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The reproductive cycle and development of *Crepipatella fecunda* (Gastropoda: Calyptraeidae) from southern Chile

O.R. Chaparro*‡, C.L. Saldivia*, S.V. Pereda*, C.J. Segura*, Y.A. Montiel* and R. Collin†

*Instituto de Biología Marina, Dr Jürgen Winter, Universidad Austral de Chile, Casilla 567, Valdivia, Chile. †Smithsonian Tropical Research Institute, Unit 0948, APO AA 34002, USA. ‡Corresponding author, e-mail: ochaparr@uach.cl

Crepipatella fecunda is a benthic, primarily suspension-feeding gastropod that occurs in great abundance along the Chilean coast. It is a protandrous species whose reproduction involves brooding of an encapsulated embryonic stage followed by the release of free-living planktotrophic larvae. Because its close sister species, *C. dilatata*, co-occurs with *C. fecunda*, understanding the details of reproduction in this species might shed light on differences in reproductive features that correlate with divergences in mode of development. In southern Chile, brooding occurs throughout the year except for May and June, and each female produces 3–7 broods. The smallest brooding female was 28.2 mm in shell length and the largest was 56.3 mm. All full-grown eggs from the ovary are deposited at one time in a single brood, and only smaller oocytes remain in the gonad after the female finishes ovopositing. Those females that host pinnotherid crabs do not deposit eggs. All the eggs develop into embryos whose intracapsular development is similar to *Crepidula fornicata* and *Crepipatella lingulata*. Planktotrophic larvae hatch at a mean shell length of 329.5 μm (SD=27.09) after 4–5 weeks. During the pelagic stage the shell and velum of the larvae grow, but little other morphological development is visible externally. The pelagic stage lasts for 15–16 days at 17°C, during which the larvae grow $\sim$20.7 μm d^{-1}. Observations of cultured larvae and protoconchs of field-collected juveniles show that settlement occurs when the larvae reach a shell-length of 650 μm (SD=28.3 μm).

INTRODUCTION

Brooding in calyptraeids involves the physical care of a cluster of thin-walled capsules which are positioned above the substrate and the propodium, and below the neck and neck lappets of the female (Hoagland, 1986). In those species with direct development, all embryonic development occurs within the capsule, before the crawling juveniles hatch. Embryonic development may be sustained by the ingestion of nurse eggs or nurse embryos, or by endogenous yolk supplies (Gallardo, 1977, 1979; Hoagland, 1986; Collin, 2000, 2003a; Chaparro et al., 2002a). Other species have mixed development, in which the early stages occur in the brooded egg capsules after which free-swimming larvae spend hours to weeks in the water column prior to settlement and metamorphosis (Gallardo, 1979; Hoagland, 1986; Collin, 2000, 2003a). Those larvae that spend more than a few hours in the water column feed on phytoplankton and grow significantly before metamorphosis.

A recent detailed phylogenetic analysis of calyptraeid development shows that divergence in mode of development can occur very rapidly and that the planktotrophic *Crepipatella fecunda* (Gallardo, 1979) is the close sister to the direct developing *Crepipatella dilatata* (Lamarck, 1822) (Collin, 2004). Therefore understanding the details of reproduction and development in *C. fecunda* and *C. dilatata*, not only adds to the comparative dataset already available for calyptraeid gastropods but might shed light on patterns of divergence in reproductive features that correlate with divergences in mode of development. Here we focus on describing the reproduction of *C. fecunda* as a step to understanding the differences between the two species.

Crepipatella fecunda (Gallardo, 1979) (previously *Crepidula fecunda*; see Collin, 2003b) is a species that occurs commonly in the intertidal and shallow subtidal from Lima, Peru in the north (Collin, 2003a) to Quitralco Fjord (45°S) in the south (Gallardo & Penchaszadeh, 2001). *Crepipatella fecunda* is extraordinarily abundant in some places and may impact the aquaculture of other sedentary filter-feeders with which they compete for space and probably for suspended food. Despite the fact that more information is available on the development of calyptraeids than for most other families of gastropods (Collin, 2003a), and despite the ecological importance of *C. fecunda* in Chile, little information is available on the reproduction and development of this species. The available published information is limited to a morphological description of the species (Gallardo, 1979), and, with respect to reproduction, the relationship between female size and capsule size, and eggs per capsule (Gallardo, 1977; Chaparro & Flores, 2002; Véliz et al., 2003). An understanding of the population biology, genetic structure and ecology of a species requires information on the development and reproductive biology, especially, seasonality of reproduction, duration of the larval phase and number of broods produced per year. Here we report data on these

characteristics of populations of *C. fecunda* from southern Chile.

MATERIALS AND METHODS

Specimens of *Crepipatella fecunda* were collected from a monospecific population in the intertidal of the Bahía de Yaldad, Isla de Chiloé, southern Chile (43°08′S 73°44′W).

Spawning and brooding

Two hundred adult females were collected from the field, removed from the small rocks they were attached to, and allowed to reattach to transparent glass plates. Each animal was measured and the plates with the animals on them were returned to the location from which they were originally collected. This allowed individual females to be followed without disturbing them and disrupting the broods. Every week for a year each individual was examined through the glass and the presence or absence of egg capsules, the stage of development, and the presence of pinnotherid crabs, which live in the area that would normally be occupied by the brood was noted (Chaparro et al., 2001a). The following criteria were used to determine the stage of development: (a) yellow coloured capsules, early stages of development; (b) orange capsules, mid-stage embryos; (c) dark grey capsules, embryos near hatching; and (d) empty capsules, recent hatching. Since we followed individual females we could use this information to determine the duration and frequency of brooding and the duration of the period between broods. Mortality was minimal and we do not expect sperm to be limiting in this experiment because females can store sperm for at least a year and males were free to crawl onto the plates.

To determine how the maturation and release of oocytes from the gonad is related to the production of broods and the inter-brood period we examined paraffin embedded histological sections of the ovary stained with haematoxylin–eosin. Samples were taken from females with broods at the four stages outlined above. Egg size for all the oocytes in each section was quantified on the basis of the maximum area in sections that included the nucleus.

Intracapsular development

Various stages of intracapsular development were observed with both inverted light microscopy and scanning electron microscopy (SEM). For SEM the embryos were fixed in buffered 2% gluteraldehyde and post-fixed in 1% osmium tetroxide. Following fixation they were dehydrated with a graded series of ethanol followed by acetone, critical point dried using CO_2 as the transitional fluid, mounted on stubs, and coated with gold prior to viewing with the microscope.

Size at hatching

Numerous stacks of adults were collected from the field and placed in aquaria with filtered water bubbled with air, and fed *ad libitum* with cultured *Isochrysis galbana*. Females that did not release larvae after two days were discarded and new individuals were collected. The recently liberated larvae were collected and fixed in 4% formaldehyde in seawater. The shell length of each of 296 larvae was measured to determine size at hatching. Because several hundred adults were present and larvae were collected over several days, these larvae probably came from several broods, although the exact number of broods represented in this sample is unknown.

Pelagic development

Larvae that hatched naturally from *C. fecunda* females maintained in large (300-l) aquaria were collected with a 100 μm sieve and transferred to four 100-l aquaria. These aquaria were maintained with 1 μm filtered seawater at 30 psu salinity and approximately 17°C (conditions similar to those in the Bahía de Yaldad in the summer).

Larvae were fed daily *ad libitum* with *Isochrysis galbana*. Every two days the water was changed and a sample of larvae was collected to measure the shell length. To induce settlement we introduced conspecific adults and glass plates that had been submerged in seawater for at least a week to allow the formation of a biofilm. The plates were removed daily to determine if they held recently settled juveniles. The shell length of each juvenile was measured to determine the size at settlement.

To determine if the size at settlement observed in cultured animals corresponded to size at settlement in the wild, we examined the protoconchs of wild-caught juveniles. Small juveniles were collected from the intertidal at Yaldad and the protoconch examined under a light microscope. The line demarcating the protoconch from the teleoconch represented the size at settlement and could be clearly seen in the shells of small juveniles.

RESULTS

Brooding females ranged from 28.2 to 56.3 mm in shell length (mean=42.8 mm SD=5.9). Brooding occurred throughout most of the year, with the highest frequency of reproductive activity between September and March, when 70% of the females were brooding (Figure 1). In May and June none of the experimental females had broods, but some individuals were found brooding during every other month (Figure 1).

Approximately 98% of the individuals that we followed produced at least three broods in the year. The remaining 2% were females that did not produce a single brood. These females all had pinnotherid crabs living in the area where the eggs would have been deposited. These animals were excluded from the subsequent estimates of the

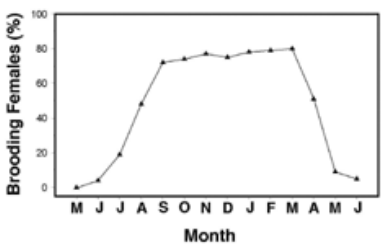

Figure 1. Proportion of females, without pinnotherids, brooding during each month from May 1998 until June 1999 (N=200).

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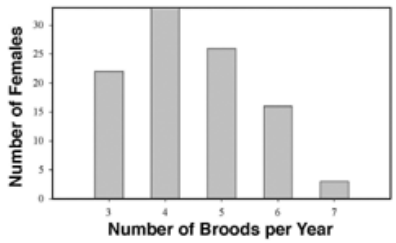

Figure 2. Histogram of the number of broods produced by each female in a year. All females that were not hosting pinnotherid crabs produced at least three broods in the year (N=100).

frequency of brooding because previous studies have shown that females hosting pinnotherids are inhibited from brooding (Chaparro et al., 2001a). Experimental females produced between three and seven broods in the year (Figure 2). Each brood took an average of 4.15 (SD=1.12) weeks to hatch and a mean of 1.95 (SD=1.07) weeks elapsed between hatching and the production of a new brood.

Examination of gonad sections showed that deposition of a brood corresponded to the complete evacuation of the large eggs from the gonad, although a second cohort of small oocytes was retained in the gonad.

Encapsulated development

All the eggs deposited in a capsule develop into embryos. Uncleaved eggs are 232.7 μm in diameter (SD=1.7; N=25). Initial cleavage is spiral (Figure 3A) and a spherical blastula is formed with the yolky macromere-derived cells at the vegetal pole and areas of more transparent cells at the periphery of the animal pole below the polar bodies (Figure 3B). Gastrulation is by epiboly and results in a slightly elongated gastrula. The foot, head and velum anlagen begin as bulges around the mouth as the embryo elongates (Figure 3C). At this stage there are no clearly organized ciliary bands although there are patches of cilia on the presumptive velum and presumptive foot.

A pair of velar lobes begin their growth as projections from the body wall just lateral to the mouth, and there is a slight thickening below the mouth that will become the foot (Figure 3D). As the velum extends out from the body wall the pre-oral, post-oral, and oral cilia can be seen in clear bands. On the dorsal side of the mouth the ciliated band appears to merge with the shorter more uniform cilia of the head vesicle. The spherical head vesicle, which is prominent during embryonic development, does not contain yolk as it does in some direct developing calyptraeids (Figure l; Collin, 2004). On each side, below the velum, there is a single round embryonic kidney (Figure 3E). Both the embryonic kidney and the head vesicle shrink late in development and are no longer visible at hatching.

The shell begins to grow over the flattening at the posterior dorsal end of the early embryo and is clearly visible as a cap over the viscera when the embryo reaches a length of 300 μm. The operculum also becomes visible at this stage, as does a row of cilia along the mid-line of the foot. The second half of embryonic development, after the

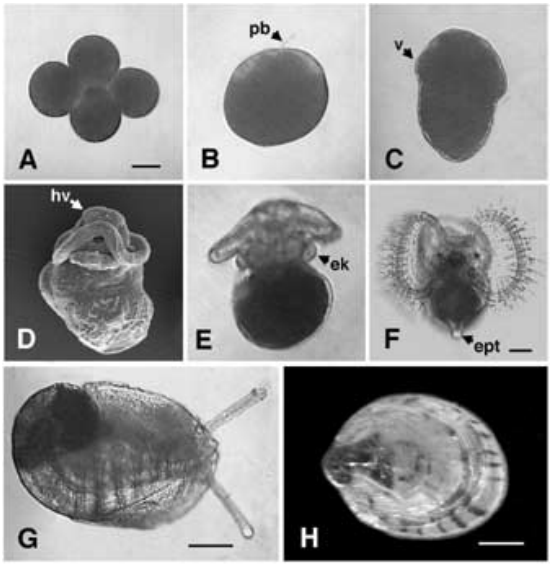

Figure 3. Developmental stages of *Crepipatella fecunda*. (A) 4-cell stage; (B) blastula showing the clear micromere derived cells at the animal pole below the polar bodies; (C) 'Trochophore' stage showing the beginning of the lateral extension of the velum; (D) SEM of an early veliger showing the head vesicle above the velum and foot below the mouth; (E) early veliger with the velum extended revealing the embryonic kidneys; (F) hatchling larva with the simple bilobed velum and the epipodial tentacle at the posterior margin of the foot; (G) recently settled juvenile viewed from below on the inverted microscope, showing the gills; (H) shell of a field-collected juvenile showing the protoconch–teleoconch boundary. pb, polar bodies; ek, embryonic kidney; ept, epipodial tentacle; hv, head vesicle; v, velum. Scale bars: A–E, 60 μm; F, 75 μm; G, 175 μm; H, 225 μm.

differentiation of the major structures, generally consists of the growth of the existing structures and the elongation and elaboration of the ciliary band.

Pelagic larvae

After hatching the larvae swim and feed in the water column for about two weeks until they metamorphose into benthic juveniles. The larvae begin their pelagic life with an average shell length of 329.5 μm (SD=27.09; N=296), and over the next 15–16 days they grow to a length of 650.2 μm (SD=28.3; N=40) before they settle.

At hatching (Figure 3F) the transparent shell is smooth and consists of a single right-handed coil. No yolk remains in the viscera and the heart, style sac, stomach, and intestine are all clearly visible through the shell. The intestine is black-pigmented and the other internal structures are all transparent prior to feeding. The bilobed velum has two red stripes along the entire periphery of the food groove. There are often several opaque spots on the velum just medial to the food groove. At hatching, both eyes are present as are two short tentacles. The medial posterior margin of the foot is slightly elongated into an epipodial tentacle that can extend beyond the margin of the operculum in a fully extended animal.

The larvae of *Crepepatella fecunda* do not change greatly in morphology during the pelagic phase, although the

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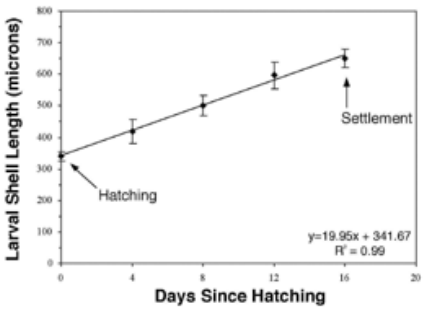

Figure 4. Growth rate of *Crepipatella fecunda* larvae in culture at 17°C. Error bars show the standard deviation of the shell length.

velum, shell, and foot grow considerably. By day 4, many (7–13) pigment spots are clearly visible along the medial edge of the food groove in almost all specimens. In 8-d-old larvae, the formation of the gill filaments can be observed through the shell. After 12 days the foot has grown considerably in size, the operculum is very obvious and five or six gill filaments can be seen.

Growth of the pelagic larvae is linear (Figure 4; ordinary least squares regression: Y=19.96X+341.67; r^2=0.99, N=153; $P<0.01$) with respect to shell length. Growth rate was 20.7 μm d^{-1} when the larvae are maintained under the experimental conditions described here.

The larvae settled after 15–16 days in culture at a shell length of 650 μm (SD=28; N=40) (Figure 3G). The recently settled juveniles had lost the velum and operculum. The size at settlement that we observed in juveniles from cultures appeared to be the normal size at settlement since the field-collected juveniles had an average settling size of 633 μm (SD=26.3; N=72) as measured from the protoconch–teleoconch boundary (Figure 3H).

DISCUSSION

Crepipatella fecunda is a protandrous hermaphrodite and, in the population in Yaldad, sex change occurs at about 26 mm. Individuals from this population have functional male gonads at shell lengths between 13 and 26 mm (Chaparro et al., 2001b) and the smallest females found brooding during the present study were 28.2 mm in length. This minimum length of brooding females is smaller than that reported for the same species from two different populations from slightly further north (31 and 34 mm; Gallardo, 1977, 1979). The same author also found that the maximum size of brooding females was 65 or 63 mm (Gallardo, 1977, 1979). This is a much larger maximum size than is found in the intertidal population of Yaldad (57.6 mm). Similar size distributions of males and females were found in a population from Coquimbo in northern Chile, where males were 19–34 mm and females were 33–56 mm (Véliz et al., 2003). Such variation in the size of sex change is not unusual for calyptraeid species, in which sex change may depend on the size distribution in the population and the size of available substrate (Franzen & Hendler, 1970).

Female *C. fecunda* from Yaldad are reproductive almost all year round, with about 70% of the females brooding at most times (except for May–June). In a more northern population of *C. fecunda* the non-reproductive period occurs earlier in the year (April), and the number of brooding animals is lower and more variable than at Yaldad (Gallardo, 1977). Gallardo also observed that *Crepidula philippiana* and *Crepipatella dilatata*, two other calyptraeid species from the same latitude in Chile, also reproduce throughout the year (Gallardo, 1996). In contrast, similar data from North America show that many species in both the Atlantic and Pacific reproduce seasonally (e.g. *Crepidula convexa* Hendler & Franz, 1971; Matusiak & Fell, 1982; *Crepipatella lingulata* but not *Crepidula adunca* Collin, 2000).

Those females of *Crepipatella fecunda* that hosted pinnotherid crabs were never observed with broods, and such crab-induced reproductive failure was observed in 2% of the population. Although they do not brood, females that host crabs are capable of generating mature gametes (Chaparro et al., 2001a). The presence of the crab appears to prevent the brood from being deposited (Chaparro et al., 2001a). Although pinnotherids cause complete reproductive failure in females their effects on male reproductive success remain unknown.

Females from the intertidal population in Yaldad have three to seven reproductive events per year. Multiple reproductive events have been identified in other species of Chilean calyptraeids such as *Trochita calyptraeformis* (previously *Calyptraea* (*Trochita*) *trochiformis*; see Collin 2003b) which broods six or seven times in a single year (Cañete & Ambler, 1992). However, the presence of several reproductive events in a year is unusual among other Chilean marine molluscs from the same latitude, whose reproduction is generally restricted to one or two broods per year. Although a strategy with multiple broods per year may be uncommon among Chilean marine molluscs it appears to be typical of calyptraeids in other regions of the world (e.g. Matusiak & Fell, 1982; Collin, 2000).

In *Crepipatella fecunda* the average time to hatching is four weeks and it takes two weeks for the females to produce a new brood after the previous brood hatches. This is considerably faster than the other Chilean calyptraeids for which information is available. Broods of *Trochita calyptraeformis* take between 49 and 51 days to hatch and a new brood is laid nine days after the previous one hatches (Cañete & Ambler, 1992). The longer duration of intracapsular development in *T. calyptraeformis* is attributed to direct development in this species. Other species of calyptraeids with planktotrophic development take approximately the same amount of time to hatch at similar temperatures as reported here (reviewed in Collin, 2003a). In general the duration of calyptraeid development depends on both temperature and egg size (Collin, 2003a). The time between broods is often shorter in other calyptraeid species where new broods may commonly be laid within a day or two of the previous brood hatching (e.g. *C. lingulata* Collin, 2000; R. Collin, personal observation).

The egg size reported here for *C. fecunda* is somewhat larger than the average for other planktotrophic calyptraeids (the mean for 26 species is 189 μm (Collin, 2003a)), and it is also larger than the mean 191.4 ±8.6 μm reported for northern populations of *C. fecunda* (Véliz et al., 2003). Despite this slightly larger egg size, the observed

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intracapsular and larval development of *C. fecunda* appears to differ little from that of other calyptraeids with planktotrophic development (e.g. *C. lingulata* Collin, 2000; *Crepidula fornicata* Werner, 1955). The most obvious differences are in the pigmentation of the larval velum, which may provide useful characters for identification of larvae.

The mean hatching size of 329 μm is close to the average for the family (343 μm for 26 planktotrophic species) and somewhat larger than the 294 μm reported by Véliz et al. (2003). However, both studies recorded a significant range in hatching size. The hatching size in the Yaldad population ranges between 265 and 415 μm shell-length. Such large variation allowed Chaparro et al. (2002b) to compare recently hatched larvae with embryos with the same shell length that had not yet hatched. They found differences between pre- and post-hatching veligers in the velar area, the length of the ciliated band, the length of the preoral cilia, the potential filtering area, and the width of the food groove. This suggests that the size at hatching is controlled by a factor other than the size of the shell. The high variation in hatching size recorded in this study may be due to differences in hatching size between broods or between mothers. There is also a possibility that hatching size may vary among populations as Gallardo (1979) reported a hatching size of 500 μm for another population of *Crepipatella fecunda* in the south of Chile. The factors responsible for variation among populations of *C. fecunda* in reproductive investment and hatching size, and the consequences of such variation, require further study.

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