DESCRIPTION OF THE EMBRYONIC DEVELOPMENT OF
*CHERAX QUADRICARINATUS* (VON MARTENS, 1868)
(DECAPODA, PARASTACIDAE), BASED ON THE STAGING METHOD

BY

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ABSTRACT

The complete embryonic development of *Cherax quadricarinatus*, the redclaw crayfish, is
described based on external morphological changes. Substages prior to hatching are defined in
relation to the percentage time of embryonic development. Ten prehatching and three posthatching
stages are described and illustrated. Embryo development, from recently spawned eggs to the first
juvenile stage, lasted 42 days at 26.0°C.

RÉSUMÉ

Le développement embryonnaire complet de *Cherax quadricarinatus* l’écrevisse à pinces
rouges, est décrit à partir des changements morphologiques externes. Les stades antérieurs à
l’éclosion sont définis par rapport au pourcentage de temps du développement embryonnaire. Dix
stades avant éclosion et trois après éclosion sont décrits et illustrés. Le développement embryonnaire
des œufs récemment pondus au premier stade juvénile, a duré 42 jours à 26.0°C.

INTRODUCTION

*Cherax quadricarinatus* (Von Martens, 1868), a crayfish occurring widely
across northern Australia, is highly attractive for aquaculturists (Austin, 1998).
A variety of recent studies demonstrate the increasing interest in this species
(Fletcher & Warburton, 1997; Richardson et al., 1997; Byrne, 1999; Levy et al.,
1999; Romero et al., 2000; Yehezkel et al., 2000; Abdu et al., 2001; Barki &

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Karplus, 2001; Figuereido et al., 2001; Jones & Ruscoe, 2001; Kalaila et al., 2001; Meade & Watts, 2001). However, some critical aspects of its culture are still in need of improvement. In particular, the embryonic development is not perfectly known. To date, few attempts to describe its egg stages have been made. Yeh & Rouse (1994) separated stages by egg colour changes. Such an approach is inexact, since the egg colour is not an unvarying feature of the species. Levi et al. (1999) divided the development of this species in egg, young stage 1, young stage 2, and young stage 3. Fan Li et al. (2000) described stages based only on major external embryonic changes, by which stages are separated only in cleavage, nauplius, meta nauplius, and embryo, followed by only one posthatching stage. A reliable guide must provide all persistent features that recognize every step of the process with specific details of events inside the egg, but this is not available for all species of Cherax. Cherax destructor (Clark, 1936) is the only species with a previously described embryonic development (Sandeman & Sandeman, 1991).

The study of ontogeny is important in resolving some of the problems involved when eggs of commercially important species are incubated (Reynolds, 2002). The understanding of basic changes during embryonic or larval development might be critical for improving rearing conditions of gravid females (Charmantier et al., 1991).

When describing the ontogeny of species that change habitat during development, it is possible to separate stages using external morphological features. This is a reasonable technique because the organism acquires the necessary structures to prosper in a particular habitat. This occurs in shrimp (Yang & Kim, 1999). As parastacid crayfish became adapted to freshwater, selection would have progressively eliminated free-living larvae (hatching from numerous, small eggs) and favour the development of larger and fewer, lipid-rich lecithotrophic eggs (Reynolds, 2002). In members of this family, fecundity ranges generally from 300 to 700 eggs and the burrowing females brood their eggs until hatching, with a degree of parental care (Levi et al., 1999). Eggs are centrolecithal and cleavage is followed by a continuous process of embryonic development (Anderson, 1982). All stages are retained in an embryonic form, which means direct development to hatching as a post-embryo, which is the stage preceding the juvenile stage (Sullivan & Macmillan, 2001). For C. quadricarinatus, as in other crayfish, this kind of development involves no habitat changes during ontogeny or even in the life cycle (Loya-Javellana et al., 1994).

As shown by Sandeman & Sandeman (1991), the staging method is the most suitable for this kind of development. There are three recognized ways to label the stages in the development: by age, by events, or by percentage of the total development period (Sandeman & Sandeman, 1991). The most appropriate way will ultimately depend on the nature of the ontogenic changes in the species.
studied. Age could be different for eggs of the same species at the same stage if affected by temperature or other environmental conditions. To label stage or substage by major events within the eggs could be inaccurate when there is a continuous developmental process. However, there are studies that propose reliable ways for describing this process (see Bentley, 1979; Sandeman & Sandeman, 1991). A good option is to divide the period of development into percentages, describing each stage or substage using the most relevant features that can be consistently seen in every embryo and without resorting to histological procedures (Sandeman & Sandeman, 1991).

This paper describes the external embryonic development of *C. quadricarinatus*, based on the percentage-staging method. This information will help to improve the handling and maintenance of gravid females by using an identification guide as a tool to monitor critical periods during development.

**MATERIALS AND METHODS**

Specimens (20 females and 5 males) of *Cherax quadricarinatus* weighing $50 \pm 7.5\, \text{g}$ were placed in a 1500-l fiberglass tank at $28 \pm 0.5\, ^\circ \text{C}$ with permanent aeration. The tank was provided with PVC tubes (15 cm diameter, 25 cm long) to be used as a shelter. Animals were exposed to a 12 : 12 h light/dark cycle and fed a commercial shrimp pellet diet. Berried females, when detected, were individually placed in 40-l tanks with water of $26 \pm 0.5\, ^\circ \text{C}$ with shelter and continuous aeration. Six berried females were sampled. The first sample, which corresponds to recently spawned eggs, was obtained just before the females were transferred. Samples were taken every 72 h during the experiment. Ten eggs were removed from the female swimerets for each sampling. The developmental stage of the embryos was determined using a modification of the percentage staging system described in Sandeman & Sandeman (1991), in which egg-laying is defined as 0% and hatching as 100%. In this study, development was divided into 10% intervals prior to the hatching stage. Egg features were observed in live specimens. In early stages (prior to 40% of development), the egg chorion had to be broken to observe embryonic progress by carefully separating tissue from yolk. An optical microscope (10× magnification) was used to observe early stages. Intact eggs from the same sample were used to obtain photographs of the same stages. After 40% development, morphological features were observable in entire eggs by placing them in a Petri dish with water and observing them through a dissecting microscope (6× magnification). Maximum length was determined by measuring the eggs longest diameter. Post-embryonic and juvenile maximum length were determined by measuring the distance from the tip of the rostrum to the tip of the largest uropod.
All photographs were taken at 4× with a colour camera (Cool Snap Pro Plus, Media Cybernetics) attached to the dissecting microscope. Images were processed with Image Pro Plus v. 4.0 software.

RESULTS

At spawning, females produced 350 to 700 eggs. At 26.0 ± 0.5°C, *Cherax quadricarinatus* reached the juvenile stage in 42 days. Embryos hatched, on the average, on day 31. We recognized that 13 developmental stages (including two post-embryos and one juvenile stage) occurred. Lengths and widths of specimens at each developmental stage are summarized in table I.

Developmental stages

Stage 1 (days 1, 2, 3; 0 to 10%, fig. 1A, B). — Fertilized eggs are oval, filled with yolk. No evidence of cells in recently-spawned eggs by observation with stereomicroscope, even by puncturing the egg. At day 3, yolk starts to separate into smaller droplets; and some tissue is forming. Continuous cell division forms light patch of cells on ventral surface of egg after three days; this is the germinal disk (see Anderson, 1982).

Stage 2 (days 4-6; 10 to 20%, fig. 1C, D). — The yolk is completely divided into small droplets. Layer of cells starts to spread and form a depression corresponding to gastrulation (see Anderson, 1982). Ventral plate of gastrula starts to sink and forms groove. As groove unfolds, blastopore appears, and fore portion of caudal papilla starts to develop. Later, blastopore closes and hind portion of caudal papilla appears.

Stage 3 (days 7-9; 20 to 30%, fig. 1E, F). — Primordial eyes appear as two round elevations with dark surfaces, one at each side of the embryo (see Hafner & Tokarski, 1998). Behind primordial eyes, three pairs of similar transparent evaginations correspond to primordia of antennules, antennae, and mandibles, respectively. Post-naupliar somites appear in middle, between cephalic primordia and caudal papilla, which gets thicker at end of stage 3.

**Table I**

Average lengths and widths (mm) of *Cherax quadricarinatus* (Von Martens) eggs and carapaces of post-embryo I (POI), post-embryo II (POII), and juvenile (J). Stages to hatching as % time

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<th>Stage</th>
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Fig. 1. Eggs of *Cherax quadricarinatus* (Von Martens, 1868) in stages 1-3. A, B, stage 1, 10% development, no evidence of tissue formation; C, D, stage 2, 20% development, yolk splits into large droplets, white patch of cells (arrow) visible in lower part; E, F, stage 3, 30% development, caudal papilla forming, primordial eyes and post-naupliar somites (arrow) visible.

Stage 4 (days 10-12; 30 to 40%, fig. 2A, B). — Eye lobes more pronounced, with edges thicker and darker, but eyes still undifferentiated. Antennules, antennae, and mandibles larger and more defined; maxillules and maxillae growing. Caudal papilla increases in length and folds forming a horseshoe-like shape. In most embryos, post-embryonic ectoderm is present and covers entire yolk.

Stage 5 (days 13-15; 40 to 50%, fig. 2C, D). — Antennules longer, each bears a flagellum. Antennae longer, reaching thoracic section with small exopodite. Maxillules, maxillae, and maxillipeds elongated, limb buds shaped. Caudal papilla larger, thicker, and folded forward. Behind caudal papilla, a transverse ridge corresponds to opening of free edge of the carapace (see Huxley, 1880). The dorsal heart vessel beats regularly.

Stage 6 (days 16-18; 50 to 60%, fig. 2E, F). — Eyes larger, covered with dark layer. Very short rostrum between eyes. Mandibles longer. Transverse groove in yolk, across middle of egg. Continuous embryo and yolk contractions observed.
Fig. 2. Eggs of *Cherax quadricarinatus* (Von Martens, 1868) in stages 4-6. A, B, stage 4, 40% development, eyes lobes thickening, caudal papilla larger, horseshoe shape (arrow); C, D, stage 5, 50% development, eyes forming pronounced bulges (arrow), antennules and antennae visible, caudal papilla larger, folded, all abdominal somites forming; E, F, stage 6, 60% development, eyes spherical, a medial horizontal groove present (arrow), rudimentary pereiopods present.

Edge of the carapace extends along embryo. Antennae longer, folded towards back of head. Abdominal somites and rudiments of pereiopods, including visible chelae. Caudal papilla folded forward, covered by pereiopods, almost reaches head.

Stage 7 (days 19-20, 21; 60 to 70%, fig. 3A, B). — Eyes face forward, external layer darker, particularly at edge. Basal portion and flagella of antennae differentiated, folded towards chelipeds. Mouthparts clearly differentiated and larger. Three pairs of chelipeds visible in front of body. Lateral portion of carapace deeper, forming cavities corresponding to branchial chambers.

Stage 8 (days 22-24; 70 to 80%, fig. 3C, D). — Grooves across yolk deeper along dorsal midline. Embryo now able to move. Cornea on external dark zone of eyes; inner lighter area more complex. Basal joints and flagella of appendages differentiated. Chelipeds cover mouthparts; pereiopods larger, thicker, and reach posterior edge of labrum.
Stage 9 (days 25-27; 80 to 90%, fig. 3E, F). — Eyes larger, extending beyond cephalothorax. Rostrum thicker and longer. Embryo occupies half ventral side of egg. Thoracic appendages more developed; chelipeds now completely formed. Abdomen and external region of thorax larger.

Stage 10 (days 28-31; hatching, fig. 4A, B). — Embryo occupies 3/4 of available space inside egg. All thoracic appendages completely formed and occupy entire ventral side of egg. Embryo hatches and chorion is expelled. Some recently hatched embryos often remain attached to chorion by membranous strands. Pereiopods extend. Appendages similar to adult, but chelae more slender.

Stage 11 (days 32-36; post-embryo I, fig. 4C, D). — First hatched stage. Cephalothorax convex, larger in proportion to abdomen due to big hunchback containing yolk. Body almost transparent. Eyes appear sessile. Antennules and antennae now released and curved backwards. The three pairs of chelae are
Fig. 4. Egg hatching and post-embryo I in *Cherax quadricarinatus* (Von Martens, 1868) at stages 10-11. A, B, stage 10, hatching, thoracic appendages functional, pereiopods moving, no hair or setae, embryo still attached to chorion; C, D, stage 11, post-embryo I, chorion lost, cephalothorax with yolk hunchback, chelae larger, pereiopods with dactylus hooks, abdomen curling, pleopods present, telson and uropods fused.

larger; pereiopods 4 and 5 have dactylus hooks. Abdomen completely formed, curved, pleopods present on each somite, except first (pleopod 1 is not present in Parastacidae; Hobbs, 1974). All adult appendages present and fully formed, except uropods, enclosed in paddle-shaped tail. No feeding activity. No independent locomotion since this stage remains attached to swimmerets of mother.

Stage 12 (days 37-41; post-embryo II, fig. 5A, B). — Hunchback is smaller and cephalothorax is acquiring its final shape. Almost all major adult characteristics are evident. Scattered hairs present on pereiopods. Pigmentation appearing on upper portion and both sides of carapace. Eye-stalk developing and rostrum emerging between eyes. Antennules and antennae larger, extending forwards. Abdomen straightening out; pleopods all formed and functional. Telson and uropods appear, as membrane that bound them gradually disappears. Gastroliths forming inside
Fig. 5. Post-embryo II and juvenile of *Cherax quadricarinatus* (Von Martens, 1868) in stages 12-13. A, B, stage 12, post-embryo II; hairs on pereiopods and pigmentation on carapace, eye-stalk and rostrum developed, segmentation of antennules and antennae visible, abdomen stretched, telson and uropods separating; C, juvenile, yolk depleted, abdomen fully extended, uropods and telson separated and hairy, hairs on antennae.
exoskeleton. No feeding activity. No independent locomotion and the post-embryo II remains on the swimmerets of the female.

Juvenile (day 42; fig. 5C). — Yolk depleted, and the cephalothorax assumes final shape and proportions. Abdomen extended; pleopods and pereiopods in continuous movement. Gastroliths visible inside cephalothorax, one on each side. Uropods and telson separated, rounded, and bearing numerous setae. All pereiopods hairy. Eye-stalk completely developed and rostrum longer. Hairs present on antennae and rostrum. Carapace still translucent, but red spots are appearing on entire body, thus indicating beginning of pigmentation. This is the first stage capable of independent locomotion. Some juveniles leave female and look for shelter and food, but others stay on swimmerets of mother for another week.

DISCUSSION

The external changes in *Cherax quadricarinatus* during ontogeny can be accurately described by the staging method. Sandeman & Sandeman (1991) divided the embryogenesis of *Cherax destructor* into percentage stages that clearly consider and describe all ontogenic events. This information was very helpful while monitoring the embryonic development in *Cherax quadricarinatus*. Since *C. destructor* and *C. quadricarinatus* are very closely related species (Hobbs, 1974; Austin, 1995), it was no surprise that an analogous embryonic development was found. In *C. quadricarinatus*, as in *C. destructor*, the most important ontogenetic events occur within the chorion. Perhaps the most striking difference in embryonic morphology between these species is comprised in the size of the eyes in comparison to the rest of the body. Eyes are larger and more widely separated in *C. destructor* during the final 20% of pre-hatching development. After hatching, only minor changes take place (e.g., setation on almost all appendages, hunchback disappears, development of uropods), compared to those occurring before hatching. At hatching, *C. quadricarinatus* is virtually bare of external receptor hairs, as observed in *C. destructor* (cf. Sandeman & Sandeman, 1996). The appearance of sensory hairs is an indication of the readiness of the organism for independent life.

It is important to emphasize that not all described changes are easily seen by dissection techniques. Because cleavage and blastoderm formation are intralecithal in large eggs (Anderson, 1982) such as those produced by crayfish, these processes are difficult to study without special techniques. The description of *C. quadricarinatus* external development to the juvenile stage will be useful for future studies dealing with ontogenic processes, including the use of histological techniques that will provide a more detailed description of very early stages (before 40% development) and describing the development of internal organs. Internal events are
closely correlated with external ontogeny, and, if a parallel recording of internal and external events is established, the ontogeny of *C. quadricarinatus* could be better understood.

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