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Larval development and metamorphosis of the Australian diadematid sea urchin *Centrostephanus rodgersii*

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Summary

The complete larval development through to metamorphosis of the sea urchin *Centrostephanus rodgersii* is described for the first time. Embryos developed from small eggs (113 μm) to large echinopluteus larvae (3250 μm arm length) over a period of approximately 4 months. Fully developed larvae are two-armed echinoplutei with densely pigmented postoral and anterolateral arms and oral hood. The posterodorsal and the preoral arms do not appear to form. The skeletal body rods form a basket-like structure posteriorally, and fenestrated skeletal rods support the postoral arms. Five primary podia emerge from the vestibule, at around 100 days old, and attach to the substrate at settlement. The larval epidermis recedes from the arm rods and collects on the aboral surface of the juvenile, and the adult rudiment emerges as the larva metamorphoses to the juvenile stage.

Key words: Larval development, metamorphosis, Diadematoida, *Centrostephanus rodgersii*

Introduction

Sea urchins in the Order Diadematoida, particularly those in the genus *Diadema,* are among the most ecologically important echinoids in tropical regions (Lessios et al., 2001). Temperate and subtropical members of this order in the genus *Centrostephanus* are, however, much less familiar (Andrew and Byrne, 2001) and the larval development of echinoids in the order is not well known. Within the family Diadematidae*,* there are several reports of development via a two-armed larva in the genera *Diadema* (Mortensen, 1921; Emlet, 1988; Emlet et al., 2002) and *Centrostephanus* (King, 1992; Emlet et al., 2002). The larval form is generally referred to as a two-armed larva because of the very long postoral arms in comparison to the short anterolateral arms, giving the appearance of only one pair of larval arms. Confusingly, Eckert (1998) refers to a four-armed larva, whereas Mortensen (1921, 1931, 1937) and Emlet (1988) refer to a two-armed larva. In agreement with Mortensen (1921, 1931, 1937) and Emlet (1988) we also refer to this larval form as "two-armed". This highly derived (Emlet et al., 2002) larva appears to be a characteristic form of many of the diadematids (Emlet et al., 2002). However, a very different larval form with 10 arms and posterior process has been reported to occur within the family Aspidodiadematidae (Young and George, 2000). There are only two reports of larval develop-

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ment within the genus *Centrostephanus* — a photograph of *C. coronatus* (Emlet et al., 2002) and a brief description and single photograph of *C. rodgersii* (Andrew and Byrne, 2001) based on King (1992). For both species there is no further information regarding the larval development.

C. rodgersii (Order Diadematoida, Family Diadematidae) is the dominant sea urchin in shallow subtidal reef assemblages along the southeast coast of Australia (Edgar and Barrett, 1997). This species plays an important role in structuring subtidal benthic communities through its intensive grazing on foliose macroalgae, and creates areas dominated by crustose coralline algae known as "barrens" (Fletcher, 1987; Andrew and Underwood, 1989). The magnitude and continuance of areas of barrens in southern Australia and the dominance of sea urchins in such habitats have important implications for the ecology of many species of algae, fish and invertebrates (Shepherd, 1973; Andrew and Underwood, 1992).

Despite the abundance and ecological and commercial importance of *C. rodgersii*, little is known about its early life history. Reproduction occurs throughout winter with spawning commencing in June and extending over several months, depending on the latitude of the population (King et al., 1994; Byrne et al., 1998). While details of its larval developmental biology have not previously been described, it is known to develop via a feeding pluteus larva, before settling and metamorphosing into a benthic juvenile (Andrew and Byrne, 2001). This study was undertaken to confirm predictions that *C. rodgersii* undergoes planktotrophic development and to examine its development through to metamorphosis.

Materials and Methods

Seawater

Seawater was obtained from a 30,000 L recirculating seawater system at the University of New South Wales. The seawater in this system is regularly obtained from Sydney Harbour. Prior to its use, it was 0.2 μm filtered and sterilised by autoclaving and by the addition of 36.5 mg/L streptomycin sulphate and 21.9 mg/L penicillin. This filtered and sterilised seawater (FSW) was used for collection of gametes, fertilisation and the subsequent culture of embryos and larvae.

Collection of adults and gametes

Adults of *C. rodgersii* were collected while snorkelling at Bare Island in Botany Bay and Shark Bay in Sydney Harbour during the species' spawning season (June–October) in 2000 and 2001. Urchins were induced to spawn by intra-coloemic injection of 5 mL 0.5 M KCl. Spawning was immediate in the majority of urchins, and gametes were collected in the first 10–15 min of spawning only. Sperm were collected "dry" directly from the surface of the urchin to prevent activation by water and were held in Petri dishes on ice. Eggs were collected by inverting females over beakers containing FSW. Eggs were washed several times in FSW and their condition checked. Sperm were diluted with FSW and their motility checked immediately prior to being added to the eggs. To determine the size of gametes, 10 spawned gametes from each of five adults of each sex were randomly selected and measured.

Eggs and sperm from a minimum of two females and two males were used to establish each batch of embryos. Several drops of dilute sperm were added to a suspension of eggs in FSW and gentle aeration was applied for approximately 15 min to allow fertilisation to occur. A subsample of eggs was examined under a microscope for the presence of an elevated fertilisation envelope, which is indicative of successful fertilisation. Cultures with >95% of eggs fertilised were rinsed twice in FSW by gentle reverse siphoning. Cultures with <95% of eggs fertilised were discarded.

Culture

For each batch of successfully fertilised eggs, four replicate cultures were established in sterile, 2 L glass beakers containing 1.8 L of FSW. A maximum density of five embryos/mL was used to prevent overcrowding. Cultures were stirred constantly by rotating paddles (Strathmann, 1987) and maintained in a constant temperature room at 19 ± 1 °C with a 12 h light:12 h dark lighting regime. Once fully formed guts were observed in larvae (two-arm pluteus stage at 55 h), cultures were fed a mixture of microalgae including *Chaetocerus muelleri, Isochrysis galbana* (Tahitian strain) and *Pavlova lutheri* at a concentration of 5×10^4 cells/mL every second day. FSW was changed every 3–4 days by reverse siphoning. If a biofilm was visible on the beaker, the culture was gently poured into a clean sterile 2 L beaker.

During the first 4 days of development, embryos and larvae were examined under a microscope at intervals as short as 15 min. Times to key developmental stages up to the feeding larval stage were recorded and photographs of at least 10 haphazardly selected embryos or larvae were taken for measurements of their size. After 4 days, observations were reduced to every 1–2 days and photographs of randomly selected

larvae were taken at 1, 2, 3, 4, 6, 7, 10, 11 and 13 weeks. Several larvae were also photographed during metamorphosis and after they settled as juveniles. To induce metamorphosis in competent larvae, small pieces of rock covered with encrusting coralline algae were placed with individual larvae in Petri dishes containing 5 mL FSW. Encrusting coralline algae are known to induce metamorphosis and settlement in many species of invertebrates including echinoids (Johnson, et al. 1991; Lambert and Harris, 2000).

Results

Gametes and fertilisation

The timing of development to key embryonic and larval stages is shown in Table 1. Mature ova of *C. rodgersii* are small, orange in colour, negatively buoyant and spherical with a mean diameter of 113 μm (SE ±2 μm). Spermatozoa have a conical head region approximately 5 μm long and flagellum greater than 20 μm in length. Following fertilisation, an envelope with a mean thickness of 0.7 μm (SE ±0.06 μm) is raised from the egg surface, forming a perivitelline space of approximately 10–20 μm wide.

Table 1. Age of key developmental stages in the echinoid *Centrostephanus rodgersii* cultures reared at 19 ±1 °C)

Developmental stage	Time since fertilisation (h)
2-cell	2
4-cell	2.5
8-cell	3
16-cell	3.5
32-cell	6
64-cell	7.5
Morula	8
Early blastula	10
Hatched blastula	18
Early gastrula	26
Late gastrula	40
Prism	42
Early pluteus (two-arm)	55

Blastula and gastrula

Following fertilisation (Fig 1A), cleavage is equal and radial up to the eight-cell stage. The fourth cleavage is unequal and forms four micromeres and four macromeres at the vegetal pole, and eight mesomeres at the animal pole (Fig. 1.B). Cleavage continues and blastomeres become arranged in a single layer epithelium around a central blastocoel, forming the hollow blastula (Fig. 1C). Ciliation becomes pronounced, and embryos hatch from the fertilisation envelope as swimming blastulae, 18 h after fertili-sation. Blastulae undergo gastrulation (Fig. 1.D), with the invagination of the vegetal plate and elongation of the archenteron into the blastocoel. The oral ectoderm of the gastrula flattens as the gastrula becomes roughly triangular, forming the prism larva (Fig. 1E).

Echinopluteus larva

The prism larva develops into a feeding two-arm pluteus larva within 55 h (Fig. 1F). From as early as 50 h after fertilisation, scattered red pigmentation occurs throughout the larval body. As the larvae develop, the pigment becomes concentrated in dark bands around the oral hood and at the end of the arms; the rest of the larva is transparent. A ciliated band used in swimming and suspension feeding develops and thickens in a continuous loop on the larval arms and preoral lobe. By the third day after fertilisation, larvae develop long postoral arms and short anterolateral arms. The preoral and posterodorsal arms and skeleton do not form and development of the anterolateral arms is minimal with the oral hood encompassing them. This gives the larva the appearance that it only has two arms (Fig. 2A). After 2 weeks, the appearance of the larvae does not change, except for the thinning and lengthening of larval arms (Fig. 2B). The mean maximum single postoral arm length for all larvae was 3250 μm (SE ±764 μm, n = 10) and the maximum arm length achieved by an individual was 4660 μm. The arms become so long and thin that many larvae suffered broken arms in our cultures. This was most pronounced in larvae between 7 and 10 weeks old.

Metamorphosis

On settlement, five long primary podia emerge from the vestibule to explore the substrate (Fig. 2C and D). Once these podia are firmly attached to the substrate, metamorphosis occurs. Mass mortality was observed in many batches of larvae throughout culturing, with the vast majority of larvae dying before metamorphosis occurred. From a total of at least 25 cultures, approximately 50,000 larvae, only six individuals metamorphosed, despite the presence of well-developed rudiments in the majority of larvae. Larvae that did metamorphose did so between the ages of 105 and 126 days.

During metamorphosis, the rudiment emerges (Fig. 2C) on the left side of the body as the larval epidermis recedes, beginning with the epidermis on the larval arms (Fig. 2D). At the same time, the larval rods begin to bend downwards — through an angle of

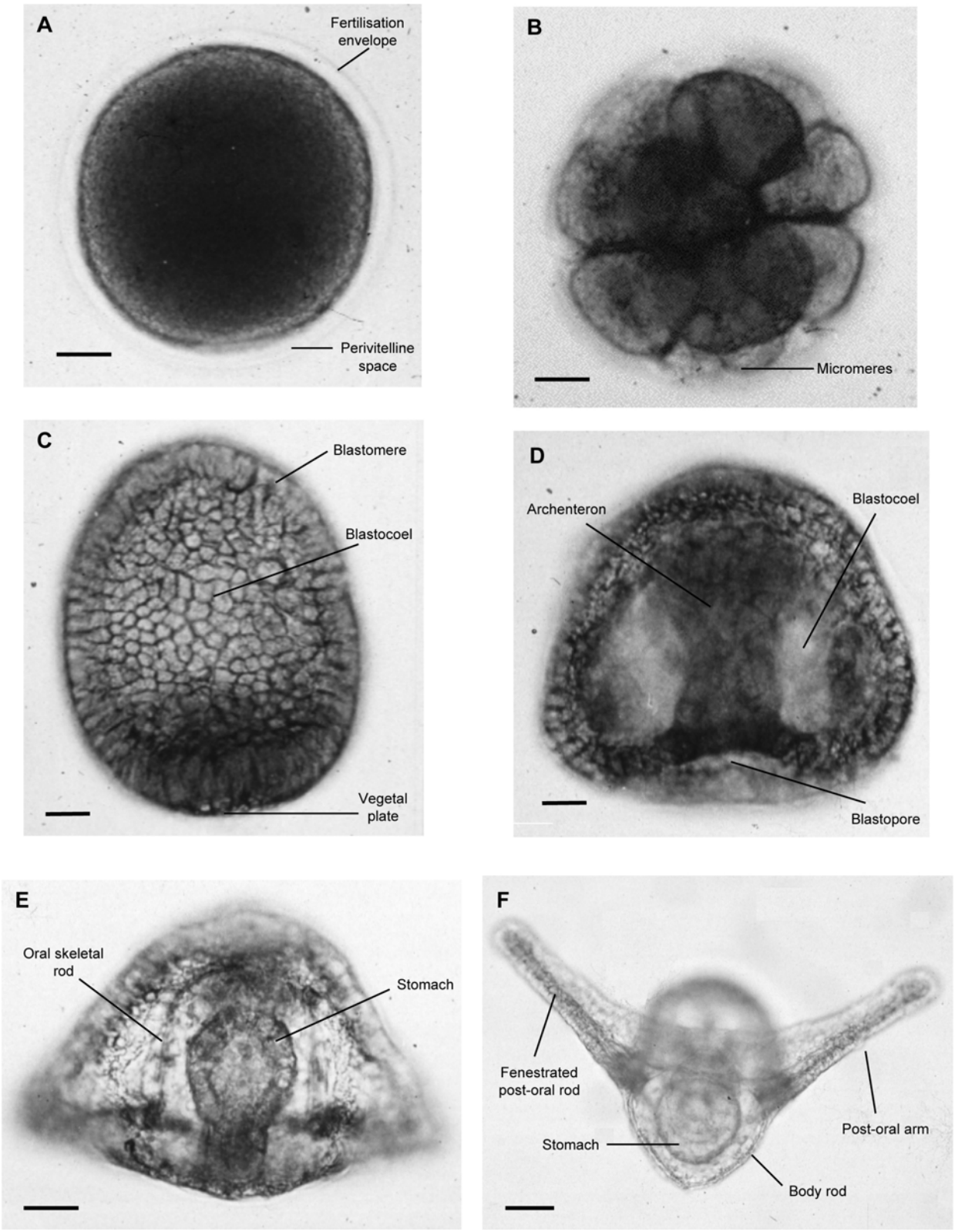

Fig. 1. Early development stages of *Centrostephanus rodgersii*. (A) fertilised egg; (B) 16-cell stage; (C) late stage blastula; (D) gastrula; (E) early prism larva; (F) 55 h old pluteus larva (ventral view). Scale bars: (A)–(D) = 20 μm; (E), (F) = 50 μm.

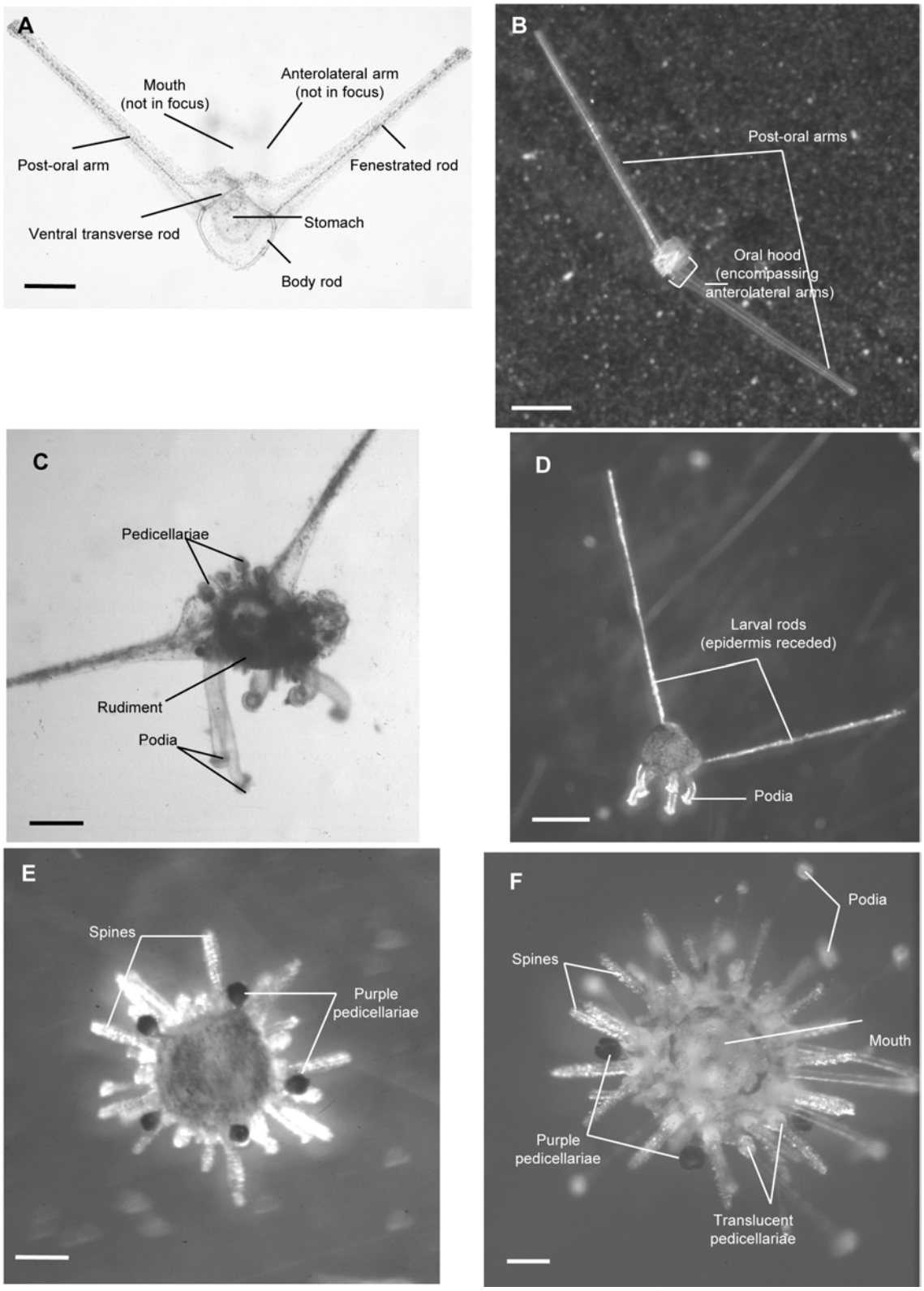

Fig. 2. Larval and juvenile *Centrostephanus rodgersii*. (A) 2-week-old larva; (B) 4-week-old larva; (C) Metamorphosing larva with rudiment emerging from the left side of the body; (D) Metamorphosing larva; (E) 5-day-old juvenile (aboral surface); (F) 2-week-old juvenile (oral surface). Scale bars: (A) = 100 μm; (B) = 1000 μm; (C) = 300 μm; (D) = 500 μm; (E) = 300 μm; (F) = 200 μm.

approximately 90° — and eventually the larval rods drop off. The larval epidermis recedes completely from the arm rods and collects on the aboral surface of the juvenile. The imaginal disk fully emerges, and the juvenile became radially symmetrical (Fig. 2D). The timing of spine formation in the rudiment varies. For some individuals, pedicillaria and definitive spines form as the rudiment emerges (Fig. 2C), while in others the spines do not form until after the larval epidermis has completely receded from the arm rods and collected on the aboral surface and radial symmetry has been fully achieved (Fig. 2D).

Juveniles

The mean diameter of the six juveniles that successfully metamorphosed was 550 μm (SE ± 54 μm). Juveniles have a flattened oral surface and dark purple pedicellaria arranged radially and regularly near the aboral surface (Fig. 2E). Five translucent pedicellaria also form (Fig. 2F), and more are added as the juvenile ages. Juveniles are covered primarily with dark red pigment, with some dark purple pigment scattered throughout (Fig. 2E and F). A number of definitive spines develop also, and remained transparent for up to 2 weeks following metamorphosis (Fig. 2E and F). In the following weeks, additional tube feet, juvenile and definitive spines developed.

Discussion

C. rodgersii differs from members of most other families of sea urchins in that it develops via a highly derived two-arm echinopluteus larva. The preoral and posterodorsal arms and skeleton do not form and thus this and other diadematid larvae, are described as "two armed" (Emlet, 1988; Young and George, 2000). It does not develop via an eight-armed larvae as previously suggested by Andrew and Byrne (2001). Larval development of *C. rodgersii* and of several *Diadema* larvae (Mortensen 1931, 1937; Eckert, 1998) appears to be very similar, with the postoral arms developing into very long arms, only short anterolateral arms, and no preoral or posterodorsal arms at all. The phylogenetic implications of this unusual two-armed larval form have previously been discussed (Mortensen, 1921, 1931, 1937; Emlet, 1988). Emlet (1988) concluded that it has only arisen once. Larvae are large compared to other planktotrophic echinoid larvae, with maximum arm lengths of over 4,000 μm. These features of the development of *C. rodgersii* are similar to those described for a related species, *Diadema antillarium* (Mortensen, 1921; Eckert, 1998), and may be common traits throughout the Diadematidae family (Emlet et al., 2002). However, not all larvae within the Order Diadematoida develop via a two-arm larva. For example, larvae from the family Aspidodiadematidae develop via a complex 10-arm planktotrophic echinopluteus larva (Young and George, 2000).

Development to metamorphosis of *C. rodgersii* was found to be slow relative to other planktotrophic echinoids, with larvae spending a minimum of 3 months in the water column before settlement. Larvae could also prolong their larval life to almost 5 months in laboratory cultures. This extended period of larval development may be a characteristic of this species, but may also be due in part to the seawater that was used for culturing which was both sterilised and filtered. Alternatively, slow development may be a common feature within the Order Diadematoida, with both *C. rodgersii* and *Aspidodiadema jacobyi* developing over a period of up to 5 months in the laboratory (this study; Young and George 2000).

Although data are only available for two species, temperature may play a role in the timing of larval development within the Diadematoida. The embryonic and early larval development of *C. rodgersii* occurs at a slightly slower rate than its tropical counterpart *D. antillarium* (Eckert, 1998). *C. rodgersii* develops into an early two-arm pluteus larva within 55 h, compared to 30 h for *D. antillarium*. The time to metamorphosis is also significantly shorter in the tropical diadematid, reaching metamorphosis within only 34 days (Eckert, 1998), compared to between 105 and 126 days for *C. rodgersii*. Temperature is widely recognised to affect strongly the developmental rates of sea urchins (Emlet, 1995) and may account for the faster development of *D. antillarium* as compared to *C. rodgersii.* Cultures of *D. antillarium* were reared at 23 ±1 °C (Eckert, 1998) while *C. rodgersii* cultures were reared at 19 ±1 °C. In addition, differences in the culture media used in these two studies are likely to account for some of the differences in developmental rates of these two species. Despite differences in the duration of larval development, larval body size and arm length are comparable between the two species (see Eckert, 1998, for *D. antillarium* and Fig. 2B for *C. rodgersii*).

Long larval development of *C. rodgersii* may have important consequences for the distribution and abundance of the species. Extended planktonic periods in the water column increases larval exposure to predators (Pechenik, 1999), offshore transport and the potential for wider distributions. Extended periods of time spent in the plankton were thought to facilitate the existence of an offshore population of *D. antillarium* (Eckert, 1998) and may also facilitate the expansion of the geographic range of *C. rodgersii* along the south

east coast of Australia. *C. rodgersii* was previously reported to occur from northern New South Wales to southern Victoria (Australian Museum, Sydney, collection records). This species now occurs as far south as Tasmania in Australia where it has formed extensive patches of barrens midway down the east coast, as well as in New Zealand (Craig Johnson, University of Tasmania, personal communication).

The timing of spawning of different populations of *C. rodgersii* varies latitudinally along the New South Wales coast (Byrne et al., 1998). Using the time to metamorphosis and settlement in our study, we can predict the larval settlement periods for *C. rodgersii* in various locations on the New South Wales coastline. In northern New South Wales (Solitary Islands region), settlement should occur roughly from September to October, in mid-New South Wales from October to January, and in southern New South Wales, from November to February. These predictions are only approximate as cultures were reared under controlled laboratory conditions at a constant temperature of 19 ±1 °C. In their natural environment, larvae would be exposed to a range of temperatures and more variable conditions. Andrew and Underwood (1989) sampled large areas of the New South Wales coast from 1985 to 1988 and observed two recruitment events: one in December 1985 and the other in January 1988, in Cape Banks and Bare Island, Botany Bay, respectively. These recruitment events in Sydney are consistent with our predictions for mid-New South Wales. However, the small number of recruitment events recorded by Andrew and Underwood (1989) despite a huge sampling effort, suggest that successful recruitment along the New South Wales coast is highly variable. Such high variability in recruitment is well documented in a number of species of echinoids (Cameron and Schroeter, 1980, Ebert and Russell, 1988; Rowley, 1989).

This study has revealed that larval development of *C. rodgersii* is similar other sea urchins within the family Diadematidae, with larval development of urchins within this family occurring via a two-armed planktotrophic echinopluteus larva. The information provided here contributes to the current understanding of larval development and form of *C. rodgersii* and also to the possible timing of recruitment events of this species along the south east coast of Australia.

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