

Effects of acute salinity stress on embryos of the sacoglossan sea slug, *Alderia modesta*

Thomas Parker¹²

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¹ Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 92850

² Department of Biological Sciences, California State University Fullerton, Fullerton, California 92831, United States
Correspondence – tparker@csu.fullerton.edu

Abstract

Intertidal organisms face highly variable environments with a multitude of stresses placed on them. Freshwater input from rain and run off is one such stress. Salinity changes have been shown to have adverse developmental effects on embryos of intertidal organisms. Although these organisms have developed different mechanisms by which larvae and embryos cope with stresses, relatively little has been demonstrated on the range of environmental stresses the eggs are able to tolerate. In this study adults and egg masses of the sacogloassan sea slug, *Alderia modesta* (Lovén 1844), were subjected to acute salinity stress to determine the consequences for reproduction and embryonic development to hatching, respectively. New egg masses that had been laid at constant salinity were exposed to varying salinity (7-37ppt) stress within 12 hours of deposition. Adults were exposed to varying salinity while reproducing and the egg masses they subsequently produced were compared between ambient and experimental conditions. Hatching success of eggs was significantly higher at intermediate salinities, with zero hatching success at the lowest salinity treatment (7ppt), and delayed development with low (10%) success at the highest treatment. When exposed to the same acute salinity stresses, adults did not lay egg masses so no comparative data was collected. These results show tolerance to and development in lower salinity than previous studies, providing evidence that successful reproduction might be less restricted by conditions of embryonic development.

Introduction

Intertidal organisms face highly variable environments with a multitude of stresses placed on them. Predation, desiccation, temperature and salinity can vary both spatially and temporally giving rise to detrimental effects on the organisms such as decreased growth or death. Salinity influences developmental rates of various marine organisms (Fretter and Graham, 1962; Tettelbach and Rhodes, 1981; Pechenik, 1982, 1983). Freshwater input from rain (Tettelbach et al., 1985) and runoff (Sanders et al., 1965; Drouin et al., 1985) can cause extreme low salinity in intertidal areas. Conversely, seasonal changes in rain fall can create relatively high salinity environments for the same organisms (Levin 1982). These fluctuations in salinity are thought to be more pronounced in intertidal areas occupied by embryos and larvae than in pelagic habitats (Spight, 1975; Pechenik, 1978, 1979).

Intertidal benthic organisms have multiple ways to help developing embryos and larvae cope with these stresses (Thorson, 1950; Strathmann, 1985). Many gastropods encase their eggs in gelatinous or encapsulated masses which are thought to provide protection against stresses such as predation, desiccation, and salinity fluctuations (Thorson, 1950; Pechink, 1979; Strathmann, 1985). The relative protection of encased embryos and larvae from temperature and desiccation has been studied in a few species (Spight, 1977; Pechenik, 1978; Strathmann, 1999), while comparative studies of protection against predation has been limited (Rawlings, 1990). Gastropod egg masses are encased and protect against salinity fluxes (Pechenik, 1983; Woods and DeSilets Jr., 1997), but effects of acute salinity changes over the full developmental period is not well understood (Roller and Stickle, 1989).

In this study, I examined the effects of acute salinity change, or rapid exposure to large salinity changes over long periods, on hatching success and biomass of fertilized egg masses on

Alderia modesta (Lovén, 1844), an intertidal sacoglossan sea slug, *A. modesta* is an obligate feeder on the yellow-green algae *Vaucheria sp.* and is found only in intertidal areas with its food source (Engel, 1940; den Hartog and Swennen, 1952; den Hartog, 1959). *A. modesta* is euryhaline, as adults are shown to survive in salinities as low as 2ppt and as high as 32ppt (Krug personal communication). Egg masses have also been shown to develop and hatch in salinities ranging from 8ppt to 32ppt (Krug personal communication). In this study, I exposed adults and egg masses to salinity treatments ranging from 7ppt to 37ppt, which coincides with the observed survival range for adult *A. modesta*, to determine effects on egg mass biomass, hatching success of veligers, and developmental differences due to salinity changes.

Methods

Egg Masses

Adult *Alderia modesta* were collected in August 2014 at Argyle Lagoon, San Juan Island, Washington. All adults were collected from *Vaucheria sp.* patches near the creek inlet for the lagoon and taken back to the laboratory. Salinity of Argyle water adjacent to the *Vaucheria sp.* patch was measured to be 28ppt. Adults were placed on *Vaucheria sp.* in seawater at ambient salinity (approx. 27ppt) and temperature (approx. 22°C) and allowed to feed and mate for 8 hours each day over the course of 5 days. After 8 hours, each adult was placed into a separate 35mm x 10mm petri dish of seawater at ambient salinity and temperature, but without *Vaucheria sp.* to allow for egg mass deposition. Egg masses were collected between 10am and 12pm the following morning and placed into treatment water. Adults were then placed back together with *Vaucheria sp.* and allowed to feed and mate.

Hatching success

Salinity treatments were created using 27ppt, 0.3 μ m mesh-filtered, seawater diluted with reverse osmosis or double strength filtered sea water to achieve desired salinity. Treatments increased by increments of five from 7ppt to 37ppt. Salinity for all water was determined with a refractometer. All experiments were run at room temperature (approx. 22°C). Egg masses were individually distributed into 35mm x 10mm petri dishes containing either control salinities (27ppt) or experimental salinities (7, 12, 17, 27, 32, 37ppt) and allowed to develop. Egg masses were checked daily and hatching success was quantified by counting hatched veligers and unhatched eggs for all treatments when first hatching of a cohort (all egg masses that were placed into treatments on a single day) was observed. Daily checks of egg masses that had not hatched were continued to determine potential delayed development and hatching times.

Adults and Egg Mass Biomass

Adults were separated into groups of 3 and placed into 80mm x 12mm petri dishes with varying salinity seawater (7-37ppt) and *Vaucheria sp.* to feed on for 8 hours. After 8 hours each adult was placed into separate 35mm x 10mm petri dishes, with the same salinity treatment in which they were allowed to feed, for egg deposition. Dishes were checked the following day for eggs and adults were placed back with *Vaucheria sp.* for feeding and mating. Biomass of eggs deposited at 27ppt were taken as a control prior to exposing adults to their respective treatment levels.

Results

Hatching success was quantified as the proportion of hatched veligers to the total egg count per mass. Egg masses contained 249 \pm 98 ($\bar{x} \pm$ SD; n = 63) embryos, and clutch size was

determined to not have a significant effect on hatching success ($p = 0.558$, $df = 60$, $n=63$). Clutch size and salinity were also analyzed to look for any interaction, but it was also insignificant ($p=0.781$). Salinity was found to have a significant effect on hatching success ($p<0.001$, $df = 60$, $F\text{-stat} = 24.04$) with the highest hatching success coming from intermediate salinity levels (Figure 1).

When adults were placed into other salinities besides ambient egg masses were not laid. No data was available for comparison of egg mass biomass.

Discussion

The results of this study show that egg masses at both low and high salinities significantly lowered hatching success compared to the intermediate salinities. At a salinity of 7ppt, no hatching occurred. High salinity caused a delay in hatching, while very low salinity stopped development entirely. The low salinity results seem to be consistent with work on other gastropod species (Roller and Stickle 1989), as well as studies on the same species in the southern part of its geographic range (Krug personal communication).

In a previous study, development occurred at 8ppt but only if the egg masses were placed into the treatment 2-3 days after developing in intermediate salinity water. Any masses placed into the treatment within 1 day of development ceased to continue developing (Krug personal communication). The results of this study are consistent with those of the previous study, as egg masses placed into 7ppt did not develop at all. As embryos develop they have a more robust salinity tolerance (Krug personal communication), and therefore embryos at different stages of development could withstand varying degrees of salinity stress. This aspect of development

should be further studied to discover how timing of stresses could impact populations differentially.

Even with increased tolerance from longer developmental times in intermediate water, it is unlikely that embryos would be as euryhaline as their adult counterparts. When salinity rapidly changed for adults they stopped laying egg masses altogether, but continued to feed and lay egg masses after returning to intermediate salinity water. Although adults can survive the full range of stress, embryos could still provide a limitation on distributional range for the species through decreased resistance.

Prolonged, abundant fresh water input into intertidal habitats could have huge detrimental effects on developing egg masses, but the consequences of less severe fluxes still need to be determined. Work with other gastropods has shown moderate to high survival in egg masses exposed short fluxes in fresh water input (Pechenik, 1983; Wood and DeSilets Jr., 1997). Roller and Stickle (1988) showed that temperature also impacted survival to hatching in embryos subjected to salinity stress. The increase in survival at higher temperatures (28 vs. 22°C) could be attributed to increased developmental rate and subsequent increased salinity tolerance while progressing through stages. Future work on salinity effects on *A. modesta* should include temperature variability, as temperature habitats vary by ~5°C in a matter of hours within the same day (personal observation) and probably vary much more between high tide and low tide or seasonally.

Data from this study suggest that embryonic stages of *A. modesta* are quite euryhaline, developing and hatching at salinities ranging from 12ppt to 37ppt. While embryos are not quite as tolerant as adults, they are still robust, and may be even more so with increased

development time prior to exposure. The low tolerance of embryos could limit reproductive success of adults, but not drastically.

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Figure captions

Figure 1. Average hatching success of embryos (n=9/salinity) under different acute salinity treatments ranging from 7ppt to 37ppt. Data was analyzed using regression analysis (df=60, F-stat = 24.04, p<0.001).

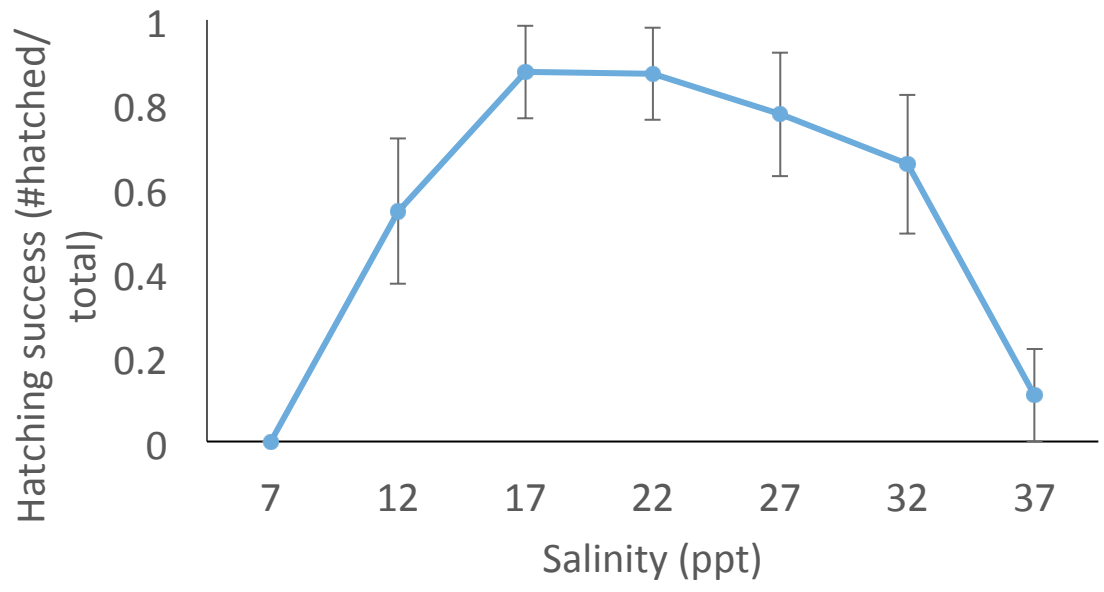


Figure 1.