Vol. 643: 87–97, 2020
https://doi.org/10.3354/meps13354

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published June 11

Carryover effects of brooding conditions on larvae in the slipper limpet *Crepidula fornicata*

Kirstin Meyer-Kaiser*

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

ABSTRACT: Larval dispersal is a critical step in the life-histories of sessile benthic invertebrates. There is a growing body of research showing plasticity in marine invertebrate larvae, but the causes and ranges of intraspecific variation in larvae are not completely understood. In this study, field-based collections of *Crepidula fornicata* larvae in 2017 motivated a laboratory experiment on carryover effects in 2019. Experimental conditions that approximated environmental conditions experienced by mothers in the field were used to test whether seasonal environmental variations during brooding could lead to differences in larval size and the time to develop to competency. Mothers were kept in 2 different temperature and feeding treatments during brooding, but larvae were cultured in a common garden. Larvae that were brooded at spring temperatures (~13°C) took longer to develop to competency in the common garden and grew larger before becoming competent than larvae brooded at warmer summer temperatures (~21°C). There was no effect of maternal feeding (fed or not fed) on time to develop to competency or larval size. Thus, *C. fornicata* larvae released earlier in the year are likely to spend longer periods in the water column. They may disperse farther and grow to larger size before settlement. *C. fornicata* is a model species for larval biology. The results of this study can be used to inform biophysical modelling efforts and refine predictions of connectivity or species range shifts in a changing climate.

KEY WORDS: Invertebrate · Dispersal · Competency · Invasive species · Brooding · Mollusc

1. INTRODUCTION

For sessile marine invertebrates, the larval phase represents the main opportunity for dispersal to new environments. Studying population connectivity and predicting how species ranges may shift in a warming climate requires a refined understanding of factors influencing larval dispersal. One major parameter that limits larval dispersal is the pelagic larval duration (PLD), or the amount of time a larva spends in the water column before settlement and metamorphosis (Pineda et al. 2007). Generally speaking, species with longer PLD will disperse farther, though larval behaviors such as remaining near the seafloor can limit dispersal distance (Shanks 2009).

In many cases, PLD is treated as a static, species-specific parameter (Metaxas & Saunders 2009), but there is a growing understanding that the larval phase is plastic. PLD varies as a result of temperature (Lima & Pechenik 1985), feeding conditions for planktotrophic larvae (Welch & Epifanio 1995, Bertram & Strathmann 1998, Hentschel & Emlet 2000, Howard & Hentschel 2005), and maternal yolk investment for lecithotrophic larvae (Marshall & Keough 2003a). Many larvae can delay metamorphosis if a suitable habitat is not available, sometimes for years in laboratory conditions (Strathmann & Strathmann 2007). Some species, especially bivalves and echinoderms, engage in post-larval drift, effectively extending their PLD (Lane et al. 1985, Sumida et al. 1998). More recent studies have shown that some larvae can rapidly develop to competency in the presence of settlement cues (Gaylord et al. 2013) or engage in settlement behavior when pre-competent, suggesting an

*Corresponding author: kmeyer@whoi.edu

ability to abbreviate the larval period (Meyer et al. 2018). Poecilogony also leads to intraspecific variation in PLD (Gibson & Gibson 2004).

Environmental conditions experienced during one life stage can carry over into the next. This can occur within one generation, as stressful conditions such as low food, low pH, and delayed metamorphosis during the larval phase affect mortality and growth of juveniles (Phillips 2002, 2004, Marshall & Keough 2003b, 2004; reviewed by Pechenik 2006), fecundity of adults, and the connectivity of invertebrate populations (Marshall et al. 2003a, 2010). Carryover effects also occur between generations, and this transgenerational plasticity can be an adaptation to adverse environmental conditions, such as ocean acidification (Wong et al. 2018, Clark et al. 2019; reviewed by Ross et al. 2016). There has been less research on carryover effects when mothers have experienced normal, present-day conditions, such as seasonal variations. Carryover effects have previously been demonstrated under normal environmental conditions with respect to nutrition in echinoid larvae (Bertram & Strathmann 1998) and temperature in bryozoan larvae (Burgess & Marshall 2011), but our understanding of maternal effects in marine invertebrate larvae is far from complete.

The present study aimed to explain how normal seasonal variations contribute to plasticity in larvae of the slipper limpet *Crepidula fornicata*. This species reproduces in the spring and summer months, with gametogenesis beginning in February and females brooding egg masses through August or September (Valdizan et al. 2011, Bohn et al. 2012, Pechenik et al. 2017). Such a long reproductive period means that brooding females are exposed to wide ranges of temperature and primary productivity, which may have carryover effects on their larvae. Using experimental conditions similar to those *C. fornicata* adults may experience during the reproductive season in the field, carryover effects of brooding temperature and maternal feeding on larval size and time to develop to competency were analysed. The intention was to quantify plasticity that might occur naturally in the field rather than to push individuals to their tolerance limits.

C. fornicata is the ideal organism for carryover effect studies on pelagic larvae because its larvae are brooded for a period and then released to undergo further development and dispersal in the water column. Egg masses are brooded in the mantle cavity of the mother, with larvae hatching and entering the water column a few weeks later. This species is easily kept in culture with minimal mortality (reviewed by Pires 2014, Pechenik 2018) and it is a model organism for larval biology (Henry et al. 2010, Henry & Lyons 2016). Using *C. fornicata*, answers to the following questions were sought: (1) How do brooding temperature and maternal feeding affect the time to develop to competency? (2) How do brooding temperature and maternal feeding affect larval size? (3) Is there a relationship between larval size and time to develop to competency?

2. MATERIALS AND METHODS

2.1. Monitoring *Crepidula fornicata* larval abundance

In 2017, abundances of *Crepidula fornicata* larvae were observed throughout the summer months using larval traps. These collections motivated the subsequent laboratory experiment in 2019 to explore carryover effects of brooding conditions on larvae. Cylindrical larval traps were constructed from 3 stacked, 50 ml centrifuge tubes, using the conical portion of the tubes to create internal funnels following Todd (2003). Traps were filled with 20% dimethyl sulfoxide in water saturated with NaCl and deployed in PVC housings (following C. M. Young unpubl. data) along with temperature loggers (HOBO Tidbit v2, Onset; set to record temperature every 15 min) and suspended under floating docks at 2 locations in Woods Hole, MA, USA: Eel Pond (41° 31′ 29″ N, 70° 40′ 11″ W) and Great Harbor (Woods Hole Oceanographic Institution [WHOI] pier, 41° 31′ 25″ N, 70° 40′ 19″ W). Traps were recovered every 2 wk in an alternating schedule (i.e. Eel Pond trap recovered one week, WHOI pier trap recovered the following week) from late March to early August 2017. Following recovery, traps were emptied, and all captured *C. fornicata* larvae were counted by hand using a dissecting microscope.

2.2. Carryover experiment

2.2.1. Adult collection and culturing

Adult *C. fornicata* were collected from Little Harbor, Wareham, MA, USA (41° 43′ 32″ N, 70° 40′ 27″ W), under Massachusetts Division of Marine Fisheries Scientific Collection Permit no. 17847 to K.M.-K. Stacks were collected by hand from the shallow subtidal by wading at low tide, and wrapped in *Codium* algae for transport to WHOI. Water temperature was measured in the field during collection, and adults

were kept in flow-through seawater tanks at field temperature for >24 h to acclimate to laboratory conditions before the onset of experimental treatments. Adults were collected on 2 May (placed in treatments on 6 May) and 22 June 2019 (placed in treatments on 23 June) for 2 runs of this experiment (n = 20 females per run), but some females failed to release larvae during the experimental period and were discarded. A total of 17 females released larvae in Run 1 (May), while 8 females released larvae in Run 2 (June). Three of these broods died shortly after release, giving a total of 16 larval cultures (n = 3–5 in each treatment) for Run 1 and 6 cultures (n = 1–2 in each treatment) for Run 2 (Fig. 1).

Adults and larvae were cultured in WHOI's Environmental Systems Laboratory. Flow-through seawater tables were used as water baths. Adults were kept at 13°C (mean ± SE: 13.67 ± 0.13°C, hereafter 'cool') and 21°C (21.35 ± 0.13°C, hereafter 'warm'), representing field conditions experienced by brooding *C. fornicata* females in spring and summer, respectively (see Fig. 2) (Diederich & Pechenik 2013). The bottom female was separated from each stack and kept along with her substratum (empty shell or small stone) in a clean 2 l glass jar filled with 1.5 l filtered seawater. Water in adult culture jars was obtained from seawater lines at the appropriate experimental temperature to avoid thermal shock, filtered to 5 µm, changed every third day, and lightly bubbled with air throughout the experiment. Females were randomly assigned to 'warm' and 'cool' temperature treatments (n = 10 females per temperature treatment) and 'fed' or 'not fed' treatments (n = 10 females per feeding treatment) for a 2-way crossed experimental design (Fig. 1). 'Fed' females were given 100 µl Shellfish Diet 1800 (Reeds Mariculture) every day, or 1.3×10^5 cells ml^{-1}. This concentration is within one order of magnitude of phytoplankton concentrations measured by Turner et al. (2009) in Buzzards Bay, MA, during the summer months ($0.5–2.5 \times 10^5$ cells ml^{-1}). It is difficult to compare food availability between lab and field conditions, as cells were cleared from the jars by adults each day. The intent was to approximate phytoplankton concentrations in the field, though a more realistic simulation would include a constantly replenished food supply. 'Not fed' females were not given any additional food and could only feed on small particulates (<5 µm) in the culture jars, simulating early spring (pre-bloom) conditions. Individuals in both treatments produced fecal matter (potentially including pseudofeces and true feces).

Many *C. fornicata* females released larvae after a few days in captivity, with similar proportions across

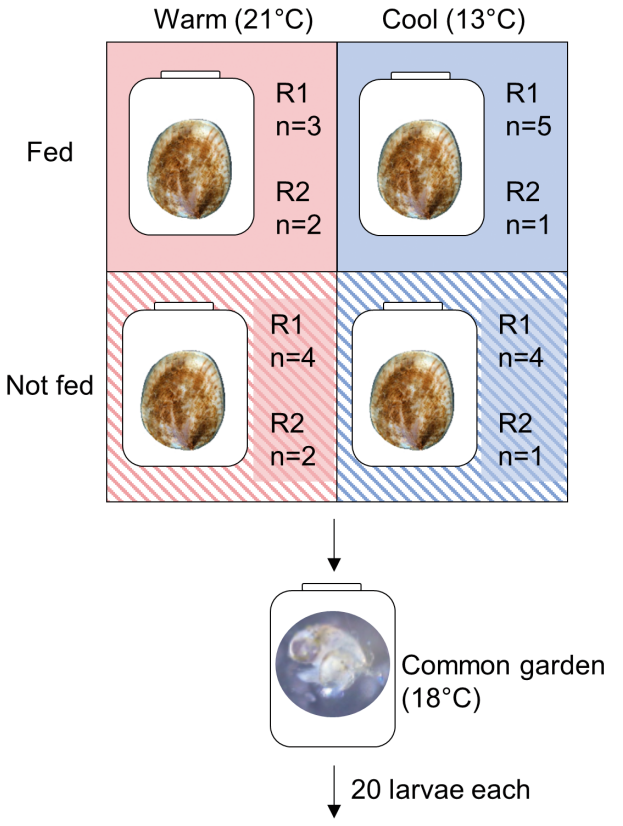

Fig. 1. Experimental design for the carryover experiment with brooding and larval *Crepidula fornicata*. Mothers were kept during the brooding period in temperature (warm or cool) and feeding (fed or not fed) treatments. After release, cultures of 400 larvae from each mother were kept in a common garden and sub-sampled for competency tests using 20 mM KCl every 2 d and size measurement every 4 d. R1: first run of the experiment (started 6 May 2019); R2: second run of the experiment (started 23 June 2019); n: number of replicates

treatments (40–60%). These first larval releases were discarded because it was assumed the mother had experienced field conditions rather than experimental conditions for the majority of the brooding period. Females that released larvae early were retained until a full brooding period had been completed in captivity. Because they had been separated from accompanying males, the second releases were most likely fertilized using stored sperm (Dupont et al. 2006). The date of each larval release was recorded, and for mothers that released 2 broods in captivity,

the time between releases was calculated as the minimum brooding time at the given experimental temperature. After the final larval release, all females were frozen at –20°C for temporary preservation, then later removed from their substrata to assess whether a new egg mass had been deposited.

2.2.2. Larval culturing conditions

Larvae released after >1 wk in captivity were retained and cultured in a common garden flow-through seawater table at 18°C (17.86 ± 0.12°C) (Fig. 1). This temperature was chosen because it is non-stressful to *C. fornicata* larvae (Pechenik & Lima 1984, Lima & Pechenik 1985), was between the temperatures experienced by cultured adults (to simulate well-mixed coastal waters), and approximated the sea surface temperature of Buzzards Bay during June, when *C. fornicata* larvae were most abundant in the water column (see Fig. 2) (Fisher & Mustard 2004).

A larval culture consisted of 400 larvae from a single mother in 0.2 µm filtered, UV-sterilized seawater (hereafter FSW) in a clean 2 l glass jar. Jars were cleaned by manually scrubbing inside and outside surfaces with a dish scrubber under running warm tap water, rinsed twice with FSW, and pre-filled to 1.5 l with FSW. Larvae were counted by hand and added to the culture jar with a Pasteur pipette. Water in larval culture jars was changed every 2 d following Pechenik & Heyman (1987), with larvae being gently removed from the jar via reverse filtration, then transferred to a clean, pre-filled jar. Larvae were fed live *Isochrysis galbana* after each water change at a concentration of 1.2–1.5 × 10^5 cells ml^{-1} (1.41 ± 0.04 × 10^5 cells ml^{-1}), following Pires et al. (2000).

2.2.3. Data collection

Larvae were cultured and sub-sampled throughout the culturing period to assess competency and size (Fig. 1). Size was measured every 4 d, and competency to metamorphose was assessed every 2 d beginning when the larvae were 8 d old. Because different females released larvae on different days, age was tracked separately for each larval culture. A total of 20 larvae were sampled on the day of release (Day 0) and on Day 4 for size measurement. Subsequently, on Days 8–28, a total of 20 larvae were removed from the culture every 2 d and exposed to a solution of 20 mM excess KCl in seawater to assess competency to metamorphose (following Pechenik & Heyman 1987). Metamorphosis was defined as complete loss of the velum (Pechenik & Heyman 1987). KCl tests were conducted for 24 h at 18°C by floating a 6 well plate on the surface of the seawater table. Larvae were retained for size measurement every 4 d (Days 8, 12, 16, etc.) and frozen at –20°C for temporary preservation. Larval size was measured using an ocular micrometer in conjunction with a dissecting microscope, measuring from the origin of the whorl to the shell brim with a larva laying on its side (Pechenik & Lima 1984).

Many larval cultures in the cool treatment did not reach 100% competency (defined as 100% metamorphosis of a sub-sample exposed to 20 mM KCl for 24 h) during the experiment (in 26 d). Some adults never released larvae in captivity, especially in the second run of the experiment. These factors, in combination with the difficulty in maintaining clean larval cultures for long periods, led to lower than the expected replicates (n = 40; Fig. 1, Table 1). The present study only reports data for time-points that had ≥3 replicates in a given treatment.

2.3. Data analysis

The experimental design in this study can best be described as a repeated-measures (RM) design, because larvae used for competency tests and size measurements were sub-sampled from cultures repeatedly over time. For statistical analysis, each larval culture was treated as a replicate 'subject' and runs of the experiment as blocks. RM-ANOVA tests were used to test for significant differences in larval size and percent competence between treatments. In order to determine whether there was any significant interaction between runs of the experiment and treatments, RM-ANOVA was first conducted using 'run' and 'run × treatment' as fixed block factors. The interactions of run × treatment were not significant ($p > 0.05$), so each RM-ANOVA was re-ran with run as a fixed block factor but without the interaction, following the recommendations of Quinn & Keough (2002) for multiple runs of an experiment separated by time. Mauchly's tests were used to test for sphericity, and if the data did not fit this assumption, a Greenhouse-Geisser adjustment was used for the df and p-values. Results were also confirmed with a non-parametric Friedman's test if the assumption of sphericity was violated. For post hoc tests, pairwise RM-ANOVA tests were performed with a Bonferroni correction for α (0.05 / 6 = 0.008).

Table 1. Timeline of larval *Crepidula fornicata* cultures. Adults that never released larvae or only released larvae after a few days in captivity were excluded. Run 1 adults were placed in experimental treatments on 6 May; run 2 adults on 23 June. –: no early larval release; these individuals only released larvae once in captivity, and these larvae were used in the experiment

Maternal treatment	Replicate no.	Early larval release	Larval release for experiment	Culture terminated (age in days)
Run 1				
Warm, not fed	2	8 May	20 May	9 June (20)
	3	8 May	27 May	16 June (20)
	4	–	23 May	12 June (20)
	5	8 May	20 May	9 June (20)
Warm, fed	1	8 May	22 May	26 May (4)
	2	–	15 May	25 May (10)
	3	9 May	21 May	10 June (20)
	5	10 May	22 May	11 June (20)
Cool, not fed	1	10 May	9 June	5 July (26)
	2	10 May	9 June	5 July (26)
	4	9 May	9 June	5 July (26)
	5	–	25 May	14 June (20)
Cool, fed	1	8 May	8 June	6 July (28)
	2	9 May	9 June	29 June (20)
	3	10 May	11 June	3 July (22)
	4	12 May	14 June	6 July (22)
	5	12 May	14 June	6 July (22)
Run 2				
Warm, not fed	7	27 June	9 July	29 July (20)
	8	–	30 June	28 July (28)
	10	27 June	23 July	25 July (2)
Warm, fed	8	–	3 July	23 July (20)
	9	–	10 July	30 July (20)
Cool, not fed	9	–	10 July	30 July (20)
Cool, fed	6	–	9 July	10 July (1)
	8	–	10 July	28 July (18)

For a slightly different view on larval competence and size, the first time-point was determined when ≥50% of larvae from a culture metamorphosed in the presence of KCl (Zimmerman & Pechenik 1991) and larval size at that time-point of 50% competence. In some cases, a larval culture reached 50% competence on a day when size was not measured (because size was only measured every 4 d, while competence was tested every 2 d), so larval sizes were interpolated based on measurements from the time-points before and after.

Differences in the time to 50% competence, larval size at the time of 50% competence, and brooding time between treatments were tested using 2-way crossed ANOVAs. In order to determine whether there was a significant interaction between runs of the experiment and treatments, the test was first conducted using run and treatment as factors. Run and the interaction run × treatment were not significant in any case ($p > 0.05$), so ANOVAs were then conducted using fed (mothers fed or not fed) and temperature (warm or cool brooding temperature) as factors. Levene's tests were used to test for homoscedasticity, and non-parametric Mann-Whitney tests were used to verify results if heteroscedasticity was detected. A Spearman correlation was used to test for a correlation between time to 50% competence and size at 50% competence. All statistical tests were run in Matlab v.2017b.

3. RESULTS

Crepidula fornicata larvae were most abundant in the water column in late May and early June 2017, when water temperatures were ~18°C (Fig. 2). The peak in *C. fornicata* larval abundance occurred during a single sampling period at each site and was followed by a prolonged decline that lasted until early August (Fig. 2).

The minimum brooding time was much longer for *C. fornicata* adults in the cool treatment (~13°C) than the warm treatment (~21°C) but did not differ between fed and not fed treatments or have a significant interaction of temperature and feeding treatments (Table 2). The significant difference in brooding time between temperature was supported by a Mann-Whitney test ($U = 36$, $p < 0.001$). Cool-treatment adults that released 2 broods in captivity had 30–33 d between releases, while warm-treatment adults had just 12–14 d, except for 2 individuals with 19 and 26 d between releases (Fig. 3). Thus, 30 d is the minimum brooding time for *C. fornicata* at ~13°C, and 12 d is the minimum brooding time for this species at ~21°C. After releasing larvae in captivity, 67% of cool-treatment adults had new egg masses under their shells (which had to have been deposited before adults were frozen, i.e. within 24 h of larval release), while only 15% of warm-treatment adults had new egg masses. These observations indicate that more *C. fornicata* females have back-to-back broods at cooler temperatures.

Larval size data met the assumption of sphericity (Mauchly's test, $p > 0.05$) and were not significantly different between experimental runs (Table 2). There were no differences in larval sizes over time based on maternal treatment, as revealed by RM-ANOVA

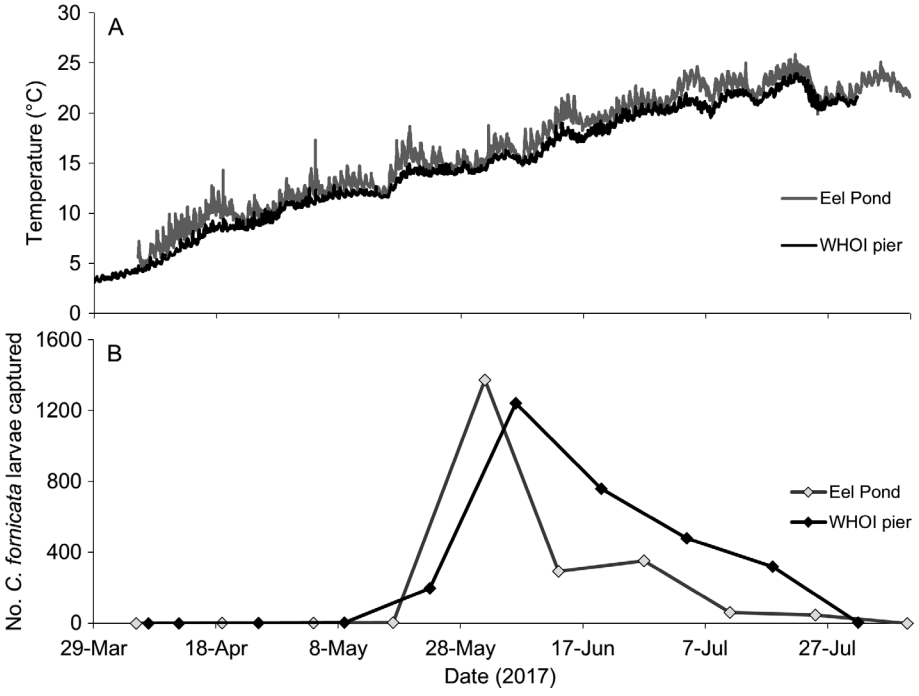

Fig. 2. (A) Water temperature just below floating docks in Eel Pond and Great Harbor (Woods Hole Oceanographic Institution [WHOI] pier), Woods Hole, MA, USA, in April–August 2017; (B) abundance of *Crepidula fornicata* larvae captured in cylindrical tube traps at the same locations. Each point represents one sampler deployed for a 2 wk period

(Table 2, Fig. 4). However, larvae brooded at 13°C were significantly larger at the time-point when 50% were competent to metamorphose than larvae brooded at 21°C (Table 2).

The percentage of larvae that were competent to metamorphose over time did not significantly differ between runs of the experiment (Table 2, Fig. 5). There was a significant effect of treatment (Table 2), but larval competency data violated the assumption of sphericity (Mauchly's test, $\chi^2 = 56.8$, df = 20, $p < 0.001$). A non-parametric Friedman's test revealed a significant difference in percent larval competence between treatments and supported the RM-ANOVA result ($\chi^2 = 92.08$, df = 6, $p < 0.001$). Post hoc pairwise RM-ANOVA revealed a significant difference between the warm, fed and cool, not fed treatments, while a pairwise test between the warm, fed and cool, fed treatments was marginally significant (Table 3). Thus, a greater proportion of larvae brooded by warm, fed mothers were competent to metamorphose over time compared to larvae brooded by cool mothers.

Table 2. Results of ANOVA and repeated-measures (RM)-ANOVA tests for brooding and larval *Crepidula fornicata*. Run: run of the experiment; Treatment: maternal treatment (warm, fed; warm, not fed; cool, fed; or cool, not fed); Temp: temperature treatment during brooding (13 or 21°C); Fed: maternal feeding treatment (fed or not fed). Significant p-values ($p < 0.05$) are shown in **bold**

Parameter	Statistical test	Factor	df	*F*	p
Brooding time	2-way crossed ANOVA	Temp	1	76.7	**<0.001**
		Fed	1	0.24	0.63
		Temp × Fed	1	1.85	0.19
Larval size	RM-ANOVA	Run	5	0.16	0.97
		Treatment	5	1.94	0.09
Larval size at 50% competence	2-way crossed ANOVA	Temp	1	11.4	**0.003**
		Fed	1	0.88	0.36
		Temp × Fed	1	0.15	0.70
Percent of competent larvae	RM-ANOVA	Run	2.63[a]	0.96	0.41[a]
		Treatment	2.63[a]	4.39	**0.01**[a]
Time for 50% of larvae to become competent	2-way crossed ANOVA	Temp	1	4.48	**0.05**
		Fed	1	0.33	0.57
		Temp × Fed	1	0.50	0.49

[a]Greenhouse-Geisser-adjusted values (ε = 0.43)

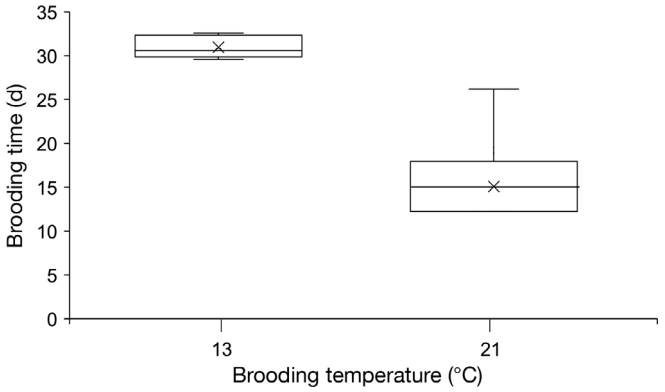

Fig. 3. Time between larval releases (brooding time) for *Crepidula fornicata* mothers kept in different temperature treatments (warm and cool) in the carryover experiment. Whiskers: minimum and maximum values; box: first and third quartiles; inner line: median; (×) mean

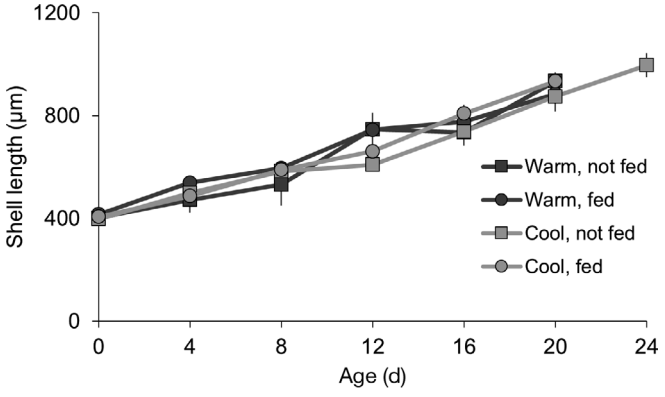

Fig. 4. Average size of *Crepidula fornicata* larvae released by mothers in different temperature (warm or cool) and feeding treatments (fed or not fed). Larvae were cultured in common garden conditions. Each point represents the average of 3–6 replicate cultures, with 20 larvae measured from each culture. Error bars: ±SE

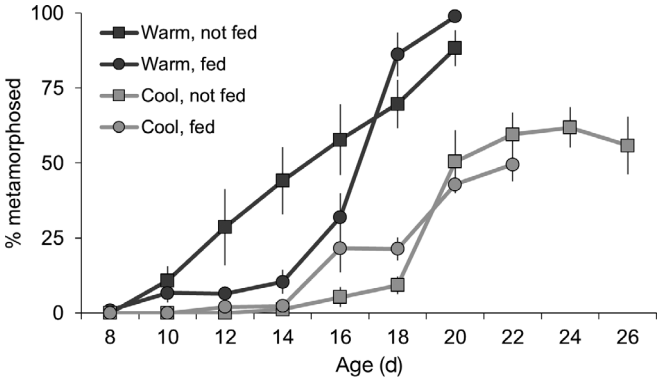

Fig. 5. Percent of *Crepidula fornicata* larvae metamorphosed after 24 h exposure to 20 mM excess KCl in seawater. Larvae were released by mothers kept in different temperature (warm or cool) or feeding treatments (fed or not fed) and cultured in common garden conditions. Each point represents the average of 3–6 replicate cultures, with 20 larvae tested for competency from each culture. Error bars: ±SE

Table 3. Post hoc pairwise repeated-measures (RM)-ANOVA tests for the proportion of competent *Crepidula fornicata* larvae. The df and p-values are Greenhouse-Geisser-adjusted values; the ε for each of these adjustments are shown. A Bonferroni correction was used for significance ($\alpha = 0.05 / 6 = 0.008$). Significant p-values are shown in **bold**

Treatment 1	Treatment 2	ε	df	*F*	p
Warm, not fed	Warm, fed	0.27	1.66	3.42	0.11
Warm, not fed	Cool, not fed	0.29	1.79	3.95	0.08
Warm, not fed	Cool, fed	0.37	2.24	3.78	0.05
Warm, fed	Cool, not fed	0.34	2.07	35.1	**<0.001**
Warm, fed	Cool, fed	0.25	1.51	12.2	0.009
Cool, not fed	Cool, fed	0.27	1.63	1.18	0.34

There was a linear increase over time in the average percent of competent larvae from warm, unfed mothers, while the percent of competent larvae from warm, fed mothers seemed to fit a logistical curve (Fig. 5). Larvae from fed and unfed cool-treatment mothers became competent at about the same rate, but larvae from cool-treatment mothers did not reach 100% competency before high larval mortality necessitated termination of the cultures (Fig. 5). The amount of time required for 50% of larvae in a culture to reach competency was longer for larvae brooded at cool temperatures (Table 2).

There was no significant correlation between the amount of time for a larval culture to reach 50% competency and the size of larvae at that time-point (Spearman correlation, $\rho = 0.30$, $p = 0.19$).

4. DISCUSSION

In the present study, the carryover experiment showed distinct differences in the time to develop to competency for *Crepidula fornicata* larvae released by cool- and warm-treatment mothers. Carryover effects were observed in this study under common garden conditions, but in the field, maternal carryover effects will interact with temperature conditions experienced by larvae, which may also affect PLD (Pechenik 1984, Zimmerman & Pechenik 1991).

The longer time to develop to competency for larvae brooded at cooler temperatures means that larvae released earlier in the spring may be exposed to pelagic predators for longer periods than larvae released later in the summer. While little is known about the identities or seasonal patterns of pelagic predators of *C. fornicata*, vulnerability to pelagic predators is one of the main disadvantages to having

a pelagic larval phase (Pechenik 1999, Burgess et al. 2016). However, larvae with a longer PLD are also likely to disperse farther from their parents. Longer-duration larvae will likely contribute to the gene flow among *C. fornicata* populations (e.g. Collin 2001) and may be able to colonize new habitats. The first species to establish a population in a newly opened habitat could come to dominate in the absence of its usual predators or competitors and exclude later-arriving species from the community (Harley et al. 2006). *C. fornicata* is already an incredibly successful invasive species in northern European habitats (Thieltges 2005, Blanchard 2009) and could spread northward on both sides of the Atlantic, facilitated by climate change (Thieltges et al. 2004, Valdizan et al. 2011).

Interestingly, there was no apparent relationship between larval size and development to competency. All larvae were approximately the same size at release and throughout the larval period despite becoming competent at different times. These results correspond to previous observations in *C. fornicata* and its congeners — that the size of newly released larvae is consistent (Pechenik & Lima 1984, Lima & Pechenik 1985) and that size is a poor predictor of competency (Pechenik & Heyman 1987, Zimmerman & Pechenik 1991) — but contrasts with studies in other invertebrate species where larval size is related to PLD (e.g. Marshall & Keough 2003a). Because larvae are provisioned with yolk at the beginning of brooding (Pandian 1969), the experimental treatments used here may not have been maintained long enough to affect provisioning in the lab. Nevertheless, nutrition seems to be decoupled from reproductive success in all life stages of *C. fornicata*. Adult *C. fornicata* do not follow expected trends for reproductive output, with higher fecundity in intertidal populations, which are exposed to lower food supplies and greater environmental stress than subtidal populations (Pechenik et al. 2017). Larvae can survive and develop to competency on low food (Bogan et al. 2019), and diet composition has no effect on development to competency (Padilla et al. 2014, Pechenik & Tyrell 2015). While there are carryover effects of larval nutritional deprivation on juvenile growth (Pechenik et al. 1996a, 2002), under normal feeding conditions, larval and juvenile growth rates are not correlated (Pechenik et al. 1996b). The decoupling of nutrition from reproduction and larval development may represent an element of resiliency that contributes to *C. fornicata*'s success as an invasive species.

The different time to develop to competency for *C. fornicata* larvae brooded at cool and warm temperatures cannot be explained by larval size, so the physiological mechanism underlying this difference is unknown. Competency is primarily an internal physiological change, in which the larva retains its planktonic characters but is ready to settle and metamorphose when induced by external cues (Hadfield et al. 2001). While the physiological mechanisms underlying competency are not completely understood in *C. fornicata*, some chemicals that induce or inhibit metamorphosis are known (Pechenik & Heyman 1987, Pires et al. 2000, Pechenik et al. 2007, Taris et al. 2010). Future studies on carryover effects in *C. fornicata* should include biochemical or transcriptomic analyses to understand the mechanisms underlying variation in competency.

Larval *C. fornicata* released by mothers under cooler conditions were larger at the time-point when 50% of larvae were competent to metamorphose. For many invertebrate species, larger larvae metamorphose into juveniles with lower post-settlement mortality and faster growth, and this has been demonstrated in bryozoans (Marshall et al. 2003b), ascidians (Marshall & Keough 2003b), and gastropods (Ray-Culp et al. 1999). For *C. fornicata*, post-settlement mortality plays a strong role in determining adult population sizes (Bohn et al. 2013) and is likely to mediate the secondary spread of this species in its invasive range in Europe as temperatures warm (Valdizan et al. 2011, Bohn et al. 2012). Because of their potential for greater dispersal and lower post-settlement mortality, *C. fornicata* larvae released early in the season may play a disproportionate role in the spread of this species to more northern habitats.

Phenological patterns observed in this study show most *C. fornicata* reproductive effort in spring. Brooding in *C. fornicata* is triggered by temperatures rising above 10°C (Valdizan et al. 2011), and the peak in larval abundance shows most mothers release larvae in late May. Mothers preferentially provision embryos with Ω3 fatty acids in May and June, contributing to the success of these larvae over conspecifics released earlier or later in the reproductive season (Leroy et al. 2013). Based on brood times recorded here, a single *C. fornicata* female could support 3–4 broods in a season, but the decline in larval abundance suggests that many mothers cease reproduction earlier. The number of mature oocytes produced by *C. fornicata* females during the fall–winter non-brooding season is limited, so individual females may cease brooding once all available offspring have been released (Beninger et al. 2010, Valdizan et al. 2011).

In order for maternal effects to increase offspring fitness, 3 criteria must be met: the environment must be variable; variations must be predictable based on

reliable cues; and the cost of plasticity must be low (Marshall & Uller 2007). Brooding temperature cannot be considered a maternal effect sensu stricto because larvae also experience the warm or cool temperature in the egg mass. The experimental design did not allow for a test of adaptive parental effects (APEs) because not all mothers released larvae and therefore plasticity cannot be separated from selection (Burgess & Marshall 2014). Nevertheless, the effects observed fit some of the criteria for APEs. Seasonal temperature variations are very predictable, and in the case of *C. fornicata* development to competency, the cost of plasticity is also low (i.e. there is no apparent physiological trade-off, though future studies should confirm this). As discussed above, longer time to develop to competency for larvae released earlier in the season may be adaptive because these larvae can attain larger size before settlement. For larvae released later in the summer, shorter development times may allow these larvae to reach competency before the onset of cool autumn temperatures. No *C. fornicata* larvae were observed in traps after mid-August, and elsewhere in *C. fornicata*'s range, no settlement was observed after 1 October, when water temperatures dipped below 14°C (Bohn et al. 2012). Plasticity in pelagic larval duration based on brooding temperature in *C. fornicata* may be an example of an APE or merely a consequence of temperature effects on an ectothermic invertebrate, and future studies could explore this question.

The extended time to develop to competency for larvae from mothers under cooler conditions occurred in artificial laboratory conditions, so results require ground-truthing with field observations. In the carryover experiment, many larvae grew conspicuous flat brims on their shells as they became older, especially those released by cool-treatment mothers. Larvae with these brims were also collected in the larval traps and have previously been observed in plankton tows by Pechenik (1986). Thus, the extended PLDs observed in the lab are likely to be experienced by *C. fornicata* larvae in the field. The conditions in the carryover experiment approximated field conditions, so the results can be translated into parameters for dispersal studies using modelling.

Biophysical modelling is a powerful tool for studying potential dispersal, connectivity, and range shifts in a warming climate. At present, the major impediment to refined predictions of dispersal is measurement of biological parameters, with many studies including just a single PLD for a given species (Metaxas & Saunders 2009). PLD is a major factor constraining dispersal of larvae in coastal environments (Pineda et al. 2007), so it is imperative that future modelling efforts allow for variation in PLD of a given species in order to refine dispersal predictions. In the present study, PLD not only varied between but also within treatments and within replicates, as competency was quantified as the percentage of larvae metamorphosing at each given time-point. PLD could be expressed as a probability density function rather than a single parameter in future modelling studies using *C. fornicata*.

Acknowledgements. I warmly thank Anthony Pires (Dickinson College) for culturing advice, experimental assistance, and comments on an early version of the manuscript. Three anonymous reviewers also provided thoughtful comments that substantially improved the manuscript. Isabella Horstmann (University of Miami) and Carl Kaiser (WHOI) assisted with the carryover experiment, while Sabine Angier (University of Puget Sound) assisted with larval trap samples. This study was supported by a Postdoctoral Scholarship and the Independent Research & Development Fund at WHOI.

LITERATURE CITED

Beninger PG, Valdizan A, Decottigies P, Cognie B (2010) Field reproductive dynamics of the invasive slipper limpet, *Crepidula fornicata.* J Exp Mar Biol Ecol 390: 179–187

Bertram DF, Strathmann RR (1998) Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. Ecology 79:315–327

Blanchard M (2009) Recent expansion of the slipper limpet population (*Crepidula fornicata*) in the Bay of Mont-Saint-Michel (Western Channel, France). Aquat Living Resour 22:11–19

Bogan SN, McMahon JB, Pechenik JA, Pires A (2019) Legacy of multiple stressors: responses of gastropod larvae and juveniles to ocean acidification and nutrition. Biol Bull 236:159–173

Bohn K, Richardson C, Jenkins S (2012) The invasive gastropod *Crepidula fornicata*: reproduction and recruitment in the intertidal at its northernmost range in Wales, UK, and implications for its secondary spread. Mar Biol 159: 2091–2103

Bohn K, Richardson CA, Jenkins SR (2013) The importance of larval supply, larval habitat selection and post-settlement mortality in determining intertidal adult abundance of the invasive gastropod *Crepidula fornicata.* J Exp Mar Biol Ecol 440:132–140

Burgess SC, Marshall DJ (2011) Temperature-induced maternal effects and environmental predictability. J Exp Biol 214:2329–2336

Burgess SC, Marshall DJ (2014) Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. Oikos 123:769–776

Burgess SC, Baskett MA, Grosberg RK, Morgan SG, Strathmann RR (2016) When is dispersal for dispersal? Unifying marine and terrestrial perspectives. Biol Rev Camb Philos Soc 91:867–882

Clark MS, Suckling CC, Cavallo A, Mackenzie CL, Thorne MAS, Davies AJ, Peck LS (2019) Molecular mechanisms

underpinning transgenerational plasticity in the green sea urchin *Psammechinus miliaris.* Sci Rep 9:952

Collin R (2001) The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Mol Ecol 10: 2249–2262

Diederich CM, Pechenik JA (2013) Thermal tolerance of *Crepidula fornicata* (Gastropoda) life history stages from intertidal and subtidal subpopulations. Mar Ecol Prog Ser 486:173–187

Dupont L, Richard J, Paulet YM, Thouzeau G, Viard F (2006) Gregariousness and protandry promote reproductive insurance in the invasive gastropod *Crepidula fornicata*: evidence from assignment of larval paternity. Mol Ecol 15:3009–3021

Fisher JI, Mustard JF (2004) High spatial resolution sea surface climatology from Landsat thermal infrared data. Remote Sens Environ 90:293–307

Gaylord B, Hodin J, Ferner MC (2013) Turbulent shear spurs settlement in larval sea urchins. Proc Natl Acad Sci USA 110:6901–6906

Gibson GD, Gibson A (2004) Heterochrony and the evolution of poecilogony: generating larval diversity. Evolution 58:2704–2717

Hadfield MG, Carpizo-Ituarte EJ, Del Carmen K, Nedved BT (2001) Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. Am Zool 41: 1123–1131

Harley CDG, Hughes AR, Hurltgren KM, Miner BG and others (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228–241

Henry JJ, Collin R, Perry KJ (2010) The slipper snail, *Crepidula*: an emerging lophotrochozoan model system. Biol Bull 218:211–229

Henry JQ, Lyons DC (2016) Molluscan models: *Crepidula fornicata.* Curr Opin Genet Dev 39:138–148

Hentschel BT, Emlet RB (2000) Metamorphosis of barnacle nauplii: effects of food variability and a comparison with amphibian models. Ecology 81:3495–3508

Howard SC, Hentschel BT (2005) Effects of short-term food variability on the plasticity of size and age at metamorphosis of porcelain crab larvae. Limnol Oceanogr 50: 1960–1971

Lane D, Beaumont A, Hunter J (1985) Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis.* Mar Biol 84:301–308

Leroy F, Meziane T, Riera P, Comtet T (2013) Seasonal variations in maternal provisioning of *Crepidula fornicata* (Gastropoda): fatty acid composition of females, embryos, and larvae. PLOS ONE 8:e75316

Lima GM, Pechenik JA (1985) The influence of temperature on growth rate and length of larval life of the gastropod, *Crepidula plana* Say. J Exp Mar Biol Ecol 90:55–71

Marshall DJ, Keough MJ (2003a) Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Mar Ecol Prog Ser 255:145–153

Marshall DJ, Keough MJ (2003b) Effects of settler size and density on early post-settlement survival of *Ciona intestinalis* in the field. Mar Ecol Prog Ser 259:139–144

Marshall DJ, Keough MJ (2004) Variable effects of larval size on post-metamorphic performance in the field. Mar Ecol Prog Ser 279:73–80

Marshall DJ, Uller T (2007) When is a maternal effect adaptive? Oikos 116:1957–1963

Marshall DJ, Pechenik JA, Keough MJ (2003a) Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum.* Mar Ecol Prog Ser 246:153–162

Marshall DJ, Bolton TF, Keough MJ (2003b) Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131–3137

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea. Ecol Lett 13:128–140

Metaxas A, Saunders M (2009) Quantifying the 'bio-' components in biophysical models of marine benthic invertebrates: advances and pitfalls. Biol Bull 216:257–272

Meyer KS, Wheeler JD, Houlihan E, Mullineaux LS (2018) Desperate planktotrophs: decreased settlement selectivity with age in competent eastern oyster *Crassostrea virginica* larvae. Mar Ecol Prog Ser 599:93–106

Padilla DK, McCann MJ, Glenn MM, Hooks AP, Shumway SE (2014) Effect of food on metamorphic competence in the model system *Crepidula fornicata.* Biol Bull 227:242–251

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

Pechenik JA (1984) The relationship between temperature, growth rate, and duration of planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 74:241–257

Pechenik JA (1986) Field evidence for delayed metamorphosis of larval gastropods: *Crepidula plana* (Say), *C. fornicata* (L.), and *Bittium alternatum* (Say). J Exp Mar Biol Ecol 97:313–319

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 (2006) Larval experience and latent effects — metamorphosis is not a new beginning. Integr Comp Biol 46:323–333

Pechenik JA (2018) Latent effects: surprising consequences of embryonic and larval experience on life after metamorphosis. In: Carrier T, Reitzel A, Heyland A (eds) Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford, p 208–225

Pechenik JA, Heyman WD (1987) Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 112:27–38

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

Pechenik JA, Tyrell AS (2015) Larval diet alters larval growth rates and post-metamorphic performance in the marine gastropod *Crepidula fornicata.* Mar Biol 162:1597–1610

Pechenik JA, Hammer K, Weise C (1996a) The effect of starvation on acquisition of competence and post-metamorphic performance in the marine prosobranch gastropod *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 199: 137–152

Pechenik J, Hilbish TJ, Eyster LS, Marshall D (1996b) Relationship between larval and juvenile growth rates in two marine gastropods, *Crepidula plana* and *C. fornicata.* Mar Biol 125:119–127

Pechenik JA, Jarrett JN, Rooney J (2002) Relationships between larval nutritional experience, larval growth rates, juvenile growth rates, and juvenile feeding rates in the prosobranch gastropod *Crepidula fornicata.* J Exp Mar Biol Ecol 280:63–78

Pechenik JA, Cochrane DE, Li W, West ET, Pires A, Leppo M (2007) Nitric oxide inhibits metamorphosis in larvae of *Crepidula fornicata*, the slippershell snail. Biol Bull 213: 160–171

Pechenik JA, Diederich CM, Chaparro OR, Montory JA, Paredes FJ, Franklin AM (2017) Differences in resource allocation to reproduction across the intertidal-subtidal gradient for two suspension-feeding marine gastropods: *Crepidula fornicata* and *Crepipatella peruviana.* Mar Ecol Prog Ser 572:165–178

Phillips NE (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. Ecology 83:2562–2574

Phillips NE (2004) Variable timing of larval food has consequences for early juvenile performance in a marine mussel. Ecology 85:2341–2346

Pineda J, Hare J, Sponaugle S (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography (Wash DC) 20:22–39

Pires A (2014) Artificial seawater culture of the gastropod *Crepidula fornicata* for studies of larval settlement and metamorphosis. In: Carroll DJ, Stricker SA (eds) Development biology of the sea urchin and other marine invertebrates. Humana Press, Totowa, NJ, p 35–44

Pires A, Guilbault TR, Mitten JV, Skiendzielewski JA (2000) Catecholamines in larvae and juveniles of the prosobranch gastropod, *Crepidula fornicata.* Comp Biochem Physiol C Toxicol Pharmacol 127:37–47

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge

Ray-Culp M, Davis M, Stoner AW (1999) Predation by xanthid crabs on early post-settlement gastropods: the role of prey size, prey density, and habitat complexity. J Exp Mar Biol Ecol 240:303–321

Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms to changing ocean conditions. ICES J Mar Sci 73:537–549

Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–385

Strathmann MF, Strathmann RR (2007) An extraordinarily long larval duration of 4.5 years from hatching to metamorphosis for teleplanic veligers of *Fusitriton oregonensis.* Biol Bull 213:152–159

Sumida PYG, Tyler PA, Gage JD, Norrevang A (1998) Post-larval development in shallow and deep-sea ophiuroids (Echinodermata: Ophiuroidea) of the NE Atlantic Ocean. Zool J Linn Soc 124:267–300

Taris N, Comtet T, Stolba R, Lasbleiz R, Pechenik JA, Viard F (2010) Experimental induction of larval metamorphosis by a naturally-produced halogenated compound (dibromomethane) in the invasive mollusc *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 393:71–77

Thieltges DW (2005) Impact of an invader: epizootic American slipper limpet *Crepidula fornicata* reduces survival and growth in European mussels. Mar Ecol Prog Ser 286: 13–19

Thieltges DW, Strasser M, Van Beusekom JEE, Reise K (2004) Too cold to prosper — winter mortality prevents population increase of the introduced American slipper limpet *Crepidula fornicata* in northern Europe. J Exp Mar Biol Ecol 311:375–391

Todd CD (2003) Assessment of a trap for measuring larval supply of intertidal barnacles on wave-swept, semi-exposed shores. J Exp Mar Biol Ecol 290:247–269

Turner JT, Borkman DG, Lincoln JA, Gauthier DA, Petitpas CM (2009) Plankton studies in Buzzards Bay, Massachusetts, USA. VI. Phytoplankton and water quality, 1987 to 1998. Mar Ecol Prog Ser 376:103–122

Valdizan A, Beninger PG, Decottignies P, Chantrel M, Cognie B (2011) Evidence that rising coastal seawater temperatures increase reproductive output of the invasive gastropod *Crepidula fornicata.* Mar Ecol Prog Ser 438: 153–165

Welch JW, Epifanio CE (1995) Effect of variations in prey abundance on growth and development of crab larvae reared in the laboratory and in large field-deployed enclosures. Mar Ecol Prog Ser 116:55–64

Wong JM, Johnson KM, Kelly MW, Hofmann GE (2018) Transcriptomics reveal transgenerational effects in purple sea urchin embryos: adult acclimation to upwelling conditions alters the response of their progeny to differential pCO_2 levels. Mol Ecol 27:1120–1137

Zimmerman KM, Pechenik JA (1991) How do temperature and salinity affect relative rates of growth, morphological differentiation, and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana?* Biol Bull 180:372–386

Editorial responsibility: Steven Morgan, Bodega Bay, California, USA

Submitted: December 10, 2019; Accepted: May 4, 2020
Proofs received from author(s): May 27, 2020