

A COMPARATIVE STUDY OF THERMAL TOLERANCE AND PERFORMANCE BETWEEN TWO MORPHOTYPES OF THE BLACK SEA CUCUMBER, *HOLOTHURIA ATRA*

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Abstract. With the increasing rate of climate change and ocean warming at the present time, it is important to understand how marine organisms may respond to temperature increases and the biological bases for these responses in order to better predict how ocean ecosystems and ecological interactions may change in the future. In particular, there has been a notable lack of knowledge concerning specific responses of benthic tropical invertebrates to warming events. This study primarily sought to examine the response of an ecologically important species of sea cucumber, *Holothuria atra*, to heat stress and compare the responses between the large and small morphotypes of *H. atra* in two different populations – Cook’s Bay and Haapiti respectively. In addition, their contribution to the local habitat as bioturbators was estimated to confirm their ecological importance in the tropic coral reef ecosystem. Groups of five sea cucumbers were placed in temperature-controlled water baths, with each group experiencing a higher temperature than the last. Respiration rate and sediment turnover rate were measured. The small morphotype population was observed to have a wider thermal performance curve with a maximal respiratory performance at a higher temperature (37.95°C) than that of Cook’s Bay (33.49°C), suggesting that the small morphotype may have adapted to live in warmer conditions. The Haapiti population also had a higher average maximum respiration rate at 9.02ppm/kg/hr compared to 7.73ppm/kg/hr of Cook’s Bay. Biomass did not seem to be a good proxy for rate of sediment turnover (Spearman’s rank correlation $\rho = -0.041$, $p = 0.734$), but based on estimates of average turnover rate and density at each location with each population, bioturbation at Cook’s was approximately 337.5kg of sediment per day and 1975kg per day at Haapiti, which quantifies the huge role that these abundant sea cucumbers play in their local ecosystem. The results from this study provide optimistic evidence of potential long-term resilience of *H. atra* sea cucumbers to climate change in the future.

Key words: sea cucumber, *Holothuria atra*, climate change, ocean warming, thermal tolerance, thermal performance curve, heat stress, sediment turnover, bioturbation, Cook’s Bay, Haapiti, Moorea, French Polynesia

INTRODUCTION

The rate at which the global climate has been warming has increased to an unprecedented point in the last thousand years (Walther et al. 2002). Regional changes have been even more drastic and variable, impacting ecological communities to varying degrees all around the world (Przeslawski et al. 2008). The ocean in particular has become susceptible to a battery of changes, including sea level rise, decreased ocean pH, increased sea surface temperature, increased meteorological disturbances, and greater amplitude ocean oscillation anomalies (Walther et al. 2002, Byrne and Przeslawski 2013). These physical and chemical changes, while not yet large in magnitude, have already elicited significant ecological responses from

many marine community assemblages. Larval recruitment, trophic dynamics, animal behavior and distribution have all been visibly affected as a consequence of the changing climate (Braud et al. 1995, Lawrence and Soame 2004, Edwards and Richardson 2004). In addition, the physiology of individuals within many populations is being influenced heavily by environmental conditions (Walther et al. 2002, Przeslawski et al. 2008)

Because these organismal responses are linked to broader ecological impacts, a more thorough understanding of them would allow for better-informed predictions of ecosystem shifts that may occur in the future (Hoegh-Guldberg et al. 2007). This poses a challenge however, due to the specific nature of thermal tolerance and resilience to temperature fluctuations within taxa, with even closely

related species having differing levels of tolerance (Przeslawski et al. 2008). Furthermore, there is a paucity of knowledge concerning specific responses of tropical invertebrates, especially benthic ones, to heat stress in relation to their life histories (McEdward 1995, Sewell and Young 1999, Chan et al. 2001, Przeslawski et al. 2008). Researchers have been addressing this by observing present coping abilities of organisms in thermal tolerance and acclimatory studies, and exploring the underlying biological mechanisms which may give rise to such responses (Stillman 2003, Calosi et al. 2008, Miller et al. 2013).

One well-studied physiological mechanism is metabolic suppression by aestivation, which refers to the energetic inactivity of organisms at raised temperatures (Navas and Carvalho 2010). While aestivation is generally associated with thermal extremes and drought situations, this behavior may also be a response to hypoxia, a secondary consequence to ocean warming, caused by a reduction in oxygen solubility and increases in upper ocean stratification (Pörtner and Knust 2007, Seibel 2011). By suppressing their metabolism, organisms can passively tolerate raised temperatures and lowered dissolved oxygen. This minimization of energy expenditure as a temporary method of coping comes with the cost of limited general activity and cannot be sustained for great lengths of time, but it exists as an important adaptive strategy for animals living in variable oxygen content environments (Pörtner 2010).

In particular, aestivation has been observed in an economically important species of sea cucumber (*Apostichopus japonicus*) that lives in shallow waters and experiences seasonal temperature fluctuations (Yang et al. 2006). *Apostichopus japonicus* begins to suppress its metabolism when a certain temperature threshold is reached, at which point oxygen consumption rate and excretion rate drop significantly, and can be maintained in a dormant state for as long as a third of a year (Yang et al. 2006, Yuan et al. 2007). Although sea cucumber aestivation has only been carefully studied in this one species, it is likely that other species may exhibit similar behaviors, given similar life history traits and physiology. It is worthwhile to pursue this possibility, given the sheer density of sea cucumbers (Holothuroidea) in the Indo-Pacific and their valuable role in the ecosystem as bioturbators (Uthicke 1999, Schneider et al. 2013). Their ability to tolerate

warm and hypoxic water conditions may have a potentially significant impact on the local ecosystems in which they reside, and furthermore, studying their physiological response of aestivation may provide insight on how benthic echinoderms might cope with the changing climate as well as contribute knowledge to the resilience of the tropical marine ecosystem as a whole (Przeslawski et al. 2008, Collard et al. 2014).

In this study, I focused on *Holothuria atra*, the black sea cucumber, which is abundant and prominent throughout the tropical Indo-Pacific. This is a species of interest because of its particularly wide geographic distribution and habitat range (Paulay 2014). *Holothuria atra* has two distinct morphotypes – the large morphotype, the sexual form found in deeper areas with higher current such as reef fronts and passes where water tends to be cooler; and the small morphotype, the asexual form found in shallow reef flats and lagoons with little wave action and warmer water (Uthicke et al. 2010). Using populations of each morphotype, I compared the two and investigated whether *H. atra* has evolved adaptive tolerance to the warmer, more hypoxic waters of the reef flats occupied by the small morphotype, and if its local densities have a significant impact on their surrounding ecosystem in terms of bioturbation. To address the goal of my study, I: (1) compared thermal tolerance ranges of the two morphotypes by examining changes in sediment turnover rate and respiration rate of holothurians over a range of temperatures, (2) determined the reliability of biomass as a proxy for bioturbation, and (3) surveyed the density of *H. atra* where the two morphotypes are found. I hypothesized that the small morphotype has a shifted thermal performance curve relative to the large morphotype, to be more tolerant of warming temperature and lower levels of oxygen, and will have a significant ecological contribution to the reef habitat proportional to body size through their sediment processing.

MATERIALS & METHODS

Study site and animal subjects

The field portion of this study was conducted at two locations in Moorea, French Polynesia – the Haapiti reef flat (17°55'50"S, 149°87'91"W), where it is warm, shallow, and sandy with low current; and the back reef of Cook's Bay (17°47'83"S, 149°82'86"W) near Avaroa Pass, which is deeper, cooler, and has

medium to high current. The experimental portion was conducted at the Richard B. Gump Moorea Field Station in Cook's Bay. The study organism is *Holothuria atra*, commonly known as the black sea cucumber.

Experiment to compare the thermal tolerance ranges between the small and large morphotypes

Sea cucumbers (n=70) were haphazardly collected in large plastic containers from Haapiti reef flat and the back reef of Cook's Bay and transported back to the lab to be placed in sea tables with regular flow of filtered seawater. The sea cucumbers from Haapiti are of the small morphotype, whereas those from the Cook's Bay are of the large morphotype. The morphotypes were determined based on habitat types. Prior to each experiment, sea cucumbers remained in the sea tables for at least 24 hours for acclimation. Immediately after each experiment, each sea cucumber was lifted out of the water for 30 seconds, during which the sea cucumber should expel out any water that was held in its cloacal cavity, and then placed on a PESOLA MS500 scale to be weighed and measured for length.

The sea cucumbers from each location (n=35 from each) were split evenly into seven groups, one group for each temperature treatment – ambient temperature and six increasing increments of about 2°C. The ambient condition used seawater with the current temperature of seawater from Cook's Bay. Each subsequent increased temperature treatment was warmed and maintained by a water bath, in which boiling water was poured into the bath and mixed with ambient seawater to keep the temperature within 1°C of the designated treatment temperature. To control the amount of sediment intake before the experiment, each group of five sea cucumbers were purged of any residual sediment from their gut by undergoing a 24-hour starvation period during which they were placed in a large container within the sea table containing no sediment. All fecal pellets released during the starvation period were removed periodically from the tanks using a baster to prevent re-ingestion of excreted pellets. Following the starvation period, each sea cucumber was transferred to the sediment-covered area of the sea table for the feeding period where sand was in excess, and given 5 hours to feed on the substrate. The sea cucumbers were then transferred into individual plastic 2-liter containers. One

container without a sea cucumber was used in each treatment as a control to monitor for background changes in oxygen concentration. Measurements of dissolved oxygen were taken using an American Marine Inc. PINPOINT II Oxygen Monitor before the containers were sealed. Each container was then submerged in a tank of seawater from the sea table and covered with a lid to eliminate air pockets. The containers were placed in the water bath container, and the water bath was brought up to 1°C above the treatment temperature and maintained within 1°C of treatment temperature for the duration of the experiment by addition of boiling water as necessary. The containers remained in the water bath for 3 hours, after which each container was opened and measured for dissolved oxygen, which allowed a determination of the respiration rate. The respiration rate was weight-corrected and was calculated by dividing the difference of the final and initial dissolved oxygen concentration readings by the weight of the cucumber divided by 3 hours. The fecal excretions released during the experiment were removed using a baster and placed in 100-mL Nasco WHIRL-PAK bags. These sediment samples were then removed from the pouches, rinsed with freshwater, and poured into small aluminum weigh boats. Once excess liquid was siphoned off using a baster, the weigh boats were placed in a model 40 GC Quincy Lab oven at 100°C for 5 hours to dry the sediment. The dry weight of the sediment samples was then obtained using a portable PESOLA PPS200 scale. Thermal performance curves were extrapolated from the respiration data for comparison.

Sea cucumber distribution survey

The two collection sites – back reef of Cook's Bay and reef flat of Haapiti – were surveyed for sea cucumber distribution. At the Haapiti site, 50-meter transects were laid out with about 25 meters between each 0-meter mark. The surveys were conducted on two separate days, with two transects surveyed per day. Transects were laid out perpendicular to the shoreline, with the 0-meter mark starting at the shore and extending into the ocean. At every other meter from the 1-meter mark to the 49-meter mark along each transect line, the abundances of *H. atra* were measured within a 1 x 1 meter quadrat by snorkeling, with a total of 25m² sampled at each transect. There were no

duplicate counts with the assumption that sea cucumbers cannot travel between transects within the surveying time frame. At the Cook's Bay site, survey data was taken from a previous study (Filiatrault 1996). Water temperature at both sites was logged at 10-minute increments using HOBO UA-002-64 pendant temperature loggers tied down near the substrate for 2 weeks. The data from Cook's Bay and Haapiti surveys were used to extrapolate sea cucumber density at each site. This information was then used to estimate the average impact of natural populations of sea cucumbers on local coral reef habitats at each surveyed location.

Analyses

The distribution of sea cucumbers from the survey data at Haapiti were visualized on a graph of average abundance at each transect meter. The average densities of Haapiti and Cook's Bay and the average sediment turnover rates of each morphotype of *H. atra* were used to calculate the average contribution of bioturbation *H. atra* has at each site.

To create the thermal performance curves, respiration rate of both morphotypes were graphed as a function of temperature. Each set of data points was then fitted to a polynomial function. Because the Haapiti population did not undergo a temperature treatment that allowed the respiratory performance to reach

a marked decline, a stand-in data point was estimated using the shape of the curve from the Cook's Bay population to provide a more congruent comparison between the two curves.

Using data from the experiment, respiration rate and weight of fecal pellets were plotted against the weight of each sea cucumber within each population of sea cucumbers on a graph. Spearman's rank correlation coefficient was calculated and used to determine whether body size is reliable as a proxy to sediment turnover (R 2.15.1).

RESULTS

Thermal tolerance and performance

The natural range temperature fluctuations measured at the back reef of Cook's Bay and the reef flats of Haapiti were 27.17°C to 33.33°C and 26.39°C and 34.90°C respectively, with regular daily fluctuations of temperature maximums around noon time and minimums around midnight.

During the experiment, both populations of sea cucumbers exhibited gradual increases in respiration rate at temperatures beyond their natural temperature ranges, save for at the highest temperature treatment for Cook's Bay, which exhibited a drastic drop. The respiration rate of all the individuals ranged from 3.07ppm/kg/hr to 11.11ppm/kg/hr. The Haapiti population had a higher average

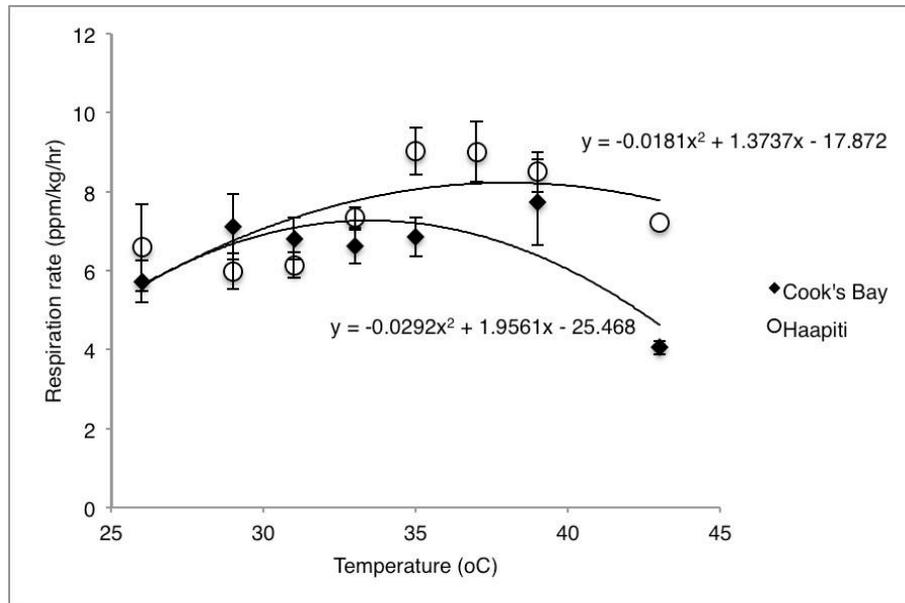


FIG. 1. Thermal performance curves of respiration of sea cucumbers at each temperature treatment, with a fitted polynomial model

respiration rate at 7.51ppm/kg/hr, compared to Cook's Bay at 6.414ppm/hg/hr. However, this difference may be amplified due the additional temperature treatment that Cook's Bay underwent which resulted in lower respiration rates. The Haapiti population also had a higher average maximum respiration rate at 9.02ppm/kg/hr compared to 7.73ppm/kg/hr of Cook's Bay.

Based on the simple quadratic curves fitted to the data, Haapiti seemed to have a wider thermal performance curve with a maximal performance at a higher temperature than that of Cook's Bay. The temperature at which Haapiti had maximal respiration rate performance was 37.95°C, whereas the Cook's Bay experienced maximal performance at 33.49°C. This is based on the assumption that the curve of the Haapiti population would follow the trajectory of average respiration rates at increasing temperatures.

It is worth noting several qualitative observations during the experimental procedure. At higher temperatures (>37°C), the sea cucumbers tended to attach to the lids of the containers. Upon removal, reddening of the skin and tube feet at parts in contact with the lid was often visible, likely caused by heat from the boiling water that splashed on top of the lids during pouring. This was particularly noticeable in the 43°C treatment of the Cook's Bay population, wherein the large portions of the ventral side of the sea cucumbers had reddened. At this temperature only, the sea

cucumbers exhibited an exceptional behavior where they extended and flared all oral feeding tentacles in the upward direction. Upon completion of the trial, the sea cucumbers were placed back in the sea tables. Three sea cucumbers from this trial extruded their entire gut and maintained their flared tentacle position until death two days later.

Sediment turnover and biomass

The range of absolute sediment turnover rate among all the sea cucumbers was 0g/hr to 4.25g/hr. The average turnover rate for the Cook's Bay population was 0.71g/hr and 1.53g/hr for the Haapiti population. The weight of the individual sea cucumbers ranged from 42.0g to 268.5g. The Haapiti sea cucumbers tended to be smaller than the Cook's Bay sea cucumbers, although similar size sea cucumbers were collected from both sites as much as possible to minimize any size differences. The average mass of sea cucumbers from the Cook's Bay population was 100.37g and the average mass for Haapiti's was 72.64g.

There was no significant correlation between body size and absolute turnover rate (Spearman's rank correlation $\rho = -0.041$, $p = 0.734$).

Distribution and estimated impact

In Haapiti, the average density of sea

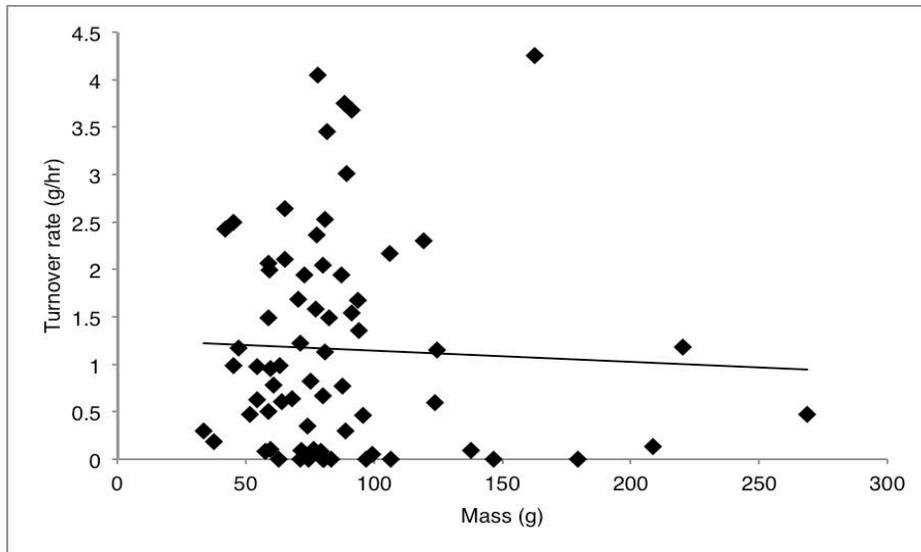


FIG. 2. Absolute turnover rate as a function of mass (Spearman's rank correlation $\rho = -0.041$, $p = 0.734$)

cucumbers at the reef flat was 2.45 individuals/m², and the maximum density recorded was 13 individuals/m². There was a general pattern of increasing density of sea cucumbers with distance from shore. The reef flat of Haapiti was dominated by *H. atra*, with no other species observed in all of the transects. In Cook's Bay, the average density of sea cucumbers was 0.63 individuals/m² and the maximum was 5 individuals/m². The average contributed bioturbation by sea cucumbers based on the average sediment turnover rate was 0.45g/m²/day at the back reef of Cook's Bay and 3.75g/m²/day at the reef flat of Haapiti. With a rough estimation of the back reef of Cook's Bay being around 0.75km², there is about 337.5kg of sediment processed by sea cucumbers per day. The reef flat of Haapiti is approximately 0.5km², so there is a turnover of about 1975kg of sediment per day.

DISCUSSION

Thermal tolerance ranges

Sea cucumbers of the small morphotype in Haapiti exhibited a higher temperature tolerance and a greater maximum respiration rate than the large morphotype sea cucumbers of Cook's Bay, suggesting that the small morphotype of *H. atra* may have adapted to living in warmer conditions in which they are generally found. This is shown in the comparison of thermal performance curves, where the Haapiti population seems to have experienced a thermal range expansion with an increased maximum relative to the Cook's Bay population. The two populations share about the same critical thermal minimum, but the Haapiti has a significantly greater thermal maximum. Because of the significant difference between the two curves, the observed temperature tolerance is likely not the result of plasticity but of adaptation (Van Damme et al. 1990). This is slightly different from the thermal range shift that was originally hypothesized. These results suggest that the small morphotype of *H. atra* may generally tend to perform better metabolically than the large morphotype at higher temperatures, suggesting a higher resilience to future ocean warming events.

Unlike the *Apostichopus japonicus*, there does not seem to be evidence of aestivation, as seen from the lack of metabolic suppression prior to mortality (Yang et al. 2006). Although there is a decrease in performance at the

highest temperature treatments, this is likely due to organ failure as the body begins to physiologically shut down as a result of high thermal stress. This is further supported by the high mortality that resulted from exposure to high temperatures during the experimental trial. Aestivation is merely a short-term coping mechanism to temperature increases. The Haapiti population that exhibited a temperature tolerance range expansion, if it were to be an adaptation to warmer temperatures, would probably be more resilient to temperature increase due to the enduring nature of adaptations.

It is interesting to see that while the range of natural temperature fluctuations in Haapiti is only ~2°C greater than that of Cook's, the difference in temperature at optimum performance between the two populations is ~5°C, far exceeding the observed habitat temperature differences. It would seem strange that the Haapiti population would overcompensate in adapting to warmer temperatures, but this may not necessarily be the case. In the field in Haapiti, sea cucumbers were found near the shore in ~0-5cm of water under direct sunlight, likely experiencing much hotter temperatures than in the deeper areas (~0.5m) where the data loggers were placed. It is thus reasonable to infer that the sea cucumbers in Haapiti experience much greater temperature variations than what the data loggers reported. Although all the sea cucumbers collected for experiment trials were at around the same depth as the data loggers and none were taken from near the shore in warmer waters, they could still tolerate such high temperatures. Perhaps this is because the sea cucumbers move between the shore and the reef flat. Natural history observations of individual sea cucumber movement would confirm this supposition.

Because the morphotypes were determined based on different habitats and locations, it is possible that the differences in physiological response observed between the two populations may have been the result of other differences. Since there were only two populations studied in this experiment, the differences in may just be confined to the two populations and would require a broader range of sampling to generalize to morphotypes. There was a biased selection for similar size sea cucumbers in both populations to standardize for body size and volume, but at the same time may introduce the confounding factor of age, which maturity may affect tolerance to heat. In addition, the

differences in response between temperature treatments and populations may be magnified by effects of pseudoreplication, which were difficult and impractical to avoid in such studies as these.

Biomass as proxy for bioturbation

From the non-significant correlation between mass of sea cucumber and sediment turnover rate, mass of individual sea cucumbers is a poor predictor for sediment turnover rate and would not provide accurate estimates of the amount of bioturbation based on biomass of sea cucumbers in a surveyed region. This is contrary to a previous study, which found a significant and high correlation between sediment turnover rate and body size (Brusch 1999). This may be because the size range of sea cucumbers used in that study was much broader than in this study, which had a very narrow size range relative to what is seen in nature. In the future, if the size range extended, it is possible that the observation made by Brusch (1999) could be replicated.

Sea cucumber distribution

The Haapiti reef flats, like other sandy and shallow reef flats, support a large density of *H. atra* sea cucumbers relative to the average coral reef habitat. The *H. atra* sea cucumbers were scattered all over the reef flats and tended to increase in density with increased distance from shore, although there were also sea cucumbers found above the tide line on the shore. The sea cucumber diversity of Haapiti was completely homogeneous, containing only the species *H. atra*. Based on a previous study, the density of *H. atra* sea cucumbers, assuming a threshold turnover rate, would make a fairly large impact on the surrounding reef flat and mangrove habitat (Uthicke 1999). The difference in substrate between Haapiti and Cook's Bay may be in part due to the much higher density of sea cucumbers in Haapiti. Cook's Bay is predominantly a coral habitat with densely distributed coral heads and coral rubble substrate with sand. *Holothuria atra* was again the most abundant species, but *Bohadschia argus* was also present in Cook's Bay. While the survey data seemed to reflect what was observed in the field – that Haapiti had a higher density of sea cucumbers than Cook's Bay – we must take into account the variability in observable density of sea cucumbers in Cook's Bay. That is, the number

of visible sea cucumbers varies from day to day, from what I observed through repeated visits to the back reef. It seems that during times of strong current and high surge, there would be almost no visible sea cucumbers, whereas during times when the water was calm, sea cucumbers could be easily spotted. Larger sea cucumbers tended to be the only visible sea cucumbers during times of strong current. This is probably because sea cucumbers, especially the smaller ones, would be more vulnerable to being picked up by the current if they were out in the open.

Implications: coral health and ocean acidification

Given the abundance and wide distribution of sea cucumbers, their contribution as bioturbators to their local habitat is very large and their role is essential in the maintenance of a healthy coral ecosystem. Bioturbation destabilizes sediment stratification and distributes organic material more evenly across the substrate (Massin, 1982, Uthicke 1999). By processing the sediment, sea cucumbers may reduce bacterial and microalgal production within the sand, preventing overgrowth and eutrophication (Uthicke 1999). In addition, sea cucumbers may also play a large role in the coral reef ecosystems in the dissolution and cycling of calcium carbonate, making it more available for calcifying organisms (Schneider et al. 2013, Collard et al. 2014). This dissolution may also increase the coral reef CaCO₃ budget, potentially producing enough to create a local chemical buffer for the reef habitat against ocean acidification (Schneider et al. 2011). With the continual rise of CO₂ in the atmosphere and in the ocean, it is important not only to understand how coral reefs will be impacted, but also to what capacity the coral and its dependent populations will be able to cope and adapt to ocean acidification (Hoegh-Guldberg et al. 2007).

Implications: climate change and ocean warming

The observation that the Haapiti population of sea cucumbers exhibited adaptation to warmer temperatures while maintaining a relatively high metabolic performance provides evidence that marine invertebrates can be capable coping and even thriving in ocean warming events. While the short-term coping mechanism of aestivation is not observed in this species, the fact that the expansion of thermal tolerance range was

exhibited is an optimistic finding because there is potential long-term resilience of the sea cucumbers to climate change. Sea cucumbers may play an increasingly important role, given the combined ocean acidification and ocean warming that has been occurring in recent years. They will likely endure even in warmer conditions and be able to buffer the coral reefs against ocean acidification to ameliorate potential synergistic negative effects (Schneider et al. 2011, Byrne and Przeslawski 2013)

Future research

While this study did find potential adaptive differences between the two morphotypes of *H. atra* sea cucumbers, more thorough studies of a broader scope would be needed to confirm this observation. A transplantation study would be of interest, since individuals of *H. atra* have been known to change morphotype to suit the habitat they are moved to (Thorne et al. 2012). Also, studies comparing multiple populations of small and large morphotypes from different locations in the Indo-Pacific would help to minimize effects of differences between populations and allow for better comparison of the two morphotypes. With climate change and ocean acidification occurring simultaneously, measures of metabolic rate and sediment processing performance of sea cucumbers in water of combined lowered pH and increased temperature would better predict the coping ability and performance of sea cucumbers in the future, as well as elucidate potential synergistic effects that warm acidified water may have on holothurian physiology.

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APPENDIX A

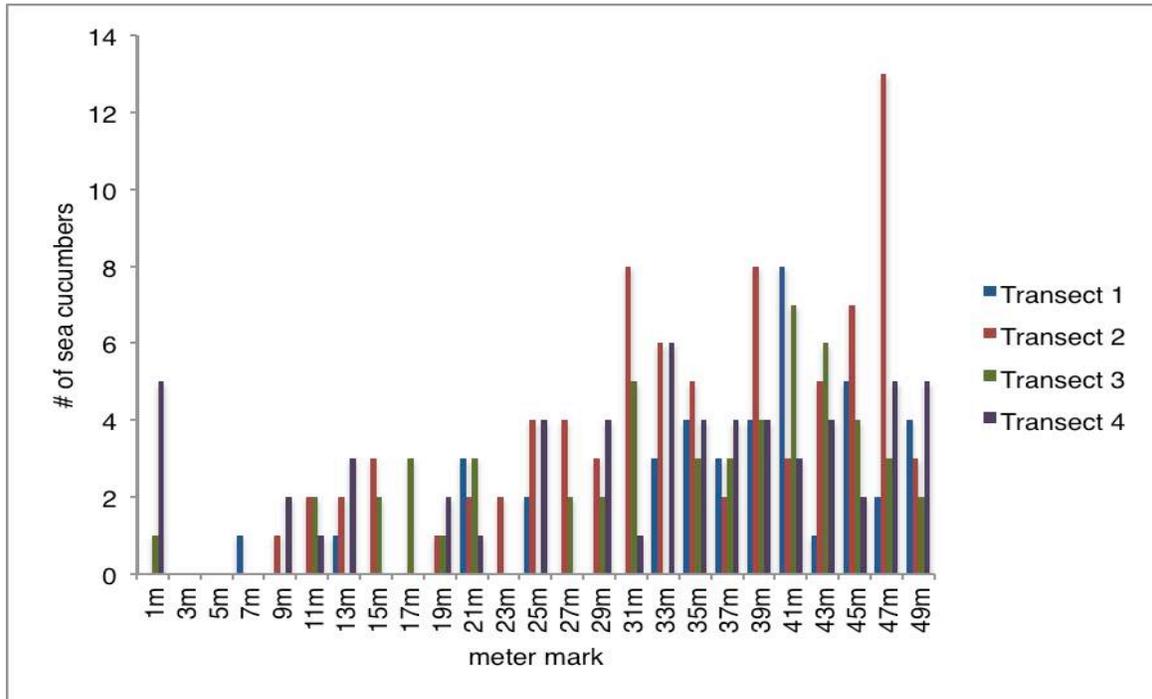


FIG. 1. Distribution of sea cucumbers from the Haapiti transect field survey



FIG. 2 Sea table setup



FIG. 3. Experimental setup of temperature-controlled water bath



FIG. 4. Expelled organs from cloaca of sea cucumber