Effect of Heat Stress and Starvation on the Respiration Rates of *Nucella ostrina*

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Nearshore Ecology Research Experience Apprenticeship
Spring 2013

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Key Words: Nucella Ostrina, Respiration, Heat Stress, Starvation, Behavior

Abstract:

This study tested if intertidal snails *Nucella ostrina* would feel more stress after undergoing a heat treatment designed to simulate a low tide condition if they were fed or starved. Our predictions were that *N. ostrina* would recover more quickly from stress if they had recently eaten, possibly due to the metabolic energy available for tissue regeneration and thermotolerance from accessible heat shock proteins. Respiration rates were measured for snails that had undergone no heat treatment, then they were exposed to a heat treatment (30 degrees Celsius for one hour) and put back in a respiration chamber to once again measure their respiration rate. Half of the snails in the treatment had been constantly exposed to food for a full two weeks, while the other half had been starved for two weeks. We found no significant difference between fed and starved snails in terms of recovery from stress, as measured by respiratory rates. Both fed and starved snails appeared to have similar responses after being exposed to a heat treatment, which could be an indication that heat does not cause stress to snails or that respiration rate is a poor metric of stress levels.

Introduction:

Climate change is raising temperatures globally; however it is unclear how organisms will respond to these changing conditions (Somero 2002). The rocky intertidal is an especially interesting area to investigate these changing conditions because it experiences both terrestrial and marine conditions and a range of temperatures. As climate conditions continue to change, studies similar to ours will give scientists an idea of how snails and other intertidal invertebrates will react to global warming. Numerous biotic and abiotic factors can affect any organism living in the rocky intertidal. Biotic conditions include predation, food availability, competition, etc.

while abiotic conditions include temperature, wave energy, salinity, etc. (Sorte and Hofmann 2005). Each of these conditions could be affected by global warming adding more stressors to organisms of the intertidal, so finding out how organisms respond to a single stressor may help us understand how they might respond to other factors.

In the Pacific Northwest the snail, *Nucella ostrina*, is commonly found in the rocky intertidal preying on barnacles *Balanus glandula, Semibalanus cariosus,* and *Chthamalus fissus* (Connell 1970). Since barnacles are sessile, they are frequently exposed to extreme thermal conditions during low tide. Snails are also exposed to these stressful conditions when feeding on exposed barnacles. When *N. ostrina* are not feeding they can be found in subtidal areas, though their distribution may be limited by predation (Hayford et al. *unpublished manuscript.).* Non-feeding *N. ostrina* are therefore, found in damp, cool, protected crack and crevices. It has been hypothesized that barnacles have adapted to endure larger gradients of heat compared to their mobile predators (Yamane and Gilman 2009). Given the distribution of *N. ostrina* it is interesting to study how they cope with heat stress while hunting at low tide, as well as how their reaction to heat changes if they have recently had a meal or if they are starved (Pincebourde et al. 2008). Snails will venture away from high stress environment when they do not need to risk their life to find a barnacle to prey on.

If snails do experience severe thermal stress due to feeding during low tide, how rapidly and effectively can they recover from this? Previous studies on other organisms have suggested that there is a correlation between metabolic energy and stress levels. For example, unfed *Carcinus maenas (*green shore crabs) consume much more oxygen at higher temperatures than at lower temperatures (McGaw and Whiteley 2012). This study will focus on the recovery ability of *N. ostrina* after being exposed to extreme thermal conditions and starvation. Will fed snails be able to recover from high temperature conditions faster and will their body temperature play a role in recovery? We predicted that snails that have recently eaten will recover more quickly because they have the metabolic energy to move to more desirable conditions and repair any tissue damage from the heat (Zenel 2010). Fed snails will likely have more energy to crawl to the water where they can cool down and repair damage caused by the heat stress.

Methods:

Our study was conducted at the Friday Harbor Labs on San Juan Island, Washington. We collected 169 snails between 17 and 25 millimeters length, tagged, measured, and weighed 96 of them, and divided them into treatment and control groups where they were put in 10 degree Celsius water with lots of barnacles until it was time to start their trial. Twice a week we took twelve snails and separated them into individual containers, where half the containers contained cobble with barnacles (fed control) and the other half cobble without barnacles (starved treatment). Our controls distinguished if fed snails recovered faster from stress. The control snails were all fed "ad libitum" while the treatment snails were starved for a two-week period prior to experiencing a low tide setting. After two weeks of being in individual containers we exposed them to a heat stress to simulate a low tide in a rocky shore.

Of the twelve snails in each trial, we measured the respiration rate of one fed and one starved snail. This rate constituted the baseline and was used to compare to the respiration rates of snails after heat trials. For every trial we developed baseline rates for the snails the night before low tide treatments. To subject snails to a controlled heat stress, we put six snails at a time (three fed, three starved) in a bowl floating in a water bath heated to 30 degrees Celsius. After one hour we removed them from the treatment and immediately transported the snail with a baseline respiration measurement into a respiration chamber and measured its respiration rate until it returned to its baseline level. We could only measure one snail at a time so we chose a random starved snail and a fed snail that had been consistently feeding over the two-week period.

The respiration chamber was hooked up to a computer with an Oxygraph program that graphed the breathing rate of the test organism, by measuring the oxygen levels in the chamber. It was also connected to a thermopump plumbing refrigeration system that kept the 15 mL of saltwater in the chamber constant at 10 degrees Celsius. In order to collect all the readings we first prepared an electrode that fit into the respiration chamber, then calibrated it so it was set to read oxygen levels in 10 degrees Celsius sea water. During trials we needed to add oxygen if levels ever dropped below 20% of the starting value.

We measured the stress levels with a relative respiration rate: this compared the baseline respiration rate (respiration rate in a regular, stress free setting, taken before treatment) to the respiration rate taken immediately after the low tide treatment. We measured respiration by placing snails in respiration chambers that graphed oxygen levels. Finally, we saw how long it took for the snail's respiration rate to get back to the baseline rate, and we compared the time and rates of these treatment snails to the controls. This gave us correlation measure of how stressed the snail was under low tide conditions, and our controls distinguished if the snails that recently ate got more or less stressed by the same conditions. We predicted that if the snails were fed, they would get less stressed because they would have more energy and less need to find food to fill their caloric consumptive needs.

Once all the snails had been exposed to the treatments and we had collected all the necessary data we need for our study, we returned them to Colin's Cove, San Juan Island, Washington.

Results:

Overall, our data do not suggest that fed snails undergo less stress in low tide settings than starved snails, as we had predicted. Mean recovery time for respiration of the treatment snails (starved) was 48.4 minutes, whereas our control snails (fed) had a slightly higher mean recovery time, at 50 minutes These values are not significantly different (t test, $p = 0.65$); the difference between fed and starved snails is most likely due to random sampling variability. Out of all of our respiration trials, a total of eight snails came out of their shell, reaching a behavior representative of a full recovery.

Figure 2 represents recovery of a specific snail comparing respiration rates after heat treatments to baseline rates, showing how long it takes to reach normal respiration levels. This figure was chosen because it was from a snail that fully recovered. Respiration slightly dips after it reaches baseline respiration but then it bounces back up, confirming that the baseline has been met and continued. Overall there was a large variation in respiration rates and behaviors between baselines and trials. Figure 3 represents an average for all of the trials of relative respiration over the hour. It is unexpected that on average, it appears snails begin respiration around the baseline and slowly decline over the hour. This could be partially real and possibly due to baseline behaviors. Since respiration declined over time we should have measured it longer to see if the trend continued. The baseline issue could be that we divided our treatment rates by a baseline rate that was too high.

Discussion and Conclusions:

Overall our study concluded that nutritional state does not affect the ability of snails to recover from heat stress. We had hypothesized that fed snails would be less stressed by exposure to high temperatures, but this was not the case at least when stress is measured by respiration rates. There was virtually no difference between overall post-stress respiration rates in fed and starved treatments. In terms of behavior however, it is interesting to note that at temperatures high enough to result in the death of some snails (32 degrees Celsius), 75% of the individuals that survived had been fed. This suggests that fed snails somehow cope better with life threatening conditions, most likely having the metabolic energy to repair damaged tissue and enzymes or express specific heat shock proteins allowing for higher thermotolerance (Sorte and Hofmann 2005). It is possible that we collected the snails so early in the season that they had not yet adapted to elevated temperatures of 32 degrees Celsius, and it was too large of a change in temperature compared to what they experience this time of year; therefore most went into shock and died.

The data is conclusive that our results are not what we expected which suggests either we were not doing a sufficient job stressing the snails or that respiration rates may not be the best way of measuring recovery for snails; both could be important pieces of information for future analysis in this field. There may be other factors in the intertidal that cause more stress for invertebrates such as predation or food availability (Sorte and Hofmann 2005). Maybe respiration rates have nothing to do with their stress level and there is a better means of measuring it. The idea that conditions will continue to change in the intertidal over the coming decades makes it necessary to test as many factors as possible and observe how organisms react to each and what factors have the most dominant effect. This way we can begin to predict behaviors of organisms, which may help us to preserve intertidal ecosystems.

Our pilot trials suggested that mortality might be affected more by nutritional state than was respiration rate. In our pilot trials we exposed the snails to a heat treatment at 32 degrees Celsius (2 degrees hotter than the rest of our trials) and eight out of the twelve snails exposed to that temperature died, but out of the four survivors three of them were fed. These observations suggest that metrics other than respiration rate might provide useful data about the importance of nutritional state.

Acknowledgements:

Many thanks go out to Hilary Hayford for her outstanding mentoring ability not only teaching how to set up and run a scientific experiment of this caliber but also for her insight in this field and all of her useful knowledge, guidance, and enthusiasm. Megan Dethier and Charley O'Kelly for their leadership and guidance in this program and their time and effort spent editing proposals teaching fundamentals of scientific writing. Dan O'Connell and Kat Barton for being extraordinary lab mates and for the hours they spent in lab working hard. Finally, to UW-Friday Harbor Laboratories for providing incredible facilities and resources making it possible for us to learn first hand, from some of the best, how to conduct experiments like this.

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Figures:

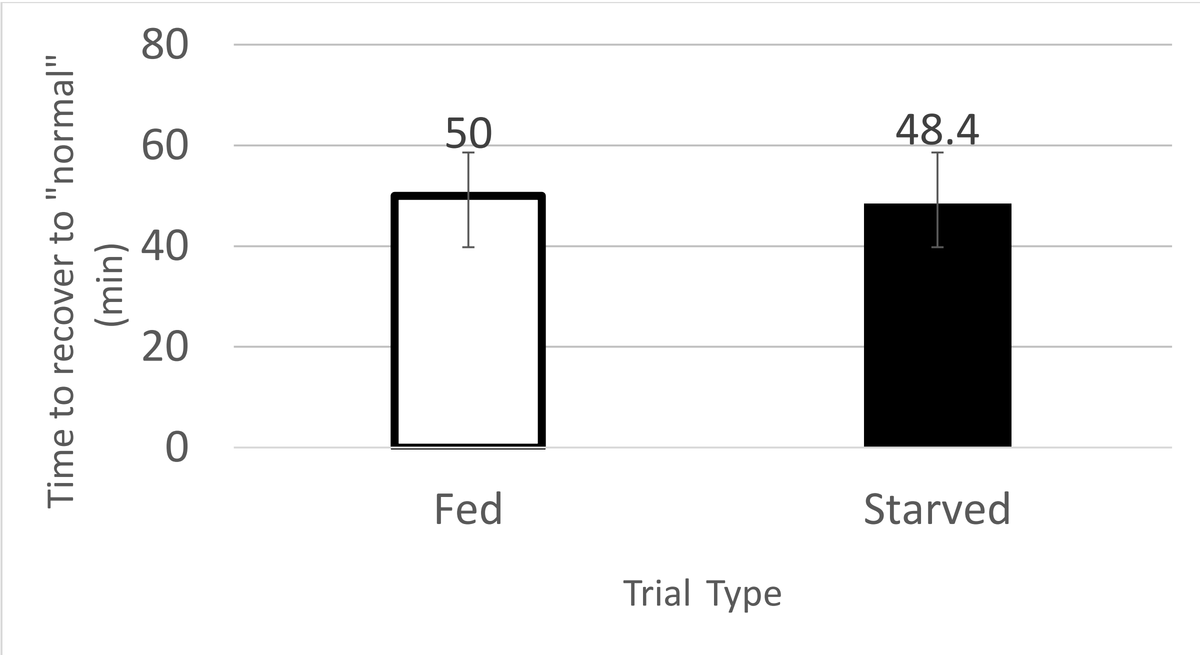

Figure 1: Mean Recovery Rates. Represents 4 snails for fed trials and 5 snails for starved trials. Error bars represent standard deviation. T-Test results: T=0.449 and P=0.655

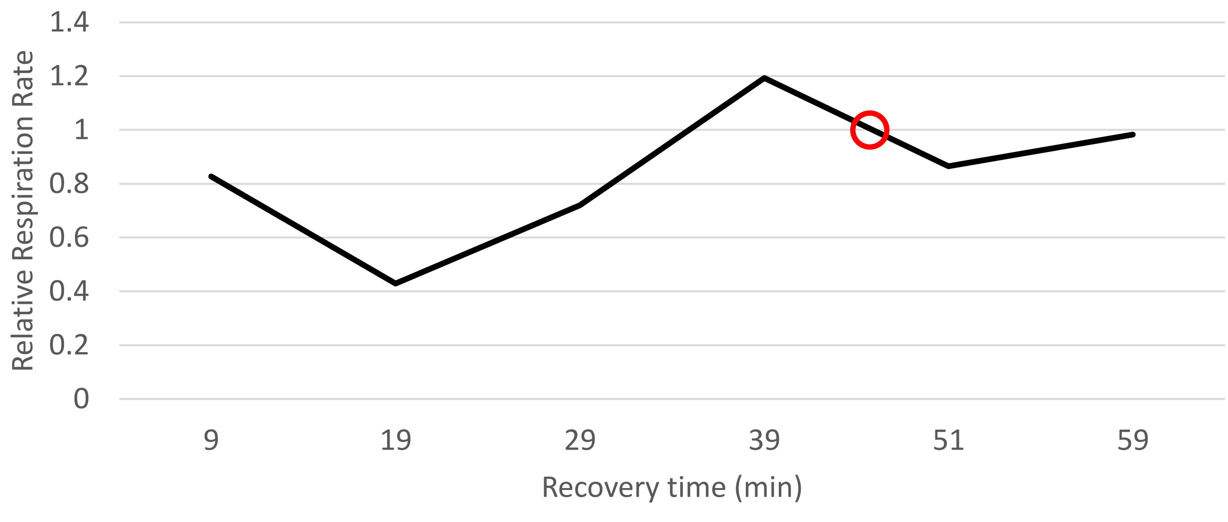

Figure 2: Relative Respiration vs. Recovery Time. Trial 10, Snail 55 (fed). Red circle represents point of recovery.

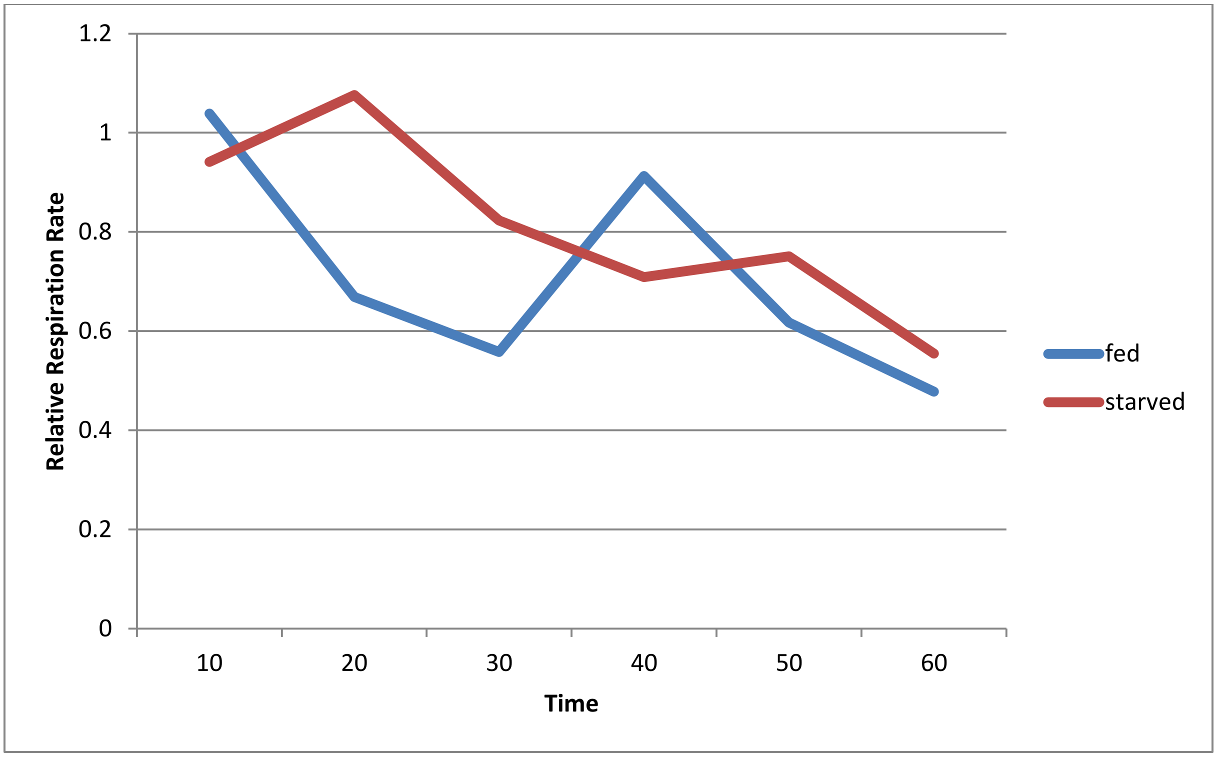

Figure 3:Average Relative Respiration Rates. Fed trials includes 4 snails, starved trials includes 5 snails