EXTERNAL DESCRIPTION OF THE EMBRYONIC DEVELOPMENT OF THE PRAWN, *MACROBRACHIUM AMERICANUM* BATE, 1868 (DECAPODA, PALAEMONIDAE) BASED ON THE STAGING METHOD

BY

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ABSTRACT

The embryonic changes during the development of the freshwater prawn, *Macrobrachium americanum* are described from observations made on live embryos based on the percentage-staging method. Eggs were observed with a stereomicroscope to obtain descriptions of embryonic periods. This prawn has an incubation time of 18 days at 24°C. Ten periods are described and illustrated. A comparison of this developmental process with those of congeneric species is included.

RESUMEN

Se describen los caracteres externos del desarrollo embrionario del langostino dulceacuícola *Macrobrachium americanum* tomando como criterio el método de estadios fijos basado en porcentajes. Los huevecillos fueron observados en vivo con un microscopio estereoscópico y se descibe cada periodo de desarrollo. Los huevecillos tardan 18 días en incubarse a una temperatura de 24°C. Diez periodos se describen e ilustran. Se compara el desarrollo con el de algunas especies cercanas.

INTRODUCTION

Freshwater prawns of the genus *Macrobrachium* (Decapoda, Caridea, Palaemonidae) constitute one of the most diverse, abundant, and widespread groups of crustaceans. They are known to be extensively distributed across tropical and subtropical regions worldwide, and comprise over 200 described species (Murphy & Austin, 2004). While certain aspects of the general biology and ecology of this large genus are relatively well known, some others, including its embryology, are

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as yet poorly studied. In palaemonids, females maintain the eggs attached to the pleopods in a clutch until hatching. The nauplius is embryonated, a crustacean marker of meroblastic development (Müller et al., 2004). There is an intralecithal cell division, formation of syncytial blastoderm, a blastoporal area, the presence of a superficial germinal disc, a caudal papilla, an embryonic post-nauplius, and an incorporation of the yolk mass by the midgut (Anderson, 1982; Müller et al., 2003). Hatching occurs as a zoea (Rabalais & Gore, 1985). The main features related to the embryogenesis of crustaceans with centrolecithal eggs and superficial cleavage are easily recognized in the embryonic morphology (Anderson, 1973, 1982; Rabalais & Gore, 1985). Several criteria have been applied to describe this pattern in lecitotrophic Decapoda, such as the eye index (Perkins, 1972), the quantitative staging index (Beltz et al., 1992), the percentage staging (Sandeman & Sandeman, 1991), and daily staging schemes (Nazari et al., 2000, 2003).

Even though the general pattern is well known, only a few works on the embryonic morphology of species of *Macrobrachium* are available in any of those schemes of description. Previous reports deal with *M. rosenbergii* (De Man, 1879), *M. olfersii* (Wiegmann, 1836), and *M. acanthurus* (Wiegmann, 1836) (cf. Caceci et al., 1996; Müller et al., 2004; Müller et al., 2007, respectively). Another study compares the embryogenesis of four Palaemonidae, i.e., *Macrobrachium olfersii*, *M. potuina* (Müller, 1880), *Palaemon pandaliformis* (Stimpson, 1871), and *Palaemonetes argentinus* (Nobili, 1901) (cf. Müller et al., 2004).

Macrobrachium americanum Bate, 1868, is distributed in the continental waters of the Pacific side of America, from Mexico to Peru. It is a large species (up to 23.5 cm total length, Kensler et al., 1974) with a significant commercial value throughout its range. It inhabits freshwater streams and occasionally enters brackish water (Kensler et al., 1974). It is common in northwestern Mexico rivers, where it is usually found under rocks, burrowing in the muddy bottom, and among submerged tree branches. It reproduces during the warm months. Because of its abundance and local importance for fishery, which results in making it an endangered species in the area, *M. americanum* was selected for our study of embryonic development of freshwater prawns. The main purpose of the study is to provide a basic guide for further studies when dealing with ovigerous prawns of this or closely related species.

MATERIAL AND METHODS

Macrobrachium americanum adults were caught with crab traps placed at 1.8 m depth in the El Naranjo River, Sinaloa (25°57′N 108°51′W), which flows in a subtropical valley. This habitat experiences extreme temperature variation during the year (i.e., average water temperature, 24.1°C; range from 0.5 to 44.5°C) and the average precipitation is high (938.5 mm per annum during the last 10 years)

(Muñoz-Sevilla & Escobedo-Urías, 2005). Prawns were transported in plastic coolers and placed in permanently aerated, round fiberglass containers (1.5 m diameter, 0.8 m height) filled with 1000 liters of filtered water. Hollow concrete bricks were utilized as shelters, one brick per two prawns. Animals were kept in a natural light/dark cycle and fed a mixture of fresh tilapia and squid meat. Soft-berried females, when detected, were separated and placed in 40 liter plastic aquariums with water at 24 ± 0.5°C, and with continuous aeration and shelter. Three berried females were separated and sampled. The first sample, which corresponds to recently spawned eggs, was obtained just before the females were detected as berried. Samples were taken every 48 hours during the experiment. A clutch of approximately 100 eggs was removed from the female pleopods for each sampling. The developmental period of embryos was determined by the staging method as proposed by Sandeman & Sandeman (1991), in which egg-laying is defined as 0% and hatching as 100%. In this study, development was divided into 10 periods, each representing 10% of the entire development time elapsed between spawning and hatching. Egg features were observed in live specimens with a stereo-microscope (10× magnification). Total length was measured as the egg diameter. All photographs were taken at 4× with a digital camera attached to the microscope. The nomenclature used in the description follows Anderson (1982).

RESULTS

At 24.0 ± 0.5°C *Macrobrachium americanum* eggs complete their development in 18 days. Eight developmental periods occurred in this time and maximum lengths and widths of specimens at each developmental period are summarized in table I.

TABLE I

Comparative data of embryonic periods (expressed in % or number of days) in *Macrobrachium* species with completely described embryonic development. D, total development duration in days; S, total number of periods considered

Species	Germinal disk	Embryonic nauplius	Eye		Heart beat	
			Pigment	Oval	Slow	Fast
M. americanum Bate (present work)	10-20%	20-30%	60-70%	80-100%	50-60%	80-90%
M. olfersii (Wiegmann) (cf. Müller et al., 2003)	14-21%	21-29%	50-64%	93-100%	50-64%	93-100%
M. acanthurus (Wiegmann) (cf. Müller et al., 2007)	15%	30%	50%	80%	60%	80%

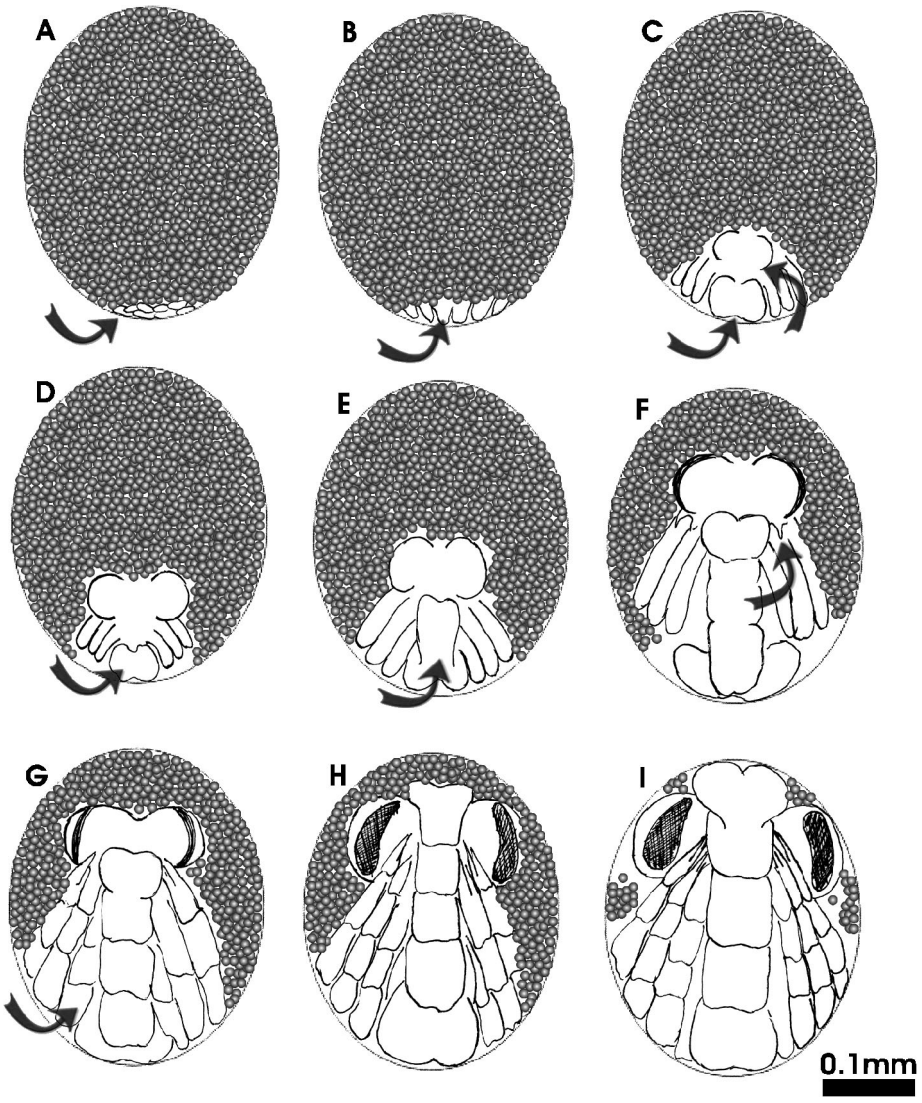

Fig. 1. The embryonic periods of development of *Macrobrachium americanum* Bate, 1868, front view. A, 10 to 20%, light patch of cells on the ventral surface of the egg (arrow); B, 20 to 30%, the six primordial cells are observed in pairs (arrow); C, 30 to 40%, eye lobes are in development as thicker and darker sections (arrow), caudal papilla is developing as a horse-shoe shape (arrow); D, 40 to 50%, cephalic appendages elongated, limb bud-shaped, caudal papilla is C-shaped (arrow); E, 50 to 60%, caudal papilla larger (arrow), rudiment of the telson is visible; F, 60 to 70%, eyes with external layer darker, flagella of antennae differentiated (arrow), abdominal somites become visible; G, 70 to 80%, telson reaches the optic lobes, cornea developed, chelipeds cover the embryo front, pereiopods larger and segmented (arrow); H, 80 to 90%, all embryonic regions clearly differentiated, thoracic appendages thicker and aligned at the front of the embryo, the abdomen has split into its five segments; I, 90% to hatching, embryo occupies all the egg, traces of yolk in most embryos, extremities of the appendages and the telson bearing setae.

Developmental periods

Period 1, days 1-2; 0 to 10% (not illustrated). — Fertilized eggs are spherical, and filled with yolk. Some egg yolks may start to split into small droplets as evidence of cleavage.

Period 2, days 3-4; 10 to 20% (figs. 1A, 2A). — A light patch of cells is visible on the ventral surface of the eggs, forming a spreading and a depression that presumably corresponds to gastrulation. Regions with high density of cells

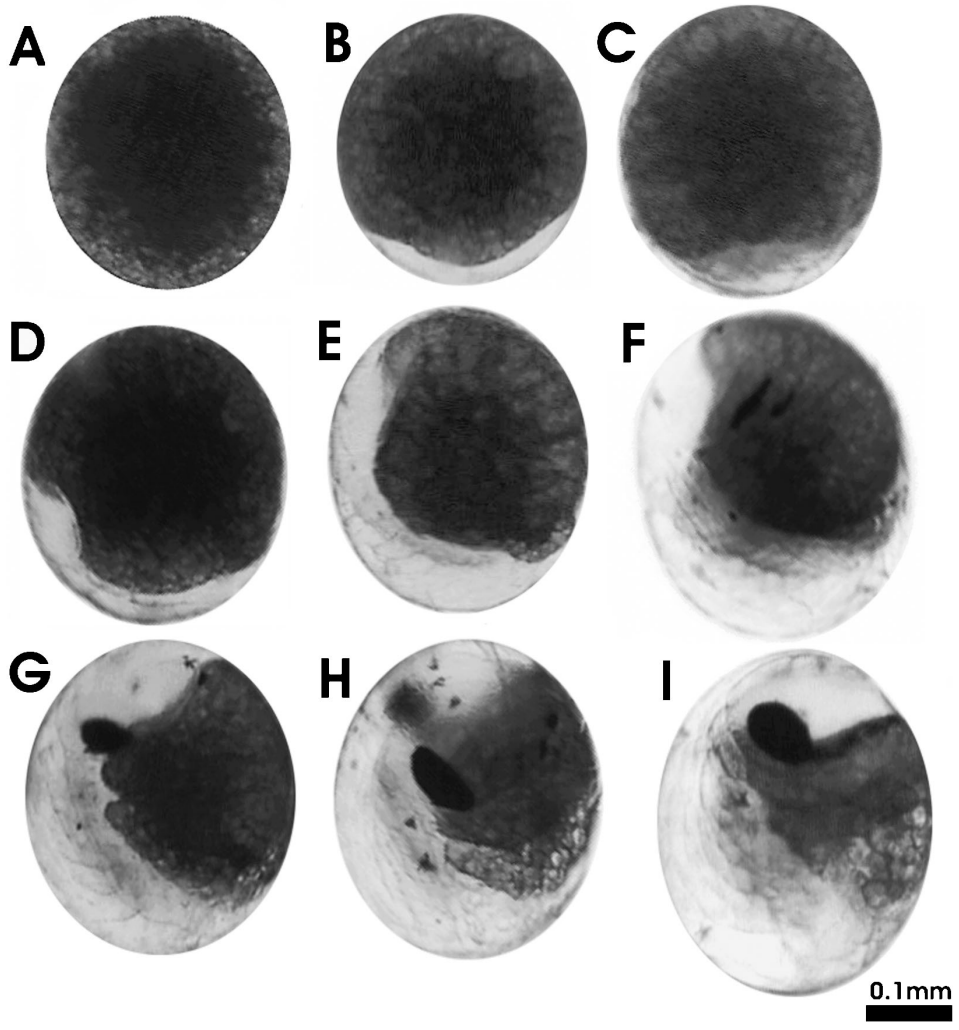

Fig. 2. External appearance of live eggs photographed at different periods of development in *Macrobrachium americanum* Bate, 1868, lateral view. A, 10 to 20%; B, 20 to 30%; C, 30 to 40%; D, 40 to 50%; E, 50 to 60%; F, 60 to 70%; G, 70 to 80%; H, 80 to 90%; I, 90% to hatching.

observed as the blastopore area appears on the surface. No differentiated structures are recognized.

Period 3, days 5-6; 20 to 30% (figs. 1B, 2B). — A germinal disk is forming and six translucid evaginations, disposed in pairs, correspond to naupliar primordial appendages. All are similar in shape giving no information about uniramous or biramous features or size. Mandibular appendages barely visible. All tissue clearly separated from yolk. Stomodaeum region located as a patch in the medial region of the embryonic tissue.

Period 4, days 7-8; 30 to 40% (figs. 1C, 2C). — Optic lobes distinguished as thicker, darker, undifferentiated structures. Antennules, antennae, and mandibles larger, more defined than in earlier period. Maxillules and maxillae larger. Maxilliped evident. Caudal papilla increasing in length, folding, and horse-shoe shaped.

Period 5, days 9-10; 40 to 50% (figs. 1D, 2D). — Maxillules, maxillae, and maxillipeds elongated, all limb buds aligned and similar in shape. Caudal papilla larger, thicker, and folded forward, C-shaped. Antennules and antennae larger in size. Dorsal heart vessel formed, beating regularly. Optic lobes bending anterolaterally due to reduced space in the egg, covering part of the yolk. Ventral portion with chromatophores.

Period 6, days 11-12; 50 to 60% (figs. 1E, 2E). — Caudal papilla extending as a lappet of tissue across median portion of egg, partly overlapping the optic lobes. All appendages long and overlapping. Abdominal somites and Anlage of pereiopods discernible. Mandibles extended. Caudal papilla folded forward, covered by forming pereiopods and almost reaching head, its extremity now seen as Anlage of telson. Continuous contractions of embryo and yolk.

Period 7, days 13-14; 60 to 70% (figs. 1F, 2F). — Considerable increase in embryo size. Optic lobes facing forward, larger, with external layer darker, particularly at edge. Basal portion and flagella of antennae differentiated, folding towards chelipeds. Mouthparts formed as elongated structures at each side of head, partially covered by the thoracic appendages, the latter starting to fold towards pleon and with distinguishable chelae. Abdominal somites visible as pleon becomes distinguishable from rest of embryo.

Period 8, days 15-16; 70 to 80% (figs. 1G, 2G). — Embryo now moving its entire body as the yolk mass decreases to 25% of the egg volume. Telson reaching the optic lobes, which are considerably larger. Cornea developed on the external dark zone of the optic lobes, inner area appearing more complex, with outer, darker layer, and inner, lighter layer. Basal segments and flagella of appendages differentiating. Chelipeds covering the embryo front; pereiopods larger, thicker.

Period 9, day 17; 80 to 90% (figs. 1H, 2H). — Chromatophores evident in most embryos. Whole embryo increased in size, occupying all space available, except for the remaining yolk storage dorsally. Optic lobes much larger, protruding beyond the cephalothorax. Heart beating continuously and regularly. Thoracic

appendage segmentation more advanced, segments ventrally aligned at the front of the embryo. Pleon now divided into five somites.

Period 10, day 18, hatching (figs. 1I, 2I). — Embryo occupying all available space inside the egg. Traces of yolk remain in most embryos. All thoracic and cephalic appendages covering entire ventral side of egg. Telson positioned beyond optic lobes. Extremities of appendages and telson bearing setae.

DISCUSSION

The present study deals with a species of Palaemonidae, as do those of Nazari et al. (2000) and Müller et al. (2003), but follows the method initially proposed by Sandeman & Sandeman (1991) for a species of Astacidea. This method is indeed considered more accurate and easier to apply in decapod embryology (Sandeman & Sandeman, 1991; García-Guerrero et al., 2003a) since it is presumed that embryonic development should be analogous in all *Macrobrachium* species, as in other congeneric species of decapod crustaceans. No significant differences are expected when comparing species.

However, a different, more accurate approach may provide additional or more detailed information. The morphological features of *Macrobrachium americanum* observed in the present study match the pattern described for congeneric species, such as *M. olfersii* (see Müller et al., 2003) and *Macrobrachium acanthurus* (see Müller et al., 2007).

Embryonic development of other lecitotrophic decapods like *Cherax destructor* Clark, 1936 (see Sandeman & Sandeman, 1991) or *C. quadricarinatus* Von Martens, 1868 (see García-Guerrero et al., 2003) are less comparable but their development is also continuous.

Thus, the embryonic development of *M. americanum* could be accurately described using the percentage staging method, which divides the process into percentage periods that consider all major changes. This method has been successfully employed in various Decapoda that have in common the presence of a lecitotrophic phase as in Astacidea (Sandeman & Sandeman, 1991; García-Guerrero et al., 2003a), brachyurans (García-Guerrero & Hendrickx, 2004, 2006a), and anomurans (García-Guerrero & Hendrickx, 2006b) given that these all go through a continuous process. In species of *Macrobrachium*, including *M. americanum*, all structures will invariably appear in a similar sequence and follow matching patterns. In *M. americanum*, this method allows the observation of the development of embryonic structures without cytological examination. This technique has been demonstrated to be suitable, because of the low degree of complexity of external structures and due to the superficial position of the embryo in the egg. However, a close match is only found in congeneric species regardless of the descriptive method. Müller

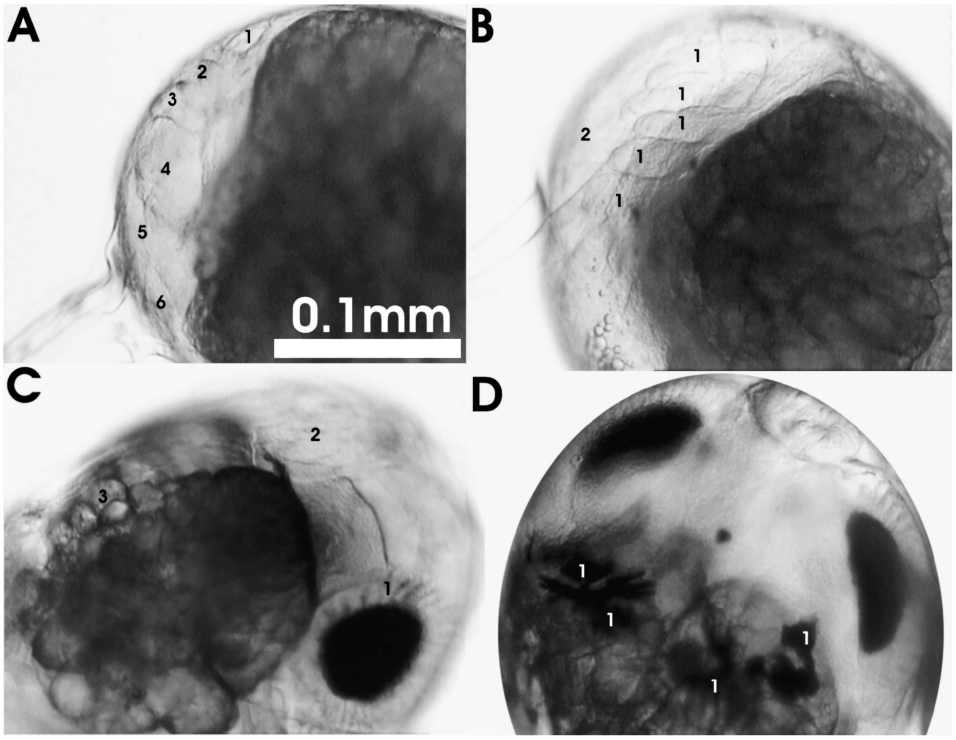

Fig. 3. Major embryonic features during the embryonic periods of development of *Macrobrachium americanum* Bate, 1868. A, six primordial structures differentiated (1-6); B, limb buds (1) and caudal papilla (2) developed; C, eyes completely differentiated (1) and telson folded reaching the head (2), yolk oily droplets (3); D, chromathophores clearly distinguished (1).

et al. (2003) described the embryonic development of *M. olfersii* based on eight different major events, called stages, with no fixed duration and separated by morphological events. However, we consider that the separation in periods of equal duration is more accurate when describing a continuous embryonic development (see Sandeman & Sandeman, 1991; García-Guerrero et al., 2003; García-Guerrero & Hendrickx, 2004, 2006a). This is due to the fact that the separation between major events of embryogenesis in crustaceans is not always easy to distinguish, and some events may start before the ending of a previous one, making the separation into events unclear when the process is a continuum.

In addition, Müller et al. (2007) also proposed criteria of complementary developmental markers, through morphometric analysis of some embryonic structures such as the optic lobe area and the eye index. However, the morphometric criteria can be useful when such structures are formed during certain periods. The major differentiation and growth events of the embryonic development of *M. americanum* and *M. olfersii* occur during matching periods (or stages), i.e., the appearance of

the germinal disk and embryonic nauplius, the emergence of chromatophores, the pigmentation of the optic lobe, and the beating of the heart (table I; see also fig. 3). Minor differences include the lack of segmentation of the mouth appendages in *M. americanum*, a feature observed at 40% development in *M. olfersii*.

We cannot consider duration of the development for comparative purposes, even in congeneric species, since other factors are involved, primarily temperature (García-Guerrero et al., 2003b). It is possible that the slight differences in development time between congeneric species (i.e., between *M. americanum* and *M. olfersii*) could be related to culture conditions.

The external description of the embryology of *M. americanum* could be useful for future studies dealing with its ontogeny, including the use of histological techniques that will provide information on the development of internal organs. Internal events are closely correlated with external ontogeny (Sandeman & Sandeman, 1991) and, if a parallel recording of internal and external events is established, the ontogeny of prawns such as *M. americanum* could be better understood as a whole.

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