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Effects of Embryonic Exposure to Salinity Stress or Hypoxia on Post-metamorphic Growth and Survival of the Polychaete Capitella teleta

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Abstract. Although a good number of studies have investigated the impact of larval experience on aspects of post-metamorphic performance, only a few have considered the potential impact of stresses experienced by brooded embryos. In this study we separately investigated the impact of salinity stress (as low as 10) and hypoxia (1 ml O2 l⁻¹) experienced by brooded embryos of the deposit-feeding polychaete Capitella teleta on hatching success, metamorphosis, post-metamorphic survival, and post-metamorphic growth. Salinity reduction from 30 to 10 or 15 reduced relative hatching success, presumably by reducing embryonic survival, but generally had no negative latent effects on juvenile survival or growth. Prolonged exposure to hypoxic conditions had no negative effects, as seen on measurements recorded, other than abandonment of brood tubes by some females. There were no negative effects on days to emergence from brood tubes, numbers of larvae emerging from brood tubes, juvenile survival, or juvenile growth. Future studies should consider the potential role of maternal behavior in protecting embryos from at least short-term exposures to hypoxia, and the capacity for anaerobic metabolism in both embryos and adults of this species.

Introduction

Latent effects occur when a stress that is experienced during early development manifests itself following metamorphosis (Pechenik, 2006). These effects have been documented in response to a variety of environmental stressors such as exposure to sublethal pollutant concentrations (e.g., Ng and Keough, 2003; Nice et al., 2003) or substantial changes in temperature, salinity, pH, food levels, or dissolved oxygen concentrations (e.g., Hettinger et al., 2012; Li and Chiu, 2013; Bashevkin and Pechenik, 2015; Vanderplancke et al., 2015). For example, when larvae of the slipper snail Crepidula fornicata (Linnaeus, 1758) were starved for several days, the resulting juveniles grew significantly more slowly, even if the larval growth rates had returned to normal after larval feeding was resumed (Pechenik et al., 1996, 2002). These effects were also observed when larvae of C. fornicata were simply fed a less nutritious food source during larval development (Pechenik and Tyrell, 2015).

Most of the studies that have documented such latent effects have focused on stressors experienced by larvae; few have examined the extent to which subsequent juvenile or adult performance may be affected by stresses experienced during embryonic development. Although brooding or encapsulation of embryos has often been thought of as protective (Gillespie and McClintock, 2007 and Chaparro et al., 2008, reviewed by Pechenik, 1979), under some circumstances brooding can expose embryos to severe stress, resulting in measurable latent effects on post-metamorphic development (e.g., Chaparro et al., 2014). For example, periods of hypoxia (Segura et al., 2014) or severe salinity stress (Chaparro et al., 2014) experienced by brooded...
embryos of the gastropod Crepipatella dilatata resulted in significantly slower juvenile growth than that of brooded embryos that did not suffer such stresses. The mechanisms accounting for these such effects are poorly understood (Pechenik, 2006).

In this study we examined the short-term and latent effects of hypoxia and salinity stresses experienced by brooded embryos of the salt marsh-dwelling, deposit-feeding polychaete worm Capitella teleta Blake, Grassle, and Eckalberger, 2009. After mating, females of C. teleta deposit their developing embryos (maximum lengths < 300 μm, Blake et al., 2009) on the interior wall of a specialized brood tube constructed by the mother from mucus and sand grains (Grassle and Grassle, 1976; Biggers and Laufer, 1999; Seaver et al., 2005; Blake et al., 2009); typically, at least 40 to 70 embryos are deposited per tube (Pechenik et al., 2001b). The brood tubes are generally about 8 to 15 mm long and about 0.6 to 1.3 mm wide (J. A. Pechenik, pers. obs.). The female does not leave the brood tube for one to several weeks (Pechenik and Cerulli, 1991; present study), until her larvae escape into the plankton. The larvae are non-feeding lecithotrophs (Blake et al., 2009) and are capable of metamorphosing within 30 minutes of their release into the plankton (Butman et al., 1988; Dubilier, 1988; Pechenik and Cerulli, 1991). Metamorphosis is easily induced by providing fine sediment with high organic content (Dubilier, 1988; Cohen and Pechenik, 1999).

Populations of C. teleta are common in the shallow mudflat areas of salt marshes and near sewage outflows and nutrient-enriched fish farms (Levin et al., 1996; Blake et al., 2009), locations in which they are likely to periodically encounter marked salinity fluctuation (Dubilier, 1988; Gray et al., 2002; Wu, 2002) and hypoxic conditions (typically defined as oxygen concentrations below 2 ml O₂ liter⁻¹, Diaz and Rosenberg, 2008). Salinity of 12 seems to be a threshold for most stages of development of C. teleta; adults survived poorly and had reduced reproductive output at reduced salinities of 10–12 (Levin et al., 1996; Pechenik et al., 2000); juveniles generally grew more slowly at salinities of 12–15 (Pechenik et al., 2000); and in short-term experiments (24–48 h), larvae became sluggish or stopped swimming at salinities of 10–12 (Pechenik et al., 2001b). Although prior studies with C. teleta have examined effects of reduced salinity on larval survival, juvenile growth, and reproductive success (Pechenik et al., 2000, 2001a, b), none has examined the impact of salinity stress or hypoxia on the development of brooded embryos, or the later consequences of such stress. In this study we exposed brooding mothers of Capitella teleta and their embryos to the separate stresses of reduced salinity or hypoxia for up to 96 hours, then examined the impact on the number of larvae emerging from the brood tubes, timing of emergence, and the potential for latent effects on juvenile survival and growth.

### Materials and Methods

#### Collection and maintenance of adult specimens

Capitella teleta broodstock were originally acquired in 2012 from Dr. Judith Grassle of Rutgers University. A population of adults was maintained at 20 °C in the lab at Tufts University, using artificial seawater (ASW; Instant Ocean Spectrum Brands, Blacksburg, VA) at a salinity of 30. Water was changed at least twice each week, and worms were fed mud that had been collected from the Little Sippewissett Marsh in Falmouth, Massachusetts (Lat. 41.574556, Long. –70.636186). The mud was sieved through a 1-mm-filter to remove debris, then frozen for several weeks before use (Cohen and Pechenik, 1999).

#### Isolating embryos

The laboratory population of Capitella teleta was visually inspected at least every other day for the presence of adult females in self-constructed brood tubes. Each brood tube was examined at 12X, using a dissecting microscope to confirm the presence of embryos. Only brood tubes containing early embryos were used in the experiments, and they were used within 24 h of their isolation.

#### Salinity experiments: effects of embryonic exposure to low salinity on hatching success, juvenile survival, and juvenile growth

Three experiments were conducted to determine the impact of pre-hatching exposure to reduced salinity on juvenile survival and growth, each testing the effects of a different exposure duration (24 h, 48 h, or 96 h). All experiments were conducted at 20 °C. On the initial day of an experiment, adult populations were examined and the requisite number of females with brood tubes were set aside. For each experiment, brood tubes containing early embryos were haphazardly assigned to either the control (30) or the reduced-salinity group. In the first experiment, brood tubes were exposed for 24 h to salinities of 30 (control), 25, 20, 15, or 10, with three replicates of one brood tube per salinity level. The second and third experiments were conducted in the same way, except that brood tubes were exposed to treatments, including the control, for 48 h and 96 h, respectively. In all cases, each brood tube was held in a glass dish containing 50 ml of oxygenated seawater at the appropriate salinity. Water was changed daily, using ASW of the appropriate salinity.

At the end of each exposure period, brood tubes were transferred to individual glass dishes containing 50 ml of aerated, normoxic ASW (salinity 30) and maintained at 20 °C. No mud was added to these dishes, as mud promotes metamorphosis (Dubilier, 1988; Pechenik and Cerulli, 1991; Cohen and Pechenik, 1999). Each dish was inspected daily for the release of larvae, which were then counted to...
Hypoxia experiments: effects of embryonic exposure to hypoxia on time to larval emergence, juvenile survival, and juvenile growth

Four experiments were conducted (all at 20 °C) to determine the effect of exposure to hypoxia during embryonic development on juvenile survival and growth. Brood tubes were collected from mass cultures, as previously described, and assigned haphazardly to the various treatments. Severely hypoxic conditions (1 ml O₂ l⁻¹, Diaz and Rosenberg, 2008) were established by bubbling N₂ gas into several liters of artificial seawater (ASW) of salinity 30 for about 10 min. The oxygen concentration in the normoxic seawater used in these studies was between 5.25–5.74 ml O₂ l⁻¹, and was obtained by vigorously bubbling air into several liters of ASW (salinity 30). Oxygen concentrations were determined using a calibrated dissolved oxygen meter (model 781; Strathkelvin Instruments, Motherwell, U.K.).

The first experiment was designed to test the effect of 24-h and 36-h exposures to hypoxic conditions on juvenile growth and survival. The second experiment tested the impact of 24-h and 48-h exposures, and Experiments 3 and 4 assessed the impact of 72-h and 96-h exposures, respectively.

In Hypoxia Experiment 1, 3 to 4 brood tubes per treatment were held in separate 300-ml Mason jars (one brood tube per container) that were filled to the top with normoxic or hypoxic ASW and tightly capped with two layers of Parafilm (Bemis Co., Inc., Neenah, WI) to prevent oxygen from diffusing from the air into the seawater. Experiments 2, 3, and 4 were conducted using 50 ml-plastic Falcon conical tubes (which were easier to seal) (Thermo Fisher Scientific, Waltham, MA), which were also filled to the top with seawater, sealed with Parafilm, and firmly capped. Each of these three additional experiments included larvae from three to nine brood tubes per treatment (see Results and, again, with one brood tube per container. Spot checks on control containers (hypoxic seawater, no brood tubes) revealed no measurable increase in oxygen content during any of the exposure durations; oxygen concentrations never exceeded 1 ml O₂ l⁻¹.

For each experiment, brood tubes were transferred to individual, 50 ml-glass dishes with well-aerated, normoxic ASW (salinity 30) after the designated exposure period, then checked daily for larval hatching. As with the salinity experiments, no mud was added to these dishes to deter metamorphosis. For each brood tube, we recorded days to hatching and the number of larvae hatched. The swimming larvae were then transferred to a glass dish containing ASW salinity 30, but no mud. From that dish, larvae (typically 50 to 150) were transferred to a second dish with ASW (salinity 30) and mud from the Little Sippewissett Marsh to stimulate metamorphosis and serve as a food supply for the juveniles, as in the salinity experiments described earlier. As was seen in our salinity stress studies, all larvae actively dove into the mud within minutes after it was placed in the jars. Water was changed every 4 days for 2 weeks, and additional mud was added to maintain excess food for the growing juveniles. Juvenile survival was determined by counting the number of survivors in each dish after 2 weeks, as we did in the salinity experiments. Juveniles were counted, rinsed quickly in several washes of deionized water to remove surface salt, then placed in pre-weighed foil pans with one worm per pan. The samples were then dried at 55 °C for 48 h and re-weighed to the nearest µg to determine the dry weight of each juvenile worm.
Effects of reduced salinity

The number of larvae that eventually emerged from the brood tubes varied with the degree of salinity stress experienced during the brooding period (Fig. 1). Significantly fewer larvae emerged after brooding females had been exposed to lower salinities (two-way ANOVA, $F_{4,32} = 9.85, P < 0.0001$), with salinity accounting for nearly 50% of the variability in the data. Although some larvae emerged from brood tubes that had been exposed to the lowest salinity (10) for only 24 h (Fig. 1), no larvae successfully emerged from any brood tubes after brooded embryos had been exposed to that salinity for 48 h or 96 h (Fig. 1). Otherwise, exposure time had no significant effect on the outcome (two-way ANOVA, $F_{2,32} = 2.65, P = 0.0865$), and there was no statistically significant interaction between salinity and exposure duration (two-way ANOVA, $F_{8,32} = 0.29, P = 0.97$; exposure time accounted for < 7% of the variability in the data). Note that we were unable to determine percent hatching per brood tube, because we could not count the initial number of embryos per tube. However, because all brood tubes were distributed haphazardly among treatments, the data represent relative hatching success following exposure to the indicated salinities.

On average, about 60%–80% of the larvae released following exposure to salinities as low as 10 for 24 h in Experiment 1 survived as juveniles for the full 2-week growth period; the level of salinity stress imposed earlier in development had no significant effect on mean percent juvenile survival ($F_{4,9} = 1.43, P = 0.30$; Fig. 2A). Similarly, at least 60% of juveniles survived after embryos had been exposed to salinities of 20 or higher for 48 h or 96 h (Fig. 2B, C), and at least 30% of juveniles survived after embryos had been exposed to a salinity of 15 (Fig. 2C). Exposing brooded embryos to low salinities for 48 h had no significant effect ($F_{3,8} = 2.17, P = 0.17$) on juvenile survival (Experiment 2, Fig. 2B). However, exposure of brooded embryos to salinities of 25, and significantly lower than in the controls following the 96-h exposure of brooded embryos to a salinity of 25, and significantly lower than in the controls following 96-h exposure of brooded embryos to a salinity of 15 (Experiment 3, Fig. 2C, Bonferroni multiple comparisons tests, $P < 0.05$). Exposing embryos to reduced salinity had no significant latent effect on mean juvenile growth, regardless of exposure level or exposure duration ($P > 0.10$ for all three tests; Fig. 3).

Results

Effects of reduced salinity

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Effects of hypoxia

Some females (4 of 12 that had been stressed for 24 h; 2 of 9 stressed for 48 h; and 1 of 10 stressed for 72 h) abandoned their brood tubes under hypoxic conditions, but no females did so under control conditions. No embryos successfully hatched from tubes that had been abandoned by their mothers.

Exposing embryos to hypoxia for up to 96 h, the maximum duration tested, had no significant effect on the mean number of days to hatching in any of the three experiments ($P > 0.11$ for all experiments; Fig. 4) or on the mean number of larvae emerging from the brood tubes afterwards ($P > 0.63$ for all experiments; Fig. 5). Embryonic experience of hypoxia also had no significant effect on subsequent

![Figure 1](https://example.com/figure1.png)

Figure 1. The impact of salinity stress during embryonic development on the mean (± SD) number of Capitella teleta larvae emerging from brood tubes. A different batch of brood tubes was used for each test duration. All brood tubes were returned to control salinity (30) after the 24 to 96 h of salinity stress, and larvae emerged 6–8 days later. Expt, experiment; N, 3 brood tubes per salinity treatment (15 brood tubes in total for each stress duration).

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Data analysis

All percent survival data were arcsin-transformed before analysis, although data in the figures are presented as percent survival. Most data were analyzed using GraphPad Prism ver. 6.0 (GraphPad Software, San Diego, CA). Data about the impact of salinity and exposure duration on the numbers of larvae emerging from brood tubes were analyzed by two-way ANOVA. Most other data were generally analyzed by one-way ANOVA, followed by Bonferroni multiple comparisons tests to determine significant differences between pairs. When the data failed to meet assumptions required for ANOVA analysis, we conducted non-parametric Kruskal-Wallace analyses, followed by Dunn’s multiple comparisons tests. Data concerning effects of hypoxia on final juvenile dry weights were analyzed using a mixed model procedure, including “parent nested in treatment” as a random factor in the model (SAS Institute, Cary, NC).
mean juvenile survival ($P > 0.20$ for all experiments; Fig. 6). Finally, hypoxic conditions experienced by brooded embryos for up to 96 h had no detrimental effects on juvenile growth (Fig. 7). In Experiment 1, in fact, final mean juvenile dry weights after embryos had been exposed to hypoxia for 24 h or 36 h were significantly higher (24 h: $t$-test = 2.33, $P = 0.023$; 36 h: $t$-test = 3.44, $P = 0.001$; mixed model analysis) than what was recorded for control individuals that had experienced no hypoxic stress (Fig. 7A). Similarly, final mean weight of individuals that had experienced 96 h of hypoxia as brooded embryos in Experiment 4 was significantly higher than that of control individuals ($t$-test = 2.56, $P = 0.011$; mixed model analysis).

Figure 2. Impact of salinity stress during embryonic development on mean (± SD) juvenile survival (3 replicates per treatment). In all treatments, juveniles were reared at the control salinity (30) following metamorphosis. Numerals below each bar indicate the total number of larvae that had been transferred to dishes of mud to induce metamorphosis. Dishes were examined 2 weeks later to assess survival. The indicated ANOVA analyses were based on data from the 3 replicates per treatment. No larvae were released after the 48-h or 96-h stress durations at salinity 10.

Figure 3. Impact of salinity stress (24–96 h) during embryonic development on mean (± SD) final juvenile weight (12 worms per salinity treatment), 2 weeks after metamorphosis was induced. Juveniles were reared at salinity 30 following metamorphosis. KW, Kruskal-Wallis analysis.
Discussion

Adults and larvae of Capitella teleta are remarkably tolerant of environmental stress (Grassle and Grassle, 1974; Pechenik et al., 2000, 2001a), which helps to explain why the species is so abundant in organically rich and polluted waters (Grassle and Grassle, 1976; Levin et al., 1996; Gray et al., 2002). The tolerance of brooded Capitella embryos to environmental stress has not been reported. In the present study, we found that brooded embryos of C. teleta were susceptible to the effects of reduced ambient salinity, but were extremely tolerant of reduced oxygen availability. The extent to which C. teleta can use anaerobic respiratory pathways at different stages of development when faced with hypoxic stress (e.g., Liu et al., 2014) has not yet been explored.

The only significant negative impact of hypoxia that we noted was the abandonment of brood tubes by some of the mothers (7 abandonments out of 31 brooding mothers). It would be interesting to determine whether the likelihood of abandonment is related to the number of embryos in each brood tube. Females of the marine gastropod Crepipatella dilatata often evict their brooded offspring under hypoxic conditions, possibly as a way of reducing the level of oxygen debt that must eventually be repaid (Segura et al., 2014). We saw no negative impact of hypoxic conditions on anything else that we measured. Indeed, in two of our experiments juveniles of Capitella teleta actually grew somewhat faster following longer periods of hypoxic stress (Fig. 7). The absence of any measurable negative consequence of hypoxic stress in this study—other than brood tube abandonment by some mothers—was particularly impressive; various life stages of many other marine species have shown detrimental effects following exposures to much lower levels of hypoxic stress than were tested in the present study (Vaquer-Sunyer and Duarte, 2008; Eerkes-Medrano et al., 2013).

In contrast, we saw a dramatic reduction in relative hatching success following exposures to even mild salinity stress for only 24 h. The extent to which this reduced relative hatching success reflects a direct effect of reduced salinity on embryonic physiology or was a secondary consequence of possible changes in female behavior is unclear. Females may stop irrigating their brood tubes if salinity decreases below a certain level. If so, the embryos might be exposed to other stresses over time, including reduced oxygen availability, decreased pH (via buildup of CO₂), and the buildup of ammonia within the brood tube (Llanso and Diaz, 1994; see also Chaparro et al., 2009 for parallel situations with a
brooding gastropod and a brooding bivalve facing reduced ambient salinity). The lack of any measurable effect of direct exposure to hypoxia on the numbers of larvae hatching in our experiments argues against a direct effect of hypoxia during salinity stress. Reduced hatching success following embryonic exposure to reduced salinity instead may reflect the combined impact of reduced salinity and hypoxia, and perhaps waste buildup within brood tubes, something that could be examined in future studies. Alternatively, the effect could have been due entirely and directly to the salinity reduction.

Note that although we collected for our experiments only females that were already brooding young embryos, we had no way of knowing whether the females deposited additional eggs after the start of our experiments. If females continued depositing eggs into their brood tubes for several days, then the salinity-related decline in the mean numbers of larvae released could have been due at least partly to a cessation of egg production following salinity stress. However, it seems unlikely that a single 24-h exposure to a salinity of 25 (Experiment 1, Fig. 1) would halt subsequent egg release into the brood tube for the next week. Note also that no larvae emerged from brood tubes following 48-h or 96-h exposures to a salinity of 10. Since all brood tubes contained many embryos at the outset of each experiment, the lack of any successful emergence following exposure to those levels of stress can only be explained by embryonic mortality. Interestingly, even 96 h of exposure to hypoxia, the other stress tested in these studies, had no impact on the numbers of larvae emerging from brood tubes (Fig. 5).

Brooded embryos of *Capitella teleta* also showed few post-metamorphic consequences (i.e., “latent effects,” reviewed by Pechenik, 2006) of early exposure to either reduced salinity or hypoxia, and were, again, even more tolerant of hypoxic conditions than of salinity stress. These results are surprising, because *Capitella teleta* has been shown to exhibit latent effects in response to some larval manipulations, including exposures to reduced salinity. In

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**Figure 5.** Impact of exposure to hypoxia during embryonic development on mean (± SD) number of larvae emerging from each brood tube. *N*, number of brood tubes per treatment.
particular, delaying their metamorphosis for as little as 72 h decreased juvenile survival dramatically (Pechenik and Cerulli, 1991); exposing larvae to a salinity of 12 for 48 h significantly decreased post-metamorphic survival; and exposing larvae to a reduced salinity of 15 for as little as 24 h led to significant reductions in post-metamorphic growth (Pechenik et al., 2001a). Yet no such latent effects were observed in the present study in consequence of exposing brooded embryos to similar or even greater levels of stress. The embryos may be more tolerant of reduced salinity than the larvae; the mother’s behavior (possible cessation of irrigation of the brood tube) may reduce the rate of salinity decline experienced by brooded embryos or the extent to which the brooded embryos were exposed to the low salinity; or individuals, for unknown reasons, may simply be less vulnerable to the latent effects of such exposure so early in development. Embryos of some species are more tolerant of salinity reductions when the change is gradual rather than abrupt (e.g., Pechenik, 1983; Woods and DeSilets, 1997). Future studies could attempt to document the range of salinities to which brooded embryos of this species are exposed in the field.

The mechanisms causing latent effects in marine invertebrates are unclear (reviewed by Pechenik, 2006), although selective DNA methylation or other epigenetic mechanisms may well be involved (Williams and Degnan, 2009; Burggren, 2014; Skinner, 2015). Individuals of C. teleta may not be vulnerable to such epigenetic modifications until later in development. Whether the likelihood of latent effects following exposure to hypoxic conditions or other stresses is somehow linked to the range of stresses naturally experienced in the field by adults of particular species may be worth considering in future studies.

In some cases, females in our study abandoned their brood tubes under hypoxic conditions, an action we never saw for control females, or for those exposed to reduced salinity. Following such abandonment, all embryos died without hatching, suggesting that adult behavior plays an important role in providing embryos with something essential or in protecting embryos from stress. That role needs to be clarified in future studies. Faced with reduced oxygen levels, for example, mothers may increase ventilation rates, as seen in some brooding decapods (Baeza and Fernández, 2002).

Figure 6. Impact of exposure to hypoxia during embryonic development on subsequent mean (± SD) juvenile survival. N, number of brood tubes from which larvae were obtained per treatment. Between 25 and 50 larvae were typically released from each brood tube.
Finally, the role of parental exposure to stress in determining embryonic or larval tolerance has yet to be explored for *C. teleta*. Exposing adults of the zebrafish (*Danio rerio*) to chronic hypoxia for 2–4 weeks substantially increased the resistance of their larvae to hypoxic stress (Ho and Burggren, 2012). Perhaps the embryos of *Capitella teleta* would be less resistant to hypoxic stress if the juveniles and adults of previous generations were maintained in well-oxygenated conditions throughout their lives.

Acknowledgments

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