yolk has fragmented into small oily droplets. Colour is lighter than in period 1. A cluster of presumptive primordial cells begins to form as a patch located in ventrolateral position.

**Period 3. Day 4 (Fig. 3A).** The cluster has differentiated into main transparent embryo structures in ventral position, separated by slits (ocular, antennule-antenna, maxillule-maxilla, maxilliped and thoracic-abdominal).

**Period 4. Day 6 (Fig. 3B).** Abdominal and cephalothoracic primordia have increased in size and are now separated. Antennule-antenna, maxillule-maxilla and maxillipeds are now tiny buds rising below and back to the optical primordial structures. Tissues and yolk appear transparent.

**Period 5. Day 8 (Fig. 3C).** Antennule-antenna, maxillule-maxilla and maxillipeds primordia have grown. Abdomen is divided incompletely into segments (metameres). Ocular lobes are oval-shaped and have developed a dark core (retina).

**Period 6. Day 10 (Fig. 3D).** Consumption of yolk is noticeable, exposing the cephalothorax. Abdomen has grown and its segmentation is almost complete. Heartbeat and contractions of the embryo are registered. Eyes fully pigmented, differentiated in cornea and retina. Chromatophores present on abdominal somites.

**Period 7. Day 12 (Fig. 3E).** Embryo occupies all the space within the egg. Yolk droplets are still stored dorsal to cephalothorax, which is entirely visible. Abdomen is fully segmented. Chromatophores have appeared on abdomen and telson. Eyes have grown and are triangle-shaped. The heart is beating continuously. Cephalic appendages and telson bear setae.

**Period 8. Day 13±1 (Fig. 3F).** Few traces of yolk in some embryos. Maxillipeds are well developed. The embryo is about to hatch.
**DISCUSSION**

When the two species included in the present work are compared, developmental events were similar, particularly during the first and second periods. Embryos of both species form their appendages during equivalent periods, approximately halfway in the developmental process in terms of ontogenetic progression, and after major primordial structures have differentiated. Growth and complexity of abdominal and ocular processes are also equivalent. In both species, eye formation clearly follows the pattern described by Cronin & Jinks (2001) for crustacean vision ontogeny. In both species, major differentiation events occur during the first week whilst growth of structures and segments prevail during the second week. Primordial cells are more evident in the eggs of *E. canalensis*. In terminal periods, the cephalothorax-abdomen proportion, larval succession of events, as previously observed by Hel-...
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REFERENCES


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