Mar Biol (2012) 159:45–56
DOI 10.1007/s00227-011-1788-2

ORIGINAL PAPER

Comparing biochemical changes and energetic costs in gastropods with different developmental modes: *Crepipatella dilatata* and *C. fecunda*

O. R. Chaparro · L. A. Lincoqueo · A. J. Schmidt · D. Veliz · J. A. Pechenik

Received: 1 March 2011 / Accepted: 25 August 2011 / Published online: 9 September 2011

Abstract The Chilean gastropods *Crepipatella dilatata* and *C. fecunda* have different development modes: brooding and direct development in *C. dilatata* and brooding and planktotrophic development in *C. fecunda*. Unlike many other congeneric invertebrate species pairs, recent genetic evidence suggests that *C. fecunda* may have evolved from *C. dilatata*. To explore the changes involved in this unusual evolutionary path, this study examined the biochemical, energetic, and morphological characters during early development of both species. Mean egg size was slightly smaller for the direct-developing species *C. dilatata*, and initial energy content was lower—by about 27%—for eggs of that species. In both species, protein content in the eggs was the principal biochemical component. Although females of *C. fecunda* produce 180 times more eggs than *C. dilatata*, females of *C. dilatata* invest 20 times more energy in each of their offspring, through nurse eggs; their embryos have approximately eight times more energy at hatching and about 5 times more energy when they enter the benthos, despite a long planktonic feeding period in the larvae of *C. fecunda*. Evolutionary switching between modes of development in these species is reflected in shifts in maternal energy investment.

Communicated by J. P. Grassle.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-011-1788-2) contains supplementary material, which is available to authorized users.

O. R. Chaparro (✉) · L. A. Lincoqueo · A. J. Schmidt
Instituto de Ciencias Marinas y Limnológicas,
Universidad Austral de Chile, Casilla 567, Valdivia, Chile
e-mail: ochaparr@uach.cl

D. Veliz
Facultad de Ciencias, Departamento de Ciencias Ecológicas,
Instituto de Ecología y Biodiversidad (IEB), Universidad de Chile,
Casilla 653, 780-0024 Ñuñoa, Santiago, Chile

J. A. Pechenik
Biology Department, Tufts University, Medford, MA 02155, USA

Introduction

Benthic marine invertebrates show a wide range of reproductive patterns. In particular, many species disperse by means of microscopic, planktonic larvae that may spend weeks or months in the plankton before metamorphosing (Thorson 1950; Vance 1973; Scheltema 1986), whereas some other species bypass a free-living larval stage and develop to the juvenile stage within egg cases, egg masses, or even protected directly by the mother (Thorson 1950; Pechenik 1986; Collin 2003; Chaparro et al. 2008a, 2011). The ecological consequences and selective forces associated with shifts in reproductive pattern have been much discussed over many years (e.g., Thorson 1950; Vance 1973; Pechenik 1999; Allen and McAlister 2007). In most cases, it seems that planktonic development is the ancestral condition, with so-called direct development being derived (Strathmann 1978, 1985, 1993; Pechenik 1999).

However, a few species with planktonic larval stages have apparently emerged from ancestors with direct, non-planktonic development (McEdward 1995; Collin et al. 2007). One of the best examples of this reverse evolutionary trajectory is two species in the genus *Crepipatella*: in a recent analysis based on cytochrome oxidase I gene sequences, *C. fecunda* nested within a clade of species with direct development, implying that it had re-evolved free-living larvae from an ancestor with direct development and it nested particularly within *C. dilatata* (Collin et al. 2007). Females of *Crepipatella dilatata* release relatively small

numbers of fully formed juveniles (Chaparro et al. 1999, 2005a; Collin 2003), while those of the sympatric (Véliz et al. 2003; Schmidt et al. 2006) species *C. fecunda* release many thousands of planktonic larvae that feed on phytoplankton for at least 2 weeks before metamorphosing (Gallardo 1977; Chaparro and Flores 2002). In both species, females package the embryos within thin egg capsules (Gallardo 1979) and brood those capsules in the mantle cavity for at least several weeks until offspring are released. The goal of this paper is to shed light on the energetic and biochemical shifts that accompany—or make possible—such life history transitions. Such a transition must involve the conversion of nurse egg production to the production of developing embryos and could also involve shifts in egg size, egg numbers, egg and embryo biochemistry, and egg energy content, all of which are considered here along with a synthesis of all available relevant information for the two species from the literature.

The two *Crepipatella* species considered here are ideal for such an investigation. The females of both species are sedentary suspension feeders (Navarro and Chaparro 2002; Chaparro et al. 2008b), so that no energy is expended in searching for suitable sites to deposit egg capsules or for seeking food. The brooding period is longer in *C. dilatata*, but energy during this extended period of development is provided to the developing embryos by a large number of specialized nurse eggs that are enclosed within the same capsules and ingested during development (Gallardo and Garrido 1987; Chaparro and Paschke 1990; Chaparro et al. 1999). Both species are found both subtidally and intertidally and co-occur at some locations, although populations of *C. fecunda* are more common intertidally than are populations of *C. dilatata*. Finally, adults of the two species are morphologically similar and, indeed, are difficult to tell apart by eye (Gallardo 1979; Véliz et al. 2003; Schmidt et al. 2006).

Materials and methods

Adult specimens were collected during the 2006–2007 reproductive seasons (November to January). Individuals of *Crepipatella dilatata* were obtained subtidally (shell length [SL] range: 20–35 mm) from the estuary of the River Quempillén, Ancud, Chiloé (41°52′S; 73°46′W), while individuals of *Crepipatella fecunda* were collected intertidally (SL range: 29–60 mm) from Pelluco beach, Puerto Montt (41°28′S; 72°56′W), south of Chile. In the laboratory, limpets were removed from the substrate to collect the egg capsules ("spawning egg mass") from which nurse eggs, embryos, and larvae were obtained for analysis.

Collection of eggs, embryos, and larvae

Crepipatella dilatata

Collected capsules of *C. dilatata* were put into a Petri dish with filtered seawater (0.45 μm), and the capsules were sorted while viewing them through a stereomicroscope. Pre-shelled stages were sorted into the following categories for separate analysis: nurse eggs (NE), embryos (E), morulae + blastulae (M), and trochophores (T). Capsules containing shelled veliger larvae were sorted by SL (shell length): 400–499 μm, 500–599 μm, 600–699 μm, etc. up to 1,300 μm SL. The nurse eggs were obtained only from capsules whose embryos had developed sufficiently to make distinctions between nurse eggs and embryos. The "embryo" category (E) included only embryos that had already cleaved at least several times, which allowed us to tell them apart from nurse eggs contained in the same capsules. Morula and blastula stages were classified in a single category (M) due to the difficulty of collecting enough material of both development stages. Samples were deposited in Eppendorf tubes and lyophilized.

Crepipatella fecunda

Encapsulated eggs (E), trochophores (T), and early veligers (V) (Ojeda and Chaparro 2004) of *C. fecunda* were analyzed separately. In addition, advanced pre-hatched veligers were divided into 5 categories based on the differences in SL (220–280, 281–340, 341–400, and 401–460 μm) for separate analysis. The samples of each developmental stage were pipetted into 1.5-mL Eppendorf tubes and lyophilized.

Biochemical changes during development were also determined for planktonic larvae of this species. Adults of *C. fecunda* were maintained in a single aquarium. All larvae that hatched naturally during a period of 12 h were carefully filtered from the water using Nitex mesh (100 μm pore opening) and transferred to 2-L aquariums of seawater (salinity 30, 15°C ± 1) that had been filtered to 0.45 μm. The initial concentration of larvae was approximate 1,000 larvae L^{-1}. Larvae were fed ad libitum daily with the microalgae *Isochrysis galbana,* and the seawater was replaced every 2 days. Dead or moribund larvae were removed at each water change, and the aquariums were washed before returning the remaining larvae to the culture aquarium. Water was continuously oxygenated by means of moderate bubbling. Cultured larvae were sampled at 4 different ages: 1 ("recently hatched"), 5, 10, and 15 days after hatching. Larvae of this species typically begin settling and metamorphosing when they are approximately 15 days old (Chaparro et al. 2002a, 2005b).

Determining length and weight of eggs, embryos, and larvae

For *C. dilatata*, egg diameters were measured on eggs that had undergone at least a few cell divisions; measurements were not made if egg capsules contained uncleaved embryos; since for newly deposited egg masses, it was not possible to distinguish eggs that will form embryos from those that will serve as nurse eggs, as described earlier. For *C. dilatata* and for *C. fecunda,* we measured the diameter of approximately 100 eggs from 5 young egg masses.

Veliger shell lengths for both species (encapsulated and/or planktonic stages) were measured for 30–80 individuals by depositing them in a small chamber with filtered seawater and then filming them using an inverted microscope equipped with a video camera (Pulnix). The tapes were processed using standard image-capturing software. SL was determined by measuring along the anteroposterior axis as described by Cubillos et al. (2007).

Total, organic, and inorganic dry weights (DW) of each development stage were determined from batches of 100–300 individuals; since the number of individuals per sample was known, we calculated those characteristics per individual. Samples were put on 24-mm-diameter washed, combusted, and pre-weighed filters of borosilicate (Advantec MFS, Inc.) and quickly rinsed with distilled water to eliminate salts. Filters with samples were dried at 60°C for 48 h, cooled in a desiccator, and then weighed. Filters with samples were then incinerated at 475°C for 4 h to combust organics, cooled in a desiccator, and re-weighed to determine the inorganic content by subtraction. Average total, organic, and inorganic DW average per individual were thus determined.

Biochemical analysis and energy conversions

Lyophilized samples were used to quantify the lipid, carbohydrate, and total protein content for individuals at each development stage. For the quantification of total lipids, we used the colorimetric method of Marsh and Weinstein (1966). Lipids from 1–2 mg of samples were extracted in chloroform/methanol, dried at 200°C in concentrated sulfuric acid, and read at 375 nm with a spectrophotometer, with cholesterol as the standard. Total carbohydrates were quantified using the phenol–sulfuric acid method described by Dubois et al. (1956). A sample of 1–2 mg was used for each analysis, and all readings were recorded in triplicate. For the extraction of carbohydrates, samples were treated using the method of Barnes and Heath (1966). The samples were boiled in a solution of 5% trichloroacetic acid containing 0.1% silver sulfate. Glucose was used as the standard. Total protein content was determined using the bicinchoninic acid (BCA) reagent (Pierce Laboratories). Samples of 1–2 mg were used for each determination, and bovine serum albumin (BSA) served as the standard.

The values of the biochemical analyses were converted to energy equivalents using the conversion factors of 24, 39.5, and 17.5 J mg^{-1} of sample for proteins, lipids, and total carbohydrates, respectively (Gnaiger 1983).

Statistical analysis

Differences among the measured variables throughout development to the juvenile stage were determined using one-way ANOVA, followed by Tukey pairwise a posteriori analysis when overall statistical significance was found. Development was split into three categories for analysis: (1) embryonic (pre-shell stage), (2) encapsulated veliger stage for both species, and (3) the pelagic larval stage for *C. fecunda* and the post-metamorphic pre-hatching stage for *C. dilatata*. Because the data for lipid, carbohydrate, and total protein content were not normally distributed, these data were arcsin transformed before analysis (Underwood 1997).

Results

Length and weight of eggs, embryos, and larvae

The average diameter of eggs for *Crepipatella dilatata* was 240 µm (SD = 18.2, $N = 100$), and the average DW was 3.0 µg (SD = 0.5, $N = 10$). Approximately 92% of the early embryonic DW was organic matter. During intracapsular development, embryonic DW increased to an average of 95.7 µg (SD = 0.1, $N = 6$) just prior to hatching, more than 10 times the weight of *C. fecunda* veligers at metamorphosis (see below). The increase in total DW for *C. dilatata* was especially substantial for encapsulated individuals growing from SL of ~650 to 1,000 µm (Fig. 1a). At that length, almost all nurse eggs had been consumed by encapsulated embryos. Encapsulated veligers showed their maximal value for organic DW (approximately 60.3 µg $veliger^{-1}$), at SL of about 1,000 µm, with organic content accounting for about 68% of total weight. However, as the encapsulated veligers continued to grow beyond about 1,000 µm in shell length, individual organic weight decreased. The DW of organic matter in the post-metamorphic but still encapsulated juvenile, which was very close to hatching, decreased from an average of 60.03 µg $individual^{-1}$ to 47.9 µg $individual^{-1}$ (Fig. 1a). Juveniles hatched at SL between about 1200 and 1300 µm.

For the nurse eggs of this species, average diameter was 242 µm (SD = 21.7, $N = 436$) and average DW was 2.58 µg (SD = 0.54, $N = 5$). The average diameter of the eggs of *Crepipatella fecunda* was 259.4 µm (SD = 19.2, $N = 100$),

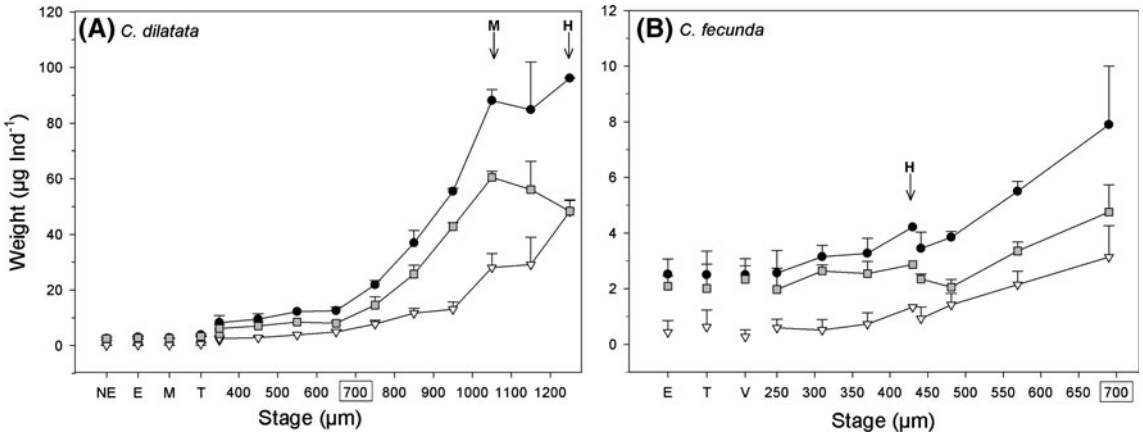

Fig. 1 Total (*circle*), organic (*square*), and inorganic (*triangle*) DW of embryos and larvae of **a** *C. dilatata* and **b** *C. fecunda*. *Vertical bars* show SD. *E* eggs, *NE* nurse eggs, *M* morula-blastulae, *T* trochophore, *V* very early veliger. *H arrow* ~ hatching SL. *M arrow* ~ metamorphosis. Note that the *X*- and *Y*-axis scales differ for the two species

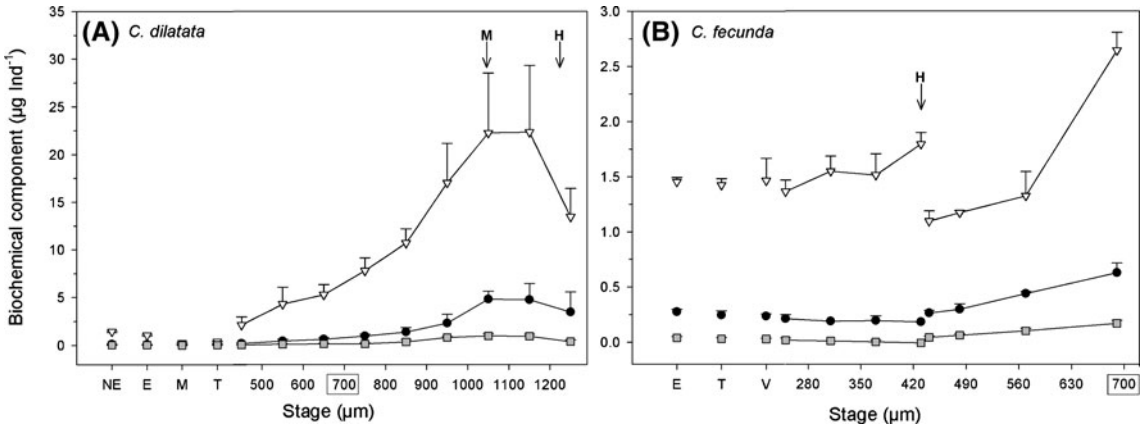

Fig. 2 Changes in lipid (*triangle*), protein (*circle*), and total carbohydrates (*square*) during the development of **a** *C. dilatata* and **b** *C. fecunda*. *Vertical bars* show SD. *H arrow* ~ hatching SL. *M arrow* ~ metamorphosis. *E* eggs, *NE* nurse eggs, *M* morula-blastulae, *T* trochophore, *V* very early veliger. Note that the *X*- and *Y*-axis scales differ for the two species

only slightly larger than those of *C. dilatata*, and the average DW was 2.5 µg egg^{-1} (SD = 0.5, $N = 6$), about 17% less than that for eggs of *C. fecunda*. Approximately 83% of total DW corresponded to organic matter. As encapsulated development progressed within the egg capsules, the most developed veligers, with average shell lengths of about 430 µm, increased their total DW by about 60% to an average of 4.2 µg veliger^{-1} just before hatching. Of this weight, 68.2% corresponded to organic matter, a decline of 18% from the initial condition. In the step from encapsulated veliger to pelagic larva, organic content decreased further and the average total DW decreased from 4.2 µg veliger^{-1} to 3.3 µg veliger^{-1} (SD = 0.5, $N = 4$). However, during the subsequent period of pelagic development, larvae increased both in organic and inorganic weight. At the end of 15 days in the plankton, the total DW of larvae averaged 7.9 µg (SD = 2.1, $N = 4$), of which approximately 60% was organic weight (Fig. 1b). Inorganic content increased by five times between early encapsulated veliger and settling pelagic veliger. The encapsulated veligers hatched with an average SL of 441 µm (SD = 36, $N = 30$) and, by the time they became competent to metamorphose 15 days later, they had reached an average SL of 690 µm (SD = 97, $N = 30$). Inorganic content also increased substantially throughout the development for both species (Fig. 1), probably reflecting increased shell calcification (Martínez et al. 2008).

Biochemical composition

In *C. dilatata,* the average concentration of proteins, lipids, and total carbohydrates in the eggs was 1.06 µg (SD = 0.08, $N = 3$), 0.09 µg (SD = 0.02, $N = 6$), and 0.044 µg (SD = 0.026, $N = 5$), respectively (Fig. 2a). During the period of pre-shelled embryonic development, protein ($F_{(3, 8)} = 190.49$, $P = 0.0001$) and total lipid ($F_{(3, 19)} = 3.96$, $P = 0.0238$) content decreased significantly, whereas no significant differences in carbohydrate content were seen during this period ($P = 0.5555$).

Comparing veligers with shell lengths of 450 µm with those at the approximate length at metamorphosis (950 µm), we documented significant differences in the content of proteins ($F_{(5, 24)} = 32.841$, $P = 0.0001$), lipids ($F_{(5, 42)} = 39.740$, $P = 0.0001$), and total carbohydrates ($F_{(5, 26)} = 35.538$, $P = 0.0001$). The maximal values of the biochemical components in the encapsulated embryos were evidenced when the veligers reached approximately 1,000–1,100 µm in shell length. At that length, the average biochemical content of these advanced, pre-metamorphic embryos was 22.27 µg (SD = 6.27, $N = 5$), 4.82 µg (SD = 0.84, $N = 7$), and 0.99 µg (SD = 0.23, $N = 3$) µg for proteins, lipids and carbohydrates, respectively.

Between the time that larvae reached a SL of 1,000 µm and the time of hatching from the egg capsule as juveniles, we documented significant declines in both protein ($F_{(2, 14)} = 5.54$, $P = 0.017$) and carbohydrate content ($F_{(2, 8)} = 12.53$, $P = 0.003$). In both, the smallest values were identified in specimens in the process of hatching. No significant changes in lipid content were identified during this post-metamorphic, pre-hatching incubation period ($P = 0.179$) (Fig. 2a).

Proteins were the major biochemical components during both encapsulated and pelagic development of *C. fecunda* (Fig. 2b). For *C. fecunda*, the average content of proteins, lipids, and total carbohydrates for uncleaved eggs was 1.45 µg (SD = 0.04, $N = 6$), 0.27 µg (SD = 0.02, $N = 19$), and 0.039 µg (SD = 0.0099, $N = 19$), respectively, so that protein was again the dominant biochemical component. Protein content was significantly higher in pre-hatching veligers than in eggs ($P = 0.0369$). Lipids and carbohydrates also showed significant increases during encapsulation (lipids $F_{(4, 57)} = 8.36$, $P < 0.0001$, carbohydrates $F_{(4, 57)} = 5.91$, $P = 0.0005$). In recently hatched larvae of *C. fecunda*, average proteins, lipids, and total carbohydrates were 1.104 (SD = 0.09), 0.27 (SD = 0.02), and 0.058 µg larva^{-1} (SD = 0.01), respectively, rising 15 days later to 2.64 µg (SD = 0.16), 0.64 µg (SD = 0.09), and 0.18 µg (SD = 0.0028), respectively. Biochemical composition changed significantly during pelagic development, with increases in protein ($F_{(3, 8)} = 75.74$, $P = 0.0001$), lipids ($F_{(3, 8)} = 29.62$, $P = 0.0001$), and carbohydrates ($F_{(2, 9)} = 9.69$, $P = 0.0057$) from the time of hatching to the pre-settlement stage.

Embryonic and larval energy dynamics

The energy contained in each egg of *C. dilatata* declined from 29.9 to 16.3 mJ toward the trochophore stage. Subsequently, however, for developing embryos that were approximately 350 µm in shell length, energy levels increased almost continuously as nurse eggs were consumed, reaching a maximum of 742.3 mJ embryo^{-1} for veligers with shell lengths of approximately 1,000–1,050 µm. The encapsulated nurse eggs had an average energy of 40.5 mJ (Table 1). During this phase of intracapsular development, energy reserves were mostly in the form of proteins, with lipids and carbohydrates present in smaller amounts. From veligers of 1050 µm SL until the end of encapsulated development (hatched juvenile), there was a 40% decrease in the energy content of each individual, eventually reaching average values of 467 mJ (Figs. 3b, 4b; Table 2).

In *C. fecunda,* the energy contained in the eggs was initially 46.4 mJ egg^{-1} (Table 1), mainly due to the high protein content. This is not only greater than that of the *C. dilatata* eggs, but even somewhat greater than that of the *C. dilatata* nurse eggs (see above). At the end of encapsulated development, pre-hatching veligers had a slightly higher equivalent total energy of 51.1 mJ veliger^{-1} (Table 2). In the transition from encapsulated veliger to pelagic larva, energy content decreased, associated with a corresponding decrease in protein content (Fig. 2b). However, energy content increased throughout the planktonic period, reaching a maximum of 89.0 mJ larva^{-1} in 15-day-old veligers. This energy increase was mostly caused by an accumulation of proteins and lipids (Figs. 3a, 4a).

Discussion

Females of *Crepipatella fecunda*, the species with free-living larvae, produce about 180 times as many embryos as *Crepipatella dilatata* does, by producing more egg capsules as well as greater numbers of embryos per capsule (Table 1). When embryos of both species are about 340–350 µm in SL, their energy content per embryo was similar. However, the total maternal energy investment for each embryo deposited in the capsules of the direct-developing *C. dilatata* was 914 mJ, of which 884 mJ per embryo is accounted for by consumption of nurse eggs. In *C. fecunda,* the comparable maternal investment was only 50 mJ per embryo. Thus, *C. fecunda* actually invests only about 10 times more energy in each egg mass than *C. dilatata* does.

For both *C. dilatata* and *C. fecunda*, early development was fueled mainly by the metabolism of proteins and lipids, as reported previously for several other marine invertebrates (e.g., Lucas et al. 1979; Petersen and Anger 1997; Moran and Manahan 2003; Martínez et al. 2008; García-Guerrero 2009). The energetic importance of carbohydrates in embryonic and larval development is, in general, very small (Pandian 1969; Mizrahi and Achituv 1991; Petersen and Anger 1997; Videla et al. 1998; Labarta et al. 1999; Moran and Manahan 2003, García-Guerrero 2009), as was also found here for *C. dilatata* and *C. fecunda*.

Table 1 Comparison of reproductive characteristics and total energy investment for *C. dilatata* and *C. fecunda*

Variables	*Crepipatella dilatata*			*Crepipatella fecunda*		
	Mean	Range	References	Mean	Range	References
Fecundity characteristics						
Capsules female^{-1}	17	15–19	Chaparro et al. (1999)	46	23–69	Chaparro and Flores (2002)
Embryos capsule^{-1}	15	0–50	Chaparro et al. (1999)	1,000	400–1,600	Gallardo (1979)
Nurse eggs embryo^{-1}	20	8–38	Chaparro et al. (1999)		–	Gallardo (1977)
Offspring female^{-1}	255		Estimated from Chaparro et al. (1999)	4.6×10^4		Estimated from Gallardo (1979), Chaparro and Flores (2002)
Maternal energy investment						
Nurse eggs (mJ capsule^{-1})	1.2×10^4		PR	–		Gallardo (1977)
Embryos (mJ capsule^{-1})	449		PR	4.64×10^4		PR
Capsule wall energy content (mJ capsule^{-1})[a]	1,096		Chaparro et al. (1999)	4,031		Chaparro and Flores (2002)
Total energy (mJ capsule^{-1})	1.37×10^4		Estimated from PR, Chaparro et al. (1999)	5.04×10^4		Estimated from PR, Chaparro and Flores (2002)
Total energy invested (mJ capsule mass^{-1})	2.33×10^5		Estimated from PR, Chaparro et al. (1999)	2.32×10^6		Estimated from PR, Chaparro and Flores (2002)
Invested energy per embryo (mJ embryo^{-1})	914		Estimated from PR, Chaparro and Flores (2002)	50		Estimated from: PR, Chaparro and Flores (2002)

Values are per reproductive event for an average-sized female (*C. dilatata*: SL 27 mm, Chaparro et al. 1999; *C. fecunda*: 42 mm, Chaparro et al. 2005b)

PR present research

[a] Value estimated assuming it represents 8% of the total energy of each egg capsule

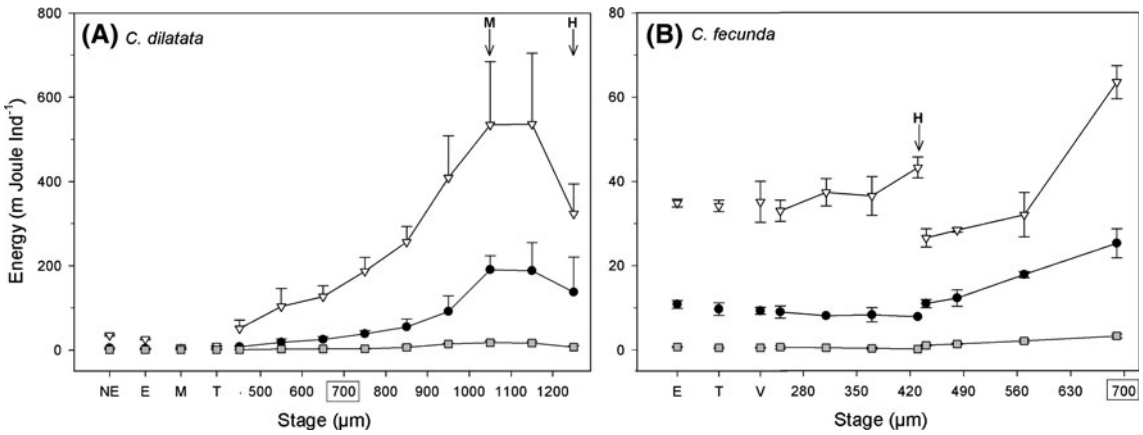

Fig. 3 Changes in the amounts of energy coming from lipid (*circle*), protein (*triangle*), and total carbohydrates (*square*) during the development of **a** *C. dilatata* and **b** *C. fecunda*. *Vertical bars* show SD. *H arrow* ~ hatching SL. *M arrow* ~ metamorphosis. *E* eggs, *NE* nurse eggs, *M* morula-blastulae, *T* trochophore, *V* very early veliger. Note that the *X*- and *Y*-axis scales differ for the two species

In other respects, development is quite different for the two species. During the intracapsular development of *C. dilatata*, from the initial veliger to juvenile phase, the veligers greatly increased their weight by consuming nurse eggs; inorganic content of the embryonic shell also increased, but to a smaller degree. Chaparro and Paschke (1990) also reported only a small increase in inorganic weight for the embryos of this species, suggesting only weak calcification of the protoconch during this phase of encapsulated life. In contrast, the organic content of *C. fecunda* embryos change little during encapsulation, with most of the increases in total weight accounted for through increased calcification of the shell. Energy content also changed little during the encapsulated stages for *C. fecunda*; the veligers hatch (at shell lengths of about 340–350 µm, Supplementary material 1) with an average energy content of only 51 mJ veliger^{-1}, about 10% higher than the initial energy content of the eggs, implying the use

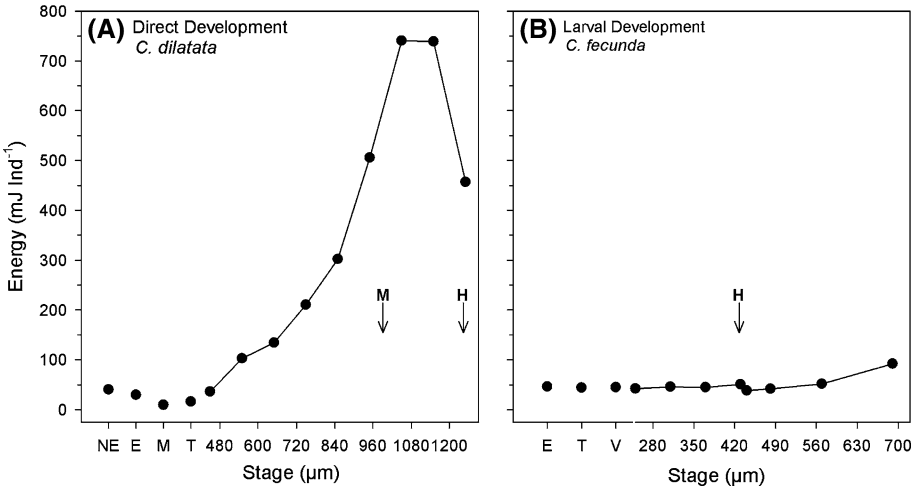

Fig. 4 Changes in energy content during the development of **a** *C. dilatata* and **b** *C. fecunda*, *NE* nurse eggs, *E* eggs, *M* morula-blastula stages, *T* trochophore, *V* very early veliger. *H arrow* ~ hatching SL. *M arrow* ~ metamorphosis

Table 2 A comparison of developmental characteristics and per individual energy investment for the direct developer *C. dilatata* and the plankton developer *C. fecunda*

Variables	*Crepipatella dilatata*		*Crepipatella fecunda*	
		Reference		Reference
Energy investment				
Egg energy (embryonic) (mJ egg^{-1})	29.94	PR	44.9	PR
Energy at hatching (mJ juvenile^{-1})	467.1 (59.43)[b]	PR	51.1[a]	PR
Extraembryonic energy gain (mJ offspring^{-1})	682.9	PR	37.9	PR
Cost for metamorphosis (mJ juvenile^{-1})	275.2	PR	NI	
Energy at settlement (mJ juvenile^{-1})	467.1[c]	PR	89.0[d]	PR
Morphology and functionality of velum				
Cilia length[a] (µm)	15[e]	Chaparro et al. (2002b)	75[f]	Chaparro et al. (2002b)
Velum area[a] (mm^2)	0.03[e]	Chaparro et al. (2002b)	0.045	Chaparro et al. (2002b)
Size at first exogenous feeding	>300 µm[g] 430 (µm)[h]	Chaparro and Paschke (1990) PR	337 (µm)[h]	Chaparro et al. (2002b)
Embryonic and larval growth				
Instantaneous shell growth rate (µm d^{-1})	49.7[e]	Chaparro and Paschke (1990)	19.95[f]	Chaparro et al. (2002b)

NI No information available, *PR* present research

[a] Veliger 375 µm shell length

[b] Energy if offspring should hatch at a size similar to that of *C. fecunda* (441 µm)

[c] Juvenile 1000 µm shell length

[d] Settlement size 690 µm shell length

[e] Encapsulated embryos

[f] Pelagic veliger

[g] Ingesting pieces of nurse eggs

[h] Planktonic feeders

of only a modest source of extraembryonic nutrition within the egg capsules. This suggests that *C. fecunda* possesses only limited extraembryonic food resources while encapsulated, and, as in many other species, its embryos must depend mainly on endogenous reserves to meet their energy requirements (Strathmann 1985; McEdward et al. 1988). However, possible energy supplements for the encapsulated embryos are intracapsular fluid (De Mahieu et al. 1974; Pechenik et al. 1984; Moran 1999), progressive disintegration of the internal capsular wall (Hendler and Franz 1971; De Mahieu et al. 1974; Ojeda and Chaparro 2004), or the consumption of dead siblings or embryos with stunted development (Miloslavich and Penchaszadeh 2001; Collin 2003; Cubillos et al. 2007). Encapsulated veligers of *C. fecunda* can capture and ingest extraembryonic particles when they have been manipulated experimentally

(Chaparro et al. 2002a, b; Cubillos et al. 2007). Ojeda and Chaparro (2004) demonstrated that the spongy internal layer of the capsule decreased in thickness during embryonic development, which would contribute mostly proteins (approx 15 μg capsule^{-1}) to intracapsular fluid. Although it has not been demonstrated directly that the dissolved proteins are ingested by the embryos of *C. fecunda*, as has been described for some other gastropod species, the continuous decrease of proteins in the intracapsular fluid during veliger development suggests that these are adsorbed by the encapsulated embryos or captured by the velum and ingested (Shilling et al. 1996; Vavra and Manahan 1999; Martínez et al. 2008). The progressive disintegration of the internal wall could represent an energy source during the encapsulated development of some gastropod species (Hendler and Franz 1971, De Mahieu et al. 1974, Ojeda and Chaparro 2004).

One advantage of a planktotrophic larval stage is that most of the development is independent of maternal energy, and embryos are not forced to compete for food with their capsule mates to fuel further development. During the transition from brooded veliger to pelagic larva in *C. fecunda,* we documented a decrease in per embryo organic content, and particularly in protein content, suggesting that proteins in particular were the major energy source during that phase of development, and that the first few hours of pelagic development are energetically expensive. Previous research has demonstrated that average clearance rates of newly hatched larvae are much larger than those for encapsulated veligers of the same length, probably because hatched larvae have a filtering surface area (area of the velum periphery covered by active velar cilia) that is approximately 80% bigger than that of pre-hatched larvae (Chaparro et al. 2002a). In this way, the energy demand during the first hours of pelagic development would basically be covered by endogenous reserves, while veligers increase their capacity for suspension feeding. The increase in inorganic weight during the pelagic phase is due to the development of the shell, but the increase in organic weight is explained by a secondary increase in protein and lipid content, as a product of the exogenous energy consumption obtained from the available microalgae in the aquarium in which larvae had been cultured.

In *C. dilatata*, the increase in total DW of the veliger stage is powered mainly by the consumption of nurse eggs, which are ingested most rapidly during the early and middle periods of development (Chaparro and Paschke 1990). Our results also show that the energy content of the nurse eggs of this species exceeds that of the eggs that develop into embryos by 30%. The energy content of *C. fecunda* eggs is closer to that of these nurse eggs than to that of the developing embryos.

In many direct-developing species (e.g., *Crepidula adunca,* Collin 2000), the velar lobes are substantially reduced in size. In the development of *C. dilatata*, however, the ciliated velum is used by developing embryos to manipulate the nurse eggs prior to their ingestion (Chaparro et al. 2002b), so that the velar lobes are well developed in both species (Chaparro et al. 2002b). Indeed, the velum plays a key role in the development of *C. dilatata:* the shorter velar cilia of *C. dilatata* (15 μm long versus 75 μm long in *C. fecunda* of similar length) seem to facilitate the manipulation of the large nurse eggs on the velar lobes and their subsequent ingestion (Chaparro et al. 2002b). Early veligers of *C. dilatata* can also capture and ingest small particles, even before reaching the development stage in which they consume the large nurse eggs, again attesting to the considerable functional capacity of the velum. Embryos have been observed capturing small particles coming from the rupture of nurse eggs, or from dead or damaged embryos, material that could constitute an important resource during early nutrition (Chaparro et al. 2002b). The substantial increase in protein content seen in early and medium stage veligers of *C. dilatata* in this study coincides with the period of active ingestion of nurse eggs. A similar situation has been also described by Martínez et al. (2008) for the gastropod *Chorus giganteus*.

The literature reports considerable variation in hatching size for *C. dilatata* (Gallardo 1977, 1979; Chaparro and Paschke 1990), probably due to the differential consumption of nurse eggs by encapsulated embryos (Spight 1976a; Rivest, 1983). Chaparro et al. (1999) also indicate that the juveniles of *C. dilatata* with the largest sizes at hatching come from the largest females, reflecting a relationship between nurse egg number and female size; in contrast, the number of developing embryos per capsule appear to be independent of the mother's shell length (Chaparro et al. 1999), which in turn affects the availability of nurse eggs per embryo (12–22 Gallardo 1979; 8–38 Chaparro and Paschke 1990). Using our estimate of 20 nurse eggs per embryo (the average of values reported previously), and using the energy value of each nurse egg reported in the present study (40.53 mJ nurse egg^{-1}), each embryo should be able to obtain about 810.6 mJ by ingesting nurse eggs. This exceeds the average energy actually quantified in embryos with shell lengths of 1,000–1,100 μm (742.3 mJ embryo^{-1}) by 68.3 mJ. This energy deficit probably reflects the energetic cost of intracapsular development. Indeed, we may be underestimating the magnitude of this energy expenditure, since, as noted earlier for the development of *C. fecunda*, we cannot rule out a possible additional nutritional contribution from the internal capsular wall (Hendler and Franz 1971; De Mahieu et al. 1974; Ojeda and Chaparro 2004) or intracapsular fluid (De Mahieu et al. 1974; Pechenik et al. 1984; Moran 1999), or intracapsular

cannibalism (Chaparro and Paschke 1990; Miloslavich and Penchaszadeh 2001; Collin 2003; Cubillos et al. 2007). Energy expenditure during lecithotrophic larval development has also been estimated for several other invertebrate species. For example, *Haliotis fulgens* and *H. sorenseni* expended 6.5 mJ larva^{-1} (Moran and Manahan 2003) and *Florometra serratissima* expended 36.1 mJ individual^{-1} (McEdward et al. 1988). Similarly, *Haliotis rufescens* had an energy cost from embryo to juvenile of 42–55 mJ (Shilling et al. 1996).

The direct-developing embryos of *C. dilatata* lose about 37% of their energy between the time that they finish consuming all nurse eggs (at shell lengths of about 1,000 μm) and the time of hatching (this study). Similarly, high energy costs during metamorphosis have been documented for some other invertebrate species. For example, the costs of metamorphosis have been estimated at 64.5% for the bivalve *Ostrea chilensis* (Videla et al. 1998) and 52, 28, 40% for the bryozoans *Bugula stolonifera*, *B. neritina, and B. simplex,* respectively (Wendt 2000).

When comparing feeding for similarly sized embryos of both species, the different energy sources—nurse eggs in *C. dilatata* and microalgae in *C. fecunda*—seem to have a large impact on veliger growth rates. For *C. dilatata*, nurse eggs generated an estimated instantaneous growth rate (IGR) 2.5 times higher than that achieved through the consumption of microalgae by *C. fecunda*. The extent to which this reflects greater nutritional value of nurse eggs and/or the lower cost of food acquisition for encapsulated embryos remains to be determined.

The veliger larvae of *C. fecunda* hatched with an energy content of only 51 mJ and began the process of metamorphosis several weeks later, with an energy content of 89 mJ larva^{-1}. Thus, the difference in energy content of 38 mJ must reflect the net energy contributed by the microalgae used as food during the pelagic phase. Larvae of *C. fecunda* used in this study were reared on a unialgal diet in the laboratory; the energy content of veligers in the field could be different.

Hatching size varies between 49 and 130% for *C. dilatata* (online resource 1). In contrast, hatching size in *C. fecunda* varies only 12–45% relative to the smallest veligers hatching from an egg capsule. The relatively narrow range of hatching sizes in *C. fecunda* probably reflects fairly small differences in per embryo energy content and in individual metabolic rates in the absence of substantial extraembryonic nutritional resources inside the egg capsules. In contrast, encapsulated embryos of *C. dilatata* can feast upon nurse eggs, with some individuals ingesting many more nurse eggs than other individuals, resulting in a wide range of sizes at hatching. Competition for nurse eggs can be intense; in the gastropod *Thais emarginata,* some embryos can be twice the size of others hatching from the same capsule (Spight 1976a).

C. dilatata has both a functional radula and functional gills at hatching (SL 1,200–1,300 μm), allowing for food collection from both the substratum and the water column starting from the first day after hatching (Chaparro et al. 2005a). In contrast, veligers of *C. fecunda* metamorphose at a SL of approximately 690 μm and must then rely on the radular rasping of biofilms for food during the first days of benthic life. The production of mucous cords, linked to an effective gill filtration system, becomes evident only 9 days post-settlement in this species when gill filaments have developed conspicuous branchial ciliation and the SL is approximately 800 μm (Montiel et al. 2005).

Not surprisingly then, juveniles of *C. fecunda* present an instantaneous growth rate (IGR) of only 16 μm d^{-1} during the first 9 days of benthic life, whereas for *C. dilatata,* the IGR is 22 μm d^{-1}, about 37.5% faster. Although the ability to feed with both the radula and the gill may explain this faster growth of early *C. dilatata* juveniles, a latent energy effect stemming from the previous consumption of nurse eggs during intracapsular development is also possibly at work, with some of the ingested energy stored in the digestive system for future use. In the field, actual growth rates may differ from what we have reported here, owing to the differences in food availability and temperature, for example.

Surprisingly, over the first 30 days of benthic life, the initial growth advantage reverses for the 2 species: over the first 30 days, juveniles of *C. fecunda* grow 3 times faster than juveniles of *C. dilatata* of the same age. This could be adaptive for *C. fecunda*, which metamorphoses at a SL of only 690 μm, since rapid growth might allow them to reach a size refuge from predation, compensating for their initially greater vulnerability with respect to that for *C. dilatata* (Spight 1976b; Griffiths and Gosselin 2008). How does *C. fecunda* achieve this eventual growth spurt? Additional studies are needed to examine this issue. In particular, the increased growth rate might be explained by increased length or number of gill filaments, increased density of cilia on the gills, or increased ciliary beat rate or capture efficiency.

In an evolutionary shift from direct development to planktonic development, as seems to have happened in the evolutionary trajectory leading to *C. fecunda* (Collin et al. 2007), one might have expected to see simply a conversion of nurse eggs to larger numbers of smaller developing embryos, but that is not the case. In fact, egg size is slightly higher for the species with planktonic development (*C. fecunda*) than for the species with direct development (*C. dilatata*), and the energy content of the eggs is 50% higher, while egg DW and percent organic content is lower. Remarkably, the average energy content of the eggs of *C. fecunda* exceeds that of the nurse eggs for *C. dilatata*. In addition, the reacquisition of free-living, planktotrophic

veligers would require increased velar surface area, increased cilia length, and a shift in hatching time from after metamorphosis to before metamorphosis.

The general direction of evolutionary change in marine invertebrate life histories has generally involved the loss of larvae from the life cycle (reviewed by Pechenik 1999; but see McEdward 1995; Collin 2004; Collin et al. 2007 for exceptions). Indeed, such a loss of planktonic larvae provides many advantages to a species, including the ability of parents to determine the benthic environment into which offspring will grow to adulthood (reviewed by Pechenik 1999). Such an evolutionary shift from an ancestral life history characterized by indirect larval development requires decreasing reliance on external food sources for development through metamorphosis and an increased reliance on internal energy stores (e.g., females must either increase the energy content per egg, or provide extraembryonic food resources to embryos, or both). When extra nutrients are included in each egg, typically, there is a decline in the development of the food-collecting organs (e.g., velar lobes and ciliation) during development. The timing of hatching must also be altered, so that hatching will now occur after metamorphosis rather than before metamorphosis. Such shifts are clearly seen in the development of *C. dilatata*, which presumably evolved from an ancestor with planktonic development (Collin et al. 2007).

A shift in the opposite direction—a reversal to planktonic development from a life cycle exhibiting direct development—is feasible for an ancestor like *C. dilatata*, since it retains a large, functional velum even though it lacks a free-living larval stage, as noted by Collin et al. (2007). However, it is difficult to understand the selective forces driving such a reversion to plankton development. Although planktonic larval development promotes dispersal and may reduce the likelihood of inbreeding (but see Li and Pechenik 2007), the prevalence of larval development in marine life histories may reflect the difficulties of losing larvae from life cycles more than the benefits of retaining them (reviewed by Pechenik 1999).

One apparent disadvantage of such a shift in energy allocation for C. *fecunda* is that the veligers now enter the benthos at a substantially smaller size than the size at which its direct-developing ancestor probably entered the benthos, possibly making the newly metamorphosed snails more vulnerable to hermit crabs and other benthic predators (e.g., Spight 1976b; Pechenik et al. 2010). One wonders why the larvae of *C. fecunda* do not simply remain planktonic for a longer time and metamorphose at sizes comparable to those at which the juveniles of *C. dilatata* emerge from their egg capsules. But they do not; at metamorphosis, they have less than 20% of the energy content exhibited by hatching juveniles of *C. dilatata* (Table 2). High planktonic mortality rates might select against such a prolonged stay in the plankton (reviewed by Pechenik 1999), but such high rates of planktonic mortality should also select against the reacquisition of free-living larvae in the first place, once such larvae have been lost from the life history. Many tropical gastropods produce long-lived larvae that spend months in the plankton and grow to shell lengths exceeding 2 mm (e.g., Scheltema 1971), arguing against both the impracticality of spending additional time in the plankton and the difficulty of attaining large size before metamorphosis. And, in fact the larvae of *Crepidula fornicata* can reach shell lengths of about 1.4 mm before metamorphosing (Pechenik and Lima 1984), again showing that there is no obvious biomechanical constraint preventing the veligers of *C. fecunda* from reaching a larger size.

We suggest that future studies should consider comparable data for more *Crepidula* species for which planktonic larvae appear to be the ancestral condition. It might also be worth considering the extent to which differences in some reproductive characteristics might be influenced by habitat within a species. In our study, individuals of one species were collected intertidally, while individuals of the other species were collected from subtidal populations. It might be interesting to gather comparable data for subtidal and intertidal populations of the same species.

Acknowledgments This research was funded by grants 1060194 and 1100335 from the Chilean Fondecyt to ORC. DV thanks the Initiative Scientific Millennium Grant ICM P05-002 and CONICYT Grant PFB-23.

References

Allen JD, McAlister JS (2007) Testing rates of planktonic versus benthic predation in the field. J Exp Mar Biol Ecol 347:77–87

Barnes H, Heath J (1966) The extraction of glycogen from marine invertebrate tissues. Helgol Wiss Meeresunters 13:115–117

Chaparro OR, Flores ML (2002) Reproductive output of *Crepidula fecunda* females: distribution of energy in the production of gametes and capsular walls. NZ J Mar Freshw Res 36:661–673

Chaparro OR, Paschke KA (1990) Nurse egg feeding and energy balance in embryos of *Crepidula dilatata* (Gastropoda: Calyptraeidae) during intracapsular development. Mar Ecol Prog Ser 65:183–191

Chaparro OR, Oyarzún RF, Vergara AM, Thompson RJ (1999) Energy investment in nurse eggs and egg capsules in *Crepidula dilatata* Lamarck (Gastropoda, Calyptraeidae) and its influence on the hatching size of the juvenile. J Exp Mar Biol Ecol 232:261–274

Chaparro OR, Soto AE, Bertran CE (2002a) Velar characteristics and feeding capacity of encapsulated and pelagic larvae of *Crepidula fecunda* Gallardo, 1979 (Gastropoda: Calyptraeidae). J Shellfish Res 21:233–237

Chaparro OR, Charpentier JL, Collin R (2002b) Embryonic velar structure and function of two sibling species of *Crepidula* with different modes of development. Biol Bull 203:80–86

Chaparro OR, Montiel YA, Cubillos VM (2005a) Direct intracapsular development: implications for feeding mechanisms in early juveniles of the gastropod *Crepidula dilatata*. J Mar Biol Ass UK 85:163–169

Chaparro OR, Saldivia CL, Pereda SV, Segura CJ, Montiel YA, Collin R (2005b) The reproductive cycle and development of *Crepipatella fecunda* (Gastropoda: Calyptraeidae) from southern Chile. J Mar Biol Ass UK 85:157–161

Chaparro OR, Matus PR, Thompson RJ, Segura CJ, Pardo LM, Cubillos VM (2008a) Gametic, morphometric, and physiological variables influencing clutch size in the Chilean oyster, *Ostrea chilensis* (Philippi, 1845). J Exp Mar Biol Ecol 359:18–25

Chaparro OR, Montiel YA, Segura CJ, Cubillos VM, Thompson RJ, Navarro JM (2008b) The effect of salinity on clearance rate in the suspension-feeding estuarine gastropod *Crepipatella dilatata* under natural and controlled conditions. Estuar Coast Shelf Sci 76:861–868

Chaparro OR, Schmidt AJ, Pardo LM, Andrade PV, Wagner CE, Cubillos VM (2011) Reproductive strategy of the semelparous clam *Gaimardia bahamondei* (Bivalvia, Gaimardiidae). Inv Biol 130:49–59

Collin R (2000) Sex change, reproduction and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae). Veliger 43:24–33

Collin R (2003) Worldwide patterns in mode of development in calyptraeid gastropods. Mar Ecol Prog Ser 247:103–122

Collin R (2004) Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. Evolution 58:1488–1502

Collin R, Chaparro OR, Winkler F, Véliz D (2007) Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. Biol Bull 212:83–92

Cubillos VM, Chaparro OR, Montiel YA, Véliz D (2007) Unusual source of food: impact of dead siblings on encapsulated embryo development of *Crepipatella fecunda* (Gastropoda : Calyptraeidae). Mar Freshw Res 58:1152–1161

De Mahieu GC, Penchaszadeh PE, Casal AB (1974) Algunos aspectos de las variaciones de proteínas y aminoácidos libres totales del líquido intracapsular en relación al desarrollo embrionario en *Adelomelon brasiliana* (Lamarck, 1811) (Gastropoda, Prosobranchia, Volutidae) (I). Cah Biol Mar 15:215–227

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Gallardo CS (1977) Two modes of development in the morphospecies *Crepidula dilatata* (Gastropoda: Calyptraeidae) from Southern Chile. Mar Biol 39:241–251

Gallardo CS (1979) Especies gemelas del género *Crepidula* (Gastropoda, Calyptraeidae) en la costa de Chile; una redescripción de *C. dilatata* Lamarck y descripción de *C. fecunda* n. sp. St Neotrop Fauna Environ 14:215–226

Gallardo CS, Garrido OA (1987) Nutritive egg formation in the marine snails *Crepidula dilatata* and *Nucella crassilabrum*. Int J Invertebr Reprod Develop 11:239–254

García-Guerrero M (2009) Proximate biochemical variations in eggs of the prawn *Macrobrachium americanum* (Bate, 1869) during its embryonic development. Aquac Res 40:575–581

Gnaiger E (1983) Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger E, Forstner H (eds) Polarographic oxygen sensors. Springer, Berlin, pp 337–345

Griffiths AM, Gosselin LA (2008) Ontogenetic shift in susceptibility to predators in juvenile northern abalone, *Haliotis kamtschatkana*. J Exp Mar Biol Ecol 360:85–93

Hendler G, Franz DR (1971) Population dynamics and life history of *Crepidula convexa* Say (Gastropoda: Prosobranchia) in the Delaware Bay. Biol Bull 141:514–526

Labarta U, Fernández-Reiriz MJ, Pérez-Camacho A (1999) Energy, biochemical substrates and growth in the larval development, metamorphosis and postlarvae of *Ostrea edulis* (L). J Exp Mar Biol Ecol 238:225–242

Li W, Pechenik JA (2007) The impact of inbreeding on reproduction and juvenile performance in two marine gastropods with contrasting reproductive patterns. Mar Ecol Prog Ser 346:219–234

Lucas MI, Walker G, Holland DL, Crisp DJ (1979) An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar Biol 55:221–229

Marsh JB, Weinstein DB (1966) Simple charring method for determination of lipids. J Lipid Res 7:574–576

Martínez G, López V, Mettifogo L, Cancino JM (2008) Energy utilization by embryos and larvae of the muricid snail *Chorus giganteus* (Lesson, 1829). J Exp Mar Biol Ecol 354:65–80

McEdward LR (1995) Evolution of pelagic direct development in the starfish (Asteroidea: Velatida). Biol J Linnn Soc 54:299–327

McEdward LR, Carson SF, Chia F-S (1988) Energetic content of eggs, larvae, and juveniles of *Florometra serratissima* and the implications for the evolution of crinoid life histories. Int J Invert Reprod Devel 13:9–22

Miloslavich P, Penchaszadeh PE (2001) Adelphophagy and cannibalism during early development of *Crucibulum auricula* (Gmelin, 1791) (Gastropoda: Calyptraeidae) from the Venezuelan Caribbean. Nautilus 115:39–44

Mizrahi L, Achituv Y (1991) Studies in the biochemistry of cirripede eggs. VIII. Changes in general biochemical composition during the development of *Chthamalus stellatus* (Poli) and *Euraphia depressa* (Poli). J Exp Mar Biol Ecol 152:135–143

Montiel YA, Chaparro OR, Segura CJ (2005) Changes in feeding mechanisms during early ontogeny in juveniles of *Crepidula fecunda* (Gastropoda, Calyptraeidae). Mar Biol 147:1333–1342

Moran AL (1999) Intracapsular feeding by embryos of the gastropod genus *Littorina*. Bioll Bull 196:229–244

Moran AL, Manahan DT (2003) Energy metabolism during larval development of green and white abalone, *Haliotis fulgens* and *H. sorenseni*. Biol Bull 204:270–277

Navarro JM, Chaparro OR (2002) Grazing–filtration as feeding mechanisms in motile specimens of *Crepidula fecunda* (Gastropoda: Calyptraeidae). J Exp Mar Biol Ecol 270:111–122

Ojeda JA, Chaparro OR (2004) Morphological, gravimetric, and biochemical changes in *Crepidula fecunda* (Gastropoda: Calyptraeidae) egg capsule walls during embryonic development. Mar Biol 144:263–269

Pandian TJ (1969) Yolk utilization in the gastropod *Crepidula fornicata*. Mar Biol 3:117–121

Pechenik JA (1986) The encapsulation of eggs and embryos by molluscs: an overview. Am Malacol Bull 4:165–172

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269–297

Pechenik JA, Lima G (1984) Relationship between growth, differentiation, and duration of larval life in individually-reared larvae of the marine gastropod *Crepidula fornicata*. Biol Bull 166:537–549

Pechenik JA, Chang SC, Lord A (1984) Encapsulated development of the marine prosobranch gastropod *Nucella lapillus*. Mar Biol 78:223–229

Pechenik JA, Ambrogio OV, Untersee S (2010) Predation on juveniles of *Crepidula fornicata* by two crustaceans and two gastropods. J Exp Mar Biol Ecol 384:91–98

Petersen S, Anger K (1997) Chemical and physiological changes during the embryonic development of the spider crab, *Hyas araneus* L. (Decapoda, Majidae). Comp Biochem Physiol 117B:299–306

Rivest BR (1983) Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia : Buccinidae). J Exp Mar Biol Ecol 69:217–241

Scheltema RS (1971) The dispersal of the larvae of shoal-water benthic invertebrate species over long distances by ocean currents. Fourth Europ Mar Biol Symp 1971:7–28

Scheltema RS (1986) On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull Mar Sci 39:290–322

Schmidt AJ, Toro JE, Chaparro OR (2006) Reproductive patterns and their influence on the population genetics of sympatric species of the genus *Crepidula* (Gastropoda: Calyptraeidae). J Shellfish Res 25:371–378

Shilling FM, Hoegh-Guldberg O, Manahan DT (1996) Sources of energy for increased metabolic demand during metamorphosis of the abalone *Haliotis rufescens* (Mollusca). Biol Bull 191:402–412

Spight TM (1976a) Hatching size and the distribution of nurse eggs among prosobranch embryos. Biol Bull 150:491–499

Spight TM (1976b) Ecology of hatching size for marine snails. Oecologia 24:283–294

Strathmann RR (1978) The evolution and loss of feeding larval stages of marine invertebrates. Evolution 32:894–906

Strathmann RR (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Ann Rev Ecol Syst 16:339–361

Strathmann RR (1993) Hypotheses on the origins of marine larvae. Ann Rev Ecol Syst 24:89–177

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol Rev 25:1–45

Underwood AJ (1997) Experiments in ecology. Their logical design and interpretation using analysis of variance. Cambridge University Press, Sydney

Vance RR (1973) On reproductive strategies in marine benthic invertebrates. Am Nat 107:339–352

Vavra J, Manahan DT (1999) Protein metabolism in lecithotrophic larvae (Gastropoda: *Haliotis rufescens*). Biol Bull 196:177–186

Véliz D, Winkler FM, Guisado C (2003) Developmental and genetic evidence for the existence of three morphologically cryptic species of *Crepidula* in northern Chile. Mar Biol 143:131–142

Videla JA, Chaparro OR, Thompson RJ, Concha II (1998) Role of biochemical energy reserves in the metamorphosis and early juvenile development of the oyster *Ostrea chilensis*. Mar Biol 132:635–640

Wendt DE (2000) Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biol Bull 198:346–356