



## Low salinity stress experienced by larvae does not affect post-metamorphic growth or survival in three calyptraeid gastropods

Casey M. Diederich<sup>a,\*</sup>, Jeremiah N. Jarrett<sup>b</sup>, Oscar R. Chaparro<sup>c</sup>, C.J. Segura<sup>c</sup>, Shawn M. Arellano<sup>d,1</sup>, Jan A. Pechenik<sup>a</sup>

<sup>a</sup> Biology Department, Tufts University, Medford, MA 02155, USA

<sup>b</sup> Biological Sciences, Central Connecticut State University, Box 4010, New Britain, CT 06050-4010, USA

<sup>c</sup> Instituto de Biología Marina, Dr. J. Winter, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

<sup>d</sup> Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

### ARTICLE INFO

#### Article history:

Received 27 June 2010

Received in revised form 22 November 2010

Accepted 23 November 2010

Available online xxxx

#### Keywords:

*Crepidula*

*Crepidatella*

Larvae

Latent effects

Salinity

Starvation

### ABSTRACT

Marine larvae that experience some sub-lethal stresses can show effects from those stresses after metamorphosis, even when they seem to recover from those stresses before metamorphosis. In this study we investigated the short and long-term effects of exposing the larvae of three calyptraeid gastropods (*Crepidula fornicata*, *Crepidula onyx*, and *Crepidatella fecunda*) to temporary reductions in salinity. Larvae of all three species showed slower larval growth rates, longer time to metamorphic competence, and substantial mortality after being stressed in seawater at salinities of 10, 15, and 20 for less than 48 h. Larval tolerance to low salinities varied widely within and among species, but longer stresses at lower salinities were generally more harmful to larvae. However, larvae in nearly all experiments that were able to metamorphose survived and grew normally as juveniles; there were no documented “latent effects.” For all three species, starving larvae in full-strength seawater was not as harmful as exposing larvae to low salinity stress, indicating that detrimental effects on larvae were caused by the salinity stress *per se*, rather than by an indirect effect of salinity stress on feeding. *C. fornicata* that were stressed with low salinity as juveniles were more tolerant of the stress than larvae: all stressed juveniles lived and showed reduced growth rates for no more than 3 days. Our data suggest that even though reduced salinity is clearly stressful to the larvae of these 3 gastropod species, metamorphosis seems to generally provide individuals with a fresh start.

© 2010 Elsevier B.V. All rights reserved.

### 1. Introduction

Larval experience can cause latent effects for juveniles and adults (reviewed by Pechenik, 2006). For example, Phillips (2002) temporarily stressed larvae of the marine bivalve *Mytilus galloprovincialis* with decreased food concentrations and monitored the performance of juveniles both in the lab and after transplanting some to the field. In both cases, juveniles that had been stressed as larvae showed significantly slower growth for at least the next 14 days compared to control individuals, even under conditions of abundant food. Thus larval experience can dramatically affect juvenile performance long after individuals have returned to benign conditions. Most studies to date have involved depriving larvae of food, exposing larvae to pollution, or delaying metamorphosis and documenting the effects on juvenile growth and survival (e.g., Cebrian and Uriz, 2007; Jacobs et al., 2008; reviewed by Pechenik, 2006). However, larvae may also

experience rapid and substantial fluctuations in salinity, particularly in intertidal, estuarine, and other shallow water environments (Richmond and Woodin, 1996). For example, offshore surface waters may drop from salinity of over 30 to 15 during heavy rains (Allen and Pechenik, 2010), estuarine waters may fluctuate from salinity of 35 to under 10 in under 12 h (Chaparro et al., 2008), and tide pools may reach near fresh water conditions after heavy rains (Pechenik, 1982).

The gastropod family Calyptraeidae contains more than 90 species, many of which live in estuaries or intertidal environments that may be periodically exposed to temporary reductions in salinity (Collin, 2003). The pelagic larvae of such species are likely to experience low salinity exposures intermittently during development. Previous studies have demonstrated latent effects of nutritional stress on juvenile growth rate for some members of the family, including *Crepidula fornicata* and *Crepidula onyx* (Pechenik et al., 2002; Chiu et al., 2007, 2008), and some data have been published on the salinity tolerance of *C. fornicata* juveniles (Pechenik and Eyster, 1989) and *Crepidula plana* larvae (Zimmerman and Pechenik, 1991). However, the salinity tolerance of most calyptraeid larvae and the consequences of short-term exposure to sub-lethal salinities on subsequent larval development and juvenile performance have not been reported.

\* Corresponding author. Tel.: +1 617 627 3199; fax: +1 617 627 3805.

E-mail address: [casey.diederich@tufts.edu](mailto:casey.diederich@tufts.edu) (C.M. Diederich).

<sup>1</sup> Present address: Woods Hole Oceanographic Institution, MS34, Woods Hole, MA 02543, USA.

In this study we examined the effects of temporary salinity stress during early larval development for three calyptraeid gastropods: *C. fornicata* from New England, *C. onyx* in Hong Kong (native to west coast of the United States), and *Crepidatella fecunda* (formerly *Crepidula fecunda*, see Collin, 2003) from Chile. These three species live in environments that are likely to experience substantial fluctuations in salinity. At our collection sites, all 3 species can be found in the intertidal zone. Depending on tidal cycles, specific location of animals (e.g., tide pool), and weather patterns (e.g., heavy rains at low tide), individuals may be exposed to rapid drops in salinity over short periods of time (e.g., Pechenik, 1982; Chaparro et al., 2008). During the monsoon season in Hong Kong and the wet season in Chile, *C. onyx* and *C. fecunda* may experience even more drastic and prolonged reductions in salinity (Yin, 2002; Chaparro et al., 2008). We asked whether salinity stress experienced by larvae of these 3 species led to latent effects on juvenile survival or growth rate. We designed some experiments to distinguish between the effects of low salinity *per se* and possible indirect effects of low salinity stress on feeding. In addition, we measured the effect of salinity stress on larval survival and growth rate to determine the extent to which larvae of these species could recover from substantial salinity stress before they metamorphosed. Finally, we investigated whether sub-lethal salinity stress affected developmental rate of larvae by measuring the impact of salinity stress on time to metamorphic competence.

## 2. Methods

### 2.1. Collecting and maintaining adults and larvae

Adults were collected at low tide from Wickford, Rhode Island in 1999, 2000, and 2009 and from Westbrook, Connecticut in 1999 and 2000 (*C. fornicata*); Victoria Harbor, Hong Kong in 2009 (*C. onyx*); and Puerto Montt, Chile in 2009 and 2010 (*C. fecunda*). *C. fornicata* and *C. fecunda* were maintained at room temperature (~23 °C) and at a constant temperature of 18 °C, respectively, in the laboratory in glass aquaria of aerated seawater (full-strength salinity, approximately 30). Those temperatures correspond to typical ambient sea temperatures at the time of collection. *C. onyx* were maintained at ambient seawater temperatures in a flow-through sea table. Adults of all 3 species were fed phytoplankton once or twice each day, mostly *Isochrysis galbana* (clone T-ISO) and *Dunaliella tertiolecta* (clone DUN), and water was changed every other day until larvae were released. Upon their release, larvae were collected on 150 µm mesh filters, rinsed with seawater, and transferred to one-gallon glass containers of filtered seawater (0.45 or 0.22 µm). Larvae were used in some experiments within 12 h of hatching, without any feeding (see below), or for other experiments

were fed for up to 5 days on T-ISO at approximately  $18 \times 10^4$  cells ml<sup>-1</sup> (e.g., Pechenik and Lima, 1984; Pechenik et al., 2002) before use.

The larvae used in each experiment were released by one female, but probably had multiple fathers (e.g., Dupont et al., 2006; Le Cam et al., 2009). In all experiments, when larvae were to be exposed to low salinity they were first transferred to a bath of seawater at that low salinity, and then pipetted from there into another set of dishes or 6-well (10 ml) microplates for the actual exposures, thus maintaining the desired final salinity in all treatment dishes. In all studies, salinity was reduced by adding appropriate amounts of deionized water to 0.45 µm-filtered seawater.

All phytoplankton cell concentrations were determined using Hauser Ultraplane hemacytometers, after the cells in 1 ml samples were killed with 0.05 ml of Lugol's iodine or a Coulter counter with aperture tube diameter of 100 µm.

### 2.2. Effect of reduced salinities on larval feeding rates for *C. fornicata*

Experiments with larvae of *C. fornicata* were conducted at room temperature (~23 °C) in dim light (to reduce the likelihood of phytoplankton fission during experiments) to determine whether larvae continued to feed at reduced salinities. Larvae were pipetted into a bath at the lowered salinity for 10 min prior to being tested. Larvae were then pipetted into test tubes containing a final volume of 5 ml seawater at salinities of 30, 20, 15 or 10, with initial phytoplankton concentrations (T-ISO) of  $18 \times 10^4$  cells ml<sup>-1</sup> (Eyster and Pechenik, 1988; Pechenik and Eyster, 1989). Each tube contained 5 ml T-ISO suspension and 20 larvae, with 3 replicates per treatment. Tubes containing phytoplankton suspension but no larvae served as controls. One milliliter samples of T-ISO suspension were taken from each tube after 3 h, to determine feeding rates (Pechenik, 1980). To determine if larvae in low salinity treatments fed initially and then stopped feeding or if they fed continually at slower rates throughout the experiment, the experiment was later repeated with extra replicates from which 1 ml samples of T-ISO suspension were taken after 1 h and 6 h of elapsed feeding time.

### 2.3. Larval stress experiments

#### 2.3.1. Stress conditions and larval growth rates

Pilot studies revealed differences in salinity tolerance among species and sometimes within a species, so we often needed to use different levels of stress in different experiments. Our original goal was to determine the magnitude of latent effects, not to document variation in salinity tolerance. Table 1 summarizes the experiments conducted for each species and gives the corresponding figure number for the results. For most experiments, larvae were pipetted

**Table 1**

Summary of experiments performed on *Crepidula fornicata*, *Crepidula onyx*, and *Crepidatella fecunda* to determine if low salinity stress causes latent effects in these species. Experiments for Fig. 1 (*C. fornicata* feeding at low salinities) and Fig. 13 (stressing *C. fornicata* juveniles) are not included because they contain different methods than these experiments.

Experiment	Species	Day after hatching that stress was applied	Stress duration (hrs)	% of larval life stressed	Stress condition (psu)	Starvation treatment included?	Mortality assessed?	Metamorphic competence assessed?	Larval growth rates measured?	Juvenile growth rates measured?
1	<i>C. fornicata</i>	0.2, or 4	48	12.5	10 or 20	No	No	Yes (Fig. 11A)	Yes (Fig. 4)	Yes (Fig. 9A)
2	<i>C. fornicata</i>	1.3, or 5	48	14.2	10 or 20	No	No	No	Yes (Fig. 5)	Yes (Fig. 9B, C)
3	<i>C. fornicata</i>	0 or 4	24	10.0	10 or 15	No	Yes (Fig. 2A)	Yes (Fig. 11B)	Yes (Fig. 6A, D)	Yes (Fig. 9E)
4	<i>C. fornicata</i>	2	12 or 24	5.0 or 10.0	15	No	No	Yes (Fig. 11C)	Yes (Fig. 6B)	Yes (Fig. 9D)
5	<i>C. fornicata</i>	2	48	20	15	Yes	Yes (data not shown) <sup>a</sup>	No	Yes (Fig. 6C)	No <sup>b</sup>
6	<i>C. onyx</i>	1	12 or 24	4.2 or 8.3	15	No	Yes (Fig. 2B)	Yes (Fig. 11E)	Yes (Fig. 7B)	Yes (Fig. 10B)
7	<i>C. onyx</i>	1	48	16.6	15	Yes	Yes (Fig. 3C)	Yes (Fig. 11D)	Yes (Fig. 7A)	Yes (Fig. 10C)
8	<i>C. fecunda</i>	2	12, 24, or 48	3.5, 7.1, or 14.2	20	No	Yes (Fig. 2C)	Yes (Fig. 12)	Yes (Fig. 8B)	Yes (Fig. 10C)
9	<i>C. fecunda</i>	2	48	14.2	15	Yes	Yes (Fig. 3A)	No	Yes (Fig. 8A)	No <sup>b</sup>
10	<i>C. fecunda</i>	2	12 or 24	3.5 or 7.1	15	No	Yes (Fig. 3B)	No	No	No <sup>b</sup>

<sup>a</sup> All larvae in the experimental treatment died.

<sup>b</sup> All larvae died before metamorphosis, so juvenile growth rates could not be measured.

into 80–100 ml phytoplankton suspensions in glass dishes (10–20 larvae per dish; see Results for details); for the experiment whose results are shown in Fig. 6, however, larvae were pipetted into 10 ml phytoplankton suspensions in microplates (1 larva per well). Larvae of particular ages were stressed in salinities ranging from 10 to 20 for 12–48 h with or without food, as detailed in Results. Control larvae were kept in seawater at a salinity of 30. All larvae were fed T-ISO at concentrations of approximately  $18 \times 10^4$  cells  $\text{ml}^{-1}$  and cell concentrations never fell below  $13 \times 10^4$  cells  $\text{ml}^{-1}$ , except in starvation experiments.

Since it has been shown that larval nutritional stress can cause latent effects on juvenile growth rates even when larval growth rates return to normal before metamorphosis (Pechenik et al., 1996a, 2002), we starved larvae in some experiments to distinguish between the effects of salinity stress and possible indirect effects of starvation stress induced by low salinity. Salinities were determined to the nearest practical salinity unit (psu) with a salinometer or handheld refractometer. After the stress period (12–48 h), larvae were returned to filtered seawater at a salinity of 30 with phytoplankton, with seawater and food replaced every other day for the duration of the study. Larvae were monitored for survival at intervals for the duration of the experiments (see Results). Using methods found in Untersee and Pechenik (2007), larvae that lacked a heartbeat (the heart is visible through the shell), swimming activity, and muscular activity were scored as dead. Larvae were periodically (see Results) either measured non-destructively (Pechenik et al., 1996a) at  $40\times$  or  $63\times$  using a calibrated ocular micrometer or photographed and examined using standard image-analysis software until metamorphosis. These measurements allowed us to determine initial larval growth rates after stress and, when the growth rates of stressed larvae were initially lower than those of the controls, the extent to which larval growth rates recovered over the next 2–6 days.

### 2.3.2. Time to metamorphic competence

In several experiments, we determined whether salinity stress prolongs the time to metamorphic competence (i.e., slows developmental rate). When larvae in control treatments reached an average shell length of approximately 900  $\mu\text{m}$  or when at least 80% of larvae in control treatments formed posterior shell brims—a crude proxy for metamorphic competence (Pechenik, 1986; Pechenik and Heyman, 1987)—all larvae were exposed to 20 mM excess KCl in seawater for 6 h to quantify the number of individuals competent to metamorphose (Pechenik and Heyman, 1987; Pechenik and Gee, 1993). Inducing metamorphosis through exposure to excess KCl does not affect juvenile growth rates (Eyster and Pechenik, 1988). Average larval size at exposure to excess KCl was not standardized because growth and onset of metamorphic competence are uncoupled in *C. fornicata* (Pechenik et al., 1996a). Larvae that did not metamorphose were returned to seawater at a salinity of 30 with phytoplankton and tested again for metamorphic competence after 5 days. In all experiments larvae were stressed for 12–48 h within the first 5 days after hatching. In one experiment with *C. fecunda*, cumulative spontaneous metamorphosis was recorded for 24 days after the end of the stress. Remaining larvae were then transferred to dishes containing adult-conditioned seawater to induce metamorphosis.

### 2.3.3. Juvenile growth rates after larval stress

Juvenile growth rate measurements allowed us to determine if there were latent effects due to salinity stress or starvation stress experienced in the larval stage. After metamorphosis, juveniles were transferred to 50 ml phytoplankton suspensions in glass dishes and reared individually at T-ISO concentrations of approximately  $18 \times 10^4$  cells  $\text{ml}^{-1}$ , with water changed daily; cell concentrations never fell below  $13 \times 10^4$  cells  $\text{ml}^{-1}$  between water changes in spot checks. Juveniles were measured immediately after metamorphosis and 3–5 days later to estimate growth rates. In one experiment, some

juveniles of *C. fornicata* from each treatment were reared in the laboratory for 4 days and then transferred to the field to determine how fast juveniles could grow under natural conditions (e.g., Phillips, 2002). Individual juveniles were pipetted into 250 ml, screw-cap Nalgene bottles that had two 7 cm  $\times$  5 cm openings covered with 500  $\mu\text{m}$  mesh to allow for water exchange. Bottles with juveniles were haphazardly placed in the top two 20 cm tall compartments of a 5 compartment shellfish bag (40 cm  $\times$  40 cm  $\times$  100 cm). The bag was suspended from a floating dock at Outer Island in Branford, CT such that the juveniles were always in the upper 1 m of the surface water.

## 2.4. Juvenile stress experiments

The direct impact of low salinity stress on juveniles was examined for *C. fornicata*. In this set of experiments larvae were reared to metamorphosis in full-strength seawater; juveniles were later exposed to low salinity or to starvation stress. Several hundred juveniles were obtained from larvae that were reared in the lab and induced to metamorphose using 20 mM excess KCl as previously described; 70 of those juveniles were haphazardly chosen for the experiment. The juveniles were pooled from 3 different mothers that had released larvae within 1 day of each other. Following metamorphosis, the 70 juveniles were maintained in filtered seawater on a diet of T-ISO (about  $18 \times 10^4$  cells  $\text{ml}^{-1}$ ) for one day. They were then measured at  $40\times$  or  $63\times$  using a calibrated ocular micrometer and transferred to individual containers with 50 ml of phytoplankton suspension at approximately  $18 \times 10^4$  cells  $\text{ml}^{-1}$ , one juvenile per container, 10 juveniles per treatment. Juveniles were stressed as follows: a salinity of 15 for 6, 12, 24, or 48 h with food; a salinity of 15 for 48 h without food; a salinity of 30 for 48 h without food. Control juveniles were maintained at full-strength seawater (salinity of 30) for 48 h, with food. After stress exposure, all animals were re-measured and returned to full-strength seawater and phytoplankton suspension for another 6 days. Juvenile shell lengths were determined 3 and 6 days after the stress ended to assess growth. During this period of growth, phytoplankton was replenished daily. Phytoplankton cell concentrations never fell below approximately  $13 \times 10^4$  cells  $\text{ml}^{-1}$  in spot checks.

## 2.5. Statistical analyses

Growth rates were calculated from changes in shell length for both larvae and juveniles. For experiments in which larvae were stressed, all treatments in each experiment were compared using one-way analysis of variance (ANOVA) with Bonferroni post-tests to compare individual treatments (GraphPad Prism Software Version 4.03). Data for percent metamorphosis and percent survival at single time points

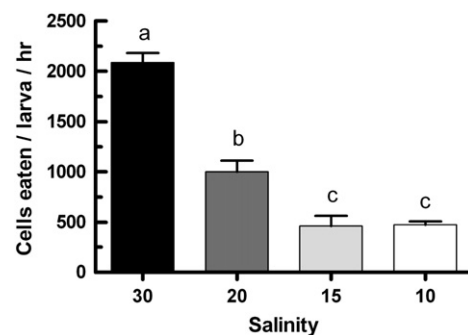


Fig. 1. Influence of decreased salinity on *Crepidula fornicata* larval feeding rate. Mean + S.E.M. shown for all treatments; N = 6 tubes, with 20 larvae per tube feeding for 3 h. Means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). Phytoplankton samples taken after 1 h and 6 h of feeding show that larvae continue to feed the entire time they are in the low salinity treatments.

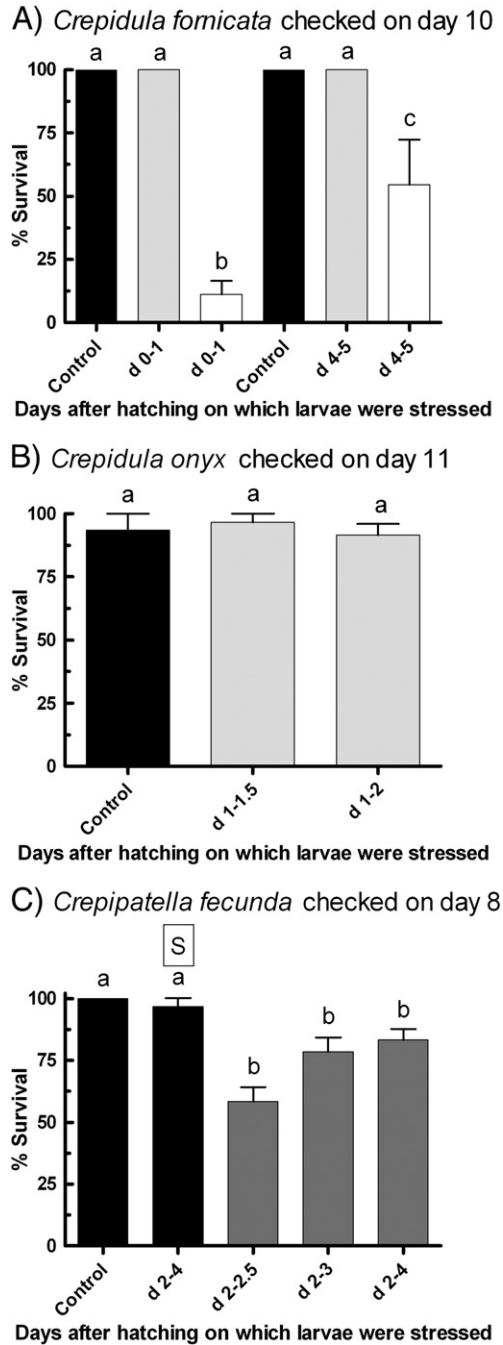
were arcsine transformed before using one-way ANOVA with Bonferroni post-tests to compare individual treatments. Where appropriate (Figs. 6C, 7A, 11D) treatments were compared using two-way ANOVA with salinity and starvation as independent variables. Kaplan–Meier survival curves (Kaplan and Meier, 1958)

were analyzed using a log-rank test to determine if the curves were significantly different. For experiments in which juveniles were stressed, because shell lengths were determined on different days after stress, individual t-tests were used to compare each treatment to the control treatment at a particular time.

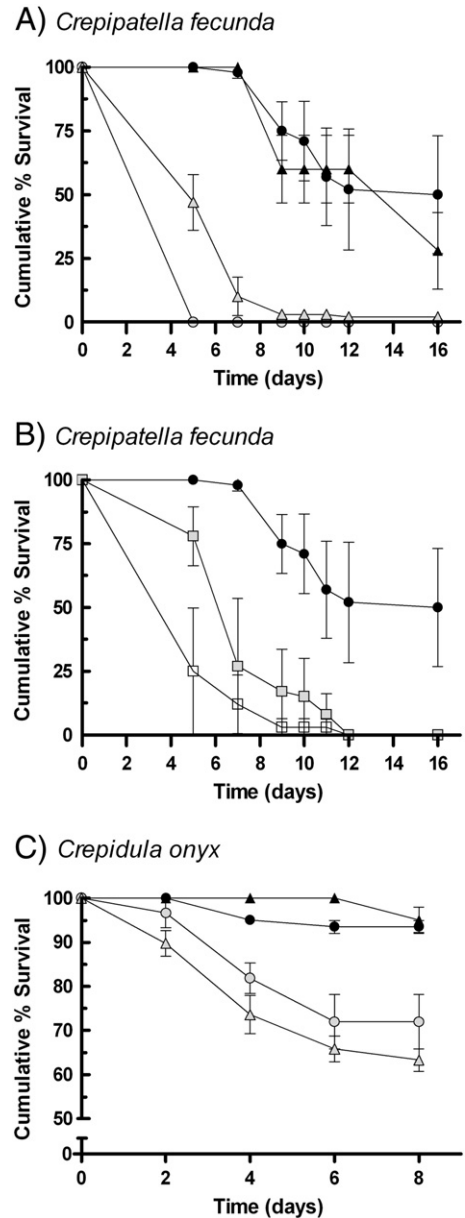
### 3. Results

#### 3.1. *C. fornicata* larval feeding rates at reduced salinities

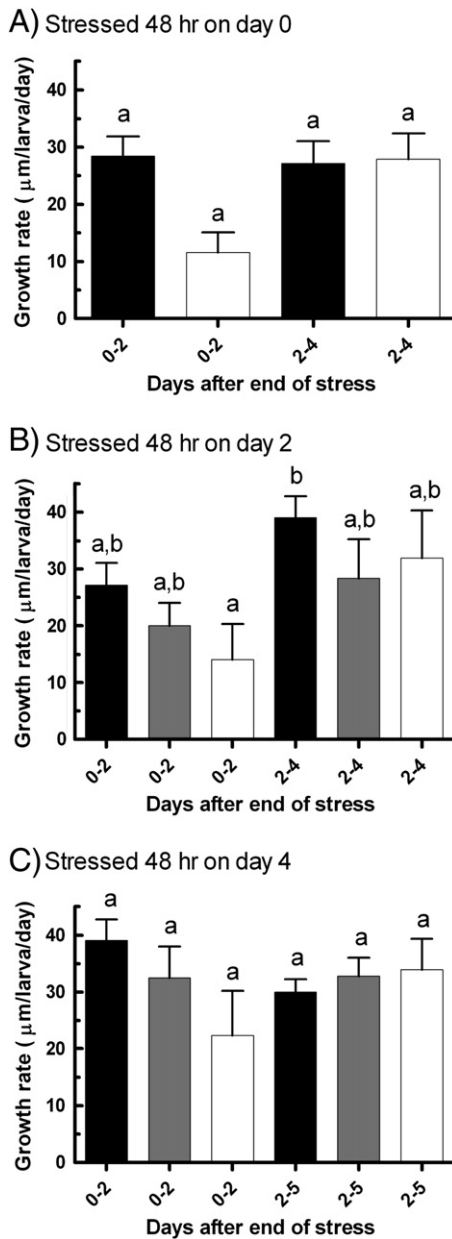
Phytoplankton concentrations in control tubes containing no larvae did not change during the 3 h experiments; i.e., reduced salinities of 20, 15, or 10 did not cause measurable cell rupture or cell



**Fig. 2.** Influence of salinity or starvation stress on larval survival. Mean ± S.E.M. shown for all treatments. Day 0 is the day that larvae hatched. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, light gray—15, and white—10). Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test).  $N = 3$  dishes for all treatments. (A) *Crepidula fornicata* larval survival on day 10; 12 larvae per dish. All larvae stressed in a salinity of 10 eventually died. (B) *Crepidula onyx* larval survival on day 11; 20 larvae per dish; treatment '1–1.5' reflects a 12-hour stress 1 day after hatching. (C) *Crepidipatella fecunda* larval survival on day 8; 20 larvae per dish; □ indicates larvae that were starved during the stress period; treatment '2–2.5' reflects a 12-hour stress 2 days after hatching.



**Fig. 3.** Influence of salinity and/or starvation stress on larval survival. Each point represents mean cumulative survival on a given day ± S.E.M. Symbols indicate the type of stress and the salinity at which larvae were stressed (black circle—control, light gray circle—15, black triangle—starvation only, light gray triangle—starvation at a salinity of 15, light gray square—15 for 12 h, and white square—15 for 24 h).  $N = 3$  dishes for all treatments; 20 larvae per dish at day 0. Within each graph curves are significantly different ( $p < 0.0001$ , Kaplan–Meier survival curves with log-rank test) (A) *Crepidipatella fecunda* stressed on day 2 for 48 h. (B) *Crepidipatella fecunda* stressed on day 2 for 12 or 24 h. (C) *Crepidula onyx* stressed on day 0 for 48 h.

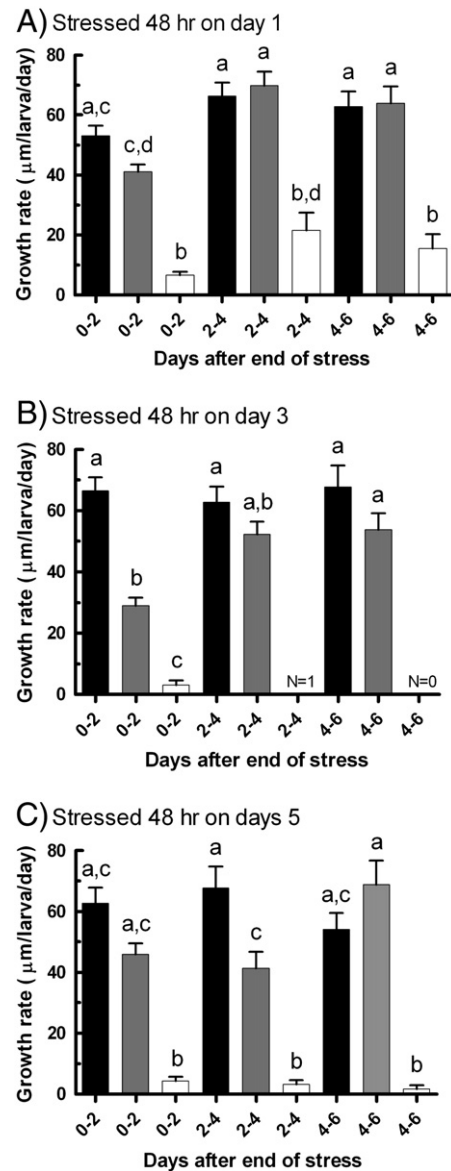


**Fig. 4.** *Crepidula fornicata* Group 1. Influence of 48 h salinity stress on larval growth rate in *C. fornicata*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, and white—10). For each treatment, N = 5 dishes, 12 larvae per dish, 6–12 larvae subsampled per dish for shell length measurements. Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). (A) Larvae were stressed immediately after hatching (day 0) for 48 h; individuals from the treatment stressed at a salinity of 20 were lost. (B,C) After rearing larvae in control conditions for (B) 2 days or (C) 4 days after hatching, larvae were stressed for 48 h.

division. At these reduced salinities, larvae of *C. fornicata* continued to feed, albeit at substantially lower rates than in full-strength seawater (Fig. 1). Feeding rates at low salinities determined 1 h, 3 h, and 6 h after the start of the experiment were statistically equivalent (two-way ANOVA,  $p < 0.0001$  for salinity,  $p = 0.11$  for time,  $p = 0.55$  for salinity  $\times$  time), indicating that stressed larvae fed at nearly constant rates for the duration of the experiment.

### 3.2. Larval and juvenile survival after salinity stress

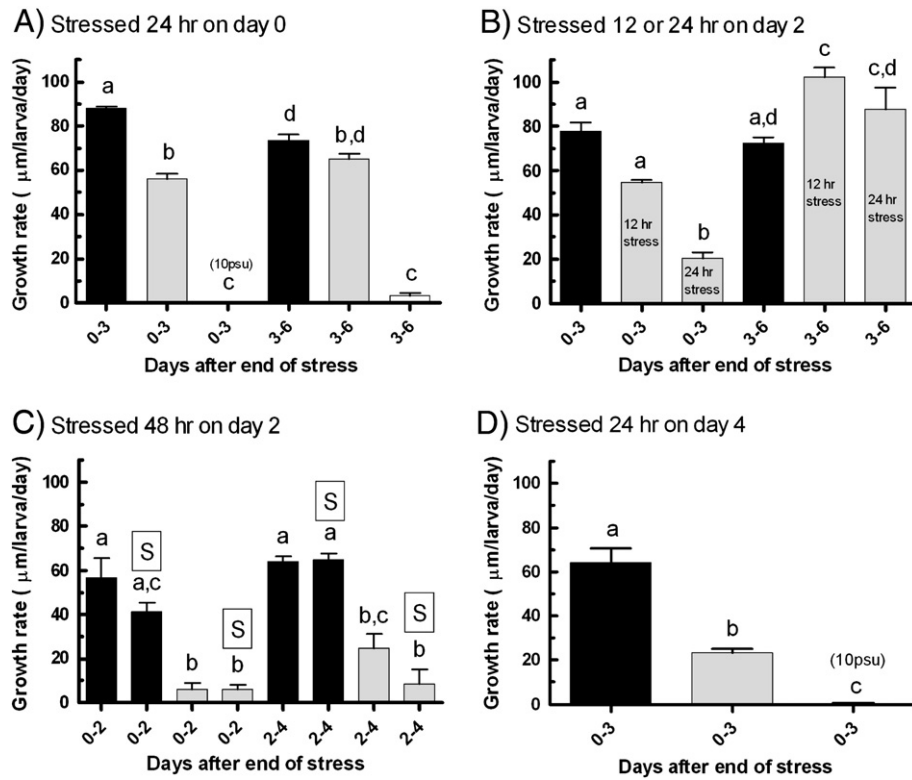
Low salinity stress often increased larval mortality substantially, with the magnitude of the effect varying with both salinity and



**Fig. 5.** *Crepidula fornicata* Group 2. Influence of 48 h salinity stress on larval growth rate in *C. fornicata*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, and white—10). For each treatment, N = 24 larvae reared individually in microplates. Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). After rearing larvae in control conditions for (A) 1 day (B) 3 days or (C) 5 days after hatching, larvae were stressed for 48 h.

species (Figs. 2 and 3). Larval age when stress was applied also affected larval mortality at a salinity of 10 in one experiment (Fig. 2A), but all larvae in these treatments (salinity of 10) from this experiment eventually died. Of the 3 species tested, *C. fecunda* larvae were the least tolerant to decreased salinities (Figs. 2 and 3): A 12-hour stress at a salinity of 15 killed nearly all *C. fecunda* larvae within the following 8 days (Fig. 3B); the same stress killed only 4% of *C. onyx* larvae by day 11 (Fig. 2B) and a 24 hour stress at a salinity of 15 did not kill any *C. fornicata* larvae by day 8 (Fig. 2A). Although larvae of *C. fecunda* also exhibited substantial mortality under control conditions in the laboratory, over 50% of control larvae were still alive after 12 days (Fig. 3B). Exposing larvae of *C. fecunda* to a salinity of 20 was less stressful than exposing them to a salinity of 10, but mortality was still significantly higher than that of control larvae (Fig. 2C).

All *C. fornicata* larvae stressed at a salinity of 10 for 24 h (Fig. 2A) or at 15 for 48 h (data not shown,  $n = 36$ ) eventually died within 21 days



**Fig. 6.** *Crepidula fornicata* Group 3. Influence of 12–48 h salinity and/or starvation stress on larval growth rate in *C. fornicata*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, light gray—15, and white—10). For each treatment, N = 3 dishes, 12 larvae per dish all of which were measured. Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). (A) Larvae were stressed immediately after hatching (day 0) for 24 h. (B) Larvae were reared in control conditions for 2 days after hatching, and then stressed for 12 or 24 h. (C) Larvae were reared in control conditions for 2 days, and then stressed for 48 h; S indicates larvae that were starved during the stress period. See Table 2 for results of two-way ANOVA. (D) Larvae were reared in control conditions for 4 days, and then stressed for 24 h.

after the stress ended, though none died within the first 5 days after the stress; no control larvae died. Larvae of *C. onyx* seemed the most tolerant of low salinity stress in our experiments: they experienced substantial mortality after a 48 h exposure to a salinity of 15 (Fig. 3C), but not to the extent shown by *C. fecunda* (Fig. 3A) or *C. fornicata* (all *C. fornicata* larvae died, data not shown). Starving larvae at full-strength salinity, on the other hand, did not affect larval survival in any of the species tested (Figs. 2 and 3, data for *C. fornicata* not shown), suggesting that mortality was due to the salinity stress *per se*, rather than to an indirect effect of low salinity on ability to feed. No juveniles died in any treatment for those individuals that survived to metamorphose.

### 3.3. Larval growth rates after salinity stress

The larvae of all 3 species showed no detectable growth while experiencing salinity or starvation stress. After larvae were returned to full-strength seawater with food, the effect of the salinity stress on growth rates varied widely, even within species. Mean larval growth rates for *C. fornicata* were either unaffected (Fig. 4—salinities of 10 and 20, all treatments; Fig. 5A and C—salinity of 20; and Fig. 6B—salinity of 15 for 12 h), slowed for a number of days but then returned to control rates before metamorphosis (Fig. 5B—salinity of 20, Fig. 6A—salinity of 15, Fig. 6B—salinity of 15 for 24 h), or slowed dramatically and then never returned to control growth rates (Fig. 5—salinity of 10, all treatments; Fig. 6A—salinity of 10; Fig. 6C—salinity of 15).

The effect of reduced salinity on subsequent larval growth rate depended on the strength and duration of the stress, with exposure to lower salinities for longer periods generally slowing growth rates more dramatically (e.g., see Fig. 5A—salinity of 20 vs. 10 and Fig. 6B and C—salinity of 15 for 24 h vs. 15 for 48 h). Interestingly, the effect

that the stress had on larval growth rate for *C. fornicata* also varied among experiments, probably reflecting genetic differences among parents.

Mean larval growth rates also varied with duration of the low salinity stress for *C. onyx* (Fig. 7).

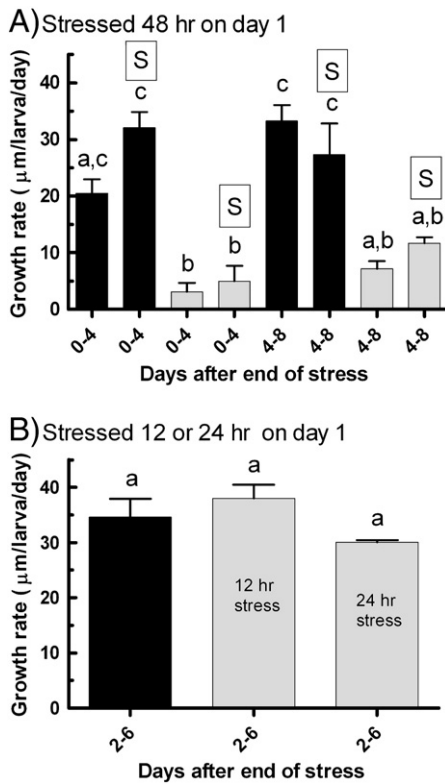
*C. fecunda* larvae stressed at a salinity of 20 grew normally after the stress was removed (Fig. 8B), but larvae stressed at a salinity of 15 grew significantly more slowly than control larvae for 2 days after the end of the stress; moreover, the larvae were all dead within 5 days after the stress ended (Fig. 8A).

Starving larvae did not slow larval growth rate like salinity stress did for *C. fornicata* (Fig. 6C, Table 2), *C. onyx* (Fig. 7A, Table 2), or *C. fecunda* (Fig. 8A, B; *C. fecunda* data were not analyzed by two-way ANOVA because they did not meet the requirements of that statistical test).

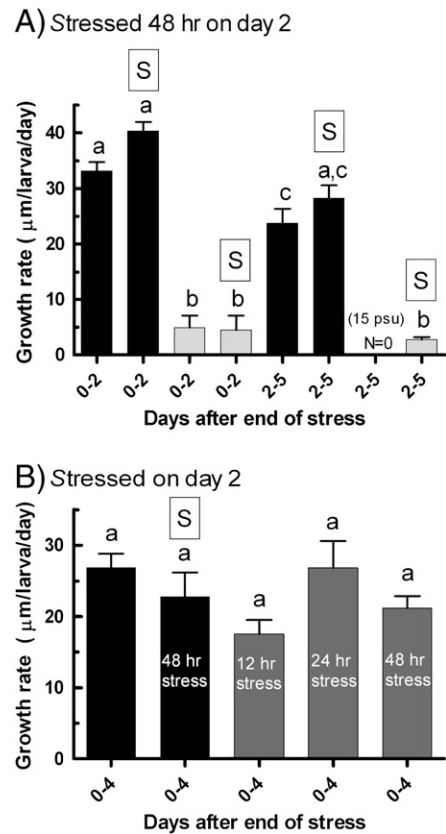
The amount of time that *C. fornicata* larvae were allowed to grow under control conditions before being subjected to salinity stress (i.e., the date that larvae were stressed after hatching) had little effect on growth rate following stress (Figs. 4–6). For larvae of all 3 species, exposure to lower salinity and longer exposure duration depressed larval growth rates to a greater extent, but the magnitude of effects varied with species and among experiments within a species.

### 3.4. Juvenile growth after stress to larvae

In nearly all experiments, larvae that were able to metamorphose had juvenile growth rates statistically equivalent to those of controls, even following the most severe salinity stress, regardless of whether or not larval growth rates recovered to control levels before metamorphosis (Figs. 9 and 10). Only when larvae of *C. fornicata* were stressed at a salinity of 10 for 48 h starting on day 4 after hatching did juveniles grow significantly more slowly than the



**Fig. 7.** Influence of 12–48 h salinity and/or starvation stress on larval growth rate in *Crepidula onyx*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control and light gray—15). For each treatment, N=3 dishes, 20 larvae per dish, 5–10 larvae subsampled per dish for shell length measurements. Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). (A) After rearing larvae in control conditions for 1 day after hatching, larvae were stressed for 48 h;  $\square$  indicates larvae that were starved during the stress period. See Table 2 for results of two-way ANOVA. (B) After rearing larvae in control conditions for 1 day after hatching, larvae were stressed for 12 or 24 h.



**Fig. 8.** Influence of salinity and/or starvation stress on larval growth rate in *Crepipatella fecunda*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, and light gray—15). For each treatment, N=6 dishes for controls, 3 dishes for all other treatments, 20 larvae per dish, 5–10 larvae subsampled per dish for shell length measurements. Means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test).  $\square$  indicates larvae that were starved during the stress period. (A) After rearing larvae in control conditions for 2 days after hatching, larvae were stressed for 48 h; (B) After rearing larvae in control conditions for 2 days after hatching, larvae were stressed for 12, 24, or 48 h.

controls following metamorphosis (Fig. 9A). In addition, no juveniles died in our study for any of the three species tested.

For *C. fecunda*, starving larvae for 48 h appeared to reduce juvenile growth rates: mean growth rate ( $\pm$  standard deviation) of control juveniles was  $93.1 \pm 33.3 \mu\text{m juvenile}^{-1} \text{day}^{-1}$  while mean growth rate for juveniles starved for 48 h as larvae was only  $61.9 \pm 35.9 \mu\text{m juvenile}^{-1} \text{day}^{-1}$  (Fig. 10C). However, the difference was not significant and a more comprehensive study needs to be done for *C. fecunda* to determine if starving larvae causes latent effects on juveniles, as it often does for *C. formicata* and *C. onyx* (Pechenik et al., 2002; Chiu et al., 2007, 2008).

**3.5. Impact of salinity stress on time to metamorphosis**

Subjecting larvae to salinity stress for 12 to 48 h prolonged the pre-competent period for all 3 species (Figs. 11 and 12). In all experiments with *C. formicata* and *C. onyx*, nearly all control larvae metamorphosed on the day that they were exposed to the metamorphic stimulant, excess KCl (Pechenik and Heyman, 1987; Pechenik and Gee, 1993). However, subjecting larvae to salinity stress earlier in development reduced the number of larvae competent to metamorphose on those same days, relative to controls; the magnitude of the delay in becoming metamorphically competent varied with the strength and duration of the salinity stress (Fig. 11). For *C. formicata* the age at which larvae were stressed had no significant effect on the rate at which larvae became competent to metamorphose (Fig. 11A, B). For *C. onyx* subjecting larvae to starvation did not affect the duration of the pre-competent period (Fig. 11D, Table 2).

Although we did not assess the effect of salinity stress on time to metamorphic competence in *C. fecunda*, we did assess the effect of salinity stress on time to spontaneous metamorphosis. Control larvae

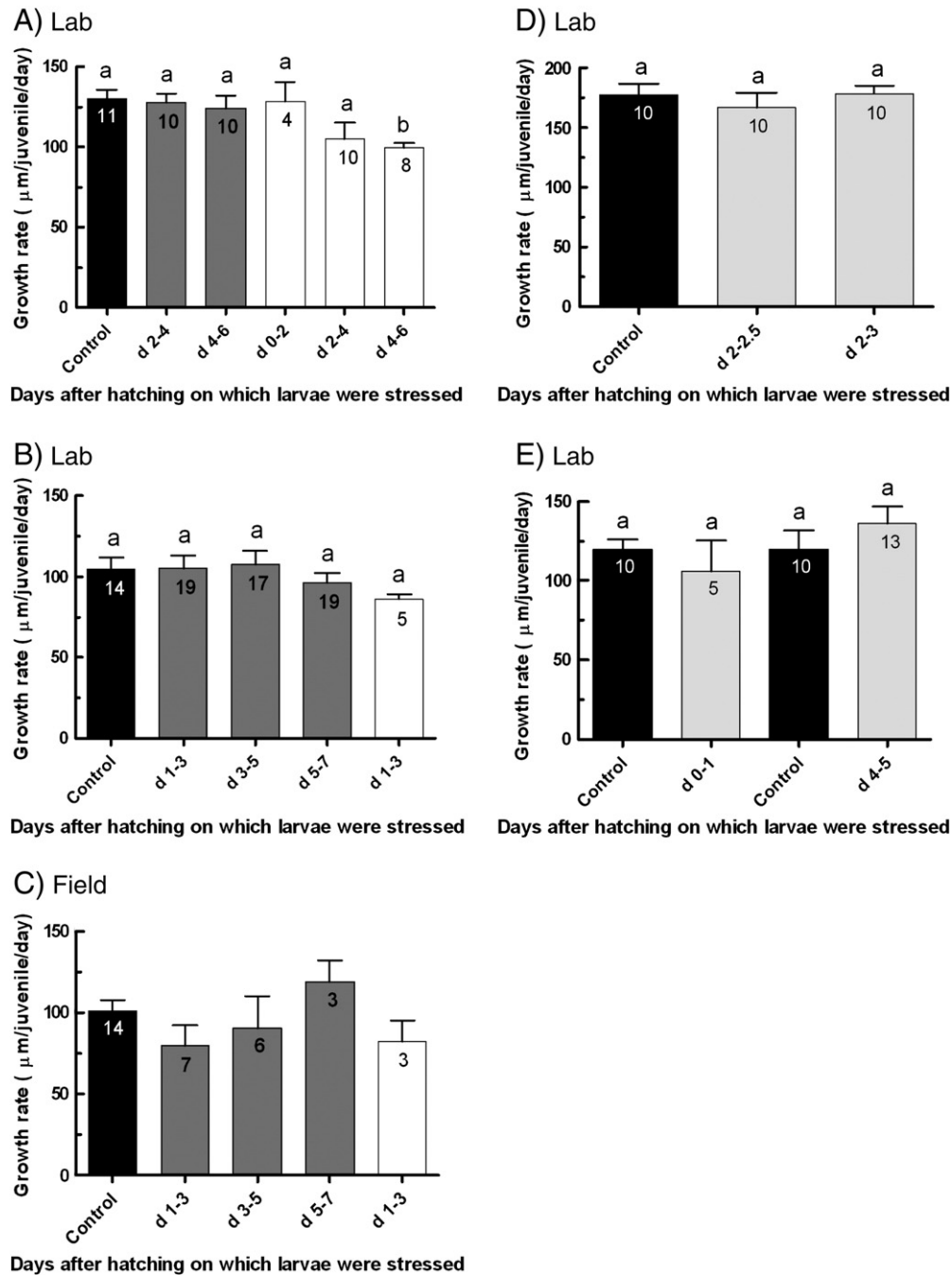
**Table 2**  
Summary of two-way ANOVAs performed on all experiments in which data met the requirements of the statistical test.

Relevant figure	Species	Days	Source	Df	ms	F	p-value
6C	<i>C. formicata</i>	0-2	Salinity	1	5586	71.53	<0.0001 <sup>a</sup>
			Starvation	1	175.6	2.25	0.172
			Salinity × starvation	1	175.6	2.25	0.172
		2-4	Salinity	1	6869	93.67	<0.0001 <sup>a</sup>
			Starvation	1	175.6	2.39	0.160
			Salinity × starvation	1	209.2	2.85	0.130
7A	<i>C. onyx</i>	0-4	Salinity	1	1479	83.28	<0.0001 <sup>b</sup>
			Starvation	1	134.7	7.57	0.025 <sup>b</sup>
			Salinity × starvation	1	69.1	3.89	0.084
		4-8	Salinity	1	1304	42.51	0.0002 <sup>b</sup>
			Starvation	1	1.688	0.055	0.821
			Salinity × starvation	1	82.89	2.67	0.13
11D	<i>C. onyx</i>	1-3	Salinity	1	4.97	109.9	<0.0001 <sup>c</sup>
			Starvation	1	0.0148	0.33	0.583
			Salinity × starvation	1	0.0825	1.83	0.214

<sup>a</sup> Indicates significantly slower growth rates than control larvae of *C. formicata* in Fig. 6C.

<sup>b</sup> Indicates significantly slower growth rates than control larvae of *C. onyx* in Fig. 7A.

<sup>c</sup> Indicates significantly longer duration of pre-competent period than control larvae of *C. onyx* in Fig. 11D.



**Fig. 9.** Influence of larval salinity stress on juvenile growth rate in *Crepidula fornicata*. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, light gray—15, and white—10). Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). Sample sizes (number of individually reared juveniles) are displayed in each bar. Juvenile growth rates were measured for 4 days after metamorphosis (A) Larvae from “*C. fornicata* Group 1.” (B) Larvae from “*C. fornicata* Group 2.” (C) Some juveniles from (B) were transferred to the field and growth rates were measured for an additional 4 days; no significant differences in mean growth rate in a treatment between lab and field were found. (D) and (E) Larvae from “*C. fornicata* Group 3”; treatment ‘2–2.5’ reflects a 12-hour stress 2 days after hatching.

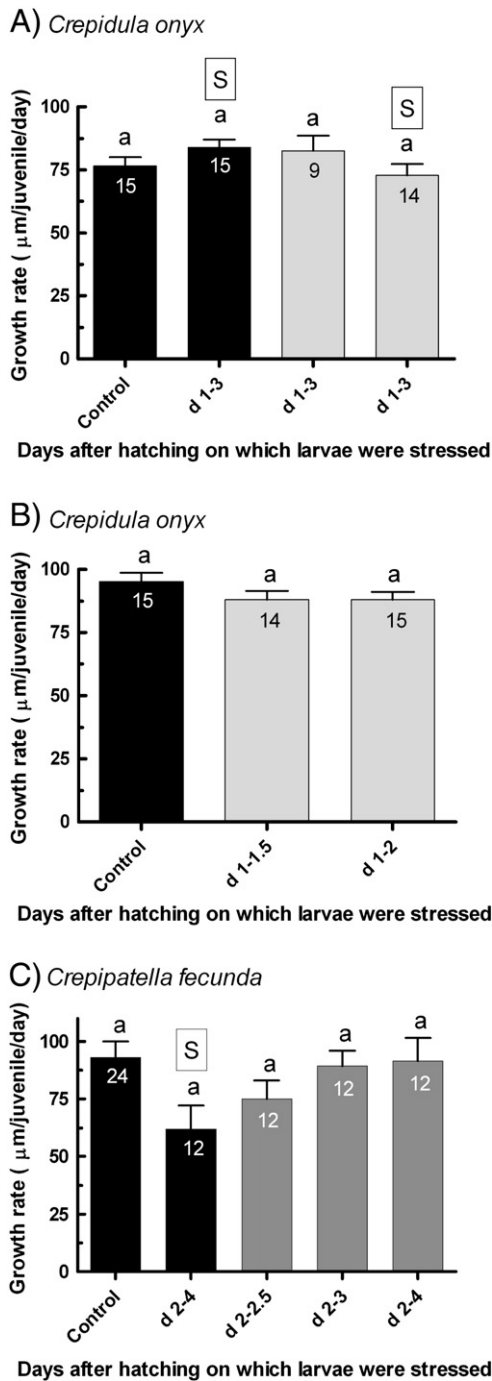
of *C. fecunda* began to metamorphose spontaneously 12 days after hatching, while larvae that were exposed to low salinity stress for 12–48 h took significantly longer to reach that point in development (less than 10% had metamorphosed by day 28 after hatching, Fig. 12).

Larvae of *C. fornicata* and *C. onyx* that did not metamorphose when first tested were allowed to grow under control conditions for 5 more days and then re-assessed for metamorphic competence. Stressed larvae from some treatments metamorphosed successfully after this second exposure to the metamorphic stimulus (Fig. 11D: 33% of larvae subjected to a salinity of 15 and 78% of larvae subjected to a salinity of 15 + starvation metamorphosed after the second

exposure), while larvae from other treatments (Fig. 11B—salinity of 10) again failed to metamorphose and in fact never produced a living juvenile. For treatments in which larvae never metamorphosed, larvae did not die immediately after the stress, but typically lived with minimal growth as long as several weeks before dying.

### 3.6. Impact of salinity stress on *C. fornicata* juveniles

Juveniles of *C. fornicata* that were stressed for 12, 24, or 48 h at a salinity of 15 had significantly slower growth rates for 3 days after the end of the stress (Fig. 13A). However, mean growth rates returned to



**Fig. 10.** Influence of larval salinity and/or starvation stress on juvenile growth rate in *Crepidula onyx* and *Crepipatella fecunda*. Mean  $\pm$  S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, and light gray—15). Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test). Sample sizes (number of individually reared juveniles) are displayed in each bar. Juvenile growth rates were measured for days 0–4 after metamorphosis. **S** indicates larvae that were starved during the stress period. (A) *C. onyx* larvae from Fig. 7A; (B) *C. onyx* larvae from Fig. 7B; treatment '1–1.5' reflects a 12-hour stress 1 day after hatching. (C) *C. fecunda* larvae from Fig. 8B; treatment '2–2.5' reflects a 12-hour stress 2 days after hatching.

control levels for juveniles from all treatments during the following three days (Fig. 13B), although stressed individuals remained smaller than control individuals during this time. Even when juveniles were exposed to a salinity of 15 for 48 h, juvenile growth rates were only slowed for 3 days; exposing larvae to the same stress halted growth and eventually killed all the larvae. No juveniles died following any

treatment. Although juveniles stopped growing while subjected to starvation stress, the juveniles that were starved for 48 h resumed normal (control) growth immediately after the stress was removed (Fig. 13A).

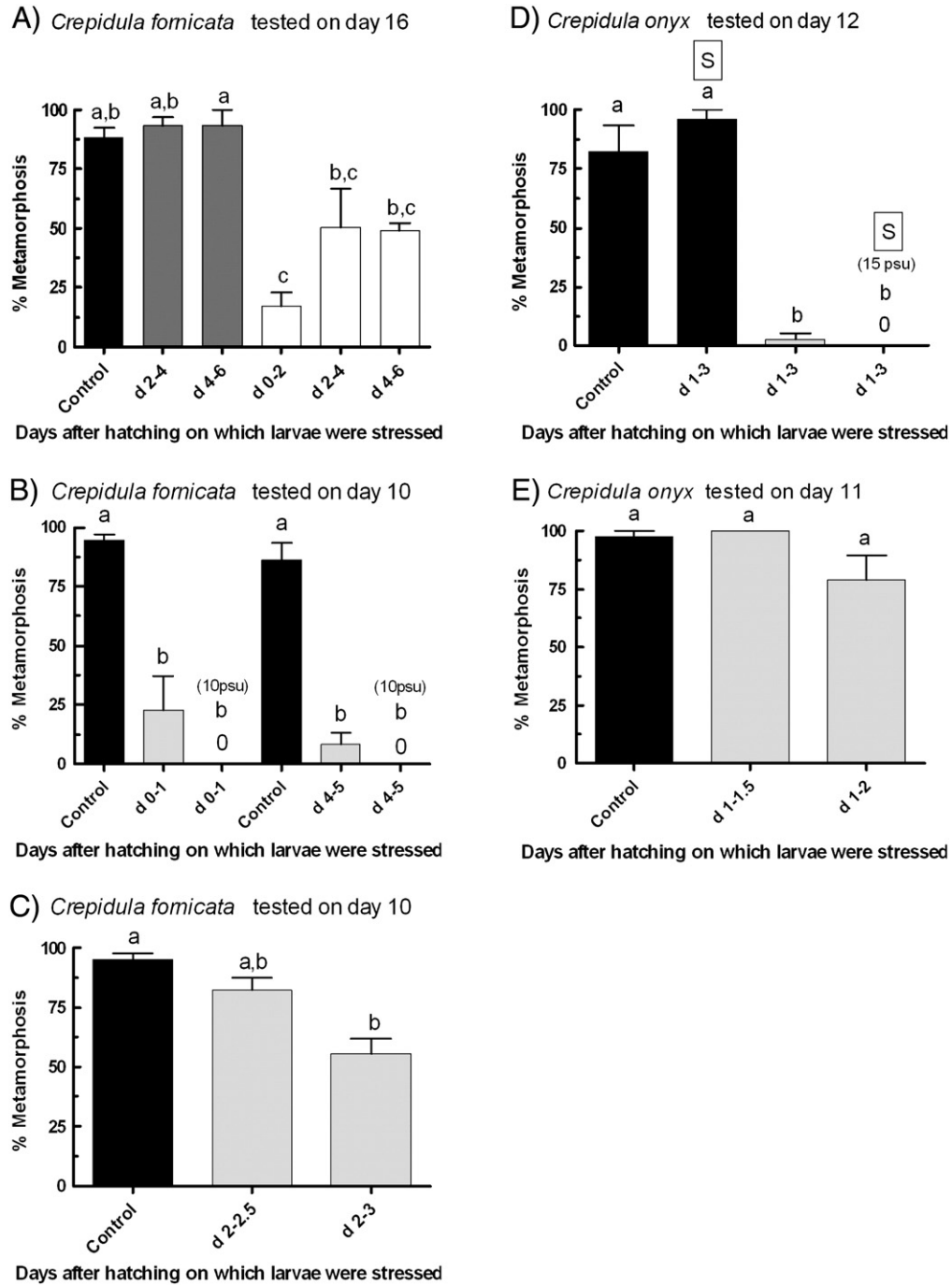
#### 4. Discussion

The direct effect that low salinity stress may have on osmoconformers like the calyptraeid species studied here may vary depending on the organism's ability to manipulate intracellular solute concentrations in order to regulate cell volume (Bradley, 2009). When faced with hypoosmotic stress, osmoconformers must "dump" solutes or increase protein synthesis (converting many osmotically active amino acids into one osmotically active protein molecule) to prevent the cells from swelling and bursting (Bradley, 2009). Even if volume regulation is successful, cells may have discarded molecules important for cellular function, causing long-term or permanent damage (Bradley, 2009). Thus, the degree to which the salinity in the environment is reduced should be of considerable importance to the animal: the more the salinity drops, the more likely that the animal will be permanently injured. Indeed, *C. fornicata* larvae were often not able to recover from the lowest salinities that we subjected them to (e.g., a salinity of 10 for as little as 24 h) (Figs. 5 and 6), but were able to recover from more moderate stresses (e.g. salinities of 20 and 15 for 12–48 h) (Figs. 4–6). Similarly, although larvae of *C. fecunda* were less tolerant of salinity stress than those of *C. fornicata*, they were also able to recover from a stress at a salinity of 20, although not from a stress at a salinity of 15 (Fig. 8).

Survival of organisms in reduced salinity and the immediate effects of low salinity stress on characteristics such as growth rate have been widely studied, especially in estuarine species (e.g., Lance, 1963—copepods; Lyster, 1965—polychaete larvae; Griffith, 1974—killifish; Brand, 1984—phytoplankton; Dunson and Seidel, 1986—reptiles; Deaton et al., 1989—bivalves; Kirst, 1990—algae). Some of the larvae that we tested could not even tolerate a salinity of 15 for 12 h (*C. fecunda*, Fig. 3B), while those of other species were able to tolerate that same reduction in salinity for 48 h (*C. onyx*, Fig. 3C), or even a reduction to a salinity of 10 for 48 h (*C. fornicata*, Fig. 4). The focus of the present study, however, was on the possibility of latent effects from salinity stress, not the determination of larval salinity tolerance *per se*. Though larvae likely had multiple fathers (e.g., Dupont et al., 2006; Le Cam et al., 2009), they came from a limited number of females and some (or much) of the variation seen among species in this study may actually reflect among-female variation; additional work is needed to explore the variation in salinity tolerance and the genetic basis for that variation.

Indeed, for *C. fornicata* we found a surprising amount of within-species variation in salinity tolerance for larvae released by different females (e.g., compare Figs. 4–6), suggesting that there is a substantial amount of genetic variation for traits conferring resistance to the detrimental effects of reduced salinity. A genetic effect has previously been seen in *C. fornicata*: Hilbish et al. (1999) showed highly significant differences in both growth rate and swimming speed among larvae from different *C. fornicata* families. In our experiments, mean ( $\pm$  standard deviation) growth rates of control larvae from different parents ranged from  $28.4 \pm 3.5$   $\mu\text{m}/\text{larva}/\text{day}$  (Fig. 4B) to  $87.9 \pm 1.6$   $\mu\text{m}/\text{larva}/\text{day}$  (Fig. 6A), which is within the range reported by Hilbish et al. (1999). However, mean growth rates of control larvae within an experiment stayed fairly constant over time, which agrees with Pechenik's (1980) finding that larvae of *C. fornicata* grow at a constant rate after hatching.

Larvae of all three species that recovered from low salinity stresses generally took longer to become competent to metamorphose than control larvae (Figs. 11 and 12). Since all 3 of the species that we examined live in environments that may experience rapid and prolonged reductions in salinity (Schmidt et al., 2006; Pechenik et al., 2007), these results suggest that their current abundances and distribution in nature may be partially determined by the degree to which they encounter water of reduced salinity as larvae.

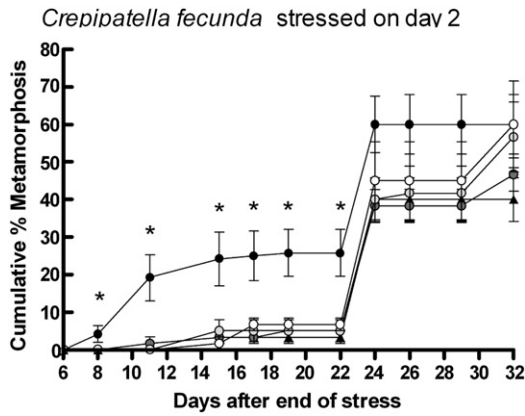


**Fig. 11.** Influence of salinity and/or starvation stress on duration of the pre-competent period for *Crepidula fornicata* and *Crepidula onyx* in separate experiments. Mean + S.E.M. shown for all treatments. Bar color indicates the salinity at which larvae were stressed (black—control, dark gray—20, light gray—15, and white—10). Within each experiment, means that have the same letter are not significantly different ( $p > 0.05$ , Bonferroni multiple comparisons test).  $N = 3$  dishes for all treatments. (A) *C. fornicata* tested on day 16; 6–12 larvae per dish from “*C. fornicata* Group A.” (B) and (C) *C. fornicata* tested on day 10; 6–12 larvae per dish from “*C. fornicata* Group C”; treatment ‘2–2.5’ reflects a 12-hour stress 2 days after hatching. (D) *C. onyx* tested on day 12 from Fig. 7A. **S** indicates larvae that were starved during the stress period. See Table 2 for results of two-way ANOVA. (E) *C. onyx* tested on day 11; 20 larvae per dish from Fig. 7B. Treatment ‘1–1.5’ reflects a 12-hour stress 1 day after hatching.

However, our data suggest that if individuals of *C. fornicata* encounter reduced salinity after they metamorphose, they will be largely unaffected after the stress is removed. Although juvenile growth rates slowed considerably for up to 3 days after juveniles were stressed at a salinity of 15 for 48 h, they returned to control growth rates after full-strength salinity was restored, and none of the stressed juveniles died (Fig. 13). In contrast, all *C. fornicata* larvae died after exposure to the same stress for the same amount of time (data not shown). It is possible that juveniles are able to seal themselves off from their external environment better than larvae, decreasing the

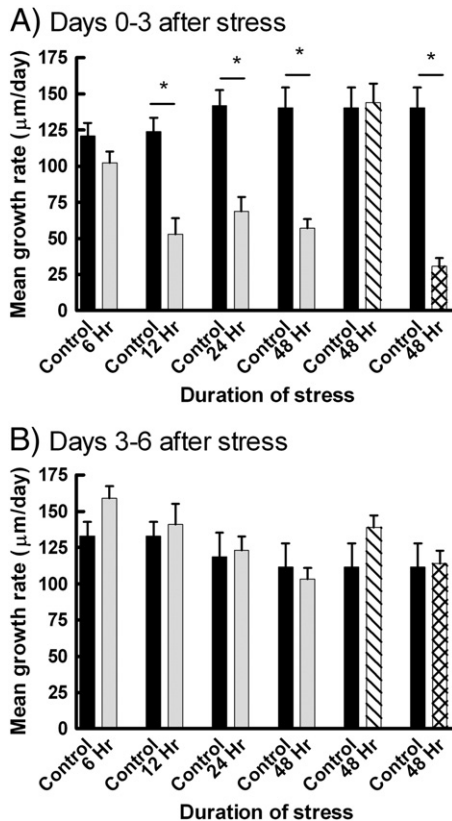
duration of exposure to low salinity. Alternatively, there may be a physiological shift during metamorphosis that provides the individual with an increased ability to tolerate salinity changes. Indeed, some marine animals do become more tolerant of environmental stress as they develop (e.g., Bambang et al., 1995; Anger, 1996; Schreiber and Specker, 1999; Anger and Charmantier, 2000), but this has not yet been documented for *C. fornicata*.

Although many marine animals demonstrate latent effects (e.g., Pechenik et al., 1996b; Wendt, 1998; Pechenik et al., 2002; Emler and Sadro, 2006; Giménez, 2010) or “carry-over effects” (e.g., Marshall et



**Fig. 12.** Influence of salinity or starvation stress on time to metamorphosis of *Crepipatella fecunda* larvae. Mean  $\pm$  S.E.M. shown for all data points. Symbols indicate the type of stress and the salinity at which larvae were stressed (black circle—control, black triangle—starvation only, dark gray circle—20 for 12 h, light gray circle—20 for 24 h, and white circle—20 for 48 h).  $N=3$  dishes for all treatments; 20 larvae per dish. Asterisks indicate a significant difference in mean metamorphosis on a particular day ( $p < 0.05$ , one-way ANOVA for each day). From hatching to day 23 larvae were checked periodically for spontaneous metamorphosis. On day 24 larvae were transferred to adult-conditioned seawater prompting an increase in metamorphosis of larvae in all treatments.

al., 2003; Ng and Keough, 2003) across life history stages from a variety of sub-lethal stresses experienced by larvae (reviewed by Pechenik, 2006), exposing larvae to salinities as low as 10 rarely



**Fig. 13.** Influence of juvenile salinity and/or starvation stress on growth rate in *Crepidula fornicata*. Mean  $\pm$  S.E.M. shown for all treatments. Bar color and pattern indicate the type of stress and the salinity at which larvae were stressed (black—control, light gray—15, striped—starvation only, and cross hatch—starvation at a salinity of 15). Asterisks indicate a significant difference between control and experimental treatments ( $p < 0.05$ , t-test).  $N=10$  individually reared juveniles per treatment. (A) Growth rates for the 1st three days after the end of the stress. (B) Growth rates for the 2nd three days after the end of the stress.

affected juvenile growth or survival for any of the three calyptraeid gastropod species that we investigated in this study (Figs. 9 and 10), even when the salinity stress caused substantial larval mortality or substantially reduced mean larval growth rate and increased time to achieving metamorphic competence (Figs. 2–8, 11, 12). This is surprising since short-term exposure to low salinity was clearly stressful to the larvae and since *C. fornicata* and *C. onyx* have previously been shown to exhibit latent effects in response to short-term nutritional stress experienced by larvae. However, the larvae of these species were affected by the salinity stress to various degrees, and the effects differed both among species and among individuals within a species. Some larvae in all 3 species never recovered from the stress and died before metamorphosing (Figs. 2, 3, 5, 6, 7, 8), while others showed initially reduced growth rates and reduced rates of development but nevertheless recovered to normal growth rates before metamorphosis (Figs. 2–7). Similarly, Pechenik et al. (2001) found no latent effects for juveniles of *Capitella* sp. I for larvae exposed to cadmium, even though some of the larvae were killed at the higher cadmium concentrations tested and even though this species showed latent effects when larvae were exposed to reduced salinity.

Many marine animals exhibit latent effects after metamorphosis from some larval stresses but not from others. For example, Thiyagarajan and Qian (2003) found that juvenile growth rates of the solitary ascidian *Styela plicata* were reduced when larvae were exposed to low salinity and low temperature, but not when the larval swimming period was prolonged. Of the species that we investigated, latent effects have also been shown to vary with the stress applied. In *C. fornicata*, juvenile growth rates were depressed following starvation stress in the larval stage (Pechenik et al., 1996b, 1996c, 2002), but latent effects were not detected when larvae were exposed to sub-lethal concentrations of cadmium (Pechenik et al., 2001) or when metamorphosis was delayed (Pechenik and Eyster, 1989). In *C. onyx*, juveniles had slower growth rates if those individuals had experienced nutritional stress as larvae in studies by Chiu et al. (2007, 2008), but did not grow more slowly in our study (Fig. 10A). Since our experiments and those of Chiu et al. (2008) were similarly designed, the difference in results suggests that there may be variation in susceptibility among offspring from different parents. There have been no previous studies on latent effects in *C. fecunda*, though the present study suggests that starving larvae may cause slower growth rates as juveniles, while low salinity stress as larvae appears to have no effect on juvenile growth rate.

The reasons why some stresses cause latent effects and others do not are unknown, largely because the mechanisms responsible for those effects are also unknown (see Pechenik, 2006). Some have offered a simple morphological hypothesis: larval stress reduces the size of the juvenile feeding apparatus, which in turn decreases the animal's ability to feed after metamorphosis, causing slower growth rates later in life (Wendt, 1996; Pechenik et al., 2002; Marshall et al., 2003). Others have suggested that stresses experienced early in development may interfere with transcriptional or translational processes that will show effects across life history stages (Pechenik et al., 1998; Pechenik, 2006). Also, it is thought that some stresses may alter DNA or damage enzymes directly, causing effects long after the stress is gone (Heintz et al., 2000). Recently, advances in the understanding of epigenetic effects across cell lineages (including cellular inheritance of changes in DNA methylation patterns) has provided a possible mechanism through which environmental stress experienced at one life history stage might affect organisms later in their lives (Jablonka and Raz, 2009). These hypotheses are not mutually exclusive and much work is needed to determine the specific mechanisms causing latent effects in particular species.

Why such a clearly stressful event for the larvae of these 3 calyptraeid species did not impact juvenile growth rate when at least some other stresses do cause such latent effects is unknown. *Crepidula* spp. (especially *C. fornicata*) have recently been suggested as a

promising model system for developmental biology (Henry et al., 2010); as more information on the genomes of these species and information on the genetic, epigenetic, enzymatic, and cellular effects of sub-lethal stress become available we may ultimately be able to understand the mechanisms underlying latent effects in these organisms, and why some stresses produce them while others do not.

## Acknowledgements

We would like to thank the anonymous reviewers for their comments on the manuscript. J. Pechenik thanks P-Y. Qian for providing lab space and financial support during his sabbatical leave in Hong Kong. O.R. Chaparro thanks A.J. Schmidt for help with experiments in Chile. Portions of the research were funded by the CSU-AAUP Research Grant to J. N. Jarrett, the Fondecyt-Chile Grant #1100335 to O.R. Chaparro, and the AoE grant (AoE/P-04/04-2-II) to P-Y. Qian. [SS]

## References

- Allen, J.D., Pechenik, J.A., 2010. Understanding the effects of low salinity on fertilization success and early development in the sand dollar *Echinarachnius parma*. *Biol. Bull.* 218 (2), 189–199.
- Anger, K., 1996. Salinity tolerance of the larvae and first juveniles of a semiterrestrial grapsid crab, *Armases miersii* (Rathbun). *J. Exp. Mar. Biol. Ecol.* 202 (2), 205–223.
- Anger, K., Charmantier, G., 2000. Ontogeny of osmoregulation and salinity tolerance in a mangrove crab, *Sesarma caracaeense* (Decapoda: Grapsidae). *J. Exp. Mar. Biol. Ecol.* 251 (2), 265–274.
- Bambang, Y., Charmantier, G., Thuet, P., Trilles, J.P., 1995. Effect of cadmium on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). *Mar. Biol.* 123 (3), 443–450.
- Bradley, T.J., 2009. *Animal Osmoregulation*. Oxford University Press, Oxford, pp. 59–71. vii.
- Brand, L.E., 1984. The salinity tolerance of 46 marine phytoplankton isolates. *Estuar. Coast. Shelf. S.* 18 (5), 543–556.
- Cebrian, E., Uriz, M.J., 2007. Contrasting effects of heavy metals and hydrocarbons on larval settlement and juvenile survival in sponges. *Aquat. Toxicol.* 81 (2), 137–143.
- Chaparro, O.R., Segura, C.J., Montiel, Y.A., Thompson, R.J., Navarro, J.M., 2008. Variations in the quantity and composition of seston from an estuary in southern Chile on different temporal scales. *Estuar. Coast. Shelf. S.* 76 (4), 845–860.
- Chiu, J.M.Y., Wang, H., Thiyagarajan, V., Qian, P.Y., 2008. Differential timing of larval starvation effects on filtration rate and growth in juvenile *Crepidula onyx*. *Mar. Biol.* 154 (1), 91–98.
- Chiu, J.M.Y., Ng, T.Y.T., Wang, W.X., Thiyagarajan, V., Qian, P.Y., 2007. Latent effects of larval food limitation on filtration rate, carbon assimilation and growth in juvenile gastropod *Crepidula onyx*. *Mar. Ecol. Prog. Ser.* 343, 173–182.
- Collin, R., 2003. Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of marine speciation. *Syst. Biol.* 52 (5), 618–640.
- Deaton, L.E., Derby, J.G.S., Subhedar, N., Greenberg, M.J., 1989. Osmoregulation and salinity tolerance in 2 Species of bivalve mollusk—*Limnoperna fortunei* and *Mytilopsis leucophaeta*. *J. Exp. Mar. Biol. Ecol.* 133 (1–2), 67–79.
- Dunson, W.A., Seidel, M.E., 1986. Salinity tolerance of estuarine and insular emydid turtles (*Pseudemys nelsoni* and *Trachemys decussata*). *J. Herpetol.* 20 (2), 237–245.
- Dupont, L., Richard, J., Paulet, Y.M., 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 (10), 3009–3021.
- Emlet, R.B., Sadro, S.S., 2006. Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). *Integr. Comp. Biol.* 46 (3), 334–346.
- Eyster, L.S., Pechenik, J.A., 1988. Comparison of growth, respiration, and feeding of juvenile *Crepidula fornicata* (L) following natural or KCl-triggered metamorphosis. *J. Exp. Mar. Biol. Ecol.* 118 (3), 269–279.
- Giménez, L., 2010. Relationships between habitat conditions, larval traits, and juvenile performance in a marine invertebrate. *Ecology* 91 (5), 1401–1413.
- Griffith, R.W., 1974. Environment and salinity tolerance in the genus *Fundulus*. *Copeia* 2, 319–331.
- Heintz, R.A., Rice, S.D., Wertheimer, A.C., Bradshaw, R.F., Thrower, F.P., Joyce, J.E., Short, J.W., 2000. Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. *Mar. Ecol. Prog. Ser.* 208, 205–216.
- Henry, J.J., Collin, R., Perry, K.J., 2010. The slipper snail, *Crepidula*: an emerging Lophotrochozoan model system. *Biol. Bull.* 218, 211–229.
- Hilbish, T.J., Sasada, K., Eyster, L.S., Pechenik, J.A., 1999. Relationship between rates of swimming and growth in veliger larvae: genetic variance and covariance. *J. Exp. Mar. Biol. Ecol.* 239 (2), 183–193.
- Jablonka, E., Raz, G., 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84 (2), 131–176.
- Jacobs, M.W., Degnan, B.M., Bishop, J.D.D., Strathmann, R.R., 2008. Early activation of adult organ differentiation during delay of metamorphosis in solitary ascidians, and consequences for juvenile growth. *Invertebr. Biol.* 127 (2), 217–236.
- Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53 (282), 457–481.
- Kirst, G.O., 1990. Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant. Phys.* 41, 21–53.
- Lance, J., 1963. The salinity tolerance of some estuarine planktonic copepods. *Limnol. Oceanogr.* 8 (4), 440–449.
- Le Cam, S., Pechenik, J.A., Cagnon, M., Viard, F., 2009. Fast versus slow larval growth in an invasive marine mollusc: does paternity matter? *J. Hered.* 100 (4), 455–464.
- Lyster, I.H.J., 1965. The salinity tolerance of polychaete larvae. *J. Anim. Ecol.* 34 (3), 517–527.
- Marshall, D.J., Pechenik, J.A., Keough, M.J., 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial, ascidian *Diplosoma listerianum*. *Mar. Ecol. Prog. Ser.* 246, 153–162.
- Ng, T.Y.T., Keough, M.J., 2003. Delayed effects of larval exposure to Cu in the bryozoan *Watersipora subtorquata*. *Mar. Ecol. Prog. Ser.* 257, 77–85.
- Pechenik, J.A., 1980. Growth and energy balance during the larval lives of 3 prosobranch gastropods. *J. Exp. Mar. Biol. Ecol.* 44 (1), 1–28.
- Pechenik, J.A., 1982. Ability of some gastropod egg capsules to protect against low-salinity stress. *J. Exp. Mar. Biol. Ecol.* 63 (3), 195–208.
- Pechenik, J.A., 1986. Field evidence for delayed metamorphosis of larval gastropods—*repidula plana* (Say), *Crepidula fornicata* (L), and *Bittium alternatum* (Say). *J. Exp. Mar. Biol. Ecol.* 97 (3), 313–319.
- Pechenik, J.A., 2006. Larval experience and latent effects—metamorphosis is not a new beginning. *Integr. Comp. Biol.* 46 (3), 323–333.
- Pechenik, J.A., Lima, G.M., 1984. Relationship between growth, differentiation, and length of larval life for individually reared larvae of the marine gastropod *Crepidula fornicata*. *Biol. Bull.* 166 (3), 537–549.
- Pechenik, J.A., Heyman, W.D., 1987. Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L). *J. Exp. Mar. Biol. Ecol.* 112 (1), 27–38.
- Pechenik, J.A., Eyster, L.S., 1989. Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. *Biol. Bull.* 176 (1), 14–24.
- Pechenik, J.A., Gee, C.C., 1993. Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L), judged by a natural and an artificial cue. *J. Exp. Mar. Biol. Ecol.* 167 (1), 59–72.
- Pechenik, J.A., 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 (1), 137–152.
- Pechenik, J.A., Estrella, M.S., Hammer, K., 1996b. Food limitation stimulates metamorphosis of competent larvae and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*. *Mar. Biol.* 127 (2), 267–275.
- Pechenik, J.A., Wendt, D.E., Jarrett, J.N., 1998. Metamorphosis is not a new beginning. *Bioscience* 48 (11), 901–910.
- Pechenik, J.A., Jarrett, J.N., 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 (1–2), 63–78.
- Pechenik, J.A., Pearse, J.S., Qian, P.Y., 2007. Effects of salinity on spawning and early development of the tube-building polychaete *Hydroides elegans* in Hong Kong: not just the sperm's fault? *Biol. Bull.* 212 (2), 151–160.
- Pechenik, J.A., Hilbish, T.J., Eyster, L.S., Marshall, D., 1996c. Relationship between larval and juvenile growth rates in two marine gastropods *Crepidula plana* and *C. fornicata*. *Mar. Biol.* 125 (1), 119–127.
- Pechenik, J.A., Gleason, T., Daniels, D., Champlin, D., 2001. Influence of larval exposure to salinity and cadmium stress on juvenile performance of two marine invertebrates (*Capitella* sp I and *Crepidula fornicata*). *J. Exp. Mar. Biol. Ecol.* 264 (1), 101–114.
- Phillips, N.E., 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology* 83 (9), 2562–2574.
- Richmond, C.E., Woodin, S.A., 1996. Short-term fluctuations in salinity: effects on planktonic invertebrate larvae. *Mar. Ecol. Prog. Ser.* 133 (1–3), 167–177.
- Schmidt, A.J., Toro, J.E., Chaparro, O.R., 2006. Reproductive patterns and their influence on the population genetics of sympatric species of the genus *Crepidula* (Gastropoda: Calyptraeidae). *J. Shellfish Res.* 25 (2), 371–378.
- Schreiber, A.M., Specker, J.L., 1999. Metamorphosis in the summer flounder, *Paralichthys dentatus*: thyroidal status influences salinity tolerance. *J. Exp. Zool.* 284 (4), 414–424.
- Thiyagarajan, V., Qian, P.Y., 2003. Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *Styela plicata* (Lesueur). *J. Exp. Mar. Biol. Ecol.* 290 (1), 133–146.
- Unterse, S., Pechenik, J.A., 2007. Local adaptation and maternal effects in two species of marine gastropod (genus *Crepidula*) that differ in dispersal potential. *Mar. Ecol. Prog. Ser.* 347, 79–85.
- Wendt, D.E., 1996. Effect of larval swimming duration on success of metamorphosis and size of the ancestral lophophore in *Bugula neritina* (Bryozoa). *Biol. Bull.* 191 (2), 224–233.
- Wendt, D.E., 1998. Effect of larval swimming duration on growth and reproduction of *Bugula neritina* (Bryozoa) under field conditions. *Biol. Bull.* 195 (2), 126–135.
- Yin, K., 2002. Monsoonal influence on seasonal variations in nutrients and phytoplankton biomass in coastal water of Hong Kong in the vicinity of the Pearl River estuary. *Mar. Ecol. Prog. Ser.* 245, 111–122.
- Zimmerman, K.M., Pechenik, J.A., 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.