2013

The Affect of Low Tide on the Digestion of Balanus glandula, the Acorn Barnacle.

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The Affect of Low Tide on the Digestion of *Balanus glandula*, the Acorn Barnacle.

A Thesis Presented

by

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To the W.M. Keck Science Department
of Claremont McKenna, Pitzer and Scripps Colleges

In partial fulfillment of
The degree of Bachelor of Arts

Senior Thesis in Biology
10 December 2012

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Abstract

The rocky intertidal zone, experiencing fully marine and fully terrestrial conditions, has become increasingly investigated as a model ecosystem for studying the future implications of climate change. The barnacle, *Balanus glandula*, a common rocky intertidal inhabitant, plays an important role as a key prey item for many organisms. Low tide can be particularly challenging for barnacles as they are marine organisms subjected to the abiotic conditions of a terrestrial environment. The most stressful of these are increased temperature and decreased oxygen availability. This study aimed to investigate how low tide impacts the energy budget, specifically the digestion, of *B. glandula*. Barnacles are unable to feed at low tide however, if they were able to digest at low tide, they could maximize their energy intake by emptying their stomach to prepare to feed at the next high tide. However, digestion is a metabolically costly activity, which could make it less energetically favorable to digest when there's less oxygen available. To test for an effect of low tide on digestion, barnacles were fed, and the time to first fecal production measured as a 'baseline'. This was repeated, but barnacles were exposed to either a 16^{o}C or 35 ^{o}C low tide immediately after being fed. The change in digestion time was calculated by comparing these two times for each barnacle. It was found that regardless of temperature, barnacles delayed their digestion by about 50-60 minutes after exposure to a one hour low tide. To determine the energetic cost of digestion, the rate of oxygen consumption was compared between starved and digesting barnacles. I was unable to detect any evidence of elevated metabolic activity during digestion. Additional testing is needed to confirm these results as the barnacles may have not fed during the trial, thus had no food to digest. While it appears that increasing temperatures associated with climate change will have little impact on the digestion of barnacles at low tide, if climate change alters the duration of low tide, there could be an energetic impact to barnacles due to the slowing of their metabolism as indicated by the delay in their digestion.

Introduction

The rocky intertidal zone, the area where the land interfaces with the sea, is a unique ecosystem that experiences both fully marine and fully terrestrial conditions (Levinton 2008). These two sets of conditions are created by the rise and fall of the sea level due to tides. Tides are created by a combination of the gravitational effects of the moon and the rotation of the earth, which push and pull on the ocean causing the sea level to rise and fall. Tides can occur once or twice a day, and can vary in height and duration (Levinton, 2008).

Organisms that live in the rocky intertidal zone must deal with a variety of abiotic stresses associated with the low tide (Helmuth *et al*. 2005). Dramatic changes in temperature, desiccation, and oxygen availability are some of the most prominent challenges organisms must cope with. As the tide falls, the temperature changes from the relatively constant water temperature, to extremely variable air temperatures and direct radiation from the sun. Exposure to air can cause desiccation, and most marine organisms enter a state of hypoxia as they can't breathe air to obtain oxygen (Levinton 2008). These factors by themselves are stressful enough, however in combination can create extreme stress these organisms must deal with at low tide.

Of these stresses, one that is particularly difficult for organisms to deal with is decreased oxygen availability. Marine organisms have specialized respiratory structures that enable them to breathe oxygen under water, however when exposed to air, many are unable to breathe air and must rely on oxygen stored in their body (Marsden and Waterhead 1998). Adenosine triphosphate, the high energy molecule that powers all cellular functions and is necessary for life, requires oxygen to be synthesized (Spoor 1946). When oxygen is not available, metabolism switches from aerobic to anaerobic, which doesn't require oxygen, but is less efficient at producing ATP and creates toxic byproducts that require oxygen to be eliminated (Connor and Gracey 2012). Some marine organisms, such as barnacles, are able to respire in air however, they must be careful since doing so increases their risk of desiccation (Davenport and Irwin 2003). At low tide, oxygen

deprivation is one of the more significant stresses to organisms in the rocky intertidal zone.

Another major stress to organisms in the rocky intertidal at low tide is temperature. Air temperatures are extremely variable, and can reach up to 40 ^{o}C during summer months (Helmuth *et al*. 2011). For organisms that are motile, this isn't as large of a problem since they can move into the water or shade. Chapperon and Seuront (2011) found that the snail, *Littoraria scabra,* chose to move to substrates with specific surface temperatures during low tide to optimize their thermoregulation. For sessile organisms, temperature regulation is much more of a problem. The temperature of sessile organisms can be affected by the substrate type, the effects of grouping, the shape and size of the organism along with a variety of other factors (Helmuth 1998). In general however, the difficulty for sessile organisms to maintain a relatively constant body temperature is linked to the ambient temperature and how stressful it becomes (Helmuth *et al* 2005).

Climate change is rapidly becoming a growing concern. The combustion of fossil fuels adds carbon dioxide, methane, and nitrous oxide to greenhouse gasses in the atmosphere, which trap solar radiation and prevent energy from escaping the atmosphere causing temperatures to increase both in the ocean and the air (Kuhlbrodt and Gregory 2012). Average global temperatures have increased 0.6 ^{o}C over the past 100 years and are expected to continue rising in the next century (Root *et al*. 2003, ipcc.ch). The effects of these increasing temperatures are wide spread from biodiversity and human health to economic impacts (Kriegler *et al* 2012).

In the rocky intertidal zone, increasing air and ocean temperatures due to climate change are of special concern. At low tide, temperature stress is already a major problem for these organisms, so if temperatures were to increase even more, this could eventually push the limits of these organisms and their ability to cope with these stresses. This makes studying the impacts of climate change on this ecosystem of immediate importance.

The acorn barnacle *Balanus glandula,* is a common ectothermic organism of the rocky intertidal zone. They range along the California coast up into the Aleutian Island in Alaska, down into Baja California (Newman 2007). As adults they are sessile suspension feeders, but have two nektonic larval stages in which they search for a suitable substrate to settle (Anderson 1994). When they find the right location, they stick their head down and secrete cement from specialized glands, permanently affixing themselves to that location. A hard shell is then built up around the rest of their body leaving a hole at the top, the operculum, that can be opened and shut with moveable shell plates.

Feathery thoracic appendages called cirri protrude out of the operculum and facilitate both respiration and feeding. The cirri beat through the water column, pumping water through the barnacles' body cavity providing oxygen and capturing floating micro-organisms, such as brine shrimp. Once the barnacle captures food in its cirri, it curls them down and pushes the food into its mouth where it is the processed and passes through the esophagus, stomach, small intestine and out the anus which is also located at the operculum.

B. glandula has a strong influence on the community structure of the rocky intertidal zone due to bottom-up effects (Morris *et al*. 1980). They are an important food source for snails, seastars and crabs, thus if something were to happen to barnacles, it would resonate up the food chain, eventually impacting marine birds.

At low tide, barnacles cannot feed since they are exposed to air. However, it is possible that they could digest during low tide, which would be an energetically favorable strategy. If they were able to digest at low tide, they would empty their stomach allowing them to take full advantage of the next feeding opportunity at the next high tide. This would allow them to maximize their food intake and give them the maximum amount of energy.

The process of digestion requires energy and also oxygen. Thus a measurement of the rate of oxygen consumption can be used to determine the metabolic activity of the barnacle. As seen in a number of other organisms, digestion elevates metabolism, therefore it is possible that barnacle digestion also elevates metabolism and in-turn be measured by the rate of oxygen consumption (McCue 2005). However, the metabolic cost of digestion has never been discovered for a barnacle.

Digestion at low tide could be affected by either the temperature or the limited oxygen supply experienced at low tide, since both impact the barnacles' oxygen consumption. As ectotherms, barnacles' metabolisms are dictated by their external environment (Wilmer *et al*. 2005). Over a certain range of temperatures organisms perform at optimal levels, but temperatures that exceed that range can

cause stress (Somero 1995). It is possible that digestion does occur at low tide under moderate temperatures, but that a stressful temperature may affect the barnacles' ability to digest as it directs it energy towards other processes to deal with the stress.

Along with temperature, the hypoxic conditions of low tide could also affect the ability of barnacles to digest at low tide. One strategy barnacles use at low tide to cope with oxygen deprivation is the uptake of a small bubble of air (Davenport and Irwin 2003). This small bubble can supplement the barnacles' oxygen supply, but can be costly due to desiccation stress. The use of this small air bubble for oxygen, if not too costly in terms of desiccation, could provide the extra oxygen needed to perform digestion during low tide.

This experiment investigated the effects of low tide on digestion. It was hypothesized that barnacles would take advantage of digesting at a low tide that was at a non-stressful temperature, but that at stressful temperatures, barnacles would stop digesting in order to conserve oxygen to deal with the stress of the temperature. Barnacles were allowed to feed and then exposed to either a non-stressful temperature or a stressful hot temperature. The time it took for barnacles to excrete feces, signifying the end of digestion was measured. Additionally, the rate of oxygen consumption from barnacle respiration was measured to determine any energetic costs of digestion.

Materials and Methods

Study System

B. glandula attached to mussels (*Mytilus californianus*, and *Mytilus galloprovincialis*) were collected from rock pilings at Newport Beach, California (33° 35' 41.73"N, 117° 52; 42.12" W) during low tide (Figure 1). They were transported to the W.M. Keck Science Center in Claremont, California where all experiments were performed.

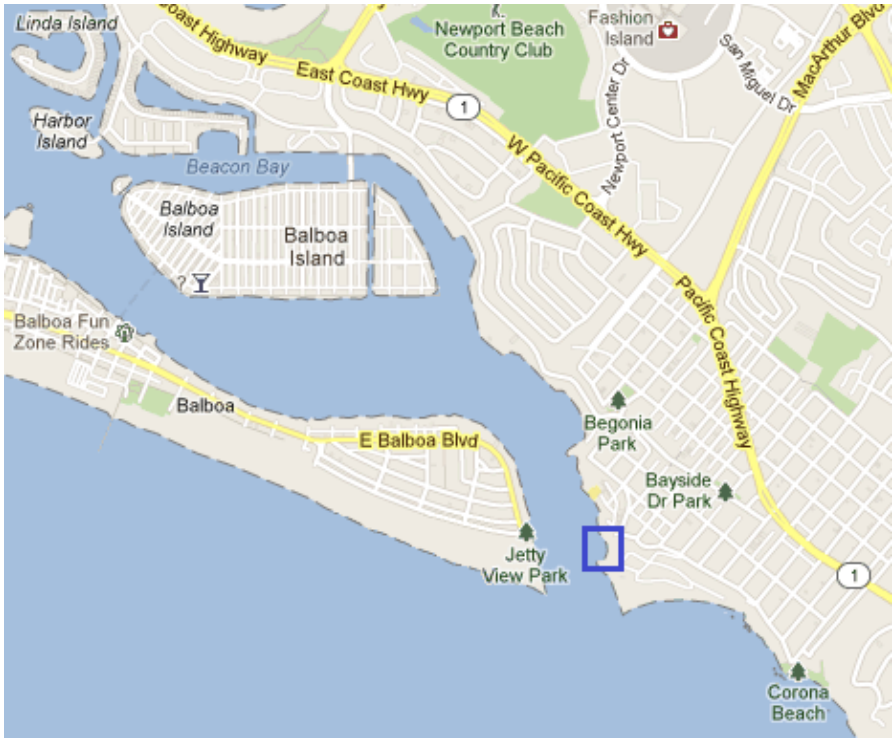

Figure 1. The collection site at Newport Beach, CA, boxed in blue (image from maps.google.com).

Mussels were shucked, and cutting pliers (No. 338, Channellock Inc, Meadville, PA) were used to cut barnacles from mussel shells so that the basal plate of the barnacle was left anchored to a fragment of the mussel shell. A 15-17 cm length of 30 lb. test fishing line was attached to each barnacle on the back side of the mussel shell fragment oriented perpendicular to the operculum with

hot melt adhesive. An identification tag made of duct tape folded in half with a hole punched in on one side was tied onto the other end of the fishing line. Each barnacle was measured across the largest diameter of the operculum using digital calipers (Digimatic Caliper Model# CD-6”CX, Mitutoyo America Corporation, Aurora, IL) and recorded. Barnacles were suspended by their tags on egg crate trays.

Tide tanks were created by attaching a pump controlled by an automatic timer to a 48-quart cooler using plastic tubing (Figure 2). When the pump was on, it filled the tide tank with water from a 75 gallon recirculating indoor storage tank, simulating high tide. Excess water pumped into the tide tank was drained back into the storage tank by a PVC pipe and plastic tubing. When the pump was off, all of the water drained out of the tide tank via the pump line back into the storage tank leaving the barnacles exposed to air, simulating low tide. The timer was set so that the immersion and emersion experienced by the barnacles were synchronized to the actual tidal cycles experienced at Newport Beach. Every 24 hours, the barnacles experienced high tide from 3:30am to 8:30am, a low tide from 8:30am to 12:30pm, a second high tide from 12:30pm to 8:30pm and a second low tide from 8:30pm to 3:30am. The lights were also synchronized to come on at 7am and shut off at 7pm. The tide tanks were covered with sheet plastic to keep humidity constant and decrease evaporation.

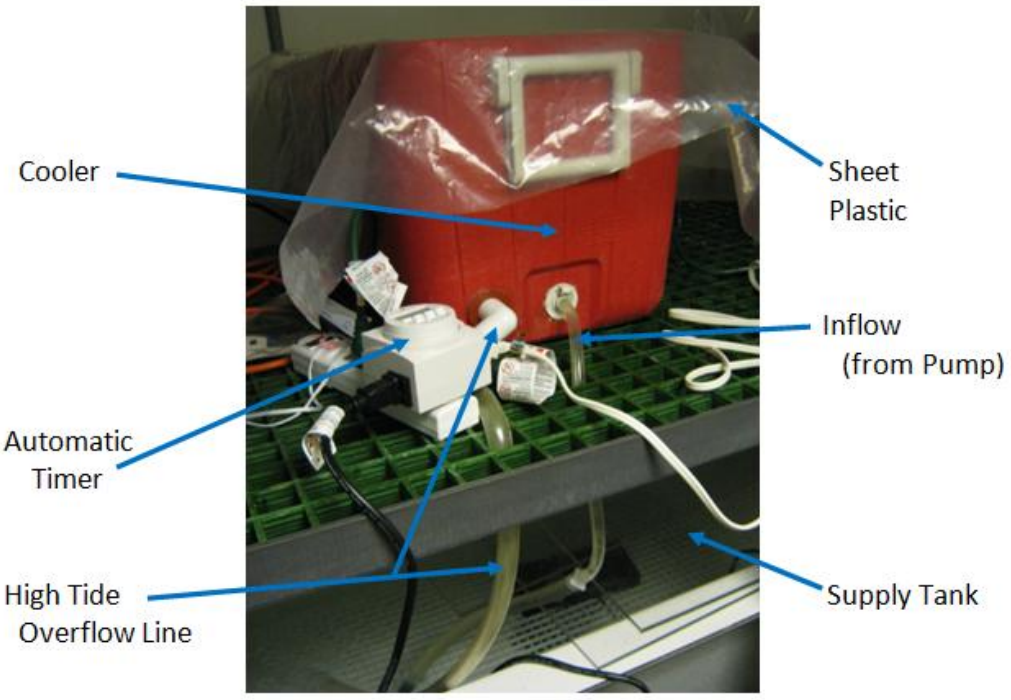

Figure 2. The tide tank set-up.

Barnacles were fed about 700 mL of hatched *Artemia spp.* larvae (INVE Aquaculture, Salt Lake City, UT) three times a week in about 5-7 gallons of sea water in a separate 10 gallon plastic storage bin (Rubbermaid, Newell Rubbermaid, Atlanta, GA) to preserve the tide tank water quality. 5.2 mL of *Artemia spp.* eggs were hatched in 1400 mL of sea water two days prior to feedings in a separatory funnel so that un-hatched eggs could be separated from live larvae before being fed to barnacles. The 1400 mL of hatched larvae was split between two bins, container A and container B, each filled with about 7 gallons of sea water to fully submerge the barnacles. This feeding will henceforth be referred to as a normal feeding and all barnacles not actively being experimented on were kept on this feeding schedule.

Clearance of the Alimentary Canal

In order to measure gut passage times, it was necessary to ensure the barnacle's alimentary canal was completely empty. This was determined by measuring how long it takes barnacles to stop excreting feces, signaling their digestive systems are completely empty. Barnacles were fed a normal feeding then immediately placed in plastic test-tubes and suspended from their egg crate trays. The tubes were checked for feces every 6 hours for 48 hours, and if feces were observed, the tube was emptied and put back to allow for identification of new feces after that check. The plastic test-tubes had holes drilled on the sides to allow for water flow and adequate oxygen ventilation; however the bottom portion of the test tube was solid to catch all feces excreted. This was repeated with second set of barnacles, but the tubes were checked for feces every 12 hours for 7 days. All barnacles stopped excreting feces after 40 hours. Based on this data, all barnacles were starved for 48 hours prior to experimentation to ensure all had a completely empty alimentary tract for experimentation.

To determine the general amount of time it took barnacles to begin excreting feces after feeding for 1 hour, the same procedure as before was used. Barnacles were starved for 48 hours, fed for 1 hour, and then monitored in the plastic test-tubes every 6 hours for 24 hours for feces. It was determined that most barnacles excreted majority of their feces within 12 hours of feeding. Thus a 12 hour monitoring period was appropriate for the measurement of digestion of barnacles in subsequent experiments.

Measuring Digestion during Low Tide

Data was collected from 6 July 2012 to 2 August 2012 in a temperature controlled room at 16°C. To determine how long digestion took without any tidal exposure ("baseline digestion time") barnacles were starved for 48 hours and fed a $4.35x10^{-7}$ g/ml concentrated solution of brine shrimp for 30 minutes in a 2 gallon clear glass tank. Each barnacle was observed continuously to ensure each individual had consumed food for a total of 30 minutes. After having fed for 30 minutes, each barnacle was transferred directly to a 2 gallon clear glass tank to be filmed for 12 hours (Figure 3).

One video camera (Handycam DCR-HC96, Sony Corporation, Japan) was suspended above the tank recording time-lapse pictures every 10 minutes. The bottom of the camera was anchored to a hard plastic sheet. This plastic sheet was then attached to two long screws which were clamped to a ring stand, suspending the camera above the tank with the camera recording a bird's eye view of the tank. The camera was covered by a plastic bag and a desiccant packet added to minimize the amount of humidity the camera was exposed to. A laminated piece of graph paper was glued to the bottom of the tank which provided contrast so the camera could focus on the feces that fell to the bottom of the tank after being excreted. These time lapse pictures were later analyzed to give a 10 minute estimate of when individual feces were excreted over the 12 hour period. The video from a second camera was used to identify which barnacles excreted feces and the exact time they did so.

The second video camera (Handycam HDR-XR260V, Sony Corporation, Japan) recorded video the entire 12 hours. This camera was mounted to a tripod and focused so that the operculum of all barnacles suspended in the tank were lined up at the top of the screen, in focus, with about 10-15 cm of blank screen below so falling feces could be observed. This camera was encased in a waterproof camcorder case (WP-D20L, DiCAPac, Korea), again with a desiccant to minimize the amount of humidity it was exposed to. The tank was surrounded by white pieces of paper, again providing contrast so the feces were more easily identified. Cardboard was suspended above the entire setup using ring stand clamps to block the direct reflection of overhead lighting that interfered with the clarity of the time-lapse pictures (Figure 3).

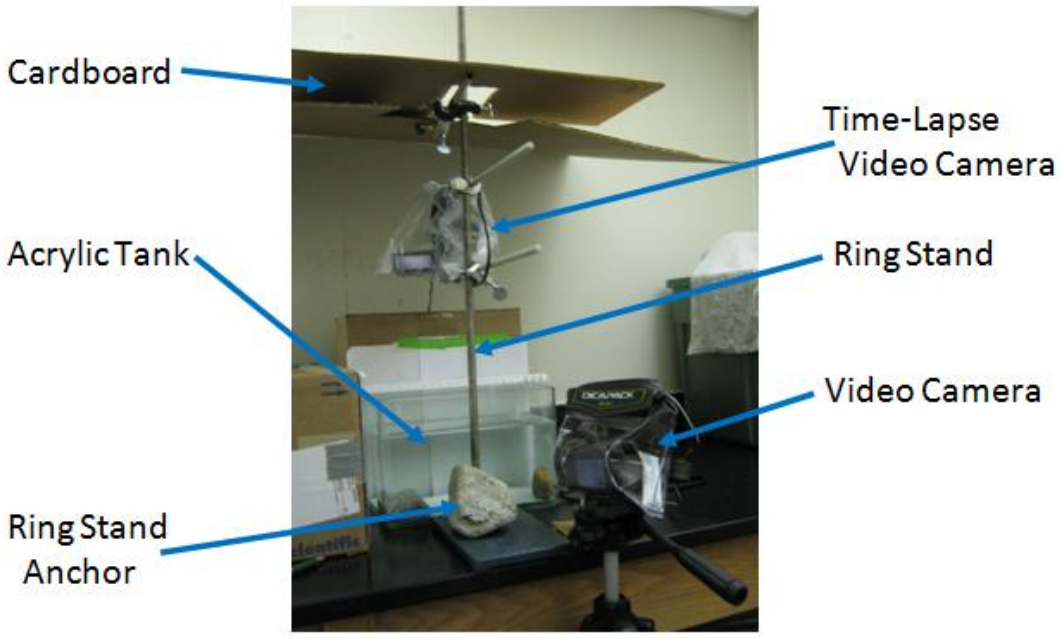

Figure 3. The video monitoring set-up.

After baseline digestion times were established, the time to digest after being exposed to a low tide was determined. Two different low tide treatments

were used in the experiment: a normal low tide at 16°C, and a hot low tide at 35°C. The normal low tide was set at 16°C, as this is the average water temperature experienced by barnacles during summer. The hot low tide was set at 35°C, as it remains just below the critical thermal maximum so it was guaranteed to be a stressful temperature without inducing death. As before, barnacles were starved for 48 hours, then fed for 30 minutes in the 2 gallon clear glass tank, continuously watched to ensure each individual fed for the full period of time. These barnacles were then transferred to one of the two low tide treatments, where they were exposed to temperature controlled air for one hour before being transferred to the second 2 gallon clear glass tank and video taped for 12 hours as before.

The 16°C low tide was simulated by placing barnacles on a plastic tray in the temperature control room set to 16°C (Figure 4).

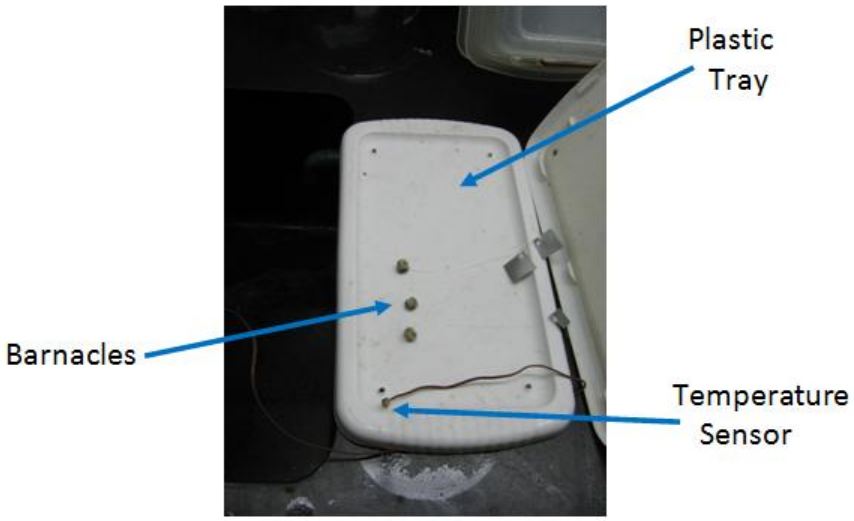

Figure 4. The normal (16°C) low tide treatment.

The 35°C low tide was simulated by placing barnacles in plastic test tubes attached to weighted egg crate and submerged in a hot water bath set to 41°C. With the water bath set at 41°C, the air temperature inside the test tubes was held at 35°C as indicated by the temperature sensor kept lowered in one of the test tubes (Figure 5). The temperature sensor imitated a barnacle by attaching the temperature probe to the inside of an emptied out barnacle shell.

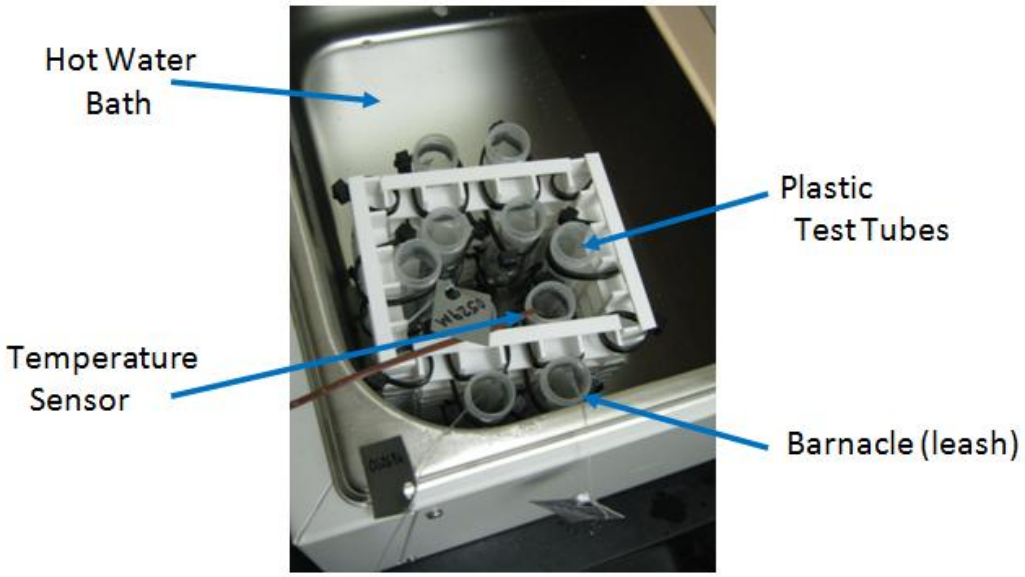

Figure 5. The hot (35°C) low tide treatment.

Respiration Measurements

Data was collected from 12 June 2012 to 28 June 2012 at the W.M. Keck Science Center in Claremont, California. Oxygen consumption was measured using a fluorometric oxygen sensor, factory calibrated for 12-25°C (Neofox system: Ocean Optics, Dunedin, Florida). Each day, the sensor was calibrated in 16°C aerated seawater to 20.9% O_2, the amount of oxygen in the atmosphere by volume. To ensure accuracy of these readings, our measurements were compared to values

calculated by the National Oceanic and Atmospheric Administration for fully oxygenated seawater at our altitude of 384 m. Seawater used with the oxygen sensor was filtered using a 45 µm HAWP filter (Millipore, Billerica, MA) to remove aerobic organisms that could alter respiration measurements and oxygenated overnight in a flask equipped with an air stone to ensure adequate aeration at 16°C.

Respiration was measured in a chamber was constructed from a 28ml glass vial (Ward's Natural Science, San Luis Obispo, CA), filled with hot melt adhesive to decrease the volume to ~10 ml (Figure 6). The bottom of the chamber was covered with a layer of black plastic to reduce any reflections from the sensor. A 10x3 mm spin bar was added to the chamber to mix the water for more accurate readings, and the whole chamber placed on a stir plate (Isotemp, Fisher Scientific, Waltham, MA). The oxygen sensor was placed through a hole drilled through a No. 3 sized rubber stopper and placed onto the chamber to create an air-tight seal. This whole chamber setup was placed into a 50 mL jacketed beaker (Kimble Chase-Kontes Glass, Vineland, NJ), connected to a circulating 15°C temperature controlled water chiller (Thermostat AC 200, Thermo Fisher Scientific, Waltham, MA). The space between the chamber and the jacketed beaker was filled with 0.45 µm filtered seawater to keep the temperature of the chamber more stable.

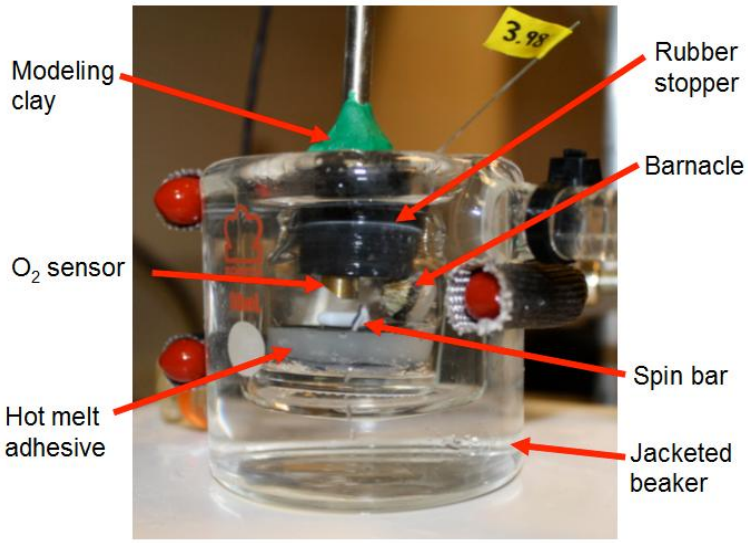

Figure 6. The respiration measurement chamber. (Photo Credit: Wong 2011)

An oxygen consumption baseline was determined by starving the barnacles for 48 hours, then inserting them into the chamber and recording their oxygen consumption levels for each defined barnacle behavior. Four barnacle behaviors were observed: closed, pumping, normal beat, and fast beat, as defined by Crisp and Southward (1961). Other behaviors such as testing, and behaviors combining two of the main four behaviors, closed/normal and fast beat/extension as defined by Wong (2011) were difficult to observe and therefore omitted from observations. At least three replicates of each behavior were recorded and only behaviors exhibited for one minute or more were included in the analysis to ensure oxygen consumption rates were consistent. Behaviors were used for comparison since individual barnacles perform different behaviors for varying amounts of time due to individual variation. Chamber water was changed every 30 minutes or after the oxygen concentration had decreased between 5-10% from fully oxygenated to

ensure that oxygen concentrations remained at a high enough level that barnacles were not stressed by oxygen limitations.

Oxygen consumption post-digestion was determined by starving the barnacles 48 hours, feeding them, then tracking the oxygen consumption and behavior for up to 8 hours post feeding. Of the three barnacles used, two were fed for one hour then respiration followed for two hours, and the third was fed for 30 minutes and followed for eight hours. Behaviors were first observed for the first 30 minutes continuously, then observed for 5 minutes every 30 minutes for the rest of the tracking period. Chamber water was changed every 30 minutes to ensure fully oxygenated water throughout the trial.

Oxygen levels were recorded in $\mu mol \bullet mL^{-1}$ so the water volume used in each trial was also measured in order to calculate the amount of oxygen consumed per minute ($\mu mol \bullet mL^{-1}$). To calculate water volume, the water mass of each trial was divided by its density ($1.022 g \bullet L^{-1}$). The water mass was determined at the end of each trial by calculating the mass difference between an empty chamber and the water-filled chamber with the barnacle removed.

Feeding Concentrations

Artemia spp. feeding concentrations were measured in accordance with Harris et al (2000). A 10ml sample from the feeding within the first five minutes of the feeding was taken using a stenson-hemple pipette. For the normal feeding, a sample from each of the two feeding containers, A and B was taken. For the experimental feeding, only one bin was used and thus only one sample taken.

These samples were filtered onto a glass fiber filter (Whatman GF/F, 47mm, Kent, UK) using a vacuum filtration system. The filtered samples were placed in a 60 ^{o}C drying oven for 48 hours to obtain their dry weight, then transferred to a 500 ^{o}C furnace for 12 hours to burn off all the soft tissue and obtain their ash weight. Their ash free-dry weight was calculated as the difference between their dry weight and their ash weight. The feeding concentration was determined by dividing the ash-free dry weight by the total volume of the sample. These feeding concentrations were used to confirm that barnacles were exposed to adequate amounts of food during experimental trials.

Data Analysis

The average time to first excretion from the baseline treatment was compared to the average time to first excretion for 16 ^{o}C and 35 ^{o}C treatments to determine the relative time to first excretion. The one hour post-feeding aerial exposure was not included in the time to first excretion. Statistical analyses were performed both including and excluding barnacles where the time to first excrement took over three hours. These barnacles were determined to be an unrealistic sampling of the data since all the other barnacles excreted feces within the first hour and thus were excluded in the final statistical analyses and figures. Additionally, three barnacles were thrown out due to one barnacle having been previously used for a stressful experiment, one having potentially spawned during the trial, and one not excreting any feces during any of the trials. The statistical program JMP (V.9, SAS, North Carolina) was used to run all statistical analyses.

An ANOVA was used to determine if there was a significant difference between the relative time to first excretion during a stressful (35 ^{o}C) low tide and non-stressful (16 ^{o}C) low tide. A Shapiro-Welk test was used to test for normality of errors, and the data was found to be non-normal. A log transformation and ANOVA were performed on the log transformed data; however the log transformation had no effect on the p-value so the untransformed data was used. A T-test was used to determine if each tide treatment was different from their baselines. An F-test was used to determine if there were any group effects.

The respiration rates from the three barnacles post feeding were compared to those barnacles' starved baseline respiration rates. Since each barnacle has different respiration rates for each behavior, a baseline respiration rate was observed for each barnacle for each possible behavior. A relative respiration rate was calculated as the ratio between the observed respiration rate post-feeding and the baseline rate for the same behavior in the same barnacle.

Results

Digestion during Tides

The first excrement for the normal (16^{o}C) low tide treatment as compared to the baseline (no tide) treatment took about 48 minutes longer. This time does not include the hour long exposure to the low tide. The time to first excrement took about an hour longer for the hot (35^{o}C) low tide treatment as compared to the baseline (Figure 7). There was no significant difference between the two temperature treatments (ANOVA: $p = 0.5337$, $F = -0.63$, $df = 29$). However, both

the 16 °C and 35 °C low tides were found to be significantly different from their baseline (t-test$_{16\ °C}$: p = 0.00087, t = 3.75, df = 26; t-test$_{35\ °C}$: p = 0.00020, t = 4.37, df = 23). There were no group effects (F-ratio = 0.3489, p = 0.5592).

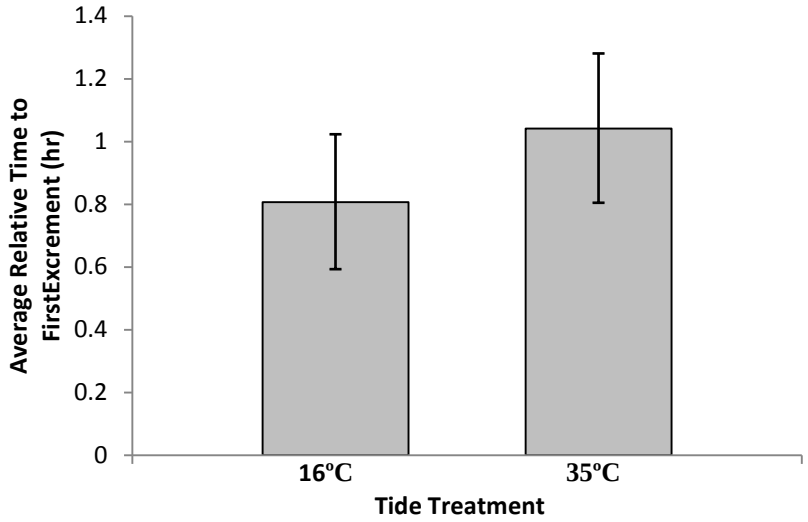

Figure 7. The average relative time to first excrement for 16°C and 35°C tide treatments ($n_{16°C}$=20, $n_{35°C}$ =16).

Respiration during Digestion

Of the three barnacles whose respiration was monitored after feeding, none of the barnacles showed any real elevation from their baseline respiration rates.

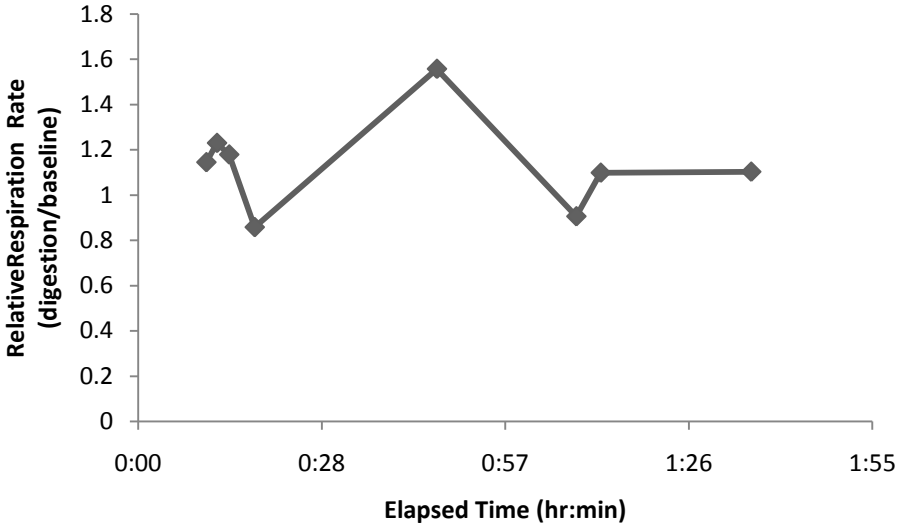

Figure 8. The relative respiration rate of barnacle 0529WW after a 1 hour feeding at 16 °C.

All rates from barnacle 0529WW, except for the fifth rate, were close to one, thus most rates were similar to their baseline rate (Figure 8). This elevated fifth rate occurred about 45 minutes after feeding.

Of the three rates measured for barnacle 0529AB, only one was slightly elevated and occurred about one hour after feeding. The other two rates were close to equaling their baseline rates (Figure 9). Only three rates were measurable because they were the only behaviors performed for at least one minute. All other behaviors lasted less than one minute and were not used for rate measurements for barnacle 0529AB.

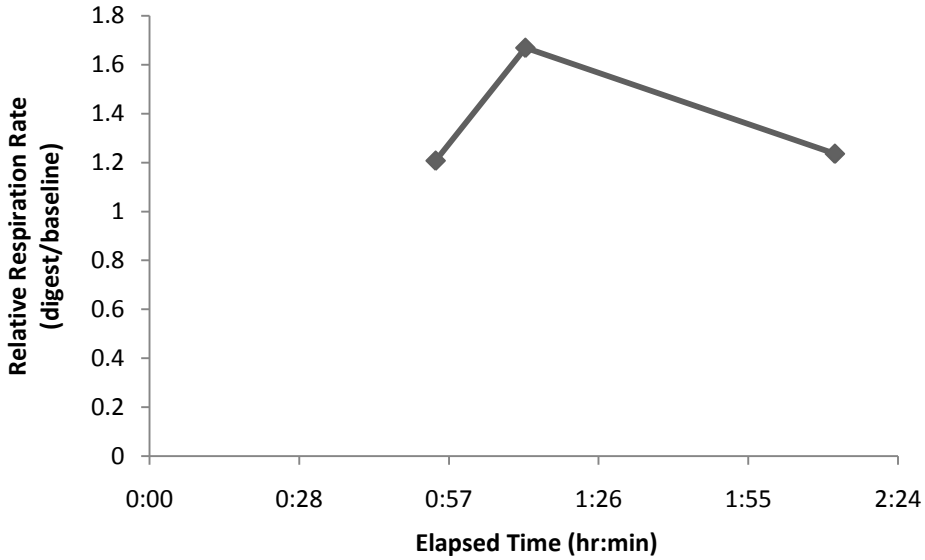

Figure 9. The relative respiration rate of barnacle 0529AB after a one hour feeding at 16°C.

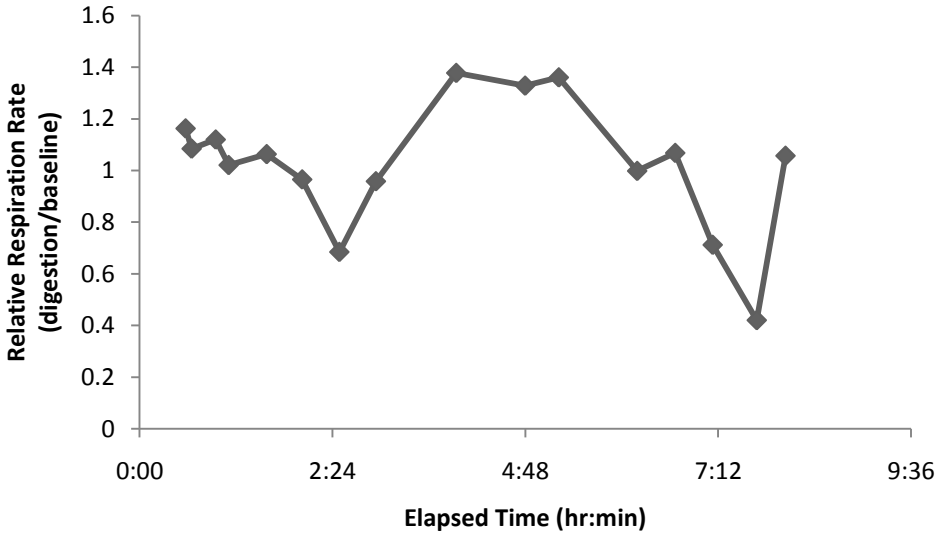

Figure 10. The relative respiration rate of barnacle 0606AM after a 30 minute feeding at 16 °C.

Similarly to the first two barnacles, the majority of the respiration rates recorded for barnacle 0606AM were close to equaling their baseline rates (Figure 10). Two rates, one around two hours and 30 minutes and the other around seven hours and 40 minutes, were lower than their baseline rates. Three rates, from about four hours to five hours were slightly elevated from the baseline rates. Just after the third elevated rate, at about five hours and 13 minutes, barnacle 0606AM excreted feces.

Unlike the barnacles from the tide treatment experiments, none of these barnacles produced any feces in the first hour. Only one, 0606AM excreted feces about five hours after feeding. The other two barnacles never produced any feces in the time they were monitored.

Feeding Concentrations

The feeding concentrations of *Artemia spp*. given to the barnacles in their normal feeding varied by a factor of two, depending on which feeding bin the

barnacles were placed in (Table 1). The average normal feeding concentration was $1.735x10^{-08}$ g/mL. The 30 minute experimental feeding concentration of *Artemia spp.* was greater than the average normal feeding concentration by a factor of 25 (Table 1).

Table 1. *Artemia spp.* feeding concentrations.

Feeding Type	Date	Dry Weight (g)	Ash-Free Dry Weight (g)	Feeding Concentration (g/ml)
Normal Feeding (Container A)	6/20/12	0.7070	0.0006	$2.321x10^{-8}$
Normal Feeding (Container B)	6/20/12	0.8626	0.0003	$1.153x10^{-8}$
30- Minute Experimental Feeding	6/21/12	0.9206	0.00085	4.35897E-07

Discussion

It was hypothesized that barnacles would digest at low tide, but only if the temperature of the low tide remained at a moderate temperature. Since barnacles can only feed during high tide, it would be an energetically favorable strategy to digest any food they had consumed during low tide to take full advantage of all feeding opportunities. However, digestion has previously been established as a costly metabolic activity in a variety of other organisms to the extent that they partition energy demands (McCue 2006). It's possible that when barnacles experience the stress of low oxygen levels or increased temperatures associated with low tide, they may stop or slow digestion to conserve oxygen and partition their energy towards other metabolic processes more necessary to survival (Owen

2001). It was found that barnacles stopped their digestion during low tide regardless of the temperature. Additional study of respiration following feeding did not reveal an energetic cost of digestion, however the sample size was small and the trial inconsistent with data from the previous digestion experiments completed.

Digestion during Low Tide

Both 16 °C and 35 °C low tides significantly delayed barnacle digestion, from digestion without any tide exposure. However, the temperature of the tide had no significant effect on this delay. The low tide exposures were one hour long at either 16 °C or 35 °C. Digestion was delayed by 48 minutes and about one hour respectively. This time delay does not include the hour that the barnacles were exposed to air simulating the low tide. This could mean that regardless of the temperature, low tide is a stressful enough event that barnacles stop digesting to deal with the stress of low tide. It was hypothesized that a non-stressful low tide would not affect the barnacles' digestion, however it appears that any temperature of low tide is stressful enough to affect digestion.

During low tide, barnacles are limited by oxygen availability, since they are out of water (Davenport and Irwin 2003). While some barnacles can take in air bubbles and continue respiration, they risk desiccation in doing so. Barnacles can be exposed to a variety of low tides, ranging from a few hours to upwards of 12 hours at varying temperatures (oceanservice.noaa.gov), so balancing the need for oxygen consumption with the potential for desiccation is a complex decision. In order to conserve the oxygen they have already stored, they may slow down their

metabolism by stopping digestion to use less oxygen while exposed during a low tide. Similar strategies have been observed in both *Mytilus californianus* and *Littorina saxatilis* (Connor and Gracey 2012; Sokolova and Portner 2001).

In this experiment, the barnacles were conditioned to two tides per day, one shorter four hour tide, and one longer seven hour tide. It could be that because the barnacles were used to these exposure times, they expected a longer tide exposure and automatically slowed down their metabolism by not digesting to conserve oxygen. This strategy has been observed in *Littorina saxatilis* in coping with abiotic stresses of low tide (Sokolova and Portner 2001). Since digestion is a metabolically costly activity (McCue 2005), shutting down digestion would help conserve oxygen stores. This may have skewed the results because if barnacles had been conditioned to only one hour low tide exposures, they may have chosen to continue digesting during the short low tide period, knowing they did not need to conserve oxygen to last a 4-7 hour low tide.

If barnacles were able to digest during low tide, they would be able to take full advantage of feeding opportunities, an energetically favorable strategy. However, it appears that the cost of low tide in terms of oxygen consumption outweighed the benefits of spending the energy to digest their food. The barnacles shut down digestion to conserve oxygen instead of using stored oxygen to digest in preparation for the next high tide. If these barnacles were conditioned to shorter, one hour tide exposures lowering their oxygen constraints, it is possible that they may choose to digest during these low tides instead of shutting down and

conserving oxygen. Further studies using barnacles conditioned to shorter low tides could be used to investigate this.

Respiration during Digestion

In addition to looking at the effect of low tide on digestion through observing fecal production, a few barnacles were used to see if oxygen consumption could be used to determine both the duration and energetic cost of digestion.

The relative respiration rate of barnacles was used to determine when the barnacles' respiration had returned to baseline levels, since each behavior for each barnacle was associated with different oxygen demands (Newell and Northcroft 1965). Of the three barnacles used, none showed any real elevation or decline from their baseline respiration rates.

Two barnacles, 0529WW and 0529AB, were fed for one hour and respiration followed for about two hours. The other barnacle, 0606AM, was fed for 30 minutes and respiration followed for about 8 hours. None of these barnacles were exposed to low tides and experiments were conducted during the time of day barnacles expected to be submerged at a high tide, so digestion was expected to have occurred immediately. Barnacles 0529WW and 0529AB showed one slightly elevated rate of oxygen consumption about one hour after feeding, but remained around their baseline rate before and after this point. Barnacle 0606AM showed decreased rates of oxygen consumption around two hours and again around seven hours, and three elevated rates from about four hours to five hours. The three

elevated rates preceded the production of feces, and could signal that producing feces is a metabolically costly activity. However, no other barnacles excreted feces during the trials, which is surprising considering that most barnacles excreted feces within the first hour after being fed during the tide treatments.

One reason the barnacles could have exhibited these surprising oxygen consumption rates and no fecal production is that they may not have actually eaten during their exposure to food. Unlike the digestion and tide experiments, these barnacles were not watched during this time to ensure that they actively fed during the entire exposure period. Thus, while food was available to the barnacles, they may not have been feeding on it, and thus not have had any food to digest. Barnacle 0606AM could have fed, however the fecal production would have been expected within the first hour as observed in the prior experiments, not after seven hours as was observed in these trials.

Although the data was variable, none of the barnacles showed any substantial elevation above baseline oxygen consumption rates. Further experimentation would be needed to confirm these results since the sample size was only three, and the barnacles should have been watched during their exposure to food to ensure consumption throughout the entire period.

Conclusions

In conclusion, barnacles shut down their digestion when exposed to a low tide, regardless of the temperature. This differed from predictions, as it was previously thought to be energetically favorable for the barnacle to digest at low

tide to take full advantage of all feeding opportunities at high tide. Additionally, this study aimed to determine the energetic cost of digestion through respiration measurements. However, no cost appeared to be associated with digestion, but the sample size was small and the barnacles measured may not have fed during the trials.

As ocean temperatures rise due to climate change, the affect on barnacle digestion will be minimal based on this study. However, if climate change impacts sea level, this could impact the duration of tides, which could have implications for barnacle digestion and oxygen limitation during low tide. This study proved to enlighten the field of barnacle ecology about the effects of low tide on digestion, however more rigorous studies are needed to determine any quantified energetic costs of digestion.

Literature Cited

Anderson DT, 1994. Barnacles: Structure, Function, Development and Evolution. Springer, New York, USA.

Chapperon C, Seuront L 2011. Behavioral thermoregulation in a tropical gastropod: links to climate change scenarios. Global Change Biology 17: 1740-1749.

Connor KM, Gracey AY, 2012. High-resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus*. American Journal of Physiology-Regulatory, Integrative, and Comparative Physiology 302: R103-R111.

Davenport J and Irwin S, 2003. Hypoxic life of intertidal acorn barnacles. Marine Biology 143:555-563.

Helmuth B, 1998. Intertidal Mussel Microclimates: Predicting the Body Temperature of a Sessile Invertebrate". Ecological Monographs 68: 51-74.

Helmuth B, Kingsolver J, and Carrington E, 2005. Biophysics, physiological ecology, andclimate change: does mechanism matter? Annual Review of Physiology 67: 177-201.

Helmuth B, Yamane L, Lalwani S, 2011. Hidden signals of climate change in intertidal ecosystems: What (not) to expect when you are expecting". Journal of Experimental Marine Biology and Ecology 400: 191-199.

Kriegler E, O'Neill BC, Hallegatte S, Kram T, Lempert RJ, Moss RH, Wilbanks T, 2012. The need for and use of socio-economic scenarios for climate change analysis: A new approach based on shared socio-economic pathways. Global Environmental Change-Human and Policy Dimensions 22: 807-822.

Kuhlbrodt T, Gregory JM, 2012. Ocean heat uptake and its consequences for the magnitude of sea level rise and climate change. Geophysical Research Letters 39: L18608.

Levinton J, 2008. Marine Biology: Function, Biodiversity, Ecology. 3rd Ed. Oxford University Press, New York, NY, USA.

Marsden ID, Weatherhead MA, 1998. Effects of aerial exposure on oxygen consumption by the New Zealand mussel *Perna canaliculus* (Gmelin, 1791) from an intertidal habitat. Journal of Experimental Marine Biology and Ecology 230: 15-29.

McCue MD, 2006. Review: Specific dynamic action: A century of investigation. Comparative Biochemistry and Physiology 144: 381-394.

Morris RH, Abbott DP, and Haderlie EC, 1980. Intertidal Invertebrates of California. 1st Ed. Stanford University Press, Palo Alto, CA, USA.

Newell RC and Northcroft HR, 1965. The relationship between cirral activity and oxygen uptake in *Balanus balanoides*. Marine Biology 45: 387-403.

Newman WA, 2007. Cirripedia. In Carlton JT (ed.) The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon 4th Ed. University of California Press 475–484.

Owen SF, 2001. Meeting energy budgets by modulation of behaviour and physiology in the eel (Anguilla anguilla L.). Comparative Biochemistry and Physiology
123: 631–644.

Sokolova IM, Portner HO, 2001. Physiological adaptations to high intertidal life involve improved water conservation abilities and metabolic rate depression in *Littorina saxatilis*. Marine Ecology 224: 171-186.

Somero GN, 1995. Proteins and temperature. Annual Reviews Physiology 57: 43-68.

Spoor WA, 1946. A quantitative study of the relationship between the activity and oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes. The Biological Bulletin 93: 312-325.

Willmer P, Stone G, and Johnston I, 2005. Environmental Physiology Animals. 2nd Ed. Blackwell Publishing, Victoria, Australia.

Wong J, 2011. The effects of behavior on the respiration of *Balanus glandula*. BA thesis. Scripps College, Claremont CA.

Acknowledgements

I would like to thank Dr. Sarah Gilman for all her support throughout the duration of this study. Her patience, guidance and personal investment was greatly appreciated and helped provide the foundation needed to complete this study. I am truly grateful for all the time and effort she put into helping design the experiment, interpret the results and write this thesis.

I am also thankful to Alicia Hendrix for working alongside me throughout the summer and providing support as a colleague.

I would also like to thank Dr. Diane Thompson for her advice and helpful comments throughout the writing process, as well as the W. M. Keck Science Department for awarding me the W. M. Keck grant to financially support this research.

Lastly, I would like to thank my family and friends for all their love and support throughout the years.