

Acclimation and growth response of the green sea urchin *Strongylocentrotus droebachiensis* to fluctuating salinity.

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ABSTRACT

Sea urchins are osmoconformers and not typically found in euryhaline habitats. Yet the green sea urchin, Strongylocentrotus droebachiensis, is often found in areas periodically exposed to low salinity, e.g., near the mouths of rivers in the Gulf of Maine. These areas represent some of the most productive sea urchin fishing grounds in the northwestern Atlantic and are currently targeted for stock enhancement programs using hatchery-produced juveniles. To assess the effects of hyposaline conditions on seed, juveniles were exposed to episodic bouts of low salinity and growth and performance were quantified. Performance was defined as the ability to hold on to the substrate, a necessity for survival in the field. Although no effect on growth was detected there was a significant response over time in the force required to remove sea urchins from the substrate.

INTRODUCTION

Echinoderms have little control over their osmolarity (Drouin et al. 1985). Traditionally, they have been viewed as strictly marine organisms (Ruppert and Barnes 1994). However, several studies suggest that some echinoderms can tolerate hypoosmotic conditions (Binyon 1966, Stephens 1972, Sabourin and Stickle 1981, Lawrence 1996). *Strongylocentrotus droebachiensis* is the most common echinoid occurring in Northern Atlantic coastal communities (Himmelman 1978, Drouin et al. 1985) and supports an active fishery in the Gulf of Maine. Some of the most productive fishing grounds occur in river mouths where salinity levels drop during heavy rains. Recently, the states of Maine and New Hampshire have granted sea urchin lease sites in these areas. However, prolonged exposure to low salinities can be fatal to green sea urchins. Roller and Stickle (1985) observed necrosis in the tissues of green sea urchins exposed to low salinities for a period of two to four weeks. Under hypoosmotic conditions, green sea urchins take in water from the environment, resulting in a swollen appearance (Lange 1964). It has also been observed that *S. droebachiensis* loses spines under low salinity conditions (Sabourin and Stickle 1981).

Size appears to be an important factor for tolerating low salinity levels. Larger individuals are better able to handle the osmotic stress than smaller ones because of the lower surface area to volume ratio (Himmelman et al. 1984). Acclimation may also be an important factor. Sea urchins that have been previously exposed to periodic changes in salinity levels may have an increased tolerance to changes in osmolarity (Himmelman et al. 1984).

Previous work in our laboratory demonstrated that juvenile green sea urchins show a growth acclimation response to periodic bouts of low salinity. Initially growth rates decreased when urchins were exposed to 24-hour bouts of hyposalinity administered every 2 weeks. However, after the first 4 treatments (8 weeks) the growth rates fully recovered relative to a control group. One aspect of the experiment presented here was to vary the hyposalinity regime to test whether the acclimation period could be reduced. The other aspect of this experiment was to assess performance by quantifying the amount of force necessary to remove a sea urchin from the substrate. It was hypothesized that it would require more force to remove the control animals than the treatment sea urchins. We also wanted to investigate whether this variable displayed a similar acclimation response. Few studies have quantified the amount of force required to remove an urchin from the substrate (Sharp and Gray 1962; Leddy and Johnson 2000) yet the ability to remain attached is critical to survival in the field and has important implications to reseeding programs aimed at restocking depleted standing stocks.

MATERIALS AND METHODS

Thirty-two green sea urchins were collected off the Isle of Shoals in the Gulf of Maine in September 2002 and shipped overnight to the laboratory at Villanova University. We measured them with knife-edge digital calipers and selected the twenty largest animals for the experiment. We divided them into two groups so the mean test diameters and variances were approximately equal (Control=16.96mm, $\sigma^2=3.070$; Treatment=17.07mm, $\sigma^2=3.149$). The ten individuals in each group were then assigned random numbers from one through ten (C1-C10 or T1-T10).

The sea urchins were placed in a system specially designed and built for this experiment (Figure 1). The system was located in an environmentally-controlled, recirculating seawater chamber at Villanova University (see Russell 1998 for details) and maintained at 10° C and a light/dark cycle approximating field conditions. Each animal was placed in a separate 30mL plastic beaker and these beakers were suspended in a sea table so the tops were above the water line. Beakers had a 2 mm mesh bottom to keep the animal and food in and allow wastes to pass through (Figure 1). Each beaker sat in a hole (38 mm diameter) punched in the Plexiglas sheet. The centers of each hole were separated by 45 mm. The Plexiglas sheet was held up with six “legs” composed of CPVC pipes. Over the top of the table was a sprinkler system also composed of CPVC. Two pumps fed seawater through Tygon® hoses to the

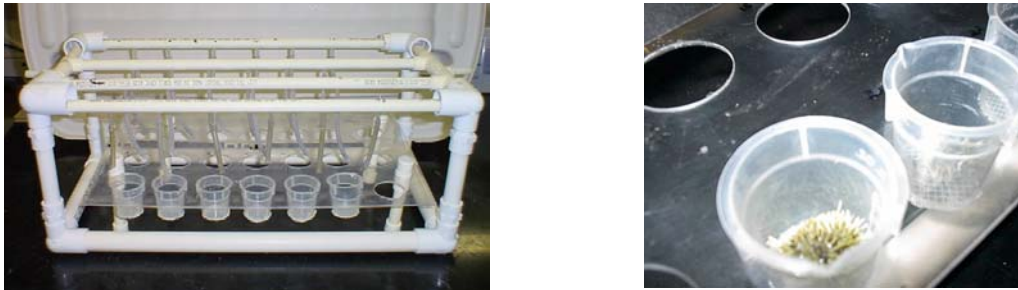


Figure 1 Individual sea urchin cages. Each 30 mL cage had a mesh bottom and was suspended in the sea table by a Plexiglas sheet so the top of the cage was above the water line. The sheet was supported by a PVC frame that was fed by two submersible water pumps on both sides of the system. Each cage had a separate plastic hose that supplied water and was plumbed from the PVC. The system was located in a sea water table that was constantly filtered.

system. The system allowed tracking individuals and provided each sea urchin with its own water supply. The beakers were rotated through the system each day to minimize variation due to different sprinkler flow rates. The sea urchins were fed a diet of the algae *Chondrus crispus* and *Laminaria spp.* From November 17, 2002 to January 19, 2003, weekly salinity treatments were conducted. The first week the experimental period consisted of twenty-four hours, while all subsequent weeks were forty-eight hours. Two, 106L plastic containers were designated “Control” and “Treatment”. Identical sprinkler systems were made for each container. Over a period of two hours, the salinity in the Treatment container was lowered from 32.5‰ to 21.5‰ through the addition of de-ionized water every ten minutes. An equal amount of 32.5‰ seawater was added to the Control container. Thus, equal amounts of water were added to each, with the salinity being the only difference between them (the containers were half full ~ 50L). After the forty-eight hour period, hypersaline water was added every ten minutes for two hours to restore the treatment salinity to 32.5‰. During this period, the Control animals were treated in the same manner.

Test diameter measurements of each individual sea urchin were recorded with digital calipers every week before the salinity change. Force measurements were also taken both before the salinity change and approximately 2 hours before the restoration to 32.5‰. Individual force was measured twice for each sea urchin. The sea urchin was placed in a harness of 6.8 kg monofilament, which was held in place by small slits in Tygon® hose (Figure 2). The 2cm pieces of hose were glued to a small funnel, which had been roughened with sandpaper and soaked overnight in seawater in order to develop a biofilm. The funnel sat in a 250 mL plastic beaker that was filled with seawater (Figure 2). To take a force measurement, the animal was placed in the harness and given time to attach to the funnel surface with its tubefeet. Measurements were taken with 1N and 5N Pesola® Lightline scales. Initial measurements were taken with the 1N scale and then if the force was greater than the scale’s range, the 5N scale was used. During the salinity treatments, measurements

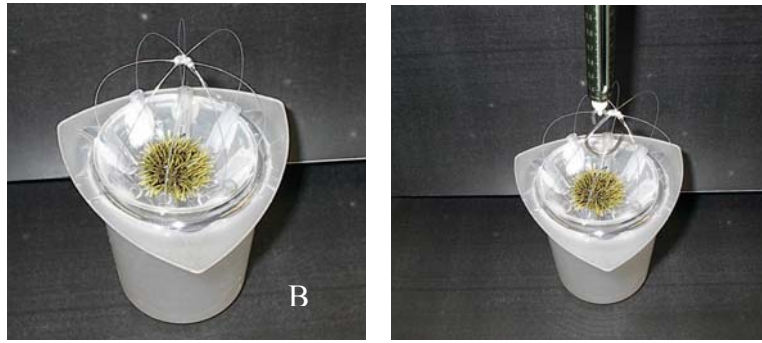


Figure 2 Apparatus used to obtain force measurements. **A.** The animal would rest in a harness of fishing line and attach its tube feet to the funnel surface. **B.** The hook of the scale was used to pick up the harness (removing the sea urchin from the substrate) and force readings were observed directly from the scale. Each individual was measured twice in an alternating sequence (C1, T1...C10, T10) to give them a recovery period.

were also taken. The 250mL beakers were filled with water from the Control and Treatment containers so that the sea urchins remained in water of the same salinity of their exposure (Control animals were in 32.5‰ seawater while Treatment animals were in 21.7‰ seawater).

RESULTS

Two control animals died during the experiment and were eliminated from the analyses of size and force data. Over the ten-week period there was no significant difference between the growth of Control and Treatment animals ($t=1.733$, $df=16$, $P=0.100$; Figure 3). However, we observed that the Treatment sea urchins had a swollen appearance and lost a number of spines during the 48 hour exposure period.

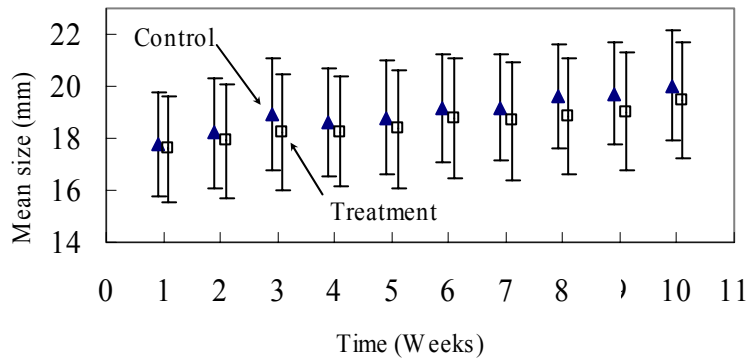


Figure 3 Mean size (\pm standard error) for Control (shaded triangles) and Treatment (open squares) samples each week. Test diameter was measured before the animals were isolated for forty-eight hours.

There was no significant difference between Control and Treatment animals in the force measurements taken before the exposure to lowered salinity ($t=0.833$, $df=16$, $P=0.416$). There was also no significant difference between the two groups during the exposure period ($t=1.116$, $df=16$, $P=0.279$); however, the force measurements for the smaller animals showed the greatest variability among measurements. In addition, there was a correlation between force and size, i.e., the larger animals required more force to remove from the substrate. To account for greater measurement error of the smaller animals and at the same time adjust the force measurement for differences due to size, we focused on the five largest animals in each group. To adjust for size we divided each force measurement by an estimate of the oral surface area for each animal (using the equation for the area of a circle):

$$\text{Adjusted force} = (F_{\text{mean}}) / ((d/2)^2)(\pi) \quad (1)$$

Where F_{mean} = average force of each individual and d = diameter.

For each exposure period the difference between the Treatment and Control (adjusted mean force measurements) was calculated both before and during the hyposalinity exposure. Before the exposure there was no significant relationship over time; however, the difference between the treatment and control force measurements showed a highly significant pattern during the exposure (Figure 4).

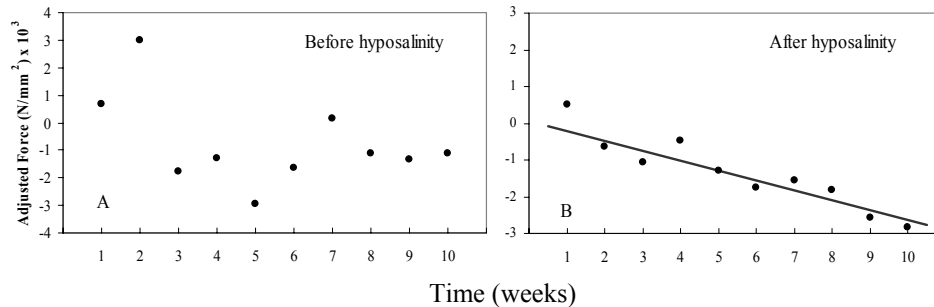


Figure 4 The difference (Treatment – Control) between the mean adjusted forces of the largest sea urchins (N=5). These adjusted forces were taken both before (A) and after (B) the forty-eight hour isolation and hyposalinity exposure period. The regression for the before-exposure data is not significant ($P = 0.40$) whereas the slope of the linear regression analysis is highly significant ($P=0.0003$) for the after-exposure data. Less force was required to remove the Treatment animals from substrate over the course of the experiment.

DISCUSSION

The sea urchins were collected from an area where they had not been exposed to low salinity so they were not acclimated. We expected to see an initial difference in growth with the Treatment group growing slower (as we did in a previous study) but there was no difference in growth between the treatment and control over the course of the 10-weeks long experiment. We also expected to see a difference in the force measurements but again there was no difference. Two aspects of the experimental design that differed from previous work are probably responsible for these results: the longer duration and more frequent hyposalinity exposures.

In our previous hyposalinity experiments we exposed treatment groups for 24 – 36 hours every 2 – 3 weeks. In this experiment we exposed them for 48 hours once each week (the animals had only 5 days between treatments). The tanks used for this experiment held significantly less volume (50L as opposed to 200L) as well. In addition, during the hyposalinity treatments animals were not fed so the sea urchins in this experiment received much less food (starved for 2 days out of 7 for 10 weeks). All or some combination of these factors contributed to the failure to detect growth acclimation.

Although there was correlation between size and the force required to remove an individual from the substrate, the variation between measurements on the same individuals were highly variable – especially for the smallest animals. For example, in the third week of the experiment, one control sea urchin had force measurements of 0.490N and 0.190N before being isolated for 48 hours. When the largest animals were analyzed using the adjusted force metric (eq. 1), a striking pattern emerged from plotting the differences between the Treatment and Control means over the course of the experiment (Fig. 4). At the conclusion of the hyposalinity exposure it took less force to remove the treatment animals from the substrate over the 10-week period. This result strongly suggests that the 5-day interval between treatments was not long enough for the sea urchins to recover. The effects of the exposures appeared cumulative. Protocols for conditioning hatchery-produced juveniles for planting in areas where low salinity conditions occur should take these observations into account. Future work on conditioning regimes should focus on varying the duration and interval between hyposalinity exposures.

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