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Selective Filtration in the Tropical Marine Sponge Rhopaloeides Odorabile: Impacts of Elevated Seawater Temperature on Feeding Behavior

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SIT Study Abroad

Selective filtration in the tropical marine sponge *Rhopaloeides odorabile*: impacts of elevated seawater temperature on feeding behavior

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Abstract

Climate change currently represents the most significant and increasing threat to coral reef ecosystems worldwide as sea surface temperatures are predicted to increase by up to 4°C by the year 2099. Sponges that rely on strong microbial symbioses are particularly sensitive to elevations in seawater temperature. In this study, the impacts of elevated seawater temperature on feeding behavior in the tropical marine sponge *Rhopaloeides odorabile* were assessed. Sponges were exposed to temperatures ranging between 27 and 32°C. At four time points, filtration rate and volume flow rate of each sponge were measured, and feeding efficiencies on both heterotrophic and phototrophic bacteria were determined. No differences in volume flow rate or feeding efficiencies on both bacterial types were detected in 27 and 30°C treatments. In contrast, sponges exposed to 31°C exhibited significantly reduced volume flow rates and feeding efficiency on heterotrophic bacteria after 24 hours but maintained normal feeding efficiency on phototrophic bacteria through 3 days. Sponges exposed to 32°C exhibited major cellular necrosis and dramatically reduced volume flow rates and feeding efficiencies on both bacterial types after 24 hours. The threshold for normal sponge feeding behavior was 31°C, and the shift in feeding efficiencies at 31°C is clear evidence of selective filtration of phototrophic bacteria by *R. odorabile* in response to thermal stress. This thermal threshold is identical to the symbiosis threshold for corals and their zooxanthellae, indicating that sponges may be similarly threatened by climate change.

Keywords: sponge; temperature; feeding; flow rate; climate change

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1. Introduction

1.1 Sponge Biology

Sponges (Phylum Porifera) are the oldest and most primitive living group of metazoans on the planet (Colin and Arneson 1995). The diverse group of sessile organisms is classified into four classes: Calcarea, Hexactinellida, Demospongiae, and Sclerospongiae, with an estimated 15,000 species worldwide (Hooper and van Soest 2002). Sponges lack organs and true tissues, instead relying on a complex system of water canals and a suite of specialized mobile cells to carry out all bodily functions, including feeding, respiration, and reproduction (Simpson 1984) (Fig. 1). As efficient filter feeders, sponges filter and retain various bacteria, phytoplankton, and ultraplankton and often incorporate these microorganisms into their tissues as symbionts (Ribes et al 1999). Water is inhaled through tiny pores called ostia, filtered for food particles and oxygen in flagella-lined cavities called choanocyte chambers, and exhaled through larger pores called oscula (Webster 2007).

Despite their simplistic body plan, sponges exhibit a diverse range of morphologies, including encrusting, boring, foliose, massive, and branching forms (Barnes and Bell 2002). Hard skeletal support is provided by a network of hard crystalline spicules of calcium carbonate or glass, and the "spongy" skeleton is made up of collagen and spongin fibers (Colin and Arneson 1995). Sponges are important components of the benthic environment, contributing to benthic-pelagic coupling (Pile et al 1996), serving as crucial reef-building organisms by providing structural rigor (Wulff 1984), providing substrate for settlement (Barthel and Gutt 1992), and providing refuge for other marine organisms (Wulff 2006).

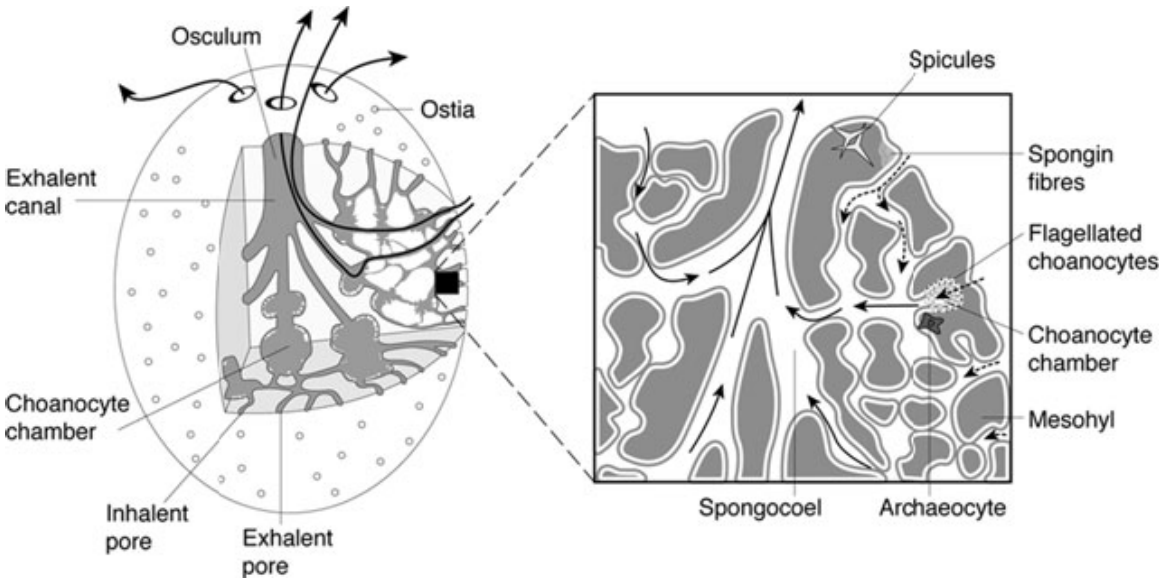

Figure 1 Cross section through a typical marine sponge showing general morphology and internal cell types (Webster 2007).

1.2 Sponge Distribution

Sponges occupy a diverse range of marine and freshwater habitats, from the polar waters of Antarctica to tropical reef systems around the world (Webster 2007). Marine sponges are widely distributed, from shallow temperate waters to tropical coral reefs to deep-sea polar benthic habitats (Hooper and Van Soest 2002). A study by Wilkinson and Cheshire (1989) examined the distribution patterns of the sponge community across the continental shelf in the central Great Barrier Reef. The study found that sponge biomass, abundance, and species richness increase with increasing depth and decrease with increasing distance from shore. Higher levels of increased turbulence and exposure to ultraviolet light are the major factors limiting sponge growth in shallow and offshore waters. Many sponges produce secondary UV-blocking compounds, enabling them to tolerate high levels of exposure to UV radiation (Wilkinson and Cheshire 1990). Sponges are more abundant and larger in size, on average, on inner-shelf reefs than offshore, which is largely due to higher levels of terrestrial nutrient run-off

and food availability in coastal habitats (Cleary et al 2005, Wilkinson and Cheshire 1989). On the Great Barrier Reef, Wilkinson and Cheshire (1989) recorded 88 and 90 sponge species on the inner- and middle-shelf respectively and only 75 and 65 species on the outer-shelf and in Coral Sea reefs.

1.3 Microbial Symbioses

Many sponges have developed close associations with microbial symbionts, which comprise 40-60% of total tissue volume in some species (Taylor et al 2007, Hentschel et al 2006). Sponge-microbe associations involve a diverse range of heterotrophic bacteria, cyanobacteria, zooxanthellae (*Symbiodinium* sp.), facultative anaerobes, and Archaea, and these symbioses are often important aspects of the host sponge's ecology and behavior (Webster and Hill 2001). Symbiotic microbes provide sponges with many benefits, including enhanced growth rates, UV protection, removal of toxic metabolic by-products, and defense against predators and pathogens (Hill 1996, Taylor et al 2007). One of the more important proposed benefits of these symbionts is nutritional requirements through the translocation of photosynthate from symbiotic cyanobacteria or zooxanthellae to host sponge (Wilkinson 1983). Weisz et al (In Press) showed this translocation in *Cliona varians forma varians*, a common tropical reef sponge in the Caribbean, using a stable isotope pulse-chase experiment. The study showed that unprocessed carbon is taken up from the water column by zooxanthellae, which then undergo photosynthesis and translocate fixated carbon to the host *C. v. f. varians*. In addition to zooxanthellae, symbiotic cyanobacteria often play a major role in contributing to the host sponges' nutrition, with the rate of carbon production being sufficient in some cases to provide over 100% of the combined carbon requirements of sponge and symbionts (Wilkinson 1983).

Since these microbial symbionts contribute significantly to the nutrition of the host sponge, it is likely that their presence, or absence for that matter, impacts host sponge metabolism and feeding behavior. A study by Weisz et al (2008) showed that sponges with low microbial abundance (LMA) had higher pumping rates than did sponges with high microbial abundance (HMA). In addition, using a novel method of measuring sponge feeding efficiency (Yahel et al 2005), A. Massaro et al (unpubl.) showed that an azooxanthellate (without zooxanthellae) sponge species in the Florida Keys had higher feeding efficiencies on three different bacterial types than did two zooxanthellate species from the same reef. Both studies hypothesized that these phenomena are due to that fact that HMA sponges are getting a significant portion of their diet directly from their symbiotic zooxanthellae, and as a result, these sponges do not need to spend as much energy pumping and feeding heterotrophically. Thus, the presence or absence of microbial symbionts has a significant influence on sponge metabolism and feeding efficiency.

1.4 Disease and Climate Change

Recent studies have shown a global increase in the prevalence of disease in marine organisms over the past several decades (Lafferty et al 2004). The role that environmental factors may have as disease-causing agents for marine organisms on the reef is a current topic of research, with factors such as anthropogenic pollution, nutrient enrichment, and introduced species all having been linked to marine diseases (Webster 2007). One of the primary factors in promoting disease outbreaks among marine organisms is global climate change (Webster 2007), which currently represents the most significant and increasing threat to coral reef ecosystems (Coker et al 2009). Models produced by the Intergovernmental Panel on Climate Change predict a 1.8-4^{o}C increase in global sea surface temperature by the year 2099 (IPCC 2007) (Fig. 2).

Marine microbial communities and associated organisms are particularly sensitive to elevations in seawater temperature (White et al 1991), as environmental stress compromises the physiological fitness of marine microorganisms and their invertebrate hosts (Webster 2007). Thermal stress provides conditions conductive to disease outbreaks by increasing the prevalence and virulence of pathogens, facilitating invasions of new pathogens, and reducing host resistance and resilience (Sutherland et al 2004).

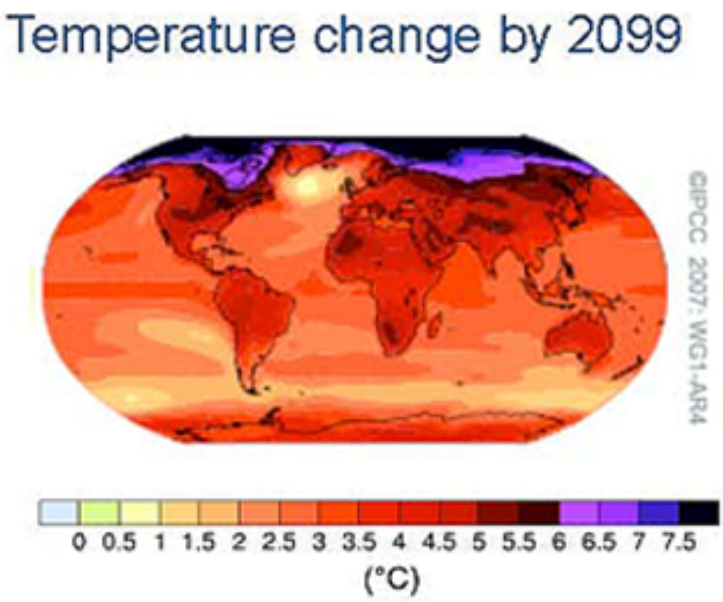

Figure 2 Predicted land and sea surface temperature changes by the year 2099 (IPCC Report *Climate Change 2007: The Physical Basis*).

Sponges are a particular group of marine organisms that is currently threatened by climate change because of their strong associations with microbial symbionts (Harvell et al 1999). In a previous study, sponges in the Mediterranean showed obvious signs of disease linked to a 2–4°C elevation above normal seawater temperature (Cerrano et al 2000). Without additional research and better means of conservation, global climate change will have a significant impact on the marine invertebrate community. A recent study by Webster et al (2008) showed that elevated seawater temperature is a current threat to the microbial communities of tropical reef sponges. Webster and coworkers investigated the seawater

temperature thresholds for bacterial symbiosis in the common Great Barrier Reef sponge *Rhopaloeides odorabile*. Webster and Hill (2001) had previously showed that the heterotrophic bacterial community associated with *R. odorabile* is dominated by a single α-Proteobacterium strain and the phototrophic bacterial community consists of a single cyanobacterium strain. The 2008 study found that sponges exposed to 33^{o}C, a 6^{o}C increase above ambient water temperature, exhibited a complete loss of these primary symbionts within 24 hours and severe cellular necrosis after 3 days. In addition, Bannister (2008) showed that the feeding behavior of *R. odorabile* may be impacted by environmental factors. His study found that high concentrations of clay sediments in the water column resulted in significantly reduced pumping rates and feeding efficiencies.

1.5 Justification for Study

Many marine organisms are very sensitive to changes in their environment, and elevated seawater temperature is clearly a current threat. Microorganisms are susceptible to very subtle changes in temperature (White et al 1991), making marine microbial communities and their host organisms particularly vulnerable. A. Massaro et al (unpubl.) showed that the presence of microbial symbionts has a significant impact on feeding efficiencies in host sponges, and Webster et al (2008) showed that elevated seawater temperatures have a drastic adverse impact on these microbial associations and lead to cellular necrosis in the host sponge. Thus, elevated seawater temperatures may have a significant impact on sponge feeding behavior. *R. odorabile* was chosen as a study species because it has been recently studied (Webster et al 2008, Bannister 2008), and it is common on the Great Barrier Reef (Bannister 2008). In addition, *R. odorabile* has shown potential for commercial aquaculture (Louden et al 2007), but little is known about its ecology and behavior. The knowledge gained from this study will help to determine optimum

sites for commercial aquaculture of *R. odorabile* as well as improve conservation and management strategies for protecting sponge communities worldwide from the adverse effects of climate change (Webster et al 2006, Louden et al 2007).

1.6 Study Aims

This study was a follow-up experiment on the work of A. Massaro et al (unpubl.) and Webster et al (2008), and the purpose was to investigate the impacts of elevated seawater temperature on feeding behavior in tropical sponges. The feeding efficiency of *R. odorabile* at four different seawater temperatures was investigated by measuring filtration and pumping rates over a 7 day exposure period. Methods of measuring and calculating feeding efficiency were based on Yahel et al (2005) and A. Massaro et al (unpubl.), and experimental water temperatures were determined based on the results of Webster et al (2008), which concluded that the temperature threshold for microbial symbiosis is between 31 and 33^{o}C. The findings of this study may further show that the tropical sponge community of the Great Barrier Reef and worldwide is currently threatened by global climate change.

2. Methods

2.1 Study Site

2.1.1 Collection Site

The collection site for organisms used in this study was Pelorus Island, North Queensland, Australia (18^{o}32.710'S, 146^{o}29.273'E). Pelorus Island is part of the coral fringed Palm Islands, located in Halifax Bay in the lagoon of the central section of the Great Barrier Reef Marine Park (Fig. 3a). The Palm Islands are approximately 16 kilometers from the coast near Lucinda and 25 kilometers from the nearest mid-shelf coral reef, located on a gently sloping continental shelf ranging from 20 meters on the coastal side to 32 meters on the seaward side.

Cloning was conducted in August 2009 and final collection was conducted in November 2009.

2.1.2 Experiment Site

The study was conducted at the Australian Institute for Marine Science (AIMS), Townsville, Australia. AIMS is Australia's leading tropical marine research agency, ideally located approximately 50 kilometers from the center of Townsville in a scientific zone surrounded by a 207 hectare national park and marine reserve (Fig. 3b). Sitting adjacent to the centre of the Great Barrier Reef, it is free from development, biosecure, and has access to clean seawater and a protected harbor. The experiment was conducted in an indoor temperature-controlled room at AIMS from November 18th, 2009 through December 5th, 2009.

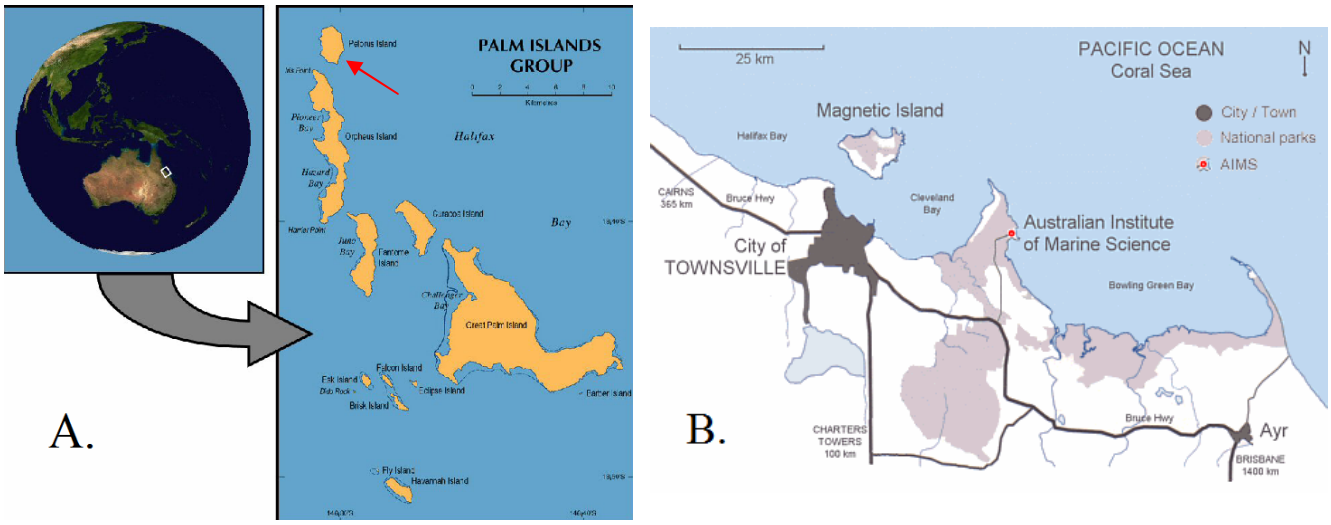

Figure 3 Location of **(A)** Pelorus Island (Webster et al 2006) and **(B)** The Australian Institute of Marine Science.

2.2 Study Species

Rhopaloeides odorabile is a dictyoceratid sponge in the family Spongiidae and is common throughout the Great Barrier Reef region (Webster and Hill 2001). It has a massive, amorphous morphology, with distinct oscula positioned on the dorsal ridge and is also

characterized by a red-brown surface pigmentation in illuminated habitats (Thompson et al 1987). The preferred habitats of *R. odorabile* are high-energy, oligotrophic environments (Wilkinson and Evans 1989), and its distribution includes inner-, mid-, and outer-shelf reefs, mostly at depths between 5 and 15 meters where strong wave induced turbulence is a regular feature (Bannister et al 2007). The species has a distinctive chemical composition, characterized by a rare group of C_{20} diterpenes, and substantial variation in the yield and composition of these chemicals for various collections of *R. odorabile* have been shown to reflect the range of environmental conditions under which they live (Thompson et al 1987). Assessments of both the reproductive (Whalan et al 2007) and microbial ecology (Webster and Hill 2001, Webster et al 2008) have also recently been undertaken for this species.

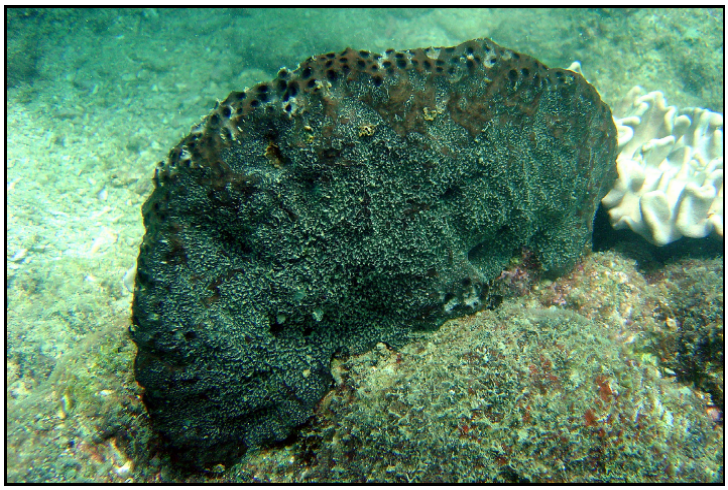

Figure 4 The sponge *Rhopaloeides odorabile* (Bannister 2008).

2.3 Collection and Cloning (Nicole Webster)

One large *R. odorabile* was collected by scuba at 15 meters from Pelorus Island. This donor sponge was cut into twelve individual clones using a sharp knife and then transferred to aquapurse racks that were secured to the reef base near the original collection site. Each sponge clone was approximately 50 cm^3 and contained one clear exhalent osculum. The clones were

allowed to heal on the reef for three months before collection and then placed in an outdoor seawater flow-through aquarium at 27°C water temperature for a one week acclimation period at the Australia Institute of Marine Science, Townsville.

2.4 Experimental Set-Up

The sponges were transferred from the outdoor aquarium to twelve 30 liter aquaria in an indoor temperature-controlled seawater flow-through system. One sponge was placed into each aquarium. Incoming seawater was filtered to 1 mm to remove large particulates yet provide the sponges with a sufficient nutritional supply in the form of small particulates and microorganisms. Sponges were be maintained under a diel cycle of 12:12 hours at light intensity reflecting 15 meters on the reef. The aquaria were evenly split into four groups, each representing a different seawater temperature (27°C, 30°C, 31°C, and 32°C), and placed in random order. Initially, water flowing into all aquaria was left at 27°C for a one week acclimation period. Temperatures were then raised gradually (0.2°C hr^{-1}) until reaching the final respective treatment levels.

2.5 Sampling

2.5.1 Inhalant and Exhalent Sampling

Four time points were used (0 days, 1 day, 3 days, and 7 days). The 0 day time point was sampled at the end of the one week acclimation period in the indoor aquaria, and the 1 day time point was sampled 24 hours after all tanks had reached their respective adjusted water temperatures. At each time point, one 10 ml inhalant water sample and one 10 ml exhalent water sample were collected from each of the three sponges at each temperature. Each inhalant sample was collected by placing the tip of a needle as close to the ostia of the sponge as possible without touching the sponge and slowly drawing up 10 ml of water into a syringe (Fig. 5). Each exhalent sample was collected by placing the tip of a needle as far into the osculum of the sponge as

possible without touching the sponge and slowly drawing up 10 ml of water into a syringe. Each 10 ml sample was fixed with 0.5 ml 37% formalin immediately after collection.

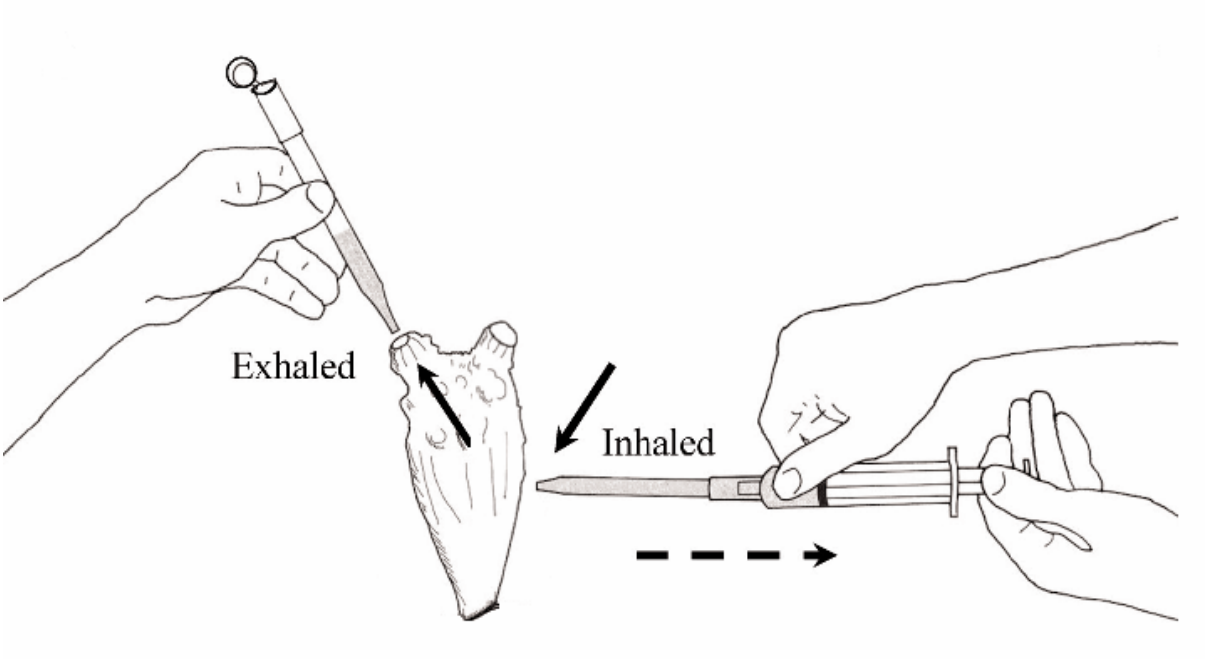

Figure 5 Schematic representation of inhalant and exhalent sampling techniques (modified from Yahel et al 2005).

2.5.2 Bacterial Cell Counts

Each inhalant and exhalent water sample was stained with 20 µl DAPI (2 µg μl^{-1}) for five minutes in the dark and then filtered onto a 25 mm black isopore membrane filter (0.22 µm) by vacuum filtration. When black filters were unavailable, identical white isopore membrane filters were stained using Irgalan Black. The white filters were soaked for two hours in a solution of Irgalan Black (0.002 g ml^{-1}) in 2% acetic acid and then rinsed in clean water and used immediately. Each filter was then viewed under an epifluorescent microscope. The total number of bacterial cells present in each sample was estimated under the DAPI filter, utilizing the fluorescence of stained DNA. Five fields of view were counted at 40x magnification for each sample. The total number of phototrophic bacteria was then estimated under the Cy3 filter,

utilizing the autofluorescence of chlorophyll *a*. Five fields of view were counted at 20x magnification for each sample. For each sponge, the mean number of phototrophic bacteria was halved and subtracted from the mean number of total bacterial cells to obtain the average total number of heterotrophic bacterial cells present in the sample. This calculation was done separately for each inhalant and exhalent sample.

2.5.3 Volume Flow Rate Measurements

At each time point, immediately after inhalant and exhalent samples were collected, the pumping rate of each of the three replicates at each temperature was measured. A clear plastic ruler was attached vertically to the inside of a 2000 ml beaker. Each sponge was transferred into the beaker, being careful to keep it completely submerged the entire time, and placed directly in front of the ruler with the oscular opening facing up, perpendicular to the ruler. Pumping rates were recorded on video, taping the movement of dye fronts in the excurrent plume. Using a syringe and needle, small 'puffs' of approximately 0.1 ml fluorescein dye (100 mg l^{-1}) were released directly into the osculum from which the exhalent feeding sample was collected. Video recordings of dye movement were analyzed, and the exact time it took for the top of the puff to travel from one mark on the ruler to another was recorded. The distance traveled was then divided by time to give a pumping rate (cm s^{-1}) for each sponge. The diameter of each oscular opening was also measured and recorded in cm. Volume flow rate (ml s^{-1}) was then calculated by multiplying the pumping rate by the cross-sectional area of the oscular opening.

2.6 Feeding Efficiency Calculations

Filtration rate on both heterotrophic and phototrophic bacteria of each sponge at each time point was calculated as [(Inhalant-Exhalent)/Inhalant] using the mean number of cells counted from each replicate. Feeding efficiency, or the amount of water the sponge can 100%

clear of bacteria per unit time, of each replicate at each time point was then computed by multiplying the filtration rate by the volume flow rate. The mean feeding efficiency on both heterotrophic and phototrophic bacteria for each temperature at each time point was calculated and recorded.

2.7 Data Analyses

Variability in volume flow rates and feeding efficiencies on both heterotrophic and phototrophic bacteria was assessed using a two-way analysis of variance (ANOVA) with time and temperature as the independent variables (Statistica 6.0; StatSoft Inc., Tulsa, OK USA). Post-hoc differences were examined with the Fisher LSD test.

3. Results

3.1 Temperature Treatment Observations

All but one 31°C sponge clone survived and remained visibly healthy (Fig. 6a) in each of the 7 day exposures at 27-31°C (the deceased clone was replaced for the 7 day exposure sampling). However, two clones exposed to 32°C exhibited minor surface necrosis (<10% surface area) after 24 hours, and one clone at 32°C exhibited major surface necrosis (50-70%) after 24 hours. All three clones at 32°C became covered in white mucus and exhibited major surface necrosis, revealing protrusions of skeletal fibers from the tissue and demonstrating significant stress after 3 days (Fig. 6b). All 32°C clones had died by 7 days.

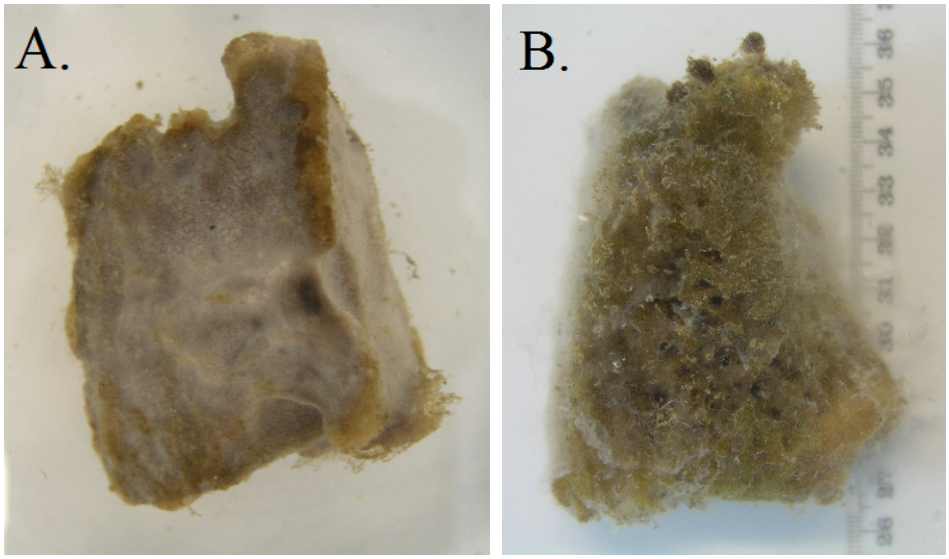

Figure 6 *R. odorabile* from T=3 days **(A)** showing healthy pinacoderm (outer layer) tissue at 27°C and **(B)** major surface necrosis and skeletal protrusion at 32°C.

3.2 Volume Flow Rates

Clones at 27°C had an average volume flow rate of 0.065±0.003 ml s^{-1} and clones at 30°C had an average volume flow rate of 0.064±0.003 ml s^{-1} throughout the 7 day exposure period. These clones exhibited no change (P>0.05) in volume flow rates over time (Fig. 7). Clones at 31°C exhibited a gradual reduction in volume flow rate throughout the 7 day exposure period, with slightly reduced (P>0.05) volume flow rates after 24 hours and 3 days and significantly lower (P<0.05) volume rates than those at 27-30°C after 7 days (see Appendix 1). Two of the 32°C clones stopped pumping completely and the third clone had a drastically reduced volume flow rate (mean = 0.031 ml s^{-1}) after 24 hours. All 32°C clones stopped pumping after 3 days.

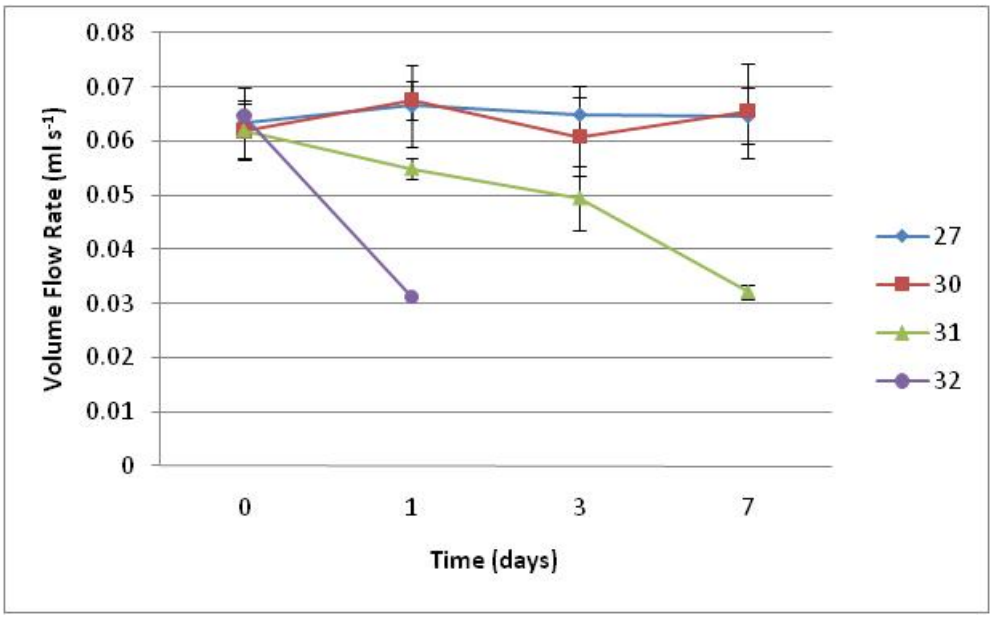

Figure 7 Mean volume flow rate (ml s^{-1}) from *R. odorabile* exposed to 27-32°C seawater over a 7 day trial. N=3 except for 31°C, T=3days (N=2) and 32°C, T=1day (N=1); bars represent ± s.e.

3.3 Feeding Efficiency

3.3.1 Heterotrophic Bacteria

Clones at 27°C had an average feeding efficiency on heterotrophic bacteria of 0.020±0.003 ml s^{-1} and clones at 30°C had an average feeding efficiency on heterotrophic

bacteria of 0.018±0.002 ml s^{-1} throughout the 7 day exposure period. These clones exhibited no change (P>0.05) in feeding efficiency on heterotrophic bacteria over time (Fig. 8). Clones at 31°C exhibited a gradual reduction in feeding efficiency on heterotrophic bacteria throughout the 7 day exposure period, with significantly lower (P<0.05) feeding efficiencies than those at 27-30°C after 24 hours (see Appendix 1). After 24 hours, the single pumping clone at 32°C had a drastically reduced feeding efficiency on heterotrophic bacteria (mean = 0.006 ml s^{-1}).

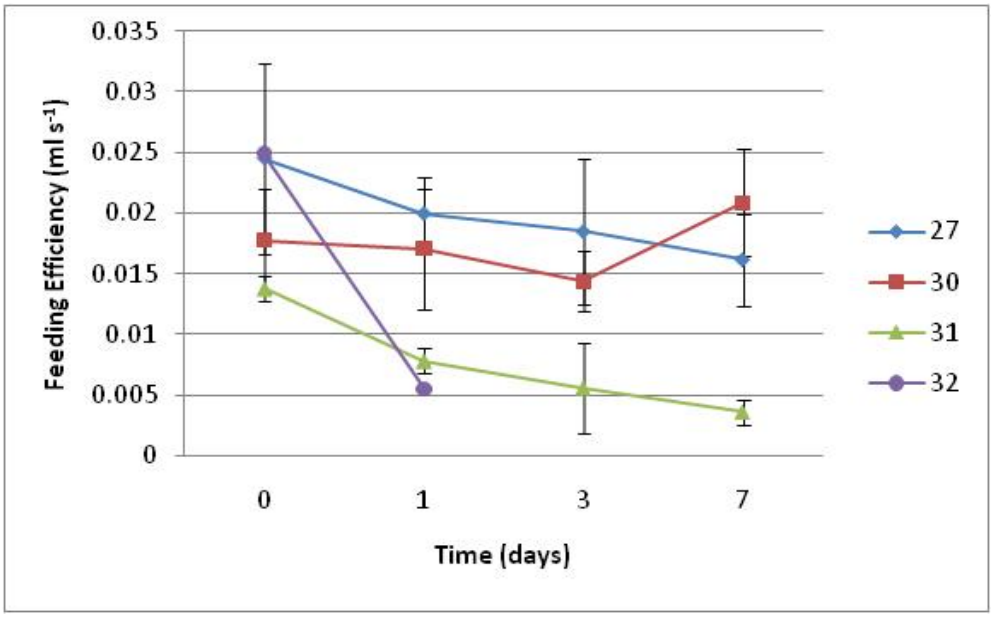

Figure 8 Mean feeding efficiency (ml s^{-1}) on heterotrophic bacteria from *R. odorabile* exposed to 27-32°C seawater over a 7 day trial. N=3 except for 31°C, T=3days (N=2) and 32°C, T=1day (N=1); bars represent ± s.e.

3.3.2 Phototrophic Bacteria

Clones at 27°C had an average feeding efficiency on phototrophic bacteria of 0.025±0.003 ml s^{-1} and clones at 30°C had an average feeding efficiency on phototrophic bacteria of 0.027±0.002 ml s^{-1} throughout the 7 day exposure period. These clones exhibited no change (P>0.05) in feeding efficiency on phototrophic bacteria over time (Fig. 9). Clones at 31°C had an average feeding efficiency on phototrophic bacteria of 0.023±0.002 ml s^{-1} with no

change (P>0.05) through 3 days with a significant reduction (P<0.05) to 0.015±0.001 ml s^{-1} after 7 days (see Appendix 1). After 24 hours, the single pumping clone at 32°C had a drastically reduced feeding efficiency on phototrophic bacteria (mean = 0.002 ml s^{-1}).

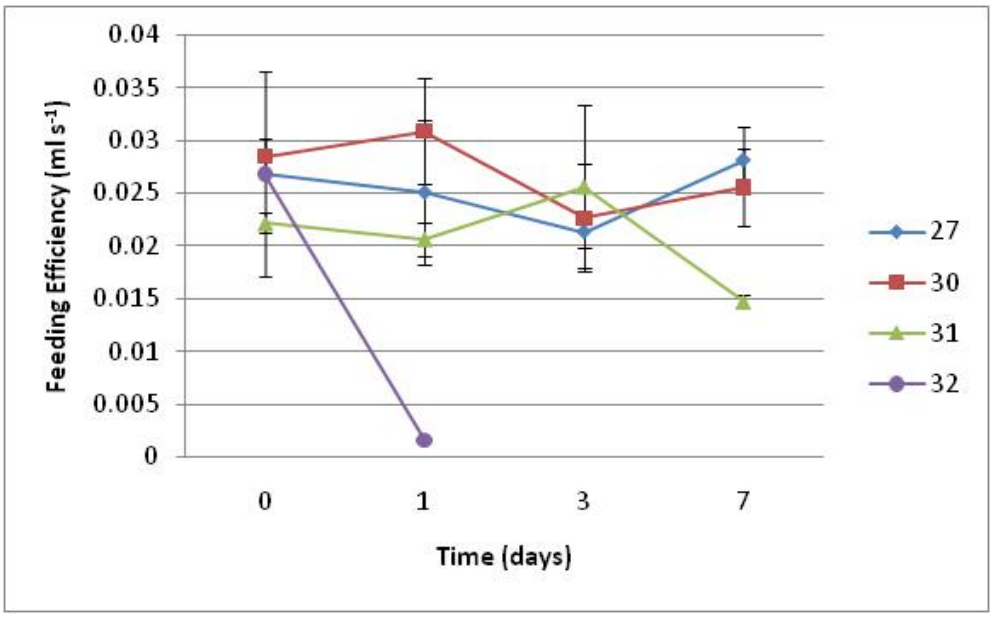

Figure 9 Mean feeding efficiency (ml s^{-1}) on phototrophic bacteria from *R. odorabile* exposed to 27-32°C seawater over a 7 day trial. N=3 except for 31°C, T=3days (N=2) and 32°C, T=1day (N=1); bars represent ± s.e.

4. Discussion

4.1 Summary of Results

This study revealed that the feeding behavior of the tropical reef sponge, *R. odorabile*, is significantly impacted by seawater temperatures of 31°C and 32°C, only 2-3°C above the mean summer water temperature at Orpheus Island, nearby Pelorus Island from where the sponges were collected (Berkelmans and Willis 1999). After 24 hours at 32°C, volume flow rate and feeding efficiencies on both heterotrophic and phototrophic bacteria were drastically reduced. Volume flow rate and feeding efficiency on heterotrophic bacteria at 31°C were significantly

reduced after 24 hours; however, feeding efficiency on phototrophic bacteria remained normal through 3 days and was not significantly reduced until 7 days.

4.2 Thermal Threshold for Microbial Symbiosis

The impact of temperature on feeding efficiency in *R. odorabile* coincides with the findings of a recent study that investigated the impact of temperature on the microbial community in *R. odorabile* with a similar setup to this study (Webster et al 2008). In the previous study, no differences in bacterial community composition or sponge health were detected in treatments between 27 and 31^{o}C. Sponges exposed to 33^{o}C, however, exhibited a complete loss of primary symbionts within 24 hours and major cellular necrosis after 3 days. These findings are consistent with the present study where sponges exposed to 32^{o}C exhibited a dramatic reduction in feeding efficiency after 24 hours and the complete inability to feed after 3 days. Furthermore, the previous study found that the thermal threshold for microbial symbiosis in *R. odorabile* was between 31 and 33^{o}C. Although no difference in bacterial community composition or sponge health was observed at 31^{o}C, this temperature is close to the thermal threshold and physiological impacts are still likely. The impact of 31^{o}C on feeding efficiency observed in the present study is likely an acute stress response to the water temperature nearing the thermal threshold.

4.3 Volume Flow Rate

Few studies have investigated the effect of seawater temperature on pumping rate in tropical sponges; however, one previous study found reduced volume flow rates due to thermal stress. Reiswig (1971) showed that pumping rates of *Mycale* sp. and *Verongia gigantea* were reduced by an average of 16.11% at water temperatures 3.5^{o}C lower than the average ambient temperature. Although this previous study did not investigate the effects of elevated water

temperatures, the findings coincide with the 4°C deviation from normal ambient temperature that caused a reduction in volume flow rate in the present study. Several other studies have shown that volume flow rate in sponges is reduced in response to other environmental factors, particularly increased concentrations of clay sediment (Gerrodette and Flechsig 1979, Bannister 2008).

4.4 Feeding Efficiency

To date, few studies have assessed the effects of seawater temperature on feeding behavior in marine sponges. A recent study found that the tropical marine sponge *Halichondria panicea* exhibited increased filtration rates of *Rhodomonas* sp. at seawater temperatures 5.5°C above the normal ambient temperature for these sponges (Riisgard et al 1993). The study proposed anatomical changes, particularly the dilation of inhalant canals and/or choanocyte chambers that may allow the sponges to filter more efficiently; however, they did not propose an ecological reason for the increased filtration rates. The study by Riisgard and coworkers only measured filtration rates of *Rhodomonas* sp., a phototrophic algae containing chlorophyll *a*. The present study found that filtration rate of phototrophic bacteria in *R. odorabile* increased (by definition, a constant feeding efficiency and reduced volume flow rate means increased filtration rate) in elevated seawater temperatures, which coincides with the findings of Riisgard et al. However, the present study also found that feeding efficiency on heterotrophic bacteria was reduced at elevated seawater temperatures.

4.5 Selective Filtration

This reduction in feeding efficiency on heterotrophic bacteria and the maintenance of normal feeding efficiency on phototrophic bacteria seen in *R. odorabile* at elevated seawater temperature is clear evidence of selective filtration. Previous studies have shown that

phototrophic cyanobacteria, *Synechococcus* sp., (470 fg C $cell^{-1}$) (Campbell et al 1994) are over twenty times as carbon-rich as are heterotrophic bacteria (20 fg C $cell^{-1}$) (Ducklow et al 1993). Thus, it is likely that when a sponge experiences thermal stress, it will selectively filter food particles, in this case phototrophic bacteria that provide it with more energy, rather than waste energy filtering less nutritional heterotrophic bacteria. Feeding efficiency on phototrophic bacteria at 31^{o}C was significantly reduced after 7 days, which most likely indicates that there is a time threshold between 3 and 7 days at which *R. odorabile* can no longer cope with the thermal stress and as a result, no longer has the ability to feed effectively.

Numerous studies have found that sponges exhibit selective filtration patterns. Stuart and Klumpp (1984) showed that the common encrusting demosponge sponge *Haliclona anonyma* exhibited effective food resource partitioning on the basis of particle size, selectively filtering smaller particles (100%) over larger particles (20%). More recently, Yahel et al (2006) showed that two common glass sponges, *Rhabdocalyptus dawsoni* and *Aphrocallistes vastus*, selectively filtered photosynthetic eukaryotic algae (86±9%) over heterotrophic bacteria (28±16%) when abundance of both was high. These findings coincide with the selective filtration of photosynthetic bacteria over heterotrophic bacteria exhibited by *R. odorabile* under thermal stress in the present study.

4.6 Other Considerations

The vast majority of previous works investigating the behavioral ecology of sponges have been conducted *in situ* in order to study the behavior of the sponge of interest in its most natural setting. Many sponge species are very sensitive to laboratory conditions and may exhibit a variety of stress responses when removed from their natural habitat (Riisgard et al 1993). Thus, behavior in laboratory settings may be deviated from natural behavior. The present study was

conducted in a temperature-controlled laboratory, and thus, the feeding behavior of *R. odorabile* may not accurately reflect its feeding behavior in its natural habitat. It is important to note, however, that the results of this study were completely dependent on differences observed between temperatures and over time. Thus, since sampling techniques were consistently applied to all replicates throughout the 7 day exposure period, the effects of temperature on feeding behavior observed in this study are valid.

5. Conclusion and Future Directions

The thermal threshold for normal feeding behavior in *R. odorabile* was 31^{o}C, a 4^{o}C elevation above average ambient seawater temperature for this species. Once this threshold was reached, sponges exhibited reduced volume flow rate and clear selective filtration for phototrophic bacteria, which is more nutritional to the sponge than heterotrophic bacteria (Campbell et al 1994, Ducklow et al 1993). The thermal threshold for effective feeding and pumping in *R. odorabile* is identical to the symbiosis threshold for corals and their zooxanthellae at the same location (Berkelmans and Willis 1999). As sea surface temperatures are predicted to increase by up to 4^{o}C by the year 2099 (IPCC 2007), these findings indicate that sponges may have a similar vulnerability as corals to changes in seawater temperatures.

Further research is required to assess the extent of the impact of elevated seawater temperature on feeding behavior in tropical sponges. An additional study to investigate damage to choanocyte chambers in *R. odorabile* in response to elevated seawater temperature will be conducted in the near future. Tissue samples from *R. odorabile* exposed to the same water temperatures as in this study will be analyzed under a light microscope in order to assess and quantify disruption of choanocyte chambers. The results should provide evidence of the

physiological impacts of temperature on the cells involved in sponge feeding, further supporting the impacts observed in the present study.

In addition, further studies should be conducted to assess the role of microbial symbiosis in sponge feeding behavior and the effects of seawater temperature on this association. Studies have shown that LMA sponges have higher volume flow rates than do HMA sponges (Weisz et al 2008) and that azooxanthellate sponges have higher feeding efficiencies for various bacterial types than do zooxanthellate sponges (A. Massaro et al, unpubl.). Since Webster et al (2008) found a complete shift of sponge-microbe associations at elevated seawater temperatures, the impact of temperature on feeding behavior may be different in sponges with different levels of microbial abundances. Future studies investigating the differences between the impacts of elevated seawater temperature on LMA and HMA sponges as well as azooxanthellate and zooxanthellate sponges will provide a greater understanding of the influence that symbiotic microbial communities have on marine sponge feeding behavior.

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Appendix 1

Table A1 Summary of two-factor ANOVA Fisher LSD test comparing the mean volume flow rates across time (T=0-7days) and temperature (27-31^{o}C). Columns 1 and 2 represent significantly different homogenous groups (P<0.05).

Time (days)	Temp (^{o}C)	Mean Volume Flow Rate (ml s^{-1})	1	2
7	31	0.032177		****
3	31	0.049537	****	****
1	31	0.054956	****	
3	30	0.060878	****	
0	30	0.061954	****	
0	31	0.062029	****	
0	27	0.063382	****	
7	27	0.064661	****	
3	27	0.064987	****	
7	30	0.065534	****	
1	27	0.066524	****	
1	30	0.067616	****	

Table A2 Summary of two-factor ANOVA Fisher LSD test comparing the mean feeding efficiencies of heterotrophic bacteria across time (T=0-7days) and temperature (27-31^{o}C). Columns 1, 2, and 3 represent significantly different homogenous groups (P<0.05).

Time (days)	Temp (^{o}C)	Mean Feeding Efficiency (ml s^{-1})	1	2	3
7	31	0.003587			****
3	31	0.005568		****	****
1	31	0.007819		****	****
0	31	0.013816	****	****	****
3	30	0.014382	****	****	****
7	27	0.016163	****	****	
1	30	0.017074	****	****	
0	30	0.017766	****	****	
3	27	0.018497	****	****	
1	27	0.019978	****		
7	30	0.020857	****		
0	27	0.024521	****		

Table A3 Summary of two-factor ANOVA Fisher LSD test comparing the mean feeding efficiencies of phototrophic bacteria across time (T=0-7days) and temperature (27-31^{o}C). Columns 1 and 2 represent significantly different homogenous groups (P<0.05).

Time (days)	Temp (^{o}C)	Mean Feeding Efficiency (ml s^{-1})	1	2
7	31	0.014745		****
1	31	0.020672	****	****
3	27	0.021289	****	****
0	31	0.022153	****	****
3	30	0.022644	****	****
1	27	0.025026	****	****
7	30	0.025534	****	****
3	31	0.025624	****	****
0	27	0.026833	****	****
7	27	0.028097	****	****
0	30	0.028489	****	
1	30	0.030874	****	