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

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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 *Crepipatella 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.

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.

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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 *Crepipatella 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 18×10^4 cells ml^{-1} (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 18×10^4 cells ml^{-1} (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 *Crepipatella 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	*C. fornicata*	0.2, or 4	48	12.5	10 or 20	No	No	Yes (Fig. 11A)	Yes (Fig. 4)	Yes (Fig. 9A)
2	*C. fornicata*	1.3, or 5	48	14.2	10 or 20	No	No	No	Yes (Fig. 5)	Yes (Fig. 9B, C)
3	*C. fornicata*	0 or 4	24	10.0	10 or 15	No	Yes (Fig. 2A)	Yes (Fig. 11B)	Yes (Fig. 6A, D)	Yes (Fig. 9E)
4	*C. fornicata*	2	12 or 24	5.0 or 10.0	15	No	No	Yes (Fig. 11C)	Yes (Fig. 6B)	Yes (Fig. 9D)
5	*C. fornicata*	2	48	20	15	Yes	Yes (data not shown)[a]	No	Yes (Fig. 6C)	No[b]
6	*C. onyx*	1	12 or 24	4.2 or 8.3	15	No	Yes (Fig. 2B)	Yes (Fig. 11E)	Yes (Fig. 7B)	Yes (Fig. 10B)
7	*C. onyx*	1	48	16.6	15	Yes	Yes (Fig. 3C)	Yes (Fig. 11D)	Yes (Fig. 7A)	Yes (Fig. 10C)
8	*C. fecunda*	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	*C. fecunda*	2	48	14.2	15	Yes	Yes (Fig. 3A)	No	Yes (Fig. 8A)	No[b]
10	*C. fecunda*	2	12 or 24	3.5 or 7.1	15	No	Yes (Fig. 3B)	No	No	No[b]

[a] All larvae in the experimental treatment died.
[b] All larvae died before metamorphosis, so juvenile growth rates could not be measured.