Physiological Responses to Thermal and Hypersaline Stress in Sea Urchins *(Lytechinus variegatus)*

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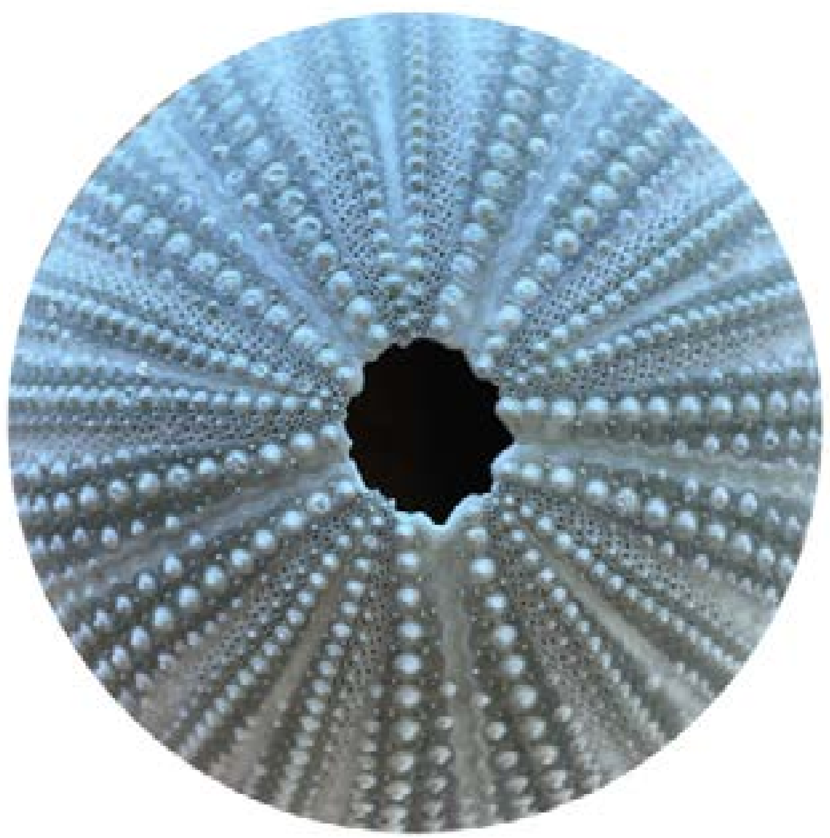
Article Synopsis

As marine heatwave events become a frequent reality in the Atlantic Ocean and globally, it is vital to understand organismal responses, particularly of species with ecosystem-wide impacts like grazing sea urchins. To protect and promote resilience of key marine species, there first must be knowledge on the impact of climate change stressors on a basic physiological level. This paper provides an insight into the decline in respiration for urchins experiencing increased temperature or increased salinity relative to urchins experiencing the typical conditions. While respiration dropped significantly by day five of exposure to both of these stressors, the righting response time, or time it takes to turn right-side-up, did not change significantly. This suggests that the decay in health associated with heatwave stressors does not occur on all fronts all at once. Alarmingly, the high heat treatment exhibited 100% mortality within 3 days. This high heat treatment's conditions were derived from the average daily maximum temperature at the site of the study's urchin population – giving a glimpse into the potential losses that come with a rapidly warming ocean.



Graphic by Ava Kocher

Physiological Responses to Thermal and Hypersaline Stress in Sea Urchins *(Lytechinus variegatus)*

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Abstract

Rising global surface and ocean temperatures threaten to drastically alter the environmental conditions for marine organisms, via ambient changes and heatwave events. To understand the impact of marine heatwaves on invertebrate physiology, this study investigated how exposure to thermal and hypersaline stress affects the physiology of *Lytechinus variegatus*, as measured by respiration rate and righting response. Urchins were collected and held in seawater tables at four heat and salinity treatments, with five replicates per treatment. The respiration rate and righting response variables were measured over the course of six days of treatment exposure. 100% mortality occurred in the high heat treatment within three days. Both the intermediate heat and hypersaline treatments exhibited significant decreases in respiration rates on day five, while the control treatment stayed steady. Righting response times showed no significant differences between treatments or between days of exposure. These results suggest *L. variegatus* tolerance to heat and saline stress events is physiologically complex and duration-dependent.

Introduction

The rate of ocean warming has doubled in the past 20 years, from 0.58 watts/m2 to 1.05 watts/m2 (von Schuckmann et al. 2024). Marine heatwaves are becoming more frequent and intense (Capotondi et al. 2024). Marine heatwaves are associated with global declines in foundation species (Smith et al. 2024). Moreover, lengthened periods of drought from climate change could produce salinization across intertidal ecosystems with potential detriments on osmotic functioning of invertebrates like echinoderms (Röthig 2023). Sea urchins, in the phylum Echinodermata, often inhabit shallow or intertidal zones, areas particularly vulnerable to intense impacts from marine heatwaves (Smith 2023).

This study investigated the impact of simulated marine heatwave and drought events on the urchin *Lytechinus variegatus*, through high temperature and hypersaline conditions. Collected at its northernmost range in North Carolina, *L. variegatus* ranges from Brazil to North Carolina (Watts et al. 2001). The species inhabits shallow waters in seagrass beds and rocky substrates (Watts et al. 2001). Commensal communities including polychaetes, nematodes, amphipods, and copepods have been found within spine canopies (Bell & McClintock 1982). There has been evidence of this species regulating seagrass growth (Valentine & Heck 1991). This species was selected based on its abundance at the study site, broad distribution in the Western Atlantic Ocean, as well the existence of research in the literature on thermal stress and tolerance for this species in its tropical range but a gap in research on climate change stressors on the species at its northern range in temperate waters (Brothers & McClintock 2015, Collin 2016).

The impact of the heat stress on the physiology of *L. variegatus* has been studied in a southern population in Florida (Brothers & McClintock 2015). This southern population has a greater exposure to high heat events and thus may be differently genetically and epigenetically equipped to deal with heatwave events than the colder clime population of this paper. This paper studies the physiological impact of heat and saline stress events on *L. variegatus* collected from a population at the northernmost range. Increased thermal stress has been shown to drastically limit the functioning and reproduction of urchins of other species, as compared to other marine climate-change stressors such as decreased pH (Byrne et al. 2009). Increased salinity has been shown to be associated with drought periods in marine environments, with the stressor becoming more frequent and intense with climate change (Tweedley et al. 2019). Respiration rate has been used as a physiological metric and proxy for metabolism (Stuart et al. 1981, Fujiwara et al. 2000, Sin et al. 2019). Righting response, the duration of return to normal position, has been used to approximate organismal health and survivability for echinoderms (Brothers & McClintock 2015, Zhang et al. 2017, Détrée et al. 2023). The study asks how exposure to thermal and hypersaline stress affects the physiology of *L. variegatus*, as measured by respiration rate and righting response.

Methods

20 urchins were collected from Atlantic Beach, NC at the site of the Duke Oyster Farm (34.8708°N, 0.7059°W). Temperature and salinity parameters for the control, heat, and saline treatments were derived from data on the conditions recorded over the course of Summer 2024 at the Duke Oyster Farm by the Bass Connections team 'Climate Change Impacts on Farmed and Wild Oysters.' The control treatment corresponded to the overall average temperature, the high heat treatment corresponded to the average daily maximum temperature, the intermediate heat treatment fell between the overall average temperature and the average daily maximum temperature, and the hypersaline treatment corresponded to the average daily maximum salinity. These site-specific parameters were chosen to represent the actual environmental conditions experienced by this population of *L. variegatus* from May - June 2024.

Over 48 hours, the urchins were acclimated from the March ambient seawater temperature of 16°C to the treatment conditions in static seawater tables. With water changes every other day, aquarium pumps were used to circulate the water and temperature was maintained with an aquarium heater. A YSI Multiparameter Meter was used twice daily to ensure constant pH, dissolved oxygen, temperature, and salinity levels.

Four experimental treatments were tested: a control treatment at 25.7°C and 35 parts per thousand (ppt) salinity, a high heat treatment at 32°C and 35 ppt, an intermediate heat treatment at 28°C and 35 ppt, and a hypersaline treatment at 25.7°C and 38 ppt. There were 5 randomly assigned replicate individuals per treatment, with the treatment lasting 6 days. To avoid stressing the specimens by collecting two sets of data daily, an alternating approach was taken, with respiration rate measured on days 1, 3, and 5 and righting response measured on days 2, 4, and 6.

To collect respiration rate, dissolved oxygen was measured every 10 minutes for 2 hours in a sealed glass vessel with a PreSens fiber-optic respirometer. Each vessel contained one of the 5 replicates. Additionally, for each treatment, a blank vessel with seawater from the treatment tank was measured, to account for any non-urchin related respiration and oxygen consumption happening in the background seawater. When graphed, the slope of each replicate was recorded as the unadjusted respiration rate, in mg O2/L/min (Figure 1).

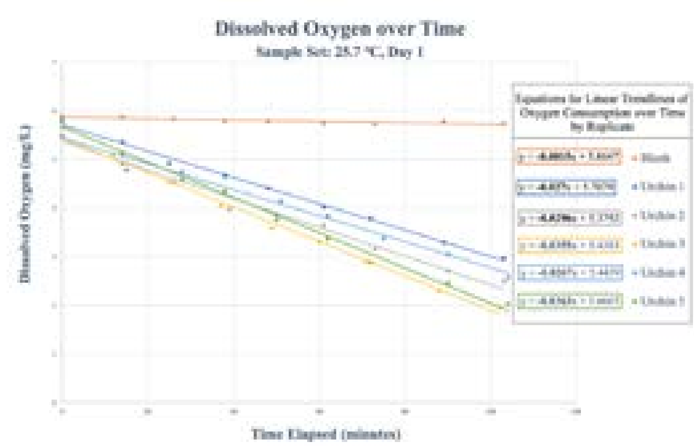

Figure 2. Decay of dissolved oxygen over elapsed time for the control treatment (25.7°C), day 1. The slopes from each replicate's linear trendline, in bold on the right, were used to calculate average mass-specific respiration rates for days 1, 3, and 5 of each treatment.

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Using the formula below, the unadjusted respiration rates were then manipulated to account for the volume of seawater in the respirometry vessel, the mass of the urchin, and background respiration rate from the blank vessel, to produce a mass-specific respiration rate in mg O2/hr/g, which was used for all data analysis and results.

$$\textit{mass specific respiration rate}\ (mg\ O_2/hr/g) = \frac{\left[(|slope| \cdot V_1) - (|blank| \cdot V_b)\right] 60}{mass}$$

slope = unadjusted respiration rate for specimen (mg O2/L/min)
V_1 = volume of seawater in respirometry vessel of specimen (L)
blank = unadjusted respiration rate for blank vessel (mg O2/L/min)
V_b = volume of seawater in respirometry vessel of specimen (L)
mass = mass of urchin specimen (g)

Righting response was measured via stopwatches capturing the duration of time taken for a specimen to "right" itself: starting upon placement on the aboral side and stopping once the urchin fully returned to its oral side completely flush with the tank substrate. Righting response was measured once per urchin for each recording.

Anova tests and paired Tukey tests were run in RStudio to determine any significant differences in respiration rate and righting response time according to both treatment and day.

Results

The high heat treatment of 32°C exhibited high mortality rates, with 100% survival on day 1, 40% survival on day 2, and 0% survival on day 3 (Fig. 2). Mortality was surmised on the basis of unresponsiveness of tube feet, rapid and widespread loss of spines, and degradation of the tissues surrounding the skeletal test. Mortality only occurred in the high heat treatment, where it was recorded for all 5 replicates, before the treatment period ended. This mortality prevented the collection of respiration rate on days 3 and 5 as well as the collection of any righting response times for the high heat treatment.

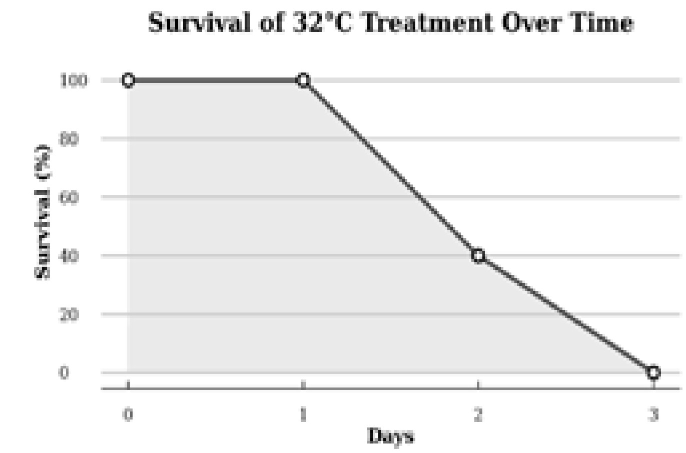

Figure 2. Survival of replicates in the high heat treatment drops from 100% on day 1 to 0% day 3.

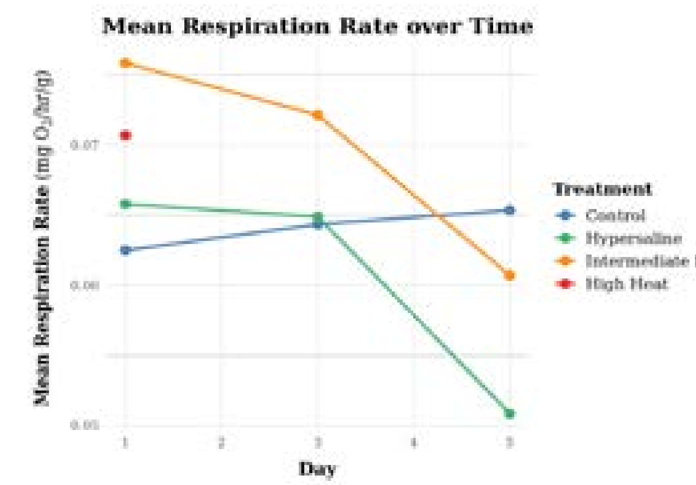

Figure 3. Mean mass-specific respiration rates across 5 days for each treatment. Note that the high heat treatment only has respiration rates from day 1 as explained by the mortality by day 3 depicted in figure 2.

The mean respiration rate was highest for urchins in the warm treatment (Fig. 3), with a mean Day 1 rate of 0.0758 mg O2/hr/g, compared to the urchins in the control treatment, with a mean Day 1 rate of 0.0625 mg O2/hr/g. The respiration rate of the control urchins rose slightly over the course of the treatment, from 0.0625 mg O2/hr/g to 0.0653 mg O2/hr/g from day 1 to day 5, a 0.028 mg O2/hr/g increase (Fig. 3), although this change was not significant (Fig. 4).

Respiration rates of both the intermediate heat and hypersaline urchins dropped slightly from day 1 to day 3 with a 0.0037 mg O2/hr/g and 0.0009 mg O2/hr/g decrease respectively, a nonsignificant difference

(Fig. 4). Respiration rates of both the intermediate heat and hypersaline urchins dropped more dramatically from day 3 to day 5 with a 0.0114 mg O2/hr/g and 0.0140 mg O2/hr/g decrease respectively (Fig. 4). The day 3 to day 5 differences were significant for the intermediate heat and hypersaline treatments (p-value = 0.049 and 0.025).

Both hypersaline and intermediate heat urchins exhibit significant differences in respiration rates between the rates for day 1 and 5 (p-value = 0.037 and 0.004) (Fig. 4). Respiration rates for urchins from combined treatments exhibit significant differences between day 1 and 5 (p-value = 0.018) and day 3 and 5 (p-value = 0.030) (Fig. 4).

Figure 5 visualizes the differences across treatments, broken down by day of treatment. Note that the high heat treatment data only appears in visualization of the combined days and of day 1, due to the complete morality by day 3 for this treatment.

The combined days exhibit a significant difference in respiration rates only between the hypersaline and intermediate heat treatments, (p-value = 0.027). There are no significant differences in respiration rates between treatments on days 1 and 3. Day 5 exhibits a significant difference in respiration rates between the control and hypersaline treatments (p-value = 0.033).

Righting response times ranged from 25.16 seconds to 1548.05 seconds, with a mean of 198.45 sec and a median of 125.99 sec (Fig. 6). The Anova test found no significant effect of either treatment or day on righting response times.

Discussion

The complete mortality of urchins within the 32°C treatment in this study compared to the survival, albeit with reduced righting response functioning, of a Florida population of the same species in a 10-day 32°C treatment by Brothers and McClintock suggests the potential for differential thermal stress tolerances between the southern range of the species in FL and

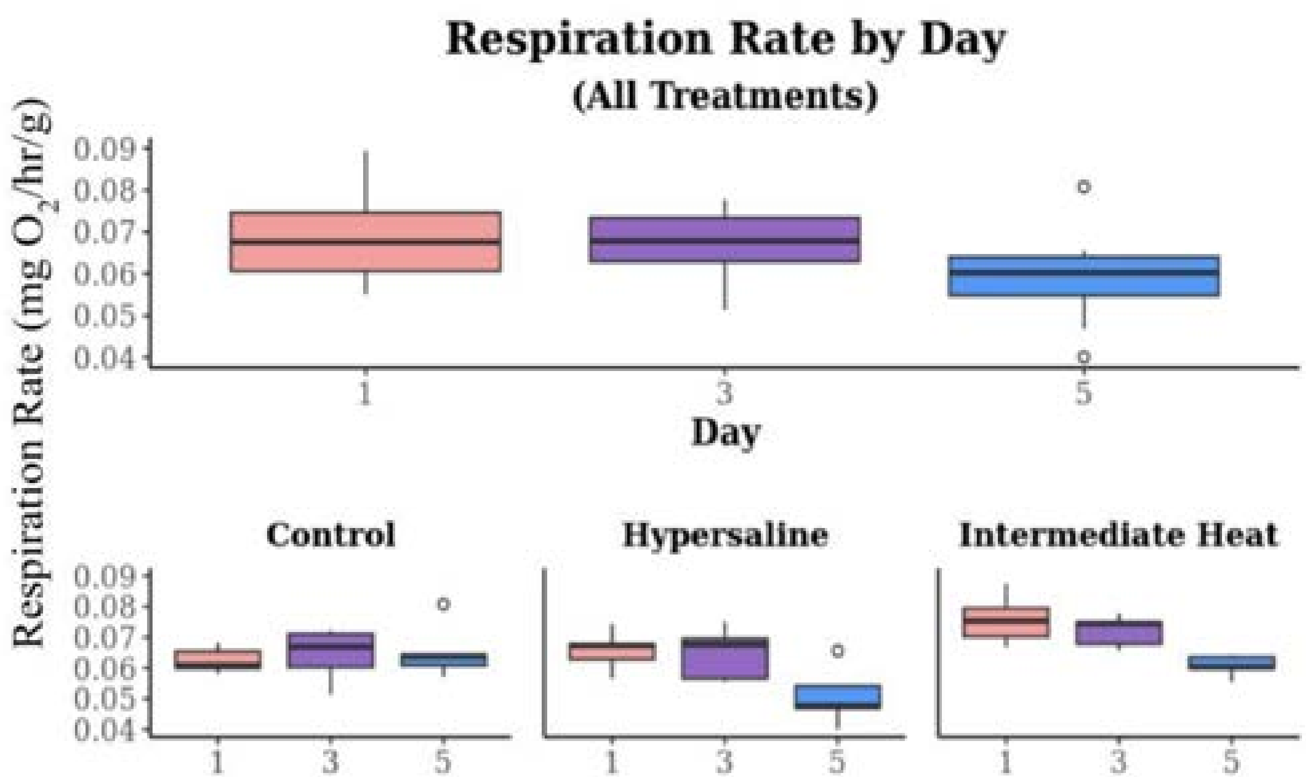

Figure 4. Respiration Rate by Day Box Plots

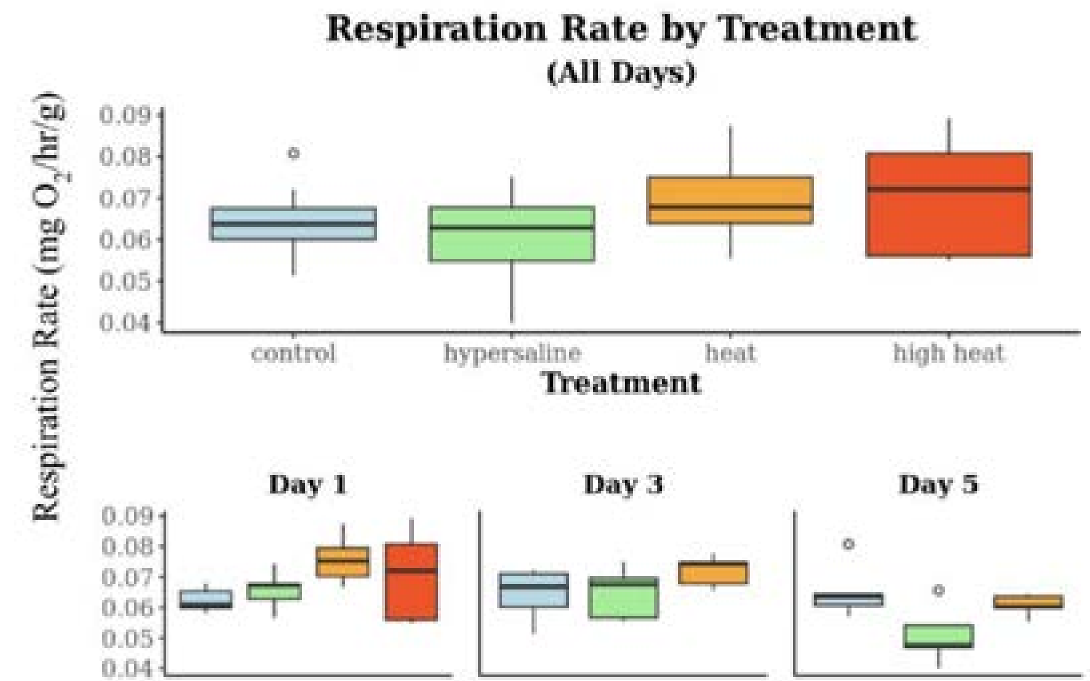

Figure 5. Respiration Rate by Treatment Box Plots

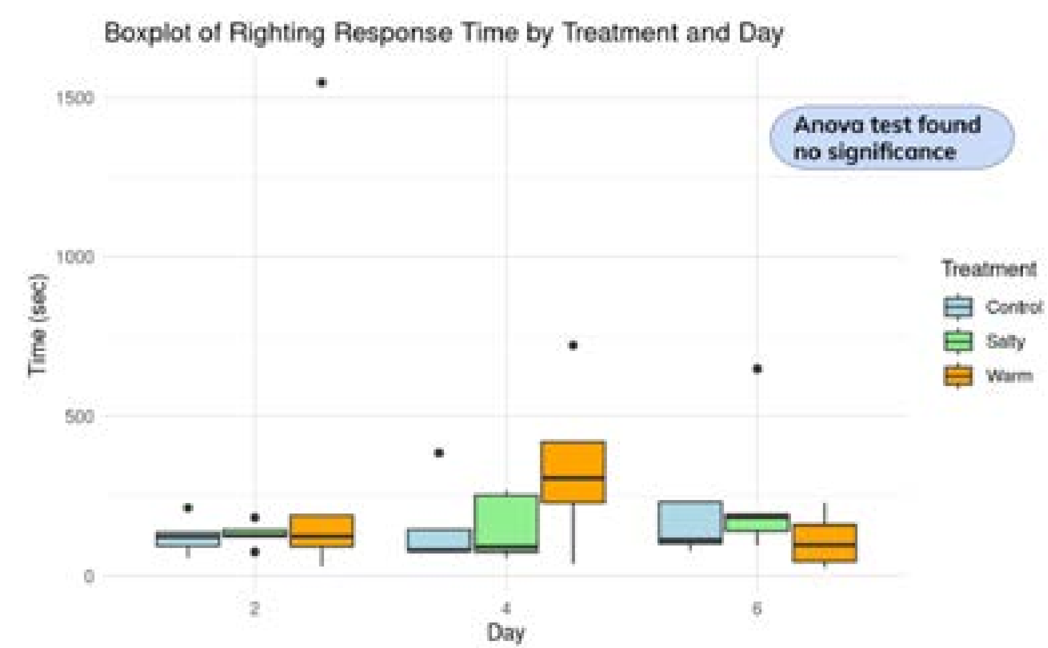

Figure 6.

the northern range of the species in North Carolina (Brothers and McClintock 2015).

The total mortality of urchins in this study at 32°C and maintained righting response functioning of urchins at 28°C suggests a thermal tolerance threshold, in which the urchins can handle intermediate heat stress events.

The higher respiration rate of the intermediate heat urchins compared to the urchins in other treatments on days 1 and 3 could be due to increased metabolic rates at higher temperatures, in which chemical reactions occur at faster rates. Both the intermediate heat and hypersaline urchins exhibited significant decreases in respiration rates on day 5, while the control urchin respiration rates stayed steady. This "day-5-dropoff" effect suggests that the duration of the stressor impacts the tolerance, with respiration rate taking a dive after 5 days of exposure.

Respirometry data found significant changes in respiration rate both across treatments and across days, whereas righting response showed no significant changes across either variable. This pattern suggests a decline cascade in urchin physiology, with respiration decreasing before failure of the righting function in the water vascular system.

A factor to consider in the interpretation of these responses to simulated heatwave conditions is the adjustment from colder March seawater to treatments representing stressors of a summer heatwave. The ambient seawater in the flow-through tanks that the urchins were kept in prior to the study was recorded at a temperature of 16°C. While 48 hours was built into the study to incrementally bring the urchins up to the control temperature of 25.7°C to avoid the shock response of a sudden transition, this still does not represent the slow warming from March average temperatures to summer average temperatures. The suddenness, or 'steepness' of thermal stress plays into the organismal response and could shift the thermal tolerance threshold.

The conclusions of this study are limited by the lack of data on individual urchins across treatments. This study did not attempt to divide the replicate individuals to produce results tied to individual identity. Moreover, different urchins were used for each treatment to avoid overstressing, meaning that individual variance complicates conclusions drawn across treatments. Additionally, only 5 replicates were used per treatment. More replicates would produce more statistically powerful conclusions.

Future research could implement dividers between replicates to connect response data to individuals. More frequent respirometry would illuminate the decay as urchins decline after prolonged exposure to stressors. A subsequent study could investigate the effect of the interaction between heat and salinity stress via treatments that adjusted temperature and salinity simultaneously, potentially adding hyposaline conditions as well. Future research could address the urchins' ability to recover after stress events by extending the period of data collection past the period of altered conditions. More work needs to be done to understand how heatwave intensity, duration, and steepness factor into organismal responses and resilience.

Conclusion

This study explored gender disparities in satTo protect and promote resilience of key marine species, there first must be a knowledge base around the impact of climate change stressors on a basic physiological level. This study examined the indicators of respiration rate and righting response to measure urchin health through five-day treatments of thermal and hypersaline stress. Complete mortality at 32°C and maintained righting response at 28°C suggest a thermal tolerance threshold relative to population within a species, as compared to the complete survival of the 32°C 10-day treatment of a Florida population of the same species. Both the intermediate heat and hypersaline conditions exhibited a decline in respiration rate on the fifth day relative to the first and third, suggesting that duration of the stressor impacts physiological response. As marine heatwave events become a frequent reality in the Atlantic Ocean and the global ocean basin, it is vital to understand organismal responses, particularly species with ecosystem-wide impacts like grazing urchins.

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