Research Article

Variation in Righting Times of *Holothuria atra, Stichopus chloronotus,* and *Holothuria edulis* in Response to Increased Seawater Temperatures on Heron Reef in the Southern GBR

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Sea cucumbers can mitigate some impacts of climate change through digestion of benthic sands and production of calcium carbonate. The projected ecological benefits of sea cucumbers in warmer, more acidic oceans are contingent on the capacities of individuals to acclimate and populations to adapt to climatic changes. The goal of this experiment was to evaluate the degree to which warming waters would impact three abundant species of sea cucumbers on the Heron Reef in Queensland, Australia. We conducted a behavioral assay using three species of sea cucumbers, *Holothuria atra*, *Stichopus chloronotus*, and *Holothuria edulis*. Individuals from each species were subjected to three conditions mimicking current summer temperatures, current winter temperatures, and an elevated temperature consistent with future ocean warming by the year 2100. Sea cucumber reactions were evaluated using righting time as a proxy for their stress levels and overall tolerance of warming events. The three sea cucumber species reacted differently to water temperature changes: *H. atra's* righting times declined with temperature, *S. chloronotus* had greater righting times at high and low temperature extremes, and *H. edulis's* righting times remained relatively constant throughout. Our results suggest that each of these species might respond differently to ocean warming and while some may be able to continue to combat climate change in benthic communities, others may decline in ecological function.

1. Introduction

Warming oceans play a dominant role in the gradual yet drastic destruction of coral reef systems on the Great Barrier Reef [1], and on a global scale [2]. Excess anthropogenic carbon dioxide in the atmosphere also leads to ocean acidification and inhibits the ability of corals to produce calcium carbonate skeletons [3]. Thus, higher water temperatures and decreased levels of calcium carbonate in the ecosystem have already reduced coral reef resiliency and are predicted to continue to do so in the future [4]. There is evidence that ocean acidification will impact the shallow marine systems more drastically than the deeper ocean environments [5]. Therefore, shallow benthic communities, like coral reef ecosystems, are some of the most vulnerable to ocean warming and acidification.

Sea cucumbers are crucial members of these benthic communities. They play an important ecological role in many coral reef ecosystems and may mitigate local climate change impacts. On the Heron reef flat, on the Great Barrier Reef in Queensland, Australia, they act as bioeroders and are considered to be a keystone species [5]. Through their digestion of benthic sands and particulate matter, sea cucumbers can decrease seawater acidity locally and aid in the production of sediment calcium carbonate. They also secrete ammonia as a byproduct of their digestive processes, which enhances coral

reef productivity by increasing the nutrient levels in the water [6].

However, the long-term ability for sea cucumbers to mitigate ocean acidification may depend on individual species' thermal reaction norms; certain species function optimally under different thermal conditions [7]. With increased temperature and acidity of the oceans, benthic invertebrates' ecological processes may be altered. Mating periods, larval dispersal, and community structures will be affected and these impacts will be species dependent [8]. Previous studies have shown that thermal stress experienced by two unrelated sea cucumber species (*Holothuria scabra* and *Apostichopus japonicus*) led to immune and antioxidant responses to attempt to avoid damage and maintain homeostasis [9, 10] but, overall, high temperature stress will impact immune function [11].

Righting time assays are a proxy for physiological function and organismal stress in crabs [12], sea urchins [13, 14], and sea stars [15, 16]; yet, this method has not been widely used among sea cucumbers. In our experiments righting time was used to determine the influence of temperature on the sea cucumber's reaction time and stress. Although not commonly used on holothuroids, the use of this type of assay is particularly relevant for sea cucumber species because they rely on their podia to secure themselves to substrates and keep them safe from predation [17, 18]. During a righting time assay, animals are flipped to expose their more vulnerable, ventral side and timed to determine their return to a more natural, ventral-down position. The amount of time taken to reach this recovered state can relate to and ultimately determine the animals' physiological capacity and stress level as a function of the environmental changes occurring around them.

To compare how holothuroids in the GBR may acclimate to climate change conditions on a more physical level, we conducted a behavioral assay evaluating righting timing of three of the most abundant sea cucumbers on the back reef of Heron Reef. We used the holothurids *Holothuria atra*, *Stichopus chloronotus*, and *Holothuria edulis* and evaluated righting times under conditions mimicking current summer temperatures, current winter temperatures, and the elevated temperature of the oceans predicted to result from climate change by the year 2100 [19].

2. Methods

The study was carried out at the research station of the University of Queensland on Heron Island in June 2017. To determine which three species of cucumbers to include, we reviewed the Heron Reef flat report of 2014 and conducted simple field surveys at the scientific collection site southwest of Heron Island, at a site centered on 23.44313°S, 151.91351°E.

Of the 127 species of sea cucumbers on the Great Barrier Reef, five have been reported to be the most abundant on Heron Reef (*Holothuria atra, Holothuria leucospilota, Stichopus chloronotus*, *Holothuria edulis*, *and Stichopus variegatus*) [5]. Of these five species, *Holothuria atra* is the most abundant allotting for 70% of the sea cucumbers in the study area surrounding Heron Island [5]. *Stichopus chloronotus* is the

second most abundant, accounting for just over 11% of the area, and *Holothuria edulis* represent 7% of the sea cucumber species in this area [5]. We chose these three species because they were not only three of the most abundant on the reef, but also the most feasible to collect due to their tendencies to situate themselves amongst coral beds and out in the open in the sands of the reef flat during the day. Sea cucumbers exhibit a temperature-dependent diel burying schedule. They are generally inactive and buried for at least eight hours a day and are most ideally surveyed during midday hours [21]. Therefore, we surveyed the collection site and gathered our test subjects during midday hours. All sea cucumbers used in our assays were larger individuals collected from the shallow subtidal zone and therefore likely sexually reproducing morphotypes [22].

Four laboratory trials were conducted using two individuals from each of the three species at a time. Sample sizes were limited due to National Park sampling permit restrictions. The sea cucumbers were collected from the inner and mid reef at a depth of about 1-2 meters MLW on the southwest side of the island. The temperature of the seawater was taken at the time and location of collection. The average seawater temperature was 23°C. Therefore, in our experiments, the 23°C tanks acted as our control and represented the general temperature that the sea cucumbers experience during the Australian winter. Before each trial, each individual's body length was measured and they were weighed (Table 1) using a calibrated spring scale (accuracy of approximately +/- 1%) with a pan attachment.

Seven tanks were arranged in flowing seawater systems with air stones: five tanks simulated the ocean temperatures experienced by the sea cucumbers during local winters (23°C, comparable to our season of study), one tank contained submersible heaters to raise the water temperature to simulate local summer (29°C), and one tank had submersible heaters to raise the water to the predicted local temperature for the year 2100 (33°C) (based on [19]). We were limited by the amount of submersible heaters available at our disposal consequently; we were only able to heat one tank to each of the desired warmer temperatures (29°C and 33°C). The remaining five tanks were maintained at 23°C. This setup allowed us to randomly distribute all six sea cucumbers to a given temperature and allow them time to acclimate on their own to each environment while also having an extra holding tank available for use when necessary.

At the start of each round of experimentation, each sea cucumber was randomly assigned to and placed in a tank and allowed to acclimate for thirty minutes. The animals were then flipped onto their dorsal side at the center of the base of the tank and timed to determine how long it took to flip back onto their ventral side. The time was measured for each sea cucumber to return all of its podia to the base of the tank [15]. Each righting time assay was repeated three times [15] and ten-minute rest time was given to each animal between each trial. All sea cucumbers were returned to the collection site at the end of each trial. By the end of the experiment, we completed four rounds of testing with six sea cucumbers in each round.

TABLE 1: Summary statistics for body length, body mass, and the righting times at the three experimental temperatures for the three species. Given are the means ± standard deviations.

Species	*H. atra*	*H. edulis*	*S. chloronotus*
Body length (cm)	16.81 ± 3.830	18.50 ± 5.507	16.88 ± 2.986
Body mass (kg)	1.38 ± 0.612	1.13 ± 0.612	1.63 ± 0.495
Righting time (s) at 23°C	97.10 ± 42.915	76.42 ± 40.377	78.65 ± 39.237
Righting time (s) at 29°C	53.83 ± 20.332	83.66 ± 51.088	51.51 ± 26.186
Righting time (s) at 33°C	59.97 ± 22.442	99.29 ± 64.953	72.19 ± 27.489

2.1. Statistical Analyses. We used linear mixed modeling to analyze the data. We analyzed righting time as a function of species, temperature, the species x temperature interaction, body length, and the residuals of the regression of body mass on length (see Results). Individuals and trials were included as random factors. We first attempted to keep the response variable on its original scale by specifying generalized models with Poisson and negative binomial error structure. However, none of these models converged to a solution. Hence, we $\log_e$-transformed righting time and ran general linear mixed models (LMM), assuming normally distributed errors. We verified model assumptions by plotting the residual distribution, as well as residuals versus fitted values and each predictor in the model [23]. All analyses were carried out in R v3.4.0 (R Core Team 2017). We used the package lmerTest v2.0-33 [24] to run our models and to conduct the stepwise model selection (function step in lmerTest, direction set to "both"). We estimated the repeatability of righting time and tested for individual differences based on the simplified model using the packages rptR [25].

3. Results

The three species did not differ significantly in body length (Table 1; one-way ANOVA; $F_{2,21} = 114.89$; $p = 0.6938$) but did differ in body mass, independent of length, indicating differences in body shape (ANCOVA with type II test; Species: $F_{2,18} = 6.08$; $p < 0.01$; Length: $F_{1,18} = 32.27$; $p < 0.001$; Species X Length interaction: $F_{2,18} = 0.33$; $p = 0.7233$). Therefore, we included body length and the residuals of the regression of body mass on length as covariates in our linear mixed models. We selected the best model through stepwise removal of nonsignificant predictors using the Akaike Information Criterion.

The effect of temperature on righting time depended on species (Table 1). Body length and length-independent body mass were not significant and were removed from the final model (Table 2, Figure 1). As indicated by the 95% confidence intervals for the effects estimated by the linear mixed model (Figure 1), for *Holothuria atra*, at 29 and 33°C the righting times were lower than at 23°C. For *Stichopus chloronotus*, the righting time was lowest at 29°C whereas the times did not differ at 23 and 33°C, and, surprisingly, for *H. edulis* the righting time did not change with temperature.

The repeatability for righting time was significant (R = 0.362; Std. Error = 0.093; 95% CI = 0.181 - 0.532), indicating that it is a behavioral trait with consistent between-individual variation, independent of other internal or external factors.

TABLE 2: Results of the linear mixed model with log-transformed righting time as the response using the function *lmer* of the R package *lmerTest* (fit by REML). The random effects are shown as given by the simple summary of the model. The fixed effects are shown as summarized by the function *mixed* of the package *afex* (type 3 tests, Kenward-Roger approximation for degrees of freedom) [20]. Number of observations = 216; number of trials = 36; number of individuals = 24.

(a) Random effects

Groups	Variance	Standard Deviation
Trial	0.001009	0.03176
Individual	0.070234	0.26502
Residual	0.122942	0.35063

(b) Fixed effects

Effect	df	F	p-value
Temperature	2, 31.53	14.56	< 0.0001
Species	2, 20.94	1.09	0.36
Temp.: Species	4, 157.33	7.55	< 0.0001

Accordingly, the random effect accounting for between-individual variation explained a significant amount of variation in our mixed model estimating temperature effects (Table 2). On the other hand, the random effect trial and hence the order of the experimental trials accounted for almost zero variation.

4. Discussion

We found that the three sea cucumber species respond differently to temperature fluctuations. *Holothuria atra* had the slowest righting time at 23°C but faster righting times at warmer temperatures (29°C and 33°C). *Stichopus chloronotus* had optimal righting times at 29°C and slower and more varied righting times at cooler and warmer temperatures (23°C and 33°C), while *Holothuria edulis* had righting times that were seemingly unaffected by the temperature changes.

Of the three species' global geographic distributions *Holothuria atra* is a tropical species that is known to inhabit the warmer equatorial, and, occasionally, subtropical regions [26–28]. *H. atra's* tropical distribution could explain some of the tendencies for this species to perform optimally in the higher temperature environments. In contrast, *Stichopus chloronotus* is geographically limited to the tropics [26–28] suggesting that this species would fare better in their expected temperature range; yet our results imply that the species'
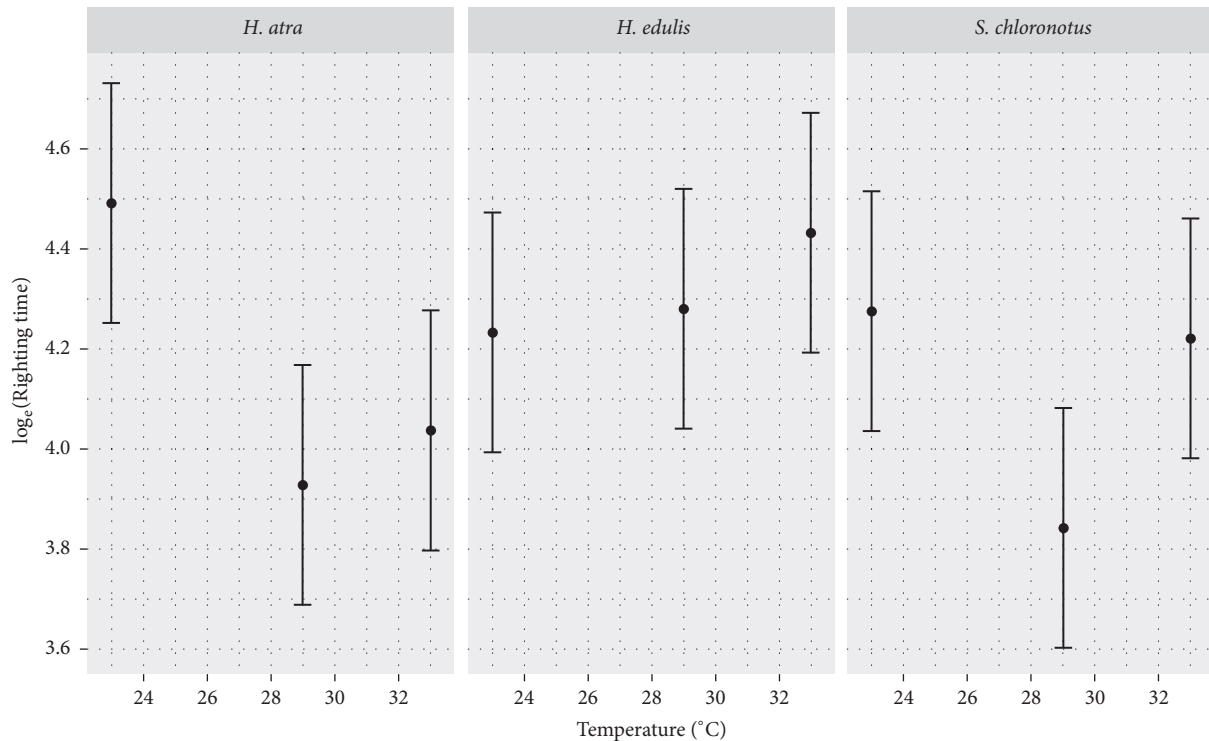
FIGURE 1: Effects of temperature on $\log_e$-transformed righting time within 95% confidence limits on the three sea cucumber species as estimated by the linear mixed model (see Table 2 for model specifics).

ability to adapt to above average warming could be limited. Lastly, while *Holothuria edulis* is most commonly present in tropical regions, it is also found in more subtropical regions than that of the two other species discussed [26–28]. *H. edulis* quite possibly experience the widest range of ocean temperatures as a species in comparison with *H. atra* and *S. chloronotus* which could explain their tendencies to perform similarly in all of the temperatures tested in this study.

The variations in righting times could also suggest that the reaction norms for key physiological functions, as approximated by our experimental assay, differ among the three species. Thus, at the future temperature ranges predicted by climate change models [19], these sea cucumbers may be affected differently. In a similar study using righting time to evaluate the stress level of sea urchins, each species tested responded to warming waters differently as well, exhibiting varied thermal tolerances [14].

In the past, ocean warming has occurred as a gradual shift that allowed individuals to acclimate and populations to evolve. However, the rapid pace of modern climate change is potentially catastrophic for many species, particularly those with slower rates of evolution [29]. The ability of certain species to adapt to climate change impacts can be determined by evaluating their upper thermal tolerance limits, which biological systems set these limits, and the species acclimatization capabilities to ultimately alter their own thermal tolerances [30]. Some species of algae have been shown to respond through adaptation to changes in temperature over short periods of time (Lohbeck et al. 2012), aiding in their ability to overcome rapid temperature shifts.

Current evidence also suggests that terrestrial ectotherms exhibit some degree of plasticity with regard to upper thermal limits and the ability to respond to selection for increased temperature tolerances, albeit to a limited extent in the light of projected temperature changes [31]. For echinoderms, despite exhibiting some adaptive capabilities, the rate and success of these responses are also somewhat dependent on the rate of the water temperature change [14]. Therefore, the potential for holothuroids to adapt to rising ocean temperatures may be dependent on not only species, but also geographic location.

Previous research on juvenile sea cucumbers, *Apostichopus japonicas*, has suggested that increased exposure of parental generations to heat variations will increase the upper thermal limit of their offspring [32]. Similarly, transgenerational mechanisms could improve the tolerance of several holothuroid species during future warming events.

Our results, while illustrative, are not comprehensive. It is important to note that there were some limitations to the experimental design. The time the sea cucumbers had to acclimate to the experimental water temperatures was limited. Clearly climate change, while fast, is occurring at a slower pace than our experiments [33], perhaps allowing sea cucumbers to acclimate to higher water temperatures. Nevertheless, regional changes in ocean currents, for example, can lead to relatively rapid shifts in local sea temperatures, and this is potentially exacerbated by global average temperature increases. Certainly, the recently documented detrimental effects of climate change-driven coral bleaching events are due to short duration and quick, intense changes

in temperature [1], conditions that are somewhat mimicked in our experiments.

As stated previously, sea cucumbers will play a role in combatting climate change in benthic marine communities. Therefore, the variety of responses to warming waters by each species documented here either may contribute to resilience of benthic communities and help maintain their ecological role or will lead to ecological imbalances in the overall coral reef system. While some species may not be robust to ocean warming (e.g., *S. chloronotus*), other species that thrive in the higher temperatures may continue their ecological roles despite ocean warming (*e.g.*, *H. atra*). The species that thrive will be able to continue to balance out the calcium carbonate budget of the oceans while the species that are not as successful will likely face a variety of consequences.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

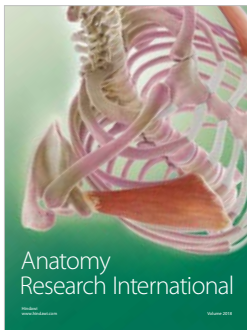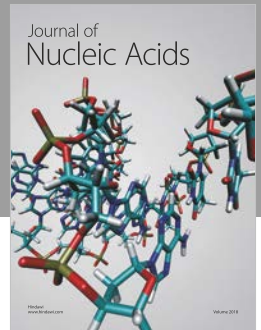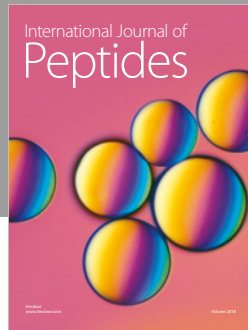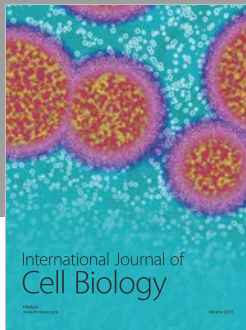The authors declare that they have no conflicts of interest.

Acknowledgments

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