Differences in resource allocation to reproduction across the intertidal–subtidal gradient for two suspension-feeding marine gastropods: *Crepidula fornicata* and *Crepipatella peruviana*

Jan A. Pechenik[1,*], Casey M. Diederich[1,4], Oscar R. Chaparro[2], Jaime A. Montory[3], Francisco J. Paredes[2], Amanda M. Franklin[1]

[1]**Biology Department, Tufts University, Medford, MA 02155, USA**
[2]**Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Casilla 567, Valdivia, Chile**
[3]**Centro i-mar, Universidad de Los Lagos, Camino Chinquihue km 6, Puerto Montt, Chile**

[4]*Present address:* **50 Fountain Plaza, Suite 600, Buffalo, NY 14202, USA**

ABSTRACT: The sedentary, suspension-feeding gastropods *Crepidula fornicata* and *Crepipatella peruviana* can be found in large numbers both subtidally and intertidally. Although intertidal animals often show reduced reproductive output compared with that of subtidal animals, we know little about how the reproductive output of marine gastropods is affected by exposure to intertidal stresses. We documented reproductive characteristics for intertidal and subtidal *Crepidula fornicata* in Rhode Island, USA (2012, 2013), and *Crepipatella peruviana* from Pelluco Beach, Chile (2010–2012). Females of both species brood encapsulated embryos for at least several weeks before releasing offspring, facilitating determinations of individual fecundity (embryos per egg mass). As expected from the environmental stresses experienced uniquely by intertidal individuals while emersed, including an inability to feed, intertidal females of *C. peruviana* had lower fecundities, producing significantly fewer egg capsules per brood; the mean number of eggs per capsule did not differ significantly by habitat. In marked contrast, the size-adjusted fecundity of *Crepidula fornicata* was significantly higher for intertidal females than for subtidal females; however, rather than brooding more egg capsules per individual or making larger egg capsules, intertidal females crowded each capsule with significantly more eggs. This study adds to previous work showing how exceptionally well-adapted *C. fornicata* is to intertidal life, and may help to explain why this species has been so unusually successful as an invasive among calyptraeid gastropods. Future studies will be required to identify the specific conditions that have provoked these adaptations in *C. fornicata*, and to understand the mechanisms through which this species achieves them.

KEY WORDS: *Crepidula fornicata* · *Crepipatella peruviana* · Slipper limpet · Reproduction · Intertidal · Invasive species · Fecundity · Egg capsules

INTRODUCTION

The intertidal zone is an extremely stressful environment for most marine organisms. When the tide recedes, organisms that cannot retreat subtidally are exposed to a suite of environmental stressors, including tissue desiccation (e.g. Garrity 1984), reduced available feeding time (e.g. Gillmor 1982), hypoxia (due to an inability to use aerial oxygen because of shell closure, for example; e.g. Brinkhoff et al. 1983), and rapid and dramatic fluctuations in temperature and, at times, salinity (e.g. Morris & Taylor

1983, Diederich & Pechenik 2013). At our study site in Rhode Island (USA), for example, tissue temperatures for a marine gastropod, the slipper limpet *Crepidula fornicata*, increased as quickly as 0.4°C min^{-1} for intertidal individuals, reaching temperatures more than 15°C warmer than those experienced by subtidal individuals living at the same site (Diederich & Pechenik 2013). Such stresses can impose severe energetic costs, in part by raising general metabolic rates and in part by inducing the expression of heat-shock proteins and thus diverting energy that might otherwise be available for growth and reproduction (Hofmann & Somero 1995, Willmer et al. 2000, Sokolova & Pörtner 2001, Dahlhoff et al. 2002, Petes et al. 2008, Han et al. 2013, Freuchet et al. 2015, Dong & Zhang 2016). Intertidal habitats are also subject to reduced salinities during heavy rains (Chaparro et al. 2008, Allen & Pechenik 2010), a condition that is also expected to reduce fecundity (e.g. Berger 2009). Not surprisingly, various intertidal barnacles, sea urchins, and mussel species that experience longer periods of aerial exposure have delayed reproduction or reduced reproductive output compared to conspecifics living lower in the intertidal zone or subtidally (e.g. Palmer 1980, Bayne et al. 1983, Page 1984, Borrero 1987, Byrne 1990, Petes et al. 2007, 2008). For example, Borrero (1987) found that not only did ribbed mussels *Geukensia demissa* living high in the intertidal zone have a delayed reproductive season compared to that of low-intertidal individuals, their gametic output was also reduced, by as much as ~60%.

How intertidal conditions impact gastropod fecundity has never been directly assessed. Calyptraeid gastropods are especially well-suited to such inquiry. Although juveniles and young males are mobile (Conklin 1897, Orton 1912a, Chaparro et al. 2001, Collin 2006), females are largely sedentary (Conklin 1897, Orton 1912a, Bohn et al. 2012) and live in stacks of individuals attached to solid substrates, including shells and rocks (Orton 1912a, Coe 1936, Chaparro et al. 2005, Collin 1995, 2006, Richard et al. 2006, Bohn et al. 2013a,b, Pechenik et al. 2015). Even the residence of males on stacks can be remarkably stable: in a paternity survey conducted on *C. fornicata* from the Bay of Brest (Brittany, France), 92% of the sires were still present on the stacks bearing their offspring, and indeed 1 male that had sired 1 brood had become a female on that same stack by the time of the sampling (Dupont et al. 2006). In short, adults — especially females — generally remain in one place.

In addition, all calyptraeid gastropods are protandrous hermaphrodites, so that the largest and oldest individuals are generally female and are found at or toward the bottom of each stack (Coe 1936, Collin 1995, 2006, Chaparro et al. 2005). Females deposit their fertilized eggs in a cluster of thin-walled egg capsules whose stalks join at a central peduncle that is attached to the solid substrate below (Conklin 1897, Collin 1995, Chaparro et al. 2005). This entire egg mass is brooded under the shell between the foot and neck of the female for approximately 20 to 30 d until the egg capsules open and the offspring emerge (Conklin 1897, Chaparro et al. 2005, Brante et al. 2008). Thus, by carefully prying females off the substrate, it is easy to document the numbers of embryos being brooded by individual females and how those embryos are packaged among egg capsules.

Moreover, calyptraeids are suspension-feeders (Orton 1912b) and are therefore unable to feed while emersed. Intertidal individuals thus have reduced daily feeding opportunities, limiting their ability to compensate for potential elevations of metabolic rate when experiencing elevated temperatures at low tide, and increasing the likelihood of reduced fecundity for intertidal individuals. Most importantly, since they are suspension feeders, both intertidal and subtidal residents found at any particular location will be exposed to the same food sources while submerged.

This study documents the consequences of intertidal life on several reproductive characteristics of 2 closely related (Collin 2003) calyptraeid gastropod species, viz. *Crepidula fornicata* and *Crepipatella peruviana* (formerly *Crepipatella fecunda*, Veliz et al. 2012). Females of both species can be found in substantial numbers both intertidally and subtidally (Chaparro et al. 2005, Bohn et al. 2012, 2013a,b, Diederich & Pechenik 2013, Diederich et al. 2015). *Crepidula fornicata* is native to the US east coast but is now a well-established invasive species in many other parts of the world (Chipperfield 1951, Blanchard 1997, Thieltges et al. 2003, Richard et al. 2006, Streftaris & Zenetos 2006, McNeill et al. 2010, Bohn et al. 2012, Doan et al. 2014), reaching population densities of thousands of ind. m^{-2} in some locations (Thieltges et al. 2004, Bohn et al. 2015). The other species included in this study, *Crepipatella peruviana,* ranges from Lima, Peru, south to Quitralco Fjord in Chile (45°S) (Chaparro et al. 2005). Fecundities for these 2 species have been estimated several times previously, but animals were collected either from the intertidal zone (e.g. Chaparro & Flores 2002) or subtidally (e.g. Proestou et al. 2008); no study has previously compared fecundities of intertidal and subtidal members of the same population for either species. In this study, we

also compared the number of egg capsules produced per brood, egg capsule sizes, and the numbers of embryos per egg capsule for intertidal and subtidal members of both species. Hereafter, we use the term 'fecundity' to mean the total number of embryos in an egg mass.

MATERIALS AND METHODS

Study site and sampling details

Crepidula fornicata

Stacks of *Crepidula fornicata* were collected at Bissel Cove in Narragansett Bay, Rhode Island (41° 32′ 50″ N, 71° 25′ 53″ W); collections were not made in the cove itself but on the portion of the coastline adjacent to the cove that faces the bay, which is subject to a diurnal tidal regime typical of much of the northeast coast of North America. At this site, *C. fornicata* is abundant both intertidally and subtidally (Diederich & Pechenik 2013, Diederich et al. 2015). All intertidal specimens were collected from ~0.5 m above (+0.5 m) the mean low lower water (MLLW) mark (hereafter, intertidal) and all subtidal specimens were collected from approximately 1 m below (−1.0 m) MLLW (hereafter, subtidal), a depth at which the substrate is never exposed to air. At this study site, the maximum low tide is about 0.37 m below MLLW, and the maximum high tide is about 1.64 m above MLLW. Intertidal and subtidal sampling sites were approximately 50 m apart. Brooding females were sampled 7 times over the course of 3 yr to examine egg mass characteristics: once in 2010, once in 2011, and 5 times in 2012, between 4 May and 15 September; in total, 71 brooding intertidal females and 51 brooding subtidal females were sampled. Females were also sampled between 13 April 2013 and 21 May 2013, to obtain additional data about the shell sizes of intertidal and subtidal brooders.

At our field site, temperatures may differ substantially between the 2 collecting areas when the tide is out: in a previous study (Diederich & Pechenik 2013), *Crepidula* biomonitors at this site exceeded 40°C in the intertidal zone in the summer of 2011, while subtidal biomonitors reached a maximum of only 27°C during the same time period. Freshwater input at Bissel Cove is typically low, and salinity levels do not fall below 27 psu (Welsh 1975, McKinney et al. 2001), especially on the bay side of the cove where we sampled (C. Diederich pers. obs.).

Crepipatella peruviana

Stacks of *Crepipatella peruviana* were collected during peak reproductive periods from Pelluco Beach, Puerto Montt, Chile (41° 29′ 28″ S, 72° 53′ 47″ W) in January 2012 (austral summer, n = 38 brooding intertidal females, 29 brooding subtidal females) and November 2015 (austral spring, n = 63 brooding intertidal females, 79 brooding subtidal females). All collections were made during low tide, with intertidal specimens being collected from the landward edge of their range and subtidal specimens being collected by divers at a depth of 3 to 4 m during low tide. Intertidal specimens were collected from approximately 1.5 m above MLLW, while subtidal specimens were all collected 2 to 3 m below MLLW, a depth at which animals are never exposed to air. At this study site, the maximum low tide is about 0.20 m below MLLW, and the maximum high tide is about 6.79 m above MLLW. Intertidal and subtidal sampling sites were approximately 60 m apart.

The intertidal and subtidal areas at this study site are both sandy, with numerous rounded rocks to which most of the slipper limpet specimens are attached.

Fecundity and related measurements

Crepidula fornicata

In order to determine the effect of habitat (intertidal or subtidal) on the reproductive output of *Crepidula*, egg masses (EMs) were collected intact from brooding females on each of the collection dates listed above. EMs were collected from all sampled brooding females in both subpopulations unless >50% of the sampled females were brooding, in which case the first 10 EMs were collected from each subpopulation (except for June 2010, when 19 brooding intertidal females were sampled).

On each sampling date, females were gently removed from their substrates so as not to damage the EMs, which remained attached to the substrate. Shell length determinations (to the nearest 0.1 mm) were made for all brooding females using calipers. EMs were carefully removed from the substrate at the peduncle-substrate attachment point with a pair of fine forceps and immediately preserved in 5% formalin buffered with sodium borate in seawater.

EMs were later examined in the laboratory using a dissecting microscope fitted with an ocular micrometer and separated into their constituent egg capsules;
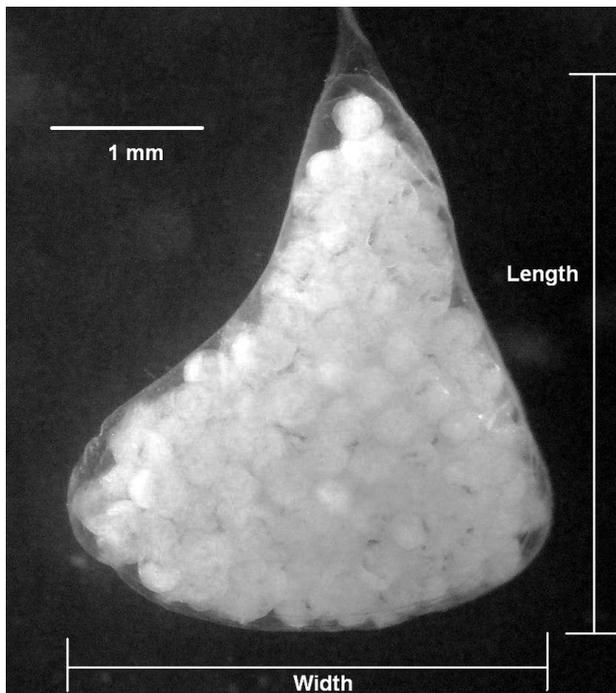
Fig. 1. Single egg capsule from an egg mass of *Crepidula for-nicata*. Capsule size was computed by measuring the length and width of each capsule and calculating the triangular area

all egg capsules were then counted. In 2012, the lengths and widths of 5 haphazardly selected egg capsules from each EM were determined at a magnification of 50×; we later estimated egg capsule sizes by computing the triangular surface area (Fig. 1).

In 2012, after dissecting all of the capsules in an EM, the embryos were mixed and the longest dimension was measured for 10 haphazardly chosen embryos, to see if intertidal and subtidal females produced embryos of similar size. Because the morphology of embryos changes markedly throughout development (see Henry et al. 2010), any analyses that we performed on embryo size were restricted to embryos in the earliest developmental stage, the morula. Finally, we determined the total number of embryos in each EM by opening all of the egg capsules in a brood and counting all of the embryos.

Crepipatella peruviana

EMs of *Crepipatella peruviana* were treated largely as described for *Crepidula fornicata*. However, rather than counting all of the eggs in every egg capsule within a brood, the number of egg capsules comprising each brood was determined, and the eggs or embryos were then counted from 5 represen-

tative capsules from each brood for a subset of the collected females (2012 subtidal, n = 29; 2015 sub-tidal, n = 57; 2012 intertidal, n = 38; 2015 intertidal, n = 49). This enabled us to estimate the number of embryos per brood for each sampled female. Capsules were photographed (software: QCapture Pro 6.0), and capsule areas were then estimated as described for *C. fornicata*, as an index of size.

Data analysis

The main goal of these studies was to compare the relative reproductive output of intertidal and subtidal snails. For both species, data examining the relationship between habitat and female reproduction parameters were analyzed using the linear model analysis in R (v3.3.1).

Five reproductive parameters were recorded for *Crepidula fornicata*: number of embryos per capsule, number of egg capsules per EM, total number of embryos per EM (fecundity), egg capsule size, and embryo size. Fecundity, embryos per capsule, and capsule size were all log-transformed before analysis to meet homoscedasticity assumptions of a linear model. All models included location (subtidal or intertidal), shell length, and dry tissue weight as predictors. Both dry tissue weight and shell length were included because individuals of a given length can vary in shell width and height. However, tissue weight had negligible impact on and was highly correlated with shell length (t_{120} = 49.1, p < 0.0001, r^2 = 0.95) and so was removed from further analyses. For parameters that were recorded over multiple years (fecundity, number of embryos per capsule, number of egg capsules per EM), year and an interaction between year and location were also included as predictors. All models also included a term for interaction between habitat and shell length. However, the interactions were removed if they were not statistically significant. As was done in some previous studies (e.g. Newell & Kofoed 1977, Honkoop & van der Meer 1998), individual fecundities in one analysis of our data from *C. fornicata* were adjusted by dividing by female shell length and then multiplying by the average sampled female shell length, 33.4 mm. This size adjustment allows the data to be compared with that from other studies; multiplying by average shell length also results in numbers within the range of what we actually observed. Marginal hypothesis tests were used to assess the significance of model terms, and model fits were assessed using QQplots and residual plots.

Three reproduction parameters were recorded for *Crepipatella peruviana*: number of eggs per capsule, number of egg capsules per EM, and fecundity (total number of embryos per EM). Data on fecundity and number of embryos per capsule were log-transformed to meet homoscedasticity assumptions of a linear model. We included location, shell length, and collection year in all models. Dry tissue weight was also included in initial analyses, but, due to negligible effects, it was later removed from all models except the one in which the response variable was fecundity. Length and dry tissue weight were not strongly correlated for this species ($t_{171} = 8.0$, p <

0.0001, $r^2 = 0.27$). We also removed 2 outliers from the analysis of fecundity. All models included an interaction between location and year, and an interaction between location and shell length. Interactions were removed if not significant. Marginal hypothesis tests were used to assess the significance of model terms, and model fits were assessed with QQplots and residual plots.

For both species, shell length distributions of intertidal and subtidal brooding females were compared using a *t*-test (corrected for unequal variances for *Crepidula*).

RESULTS

As described below, the relationship between tidal location and individual fecundity differed markedly for the 2 calyptraeid species included in this study.

Sizes of brooding females

EMs were found beneath females of *Crepidula fornicata* ranging in shell length from 16.4 to 44.6 mm (Fig. 2a). Although differences in mean shell lengths for intertidal (n = 89) and subtidal brooders (n = 89) were not significant in a simple *t*-test (corrected for unequal variances; $t = 1.76$, df = 153.9, p = 0.08), we note that 23.6% of brooding subtidal females were smaller than 25 mm in shell length, whereas only 3.4% of brooding intertidal females were below this size.

For *Crepipatella peruviana*, EMs were found beneath females ranging in shell length between 29.4 and 65.1 mm (Fig. 2b). There was no significant difference in mean shell sizes between the subtidal brooders (n = 86) and the intertidal brooders (n = 87) in a simple *t*-test ($t = 0.56$, df = 171, p = 0.58). Moreover, unlike the situation for *Crepidula fornicata*, small brooding individuals were similar in abundance at both the intertidal and subtidal sampling sites (shell lengths <40 mm: 18.8% subtidal, 26.4% intertidal).

Influence of tidal exposure on egg production and packaging by *Crepidula fornicata*

Fecundity of *Crepidula fornicata* increased substantially with increased female shell lengths for both intertidal and subtidal individuals (Fig. 3). Maximum fecundities found for *C. fornicata* in this study were
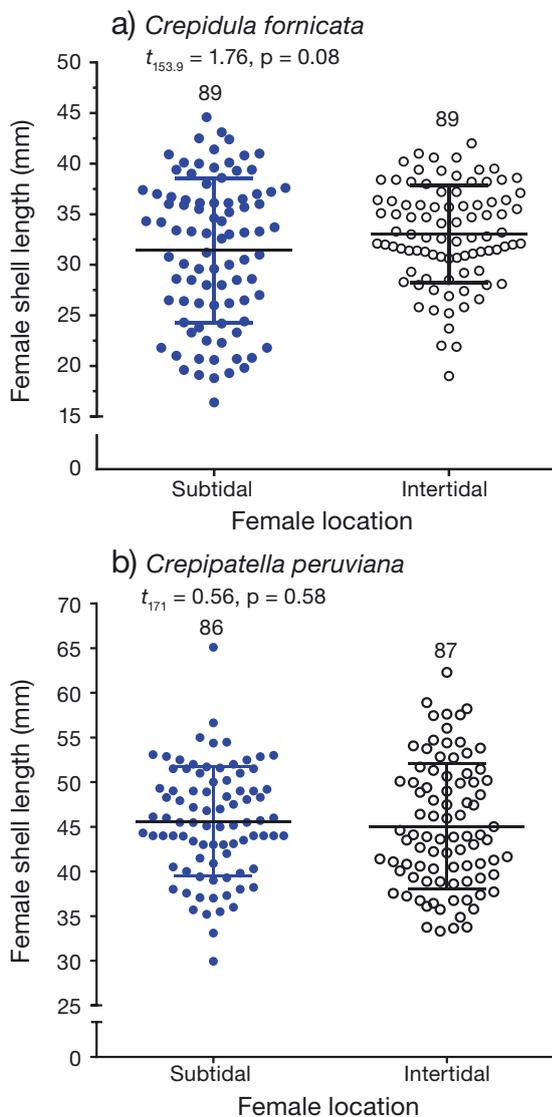

Fig. 2. Distribution of shell lengths for subtidal and intertidal females of (a) *Crepidula fornicata* and (b) *Crepipatella peruviana* bearing egg masses, for all samples collected in all years. The horizontal lines show the mean and SD for each dataset; numbers above each plot indicate sample sizes
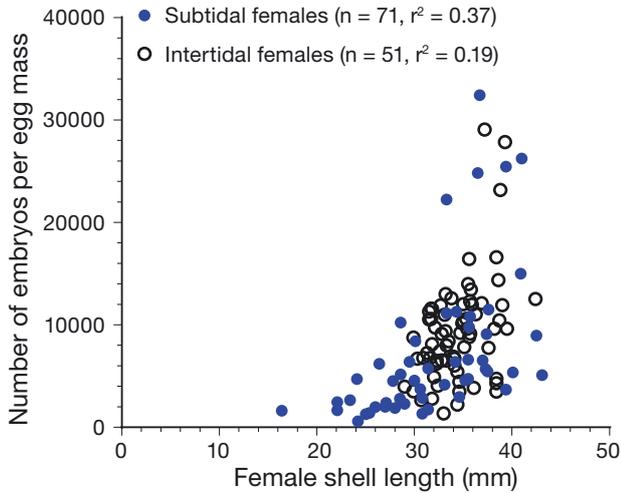
Fig. 3. Influence of shell length on the fecundity of intertidally and subtidally collected *Crepidula fornicata* from Bissel Cove in Narragansett Bay, Rhode Island (USA). The r^2 values were obtained through standard linear regression analysis

29 071 embryos for an intertidal female (shell length 37.2 mm) and 32 424 for a subtidal female (shell length 36.7 mm). The mean (± SE) fecundity for subtidal females was 7267 ± 1005 eggs (n = 51) while that for intertidal females was nearly 28% higher (9292 ±

Table 1. Statistical results relating shell length, location (intertidal vs. subtidal) and collection year (2012 vs. 2015) to various reproductive parameters for *Crepidula fornicata* in Bissel Cove in Narragansett Bay, Rhode Island (USA). The results shown are from marginal hypothesis tests. **Bold** indicates p < 0.05; EM: egg mass

Factor	F	p	n
(a) Eggs brood^{-1}			
Shell length	49.25	**<0.0001**	122
Location	5.54	**0.020**	
Year	0.89	0.35	
(b) Egg capsules EM^{-1}			
Shell length	17.84	**<0.0001**	122
Location	0.37	0.54	
Year	2.12	0.15	
(c) Embryos capsule^{-1}			
Shell length	58.89	**< 0.0001**	122
Location	10.16	**0.0018**	
Year	0.84	0.36	
(d) Capsule area			
Shell length	60.83	**< 0.0001**	73
Location	0.11	0.74	
(e) Embryo size			
Shell length	0.24	0.63	73
Location	0.69	0.41	

601.3 eggs, n = 71). Generalized linear modeling (GLM) analysis confirmed that fecundity increased with shell length (p < 0.001) and that intertidal females produced significantly more eggs per brood (p = 0.02) than did subtidal females (Table 1a).

Looking at the fecundity data for *Crepidula fornicata* by sampling month after standardizing for female shell length, intertidal females were again seen to produce significantly more embryos per EM than subtidal animals did in June 2010 (t_{36} = 4.13, p = 0.0002) and July 2012 (t_{17} = 3.17, p = 0.006; Fig. 4); the difference was also pronounced, but marginally insignificant, in August 2011 (t_9 = 2.06, p = 0.069; Fig. 4). Intertidal–subtidal comparisons could not be made in August or September of 2012 because none of the sampled subtidal females were brooding at that time, even though 30 to 40% of the intertidal individuals sampled were still brooding (Fig. 4). In only 1 case (May 2012) were size-adjusted differences in fecundity numerically lower for intertidal individuals; indeed, they were nearly always substantially numerically higher (Fig. 4). Note that all embryos were counted from each *C. fornicata* female in this study; thus these were not simply 'estimates' of fecundity.

The higher fecundities found for intertidal females of *C. fornicata* were not explained by differences in numbers of egg capsules per EM (Table 1b, Fig. 5a)
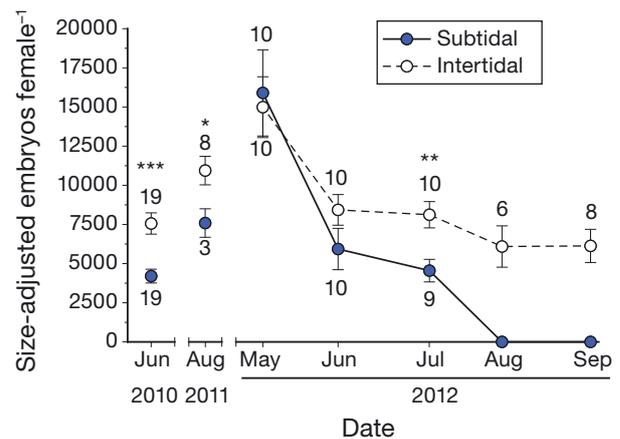

Fig. 4. Mean fecundity (embryos female^{-1}, ± SEM) of intertidal and subtidal *Crepidula fornicata* from Bissel Cove in Narragansett Bay, Rhode Island (USA), standardized by shell length. For shell-length standardization, the number of embryos per individual was first divided by female shell length and then multiplied by the average female shell length, 33.4 mm. Sample sizes are indicated above or beneath the symbols. Asterisks indicate significant differences in fecundity (*p < 0.1; **p < 0.01; ***p < 0.001; *t*-test). Statistical comparisons were not performed for August and September of 2012 because no subtidal females were found brooding on those dates
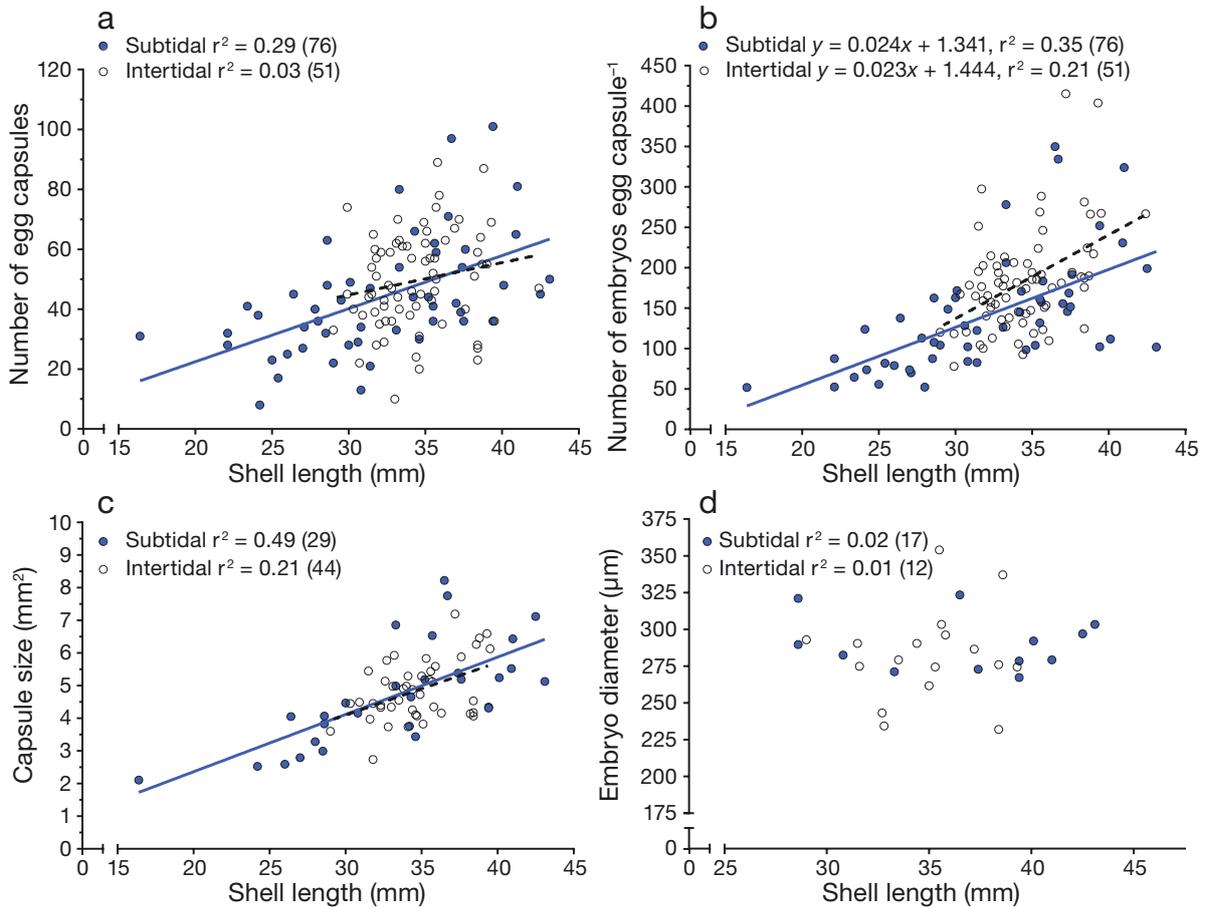
Fig. 5. Influence of female size on reproductive characteristics of *Crepidula fornicata* from intertidal and subtidal habitats at Bissel Cove in Narragansett Bay, Rhode Island (USA). (a) Total number of egg capsules in each egg mass. (b) Total number of embryos in each egg capsule. (c) Capsule size (surface area). (d) Embryo size (shell length). Details of the calculations are given in 'Materials and methods'

or egg capsule size (Table 1d, Fig. 5c). Rather, intertidal females packed significantly more embryos into each egg capsule ($p < 0.05$, Fig. 5b, Table 1c). We found no significant relationship between female size and early embryo size (Fig. 5d, $p = 0.63$), and no difference in the sizes of the early embryos found in intertidal and subtidal EMs ($p = 0.41$, Table 1e).

Reproduction of *Crepipatella peruviana*

Maximum estimated fecundities were 45 830 embryos brood[−1] intertidally (female shell length 54.7 mm) and 34 114 embryos brood[−1] subtidally (female shell length 45.1 mm). As with *Crepidula fornicata*, fecundity, the number of egg capsules per EM, the numbers of eggs per capsule, and egg capsule size all increased with female shell length in *Crepipatella peruviana* (Figs. 6 & 7; see Tables 2–4). In most other respects, however, results for *C. peruviana* differed

Table 2. Generalized linear modeling results from analyses investigating the effect of year, shell length, and tissue weight on the number of embryos per brood for intertidal and subtidal *Crepipatella peruviana* populations in Chile. Means and SEs were calculated from the raw data. Analyses were conducted on log-transformed data. **Bold** indicates $p < 0.05$

Factor	Mean (± SE)	F	n	p
Location		6.9		**0.009**
Subtidal	15019 ± 754 embryos		85	
Intertidal	14861 ± 1159 embryos		86	
Year		42.4		**<0.001**
2012	9155 ± 635 embryos		66	
2015	18575 ± 885 embryos		105	
Shell length (mm)	45.3 ± 0.5	10.6		**0.001**
Tissue weight (g)	0.87 ± 0.03	14.7		**<0.001**

markedly from those obtained for *Crepidula fornicata*. For one thing, tissue weight had a strong influence on fecundity (Table 2), but, most importantly,
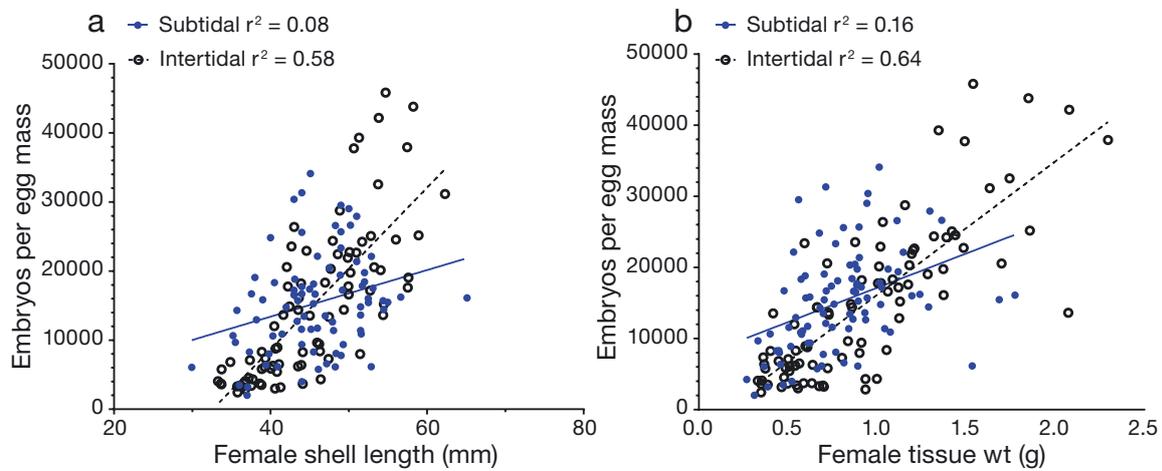
Fig. 6. Relationship between habitat (intertidal vs. subtidal) and fecundity for brooding females of *Crepipatella peruviana* from Chile as a function of (a) female shell length and (b) female dry tissue weight
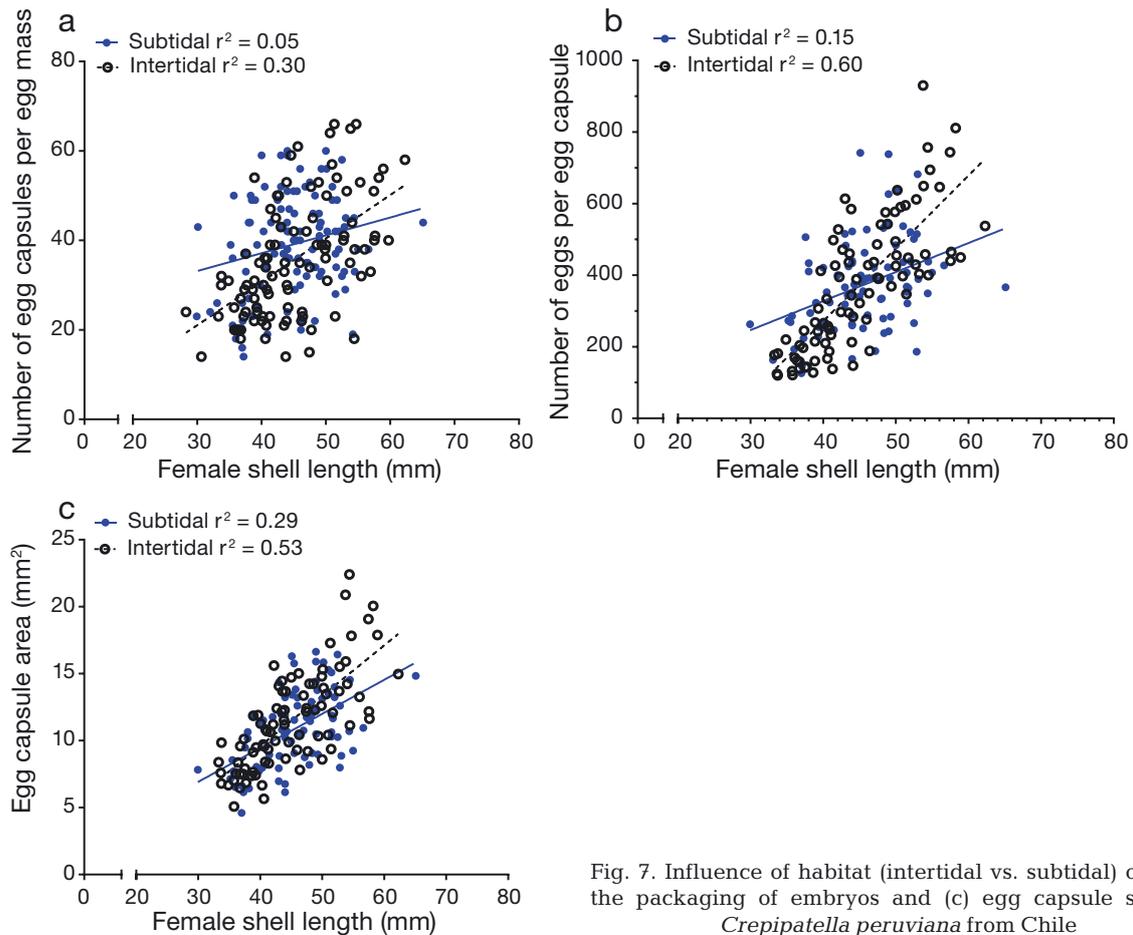

Fig. 7. Influence of habitat (intertidal vs. subtidal) on (a,b) the packaging of embryos and (c) egg capsule size for *Crepipatella peruviana* from Chile

the fecundity of intertidal females was significantly less than that of subtidal individuals for both years sampled (Table 2).

There was a strong trend towards intertidal females brooding fewer egg capsules per EM than the subtidal females (p = 0.053, Table 3). The number of embryos per capsule was not influenced by habitat in 2015 (Table 4); however, in 2012 intertidal females produced significantly fewer embryos per capsule than did subtidal females.

Table 3. Generalized linear modeling results from analyses investigating the effect of year and shell length on the number of capsules per egg mass of intertidal and subtidal *Crepipatella peruviana* populations in Chile. **Bold** indicates significance (p < 0.05)

Factor	Mean (± SE)	*F*	n	p
Location		3.8		0.053
Subtidal	39 ± 1.0 capsules		108	
Intertidal	36 ± 1.3 capsules		101	
Year		17.6		**<0.001**
2012	31 ± 1.0 capsules		67	
2015	40 ± 1.1 capsules		142	
Shell length (mm)	44.9 ± 0.5	34.2		**<0.001**

Table 4. Generalized linear modeling results from analyses investigating the effect of year, shell length, and tissue weight on the number of embryos per egg capsule for sampled intertidal and subtidal *Crepipatella peruviana* populations in Chile. Means and SEs were calculated from the raw data. Analyses were conducted on log-transformed data. **Bold** indicates significance (p < 0.05). Asterisk indicates significantly different group in the interaction

Factor	Mean (± SE)	*F*	n	p
Location		0.09		0.77
Subtidal	373 ± 13.7 embryos		86	
Intertidal	373 ± 19.8 embryos		87	
Year		51.9		**<0.001**
2012	273 ± 13.1 embryos		67	
2015	437 ± 14.8 embryos		106	
Shell length (mm)	45.3 ± 0.5	62.7		**<0.001**
Location:Year	Subtidal 2012: 339 ± 17.8	4.1		**0.04**
	Subtidal 2015: 391 ± 18.3			
	Intertidal 2012: 222 ± 14.0*			
	Intertidal 2015: 490.4 ± 21.7			

Table 5. Comparison of reproductive characteristics of *Crepidula fornicata* and *Crepipatella peruviana* with prior information from the literature

Study	Brooder shell length (mm)	Capsules brood^{-1}	Embryos capsule^{-1}	Eggs brood^{-1}
Hoagland (1986)				
C. fornicata	15–55	25–75	80–300	~2000–22500
C. peruviana	34–65	30–75	200–1200	~6000–90000
Chaparro & Flores (2002)				
C. fornicata	–	–	–	–
C. peruviana	28–56.3	?–69	~400–1200	~15000–60000
Proestou et al. (2008)				
C. fornicata	~16.5–41	15–70	50–450	~1000–26000
C. peruviana	–	–	–	–
This study				
C. fornicata	16.4–44.6	10–100	~50–400	590–32424
C. peruviana	28.3–68.2	~15–65	~100–900	2019–45830

DISCUSSION

Previous studies have found brooding females ranging in size from about 15 to 55 mm in shell length for *Crepidula fornicata* (Hoagland 1986, Collin 1995) and about 28 to 56 mm for *Crepipatella peruviana* (Chaparro et al. 2005). For *Crepidula fornicata* in our study, the smallest brooding intertidal females tended to be larger than the smallest brooding subtidal females, suggesting either that intertidal males may change sex at a larger size than subtidal males, or that intertidal females may need to reach a larger size before depositing their first EM, something that could be examined in future studies. This trend was not observed in our samplings of *C. peruviana.*

Fecundity at both of our study sites increased substantially with female size in accordance with prior data for both *Crepidula fornicata* (Collin 1995, Richard et al. 2006, Proestou et al. 2008) and *Crepipatella peruviana* (Chaparro & Flores 2002). Similarly, the numbers and sizes of the egg capsules also increased with female size. This is not surprising, since females brood their encapsulated embryos in the mantle cavity, which increases in size as the animals grow, providing larger females the space to brood a greater number of developing offspring.

The relationship between female shell length and fecundity that we found for *Crepidula fornicata* is nearly identical to that found by Proestou et al. (2008) at least 5 yr earlier at a site several miles to the south of our Rhode Island study site. Additionally, the average reproductive output of *C. fornicata* at our study site in Rhode Island was similar to the maximum reproductive output recorded for an invasive population of the same species in the Bay of Brest, France (Richard et al. 2006). Although the shell lengths of brooding individuals in this study were similar to those reported in previous studies, our study expands somewhat the maximum reported numbers of egg capsules per EM and embryos per brood (Table 5). Our general results for *Crepipatella peruviana* also agree well with prior data, although the maxi-

mum number of embryos per capsule found in our study was lower than what has been reported in prior studies (Table 5).

Both species examined in this study exhibited habitat-related differences in egg production, with intertidal and subtidal females generally producing a different number of offspring per brood than subtidal females. However, those characteristics differed remarkably in the 2 species. For *Crepidula fornicata*, intertidal females had a significantly greater size-adjusted reproductive output than subtidal females, whereas for *Crepipatella peruviana*, the opposite was true: intertidal females overall had a significantly lower reproductive output. Although we are not aware of other studies that have compared reproductive outputs of field-collected intertidal and subtidal members of the same species, several studies have looked at relative reproductive output at the extremes of a species' range, where physical stresses are likely to be greater (Lester et al. 2007). For the sea urchin *Strongylocentrotus purpuratus*, reproductive output (estimated by measures of gonadal index) at the southern edge of its range was generally greater than that farther north (Lester et al. 2007). Similarly, reproductive output for *Crepidula fornicata* was higher near the northern edge of its current range in Norway than nearer the middle of its range in Rhode Island (Pechenik et al. in press). As discussed by Lester et al. (2007), physical conditions are apparently not always the primary determinants of reproductive output.

As noted in the 'Introduction', however, the reduced reproductive output that we documented for intertidal individuals of the Chilean species is not surprising, as individuals are generally at a distinct energetic disadvantage when living intertidally (e.g. Palmer 1980, Bayne et al. 1983, Page 1984, Borrero 1987, Byrne 1990, Franz 1997, Petes et al. 2007, 2008), particularly for suspension-feeders like the calyptraeid gastropods studied here, which are unable to feed while emersed during low tides. In no case did we find intertidal individuals of this Chilean species producing larger broods than were produced by subtidal individuals of comparable size. Similarly, in a mesocosm study with the bivalves *Cerastoderma edule* and *Macoma balthica* held under subtidal and intertidal conditions, egg production was reduced for females experiencing tidal simulations at a low temperature; the differences disappeared, however, when females were maintained at a slightly higher temperature (Honkoop & van der Meer 1998).

Results for *Crepipatella peruviana* differed in a few interesting ways between the samplings made in the 2 different years: intertidal individuals of this species produced significantly fewer embryos per capsule than did subtidal snails in 2012 but not in 2015 (Table 4). Females from both habitats also produced fewer egg capsules in 2012 (Table 3). The 2012 samples were collected during the Chilean summer (January), while the 2015 samples were collected in the Chilean spring (November). As females of this species do not reproduce during the Chilean winter (Chaparro et al. 2005), energy storage may be especially high at the start of the next reproductive season. In addition, the impact of physical stress on the reproductive output of intertidal individuals in this species may be greater during the summer; if so, this would suggest that the inability to feed during low tides is not the primary factor accounting for the decreased fecundity of intertidal individuals documented for the Chilean summer of 2012. Females of this species are known to produce at least 3 broods yr^{-1} over approximately 10 mo (Chaparro et al. 2005); future studies could examine how reproductive output of subtidal and intertidal females changes with brood number.

We also recorded interesting seasonal variation in the reproductive patterns for *Crepidula fornicata*. At the start of the breeding season in May of 2012, individual size-adjusted fecundities were about double what they were later in the summer, for both intertidal and subtidal individuals. If brood size is determined by the amount of energy reserves stored by the female, a large input of phytoplankton in the spring, coupled with relatively low temperatures and correspondingly low metabolic rates, may contribute to such high spring fecundity in both zones, something that could be examined in future studies.

Although sample sizes were relatively small, the reproductive season for *C. fornicata* also seems to have lasted for a longer time for intertidal individuals: females of *C. fornicata* harboring EMs were collected well into September for intertidal individuals, but not beyond July for sampled subtidal individuals (Fig. 4). More detailed studies comparing duration of the reproductive seasons for intertidal and subtidal individuals of both species seem warranted. Future studies could also investigate the number of broods per female per year for intertidal and subtidal populations of both species, and perhaps consider the quality of the larvae produced.

Since intertidal and subtidal females were only meters apart, it is unlikely that phytoplankton quality or concentration differed among the habitats; only food access time would have differed, as intertidal individuals will have had less time each day to feed.

Intertidal *C. fornicata* may overcome this feeding-time disadvantage with their significantly larger gills (Diederich et al. 2015), giving them a larger food-collecting surface compared with that of subtidal individuals. In contrast, the gills of intertidal and subtidal *Crepipatella peruviana* showed no morphological differences, and in fact the clearance rates of intertidal individuals were significantly lower than those recorded for subtidal individuals (Diederich et al. 2015). In addition, *Crepidula fornicata* is relatively eurythermal (Diederich & Pechenik 2013), and intertidal temperatures may not induce a costly stress response for adults. Indeed, elevated temperatures during prolonged exposure to air are known to decrease metabolic rate in some intertidal species (Sokolova & Pörtner 2001, Marshall & McQuaid 2011), allowing individuals to conserve energy. This possibility has not yet been examined for either of the species included in the present study, or indeed for any other calyptraeid gastropod. Increased intertidal temperatures may positively affect food collection rates, gut clearance times, and absorption and/or assimilation efficiencies, all of which may contribute to the relatively high fecundity that we observed, issues that should be fruitful areas of study in the future (Bayne & Scullard 1978, Griffiths 1981, Dam & Peterson 1988, Sobral & Widdows 1997, Sanford 2002, Yamane & Gilman 2009). Valdizan et al. (2011) showed that rising coastal seawater temperatures have increased the reproductive output in an invasive European population of *C. fornicata.* In addition, nothing is yet known about the effect of elevated temperatures on protein damage in these species, or the ability of any calyptraeid gastropod to produce heat shock proteins in response to stress, or the minimum environmental conditions that would initiate such production in these species. Energy allocated to the production of heat shock proteins or to repairing heat-damaged proteins is energy that is no longer available for egg or egg capsule production (Hofmann & Somero 1995, Han et al. 2013).

Although, on average, intertidal *C. fornicata* females produced more embryos per female than did subtidal females, this was mediated by neither an increase in the number or size of egg capsules, nor by a decrease in the average size of embryos. Instead, a higher fecundity was achieved in intertidal females by their crowding each egg capsule with a significantly greater number of embryos. This is surprising, in that related species (*Crepipatella dilatata* and *C. peruviana*) invested only 2 to 8% of their reproductive energy into egg capsules (Chaparro et al. 1999, Chaparro & Flores 2002); thus the production of addi-

tional egg capsules should not be especially costly for calyptraeid gastropods.

At some density of embryos within a capsule, oxygen constraints on over-crowded embryos should shift the selective advantage toward production of more egg capsules rather than increasing the number of embryos per capsule (Perron & Corpuz 1982). Indeed, many species with encapsulated embryos, including *Crepidula fornicata*, experience hypoxic or anoxic conditions within egg capsules, particularly in late pre-hatching development (e.g. Cancino et al. 2000, Lardies & Fernández 2002, Moran & Woods 2007, Brante et al. 2008, Chaparro et al. 2009). Although *C. fornicata* larvae may be sensitive to hypoxia after hatching (Brante et al. 2008), encapsulated embryos are particularly tolerant of very low oxygen conditions (Brante et al. 2008, 2009). Thus, it follows that *C. fornicata* females should be able to crowd egg capsules with hypoxia-tolerant embryos to maximize fitness and the efficiency of reproductive energetic expenditure (Fig. 5b). How calyptraeid gastropods regulate the number of eggs deposited within each egg capsule is not known.

It should be noted that our research was conducted at only a single study site for each species. Future studies could compare egg production characteristics over a range of study sites, with careful measurements being taken of both subtidal and intertidal environmental characteristics. Laboratory studies might also be undertaken with these or similar species, to examine the impact of temperature, food availability, food quality, and other relevant environmental factors on reproductive output.

In summary, our results suggest that females of *Crepidula fornicata* are far better adapted to reproducing intertidally than are females of the related Chilean species *Crepipatella peruviana*, even though the intertidal *Crepidula fornicata* females that we sampled in Rhode Island are living near the upper limits of their thermal tolerance (Diederich & Pechenik 2013). Indeed, not only was reproductive output significantly greater for intertidal individuals of *C. fornicata* in our study, but the duration of the reproductive season seems to have been longer as well (Diederich 2014), adding additional support to previous indications (Diederich & Pechenik 2013, Diederich et al. 2015) that *C. fornicata* is especially well-adapted for intertidal life. In a sense, intertidal individuals of *C. fornicata* may be compensating reproductively for the higher mortality exhibited by intertidal recruits that was recently documented by Bohn et al. (2013a). In any event, although physical stressors associated with intertidal life (e.g. temperature stress, desiccation,

and fluctuating salinity) may ultimately control the upper distribution of this species (Bohn et al. 2013a,b), they do not appear to diminish its reproductive output, in contrast to their apparent impact on the reproduction of *Crepipatella peruviana*. Its resilience in the face of such stressors is a likely factor in the demonstrated ability of *Crepidula fornicata* to colonize new environments and to become such a remarkably successful invasive species outside of the western Atlantic Ocean (e.g. Blanchard 1997, Thieltges et al. 2004, Richard et al. 2006, Bohn et al. 2012). We suspect that our data on the native reproduction of this species will be useful in informing future studies on the success of this species as it continues to spread, and in predicting its fate in native habitats at the margins of its geographic boundaries in the face of climate change. How will global warming affect the reproductive seasonality and output of these intertidal calyptraeids? At what temperature will the reproductive advantage documented here for intertidal *C. fornicata* begin to be compromised? And at what point will reproduction no longer be possible for intertidal populations of *Crepipatella peruviana*? In addition, which particular environmental stress — or combination of stresses — accounts for the relatively greater (*Crepidula fornicata*) or lessor (*Crepipatella peruviana*) fecundity of intertidal individuals documented in this study? Answering that question will help us to understand some of the changes that we will likely see in future calyptraeid population sizes and geographical distributions with continued global climate confusion over the coming decades, as air and water temperatures continue to increase and as precipitation patterns continue to change (Burrows et al. 2011, Madsen et al. 2014, Oczkowski et al. 2015), and as phytoplankton species compositions and nutritional content continue to change as well (Rossoll et al. 2012, Leu et al. 2013, Wynn-Edwards et al. 2014).

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