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Submission Deadline for the Fall 2012 issue November 30, 2012

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The Effect of Temperature *on Balanus amphitrite* Feeding Rate

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Abstract

This experiment examined the relationship between feeding rate and temperature in *Balanus amphitrite* barnacles. It was found that increased temperatures, above the control room temperature of 25-26 °C, led to an increase in feeding rate, while conversely decreased temperatures below the control temperature led to a decrease in feeding rate. This data can be used to determine at what time of day and during which seasons the barnacles are most active or have the highest rate of metabolic processes.

Introduction

Barnacles, along with many other intertidal species, are subjected to a wide range of temperatures throughout the year and even throughout the day. How do these varied temperatures affect the wellbeing and physiology of organisms such as barnacles? According to the Q_{10} thermal coefficient equation, metabolic rate and chemical reactions can double or even triple for every increase of 10 °C (Wiki,Q10). It can thus be hypothesized that the feeding rate of barnacles will increase significantly to accommodate this increase in metabolic rate. Barnacles, particularly *Balanus sp*., feed using a set of 6 pairs of biramous cirri that form a scoop that flick through the water at a constant rate (Barnes et al., 2004). This experiment will examine cirri scoop rate at four temperatures: 20, 25, 30, and 35 °C. Barnacles were collected off the dock pylons of Piver's Island in North Carolina.

Materials

The temperature control experiments were performed in a 2.5 gallon glass aquarium. Water was circulated and mixed by a small powerhead pump, heated by a 200 watt heater and chilled by a freezer in the classroom. Temperature was monitored by a digital probe. The barnacles were stored in a glass bowl with fresh running seawater continuously being pumped into their enclosure. A stopwatch was used to time feeding intervals.

Methods

This experiment was conducted using a control group (at room temperature, 25°C) and three thermal variants (20, 30 and 35 °C). These temperatures were chosen arbitrarily because they are nice, round numbers. This setup was repeated three times to form three full replicates, for a total of 12 trials. Each trial was conducted in the 2.5 gallon aquarium, using fresh seawater each time. The barnacles (approximately 15 to 20 individuals), were selected arbitrarily

from the common holding tank, transferred to the experiment tank and lined up in a row, so that the before and after feeding rates could be compared. Each trial group was given 5 to 10 minutes to adjust to the new tank before feeding rates were measured over 30 second intervals. The temperature was then raised, lowered or kept the same (in the case of the control) and the barnacles were given another 5 to 10 minute acclimation period. The feeding rate was again measured in 30 second intervals. Each trial consisted of a newly selected batch of individuals. If a barnacle closed up prematurely due to external movement or some other factor, the interval was repeated until there was a constant rate achieved.

Results

. The data gathered in this experiment, presented in Figure 1, showed a positive correlation between temperature and feeding rate. As temperature increased above the control group, feeding rate increased and as temperature decreased below the control feeding rate decreased.

A linear regression was performed (slope=3.798 ± 0.6116, R^2=0.7941,$F_{1,10} = 38.57$, p<.0001) and shows that there is a moderately strong correlation between temperature and cirri beat rate.

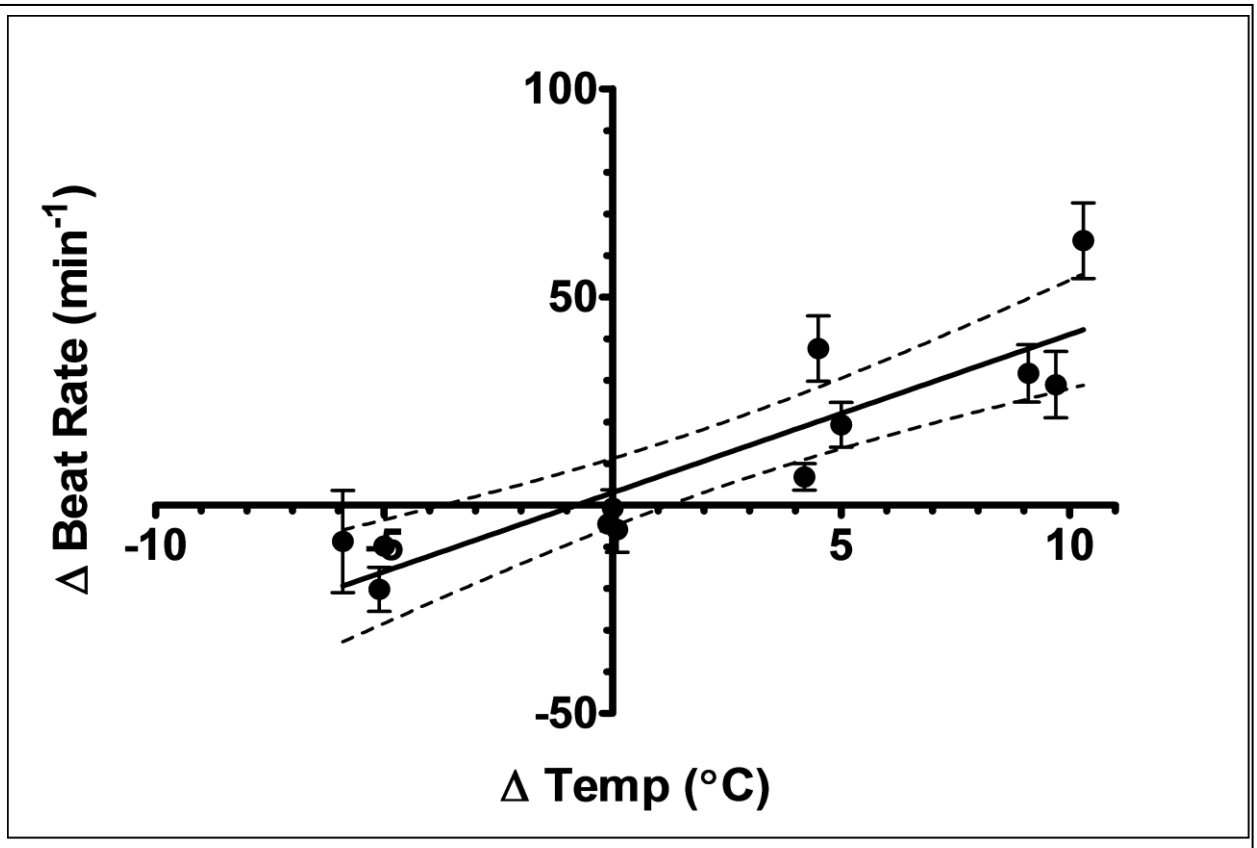

Figure 1. “Cirri Rate vs. Temperature Difference between Trials”. Standard deviation bars have been added above and below data points. The solid, black line is the linear regression line with 95% error markers represented as dashed lines surrounding it.

The extremity of the feeding rate change altered throughout the replicates in a manner that suggests other factors besides temperature were involved. The Q_{10} value was calculated, shown in Table 1, and was found to be approximately 1.655.

Temperature	Predicted Q_{10}(Avg of 3 Replicate Values)
20°C	1.471
30°C	1.878
35°C	1.616
Average	1.655

-Table 1. The Q_{10} values calculated from averaging three replicates into single values. The individual $Q_{10}'s$ were averaged to form a final value.

Throughout this experiment it was observed that the smaller, younger barnacles beat their cirri at a faster initial rate than older, larger barnacles. This led to the smaller barnacles having a more dramatic slowdown under cooler temperatures and larger barnacles having a greater speed up under higher temperatures.

Discussion

The collected data shows that there is a positive correlation between temperature and feeding rate in the *Balanus amphitrite*. There is an approximately 4 beat per minute gain in cirri beating per degree Celsius. The definite reason for this increase was not studied but it can presumably be attributed partly to the concept of the Q10 thermal coefficient and that higher temperature leads to a higher metabolic rate. This finding of the correlation between cirri beat rate and temperature can be supported by another study completed in 1955 by A.J. Southward who details similar findings as shown here (Southward, 1955, 1957). Southward studied several species of Northern Atlantic and English barnacles, including some other *Balanus sp*., but all had lower heat tolerance than Balanus … so no direct comparisons can be made. Southward claims the principle of Q_{10} as being the driver for increased cirri beat rate and claims values of 2 up to 5. These are higher values than presented here but they relate to other barnacles species and not these in particular.

The observation that size and age affect responsiveness to temperature and cirri beat rate was not significantly studied in this experiment but it was investigated by A.J Southward in 1957 who confirmed that age, and thus size and initial beat rate, in addition to temperature have a large impact on feeding rate (Southward,1957). The thermal threshold for death was not reached either on the low or the high end of the temperature extremes, showing that *Balanus amphitrite* is well adapted for a wide variety of temperatures and is perfectly suited for many areas along the US east coast.

The error in this experimented was added through variance in barnacles feeding rate due to factors other than temperature. Increased sample size would decrease this error dramatically. In addition doing several before and after trials of the same barnacles under the same conditions would reduce error. There was also marginal human error in timing and counting barnacle cirri scoops. This could be solved by having multiple people timing and counting at the same time. A final source of error was acclimation of the barnacles to laboratory settings which could have altered their thermal tolerance and normal cirri beat rate. This can be avoided by testing the barnacles immediately after capture.

Conclusion

Temperature and cirri beat rate have been definitively shown to correlate with one another. Further investigations should look into the responsiveness of small vs. large barnacles and the effects of other variables such as food supply, light and other water parameters such as salinity and pH on feeding rate.

Acknowledgements

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Citations

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DETERMINATION OF TEMPERATURE CALIBRATION FOR BENTHIC FORAMINIFERA: METHODS

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Key Words: *Ammonia beccarii*, benthic foraminifera, culturing, paleoclimate reconstructions, Mg/Ca ratio

ABSTRACT

We describe a method to culture *Ammonia beccarii,* a species of benthic foraminifera, at different temperatures in order to determine the relationship between the water temperature and the magnesium content of the proliferation test. The Mg content of the calcium carbonate tests reflects the water temperature when the organisms were living. We have records of foraminiferal Mg for the species *Ammonia beccarii* in several sediment cores from Tampa Bay, FL, but there is no temperature calibration for this species. Culturing this species in a laboratory at different temperatures will allow the determination of this relationship. Once a temperature calibration has been established, it can be applied to *Ammonia beccarii* found in sediment cores to constrain past seawater temperatures and the corresponding climatic changes.

INTRODUCTION

In order to predict future changes in climate, it is helpful to understand the magnitude and duration of past climatic changes. Global temperatures during the Holocene were relatively constant, but regional fluctuations occurred (Ruddiman 2007). The long-term objective of our work is to identify past climate changes in central Florida using a series of sediment cores from Tampa Bay.

There are many proxies for paleoclimate reconstructions including pollen, ice cores, tree rings, coral, and marine calcifying organisms (Ruddiman 2007). Foraminifera are calcareous marine organisms that are often used as a paleoceanographic proxy for the temperature and salinity of the water in which they lived. The chemical composition of foraminiferal tests reflects past environmental conditions. The ratio of magnesium to calcium content (Mg/Ca) of their calcium carbonate test indicates water temperature when the organism was living (e.g., Nürnberg et al. 1996, Hastings et al. 1998, Lea et al. 1999, Martin et al. 2002, Russel et al. 2004, Barker et al. 2005, Toyofuku et al. 2005, Marchitto et al. 2007). Foraminifera make their

tests from carbonate (CO_3^{2-}) and calcium (Ca^{2+}) in seawater to form calcium carbonate ($CaCO_3$). Occasionally Mg^{2+} substitutes for Ca^{2+} in the calcite lattice. This happens more readily as the temperature of surrounding water increases.

Foraminiferal Mg has been described as a function of seawater temperature for some species of benthic foraminifera (Martin et al. 2002, Toyofuku et al. 2005, Marchitto et al. 2008, Filipsson et al. 2010). The relationship determined by Martin et al. (2002) for *Cibicidoides* spp. is:

$$Mg/Ca = 1.22.e^{0.109*BWT}$$

The purpose of our methods is to determine such an equation for *A. beccarii*.

Tampa Bay is an estuary centrally located on the west coast of Florida. A paleoreconstruction in this area could provide a valuable description for the past climate of central Florida. Records of foraminiferal Mg/Ca for several Tampa Bay cores exist, and ages of these samples were determined by radiocarbon dating (Hoover 1998). However, there is not a temperature calibration for *A. beccarii*, a species commonly found in these estuarine sediments. Here we describe a method to culture *A. beccarii*, in the laboratory in order to develop a temperature calibration.

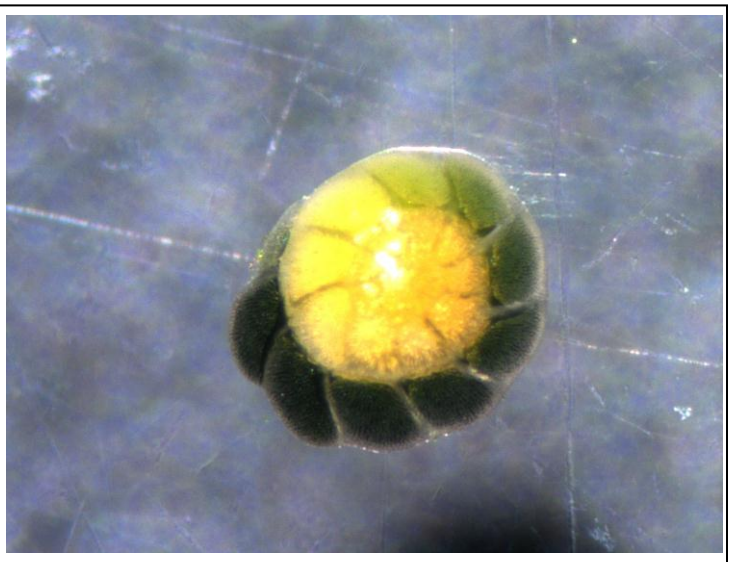


Figure 1. *Ammonia beccarii*. Photo taken by Michael Martinez, used with permission.

Ammonia beccarii is a benthic species of foraminifera generally found in shallow brackish areas (Figure 1). This species would be very useful in describing past temperatures because it has such as broad range.

It is commonly found along both coasts of the North Atlantic, as well as the west coast of Florida and the coasts of New Zealand, Japan, and Australia (Brooks 1967, Moodley and Hess 1992, Hayward et al. 1999). The species is important to the structure of benthic communities (Chandler 1998). *A. beccarii* is known to survive in a wide range of salinities, temperatures, and oxygen levels (Bradshaw 1957, Chandler 1989, Chandler et al. 1996). This should make *A. beccarii* relatively easy to culture compared to foraminiferal species with narrower temperature and salinity ranges. *A. beccarii* has been cultured in a controlled laboratory setting at multiple temperatures (Chandler 1989, Chandler et al. 1996), but Mg was not analyzed. A temperature calibration for *A. beccarii* would allow researchers to describe the climate histories of coastal regions where this species thrives.

Mg/Ca temperature calibrations have been developed for other species of benthic foraminifera by culturing in a laboratory setting (Hintz et al. 2004, Toyofuku et al. 2005, Dissard et al. 2010, Filipsson et al. 2010). Hintz et al. (2004) developed methods for culturing several deep sea benthic foraminifera species in small culture chambers under controlled conditions. Filipsson et al. (2010) also cultured many deep sea benthic foraminiferal species for five months at four temperatures to measure the relationships between temperature, $\delta^{18}O$, and Mg/Ca. Reproduction occurred in both studies (Hintz et al. 2004, Filipsson et al. 2004).

Toyofuku et al. (2005) also cultured a benthic foraminiferal species, *Planoglabratella,* at different temperatures using petri dishes in three temperature-controlled incubators. Using an electron probe micro-analyzer (EPMA), Toyofuku et al. (2005) found significant intrachamber heterogeniety. Dissard et al. (2010) cultured *Ammonia tepida*, a member of the same genus, under controlled conditions at multiple temperatures, salinities, and CO_2 concentrations.

Culturing foraminifera has additional ecological and paleoceanographic applications. For example, culturing can be used to study the effect of pollutants (Le Cadrea and Debenay 2006) and ocean acidification (Dissard et al. 2009). Hoover's undergraduate thesis (2008) describes climate in the Tampa Bay area during the Holocene, the time period from approximately 12,000 years before present to today. $\delta^{18}O$ and Mg/Ca data from *A. beccarii* in cores taken in Tampa Bay were used to constrain climate changes during the Holocene. However, a lack of species specific temperature calibration did not allow the Mg data to be fully interpreted. Hoover (2008) calls for a Mg/Ca temperature calibration specifically for *A. beccarii*, which is what these methods aim to do.

METHODS

There is no common laboratory protocol for culturing benthic foraminifera today. Therefore, flow rate, sediment type and thickness, experiment length, and feeding procedures can vary widely between laboratories. Our protocol was designed using several studies as guidance, (i.e. Chandler et al. 1996, Hintz et al. 2004, and Filipsson et al. 2010).

Field sampling

When sampling in the field for foraminifera to use in future culture experiments, it is best to collect the top 1 cm of sediment (Colburn and Baskin 1998); a simple turkey baster can be used. Samples should initially be stored in a non-metal container covered with at least an inch of seawater. It is best to sieve out fine material, then allow the sample to settle for at least 24 hours before picking out live specimens. Colored cytoplasm (usually green or brown), shiny tests, and a collection of fine materials surrounding the test are signs of vitality (Martinez, personal communication), which is later confirmed by the use of a live stain.

Using a live stain

It is important that only calcium carbonate secreted under the controlled experimental conditions is analyzed for Mg. New growth can be visually separated using a fluorescent live stain, such as calcein, that is incorporated into the test when new calcium carbonate is secreted. Calcein has been successfully used in several studies as a live foraminiferal marker (McCorkle et al. 2008, Filipsson et al. 2010). Chambers precipitated while in calcein-containing seawater appear green under an epifluorescent microscope (Bernhard et al. 2004). This proves that growth is occurring and allows the viewer to visually separate new calcite. Calcein has no effect on calcification, survival, or reproduction (Bernhard et al. 2004). Methods of calcein incubation with *A. beccarii* are described by Bernhard et al. (2004). CellTracker Green has also been successfully used in foraminiferal studies (Hintz et al. 2004, Bernhard et al. 2006). The use of a live stain is important because only living and growing individuals can be included in the experiment.

Stock culture

Stock cultures are set up to maintain live specimens and allow reproduction in order to obtain a sufficient sample size for use in the temperature experiment. An approximately 4 cm thick layer of cleaned, autoclaved, and homogenized natural mud is used (Chandler et al. 1996). Feeding is done weekly (see feeding section for details). Live mature *A. beccarii*, defined as having 15 or more chambers (Chandler 1996), are be picked out of stock-culture for use in the culture experiment.

Calcite analysis

There are several options for physically isolating newly secreted calcium carbonate for analysis. The first requires reproduction to occur during the controlled temperature experiment. If this occurs, only the second generation (completely unstained) individuals are analyzed at the experiment's completion. The second option requires laser ablation ICP-MS which uses a laser beam to ablate very small solid particles for analysis. This allows the newly precipitated chambers on a test to be targeted. Alternatively, old chambers can be physically separated from the newly precipitated chambers with a scalpel. The first method is preferred as the second magnifies intrachamber heterogeneity (Toyofuku et al. 2005). Dissard et al. (2010) used laser ablation for their culture experiments. Filipsson et al. (2010) used both methods for their multi-temperature culture experiments. A smaller starting sample size will be needed if laser ablation ICP-MS or EPMA is used because only one test is needed for each analysis.

Experimental culture set-up

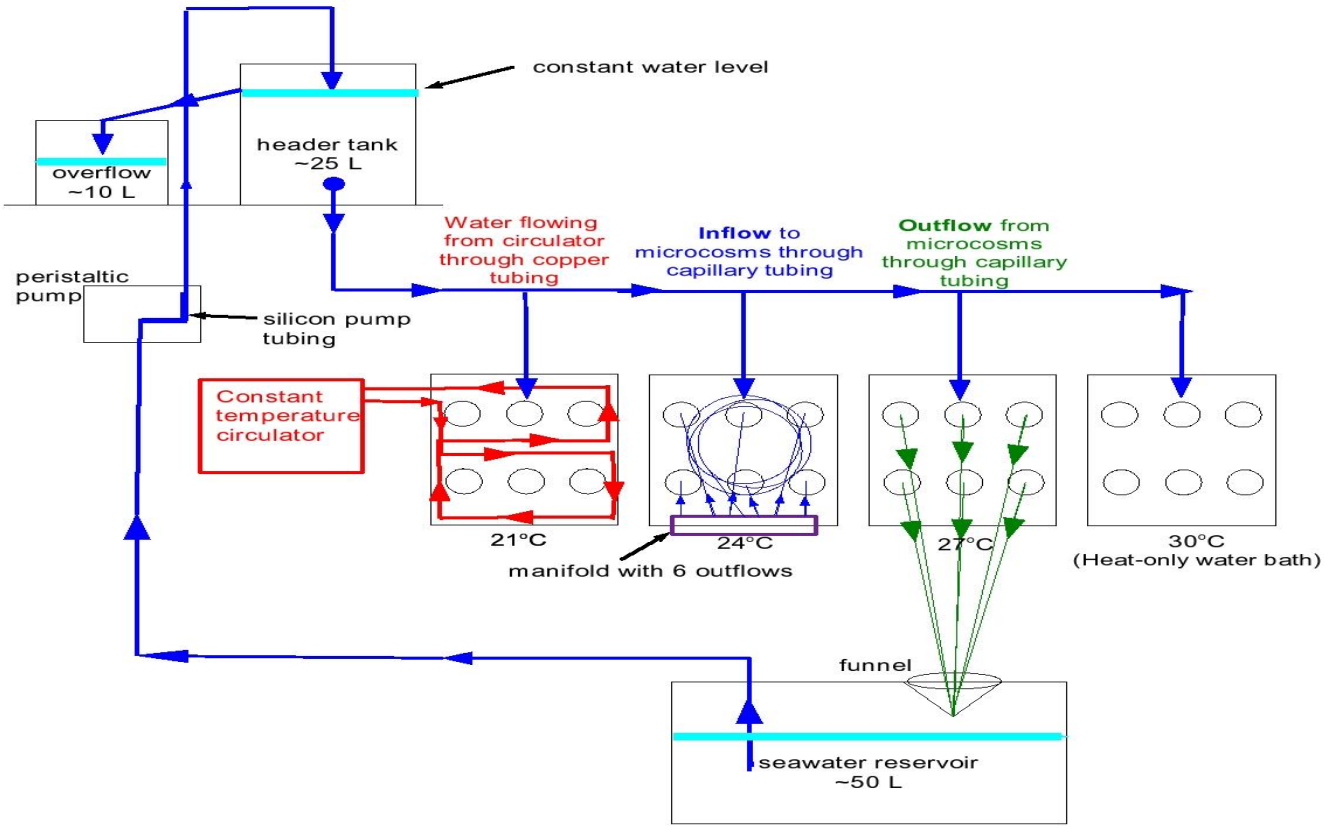
While some culture experiments have been conducted in darkness (Chandler et al. 1996), we aim to mimic a natural environment by using full spectrum light bulbs on a 12-hour timer during the experiment. The multi-temperature culture set-up (Figure 2) is as follows.

Seawater flows via gravity from a large header tank (~25 L) into four temperature controlled water baths, each containing six individual microcosms (Figure 3) holding the foraminifera, and then into a large central reservoir below (~50 L). The header tank is connected to an overflow so that its water level, and therefore the flow rate, is constant.

A peristaltic pump is used to adjust the flow to 3 mL/min (Chandler et al. 1996, Hintz et al. 2004) through each microcosm. Each set of six microcosms sits in a constant temperature bath set to 21°C, 24°C, 27°C, or 30°C. Seawater is brought to the correct temperature by running through a series of capillary tubing looping through each of the water baths before entering the microcosms.

Temperature of each water bath is maintained by constant temperature circulators. Salinity, pH/alkalinity, and flow rate must be carefully monitored throughout the experiment. Because this is a closed system, salinity variations are minimized. All parts exposed to the culture medium are metal-free and acid washed prior to the experiment to prevent Mg contamination.

Figure 2. Experimental culture design. All tubing segments in each step of the water's path (i.e. from manifold to the inflow of each microcosm) are the same length for each temperature and microcosm to prevent a "path of least resistance." Each color in a water bath is actually in each of the water baths, but separated for visual clarity.



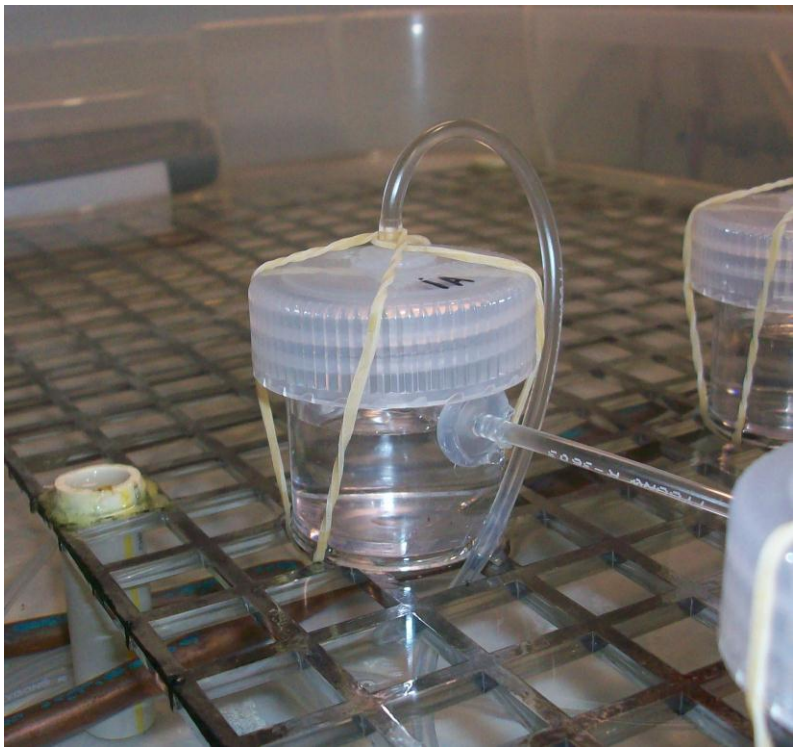


Figure 3. One of 24 microcosms with inflow from above and outflow leading to the seawater reservoir.

Salinity

Reproduction by *A. beccarii* has been observed at salinities ranging from 24 to 35 (Bradshaw 1957, Chandler 1989, Chandler et al. 1996). Bradshaw deems a salinity of 33.5 as part of the "optimum conditions" for *A. tepida*. Approximately 100 L seawater is collected and filtered for use in the culture experiment. While the experiment is running, salinity is checked daily and adjusted to a given salinity with deionized water as necessary.

Foraminiferal feeding

Feeding is done once a week (Chandler 1989, Chandler et al. 1996, Bernhard et al. 2004, Hintz et al. 2004, McCorkle et al. 2008, Filipsson et al. 2010). We provided each microcosm with 100 µL of ~$1x10^8$cells (Hintz et al. 2004) of *Isochrysis* sp. and *Dunaliella* sp. algae (Chandler et al. 2006, Filipsson et al. 2010). Flow is stopped prior to feeding and left off for an hour after feeding (Hintz et al. 2004, McCorkle et al. 2008). This ensures that algae do not flow out of microcosms before they can be consumed.

Temperature

We designed our experiment to be run at four temperatures: 21, 24, 27, and 30°C. The temperatures chosen were primarily based on the experiments of Chandler (1989), Chandler et al. (1996), and Bradshaw (1957). Temperatures were recorded continuously using HOBOware Pro temperature probes in each of the four water baths. In our laboratory at Eckerd College, room temperature varied significantly with season and time of day. It is critical to use a room with as little air temperature fluctuation as possible.

Sediment material

Although some (Chandler 1989, Chandler et al. 1996) used natural sediment (washed and autoclaved), Hintz et al. (2004) and Filipsson (2010) used clay-sized 99 % silica during experiments to minimize possible contamination and to facilitate identification of individual foraminifera at the completion of the experiment. Although sediment is not required to keep *A. beccarii* alive, it is required for reproduction (Chandler et al. 1996).

Experimental completion

In one study, it took 40 days for mature *A. beccarii* to reproduce at 25°C and 53 days at 20°C (Chandler et al. 1996). We suggest running the experiment for one month before testing to see if sufficient material exists for analysis and then at one week intervals thereafter. Hoover (2008) needed to analyze ~100 (most likely mature) *A. beccarii* tests to ensure a mass of at least 300 µg. At the completion of the experiment, all unstained individuals are picked out and cleaned prior to Mg/Ca analysis.

DISCUSSION

Our controlled temperature culture experiment was not carried out due to difficulties in obtaining adequate numbers of live foraminifera. A location for collecting marine sediments containing a sufficient sample size of A. beccarii has yet to be identified in Tampa Bay. A. beccarii's habitat has been described as "mudflat" (Chandler 1989), "low-energy" (Colburn and Baskin 1998), "sand flats with sea grass" (Colburn and Baskin 1998), "fine-sediment" (Chandler et al. 1996), and "intertidal flat" (Dissard et al. 2010). Sediment was only

collected in summer, and it is possible that the population of this species decreases then due to increased water temperatures, often >30oC, which may limit reproduction. Chandler (1989) noted that A. beccarii usually forms dense patches 2-4 cm^2 across. This was never observed in our collected sediments or attempted stock culture, leading us to believe that we did not find a suitable habitat

Filipsson et al. (2010) cultured benthic foraminifera using very similar methods, but did not use *A. beccarii.* A different species was cultured for five months at four different temperatures to measure the relationships between temperature, $\delta^{18}O$, and Mg/Ca. However, this study had some limitations. Salinity increased by 0.8 over the course of the experiment, while temperatures fluctuated by as much as 1°C. This is of special concern because temperature and salinity are two primary controls on Mg/Ca. Increasing salinity can be reduced by measuring salinity daily and diluting the seawater reservoir with deionized water as necessary. The loops of capillary tubing that the water travels though before entering the microcosms should reduce temperature variation; however, a "foraminifera free" pre-experiment is run to make sure temperatures and water chemistry in the microcosms are constant.

Bradshaw (1957) observed the growth and asexual reproduction of *Ammonia tepida*. He found that offspring emerge at two chambers and stay close to the parent for a few hours, then slowly start to move away. Growth was fastest, approximately one chamber per day, at "optimal growth conditions," which he defined as 24-30°C and a salinity of 33.5. For *A. tepida*, asexual reproduction occurred after an individual reached "critical size", which he defined as 13 chambers, and the parent died shortly after. Bradshaw (1957) observed *A. tepida*. producing every 23 days on average with a maximum of 43 days. The average number of offspring produced ranged from 28 to 32 individuals.

In Bradshaw's experiments (1957), "normal" growth and reproduction occurred at temperatures ranging from 10°C to 35°C and salinities from 20 to 40. Growth was fastest (approximately one chamber per day) and reproduction occurred earliest (after 15 days) at 30°C. The temperature range at which *A. beccarii* is able to calcify is much larger than the range at which it is able to reproduce (Bradshaw 1957). In general, the growth rate (number of chambers added per day) increased with increasing temperature. However, chambers added at warmer temperatures were generally smaller. Growth rate was similar at all salinities, and reproduction occurred earliest (after around 13 days) at a salinity of 40. This implies that salinity stress can induce reproduction, which is consistent with what we observed, but should be avoided to ensure the survival and growth of newborns.

CONCLUSION

We have developed a system that allows us to culture benthic foraminifera *A. beccarii* at various temperatures and constant salinity. Future efforts should focus on locating a reliable sample collection location in order to obtain a sufficient number of *A. beccarii* to run this experiment. The effects of temperature on Mg content of *A. beccarii* can be determined by culturing specimens at multiple temperatures using a combination of culturing techniques from several studies. Once the quantitative relationship between calcification temperature and Mg content is determined, it can be used to constrain temperatures

from the Mg/Ca ratios of the previously obtained *Ammonia beccarii* Holocene core. Wherever cores containing *A. beccarii* are available, this calibration could be used to describe past climate changes in their location.

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Research Program.

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Grazing of Bermudian Herbivores

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The objective of this study was to determine the abundance and grazing rates of several species of common herbivores on Bermuda's coral reefs. Herbivores are important in that they control macro algae populations allowing for coral's to recruit and sustain future reef growth. The coral reefs are important for fishing, recreation and to keep the island from degrading. Thus it is important to know which species are the most abundant and important grazers so that they can be protected from fishing and other adverse effects. This experiment was carried out by six buddy teams that counted fish abundance along a 30 m^2 transect and then followed predetermined species while counting bites per minute for up to ten minutes. Acts of aggression and other notable events were also recorded. Data from the 30 meter transects shows a fairly even distribution between species and sexes. It was determined that parrotfish, particularly the Queen Parrotfish, dominate the reef as they have the most bites, most abundance and most acts of aggression (intraspecific and interspecific). An ANOVA showed a p value< .0000629 and a t-test showed that Queen Parrotfish had a significant greater feeding rate than all other species. The smaller blue tangs were found to have more communal behaviors, such as schooling, which aides in deterring or diffusing aggression by other territorial species. It can be concluded that in the Bermuda reef system that parrotfish have the largest impact on algae.

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Artemia franciscana as a Model System to Test for Pathogenicity of Environmental *Vibrio parahaemolyticus* Strains.

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Vibrio parahaemolyticus is a marine bacterium that causes gastroenteritis in humans when ingested in raw or undercooked seafood. Clinical strains, found in patients suffering from *V. parahaemolyticus*-induced gastroenteritis, typically contain one or more hemolysin genes that are uncommon or not found in strains of *V. parahaemolyticus* isolated from the environment. This has led to the assumption that hemolysin gene products (toxins) are essential to *Vibrio parahaemolyticus* pathogenicity. Genes and regulatory factors other than hemolysin genes may also be involved in pathogenicity, but little direct evidence for pathogenicity in the absence of toxin production is available. No rapid, reliable and low cost method for screening *Vibrio parahaemolyticus* strains for virulence has been available. This project employed *Artemia franciscana* (a brine shrimp) in a high throughput bioassay to screen environmental *Vibrio parahaemolyticus* strains for pathogenicity. *Vibrio* strains were collected from the pristine North Inlet salt marsh at the Belle W. Baruch Institute in 2010 and 2011. Sequences of the housekeeping gene Recombinase A (*recA*) were used to confirm that the environmental strains were *V. parahaemolyticus*. The strains were screened for three hemolysin genes and were then used to infect the brine shrimp. Our results show statistically significant rates of brine shrimp mortality among *Artemia franciscana* cultures treated with *V. parahaemolyticus* strains containing known hemolysin genes.