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Early development of the limpet *Siphonaria lessonii* Blainville, 1827 in populations affected by different physical stressors

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ABSTRACT

The limpet *Siphonaria lessonii* is very common along Atlantic Patagonian intertidal rocky shores. We studied the early intracapsular embryonic development of this limpet in detail in two populations in north Patagonia, with different environmental conditions (i.e. wave exposure, wind, temperature). Early development in both populations was achieved at controlled and equal conditions (13°C). The spawn consisted of a series of enchained egg capsules embedded in a jelly mass. The development from egg to hatching veliger took 9–11 days in embryos from both populations. The developmental process at both sites was identical, differing only in the embryos' sizes at each stage. Larger adult individuals producing larger embryos were registered at the sheltered site. The differences in sizes of adult and embryos of *S. lessonii* could be attributed to distinct environmental stressful conditions between sites.

Introduction

The reproductive mode employed by marine gastropods includes a diversity of complex arrangements of parameters that can be explained by both adaptive and phylogenetic models (Gallardo & Perron 1982; Jablonski & Lutz 1983; Grahame & Branch 1985).

Among intertidal molluscs, particular developmental patterns represent an optimal solution to various ecological circumstances of the individual (Gallardo & Perron 1982; Strathmann 1986; Havenhand 1993), and may be considered adaptive (Chambers & McQuaid 1994b). Despite relevant exceptions, there is support for adaptive models based upon gastropod egg size, that species with small eggs generally have long-lived planktonic larvae that disperse widely and must feed and grow in the plankton prior to reaching metamorphosis (planktonic developers), whereas species with large eggs tend to have short-lived, non-feeding larvae or lack dispersing planktonic stages altogether (direct developers) (Thorson 1950; Moran & McAlister 2009).

The optimal balance between development time, offspring size and fecundity depends on the environment (Allen et al. 2008). Theory predicts that different environments will select for offspring of different sizes and selection should favour mothers that

provision offspring according to the environment they are likely to encounter (McGinley et al. 1987; Mousseau & Fox 1998; Marshall et al. 2006; Allen et al. 2008). A number of studies have shown that mothers can match the phenotype of their offspring to changes in the local environment and that maternal effects can be an effective means of buffering progeny from environmental stressors (Galloway 1995; Bernardo 1996a, 1996b). However, there is scarce information in the literature on the relationship between environmental conditions and intra-specific variation, their effects on developmental variability in marine gastropods and its implications for the evolutionary dynamics of eggs and offspring size (Przeslawski 2004; Marshall & Uller 2007; Marshall & Keough 2008; Krug 2009; Collin & Ochoa 2016).

Siphonariids are pulmonate gastropods that evolved to return to marine environments after their ancestors lived in terrestrial ecosystems. Known siphonariid limpets are simultaneous hermaphrodites with internal fertilization that produce gelatinous egg masses (spawn), which are usually deposited on the substratum (Allanson & Msizi 2010), although some species produce pelagic egg masses (Creese 1980; Quinn 1988). Benthic egg masses are believed to provide protection to the developing embryos from environmental

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stresses and predation (Thorson 1950; Pechenik 1979; Strathmann 1985). Nevertheless, environmental factors still affect embryonic development, sometimes deleteriously (Przeslawski 2004), i.e. exposure to desiccation, osmotic stress, temperature stress and UV radiation (Rawlings 1999).

Among species of the genus *Siphonaria* Sowerby, 1823 both planktonic larval and direct-developing juveniles occur (Chambers & McQuaid 1994a; Hodgson 1999; Pal 2003). In particular, the limpet *Siphonaria lessonii* Blainville, 1827 develops into a planktonic free-swimming veliger larva (Olivier & Penchaszadeh 1968) after hatching from egg masses. This species occur from Peru to Cape Horn in the Pacific Ocean and along the shores of Argentina and Uruguay, and the Malvinas Islands in the Atlantic Ocean (Castellanos et al. 1993). This species is common along the coasts of Patagonia in Argentina, living on intertidal rocky shores (Carcelles & Williamson 1951; Olivier & Penchaszadeh 1968; Penchaszadeh et al. 2003; Miloslavich et al. 2013; Rechimont et al. 2013; Guller et al. 2015; Miloslavich et al. 2016). Knowledge of the embryonic development in *S. lessonii* is very limited, with the exception of some aspects (density structure and feeding behaviour) published by Olivier & Penchaszadeh (1968).

The main objective of this study was to describe the early intracapsular embryonic development in the limpet *Siphonaria lessonii* in two intertidal populations with different environmental conditions and to explore differences in developmental parameters between these populations from north Patagonian rocky shores. To achieve this aim, we tested the hypothesis that adults of *S. lessonii* from sheltered environments will have offspring with more fitness (larger) than other individuals inhabiting exposed sites. This work provides a foundation upon which further hypothesis-driven research can be drawn when studying siphonariids, which are frequently used as case study organisms.

Material and methods

Study area and sampling

Field sampling was performed during low spring tides, at two rocky intertidal sites in the north of Patagonia, Argentina (Figure 1) with different exposures to extreme physical conditions. Wind circulation over the north Patagonian gulf region is characterized by strong and persistent westerly winds (Paruelo et al. 1998). Annual mean wind speed is 4.6 m/s (16.6 km/h), reaching up to 25 m/s (90 km/h). South-western and western winds occur with a frequency of approximately 38% in summer and more than 50% in winter (Rechimont 2011; Rechimont et al. 2013). The 'Barranca Blanca' site in San Matías Gulf (42° 13′29.82″S, 64°39′12.11″E) is open to the sea, with high wave action and exposed to high wind intensities typical of the region; this is directly related to high desiccation rates suffered by the intertidal biota (Arribas et al. 2016). The Punta Este site in Nuevo Gulf (42°47′09.47″ S, 64°57′30.17 E) is a more sheltered area, situated at a distance of 65 km in a straight line from Barranca Blanca, where waves are less frequent and intense and the wind action (mostly from the land) is buffered by the presence of cliffs. Barranca Blanca is considered to be in the Argentinian biogeographic province while Punta Este is in the Magellanic biogeographic province. Both are separated by the Valdes Peninsula, which is considered a natural barrier between biogeographic provinces (Boschi 2000), presenting different flora, fauna and oceanographic conditions.

Adults of *S. lessonii* (*n* = 30 per site) were hand gathered along intertidal rocky shores at each site in the spawning season, from September to December 2014 (Figure 2a) and carried alive to a conditioned aquarium at the Servicio de Acuario Experimental (CCT-CONICET CENPAT). Maximum shell length and height of adults were measured with a 0.1 mm precision digital calliper. The specimens examined in this study were larger than the individual maturity size of 11 mm in shell length (SL) reported by Di Giorgio et al. (2014).

Spawning behaviour

Once at the laboratory, adult individuals from each site were maintained and acclimatized in separate conditioned aquaria (50 l seawater) with continuous aeration at 13°C (mean annual water temperature at the sampling sites), 12:12 photoperiod and salinity of 35.

Once the adult individuals were acclimatized, and prior to spawning, each one was placed in individual containers at equal conditions.

Embryonic development

Individuals deposited their egg masses in each aquarium. Recently laid egg masses were separated and maintained in 0.5 l of 1-micron filtered and UV-treated seawater, which was changed every day. The general characteristics of egg masses were observed and photographed throughout complete development. Intracapsular development was described from recently deposited egg masses in the aquarium, randomly chosen from those spawned by the individuals
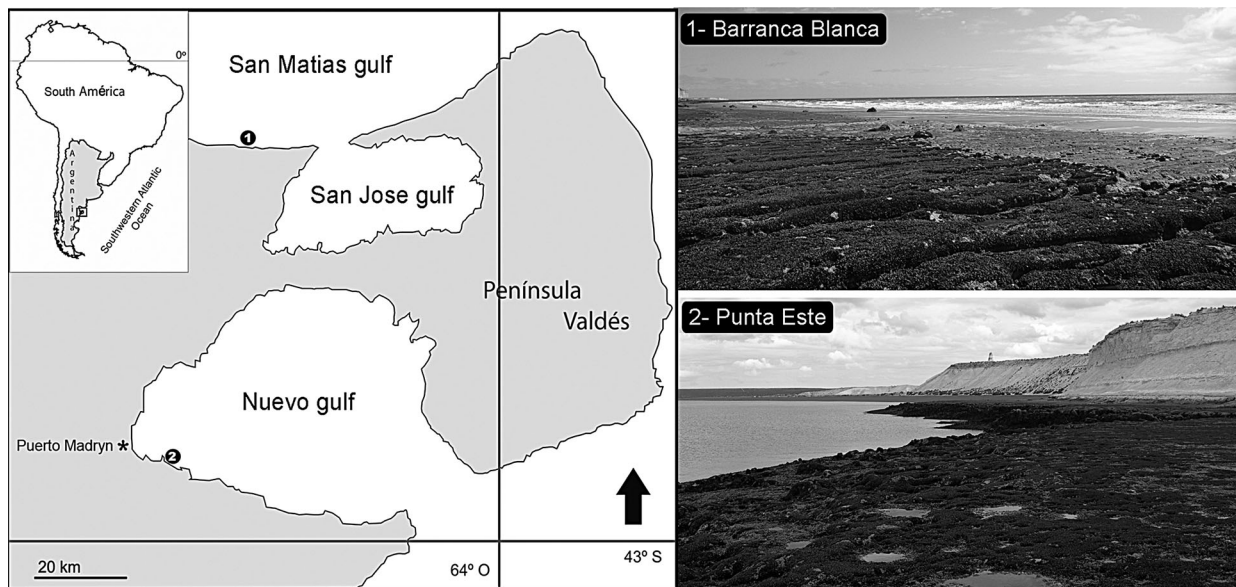
Figure 1. Sampling sites (black dots) of *Siphonaria lessonii* from (1) Exposed site 'Barranca Blanca' in San Matías Gulf. (2) Sheltered site Punta Este in Nuevo Gulf, Patagonia, Argentina.

from each site ($n_{PEste} = 20$; $n_{BBlanca} = 20$ egg masses, respectively).

One portion of each egg mass was observed daily, and the embryonic stages were characterized as described in Table I. Then, embryos were measured and photographed, using a Leica DM2500 light microscope. A second portion of each egg capsule was daily fixed in 2% glutaraldehyde for later observation in a scanning electron microscope (SEM). Embryos at different developmental stages and larval shells were observed and photographed using a JEOL JSM-6460LV SE model SEM.

Additionally, intracapsular total developmental time was recorded. Recently hatched larvae were observed and measured with a Zeiss stereoscopic microscope with a 0.1 mm precision ocular micrometer. The egg volume was calculated using the volume of a sphere equation ($V = 4/3 \, \pi \, r^3$).

Statistical analysis

The length/height of adults obtained from each site was compared using Student's *t*-tests after testing for homogeneity of variance and normality between samples. We used nested ANOVAs to test differences in egg/hatching embryo size and egg-capsule size between sites, with egg mass nested within site as a random factor. Data were square-root transformed when the assumptions of homoscedasticity and normality were not met. The egg capsule lengths of each intracapsular stage for both sites were compared using one-way ANOVA, after testing for homogeneity

of variance and normality among samples. An *a posteriori* Tukey test was used to identify differences between means. The developmental time from egg mass laying to hatching was also compared using Student's *t*-tests. All statistical analysis was done with the Statistica 7.0 statistical package.

Results

Spawning behaviour

Spawning individuals and egg masses at different stages of development were observed in the intertidal during sampling (September to December) (Figure 2a). Generally, limpets were found at high and mid-intertidal levels on rocky shores, in particular at wet areas (crevices and tidal pools). In the aquaria, the gastropods spawn after a short period of time, varying from 8 h to 7 days.

The spawn consisted of a series of enchained egg capsules embedded in a jelly mass. The egg mass length varied and the shape was irregular or curved (Figure 2b, c). A single egg was laid within each oval-shaped egg capsule, which measured on average $205.58 \pm 27.14 \, \mu m$ (means ± SD) in maximum diameter.

Embryonic development

The entire process of development from egg mass laying to veliger hatching took 9–11 days at 13°C and no significant differences were detected between sites (Student's *t*-test, t = 1.416, P = 0.184) (Table I).
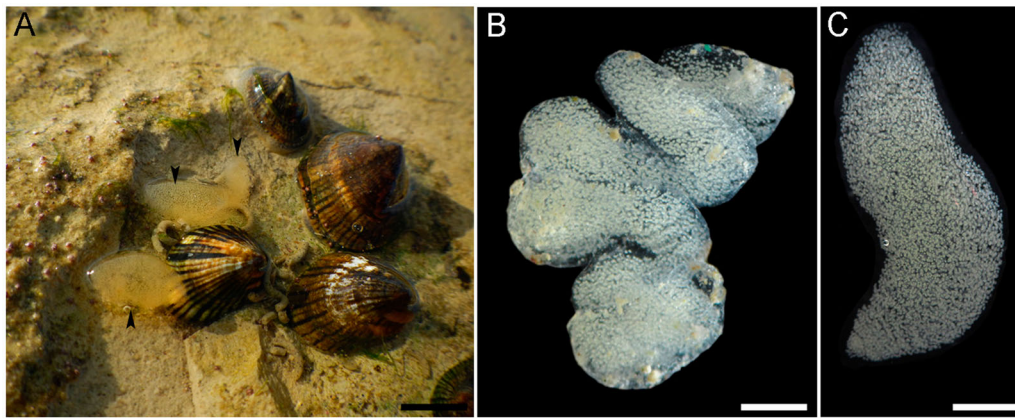
Figure 2. A, Adult *Siphonaria lessonii* spawning in the field. Note the egg masses (arrow heads). B, Irregular shaped egg mass. C, Curved shape egg mass. Scale bars: A = 1 cm; B = 0.3 mm; C = 0.2 mm.

The developmental process at both sites was identical, differing only in the embryo sizes at each stage, presented in Table I.

The uncleaved spherical eggs ranged in diameter from 78 to 84 μm. In recently laid egg capsules, the egg was immersed in a liquid with a granular aspect (Figure 3a) that became totally transparent 1–2 hours after being laid; then it was followed by the occurrence of the polar body (Figure 3b).

The first cell cleavage occurred shortly (12 h) after spawning; following cleavages up to morula stage were completed within days 2–3 from spawning (Figures 3c–e).

At the blastula stage one of the surfaces became concave and a slit-like blastopore was formed in the centre of this area, as gastrulation occurred. Also, refringent large cells or 'athrocytes', usually known as 'larval kidneys', began to develop (Figure 3f–g). The mean diameters of all these early developmental stages from egg to gastrula stages were similar (Table I).

During day 5 a band of very short cilia developed in the gastrula-shaped embryo, which transformed into an early trochophore that began to rotate slowly (Figure 3h). After that, the embryo lengthened antero-posteriorly. The blastopore moved to the upper apical section and the stomodeum was

Table I. Early embryonic development and adult size of *Siphonaria lessonii* for each site. The chronology of intracapsular development under laboratory conditions at 13°C and principal morphological changes during the different developmental stages are provided. Values represent mean size ± SD in μm unless otherwise indicated and *n* (in parentheses). *indicates significant differences for each developmental stage between sites.

Days/Hours after spawning	Developmental stage	Morphological changes in the embryos	TYPE	PUNTA ESTE (Sheltered)	BARRANCA BLANCA (Exposed)
0–12 hs	Egg	Round and whitish, before cellular cleavage.	embryo	84.19 ± 3.53 ($n = 100$)*	78.37 ± 3.99 ($n = 100$)*
			capsule	224.42 ± 19.55 ($n = 100$)	187.10 ± 15.92 ($n = 100$)
12hs–2d	First cleavages	Emission of primary and secondary polar body. First cleavages (2–4 to 16 cells).	embryo	100.57 ± 9.36 ($n = 21$)*	96.28 ± 4.35 ($n = 30$)*
			capsule	243.90 ± 20.99 ($n = 21$)	175.40 ± 10.15 ($n = 30$)
2–3d	Morula	Roundish embryo with >16 cells.	embryo	99.49 ± 6.21 ($n = 26$)*	96.20 ± 6.10 ($n = 20$)*
			capsule	246.29 ± 8.77 ($n = 26$)	197.88 ± 19.01 ($n = 20$)
4d	Blastula/ gastrula	Formation of blastopore. Post-gastrula: the embryo lengthens in the antero-posterior dimension. Formation of 'larval kidneys'.	embryo	99.38 ± 3.74 ($n = 31$)*	93.73 ± 4.56 ($n = 45$)*
			capsule	212.15 ± 20.47 ($n = 31$)	200.63 ± 12.80 ($n = 45$)
5–6d	Trocophore	Typical larvae with prototroch and apical tuft, showing first rotational and then also translational movements.	embryo	104.13 ± 13.81 ($n = 89$)*	95.99 ± 2.81 ($n= 45$)*
			capsule	228.62 ± 44.81 ($n = 89$)	194.29 ± 14.48 ($n = 45$)
7–8d	Veliger	Bilobulated velum formed. Development of a pair of statocysts. Visible foot and opercula. Larvae began to swim within the capsule. Torsion evident at the late veliger.	embryo	114.40 ± 17.42 ($n = 46$)*	106.50 ± 4.90 ($n = 35$)*
			capsule	212.73 ± 24.08 ($n = 46$)	182.46 ± 15.90 ($n = 35$)
9–11d	Hatching veliger	The velum developed a band of large cilia and the food groove. Stomach and digestive gland become visible.	embryo	151.14 ± 13.88 ($n = 100$)*	133.42 ± 9.78 ($n = 100$)*
			capsule	220.70 ± 32.36 ($n = 100$)	185.59 ± 13.02 ($n = 100$)
	ADULT SIZE (mm)		Length	19.34 ± 2.18 mm ($n = 30$)*	13.59 ± 1.56 mm ($n = 30$)*
			Height	10.07 ± 1.07 mm ($n = 30$)*	8.98 ± 0.97 mm ($n = 30$)*
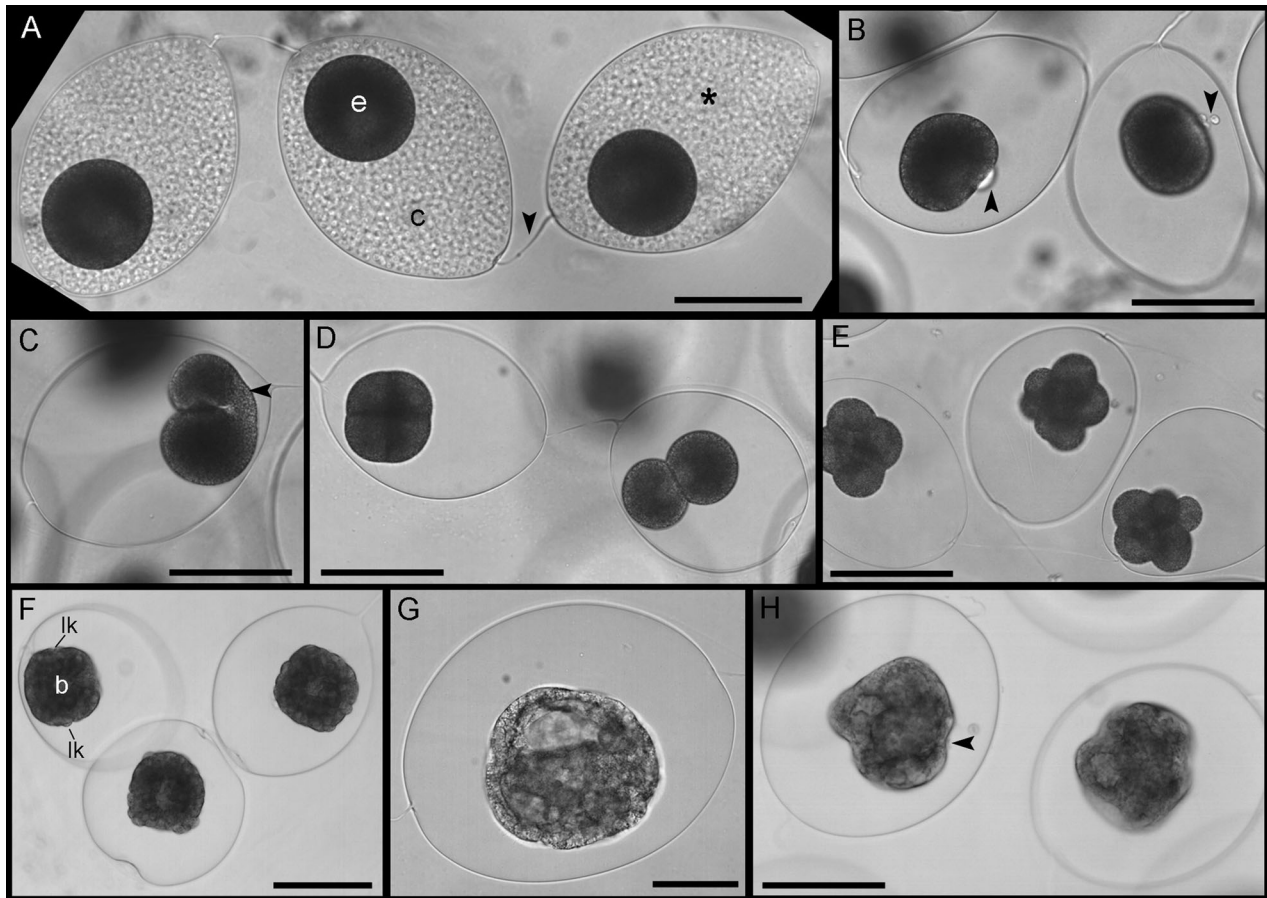
Figure 3. Intracapsular development of *Siphonaria lessonii*. A, Recently laid chained (arrowhead), uncleaved eggs; note the granular aspect of the intracapsular liquid (asterisk). B, Egg with first polar lobe extruded (arrowheads), with transparent intracapsular liquid. C, 2-cell stage. D, 2 and 4-cell stages. E, Morulae. F, Blastulae with incipient larval kidney. G, Gastrula. H, Early trochophores; cilia are present although not visible (arrow head). Abbreviations: b: blastopore, c: capsule, e: egg, lk: larval kidney. Scale bars: A–D: 100 μm; E–G: 50 μm; H: 100 μm.

developed. Cilia were formed in the region of the head vesicle (Figure 4a). At the trocophore stage the 'larval kidneys' were still present and the prototroch was completely developed, together with a visible apical tuft (Figure 4a).

At the early veliger stage, during day 7, a thin shell rudiment developed, enclosing the undifferentiated tissues that formed the visceral mass. Velar lobes started to grow out and the cilia enlarged, forming the velum (Figure 4b). The foot began to develop as a short square outgrowth beneath the velum and a very thin operculum became visible on its posterior surface (Figure 4c). Veliger larvae constantly rotated inside their capsules with vigorous movements of the velar cilia.

The late veliger stage presented a pair of conspicuous statocysts behind each velar lobe and the stomach and digestive gland were differentiated (Figures 4d–f). Cilia formed over the surface of the foot and the operculum projected beyond the foot edge. Torsion occurred and could easily be recognized by the appearance of the rectum in a dorsal position above the velum, with the anus opening into the left side of the mantle cavity (Figures 4e–f).

At the hatching veliger stage the size of the shell, velum and foot increased and differentiation of internal organs occurred, to ultimately form the advanced veliger stage ready to hatch. Prior to hatching, the larvae occupied almost all the space in the capsule and movements were reduced, although the velar cilia actively moved (Figures 4f–g). Escape from the capsule appeared to be facilitated by a softening of the wall, which was split open with the velar cilia. Around day 11, released veligers swam up to the top of the containers in which they were maintained; they survived 15 days until they died, even when they actually fed on Kent Marine Phythoplex (aqua-cultured phytoplankton).
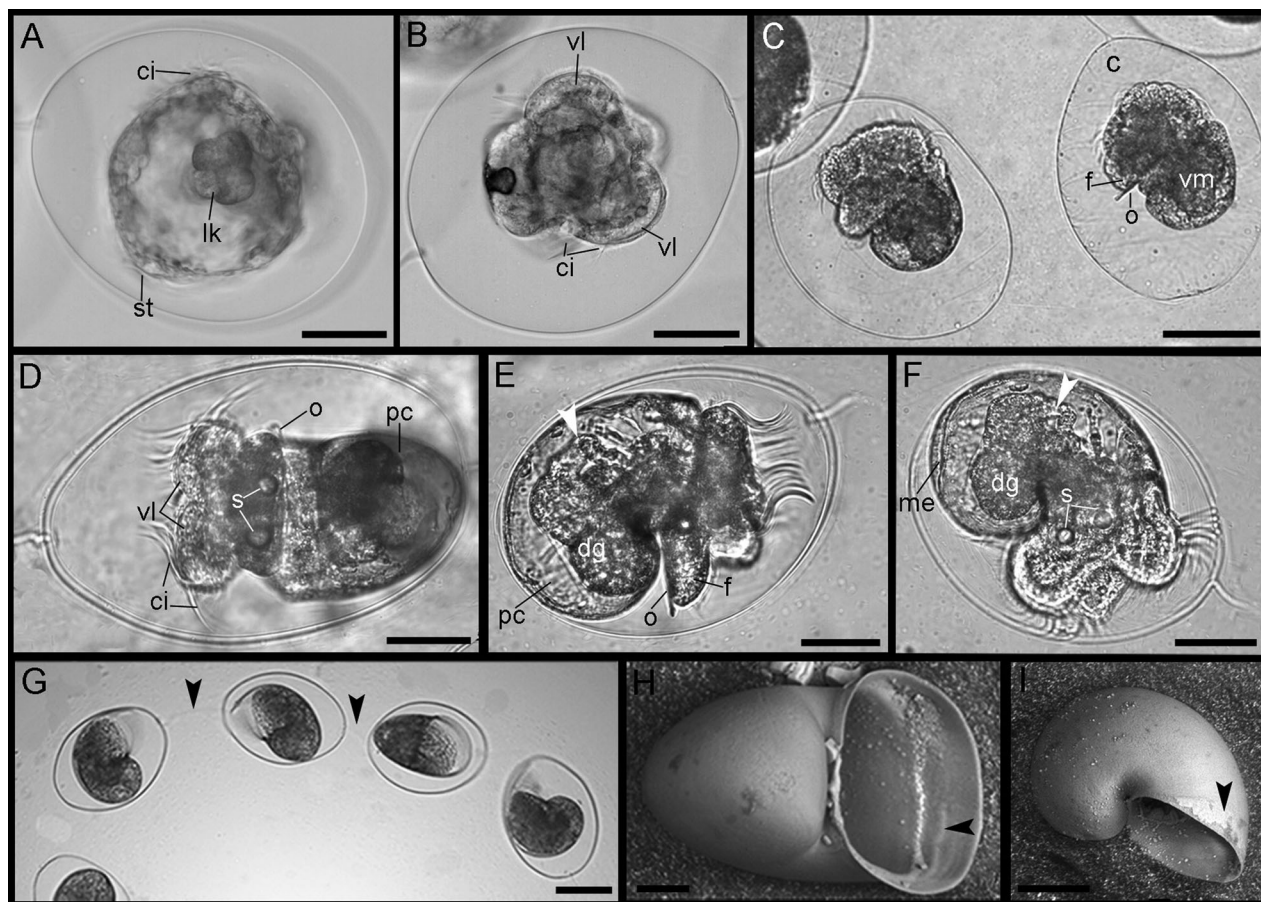
Figure 4. Intracapsular development of *Siphonaria lessonii*. A, Late trochophore. B, Early veliger with incipient velum developed. C, Veligers. Note the presence of foot and operculum. D, Pre-hatching veliger with statocyst. E, F, The velum is expanded and the stomach is visible by transparency. G, Enchained hatching veligers (arrowheads). H, I, nepionic shell of the hatching veliger larvae (SEM). Note the intracapsular shell growth (arrowheads). Scale bars: A--B, D--F, I = 50 µm; C, G = 100 µm; H = 20 µm. Abbreviations: c: capsule, ci: cilia, dg: digestive gland; f: foot; lk: larval kidney; me: mantle edge; o: operculum, pc: protoconch; s: statocyst, st: apical tuft; vl: velar lobes, vm: visceral mass.

Comparisons between sites

Adult *Siphonaria lessonii* examined from both sites ranged between 11.84 and 23.04 mm in shell length. Statistical analysis showed that individuals from the comparatively sheltered P. Este site were significantly larger and higher than individuals from the exposed B. Blanca site (length, $t = -11.08$, $P < 0.0001$; height, $t = -4.15$, $P < 0.0001$; Figure 5A). Mean values are shown in Table I.

The developmental pattern was similar between populations; however, size differences were observed in each developmental stage (Figure 6 and Table I). There was a significant effect of site on egg and hatching embryo size when egg mass was nested as a random factor within site (ANOVA for egg: F = 19.49, df = 191, $P < 0.001$; hatching embryos: F = 13.82, df = 238, $P < 0.005$). Mean uncleaved egg diameter was larger in P. Este than in B. Blanca site (Figure 5C). Similarly, mean hatching length was larger in

P. Este than in B. Blanca (Figure 5C) (Table I). In addition, the egg volume from P. Este (0.000312 mm^3) was 23% bulkier than in B. Blanca eggs (0.000252 mm^3).

Furthermore, egg capsule length considering all stages together showed significant differences between sites ($t = 12.86$, $P < 0.00$). In particular, for the early-stage and late-stage egg capsule length, the effect of site was also significant when egg mass was nested as a random factor within site (ANOVA for egg capsule egg: F = 33.14, df = 178, $P < 0.001$; egg capsule hatching embryos: F = 9.41, df = 202, $P = 0.0112$; Figure 5B).

When analysing egg capsule length throughout developmental stages within each site no significant differences (F = 1.26, df = 6, $P = 0.27$) were observed from the comparatively sheltered Punta Este site, while significant differences were observed in the exposed Barranca Blanca population (F = 9.99, df = 6, $P < 0.0001$),
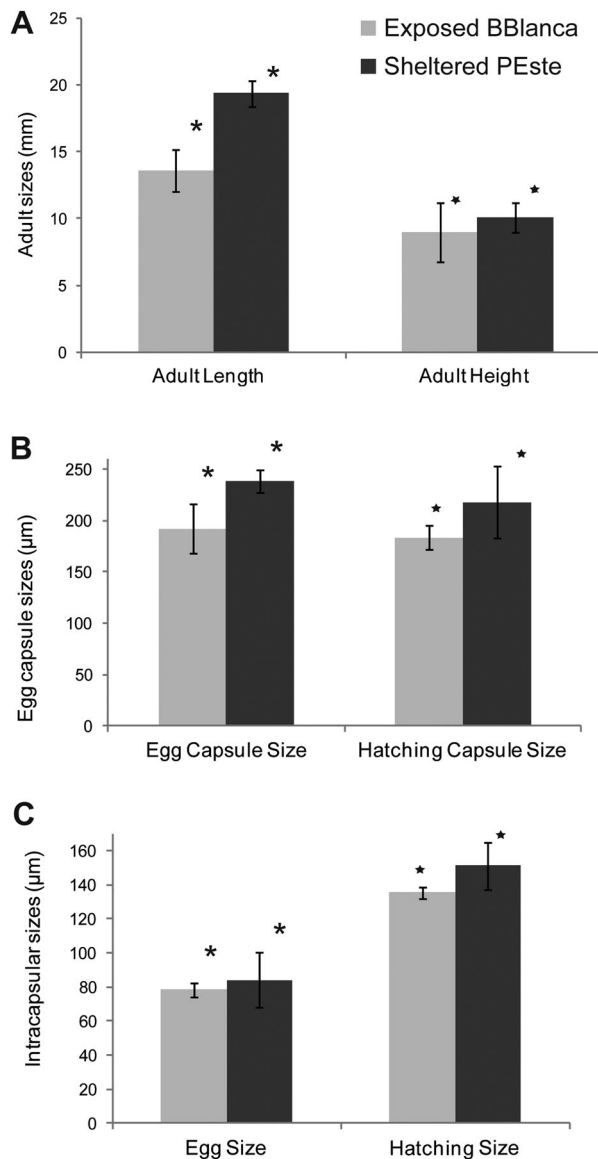
Figure 5. A, Morphometric measurements (length and height) of adults of *Siphonaria lessonii* from each location. B, Egg capsule length. C, Intracapsular sizes (egg and hatching size). Bars show SD. Asterisks and stars indicate significant differences between sampling sites.

although these differences did not correspond with a predominant pattern (Table I).

Discussion

The reproductive biology of the genus *Siphonaria* from the South-western Atlantic Ocean is under-studied. According to Olivier & Penchaszadeh (1968) and Chambers & McQuaid (1994b), and based on our results, the developmental biology of *Siphonaria lessonii* fits into the planktonic free-swimming veliger larvae type. This coincides with most studied siphonariid limpets from South Africa, which hatch as free veliger
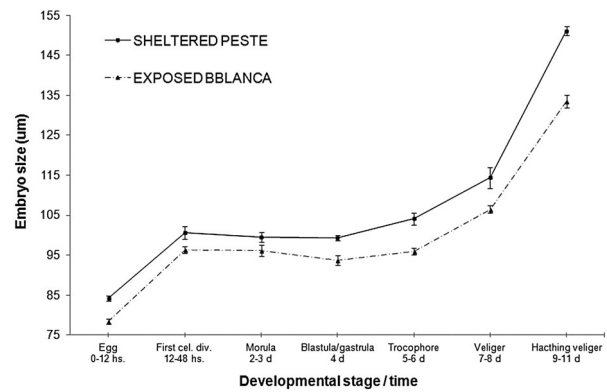
Figure 6. Size development variation and development time (days) of *Siphonaria lessoni* for each stage (bars show SE).

larvae and have egg capsules that are usually smaller than 300 microns, as observed in the present study (Mapstone 1978; Chambers & McQuaid 1994a, 1994b; Ocaña & Emson 1999; Pal & Hodgson 2003).

Olivier & Penchaszadeh (1968) registered some aspects of the reproduction of a population of *S. lessonii* from Mar del Plata, 800 km north of our study sites. Although our detailed study presented several similarities, according to those authors the developmental time involved 8–9 days after egg-laying at 17–18°C, while our results showed that the intracapsular development took 9–11 days at 13°C. A longer intracapsular development of the Patagonian populations of *S. lessonii* could be attributed to the low temperatures that often occur at these latitudes (Hoegh-Guldberg & Pearse 1995; Gillooly et al. 2002).

The spawn of *S. lessonii* contains a mass of enchained egg-shaped capsules embedded in a jelly matrix and enclosed by a labile wall, as in *S. pectinata*, *S. diemenensis* and *S. baconi* (Mapstone 1978; Ocaña & Emson 1999). The presence of two types of egg masses (irregular and curved) was observed at all the studied sites and may be related to phylogenetic differences (Pal & Hodgson 2003) or environmental conditions. Eyster (1986) reported that flowing water accelerates the embryonic developmental rate of gelatinous egg masses by decreasing hatching time and increasing embryonic activity. Therefore, it is likely that the shape of the egg mass determines, in part, the developmental effects of oxygen availability as controlled by water flow (Kranenbarg et al. 2001; Przeslawski 2004).

It is also notable that the egg masses were freely laid on rocky crevices or in tidal pools, but they are easily removed by waves and currents. This could be related to the considerably shorter period of intracapsular development (although not tested) observed in the field than in laboratory conditions (submerged

and without wave action). However, many selective pressures may be involved in reducing the period of intracapsular development (Havenhand 1993).

The fact that larger egg capsules of *S. lessonii* were observed at P. Este, compared with B. Blanca, in this study may be related to a higher content of intracapsular nutrients given by the adults (Spight 1975; Moran & Emlet 2001). An uptake of nutrients by developing embryos from perivitelline fluid was reported in the South African species *S. serrata* (Fischer, 1807) and *S. capensis* (Quoy & Gaimard, 1833), with direct and free larval development, respectively (Pal 2003; Pal & Hodgson 2005). A high fecundity in terms of egg number is achieved by adult individuals of *S. lessonii*, although it is also related to high mortality rates (Pechenik 1979; Perron 1981; Rawlings 1994, 1999). Thus, the allocation of energy in the form of extra embryonic intracapsular nutrients was not expected, but should be further studied.

Siphonaria lessonii egg capsule sizes remained similar in shape and volume throughout intracapsular development within both of the studied populations. Similarly, Mapstone (1978) reported that the egg capsule sizes of two species for *Siphonaria* remained equal while the embryos grow. Larger egg capsules, eggs and hatching larvae were observed at the Punta Este site, the locality with less environmental stress (sheltered). The lower physical stresses that limpets encounter at the protected environment of P. Este (less wind and wave action) probably also benefits adult individuals, which achieved larger sizes in comparison to the Barranca Blanca site (wave exposed and higher winds). A similar pattern was registered in Nuevo Gulf for ecomorphs of the marine rocky shore gastropod *Trophon geversianus*, which present distinct morphological and behavioural responses to physically stressful conditions, with intertidal individuals developing smaller sizes with a low-spired shell and an expanded aperture which might allow better attachment to the bottom substrate, while subtidal individuals presented a slender and narrower shell shape (Marquez et al. 2015). Gastropod intracapsular embryonic development may be influenced by a variety of environmental conditions, as well as predation and parental history. These factors usually do not operate independently (Przeslawski 2004) and their relationships should be considered when studying embryonic development within gastropod egg masses.

The fact that larger adults produced larger embryos could be related to (1) the allocation of nutrients in higher amounts in the perivitelline fluid of these females' egg capsules (Pal & Hodgson 2003) and (2)

the significantly bulkier egg volume. This is particularly true if equivalent egg quality (energy content or biochemical composition) is assumed between populations. Egg size is generally species specific, although some variability may be found within a population, e.g. related to female size, food availability or spawning season (Laptikhovsky 2006) or between populations, as occurs in this study. Other studies have demonstrated the occurrence of variability in egg diameter and in hatchling size in the family Calyptraeidae (Collin 2000, 2003; Collin & Salazar 2010), as well as in the offspring production among females from different localities (Zelaya et al. 2012). However, recent studies raised concerns about the fact that changes in egg size alone cannot account for all the physiological, ecological and evolutionary correlates of egg size (Moran & McAlister 2009). Studying factors such as changes in the biochemical composition of eggs with size, patterns of utilization of egg constituents by embryos (Moran & McAlister 2009; Collin & Salazar 2010), energetic costs of spawning and energy requirements of embryos (Averbuj et al. 2017), is required in order to better understand the complex role of egg size in the life histories of marine invertebrates.

This study represents a first approximation of the effect of environmental conditions on intracapsular embryonic development in the limpet *Siphonaria lessonii*. Further studies that explore developmental variability and its implications for adaptation should consider a wider scenario of sampling sites (environmental condition variability) and the intraspecific variability of embryonic development. Intraspecific variation in offspring size is of fundamental ecological and evolutionary importance (Bernardo 1996b; Marshall & Uller 2007; Collin 2010). In this way, these studies would allow an approach to a possible explanation of the evolutionary dynamics of marine gastropod egg and offspring sizes in relation to environmental conditions.

Disclosure statement

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