

**Effects of Salinity on Contraction, Expansion, and Locomotion Behaviors of the Sea
*Anemone Anthopleura elegantissima***

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Abstract

Intertidal organisms have been investigated heavily in studies to understand how climate change may affect marine ecosystems, communities, and even individual species. Climate change may increase the environmental stresses of intertidal such as salinity fluctuation. The sea anemone *Anthopleura elegantissima* is one of the abundant intertidal organisms along the west coast of North America. I examined whether *A. elegantissima* changes certain behaviors (contraction, expansion, and locomotion) under different salinity conditions. The results suggest that this sea anemone is better adapted to low salinity conditions than high salinities in response to disturbance. Individuals closed up faster in high salinity, and opened up slower in both low and high salinity conditions than in normal salinity. This species preferred to stay in low and high salinity conditions rather than being exposed to the air.

Keywords: Sea anemone, *Anthopleura elegantissima*, Rocky intertidal, Tide pools, Contraction and Expansion, Locomotion, Salinity stress

Introduction

Tide pools of the rocky intertidal zone are well known for their enormous biodiversity (Nielsen 2007). Organisms living in tide pools face many environmental stresses including irradiance, hypoxia, desiccation, wave exposure, and variation in temperature, pH, and salinity (Barber 2007, Amado et al. 2011). Global warming may make these stresses more extreme, which may affect the distribution and abundance of intertidal organisms. Because of the high accessibility of intertidal, scientists have started to use intertidal organisms as models to predict changes in distribution and abundance in response to climate change (Mieszkowska et al. 2005). For example, Mieszkowska and others (2005) found that *Balanus perforates* (acorn barnacles) have extended northward since the mid 1980s due to climate warming. The ranges of invertebrate fauna of a rocky intertidal community in California shifted northward consistently as the predictions related to climate warming between 1931 to 1934 (Barry et al. 1995). The expansion or migration of any species will affect the distribution and abundance of other organisms and further influence the whole community and ecosystem. Therefore, it is important to understand the ecology and behavior of intertidal organisms to make better models for predicting the effects of climate change on distribution and abundance of other marine species.

The sea anemone *Anthopleura elegantissima* is abundantly distributed in tide pools along the North America Pacific coast (Shick and Dykens 1984, Engebretson and Martin 1994). Because of its symbiotic relationship with zoochlorellae (green algae) and zooxanthellae (brown dinoflagellates) (Bates et al. 2010), its ecology and behaviors are well studied. The presence of symbionts is often used to indicate the health of individuals in *A. elegantissima* under stress because they provide nutrients to the host (Bergschneider

and Muller-Parker 2008). This species expels its symbionts under several environmental stresses including decreased salinity, and remained contracted in salinity of 19‰ or less (Engebretson and Martin 1994, Verde and McCloskey 2007). In addition to the expulsion behavior, *A. elegantissima* also contracts and expands in response to these stresses. Pearse (1974) found that *A. elegantissima* normally expanded in light and contracted in dark. However, the anemones also contracted in response to high levels of sunlight; this could prevent desiccation and decrease the amount of oxygen produced by the symbionts' photosynthesis, thus minimizing oxygen toxicity (Dyken and Shick 1984).

In nature, *A. elegantissima* lives in pools where salinity may fluctuate due to rainfall or hot sunny days during low tide. Under these conditions, the anemones might reduce the salinity stress they experience by migrating out of the pools. However, the migrating behavior of *A. elegantissima* in response to changing salinity has not been investigated. In this study, two experiments addressed the question: Does *A. elegantissima* change its behaviors under unfavorable salinity environments? Specifically, I investigated the contraction and expansion behaviors and the migrating behavior of *A. elegantissima* under both low and high salinities.

Materials and Methods

Collection and Maintenance

Anthopleura elegantissima (54 individuals) were collected from tide pools and rock surfaces at Eagle Cove, San Juan Island, WA (48° 27.809N, 123° 2.027W) on May 6th, 7th, and 9th, 2011. For removing an anemone, I first cleared the gravels and sand around it and found an edge of the pedal disc. I used a blunt knife to gently detach the pedal disc from the rocks, and placed the animal into a bucket with seawater for return to the Friday Harbor Laboratories. Anemones came from four different clones and thus were kept in

separate trays to prevent fighting with non-clonemates (Ayre and Grosberg 1966). All trays were kept in a sea table with running seawater. During the experiment period (May 18-23, 2011), all aquaria were fully submerged in a sea table of running seawater each night, allowing the anemones to recover from the stressful salinity conditions in the experiment. Anemones were not fed during the experimental period, and were returned to Eagle Cove after the experiment.

Experimental Setup

Anemones were divided into nine groups of three replicates each and were allowed to attach to rectangular acrylic sheets (25 cm L x 14.5 cm W x 0.5 cm H). The other nine groups attached to acrylic ramps that were 17 cm wide on one end and 14.5 cm on the other end (35.5 cm L x 0.5 cm H), because the aquarium was wider at the top than the bottom. This design was critical for preventing the anemones to fall through the side and interfere with the anemones on the bottom. The anemones were allowed to attach and recover for at least 24 hours before experiments began.

Three salinity treatments were used in the experiment: low (25‰), normal (32-34‰), and high (45‰). Each salinity treatment was replicated in three medium aquaria (11.75 in L x 7.75 in W x 8 in H). Low salinity seawater was made up by adding reverse osmosis (RO) water to the regular seawater. High salinity seawater was concentrated by heating and evaporation. All salinities were measured by refractometer. Each aquarium was filled to 8 cm deep with the assigned salinity treatment of fresh seawater each day. During the experiment, all aquaria were half submerged in a sea table with running seawater to keep the temperature constant in each tank (Figure 1). The temperature was measured by thermometer and was recorded as 9-11 °C during the experimental period. The lights in the lab were kept on during each experiment to keep the light condition constant.

Contraction and Expansion

This experiment tested the hypothesis that both sensitivity to disturbance and speed of recovery from disturbance would be altered by both low and high salinity conditions relative to the normal salinity condition. In each trial, one rectangular sheet with three anemones was randomly chosen and placed into each aquarium. Anemones were immersed for only 30 minutes prior to measurements because Engebretson and Martin (1994) discovered the salinity within the coelenterons of *A. elegantissima* decreasing rapidly when they were exposed to seawater with lower salinity, suggesting that this sea anemone does not have the ability to osmoregulate. After 30 minutes, I used a blunt glass rod to poke every anemone until it closed (all tentacles contracted) and recorded the number of pokes required. Then, I counted how many individuals completely re-opened (all tentacles expanded and the mouth visible) in each salinity treatment after one hour.

Locomotion

The tested hypothesis was that more anemones would migrate to the water surface or out of water in both low and high salinity conditions and all anemones would stay underwater in the normal salinity condition. Each ramp with three anemones was randomly chosen and placed inclined into each aquarium (Figure 2). The ramps were marked with a line which was 10 cm away from the bottom. The anemones were all attached to the area below the line, and counted as underwater. If an anemone moved to the line, it was counted as near the water surface. I recorded the location of anemones in each salinity treatment every two hours each trial.

Results

Contraction

The average number of pokes needed for *Anthopleura elegantissima* to close in low

(25‰) salinity treatment was 38.3 ± 8.5 , in normal (32-34‰) salinity was 36.3 ± 7.2 , and in high (45‰) salinity was 11.6 ± 1.3 (Figure 3). Due to the skewed distributions of the data (some individuals took over 100 pokes to close), I could not run a parametric ANOVA; all transformations (logarithm, arcsine, natural logarithm, and square root) failed to normalize the data. Therefore, a nonparametric Kruskal-Wallis test was used. The numbers of pokes were significantly different among three salinity treatments (Kruskal-Wallis test, $H = 21.893$, $df = 2$, $p < 0.001$). In high salinity, anemones closed after significantly fewer pokes than those in low and normal salinity treatments (pairwise Dunn's test, $p < 0.05$). The numbers of pokes to closure were similar in low and normal salinities (pairwise Dunn's test, $p > 0.05$).

Expansion

The average percentage of individuals that re-opened after one hour since being poked in low, normal, and high salinity treatments were $31.8\% \pm 9.4\%$, $88.1\% \pm 6.7\%$, and $44.4\% \pm 6.8\%$, respectively (Figure 4). There was a significant difference among three salinity treatments (one-way ANOVA, $F_{(2,18)} = 15.094$, $p < 0.001$). The average percent opened in normal salinity treatment was higher than those in low or high salinity treatment (pairwise Tukey test, $p < 0.050$), whereas response in low and high salinity treatments did not differ (pairwise Tukey test, $p > 0.050$).

Locomotion

Among the 45 total anemones in each salinity treatment, 98% stayed underwater in low salinity, 93% in normal salinity, and 91% in high salinity (Figure 5). Only one individual in low salinity water moved up to the water surface, versus three and four individuals under normal and high salinity conditions, respectively. The responses in the three salinity treatments thus were not different (Chi-square test, $p > 0.05$).

Discussion and Conclusions

The intertidal sea anemone *A. elegantissima* often lives in tide pools where salinity fluctuation is a common environmental stress (Barber 2007, Amado et al. 2011). I hypothesized that the anemones would be more sensitive to physical disturbance under the stresses of either low or high salinity, compared to normal salinity. The results did not fully support my hypothesis.

Anemones closed at similar rates in low and normal salinity conditions, but over three times faster in high salinity condition. This suggested that *A. elegantissima* was more sensitive in higher salinity seawater. According to Barber (2007), extreme low salinity conditions occur more often and faster in nature. Salinity in exposed tide pools during low tide can be easily reduced by rainfall or terrestrial runoff (Barber 2007), thus the sea anemones are more adapted to the low salinity condition. In contrast, the high salinity condition in tide pools rarely happens because evaporation is much slower (Barber 2007). It may take many hot summer days for tide pools to reduce the salinity to 45‰, or even weeks in a cold winter. Because most pools are flooded daily by tides coming in and replacing the hypersaline water, such high salinities may be very rare in nature. Thus it is not surprising that the anemones reacted rapidly in this stressful, unnatural condition.

Another hypothesis was that *A. elegantissima* would have slower recovery from disturbance in low and high salinity conditions than the normal salinity condition. The results supported this hypothesis. It is understandable that sea anemones are more willing to open up in normal salinity and less likely to open up under stressful conditions. More studies for mechanisms of *A. elegantissima* in contraction and expansion for adapting in low and high salinity conditions are needed.

The final hypothesis was that the sea anemones might migrate to the water surface or even out of the water under stressful salinity conditions. The results did not support this hypothesis. Few anemones moved near the water surface, and none of them emerged from the water. This suggested that desiccation, which they would experience if they moved out of the water, is even more stressful for the anemones than being in low or high salinity conditions. A further investigation in anemone locomotion would be to put the anemones on the top of the ramp, in the air, and observe whether they migrate into seawater in low, normal, and high salinity conditions.

In conclusion, my data suggest that the sea anemone *Anthopleura elegantissima* is better adapted to low salinity conditions than high salinity conditions. As the earth gets warmer, the chance of extreme hypersaline conditions in tide pools may increase during low tide, as will desiccating conditions outside of pools. Such climate change may affect the distribution and abundance of *A. elegantissima* as well as the community associated with it. Further investigations into the ecology and behaviors of organisms in tide pools should increase our ability to make predictions about the distribution and diversity of marine species under future climate change.

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Figures



Figure 1. A photo of experimental setup. Nine aquaria submerged in a sea table with running seawater to keep the temperature constant.



Figure 2. A photo of tank setup. The anemones on incline ramp for locomotion experiment and anemones on the bottom sheet for contraction and expansion experiment.

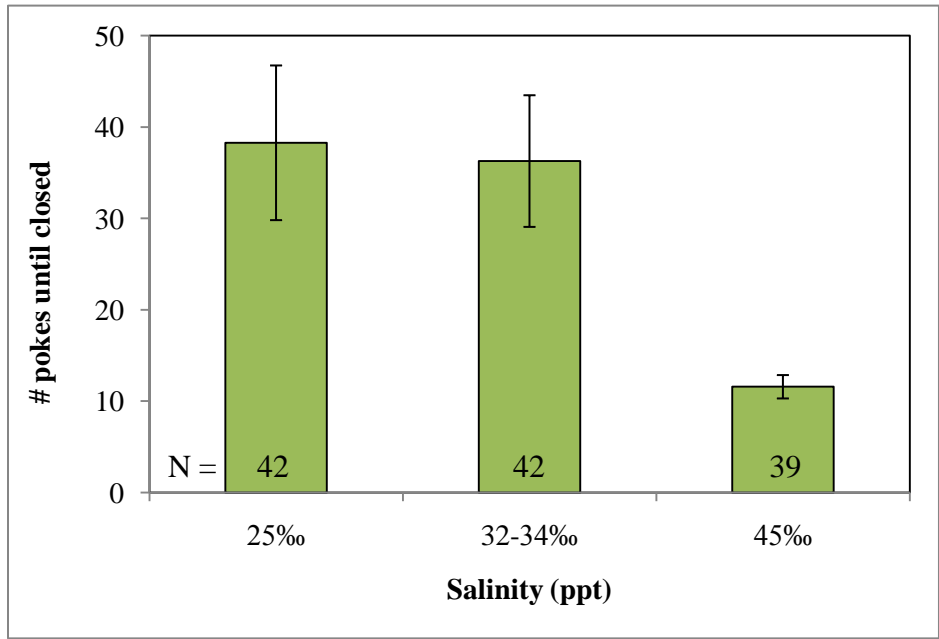


Figure 3. Average numbers of pokes until *A. elegantissima* was closed in low (25‰), normal (32-34‰), and high (45‰) salinity conditions. N = total number of anemones in all trials.

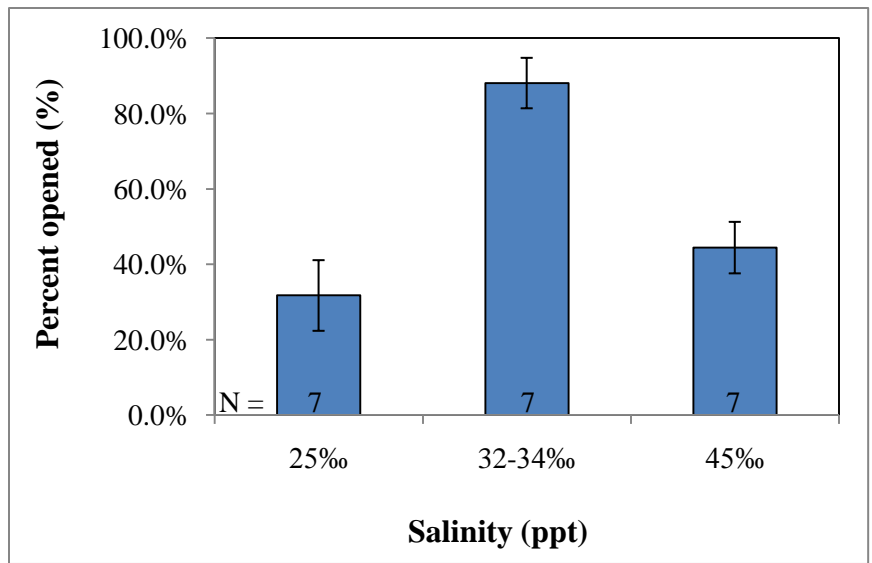


Figure 4. Average percent opened per trial after one hour since poked in low (25‰), normal (32-34‰), and high (45‰) salinity conditions. N = total number of anemones in all trials.

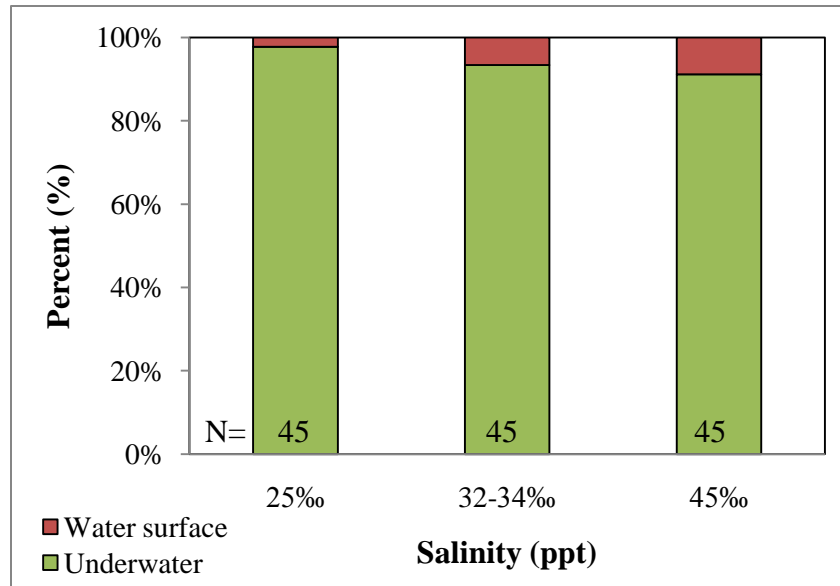


Figure 5. The proportion of *A. elegantissima* locating underwater and water surface on ramps in low (25‰), normal (32-34‰), and high (45‰) salinity conditions. N = total number of anemones in all trials.