Do Tropical Specialist Sea Urchins Have Higher Thermal Tolerances and Optimal Temperatures Than Their More Widely Distributed Relatives?

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Abstract

Warming tolerance (WT), thermal safety margins (TSM), and thermal performance curves have been documented empirically for few tropical marine invertebrates, although calculations of realized niches from biogeographic data suggest that WT is smaller for tropical organisms than for temperate organisms. To determine these characteristics for 8 species of Caribbean sea urchins, we documented the effects of acute heat stress and cold stress on righting time (a measure of performance) and survival. The upper lethal limit occurred between 35.1 and 37.1 °C for all of the species, and the upper limit for righting occurred very close to this between 34.0 °C and 36.9 °C. The lower lethal limit occurred between 4.8 °C and 7.6 °C for all species except for *Tripneustes ventricosus* where it was 14.6 °C. The lower limit for righting was significantly warmer than the lower lethal limit and occurred between 13.4 °C and 14.6 °C for all species except for *T. ventricosus* where it was 19.1°C. Within these critical limits, the thermal performance curves are broad and optimal performance windows range from 24°C and 32°C. Environmental data shows that in Bocas del Toro, Panama have warming tolerances of 6°C to 8°C for 2-hour exposures but that thermal safety margins range from -4°C to 2°C, highlighting the vulnerability of these species are to moderate environmental warming. Species with exclusively tropical ranges did not show higher optimal temperatures than species with ranges that extend into the subtropics or temperate regions.
Introduction

Caribbean sea surface temperatures (SST) are predicted to warm by 1.5-2°C before the end of the century (Nurse & Charlery 2016). It is fairly well documented how this might impact the distributions of corals and reef fishes (e.g., Bruns 1985, Precht & Aronson 2004, Carilli et al. 2010, Clauson-Kaas et al. 2017), but little information is available on how ocean warming may alter the distribution of other organisms from the region. Our goal was to compare the warming tolerance and thermal safety margins (Deutsch et al. 2008) of Caribbean sea urchins to determine if the species with exclusively tropical ranges are more heat tolerant or thermally specialized than are those species with ranges that extend through the subtropics.

To understand and predict organisms’ responses to changing environmental conditions and their biogeographic ranges it is necessary to document the breadth and shape of organism’s thermal niches or performance curves (Bozinovic & Naya 2014, Jablonski et al. 2013, Ling et al. 2009, Andersen et al. 2015, Hoffman et al. 2013; but see caveats in Sinclair et al. 2016). Thermal breadth, the range between the critical temperature limits (i.e., $CT_{\text{max}}$ and $CT_{\text{min}}$) at which the organisms fail to maintain the ability to perform or to survive (Dowd et al. 2015) is a coarse measure often assessed with short exposures to extreme temperatures (e.g., Walther et al. 2013). These are useful standard assays for comparing multiple species, and are particularly effective for species where it is logistically difficult to make more nuanced measurements (e.g., Nguyen et al. 2011, Compton et al. 2007). Critical temperatures may be good predictors of range limits for some species (e.g., Sunday et al. 2011, Andersen et al. 2015). In terrestrial organisms, critical temperatures vary among species with latitude, with upper limits often showing little variation with latitude, while lower limits change considerably with latitude producing wider thermal niches at higher latitudes (Addo Bediako et al. 2000). A similar pattern has been documented for fishes and other marine organisms (Sunday et al. 2012, Comte & Olden 2017, Stuart-Smith et al. 2017). Many of these data are based on realized thermal niches calculated from distribution data and not from empirical measurements as direct measures of $CT_{\text{max}}$ and $CT_{\text{min}}$ are both available for few species.

Direct measures of $CT_{\text{max}}$ may also be useful as predictors of which sudden extreme events could cause mortality in the field. For example, intertidal or shallow-water organisms may naturally experience short-term extreme changes in temperatures during low tides either during hot, midday exposures in the summer, or extreme cold exposures in the winter (Glynn 1968,
Anthony & Kerswell 2007, Beddingfield & McClintock 1994, Helmuth et al. 2002). Finally, warming tolerance (WT), the difference between the average temperatures experienced in the environment and the maximum critical temperature (Deutsch et al. 2008), is generally lower in the tropics than at higher latitudes (Comte & Olden 2017).

Within the thermal range of thermal tolerance, the relationship between performance and temperature is described by the thermal performance curve (Angilletta 2009). These curves are generated using easily quantified aspects of performance (sprint speed, consumption rate, etc.) measured across a range of temperatures, although in many cases too few temperatures are assayed to determine the shape of the entire curve (discussed in Dowd et al. 2015). Among marine invertebrates such curves have been used to compare: the effects of temperature on aerial and underwater performance among species (Tagliarolo & McQuaid 2015); swimming speeds and respiration rates among populations from locations with different thermal conditions (Walther et al. 2013); and developmental success among species from different latitudes (Karelitz et al. 2016). The optimal temperature $T_{opt}$ can be determined from the performance curves, and the thermal safety margin is calculated as the difference between the $T_{opt}$ and the environmental temperature (Deutsch et al. 2008). Thermal tolerance curves also provide important context to help select relevant temperature treatments for experimental work.

Tropical sea urchins play an important role as ecosystem engineers (Birkeland 1989; Steneck 2013). On reefs, grazing by herbivorous urchins suppresses macroalgal growth and helps to maintain healthy coral populations (Steneck 2013). Herbivory by urchins can be particularly important in the absence of herbivorous fishes and may help prevent phase shifts from coral dominated to algal dominated habitats (Jackson et al. 2014, Sangil & Guzman 2016). The activity of *Echinometra* species, which excavate depressions can contribute significantly to bioerosion on reefs (Asgaard & Bromley 2008, Bak 1994, Griffin et al. 2003, McClanahan and Muthiga 2013) and may also provide microhabitats and increase diversity of other invertebrates (Schoppe & Werding 1996). In seagrass meadows, *Lytechinus variegatus* may regulate seagrass biomass and meadow size, and overgrazing may permanently eliminate vegetation (Heck & Valentine 1995, Valentine & Heck 1991, Watts et al. 2013). Finally, irregular sea urchins can be important bioturbators and bioerosders, with *Clypeaster rosaceus* playing an important role in sand production (Telford et al. 1987). Their ecological importance suggests that changes in sea urchin distributions could significantly impact ecosystem function.
Our overarching goal is to understand the thermal tolerances of these species in the context of environmental conditions and species’ geographic ranges. Specifically, we test the following hypotheses: (1) Exclusively tropical species show higher warming tolerance and thermal safety margins than do species that also occur in the subtropics. (2) Species that occur in the subtropics have lower lethal limits under cooling than the exclusively tropical species. To address these questions, we documented the thermal breadth of each species by determining both the upper and lower critical temperatures (i.e., $CT_{\text{max}}$ and $CT_{\text{min}}$) for righting, a measure of performance, and for survival. To determine the shape of the performance curve we documented righting performance every 2°C between the thermal tolerance limits.

Materials and Methods

Study Species - We selected 6 species (Echinometra lucunter, E. viridis, Lytechinus variegatus, L. williamsi, Tripneustes ventricosus, and Euclidaris tribuloides) of common, ecologically important, shallow-water regular sea urchins to study righting time in detail and 2 species (Clypeaster rosaceus and C. subdepressus) of irregular sea urchins for which we tested thermal tolerance, but which can never turn themselves over due to their shape and lack of adhesive tube feet. These species all occur throughout the Caribbean but the extent of their ranges outside the Caribbean varies considerably (Table 1). Two of the species, E. viridis and L. williamsi are exclusively tropical, while their sister species E. lucunter and L. variegatus have ranges that extend significantly further south and north. Long-term monthly average sea surface temperature maps obtained from optimum interpolation (Reynolds et al. 2007) for the warmest and coolest months (August and February; Figure 1) show that the range limits for these species tend to occur where winter temperatures drop below 20°C. But that E. viridis and L. williamsi inhabit ranges where the water temperatures are unlikely to fall below 25°C (Figure 1).

All of the study species occur in shallow water in Bocas del Toro, and were collected by hand while snorkeling at less than 5 meters depth. In this area, the Echinometra species live on rocks and coral rubble in very shallow water, and may occur in only a few centimeters of water. Lytechinus variegatus lives in shallow Thalassia meadows while its sister species L. williamsi lives in crevices in shallow reefs. Euclidaris tribuloides lives under coral rubble and in crevices on reefs and Tripneustes ventricosus lives in seagrass meadows, among rubble and on shallow rocky reefs, but we collected them primarily from seagrass habitats. Animals were kept
submerged in buckets while they were transported to the Smithsonian Tropical Research Institute’s Bocas del Toro Research Station. They were maintained in 300-liter aquaria with flow-through seawater for no longer than 7 days before thermal performance trials.

_Tolerance and Performance Trials_ – Our chosen measure of performance is the speed of the righting response (Brothers & McClintock 2015, Sherman 2015, Challener & McClintock 2017). The righting response, the speed at which the animals can turn over from an inverted position, has been used for 80 years to assess the impacts of temperature on performance in echinoderms (Kleitman 1941, Farmanfarmaian & Giese 1963, Lawrence 1973, Lawrence & Cowell 1996, Brothers & McClintock 2015). Rapid righting is clearly important for fitness in animals that are dislodged. Righting involves the strong adhesion of tube feet and coordinated movement of tube feet and spines, which are also key components of other fitness related functions like movement, attachment to the substrate and covering (a behavior where the animal holds algae, seagrass or other debris over the dorsal surface) (Brothers & McClintock 2015, Sherman 2015, Challener & McClintock 2017). At extreme temperatures, animals lose the ability to right and eventually enter heat/cold-coma and ultimately die.

We tested the response of adult urchins from each species to a single 2-hour temperature exposure ranging from 6°C to 36°C. Each animal was only tested once. Prior to the test, they were maintained at ambient temperature (27-29°C) in the running seawater system. For each species, 3 sea urchins where tested in each trial and we conducted 3-6 independent trials per temperature for each species for a total of 9-18 urchins tested at each temperature. Prior to the exposure to the test temperature, the righting time of the urchin was recorded at the holding temperature. Animals were gently removed from the substrate, placed on their aboral side on a flat surface, and the time for them to turn over to the normal resting position was recorded. Animals with unusually long initial righting times were excluded from the trials.

The temperature was then raised or lowered gradually over 15 minutes to attain the desired temperature. After 2 hours at the test temperature the animal was overturned and the righting time was recorded again. If the urchin failed to right in an hour it was scored as being unresponsive. Unresponsive urchins could be in torpor (cold treatments), heat coma (hot treatments), or potentially dead. Urchins from temperatures where any animal failed to right, were allowed to slowly return to ambient temperature and given a day to recover in flow-through
holding tanks, and then scored as alive or dead. All trials were conducted in clean aquaria without materials that could be used for covering and with a smooth substrate, as these factors have been shown to influence righting times (Challener & McClintock 2017). We aimed to standardize the size of the sea urchins across trials for each species by collecting only animals in size range most typical for our collecting sites. Unusually large or small animals were excluded for the study. Because heating rate and exposure duration are not independent and because we wanted to standardize the static temperature exposure we used different heating rates to attain the experimental temperatures, which were then experienced for 2 hours regardless of treatment. This ensured that individuals in the more extreme treatments were not exposed to elevated temperatures for longer periods than those in more moderate treatments. Thermal tolerance studies use a variety of methods including ramping experiments, where the temperature increases gradually until the organisms die, to more static experiments where the test temperature is reached quickly and subsequently maintained. It should be noted that ramping experiments with low ramping rates often find lower thermal tolerance, most likely due to extended exposures (Peck et al. 2009, Vinagre et al. 2013, Nguyen et al. 2014, Faulkner et al. 2014). Studies that employ such vastly different methods cannot strictly be compared, however comparisons can be made between organisms that have been exposed to the same protocol in a single study.

To identify the $CT_{\text{max}}$ urchins were initially tested at 28 (ambient), 30, 32, 34, and 36°C. Once a temperature with 100% mortality was identified, we conducted an additional trial at 1°C below this temperature, in order to identify more precisely the $CT_{\text{max}}$. To identify $CT_{\text{min}}$ and to obtain the complete performance curve, urchins were tested at temperatures every 2°C from 28°C down to 6°C.

**Environmental Conditions** - Environmental temperature was monitored at a depth of approximately 5m (2m off the bottom) on the instrument platform at the Bocas del Toro Research Station (BRS). Data were logged at 15-min intervals by data-loggers attached to a Campbell Model 107 electronic temperature sensor. The platform is located over the seagrass bed where adult *L. variegatus* were collected for this study. Temperatures from this site are generally representative of other monitoring locations at similar depths in the region (Collin et al. 2009). To determine if extremely shallow water inhabited by *Echinometra* species is significantly warmer than the more open water over the seagrass we also recorded temperatures
at 0.25 m in a rubble patch at the base of the BRS dock, and at 0.5m depth on a shallow part of a Porites fringing reef at the small point across from the BRS. Echinometra viridis live in the rubble at the base of the dock and *E. viridis*, *L. variegatus* and *L. williamsi* all live in the *Porites* reef. Temperatures were recorded using a HOBO Stow-Away TidbiT and HOBO Water Temperature Pro V2 (Onset Computer Corporation, Bourne, MA) with an accuracy of 0.25 °C (Kaufmann & Thompson 2005) between October 15, 2015 and June 30, 2016.

To understand how temperatures measured in Bocas del Toro compare to temperatures in similar habitats throughout the Caribbean, we used temperatures measured weekly over coral, seagrass and mangrove habitats as part of the Caribbean wide CARICOMP project (Chollett et al. 2017; data available online at https://doi.org/10.1371/journal.pone.0188564.s002). These sites span much of the ranges of each species in the northern hemisphere. The maximum and minimum temperatures reported were compared to the critical temperatures of the urchins measured in this study.

**Statistical Analysis** - The CT\text{max} and CT\text{min} for righting and for survival were estimated as the LT\textsubscript{50}. That is, survival CT is the temperature at which 50% of the animals failed to survive and the righting CT is the temperature at which 50% of the animals failed to right. Each CT was estimated using logistic regression in JMP12 using the Fit Model menu and the inverse prediction option. In some cases where every temperature produced either 100% success or 100% failure the exact LT\textsubscript{50} with confidence limits could not be estimated in this way, and was therefore estimated using linear interpolation. Logistic regression with species and temperature as factors was used to determine if thermal tolerances of the species differed significantly. To find the optimal temperatures (T\textsubscript{opt}), performance curves were generated by fitting quadratic and biexponential curves to the time to right for each species using the nonlinear models implemented in JMP12. The inflection point, at the minimum time to right (i.e., optimal performance) was calculated using the first derivative of the function that provided the best fit to the data based on comparisons of AICc values. Finally, one-way ANOVA with post-hoc Tukey HSD tests was used to determine at which temperatures performance was statistically significantly reduced compared to the optimal temperature.

CT\text{max} and T\text{opt} were combined with environmental data to calculate the WT and TSM following Deutsch et al. (2008). We used 29°C, the grand average of the temperatures at the 2
reefs reported in Collin & Chan 2016), as the average environmental temperature to calculate WT and TSM. We calculated WT as the difference between the average habitat temperature and the lowest assayed temperature at which sea urchins experienced 50% failure to survive or to right. We calculated the TSM as the difference between the $T_{opt}$ identified by biexponential and quadratic curve fitting and the habitat temperature.

**Results**

**Thermal Tolerance**

*Thermal limits* – Survival $CT_{max}$ (temperature at which half of the animals died) and righting $CT_{max}$ (temperature at which half of the animals failed to right) were assayed between August 20 and October 17, 2016, when average daily water temperatures averaged 29.6°C (28.3°C - 31.0°C). Logistic regression showed that trial temperature and species both had a significant effect on the probability that the animal righted and survived (Tables 2 & 3). The survival $CT_{max}$ was 0.3-1.5°C higher than the righting $CT_{max}$ (Table 2; Figure 1). Post-hoc pair-wise comparisons among species showed that the two *Echinometra* species had significantly higher survival $CT_{max}$ (37.5°C for *E. lucunter* and 37.0°C for *E. viridis*) than any of the other species. *Eucidaris tribuloides* (36.5°C) had a survival $CT_{max}$ significantly higher than the remaining species and survival $CT_{max}$ for *Clypeaster rosaceus* (36.0°C) was higher than the last four species, which did not differ from each other (Table 2). Likewise, the righting $CT_{max}$ was significantly higher for the two *Echinometra* species than for the others, and it was significantly lower for *Tripneustes ventricosus* than for the remaining species (Table 2).

The lower thermal limits were assayed between February 18, 2016 and April 28, 2016, when the average daily water temperature was 29.2°C (27.8°C - 30.5°C). The range of low temperatures tested included the temperatures at which all urchins failed to right regardless of species. In only one species, *Tripneustes ventricosus*, did all of the animals fail to survive exposures at the coldest temperatures. Logistic regression analysis showed that trial temperature and species had a significant effect on the probability that the animal righted (Table 3). Post-hoc tests showed that the 2 species of *Lytechinus*, the 2 species of *Echinometra*, and *Eucidaris tribuloides* did not differ in the temperature at which they failed to right (righting $CT_{min} = 13.4$-14.6°C; Table 2; Figure 2), while *T. ventricosus* fails to right at a significantly warmer temperature ($LTL_{R50} = 19.1°C$; Table 2; Figure 2). *T. ventricosus* also showed a significantly
higher survival $CT_{\text{min}}$, at 14.1°C, while survival $CT_{\text{min}}$, for the two *Echinometra* species was 6.5-8°C. It was estimated to be 4.5-6.5°C for the other species. Overall the righting $CT_{\text{min}}$ is about 5°C warmer than the survival $CT_{\text{min}}$ (Table 2).

**Thermal Optima**

Thermal performance curves were determined for temperatures from 18°C to the upper thermal limit of each species. In three species (*E. lucunter*, *L. variegatus* and *L. williamsi*) both the quadratic and biexponential curves fit the data with a clear minimum righting time (Figure 4; Table 4). In *E. lucunter* and *L. variegatus* the two methods identified optimal temperatures within 1°C of each other, around 30°C and 27°C respectively. In *L. williamsi* the two methods produced quite different curves, but comparisons of AICc showed that the biexponential curve is a significant better fit and gives an $T_{\text{opt}}$ of 28°C (Figure 4; Table 4). In the other three species (*E. viridis*, *T. ventricosus*, and *E. tribuloides*) the biexponential curve was also the best fit, but minimum righting times could not be identified with confidence. Data from *E. viridis* and *E. tribuloides* both show very flat biexponential curves between 25 and 34°C (Figure 4; Table 4). Above 34°C *E. tribuloides* completely failed to right. For *E. viridis* those few that did right at 34°C did not take much longer than those that righted at cooler temperatures. Optimal temperatures estimated from the quadratic curves (which did not fit the data as well as the biexponential curves), gave optimal temperatures for righting as 28.5 and 29.5°C respectively. Finally, righting times in *T. ventricosus* were much slower than in the other species, probably due to their large size, and decreased consistently with decreasing temperature over the range where righting occurred. This suggests that the $T_{\text{opt}}$ is around 32°C, very close to the righting $CT_{\text{max}}$. As predicted based on other organisms (Dowd et al. 2015) all of the species had performance curves with $T_{\text{opt}}$ much closer to the $CT_{\text{max}}$ than to the $CT_{\text{min}}$.

Finally, one-way ANOVA showed that righting time at the optimal temperature did not differ significantly from righting time at several other temperatures in each of the species (Table 5; Figure 4). This suggests that for short exposures there is a functionally optimal window that ranges from around 26°C to 32°C in these species. However longer exposures or more challenging performance criteria may amplify differences and result in a narrower functional window.
Environmental temperatures, WT and TSM

The highest temperatures reported for seawater at the BRS and over a shallow reef in Bocas del Toro during 2006-2015 are around 31°C and seldom exceed 32°C, with 10 year average temperatures of 28.8°C and 29.3°C at the 2 sites studied (Collin & Chan 2016). Daily average temperatures registered at the BRS instrument platform during the present study in 2015-2016 ranged from 27.4-31.8°C. Comparisons of the 3 sites (platform, dock and Porites reef) between October 15, 2015 and June 30, 2016 showed little difference among sites (26.7-31.3°C and 26.7-32.3°C daily at the shallow sites; 27.7-31.3°C at the instrument platform) suggesting that microhabitat does not greatly alter thermal conditions. The CARICOMP monitoring data (Chollett et al 2017) show that Bocas del Toro is not unusually warm when compared to other Caribbean sites. Average temperature in Bocas del Toro measured weekly over coral, seagrass, and mangrove habitats with the CARICOMP protocol gives average temperatures of 28.5°C in all 3 habitats. Average temperatures at 13 of the 45 other sites were greater than or equal to those reported for Bocas del Toro.

Discussion

Are exclusively tropical species thermal specialists?

Two of the species studied here, *E. viridis* and *L. williamsi* are restricted entirely to the tropics. We wanted to test the hypothesis that their restricted ranges are due to limited cold tolerance, and that as warm-water specialists they have higher CT_max and T_opt than their sister species and the other species in the Caribbean fauna. However, our data do not support such a scenario for *Echinometra*. *E. viridis* does not differ significantly from *E. lucunter* in survival CT_max or righting CT_max (Table 2). In addition, T_opt is virtually the same when estimated with a quadratic equation and higher in *E. lucunter* when estimated with a biexponential equation (Table 4). Finally, CT_min is higher but not significantly higher in *E. lucunter* than in *E. viridis*. Similar results were obtained for *Lytechinus*. Neither CT_max or CT_min differed between *L. williamsi* and *L. variegatus* when either survival or righting were assessed. *L. williamsi* does have a higher T_opt than *L. variegatus* with the preferred biexponential fit, but the pattern is reversed with the slightly less robust fit generated by the quadratic fit (Table 4). Overall there is little evidence that the two species with the most limited tropical distributions have warmer thermal niches or performance curves, as their thermal optima and critical temperatures are not
substantively different from the other 6 species (Table 4) whose distributions extend into significantly cooler waters.

How does adult thermal performance relate to species ranges?

Distributions of the 8 species studied here overlap broadly. However, there are large differences in the species range endpoints. Many ranges end around North Carolina and Santa Catarina Island, Brazil, both of which experience average winter water temperatures around 20°C (Figure 1). For example, L. variegatus ranges from North Carolina to Southern São Paulo State in Brazil, consistent with the idea that winter temperatures limit the geographic range of this species. However, in several species one end of the range seems to be limited by winter temperatures, while the other end of the range occurs in a significantly warmer region. For example, the range of Clypeaster rosaceus ends in North Carolina in the north, but extends only as far south as Venezuela, although this species has been observed once in Brazil (Table 1; Hendler 1995, C. Cordeiro pers. com, 2016). The one species with a significantly warmer CT_{min} and high T_{opt}, Tripneustes ventricosus, does not have a particularly restricted range compared to the other species.

Recent meta-analyses have shown that, in general, marine species fill their thermal niches more completely than terrestrial species and that their range boundaries are close to those predicted by climatic data (Sunday et al. 2012). In addition, data suggest that species with large ranges the distribution is limited by temperature or climatic conditions, while those with more restricted ranges are more likely to have ranges limited by other factors (Sunday et al. 2015). Our data for Caribbean sea urchins supports this idea, as the species with restricted ranges do not show different thermal tolerances. The causes of the smaller ranges of E. viridis and L. williamsi remain unknown. However, some potential causes can be ruled out. These two species have similar larvae and larval durations to their sister species, suggesting that their dispersal abilities are roughly similar. Large river plumes have been suggested to influence sea urchin distributions in South America (Lessios et al. 2003), but depressed salinity from river outflows combined with different salinity tolerances cannot account for the differences in northern range end points of species with similar thermal tolerances.

Do environmental conditions expose sea urchins to stressful or lethal temperatures?
Extreme thermal events, either heating or cooling, can result in significant mortality of marine invertebrates, including sea urchins (Glynn 1968, Lawrence 1996, Beddingfield & McClintock 1994, Helmuth et al. 2002, Anthony & Kerswell 2007, Canning-Clode et al. 2011). It is therefore useful to know how the critical thermal tolerance limits of marine organisms relate to expected environmental temperature extremes. Our data show that tropical Caribbean sea urchins have $CT_{\text{max}}$ for righting between 34-37°C under short-term exposures, and similar $CT_{\text{max}}$ for survival. When available, assays of $CT_{\text{max}}$ for these species in other parts of the Caribbean are similar (Glynn 1968; Sherman 2015). This suggests that acclimation to local conditions does not drastically alter thermal limits, but few sites have been studied.

Likewise, detailed temperature information from sea urchin habitats are not widely available. Nevertheless, environmental monitoring data from Panama and the CARICOMP comparative dataset indicate that, as expected for locations with healthy sea urchin populations, environmental temperatures seldom exceed $CT_{\text{max}}$. However, it is likely that some shallow microhabitats with particularly low water exchange may reach or exceed these critical temperatures. For example, pools on reef flats in Puerto Rico experience temperatures $>34°C$ and invertebrate mortality has been observed when even higher temperatures are caused by mid-day low tides coincident with sunny weather (Glynn 1968). The same can be said for $CT_{\text{min}}$, which ranges from 5-15°C for survival and 13-19°C for righting. Such low temperatures are unlikely to be experienced in the Caribbean, but cold winter weather combined with low tides can drive water temperature to critical limits as far south as Florida (Beddingfield & McClintock 1994).

Understanding chronic exposures to suboptimal conditions that reduce performance may be more useful to predict population decline and/or geographic range shifts under gradual environmental warming scenarios than are critical temperatures. This idea is reflected in the thermal safety margin. Our performance curves give TSMs of -3°C to 2°C, evidence that these species are already experiencing suboptimal conditions. However, these results should be viewed with some caution. Primarily, the calculation of the TSM focuses heavily on the optimal temperature, but does not very well reflect the rate of change of performance around the optimum (Dowd et al. 2015). Since the performance curves recovered for Caribbean sea urchins are very flat the difference in righting performance over 2-3°C is not always large, making it unclear if such differences would result in notable changes in fitness, especially in light of the other environmental factors that can influence righting performance (Challener & McClintock...
However, it is clear that in many parts of the Caribbean water temperatures routinely exceed the optimal temperatures. Both the heating (or cooling) rate and the duration of the exposures used in this experiment may adequately model extreme events during low tides, but may not adequately model slower seasonal changes. Nevertheless, experiments with longer exposure durations show results consistent with our performance curves. For example, ten-day exposures of *L. variegatus* to 32°C reduce righting performance and reduce Aristotle’s lantern reflex compared to 28°C (Brothers & McClintock 2015). One-day exposures of *L. variegatus* to 31°C reduce ingestion efficiency and consumption rate compared to 29°C (Lemoine & Burkepile 2012) and one-day exposures to 34°C reduced righting compared to 28°C (Lawrence 1975). *Eucidaris tribuloides* exposed to different temperatures for 2 months showed reduced spermatogenesis at 30°C while other measures of performance were maintained at this temperature (Lares & McClintock 1991). Finally, *E. lucunter* acclimated for 7 days to 30°C showed little change in immunological function compared to those acclimated to 25°C (Branco et al. 2013). These results support our results that performance declines above 29-31°C and suggest that experiments based on short-term exposures may be fairly accurate at predicting temperatures at which longer-term studies will also detect reduced performance.

**Future Scenarios**

As Caribbean sea urchins are already living at temperatures above *T*_{opt}, it becomes important to assess WT in terms of future predicted warming scenarios. Tropical oceans have been predicted to warm by 2–3°C by the end of this century (IPCC 2007, Poloczanska et al. 2007, Ganachaud et al. 2011) and models of the Caribbean predict region-wide SST warming of 1.5-2°C (Nurse and Charlery 2016). In addition, models of sea surface temperatures for Bocas del Toro predict that average water temperatures will increase significantly with nighttime temperatures around 32-33°C in much of the shallow-water fringe of Bahia Almirante by 2080 (Li & Reidenbach 2013). This is an increase of 3-4°C over current average water temperatures. With our estimates of WT ranging from 5-8°C in Bocas del Toro, the warming predicted by Li & Reidenbach is likely to bring these species perilously close to their CT_{max} and to put them far enough above *T*_{opt} to show ecologically significant reductions in performance. Because acclimation or natural selection occurring over the course of the next few decades may be important in shaping how these
urchins respond to environmental warming (Díaz et al. 2017), more long-term studies focused on temperatures between the thermal optimum and the upper lethal limit could help us better predict if and/or when these key ecosystem engineers are likely to be extirpated from the warmest parts of their ranges.

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Collin et al., Page 21 of 29


**Table 1:** Study species, collection sites and range information

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Sites</th>
<th>Habitat</th>
<th>Northern Limit*</th>
<th>Southern Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echinometridae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Echinometra viridis</em></td>
<td>BRS Signpost (9.351N, 82.259W)</td>
<td>reef</td>
<td>Florida 29°N</td>
<td>Panama 9°N</td>
</tr>
<tr>
<td></td>
<td>Cristobal Point (9.299N, 82.291W)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital Point (9.334N, 82.218W)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Echinometra lucunter</em></td>
<td>Hospital Point</td>
<td>reef/rubble</td>
<td>North Carolina 35°N</td>
<td>Brazil 28°S §</td>
</tr>
<tr>
<td><strong>Toxopneustidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lytechinus variegatus</em></td>
<td>Cristobal Point</td>
<td>seagrass</td>
<td>North Carolina 35°N</td>
<td>Brazil 27°S §</td>
</tr>
<tr>
<td></td>
<td>Macca Point (9.256N, 82.156W)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lytechinus williamsi</em></td>
<td>BRS Signpost</td>
<td>reef</td>
<td>Florida 25° N</td>
<td>Panama 9°N</td>
</tr>
<tr>
<td></td>
<td>Hospital Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tripneustes ventricosus</strong></td>
<td>Macca Point</td>
<td>seagrass</td>
<td>Florida, ~25°N</td>
<td>Brazil 27°S §</td>
</tr>
<tr>
<td></td>
<td>Hospital Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clypeasteridae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clypeaster subdepressus</em></td>
<td>Cristobal Point</td>
<td>sand</td>
<td>North Carolina 35°N</td>
<td>Brazil 23°S §</td>
</tr>
<tr>
<td></td>
<td>STRI Point (9.347N, -82.262W)</td>
<td>sparse seagrass</td>
<td>South Carolina 33°N</td>
<td>Brazil 20°N</td>
</tr>
<tr>
<td><strong>Cideroidea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucidaris tribuloides</em></td>
<td>Hospital Point</td>
<td>reef/rubble</td>
<td>Cape Hatteras 34° N</td>
<td>Brazil 27°S §</td>
</tr>
</tbody>
</table>

* From compilations by Hendler 1995; Rodríguez-Barreras, R. (2014)

§ From ecological surveys in Netto et al. 2005; Labbé-Bella et al. (2016); C. Cordeiro, pers. com. (2016)
Table 2: Thermal tolerance window for the 8 Caribbean sea urchin species studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>LTL&lt;sub&gt;50&lt;/sub&gt; (95% ci)*</th>
<th>UTL&lt;sub&gt;50&lt;/sub&gt; (95% ci)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>survival</td>
<td>righting</td>
</tr>
<tr>
<td><em>Echinometra viridis</em></td>
<td>6.7°C (5.7-7.6)</td>
<td>13.4°C (12.5-14.4)</td>
</tr>
<tr>
<td><em>Echinometra lucunter</em></td>
<td>7.6°C (6.7-8.6)</td>
<td>14.3°C (13.3-15.3)</td>
</tr>
<tr>
<td><em>Lytechinus variegatus</em></td>
<td>6.1°C (5.1-7.1)</td>
<td>14.6°C (13.6-15.6)</td>
</tr>
<tr>
<td><em>Lytechinus williamsi</em></td>
<td>5.9°C (4.7-6.9)</td>
<td>14.3°C (13.3-15.3)</td>
</tr>
<tr>
<td><em>Tripneustes ventricosus</em></td>
<td>14.6°C (13.7-15.6)</td>
<td>19.1°C (18.1-20.2)</td>
</tr>
<tr>
<td><em>Clypeaster subdepressus</em></td>
<td>4.8°C (3.1-6.1)</td>
<td>NA</td>
</tr>
<tr>
<td><em>Clypeaster rosaceus</em></td>
<td>4.8°C (3.1-6.1)</td>
<td>NA</td>
</tr>
<tr>
<td><em>Eucidaris tribuloides</em></td>
<td>4.8°C (3.1-6.1)</td>
<td>13.7°C (12.7-14.6)</td>
</tr>
</tbody>
</table>

*LT<sub>50</sub> and 95% confidence intervals generated by the inverse prediction function from the logistic regression model for all of the species (Table 3) generated by JMP.

# In cases where no temperature generated a mix of success and failure, the confident interval cannot be estimated and the Likelihood models produce unrealistically wide estimates. The confident interval is therefore given as the range between the temperatures with 100% success and 100% failure.
Table 3: Logistic regression results for lower thermal limit (LTL) and upper thermal limit (UTL) of righting and survival. Bold highlights results with p < 0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>likelihood ratio chi-Square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTL - righting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>152.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>5</td>
<td>69.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N=243; generalized r² = 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTL - survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>95.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>7</td>
<td>154.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N=306; generalized r² = 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTL - righting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>820.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>5</td>
<td>121.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N=351; generalized r² = 0.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTL - survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>439.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species</td>
<td>7</td>
<td>178.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N=459; generalized r² = 0.93</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Thermal performance curves over temperatures from 18°C to 34°C for righting time for sea urchins that righted within an hour after a 2-hour exposure. The model preferred by the AICc is highlighted in bold for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>AICc</th>
<th>$r^2$</th>
<th>Optimum</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Echinometra viridis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>2015.06</td>
<td>0.51</td>
<td>27.4*</td>
<td>186</td>
</tr>
<tr>
<td>Quadratic</td>
<td>2071.07</td>
<td>0.42</td>
<td>29.5</td>
<td>186</td>
</tr>
<tr>
<td><em>Echinometra lucunter</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>1890.21</td>
<td>0.31</td>
<td>30.1</td>
<td>183</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1887.24</td>
<td>0.32</td>
<td>29.4</td>
<td>183</td>
</tr>
<tr>
<td><em>Lytechinus variegatus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>1908.09</td>
<td>0.41</td>
<td>27.2</td>
<td>162</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1915.10</td>
<td>0.37</td>
<td>27.4</td>
<td>162</td>
</tr>
<tr>
<td><em>Lytechinus williamsi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>1985.78</td>
<td>0.13</td>
<td>28.1</td>
<td>162</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1989.34</td>
<td>0.10</td>
<td>25.4</td>
<td>162</td>
</tr>
<tr>
<td><em>Tripneustes ventricosus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>1253.58</td>
<td>0.47</td>
<td>NA*</td>
<td>129</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1259.81</td>
<td>0.41</td>
<td>30.7</td>
<td>129</td>
</tr>
<tr>
<td><em>Euclidaris tribuloides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biexponential</td>
<td>1600.68</td>
<td>0.35</td>
<td>NA*</td>
<td>171</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1615.04</td>
<td>0.27</td>
<td>28.6</td>
<td>171</td>
</tr>
</tbody>
</table>

* Flat or virtually flat shape makes this a poor fit to the data.
NA = no inflection point present.
Table 5: One-way analysis of variance of the effect of temperature on log (righting time) for sea urchins that righted successfully during the test period.

<table>
<thead>
<tr>
<th>Species</th>
<th>DF</th>
<th>F-Ratio</th>
<th>p</th>
<th>Lower bound*</th>
<th>Upper bound#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinometra viridis</td>
<td>10</td>
<td>6.04</td>
<td>&lt;0.0001</td>
<td>30°C &gt; 24°C</td>
<td>30°C &gt; 36°C</td>
</tr>
<tr>
<td>Echinometra lucunter</td>
<td>9</td>
<td>9.35</td>
<td>&lt;0.0001</td>
<td>26-34°C &gt; 22°C</td>
<td>26-34°C &gt; 36°C</td>
</tr>
</tbody>
</table>
| Lytechinus varie
gatus | 8  | 11.04   | <0.0001| 24-32°C > 22°C | 24-32°C > 34°C |
| Lytechinus williamsi| 8  | 11.04   | <0.0001| 28°C > 26°C  | 28°C > 32°C  |
| Tripneustes ventric
cus | 8  | 10.65   | <0.0001| 28-32°C > 26°C | 28-32°C > 34°C |
| Eucidaris tribuloid
es | 8  | 4.64    | <0.0001| 24-34°C > 20°C | 24-34°C > 36°C |

* The lower limit of the optimal temperature range was evaluated by comparing down from the optimal temperature until a temperature at which the Tukey HSD post-hoc test showed a significant difference; all comparisons are shown in Figure 4.

# The upper limit of the optimal temperature range was evaluated by comparing up from the optimal temperature until a temperature at which the Tukey HSD post-hoc test showed a significant difference; all comparisons are shown in Figure 4.

% If the next temperature tested was one at which all urchins failed to right or at which so few failed to right that the confidence limits were unrealistically large, that temperature was considered to be significantly worse. The > indicates that performance is better (i.e., righting time is significantly faster).
Table 6: Calculation of Warming Tolerance (WT) and Thermal Safety Margin (TSM) for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Warming Tolerance (CT&lt;sub&gt;max&lt;/sub&gt; − T&lt;sub&gt;hab&lt;/sub&gt;) survival</th>
<th>Warming Tolerance (CT&lt;sub&gt;max&lt;/sub&gt; − T&lt;sub&gt;hab&lt;/sub&gt;) righting</th>
<th>TSM (T&lt;sub&gt;opt&lt;/sub&gt;−T&lt;sub&gt;hab&lt;/sub&gt;) biexponential</th>
<th>TSM (T&lt;sub&gt;opt&lt;/sub&gt;−T&lt;sub&gt;hab&lt;/sub&gt;) quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinometra viridis</td>
<td>8°C</td>
<td>8°C</td>
<td>-1.6°C</td>
<td>0.5°C</td>
</tr>
<tr>
<td>Echinometra lucunter</td>
<td>7.5°C</td>
<td>7°C</td>
<td>1.1°C</td>
<td>0.4°C</td>
</tr>
<tr>
<td>Lytechinus variegatus</td>
<td>5.9°C</td>
<td>5.5°C</td>
<td>-1.8°C</td>
<td>-1.6°C</td>
</tr>
<tr>
<td>Lytechinus williamsi</td>
<td>6°C</td>
<td>5.5°C</td>
<td>-0.9°C</td>
<td>-3.6°C</td>
</tr>
<tr>
<td>Eucidaris tribuloides</td>
<td>7.6°C</td>
<td>5.7°C</td>
<td>NA</td>
<td>-0.4°C</td>
</tr>
<tr>
<td>Tripneustes ventricosus</td>
<td>5.9°C</td>
<td>5°C</td>
<td>NA</td>
<td>1.7°C</td>
</tr>
<tr>
<td>Clypeaster rosaceus</td>
<td>7°C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Clypeaster subdepressus</td>
<td>6.1°C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
**Figure Legends**

**Figure 1:** Average sea surface temperature map for the western Atlantic for February and August. Image provided by Physical Sciences Division, Earth System Research Laboratory, NOAA, Boulder, Colorado, from their Web site at [http://www.esrl.noaa.gov/psd/](http://www.esrl.noaa.gov/psd/). Arrows indicate the areas where most of the ranges end points occur for the species in this study and stars indicate where the ranges of the tropical specialists, *E. viridis* and *L. williamsi* end along the continental coastlines (although the southernmost point in the ranges occurs in Panama).

**Figure 2:** $C_{T_{\text{max}}}$ of 8 species of sea urchins. Squares and diamonds show the percentage of the 9 urchins tested at each temperature that survived (white diamonds) or were able to right (black squares).

**Figure 3:** $C_{T_{\text{max}}}$ of 8 species of sea urchins. Squares and diamonds show the percentage of the 9 urchins tested at each temperature that survived (white diamonds) or were able to right (blue squares).

**Figure 4:** Performance curves for the righting of 6 species of sea urchins between 18°C and 34°C or 36°C. The blue line shows the biexponential curve fit through the data and the green line shows the quadratic curve fit through the data. Letters below each cluster of points shows the results of the Tukey HSD test, where temperatures not linked by the same letter differ significantly. Between 12 and 18 individuals were tested at each temperature.