Recovery rates of starved and fed *Nucella ostrina* after heat-induced stress

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Near Shore Ecology Research Experience-Zoobot Quarter

Spring 2013

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Keywords: *Nucella ostrina*, dog whelk, heat stress, recovery, respiration, behavior

Abstract: How much effect does nutritional state have on the recovery time of *Nucella ostrina* from low tide stresses? We predicted that fed *N. ostrina* would recover faster from low tide stress than starved ones. Fed and starved snails were exposed to a simulated low tide situation for 1 hour, and then measured for recovery based on their respiration rates and behavior. There were no significant differences in relative respiration rates of recovery between fed and starved snails.

Introduction: Organisms inhabiting the intertidal zone experience many forms of stress. Wave action, desiccation, predation, and sun exposure are all examples. Temperature increase is a common form of stress which occurs when the tide recedes and organisms go from complete submersion to exposure. Heat can cause elevated levels of stress and even kill in extreme cases (Zenel et al. 2010). This stress is especially important for individuals that need to remain moist and cool.

Nucella ostrina, a marine dog whelk of the Pacific Northwest, is an animal that is adapted to survive in the intertidal zone. These snails can survive air exposure for several hours, while feeding on the barnacles of the intertidal zone. But there can be times, especially during the summer, when the snails are exposed to the hot sun for several hours while out of the water. It is during this time that these snails can experience large amounts of stress due to higher temperatures. Prolonged exposures of eight hours to temperatures of 30°C and higher can ultimately kill these snails (Davenport et al. 2007). However, they can survive eight hours of exposure to temperatures of 16.5°C, 21.5°C, and even 26.5°C (Hofmann and Zippay 2010). After these sub-lethal temperatures, the snails can become very stressed, though the exact nature of the stress and the roles of other variables, such as hunger, are unclear.

This study measured the stress caused to *N. ostrina* by prolonged exposure to sub-lethal temperatures. We also considered the effects of hunger in *N. ostrina*'s ability to tolerate heat exposure. We hoped to learn the extent of the stress and how these snails adapt and handle it. Rising temperatures associated with climate change will affect this and many other species. *N. ostrina*'s tolerance to moderate increases in temperature may allow it to cope better than others.

One method to measure stress levels is to dissect animals and test for the concentration of a stress protein produced (Davenport et al. 2007). However, we measured stress levels using respiration chambers, so as to reduce the mortality rate of the snails. We predicted that starved snails exposed to low tide simulation would be the most stressed.

Methods: The first step in this experiment was to build pens for maintaining the snails with food and sea water at 10°C until they were needed for the stress treatment. Sea water tables in lab 1 were cleaned and filled with sea water, and Tupperware containers with mesh sides were prepared.

Snails were gathered from one site on the shore of San Juan Island. Each of 84 individuals of *N. ostrina*, with shell lengths between 18-25 mm in length, was placed in a Tupperware container. To feed these snails, we gathered several small rocks covered in barnacles from the same beach. These were brought back to the lab, placed in the

Tupperware containers, and placed in the water. Each snail was placed in each of these containers two weeks before its trial.

Prior to experiments, we labeled the snails and measured the buoyant weights and shell dimensions. Before the buoyant weights could be measured, the snails had to be completely submerged in water for 24 hours. The snails were then weighed with a hanging, buoyant scale so they could remain submerged. The measured snails were then taken out of the water and measured for length. Super glue was used to attach numbered, waterproof labels to their shells. Snails would then sit and dry for one hour before being weighed for dry weight. Odd numbered snails were classified as the "fed" group, and the even numbered snails were the "starved" group. Fed snails were placed in containers with barnacles, and starved snails were placed in containers with rocks lacking barnacles.

A rating system was devised to monitor and classify the behaviors of the snails and determine recovery. The scale was numbered from 1-6, to indicate what it was doing at a moment in time (Colpo 2011). A snail at behavior 1 was completely withdrawn into its shell. A snail with its foot partially out of its shell was at behavior 2. At behavior 3, a snail's entire foot was seen. When a snail was reaching for a substrate to grab on to, it was showing behavior 4. A fully attached snail was behavior 5, and an attached, moving snail was at 6. A snail that displayed behavior 6 was considered to have recovered.

Low tide was simulated with a hot water bath that contained three thin-glass dishes floating on the water, and was dubbed "the snail sauna" for quick reference. The target temperature for the inside of the dish surface was 30°C, and the temperature was periodically measured with a thermistor. Six snails were placed in these dishes for one hour. The respiration chamber was calibrated and prepared, and was maintained at 10°C with a water circulation system. Respiration was measured in (nmol O_2)/(g/min) over the course of one hour, and the behavior was noted periodically. There were also three chambers with cameras set up to monitor recovering snails' behaviors for one hour. Each snail was monitored twice: once for baseline rates, and second for the stress treatment. The purpose of the baseline rates was to observe a snail's respiration rate and behavior when it was not stressed.

Results: Of the first 12 snails, which were exposed to 32°C, 8 died. Of the 4 that survived, 3 of them were fed. All other trials were conducted at 30°C, and none of the snails died. The mean recovery times of respiration rate of the fed snails was 50 minutes (±7.07 s.d.), and the mean of starved snails was 48.4 minutes (±9.71 s.d.) (Figure 1). Relative respiration rates in starved snails and in fed snails were not significantly different ($t=-0.449$, $p=0.655$). Three trials were not recorded for the full 60 minutes because of human error; these were not factored into the mean recovery rates. There were many snails that reached a relative respiration rate (ratio of trial rates to baseline rates) of 1. Graphs for snails 55 and 50 best displayed the trends of recovery in relative respiration rates. Snail 55 (fed) reached a rate of 1 at 45 minutes (Figure 2), and snail 50 (starved) reached a rate of 1 in 39 minutes (Figure 3). Figure 4 displays the average relative respiration rates of all snails at each time point. The average relative respiration rates across all time points did not show any significant difference across trial types (Figure 4). Average rates decrease at 60 minutes, but no other trends were observed.

For the behavioral definition of recovery, two snails reached and remained at 2, and two snails reached behavior 3. Eight snails reached significant movement (behavior 6).

Discussion: Analysis of relative respiration rates suggests that there is no significant effect of starvation on recovery times. The mean respiration recovery times show that the starved snails actually recover slightly faster than the fed snails, by about 1.6 minutes, but this difference is not significant. Our data suggests that respiration rates may not be the best way to test for effects caused by starvation and heat stress. It is possible that the nutritional state of a snail does actually affect recovery from heat-induced stress but other parameters, such as behavior, might demonstrate such differences more effectively.

Conclusion: In conclusion, the fed snails did not recover faster than the starved snails after the trials. Respiration rates do not reveal how well a snail recovered from stresses caused by low tide conditions and consumption of food. Behavioral metrics may prove to be more revealing and could be the focus of future studies. Studies could be performed on the survivability to extreme temperatures between fed and starved snails.

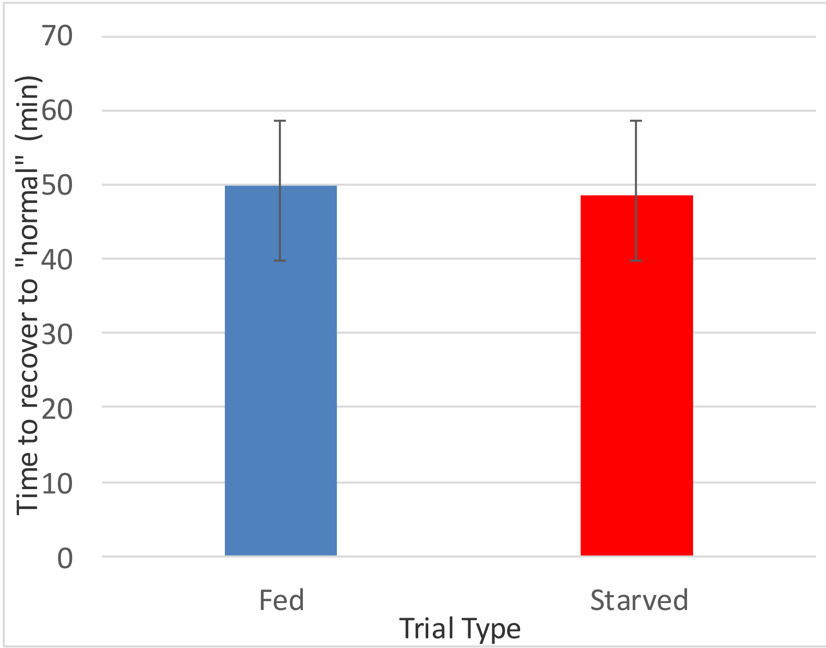

Figure 1: Mean Recovery times. Bars indicate standard deviation. Fed s.d. = 7.07. Starved s.d. = 9.71.

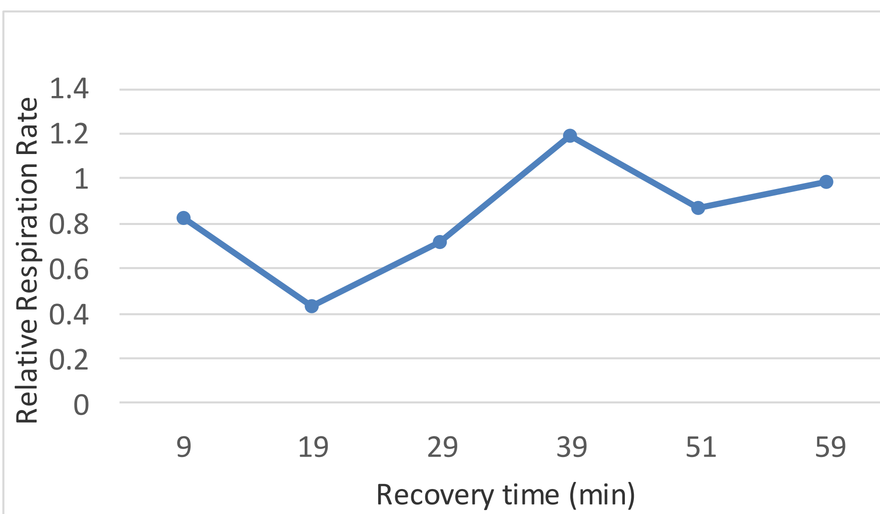

Figure 2: Trial 10 Snail 55 (fed). Recovery at 45 min

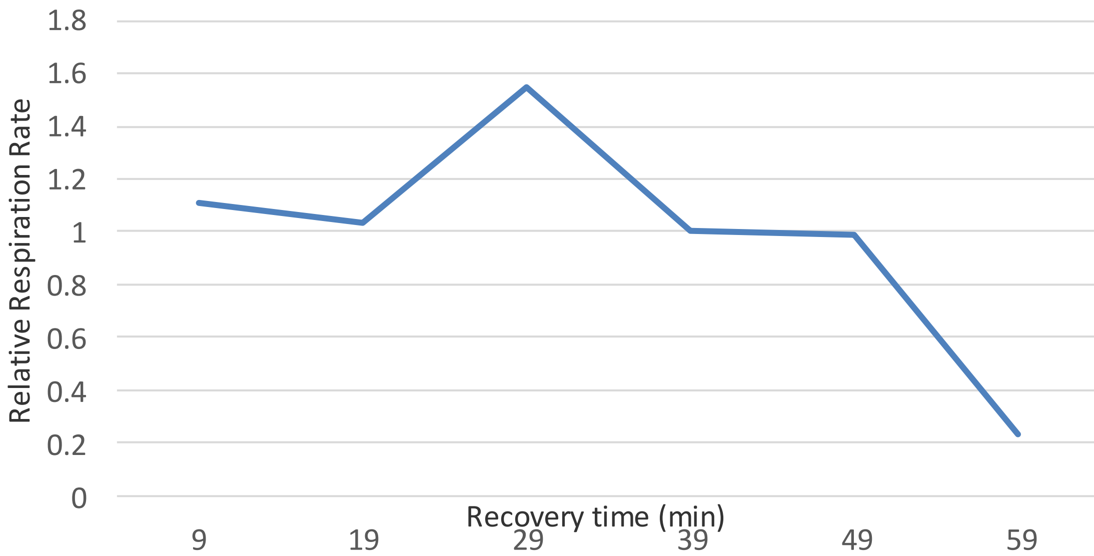

Figure 3: Trial 9 Snail 50 (starved). Recovery at 39 min.

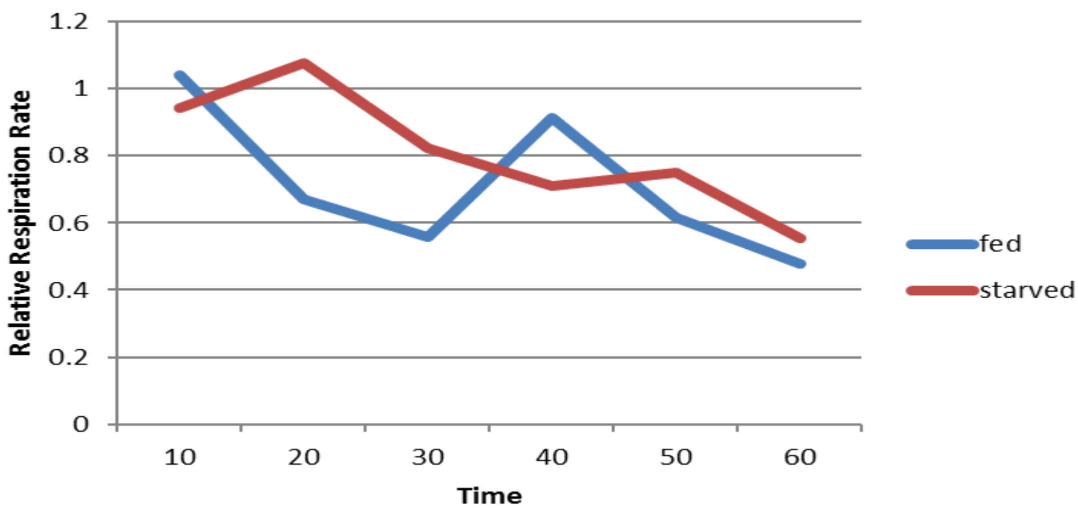

Figure 4: Average respiration rates over time for the two treatment groups. Time (min) 0 = when first put into the respiration chamber. Starved averages from 5 snails. Fed averages from 4 snails.

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