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Sponge physiology: the effects of temperature on the regeneration and reaggregation of sponges (Haliclona reniera)

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Background. Increases in oceanic temperatures are expected to affect the cellular function of ecologically important organisms, such as sponges. Sponges are important to biodiversity, coral reef systems, benthic and spongiverous organisms, and the biomedical industry. Sponges can repair wounds (regeneration) and rebuild their body from separated cells (reaggregation). The rates of regeneration and reaggregation can serve as a proxy for cellular functions. These processes are important to sponge physiology, growth and competition in reef systems. This study will examine how temperature affects the regeneration and reaggregation of *Haliclona reniera.*

Methods. This study considered the effects of temperature on growth and reaggregation. Percent of reaggregation was measured in a range of temperatures (4-34˚C) for 15 minutes and analyzed using imageJ software. Regeneration rates of wounded sponges were measured in fluctuating (28-32˚C & 28-34˚C) and non-fluctuating (28˚C and 32˚C) oceanic temperatures. The depth and size of *H. reniera* was measured with transects.

Results. Through observing growth and aggregation rates in a variety of temperatures, this study showed that sponges exposed to average fluctuations at 1m (28-32˚C) had higher regeneration rates than those exposed to high fluctuations (28-34˚C) at 0.5m. Wounded sponges regenerated faster in higher temperatures (32˚C) than in lower temperatures (28˚C). Aggregation cells fit a temperature performance curve with a peak at 29.5˚C, or just above average oceanic temperatures (29˚C). *H. reniera* was most commonly found at depths of 1m.

Discussion. Although coral and other organisms may be greatly affected by oceanic warming, sponges may persist, depending on how oceanic temperature will fluctuate in the future. *H. reniera* repaired wounds faster in average temperature with normally occurring fluctuations and aggregated most frequently at temperatures slightly above average (29.5˚C). With IPCC predictions of increased oceanic temperatures and fluctuations, *H. reniera* may not have ideal oceanic conditions, but will still endure these conditions. With coral reefs affected by climate change conditions, many organisms may die off, perhaps transitioning coral reefs into sponge reefs.

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SPONGE PHYSIOLOGY: THE EFFECTS OF TEMPERATURE ON THE REGENERATION AND REAGGREGATION OF SPONGES (*HALICLONA RENIERA*)

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ABSTRACT:

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INTRODUCTION:

Changes in environmental conditions may affect the physiology and morphology of marine organisms. The IPPC (2007) predicts that oceanic temperatures will rise by 1-5˚C based on a range of scenarios for anthropogenic carbon emissions. Temperature changes affect many organisms at the cellular level. In bacteria, at lower temperatures, molecules move more slowly, which affects the movement of cells; on the other hand, at higher temperatures, proteins denature and cannot perform in normal cellular activities (Francis & Barlow, 1988; Zwietering *et al.*, 1990). Thus, there is an optimal temperature for cellular activity.

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Many organisms have the ability to regrow cells once old cells are damaged, destroyed, or removed (Bosch, 2007; Bryant & Fraser, 1988; Carlson, 2007; Sanchez, 2000) and temperature can impede or enhance this effectiveness of regrowth (Francis, 1988). Sponges are unique in their regenerative abilities, which is also important to their survival. While damaged, sponges are able to remain competitive in coral reefs (Jackson and Palumbi, 1979), repair vulnerable wounds that expose their matrix (Leys, 1998), and regain shapes optimal for feeding (Wulff, 2010). Additionally, the growth rates of sponge cells are sensitive to temperature, and for some species, the fastest growth rates occur during time periods with the warmest waters (Koopmans and Wijffels, 2008).

In addition to repairing their wounds, sponges can also rebuild their body from separated or dissociated cells (Wilson, 1907). Dissociated cells coalesce to form masses of aggregation cells, and those masses eventually form functional adults (Simpson, 1984). The aggregation cells work together with other sponge cells; for example, choanocytes and aggregated cells work together to coalesce cells (Van De Vyver, 1976). Choanocytes are crucial to sponge survival as they pump water and nutrients through the oscula. Due to a constant flowing ocean, reaggregation is not important in nature, but aggregation cells can serve as a proxy for functional cells as they eventually create the entire adult's cell structure (Simpson, 1963). Understanding how temperature affects aggregation cells will give insight on sponge functionality and biology.

Sponges are an important aspect of coral reef ecosystems. There are many organisms that are spongiverous, or feed only on sponges, such as hawksbill sea turtles (Meylan, 1988), emperor angelfish (Andréa *et al.*, 2007), some parrotfish (Dunlap and Pawlik, 1998) some polychaete worms (Pawlik, 1983) and various nudibranchs (Belmonte *et al.*, 2015; Hubbard, 1988; Macdonald *et al.*, 2010). Sponges also act as a glue, helping bind live coral to reef systems and protect their frame from burrowing organisms (Bell *et al.*, 2013; Wulff, 2001). Sponges have also been found to control phytoplankton levels and mitigate phytoplankton blooms (Bell *et al.*, 2013; Peterson *et al.*, 2006). Sponges are important to nutrient cycling by acting as nitrogen and silica sinks (Bell *et al.*, 2013; Fiore *et al.*, 2013; Hoffmann *et al.*, 2009; Rützler & MacIntyre, 1978). They also help to protect bivalves (Chernoff, 1987; Marin & Belluga, 2005; Pitcher & Butler, 1987; Pond, 1992) and crabs (Martinelli *et al.*, 2006; McClay, 1983) and provide microhabitats for small organisms such as brittle stars and sea spiders (Bell, 2013). Sponges have many ecosystem services beneficial to reefs; predicting how sponge distribution will change is important to understanding how reef systems will be different in the future.

In addition to coral reef ecosystems, sponges are also important to the medical industry. Sponges are among the most important marine taxa to the biomedical industry thus far. Out of 13 marine taxa surveyed for biomedical compounds, sponges provided over half of the medically important compounds discovered (Reed and Pomponi, 1988). They have antiviral, antifungal, antibacterial, and antitumor compounds (Ball *et al.*, 2013). Sponges are important to coral reefs and medicine, but their long-term distribution is unknown. Scientists have been able to estimate their abundance due to tiny spicules left in fossilized coral reefs, but unlike coral, they do not leave a large, obvious footprint on coral reef communities; it is impossible to get an accurate prediction about how sponge populations have changed overtime without sponge distribution studies, which are lacking in the scientific literature (Wulff, 2006). Sponges represent a library of potentially useful medical substances. Assessing sponge distribution and possible climate-change-related threats to natural sponge diversity is important to incorporate into existing literature.

Temperature can affect growth rates of sponges, but increased temperature also has other implications. Temperature can strengthen the vulnerability of disease in marine sponges (Hummel *et al.*, 1988; Vacelet, 1994; Webster, 2007). Additionally, increased temperature can inhibit feeding mechanisms of sponges. Sponges pump water through their ostia to gain nutrients and out of their oscula to get rid of waste. In higher than average temperatures, sponges have been found to completely close their oscules, which can slow down their feeding mechanisms (Jones, 1962; Simpson, 1984). Temperature can affect physiological mechanisms, and increased temperatures may further impede functionality.

This study will focus on the species *Haliclona reniera* because it is abundant on Mo'orea and may have biomedical potential; a sponge in the same genus, *Haliclona sp.* produced a new polyunsaturated brominated fatty acid that showed cytotoxicity, or poisonous effects, against cultured cancer cells (Aratake *et al.*, 2009). This study investigates: how will natural populations of *H. reniera* respond to temperatures at and above average oceanic temperature on Mo'orea (29˚C).

The goal of this project is to understand the physiological and morphological response during reaggregation and regeneration to changing temperatures on sponges. Research questions include: (1) What is the optimal temperature for *H. reniera* to reaggregate? (2) How will temperature fluctuations affect the growth rate of *H. reniera*? (3) How will *H. reniera* respond to abnormally high temperatures? (4) What is the distribution and abundance of *H. reniera* on Mo'orea? The optimal temperature for regeneration and reaggregation would be above average oceanic temperatures (30.5ºC) speeding up cellular processes. High temperature fluctuations will reduce the growth rate of *H. reniera*. At higher temperatures, aggregation will slow and eventually stop above a certain threshold temperature. This study aims to understand how *H. reniera* reacts to increased oceanic temperature changes.

METHODS:

Study Site and Species

The collection and analysis of *Haliclona reniera* was performed from September – November of 2016 on the island of Mo'orea, French Polynesia in the Society Islands. Mo'orea is a high island surrounded by coral reef divided into four areas: outer slope, barrier reef, lagoon and back reef channel and fringing reef (Faurea, 1989). Collection and transects were performed in Opunohu Bay (Site 1:17°31'04.5"S, 149°51'04.1"W), Cook's Bay (Site 2:17°29'12.9"S, 149°49'31.8"W & Site 3:17°29'26.7"S, 149°49'33.5"W), and at Temae Beach (Site 4:17°29'52.0"S, 149°45'15.8W & Site 5:17°29'57.9"S, 149°45'38.0"W) (Fig. 1). The reefs in Cook's Bay and Opunohu Bay consisted of lagoon and backreefs, while the reef at Temae was a fringing and barrier reef. The study focused on *Haliclona reniera,* a demospongiae. It is a small, fragile, purple sponge with many oscula.

Experiment 1: Reaggregation Temperature Trial

Sponges collected from Cook's and Opunohu Bay (sites 1-3; Fig. 2) were used in this experiment. Sponges were scraped off of rocks and dead coral rubble and placed in punctured plastic containers to allow flow. While collected, sponges never touched the air to mitigate risk of sponge death (Desmet, 2009). For each experiment, a dilution of 12.56mm^3 of *H. reniera* and 7mL of water was made. The volume of sponge was cut using a plastic cylinder with a diameter and length of 2mm. 12.56mm^3 of sponge was taken through a 250 micrometer sieve three times, to ensure there were no aggregates formed before the experiment began, and added to 7mL of

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water to make the sponge dilution. Immediately after, 1mL sponge dilution was added to five different test tubes and put into a water bath at temperatures ranging from 5˚C – 35˚C for 15min. After 15min, 0.5mL of sponge aggregates was taken out and photographed. Previous has shown that "primary aggregates" can form over this time period (Müller, 1982). Aggregations were photographed and the area of each aggregation was measured in imageJ.

Experiment 2: Regeneration Field Experiment

Sponges were only collected on small, mobile rocks from the control site (site 3) in Cook's Bay to easily be placed and secured into 7x7x7mm ($343mm^3$) cages attached to concrete cinderblocks. The cages were deployed in Cook's Bay (site 2) at depths of 0.5 m and 1m, and with HOBO 64K Temperature Loggers (Onset Computer Corporation, Bourne, MA, USA). One non-wounded and three wounded sponges were placed at each depth and 3m away from each other. The wounded sponges were all systematically wounded; they were cut in half. In addition to placing the sponges near each other, one non-wounded sponge was placed with the wounded sponges to control for differences in flow and nutrient availability. Photos were taken at 8am everyday for 10 days to estimate growth rates using imageJ software (Contributors Worldwide0.

Experiment 3: Regeneration Lab Experiment

H. reniera was collected from site 2 and put into two temperature treatment tanks: 28˚C (ambient) and 32˚C (high). Each tank had new flowing sea water (both flows were at the same rate), equipped with bubblers and HOBO 64K Temperature Loggers. The high temperature treatment tank had two Deltatherm Interpet aquarium heating systems. Three wounded sponges and one non-wounded sponge (control) were analyzed for growth over a six day period. Growth was measured using a Manostat manual caliper , measuring five different lengths to the nearest 0.01mm on each sponge. The lengths were then averaged together for total growth rates.

Experiment 4: Depth Transect Experiment

Transects were performed at five sites along the northern and northeastern coast of Mo'orea (sites 1-5; Fig. 2). Sponge size and depth was measured along transects near shorelines in Cook's and Opunohu Bay with depths from 0.25 – 3m. Two 30 min swimming transects were performed at each site with two observers looking for *H. reniera*. Each specimen found in the 30min was measured for volume and the depth at which it was found was recorded. An additional two transects were performed at Temae beach (Site 4:17°29'52.0"S , Site 5: 149°45'15.8"W; 17°29'57.9"S, 149°45'38.0"W). This site differed from Cook's and Opunohu Bay because it was, on average, deeper than 3m.

Statistical Analyses

All statistical tests were conducted using the platform *R* (R Core Team 2013). A whisker-box plot was used to show the overall mean and frequency of depths occupied by sponges. Linear regression analysis was used to understand the relationship between depth and volume of sponge specimens. For the reaggregation experiment, an ANOVA was performed to test for differences among aggregation rates across temperatures. For the field growth experiment, a Welch Two Sample t-test was conducted to determine if depth/temperature fluctuations predicted *H. reniera*'s growth response to wounding. For the laboratory growth experiment, an additional Welch Two Sample t-test was conducted to understand if temperature affected *H. reniera's* growth response to wounding.

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RESULTS:

1. Reaggregation Temperature Trial

Across a range of temperatures (4-34˚C), *Halicona reniera* aggregated most frequently between 29-30˚C (Fig. 3: ANOVA: $p<0.005$; $F=9.198(1,67)$). A loess curve was fit on the temperature versus percent of cells aggregation data, which shows that there was a peak growth at 29.5˚C (Fig. 3: residual standard error=<0.01). This curve was similar to a temperature performance curve, peaking at an optimal temperature (29.5˚C) and offset towards high temperatures.

2. Regeneration Field Experiment

Temperatures at both depths fluctuated. Average temperature at 1m was 29.03˚C±0.74 but ranged from 28-32˚C, and at 0.5m was 29.44˚C±1.33 but ranged from 28-34˚C (Fig. 4. The growth rates of sponges differed at two depths (Fig. 5: t-test: df = 31.6, $p<0.005$). The growth rate was greater at 0.5m: .8µm/Day, than at 1.0 m: 1.6µm/Day.

3. Regeneration Lab Experiment

The mean of the growth rate (µm/Day) for 31˚C was higher at 13µm/Day than at 25˚C, which was 11µm/Day (Fig. 6: t-test: df = 23.2, $p=0.001$).

4. Depth Transect Experiment

The average depth across all transects for *H. reniera* was 0.95 ± 0.03m. Out of all four sites, sponges were present only on sites 1-3. At sites 1-3, *H. reniera* occurred most frequently at depths from 0.8m-1.20m (Fig. 7). The average sponge depth found at Site 1 was 1.05m ± 0.04, at Site 2 was 0.94m ± 0.05, and at Site 3 was 0.91m ± 0.05. The average sponge size at all sites was $746mm^3 \pm 420$. The average sponge size at Site 1 was $1819mm^3 \pm 700$, at Site 2 was $1000mm^3 \pm 327$, and at Site 3 was $869mm^3 \pm 235$. There was no relationship between size of the sponges and the depth at which they were found (Fig. 8: $y= -23x + 789$, $R^2 <0.001$, $F=0.001$; (1,114); $p>0.05$).

DISCUSSION:

Temperature and depth had an effect on *H. reniera* regeneration rates; increased temperature and decreased temperature fluctuation increased growth rates. The combined results of these experiments have implications with the IPCC's temperature predictions. According to the IPCC, temperature will increase by 1-5˚C in the next 100 years and temperature will fluctuate more dramatically (IPCC, 2007). With these predictions, increased oceanic temperatures will increase growth rates of sponges, however; increased thermal variability will slow growth rates. The growth rates slowed under variable fluctuating temperatures, but *H. reniera* was still able to regenerate in fluctuating temperatures. Sponges at deeper depths, however, may not persist. Shallow water sponges are exposed to variable temperatures, which may make them be able to adapt to temperature fluctuations better than sponges found at deep depths (Guzman and Conaco, 2016).

H. reniera was able to aggregate at temperatures above average (up to 34˚C), but aggregated most frequently at average oceanic temperatures (29˚C). Reaggregation cells can be compared to functioning adult sponge cells because they will eventually form to make up the

adult cell (Simpson, 1963). This implies that sponge cells may be functional at these temperatures, and global warming may not have an effect on cellular mechanisms. This experiment, however, did not explore how temperature fluctuation affected *H. reniera* reaggregation. Future studies could look at temperature fluctuations on reaggregation rates to gain more insight on the cellular biology of sponges.

Increased carbon emissions are changing the planet. Among many side effects, temperature increases and fluctuation changes are apparent. If the IPCC's predictions both with increasing temperatures and more drastic temperature fluctuations, many organisms will be marginalized. Understanding the implications of these changes now is important to help predict how the earth will change. Perhaps some organisms will adapt, or some will not be affected. Sponges may fall into both categories, or they may not persist. Sponges have many beneficial functions and could affect ecosystems if wiped out. There is a wide variety of literature on sponge biology, chemistry and medical uses, but sponge distribution and climate change effects are limited. This study aimed to fill the gap in literature about both sponge distribution and temperature effects on sponges.

Higher growth rates in *Haliclona oculata* were reported when exposed to higher temperatures; this sponge was measured for a year and the highest growth rate coincided with hotter temperatures (Koopmans & Wijffels, 2008). This study also took into consideration nutrient fluxes; however it has been proven that sponge growth is more dependent on temperature than nutrient availability (Barthel, 1986). Additionally, Koopmans *et al.*, 2008 did not take variable fluctuations into account, and reported that negative growth via predation occurred. Even despite these limitations, the growth rates of these sponges were reported to be the highest during warmer months. Oceanic temperatures from the Koopmans & Wijffels (2008) ranged from 3˚C-23˚C, which does not reflect climate change conditions in Cook's Bay, but it does show that this sponge grew faster in higher temperatures. Additionally, future studies should look at the impacts of temperature fluctuations. In the future, with higher oceanic temperature conditions, sponges may be more abundant in coral reefs, as increased temperatures will affect other organisms, and sponges could have little or no competition.

Although the ocean is the most thermally stable ecosystem on Earth, oceanic thermal variability influences physiological processes and temperature tolerances of marine organisms (Angilletta, 2009). Short-term temperature fluctuations, or 'Pulse Events,' would ultimately wipe out organisms, but long-scale temperature increases affect growth, as growth is temperature dependent (Johnston & Bennett, 1996). Measured in constant and fluctuating temperatures, there is evidence that organisms experience higher growth in variable conditions. Organisms such as sea scallops (Pilditch & Grant, 1999), sea cucumbers (Dong et al., 2006) and tobacco hornworms (Kingsolver et al., 2015) grew faster in fluctuating rather than constant temperatures. Fluctuating temperatures affect the physiology of marine organisms, and the magnitude of fluctuation matters. Higher than average temperature fluctuations caused sex reversal in minnows (Coulter *et al.*, 2015), stopped growth in planktonic ciliates (Montagnes & Weisse, 2000), impeded the acclimation after heat shock in porcelain crab muscle tissues (Garland et al., 2015) and reduced metabolic rate but increased stress tolerance in water fleas (Chen & Stillman, 2012). Fluctuating temperatures are physiologically important to animals, and average fluctuating temperatures were important to high sponge growth. Sponges grew slower in high fluctuating environments, but they still persisted.

The results of this experiment proved that high fluctuating conditions reduce growth rates in sponges, but there may be time for tolerances or adaptations. Future long-term experiments

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testing temperature fluctuations are important to uncover these adaptations. Events that cause temperature fluctuations go beyond the span of simple oceanic temperature rise. There are some events such as El Niño Southern Oscillation (ENSO) and the North Atlantic Oscillation that are getting stronger every year, which may be linked to increasing anthropogenic carbon emissions (Timmermann et al., 1999). These events cause irregularity in sea surface temperatures usually last a few months (IPCC, 2007). As we enter a new era, incidentally coined the "Anthropocene," organisms will be more and more affected by thermal variation in 'Pulse' and long-term change events. The physiology of organisms may change with these future projects; however, sponges may persist. During the 1997-1998 ENSO year, while many organisms were negatively affected, sponges emerged unchanged (Kelmo, Bell, & Atrill, 2013). There may be hope for these sessile organisms. Bell *et al.,* (2013) predicted that, in the future, coral reefs could emerge as "sponge reefs" because many coral reefs and coral reef organisms may be marginalized in the future, and sponges much less susceptible to climate change conditions than these organisms.

The distribution of *H. reniera* is dependent on depth, as it was found in a very small depth range (0.4-1.4) with an average depth of 0.94m ± 0.05. Depth is a large factor in sponge distribution (Wilkinson & Evans, 1989). Many sponges have a specific range: *Hippospongia communis* – 0.5-30m, *H. lachne* – 2-10m, *Spongia agaricina* – 5-60m, *S. barbara* – 2-15m, *S. graminea* – 2-5m, *S. officinalis adriatica* – 0.5-40m, *S. officinalis mollissima* 10-30m, *Tubigera* 2-10m (Josupeit, 1990), but only a few sponges occur within one depth. Out of 73 species sampled for depth in Ireland, only a few were exclusive to one specific depth (J. Bell & Barnes, 2000). Most sponges have ranges much greater than *H. reniera*, so it is interesting to find such a specific range. This may have implications related to *H. reniera's* physiology. Perhaps *H. reniera* does not survive at other depths, or *H. reniera* is a habitat selective organism and mostly found where it can maximize growth, at 1m, as high regeneration rates are beneficial for sponge survival (Jackson & Palumbi, 1979; Leys, 1998; Wulff, 2010). As temperatures increase and fluctuate, *H. reniera* could adapt by changing its distribution to deeper, cooler and less fluctuating depths; however, since this range is so narrow, *H. reniera* may not be able to change its distribution because it is a sessile organism and would make this change through reproduction.

The effects of temperature on sponge physiology and morphology can be further studied. It would be informative to observe growth rates throughout all seasons. Follow up studies could include more species to get a better understanding of how different species will respond to increased temperatures because sponge species differ in morphology and physiology, expanding the time period for measuring growth rates, or looking at how multiple factors affect *H. reniera*, increased temperature and salinity, for instance. Despite its limitations, this study has strong evidence on the depth distribution, reaggregation temperature limits and optimal conditions for regeneration growth rates of *H. reniera.*

In the future, as climate change conditions persist, more and more organisms will be affected by increasing oceanic temperatures and temperature fluctuations. If temperature fluctuations stay constant, despite IPCC predictions, *H. reniera* may persist and consequently grow faster. If the IPCC predictions are correct, and temperature fluctuations become more drastic, *H. reniera* and other organisms may be affected. Future studies should take into consideration temperature fluctuations as well as how multiple stressors, such as disease, ocean acidification and increased salinity, will affect sponges. Assuming the IPPC predictions, future coral reefs will be altered and perhaps sponge reefs will be the new coral reefs.

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REFERENCES:

Andréa BR., Batista D., Sampaio CLS., Muricy G. 2007. Spongivory by juvenile angelfish (Pomacanthidae) in Salvador , Bahia State , Brazil. *Atlantic*:131–137.

Angilletta MJJ. 2009. Thermal Adaptation: a Theoretical and Emprical Synthesis. *Oxford University Press*:320.

Aratake S., Trianto A., Hanif N., Voogd NJ De. 2009. A New Polyunsaturated Brominated Fatty Acid from a. *Marine Drugs* 7:523–527. DOI: 10.3390/md7040523.

Ball M., Gaunt MJ., Hook DF., Jessiman AS., Kawahara S., Orsini P., Scolaro A., Talbot AC., Tanner HR., Yamanoi S., Ley S V. 2005. Total Synthesis of Spongistatin 1: A Synthetic Strategy Exploiting Its Latent Pseudo-Symmetry**. *Angewandte Chemie International Edition* 44:5433–5438. DOI: 10.1002/anie.200502008.

Barthel D. 1986. On the ecophysiology of the sponge Halichondria panicea in Kiel Bight . I . Substrate specificity, growth and reproduction. *Marine Ecology* 32:291–298.

Bell J., Barnes DKA. 2000. The distribution and prevalence of sponges in relation to environmental gradients within a temperate sea lough: inclined cliff surfaces. *Diversity and Distributions* 6:283–303. DOI: 10.1046/j.1472-4642.2000.00091.x.

Bell JJ., Davy SK., Jones T., Taylor MW., Webster NS. 2013. Could some coral reefs become sponge reefs as our climate changes? *Global Change Biology* 19:2613–2624. DOI: 10.1111/gcb.12212.

Belmonte T., Alvim J., Padula V., Muricy G. 2015. Spongivory by nudibranchs on the coast of Rio de Janeiro state, southeastern Brazil. *Spixiana* 38:187–195.

Bosch TCG. 2007. Why polyps regenerate and we don't: Towards a cellular and molecular framework for Hydra regeneration. *Developmental Biology* 303:421–433. DOI: 10.1016/j.ydbio.2006.12.012.

Bryant PJ., Fraser SE. 1988. Wound healing, cell communication, and DNA synthesis during imaginal disc regeneration in Drosophila. *Developmental Biology* 127:197–208. DOI: 10.1016/0012-1606(88)90201-1.

Carlson BM. 2007. *Principles of Regenerative Biology*. Amsterdam: Elsevier Inc.

Chen X., Stillman JH. 2012. Multigenerational analysis of temperature and salinity variability affects on metabolic rate, generation time, and acute thermal and salinity tolerance in Daphnia pulex. *Journal of Thermal Biology* 37:185–194. DOI: 10.1016/j.jtherbio.2011.12.010.

Chernoff H. 1987. Factors affecting mortality of the scallop Chlamys asperrima (Lamarck) and its epizootic sponges in south Australian waters. *Journal of Experimental Marine Biology and Ecology* 109:155–171.

Coulter DP., Ho TO., Mahapatra CT., Guffey SC., Sepulveda MS. 2015. Fluctuating Water Temperatures Affect Development, Physiological Responses and Cause Sex Reversal in Fathead Minnows. *Environmental Science and Technology* 49:1921–1928. DOI: 10.1021/es5057159.

Desmet DN. 2009. Seeking Sponges: Distribution of Exposed Porifera in Mo'orea, French Polynesia. *UCB Moorea Class: Biology and Geomorphology of Tropical Islands*.

Dong Y., Dong S., Tian X., Wang F., Zhang M. 2006. Effects of diel temperature fluctuations on growth , oxygen consumption and proximate body composition in the sea cucumber Apostichopus japonicus Selenka. 255:514–521. DOI: 10.1016/j.aquaculture.2005.12.013.

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414 Dunlap M., Pawlik J. 1998. Spongivory by parrotfish in Florida mangrove and reef habitats.
415 *Marine Ecology* 19:325–337.
416 Faurea G. 1989. Degradation of Coral Reefs at Moorea Island (French Polynesia) by
417 Acanthaster planci. *Journal of Coastal Research* 5:295–305.
418 Fiore CL., Baker DM., Lesser MP. 2013. Nitrogen Biogeochemistry in the Caribbean Sponge,
419 Xestospongia muta: A Source or Sink of Dissolved Inorganic Nitrogen? *Plos One* 8:1–11.
420 DOI: 10.1371/journal.pone.0072961.
421 Francis D., Barlow PW. 1988. Temperature and the cell cycle. *Symposia of the Society for*
422 *Experimental Biology* 42:181–201.
423 Garland MA., Stillman JH., Tomanek L. 2015. The proteomic response of cheliped myofibril
424 tissue in the eurythermal porcelain crab Petrolisthes cinctipes to heat shock following
425 acclimation to daily temperature fluctuations. *The Journal of Experimental Biology*
426 218:388–403. DOI: 10.1242/jeb.112250.
427 Guzman C., Conaco C. 2016. Gene Expression Dynamics Accompanying the Sponge Thermal
428 Stress Response. *PLOS ONE* 11:1–15. DOI: 10.1371/journal.pone.0165368.
429 Hoffmann F., Radax R., Woebken D., Holtappels M., Lavik G., Rapp HT., Schläppy M., Schleper
430 C., Kuypers MMM. 2009. Complex nitrogen cycling in the sponge Geodia barretti.
431 *Environmental Microbiology* 11:2228–2243. DOI: 10.1111/j.1462-2920.2009.01944.x.
432 Hubbard EJA. 1988. Larval growth and the induction of metamorphosis of a tropical
433 sponge-eating nudibranch. *Journal of Molluscan Studies* 54:259–269.
434 Hummel H., Sepers A., de Wolf L., Melissen F. 1988. Bacterial growth of the marine sponge
435 Halichondria panicea induced by reduced waterflow rate. *Marine Ecology Progress*
436 *Series* 42:195–198.
437 IPCC. 2007. *Climate Change 2007 Synthesis Report*. DOI: 10.1256/004316502320517344.
438 Jackson J., Palumbi S. 1979. Regeneration and partial predation in cryptic coral reef
439 environments: preliminary experiments on sponges and ectoprocts. *Colloq. Int.* 291.
440 Johnston IA., Bennett AF. 1996. Animals and Temperature: Phenotypic and Evolutionary
441 Adaptation. *Cambridge University Press*:436.
442 Jones W. 1962. Is there a nervous system in sponges? *Biological Review* 37:1–50.
443 Josupeit H. 1990. *Sponges: World Production and Markets*. Suva, Fiji: South Pacific
444 Aquaculture Development Project, Food and Agriculture Organisation of the United
445 Nations.
446 Kelmo F., Bell JJ., Atrill MJ. 2013. Tolerance of Sponge Assemblages to Temperature
447 Anomalies: Resilience and Proliferation of Sponges following the 1997–8 El-Niño
448 Southern Oscillation. *PLOS ONE* 8.
449 Kingsolver JG., Higgins JK., Augustine KE. 2015. Fluctuating temperatures and ectotherm
450 growth: distinguishing non-linear and time-dependent effects. *The Journal of*
451 *Experimental Biology* 218:2218–2225. DOI: 10.1242/jeb.120733.
452 Koopmans M., Wijffels RH. 2008. Seasonal Growth Rate of the Sponge Haliclona oculata
453 (Demospongiae : Haplosclerida). *Marine Biotechnology* 10:502–510. DOI:
454 10.1007/s10126-008-9086-9.
455 Leys S. 1998. Hexactinellid sponge ecology: growth rates and seasonality in deep water
456 sponges. *Journal of Experimental Marine Biology and Ecology* 230:111–129.
457 Macdonald TA., Burd BJ., Macdonald VI., Roodselaar A Van. 2010. Taxonomic and Feeding
458 Guild Classification for the Marine Benthic Macroinvertebrates of the Strait of Georgia,
459 British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences 2874*.

PeerJ Preprints NOT PEER-REVIEWED

460 Marin A., Belluga M. 2005. Sponge coating decreases predation on the bivalve Arca noae.
461 *Journal of Molluscan Studies* 71:1–6.
462 Martinelli M., Calcinai B., Bavestrello G. 2006. Use of sponges in the decoration of Inachus
463 phalangium (Decapoda, Majidae) from the Adriatic Sea. *Italian Journal of Zoology*
464 73:347–353.
465 McClay C. 1983. Dispersal and use of sponges and ascidians as camouflage by Cryptodromia
466 hilgendorfi. *Marine Biology* 76:17–32.
467 Meylan A. 1988. Spongivory in Hawksbill Turtles: A Diet of Glass. *Science* 239:393–395.
468 Montagnes D., Weisse T. 2000. Fluctuating temperatures affect growth and production
469 rates of planktonic ciliates. *Aquatic Microbial Ecology* 21:97–102.
470 Mulller WE. 1982. Cell membranes in sponges. *International Review of Cytology* 77:129–
471 181.
472 Pawlik J. 1983. A Sponge-Eating Worm from Bermuda: Branchiosyllis oculata (Polychaeta,
473 Syllidae). *Marine Ecology* 4:65–79. DOI: 10.1111/j.1439-0485.1983.tb00288.x.
474 Peterson BJ., Chester CM., Jochem FJ., Fourqurean JW. 2006. Potential role of sponge
475 communities in controlling phytoplankton blooms in Florida Bay. *Marine Ecology*
476 *Progress Series* 328:93–103. DOI: 10.3354/meps328093.
477 Pilditch CA., Grant J. 1999. Effect of temperature Fluctuations and food supply on the
478 growth and metabolism of juvenile sea scallops (Placopecten magellanicus. *Marine*
479 *Biology* 134:235–248.
480 Pitcher C., Butler A. 1987. Predation by asteroids, escape response, and morphometrics of
481 scallops with epizoic sponges. *Journal of Experimental Marine Biology and Ecology*
482 112:233–249.
483 Pond D. 1992. Protective-commensal mutualism between the Queen Scallop Chlamys
484 opercularis (Linnaeus) and the encrusting sponge Suberites. *Journal of Molluscan*
485 *Studies* 58:127–134.
486 Reed JK., Pomponi SA. 1988. Biomedical Research in the Sea, A Search For Drugs and Novel
487 Compounds. *American Academy of Underwater Sciences*:273–287.
488 Rutzler K., MacIntyre I. 1978. Siliceous sponge spicules in coral reef sediments. *Marine*
489 *Biology* 49:147–159.
490 Sanchez AA. 2000. Regeneration in the metazoans: why does it happen? *BioEssays* 22:578–
491 90.
492 Simpson TL. 1963. The Biology of the Marine Sponge Microciona prolifcra (Ellis and
493 Solande). *Journal of Experimental Zoology* 154:135–151. DOI:
494 10.1002/jez.1401540109.
495 Simpson TL. 1984. *The Cell Biology of Sponges*. New York: Springer-Verlag.
496 Timmermann A., Oberhuber J., Bacher A., Esch M., Latif M., Roeckner E. 1999. Increased El
497 Niño frequency in a climate model forced by future greenhouse warming. *Nature*
498 398:694–697.
499 Vacelet J. 1994. *Control of the sever sponge epidemic. Near East and Europe: Algeria, Cyprus,*
500 *Egypt, Lebanon, Malta, Morocco, Syria, Tunisia, Turkey, Yugoslavia*. Rome, Italy.
501 Van De Vyver G. 1976. Chapter 4: Phenomena of Cellular Recognition In Sponges. In:
502 Moscano A, Monroy A eds. *Current Topics in Developmental Biology*. London: Academic
503 Press, 134–135.
504 Webster NS. 2007. Sponge disease: A global threat? *Environmental Microbiology* 9:1363–
505 1375. DOI: 10.1111/j.1462-2920.2007.01303.x.

PeerJ Preprints NOT PEER-REVIEWED

Wilkinson CR., Evans E. 1989. Sponge distribution across Davies Reef, Great Barrier Reef, relative to location, depth and water movement. *Coral Reefs* 8:1–7.

Wilson H V. 1907. On Some Phenomena of Coalescence and Regeneration in Sponges. *Journal of Experimental Zoology* 5:245–58.

Wulff JL. 2001. Assessing and Monitoring Coral Reef Sponges: Why and How? *Bulletin of Marine Science* 69:831–846.

Wulff JL. 2006. Rapid diversity and abundance decline in a Caribbean coral reef sponge community. *Biological Conservation* 127:167–176. DOI: 10.1016/j.biocon.2005.08.007.

Wulff JL. 2010. Regeneration of Sponges in Ecological Context: Is Regeneration an Integral Part of Life History and Morphological Strategies? *Integrative and Comparative Biology* 50:495–505.

Zwietering MH., Jogenburger I., Rombouts FM., Riet KV. 1990. Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology* 56:1875–1881.

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Figure 1(on next page)

Mo'orea, French Polynesia, Line Map

Fig. 1A: Line map of Mo’orea showing the full coastline of the island.

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Figure 2(on next page)

Mo'orea, French Polynesia, Line Map

Fig. 2B: close-up view of the location of sites 1-5 surveyed in this study.

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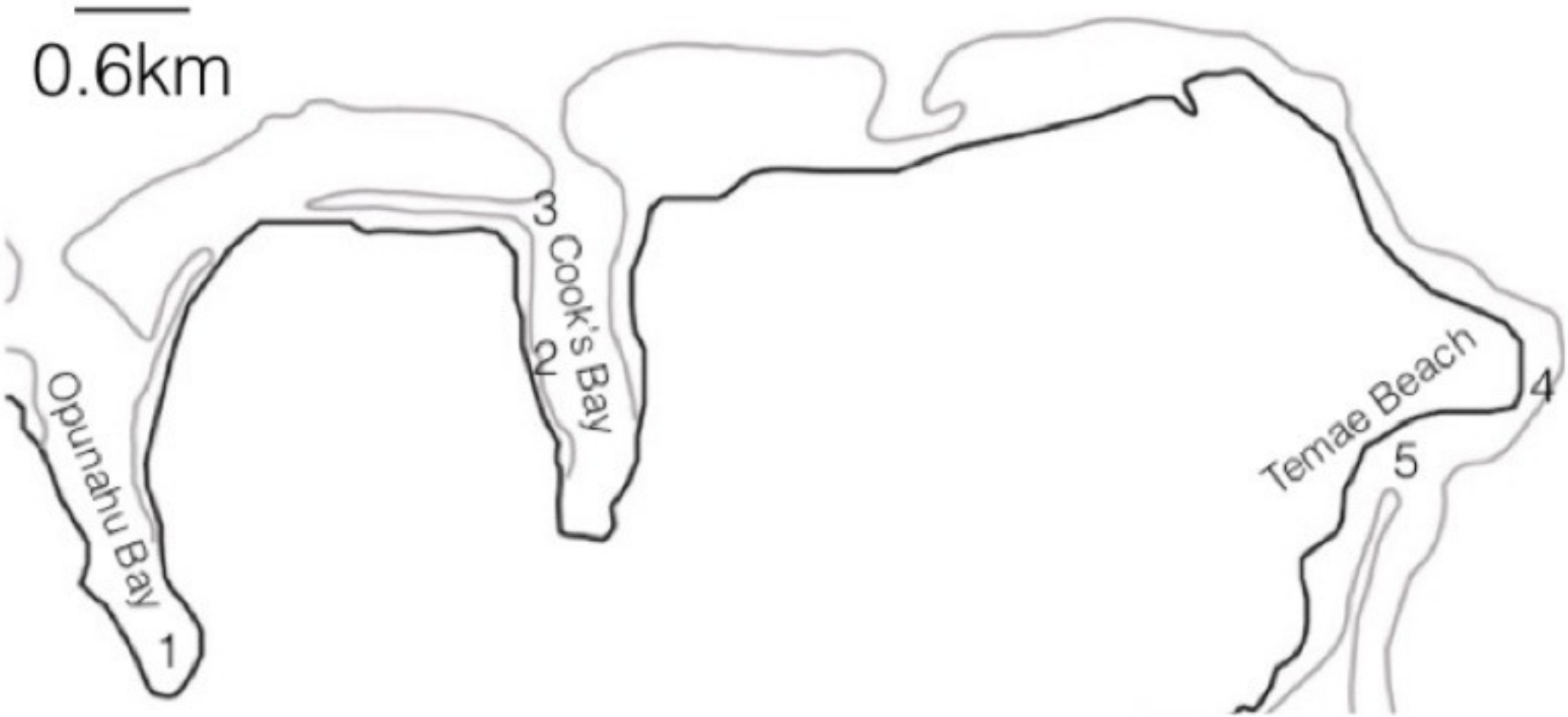

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Figure 3(on next page)

Map of regeneration field experiment

Fig. 2: Line map indicating the collection site (red) and cage site (black) for *Haliclona reniera* in Cook's Bay.

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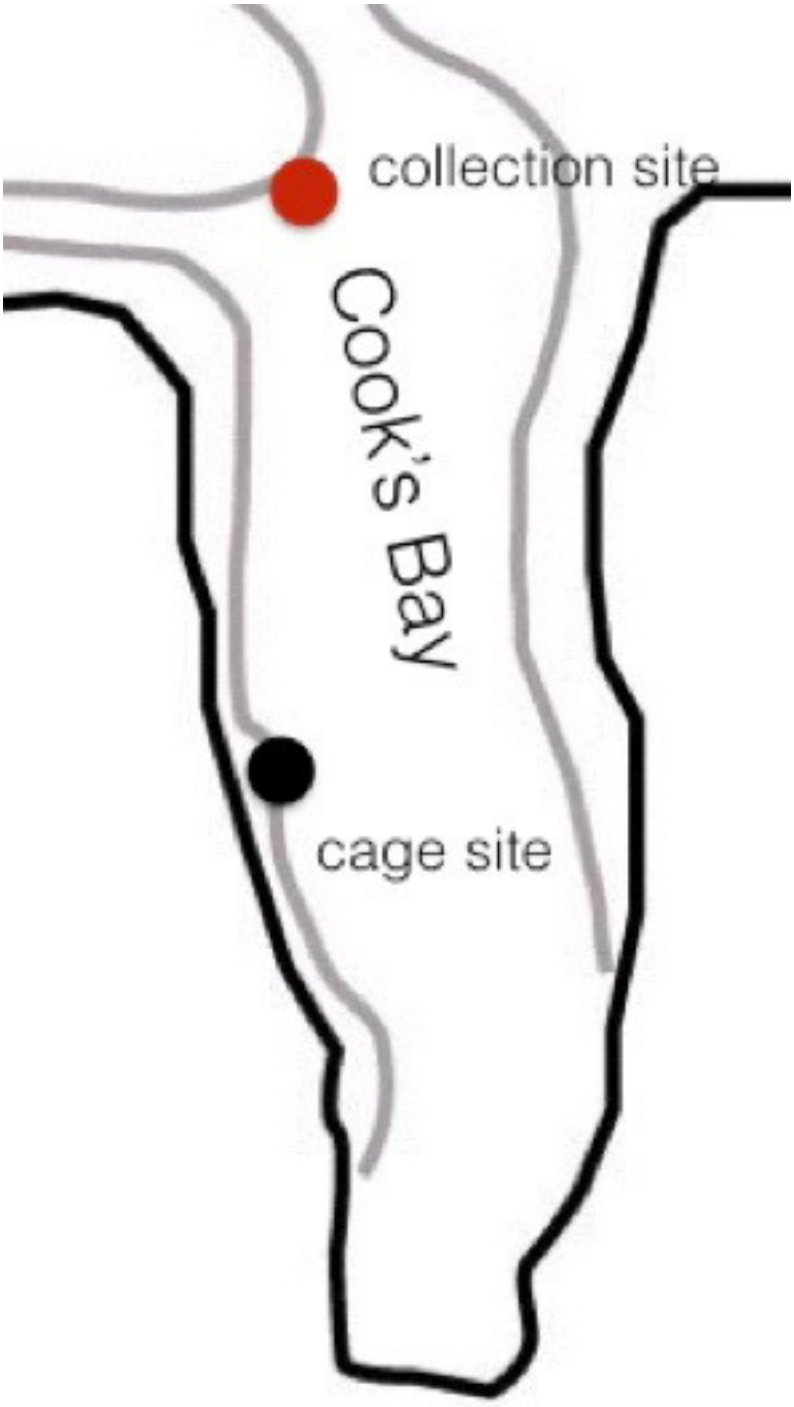

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Figure 4(on next page)

Regeneration temperature performance curve

Fig. 3: Percentage of *H. reniera* cells aggregated versus temperature (ºC) (ANOVA: $p<0.005$; $F=9.198(1,67)$, residual standard error<0.01).

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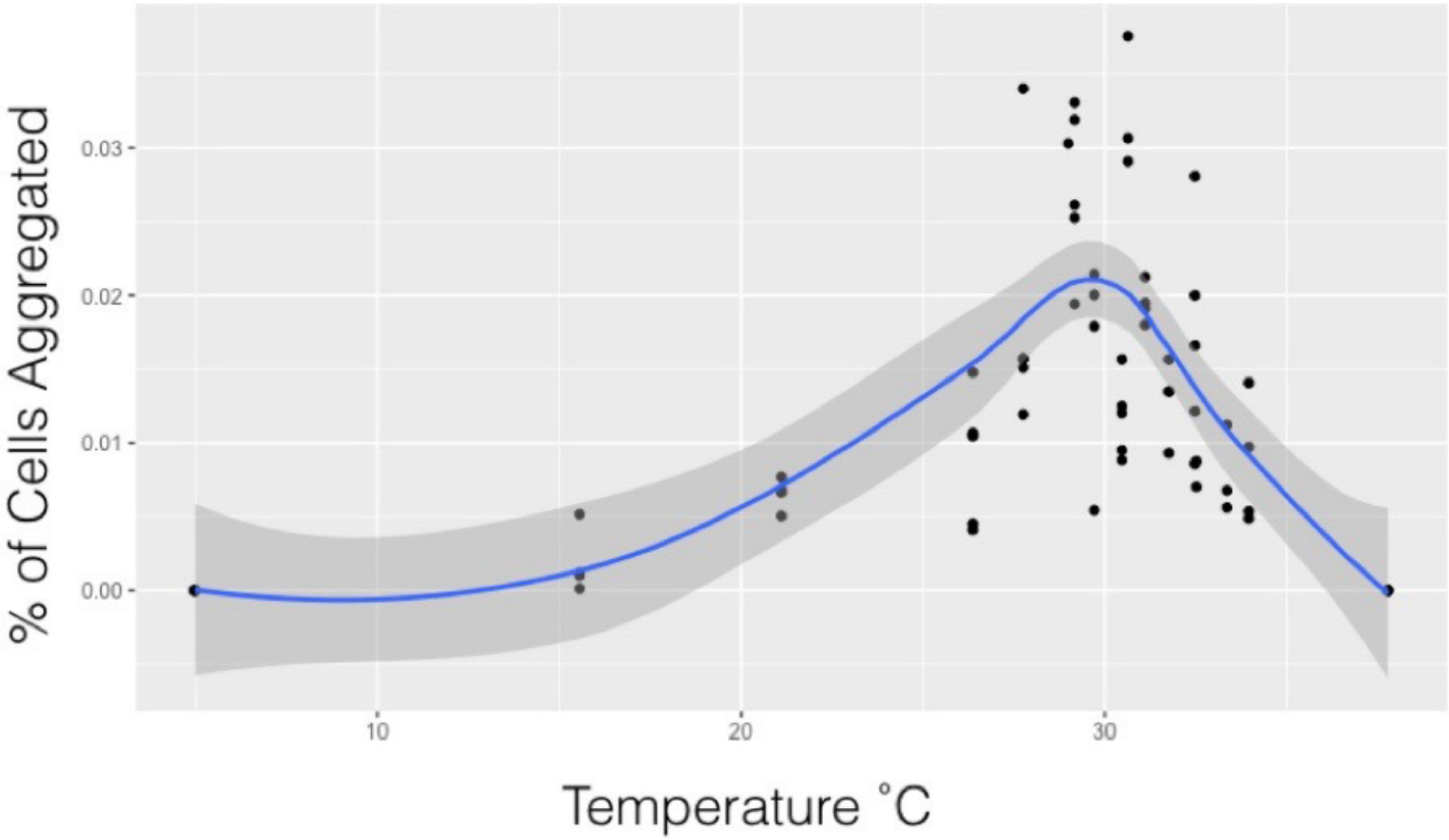

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Figure 5(on next page)

Temperature fluctuation per depth

Fig. 4: Temperature fluctuations per depth: 1m - 29.03˚C±0.74 ranging from 28-32˚C, 0.5m - 29.44˚C±1.33 ranging from 28-34˚C.

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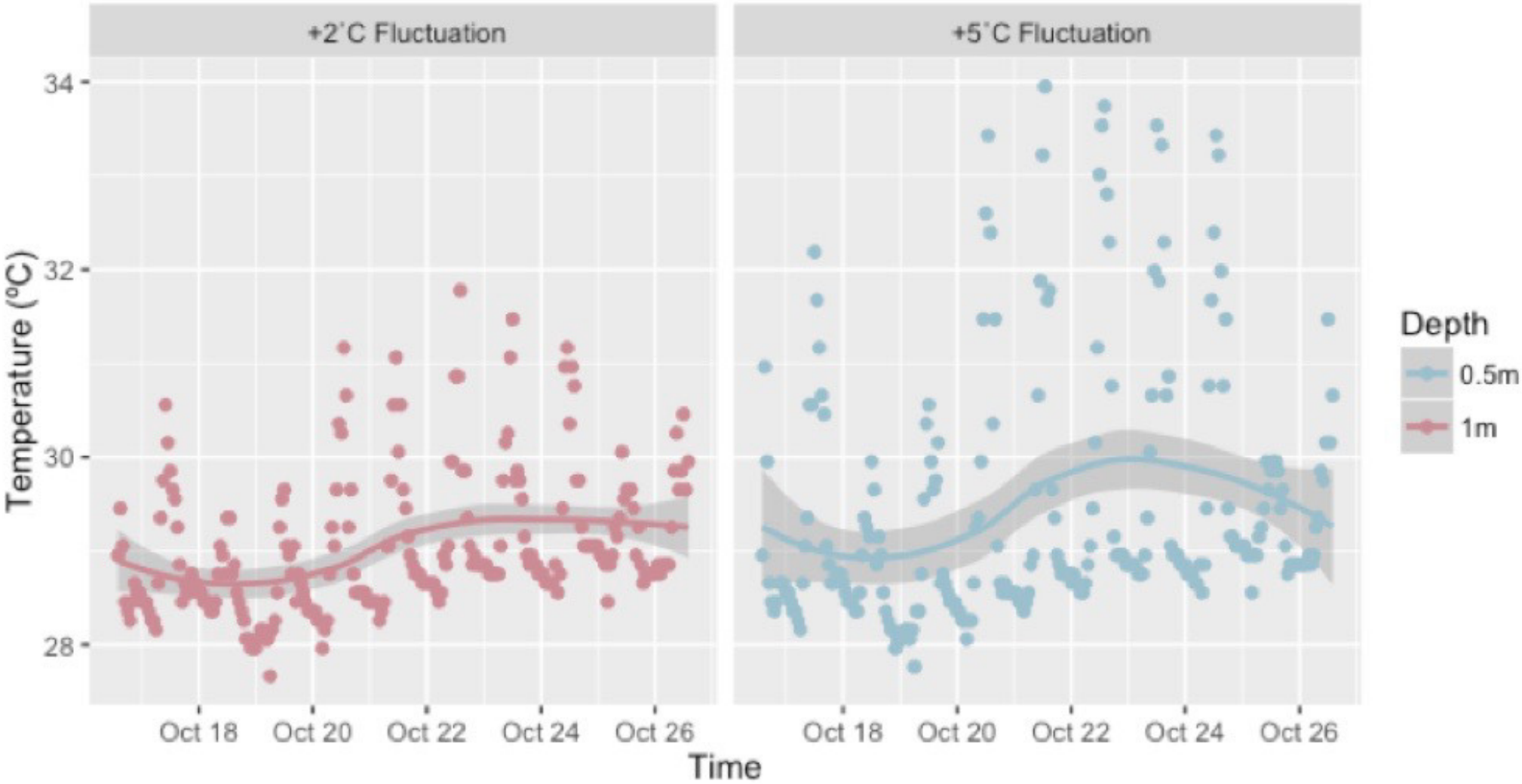

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Figure 6(on next page)

Sponge growth rate per depth

Fig. 5: Average growth (µm/Day) at 1m (28-32˚C) and 0.5m (28-34˚C). The slopes were different at each depth (t-test: df = 31.6, p<0.005). The slopes at 0.5m and 1m were .8µm/Day and 1.68µm/Day, respectively.

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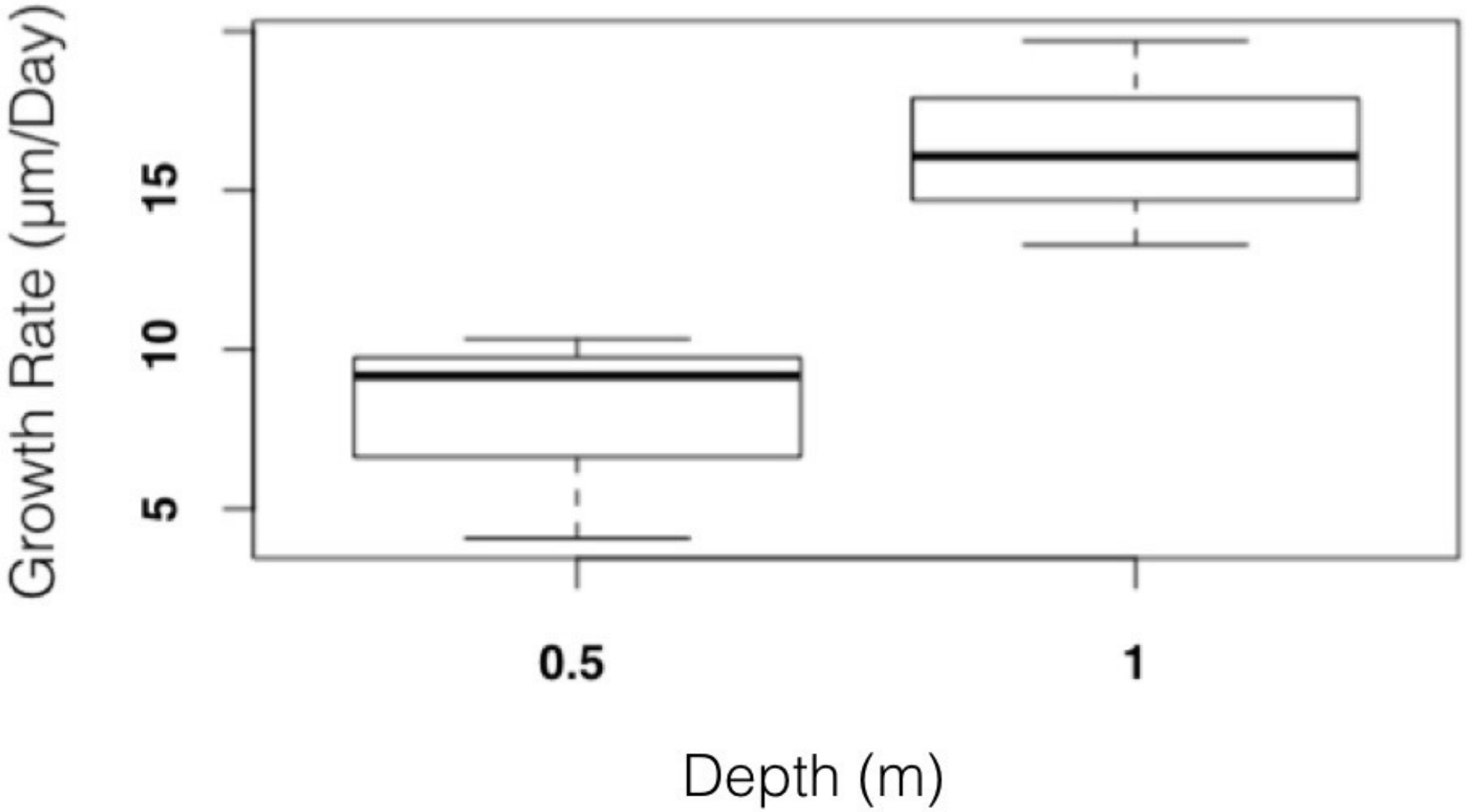

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Figure 7 (on next page)

Sponge growth rate per temperature

Fig. 6: Average growth (µm/Day) for each temperature (t-test: df = 23.2, p=0.001). The overall average slopes were 11µm/Day for 25˚C and 13µm/Day for 31˚C.

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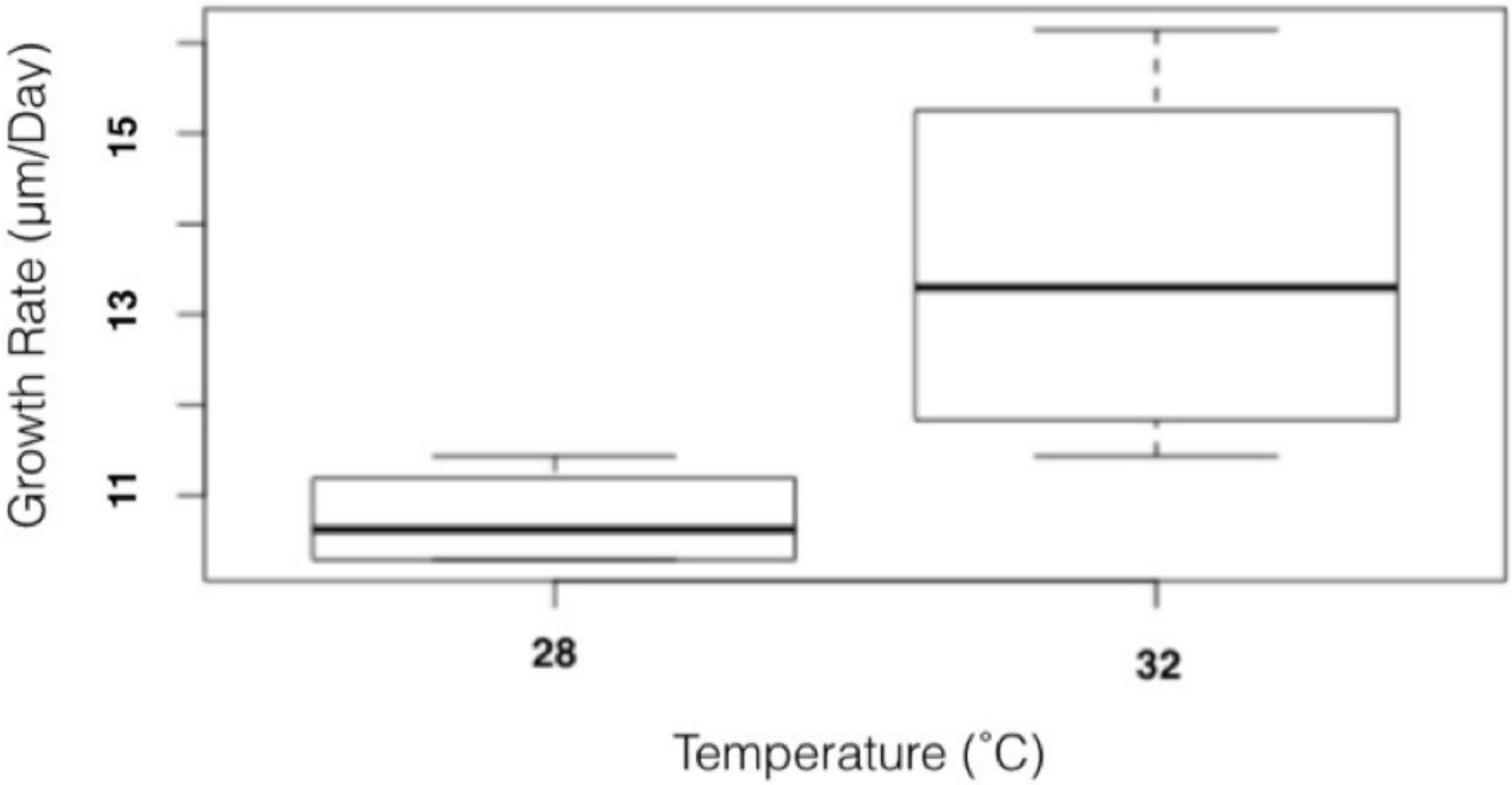

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Figure 8(on next page)

Sponge depth transect data

Fig. 7: Whisker box plot of *Haliclona reniera* found at all sites. *H. reniera* was found most frequently between 0.8 and 1.12m. The average sponge depth found at Site 1 was 1.05m ± 0.04, at Site 2 was 0.94m ± 0.05, and at Site 3 was 0.91m ± 0.0.05.

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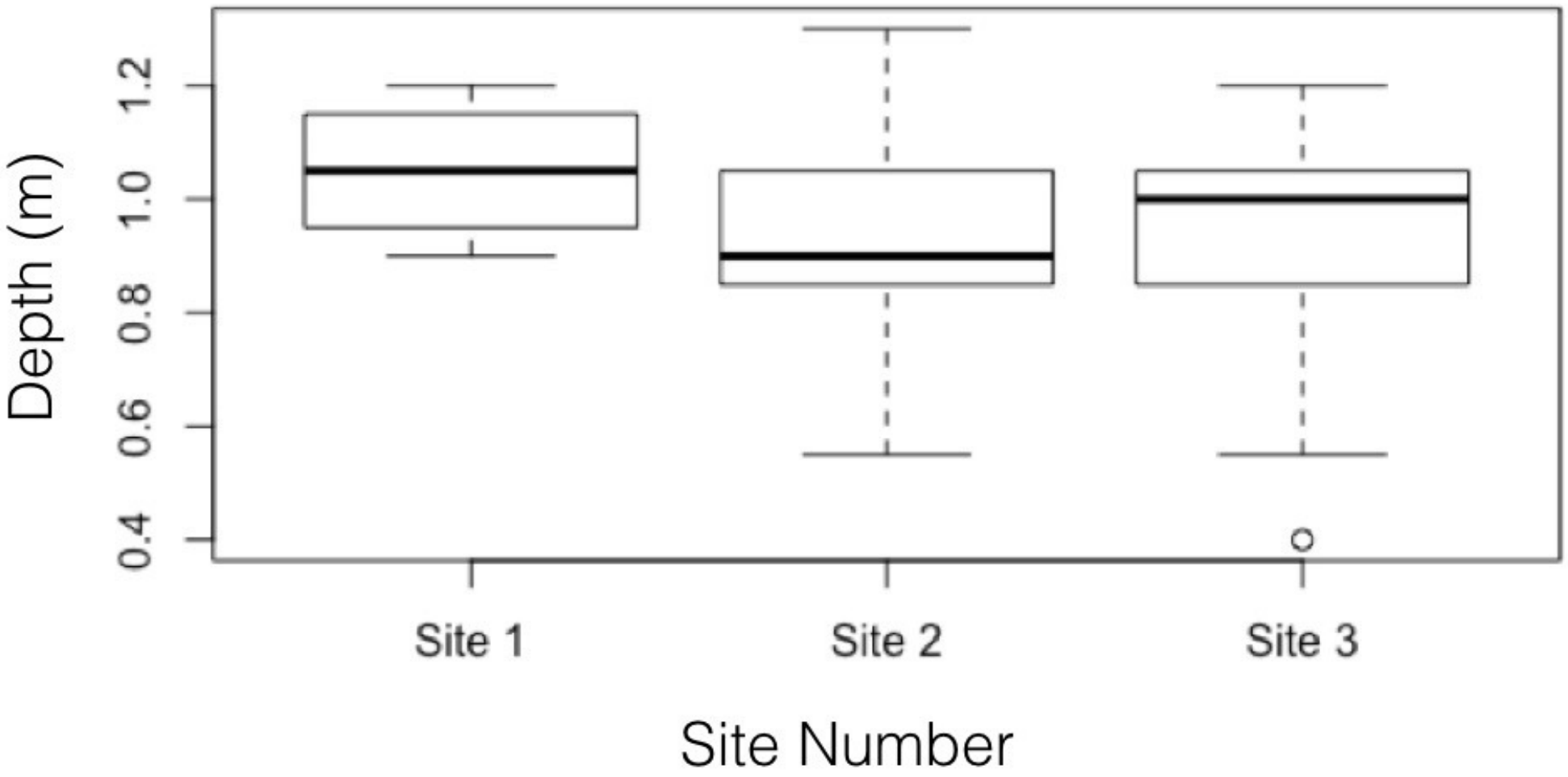

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Figure 9(on next page)

Depth versus size

Fig. 8: Linear regression of depth versus size (y=127.48X – 723.14, F=0.01956; (1,58); p>0.8)

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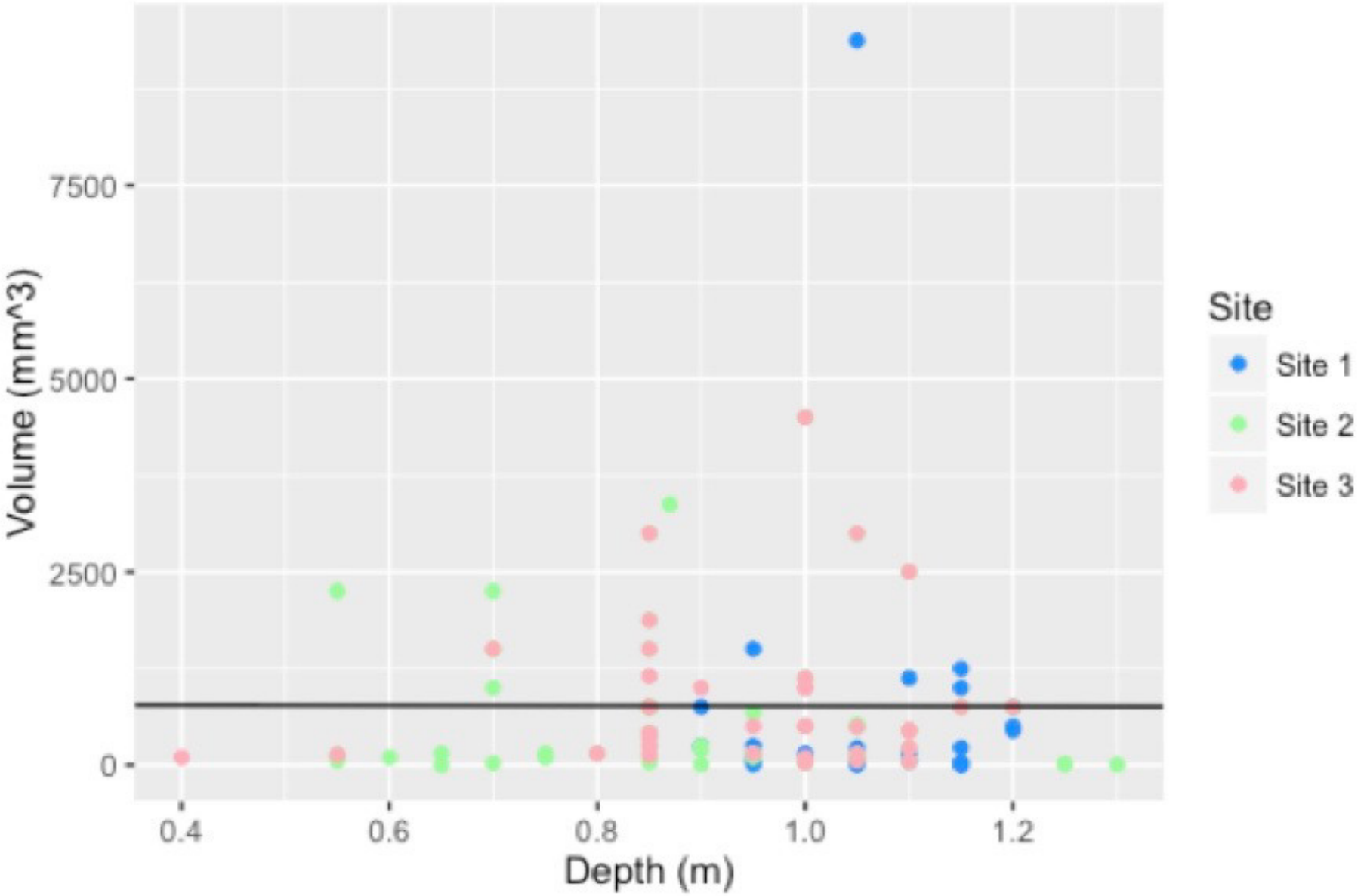

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