Running title: Compound extremes induce mortality

Title: Compound extreme events induce rapid mortality in a tropical sea urchin

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Biological Bulletin, July 6, 2022

Keywords: marine heatwaves, hypoxia, ocean deoxygenation, metabolism, global change biology, Caribbean, *Echinometra*, extreme events, multiple stressors, duration

Frequently used abbreviations and definitions:

LT - lethal time, where LT_{05} and LT_{50} are timepoints when either 5% and 50% of a test population experiences mortality under a given treatment condition, respectively

SMR – Standard Metabolic Rate

P_{crit} –Critical oxygen limit

MHW – marine heatwave

GLM – Generalized Linear Model

DO – Dissolved Oxygen, unit of oxygen concentration, presented in milligrams per liter (mg/L)

% AS– percent air saturation

kPa – Kilopascal, unit of oxygen partial pressure

SD – standard deviation

SE – standard error

STRI – Smithsonian Tropical Research Institute

BRS – Bocas Research Station

Abstract

The frequency, magnitude and duration of marine heatwaves and deoxygenation events are increasing globally. Recent research suggests their co-occurrence is more common than previously thought and that their combination can have rapid, dire biological impacts. We used the sea urchin *Echinometra lucunter* to determine if mortality occurs faster when deoxygenation events are combined with extreme heating (compound events), compared to deoxygenation events alone. We also tested if prior exposure to local heatwave conditions accentuates the impacts of compound events. Animals were first exposed for 5 days to either ambient temperature (28°C) or a warmer temperature that met the minimum criteria for a local heatwave (30.5°C). Animals were then exposed to hypoxia, defined as oxygen levels 35% below their average critical oxygen limit, combined with ambient or extreme field temperatures (28°, 32°C). Subsets of animals were removed from the hypoxic treatments every 3 hours for 24 hours to determine how long they could survive. Prior exposure to heatwave conditions did not help or hinder survival under hypoxic conditions, and animals exposed to hypoxia under ambient temperatures experienced little mortality. However, when hypoxia was coupled with extreme temperatures (32°C), 55% of the animals died within 24 hours. On local reefs in our Panama study site, compound conditions always manifested during marine heatwaves, with all four events occurring during the heatwaves in 2018. These results show short durations (~1 day) of compound events can be catastrophic and warming-driven increases in their duration will severely threaten sea urchin populations.

Spanish Abstract

La frecuencia, magnitud y duración de las olas de calor marinas y los eventos de desoxigenación están aumentando a nivel mundial. Investigaciones recientes sugieren que su coocurrencia es más común de lo que se pensaba anteriormente y que su combinación puede tener impactos biológicos rápidos y nefastos. Usamos el erizo de mar *Echinometra lucunter* para determinar si la mortalidad de estos ocurre más rápido cuando los eventos de desoxigenación se combinan con un calentamiento extremo (eventos compuestos), en comparación con los eventos de desoxigenación solos. También probamos si la exposición previa a las condiciones locales de olas de calor acentúa los impactos de los eventos compuestos. Primero se expusieron a los animales durante 5 días a temperatura ambiente (28 °C) o una temperatura más cálida que cumpliera con los criterios mínimos para una ola de calor local (30.5 °C). Luego, los animales se expusieron a hipoxia, definida como niveles de oxígeno un 35 % por debajo de su límite de oxígeno crítico promedio, combinado con temperaturas ambientales o de campo extremas (28°, 32°C). Se retiraron los subconjuntos de animales de los tratamientos hipóxicos cada 3 horas durante 24 horas para determinar los tiempos de supervivencia. La exposición previa a condiciones de olas de calor no ayudó ni obstaculizó la supervivencia en condiciones hipóxicas, y los animales expuestos a hipoxia en temperatura ambiente experimentaron poca mortalidad. Sin embargo, cuando la hipoxia se combinó con temperaturas extremas (32 °C), el 55 % de los animales murió en 24 horas. Las condiciones compuestas en nuestro sitio de estudio en Panamá fueron poco frecuentes y cortas (3 h). Pero cuando ocurrieron, fueron durante olas de calor marinas, con un total de cuatro eventos compuestos observados en los arrecifes locales durante las olas de calor en 2018. Estos resultados muestran que las duraciones cortas (~1 día) de eventos

compuestos pueden ser catastróficas y aumentar debido al calentamiento, en su duración amenazarán severamente las poblaciones de erizos de mar.

Introduction

In coastal waters low pH, low oxygen and high temperature, the three marine environmental parameters that are changing most with climate change are now commonly co-occurring stressors (Gobler and Baumann, 2016; Bopp *et al.,* 2013; Boyd *et al.,* 2014; Breitburg *et al.,* 2015), and they are often correlated in their severity (e.g., Guadayol *et al.,* 2014; Cyronak *et al.,* 2019). It is difficult to predict organismal responses to these co-occurring stressors. This is in large part because organismal responses to stressors are context dependent. Data are urgently needed to understand how organisms' environmental histories impact their responses to stressors, how stressors, which do not occur in isolation, interact in complex ways with each other, and how organisms respond to infrequent extreme stressor events that mimic real-world intensities and durations (Bopp *et al*., 2013; Jackson *et al*., 2021). Such knowledge is particularly lacking for tropical marine systems and for marine invertebrates in general.

Prior experience of a stressor can alter an organisms' subsequent response to that stressor. It can either help or hinder an organism's ability to cope with that stressor through a broad suite of mechanisms including but not limited to phenotypic plasticity, transgenerational plasticity, and genetic adaptation (Merilä and Hendry, 2014; Chevin and Hoffmann, 2017). Even after prior exposures that are short in duration, these mechanisms can improve the outcome of an organism to subsequent exposures. For example, after a 7-10 day exposure to elevated temperatures, the reef-building coral *Acropora nana* is more likely to survive subsequent exposure to extreme high temperatures (Bay and Palumbi, 2015). A recent meta-analysis of within species variation in 19 species of marine, terrestrial and freshwater ectotherms demonstrated that the magnitude of this kind of plastic response to temperature was related to the overall thermal tolerance of the population, such that that populations with greater thermal tolerance had lower plasticity in response to warming (Barley *et al.*, 2021). A similar pattern was found among species, with nine nudibranch species from the Pacific coast of North America showing with thermal plasticity negatively correlated with heat tolerance (Armstrong *et al.*, 2019). These studies are limited in their geographic extent, but as thermal tolerances are generally high in the tropics, these results might suggest, in contrast to the results of Bay and Palumbi (2015), that tropical species may have limited plasticity in thermal tolerances.

In addition to prior exposure altering organismal performance under stressful conditions, experiencing a stressor in combination with other stressors can also alter responses (Breitburg and Reidel, 2004; Crain *et al.*, 2008; Gunderson *et al.*, 2016). Multiple stressors can act antagonistically, additively, or synergistically. For example, stress experienced during the early post-settlement stage of the bryzoan *Bugula neritina* resulted in lower survival, but bryozoans experiencing multiple stressors were more likely to survive than those subjected to single stressors (Lange and Marshall, 2017), supporting the conclusion that there is an antagonistic response. Likewise, a prior 2-week exposure to reduced pH in the sea urchin *Echinometra lucunter* resulted in improved righting performance under low oxygen conditions compared to animals who were kept under ambient pH (Lucey *et al.,* 2020a). However, growing evidence suggests that the combination of warming and deoxygenation is more likely to result in synergistic responses (Vaquer-Sunyer and Duarte, 2011; Sampaio *et al.*, 2021), i.e., responses that are greater than the sums of the responses to each stressor alone (see Przeslawski *et al.* (2015) and discussion in Lange and Marshall (2016)). This is because their mode of action is linked (Pörtner and Langenbuch, 2005). In ectotherms, metabolic rate generally scales with temperature (Fry, 1947) so that increased metabolic rates indicate increased metabolic demand for oxygen (Pörtner and Knust, 2007; Pörtner, 2010) which translates into lower tolerances of low oxygen conditions.

Despite the known relationship between environmental oxygen availability and organismal oxygen demand as it relates to temperature, our understanding of how acute environmental heat stress impacts oxygen demand is limited (Woods *et al.,* this issue). This is particularly important as marine heatwaves (MHW), defined here as a period of five or more consecutive days where temperatures are over the 90th percentile of the local temperature records (Oliver *et al.*, 2018), are increasing in frequency, duration, and intensity. Examples are becoming more readily available that show these acute heat stress events are responsible for causing mass mortality of many species, including but not limited to kelp forests (Wernberg *et al.*, 2016), fisheries (Lehodey *et al.*, 1997), and coral reefs (Hughes *et al.*, 2017; Leggat *et al.*, 2019). These events have caused severe restructuring of ecosystems globally (Smith *et al.*, 2021), and as such, are gaining greater appreciation as novel drivers of range limits, habitat occupancy, and population dynamics (Frölicher and Laufkötter, 2018; Oliver *et al.,* 2018; Holbrook *et al.,* 2019; Brauko *et al.*, 2020). Recent work has also shown that MWHs can co-occur with deoxygenation (Gruber *et al.*, 2021), resulting in simultaneously lower environmental oxygen supply for organisms and higher organismal oxygen demands due to increased metabolic demand from warming. The role of these compound stressors in severe and large-scale mortality events has so far been decoupled in the literature to date.

The biological implications of the compound stress of deoxygenation and warming may best be understood through a measure of hypoxia tolerance. When environmental oxygen levels fall below organisms' temperature-dependent critical oxygen partial pressure, it is widely thought that the organism can no longer maintain its metabolism, i.e., P_{crit} (Pörtner and Grieshaber 1993).

Organisms can only survive for a limited time after this happens and will likely die if temperatures do not decrease and/or oxygen levels increase thus allowing them to return to aerobic metabolism. Therefore, it is possible that oxygen-limitation is responsible for the mortality observed from extreme heating events, more than the heating itself. To understand the role oxygen plays in these mortality events, high resolution coastal oxygen-temperature timeseries are needed (Grégoire *et al.*, 2021), as well as data on the physiological thresholds of ecologically important species under acute heat and hypoxic stress.

This study examines how prior exposure to MHWs impacts the responses of an important coral reef herbivore, *Echinometra lucunter*, to hypoxia and extreme heating. Sea urchins are important drivers of benthic community structure (Steneck, 2020) and are important tropical herbivores (Kuempel and Altieri, 2017). Ranging from North Carolina, USA through the tropical Caribbean to Brazil (Hendler, 1995) *Echinometra lucunter* is one of the most abundant coral reef herbivores and important ecosystem engineers on Caribbean coral reefs (McLean, 1967). They primarily live on rocky reef flats, preferring exposed reef habitats with high wave action primarily between a 1- 5 m depth (McPherson, 1965). In these habitats, they act as important bioeroders, creating burrows in the reef and rocks which contribute to increased habitat complexity and biodiversity (McLean, 1967; Schoppe and Werding, 1996). Increases in *E. lucunter* density are often associated with overfishing due to reduced predation and can result in reef erosion that exceeds reef growth, while decreases in their density are often associated with decreased coral cover and macroalgae overgrowth (McClanahan and Muthiga, 2006).

Echinometra lucunter is a highly abundant species on reefs in and around Bahia Almirante Bocas del Toro, Panama, and they are predictably found on open ocean facing reefs (NML, pers. obs.). Despite being well mixed, recent studies have showed that shallow reefs in the bay experience periodically hot and hypoxic conditions and that episodic low oxygen occurs on the reef at night during the warmest months of the year (Lucey *et al.,* 2020b). Figuerola *et al.,* (2021) used paleo-proxies to infer that acute hypoxia on nearby shallow reefs may have increased in frequency during the last few decades. Their model predicts a hypoxia-induced decrease in herbivores could be coupled with reef shutdown in the future. Together this emphasizes the likelihood that environmental hypoxia and warming are local threats to *E. lucunter* populations.

But there is little empirical data on how *E. lucunter*, or sea urchins more generally, are physiologically impacted by the combination of hypoxia and temperature stressors. There have been numerous studies on temperature, demonstrating that warming increases sea urchin metabolic rates (Ulbricht and Pritchard, 1972; Uthicke *et al.*, 2014), decreases performance (Sherman, 2015), and decreases feeding rates (Harianto *et al.*, 2021). There are very limited studies on the effects of hypoxia on sea urchins. These have demonstrated problems with adult growth and gonad production (Low and Micheli, 2018, 2020) and deleterious consequences for larval development (Layous *et al.*, 2021). There are even fewer studies on the combined effects of warming and hypoxia. Lucey *et al.* (2021) found the combination of 2-hour exposures to high temperature and hypoxia reduces righting performance more than either stressor alone. The performance of the tropical Pacific sea urchin *Colobocentratus atratus* is also limited by oxygen supply more under warming (Wilbur and Moran, 2018).

To our knowledge no research has yet examined the combined effect of warming and deoxygenation on tropical sea urchin metabolism and survival, or their exposure to compound extreme conditions that occur in their coral reef habitats. Here we address these research gaps. We used the sea urchin *E. lucunter* to document how these two stressors influence survival, examining how recent thermal histories impact their responses to hypoxia alone or hypoxia in combination with an extreme heating event. We take a physiological approach by maintaining constant oxygen metabolism across experimental treatments by first determining the critical oxygen limits of *E. lucunter* (P_{crit}). We use oxygen levels 35% below the species' average P_{crit} to define hypoxia as it is experienced by the organism, and factorially combine this hypoxia exposure with two temperature treatments: 1) prior exposure to a heatwave, and 2) subsequent extreme warming. We then use a high-resolution oxygen and temperature timeseries collected from local coral reefs to determine the frequency and duration of MHWs, maximum temperatures, prevalence of hypoxia, and their co-occurrences. The duration of compound stressors occurring on reefs in Bahia Almirante is then compared to the duration of sea urchin survival in compound stressor experiments.

Methods

We used respirometry on a subset of *E. lucunter* animals to obtain estimates of their critical oxygen limits (P_{crit}) at both ambient and extreme temperatures. We then performed an experiment to determine if prior thermal exposure mimicking a MHW impacted the ability of sea urchins to survive subsequent exposure to hypoxia under both ambient and extreme temperatures. We did this by exposing new animals to MHW or non-MHW conditions for 5 days, and then, subsequently exposing them to hypoxia and extreme temperature to assess their survival (Figure 1). The treatment conditions for this experiment were selected based on environmental data, the results of previous tolerance experiments, and estimates of P_{crit} derived from the respirometry trials. All trials occurred between July and November 2019 at the Smithsonian Tropical Research Institute (STRI), at the Bocas Research Station (BRS) on the Caribbean coast of Panama.

Environmental basis for experimental temperature conditions

We selected experimental temperatures based on environmental monitoring data from sites in Bocas del Toro, Panama (Figure 2). The prior thermal exposure was chosen to meet the minimum temperature value and duration to be considered a local MHW, as defined by Oliver *et al.,* (2018). We based this on the longest available timeseries available, a 19-year record of temperature measurements from loggers deployed on reefs at 3 m (STRI's Physical Monitoring Program; Dec. 2000 – May 2019). These temperatures recorded measurements every 30 min on 2 shallow reef sites: Isla Roldan Reef (-82.3376, 9.29164) and Isla Cayo Agua Reef (-82.110639, 9.242583) using HOBO Stow-Away TidbiT or HOBO Water Temperature Pro V2 (Onset Computer Corporation, Bourne, MA) with an accuracy of 0.25°C (Kaufmann and Thompson 2005). We took daily measurements made at noon and calculated the 90th percentile temperature from the combined data from both sites. This was 30.5°C and used as our prior MHW exposure treatment. We used the maximum temperature, 32°C, as the *extreme* temperature in the subsequent hypoxia exposure (Figure 1; Table 1). The ambient control temperature was 28°C, which was slightly below the average bay temperature.

Determination of experimental hypoxic conditions

Our goal in the hypoxia treatments was to ensure that the oxygen levels were lower than the species' critical oxygen limits (P_{crit}) across the experimental treatments. Therefore, we found the average P_{crit} under each temperature treatment (28 and 32°C) across a range of sizes (n= 31). In late June/early July 2019, we snorkeled to collect animals from just below the surface to depths of 3 m in a well-exposed mixed rocky coral reef environment (Hospital Point: -82.2164, 9.3326). Animals were opportunistically chosen from the reef based on their accessibility and size. They were quickly dislodged from the rocky reef substrate by wedging a teaspoon in between the reef-rock and animal and transported to the lab in large coolers filled with 70 L seawater within 30 min of collection. They were placed in 150 L seawater tables with flowing seawater and allowed to adjust to laboratory aquaria conditions for at least 24 h before use in respirometry trials.

Closed chamber P_{crit} respirometry trials were conducted at 28°C or 32°C with each animal participating in a single trial. For trials at 32 °C, the seawater was ramped from ambient to 32°C over 1 h in an isolated 10 L tank before the trial. Each animal was then individually placed in a closed glass respirometry chamber filled with approximately 0.19 L of ~ 100% air saturated 45 μm filtered seawater. Chambers were hermetically sealed glass jars equipped with a magnetic stirring bar, which was separated from the animal with a mesh screen. Chambers were placed in a 12 L water bath over a magnetic stir plate to keep temperatures constant. Oxygen in the chamber was recorded with fiber-optic sensor technology (Fibox 4 and Pst-3 sensors, PreSens, Regensburg, Germany), every 10 seconds until the oxygen level fell to 5% (1.0 kPa or 0.3 mg L^{-1}). Temperature in the water bath was maintained with aquarium heaters and a chiller, and recorded simultaneously with the Fibox 4, as well as a thermocouple thermometer (HH802U, OMEGA, Canada – accuracy 0.1 °C). Upon reaching the oxygen depletion target in the chamber, each animal was promptly moved into ambient, oxygenated water. Wet weight was measured using a precision balance (PL6001E, Mettler Toledo – accuracy 0.1 g) and volume was measured via displacement using a graduated cylinder and beaker. The animals were then euthanized by rapid cooling in seawater, and dissected to determine the presence of gonads, and thus maturity. All urchins collected were mature.

We determined critical oxygen limits (P_{crit}) for each animal using the broken stick regression approach (Yeager & Ultsch 1989). In this approach, two segments of the oxygen consumption timeseries data are iteratively fit until the intersection with the smallest sum of the residual sum of squares between the two linear models is found. This is defined as the 'breakpoint' or P_{crit}, which we found using the *respR* package in R, and we report the intercept (Harianto *et al.*, 2019). Average P_{crit} values were generated for binned size groups (small and large) due to the broad range of sizes collected (6.51 to 26.17 g), in each of the two temperatures (28 and 32°C). Additionally, we determined the resting standard metabolic rates (SMR) from these P_{crit} trials. We calculated the rate of oxygen consumption inside the respirometry chambers from the initial saturation level of approximately 100% to approximately 65%, with all lower bounded values well above the respective individual's P_{crit}. From this subset of the data, we calculated both absolute (μmol O_2 h^{-1}) and mass specific oxygen consumption rates for each individual (μmol O_2 h^{-1} g^{-1}), with the latter accounting for animal mass (g), corrected-chamber volume, subtracting animal volume (L), trial temperature and salinity (see supplemental data and code).

All respirometry trials occurred between July 1st and July 20th in 2019 on 11 different days. No animal was in the laboratory for more than 5 days prior to being used in a respirometry trial. Oxygen sensors (Pst-3 sensor spots) inside each respirometry chamber were calibrated one time before all trials using 100- and 0%-point air saturation calibration standards. Additionally, no animals were fed before trials, and they were thoroughly rinsed with 0.45 μm filtered seawater to reduce attached bacteria, sediment, or microalgae that could interfere with the respirometry measurements. To account for microbial respiration, we included an additional chamber filled with the seawater used to rinse the animals' undergoing measurements. These blank chambers were run simultaneously during each trial on each day. Microbial respiration was effectively contained to marginal fluxes (and the animals' oxygen consumption was constant, i.e., linear decrease in oxygen concentration during trials, see supplemental data). Chamber volumes were 0.19 L, animal volumes ranged from 0.03 – 0.01 L (0.014 ± 0.005 L mean ± SD), and trials lasted 4.41 h on average (±1.80 SD), with durations ranging from 2.16 to 7.90 h.

We determined the effect of temperature on P_{crit} and SMR using a 2-way ANOVA with 'temperature', 'size', and their interaction as factors. We removed interactions from models when interactions were not significant, and checked for overdispersion and normality by building histograms, and plotted residuals against fitted values to ensure linearity. We also used Levene's and Shapiro test to ensure homogeneity of variance and normality of residuals. All assumptions were met for SMR, as well as P_{crit} after it was log-transformed. Pairwise comparisons to check for differences in responses for each treatment were conducted using the Estimated Marginal Means test with Least Significant Difference test correction using the *emmeans* package in R (Lenth, 2022).

Experimental MHW exposure

We collected 376 *Echinometra lucunter* for the main experiment. Trials were run between August 19th and Nov. 19th, 2019. Prior to each trial new animals were collected from the same site and in the same way as described above for respirometry trials. Animals were brought back from Hospital Point reef to the BRS and allowed to rest after the collection for 24 h in outdoor 150 L seawater tables. They were then evenly distributed into 8 indoor 50 L tanks (20-30 animals per tank), 4 tanks which were held at the ambient control temperature 28.5°C (28.54 ± 0.29°C) and 4 which were held at 30.5°C (30.53 ± 0.14°C) to simulate a MHW. Water temperatures in all eight tanks were maintained using aquarium heaters for 5 d. During this period, each tank was bubbled with an air stone and constantly refreshed with flowing unfiltered seawater. Animals were not fed but could have scraped biofilm from the sides of the tanks or filtered particles from the water (Schoppe and Werding, 1996). They were provided with brick substrate for shelter and ambient lighting. No mortality occurred due to the 5-d thermal acclimation, but 6 animals showed evidence of skeletal trauma from collecting and died during this time. As all tanks were supplied with flowing seawater during these trials, salinity matched the bay's which was on average 35 ± 2 ppt. To ensure that the treatments were even with respect to animal body size, small and large size classes were chosen with respect to the mean weight (14.39 g), with animals weighing between 3.09 - 14.39 g in the small category, and those between 14.40 - 38.96 g in the large category. Small and large animals were evenly distributed among tanks, treatments, and time categories.

Experimental hypoxia exposure

After the 5-d prior exposure treatment, cohorts of 3-4 animals were binned together in plastic mesh cages labelled by MHW treatment, and equally distributed across two new 50 L hypoxia tanks, one each at 28 or 32°C (~60 individuals per tank). Cages served to keep track of individuals, i.e., which treatment they were from, as well as keeping them from climbing up out of the hypoxic water. Temperatures in the hypoxia tanks were set before moving the animals, while the oxygen level in each tank was gradually lowered over the course of 1 h after adding the animals. This was done by bubbling nitrogen until the oxygen concentration reached 16.5% (3.4 kPa or 1.01 ± 0.22 mg L^{-1}). This is on average 35% below the P_{crit} regardless of body size or temperature. Oxygen levels were maintained in the tanks with an automated oxygen regulator using an Atlas Scientific galvanic dissolved oxygen (DO) probe (DO Controller, Barreleye Designs, USA). This functioned as a solenoid controller which bubbled nitrogen into tanks while monitoring temperature and DO every 30 seconds (DO precision = 0.1 mg L^{-1}). Flowing, fresh seawater was constantly introduced into both tanks at a rate of 90 L h^{-1} and a circulation pump was positioned in each tank to ensure high flow and consistent oxygen throughout each tank.

After the first hour, animal cohorts (2 cages with 4-6 animals each), were removed from each of the hypoxic tanks. They were patted dry, weighed, and placed in one of 6 outdoor, fully oxygenated 150 L recovery tanks with high flowing seawater at ambient temperature. Animals were removed from the hypoxic tanks in this way every 3 h for 24 h until all individuals in the trial were added to the recovery tanks, at which point the trial ended. Due to limitations in the number of tanks and equipment malfunction these trials were spread across 3 months; we were nevertheless able to obtain sample sizes of 6-22 animals at each time point in each of the 4 treatments (Table S1).

Survival was determined 24 h after animals were transferred to their recovery tank. All animals were isolated from each other in the recovery tanks using mesh dividers. Animals were considered dead when they were not attached to the tank and had no signs of spine or tube foot movement when gently prodded. Most deaths were extremely clear, with many of the dead animals displaying massive spine and tissue loss. Dead and/or sick animals were isolated/removed from recovery tanks periodically throughout the 24 h post-trial period to avoid impacting other individuals. Living animals were returned to the collection site.

To determine if there was an effect of prior thermal exposure, temperature, size, exposure time, or their interaction on survival, we constructed generalized linear models (GLMs) with a binomial distribution and log-link function. The full model was analyzed with stepwise removal of non-significant interactions based on p-values *(p* > 0.1) (Crawley, 2012). The only interaction that was significant was between time and temperature, therefore the other interactions were removed from the final model. Models were assessed for balance, and non-linearity, unequal error variances and outliers with a residual analysis (Zuur *et al.*, 2010).

We determined what duration of hypoxia exposure caused 5% and 50% mortality (lethal time; LT) under each treatment combination. We did this by generating GLMs fitted with a binomial distribution for each treatment. The dose.p function in the *MASS* library in R was then used to estimate an LT_{05} and LT_{50} for each model (Ripley *et al.,* 2013). To check if LT values differed between treatments, a 95 % confidence interval was calculated using Student's t value. We also calculated LT values for the two temperature treatments (28 and 32°C) without prior heatwave exposure as a factor.

Hypoxia and heatwave occurrences on the reef

To determine the history of past MHWs in the area, we used the 19-y temperature dataset to calculate how frequent and long MHWs were in both Cayo Agua and Cayo Roldan. Based on the full years of temperature data in both sites, we determined the number of MHW occurrences, their duration, and the total number of MHW days in each year since 2001. These two sites are equidistant from Hospital Point, the collection site for the animals used in the above experiments. Based on other published monitoring data throughout the bay, we expect these sites to represent the far ends of a natural thermal gradient, with Cayo Roldan on the hot end and Cayo Agua on the cool end (Lucey et al., 2020a; Johnson et al., 2021). Therefore, we expect an average of conditions from these sites to be close to what Hospital Point experiences, although long-term monitoring records from Hospital Point would be the only way to verify this accurately.

To determine if MHWs, hypoxia and extreme temperatures co-occur, we analyzed a 9-month record from Figuerola *et al.,* (2021), with high-resolution dissolved oxygen and temperature data from a logger deployed on the seafloor at 3 m. The logger was placed in a coral reef habitat in a protected back bay site, Tierra Reef (Figure 2). Measurements were made every 10 min from March 2018 to November 2018 with a Minidot logger (DO accuracy ± 5%, PML, USA). From this March-November 2018 timeseries record, we documented the days when temperature on the reef was equal to or above our maximum or extreme 32°C temperature threshold, as well as days when the minimum oxygen level was under the average P_{crit} of *E. lucunter* (~ 4.8 kPa; 1.5 mg L^{-1}). We determined how many consecutive hours these hypoxic and extreme high temperatures persisted, both alone and in combination. We also documented when MHWs occurred during this 9-month period.

All statistical analyses were performed using the statistical software R version 4.2.0 (R Core Team, 2022).

Results

Determination of metabolic rates and critical oxygen limits

There was no effect of the interaction between temperature and body size on standard metabolic rate per unit mass (SMR). However, SMR was lower at 28°C than at 32°C for both large and small sea urchins (SMR = 0.69 ± 0.13 at 28 compared to 0.78 ± 0.11 at 32, mean ± SD μmol O_2 h^{-1} g^{-1}, $p = 0.03$, n = 31). The SMR of larger animals was on average lower than that of smaller animals (0.66 ± 0.11 compared to 0.81 ± 0.10 respectively; $p = 0.0004$, n =31, Figure 3A; Table 2). The greatest difference in SMR was at 28°C, with the mass-specific metabolic rate of small animals 28% faster than the metabolic rate of larger animals. The absolute metabolic rates of these animals increased with body size by a factor of ~0.72, e.g., the slope of log-transformed SMR by log-transformed wet weight regression analysis (Figure 3B) (Carey and Sigwart, 2014). The scaling factor was slightly, but not significantly higher at the warmer temperature ($p = 0.31$).

There was no effect of size, temperature, or their interaction on P_{crit} (Figure 3C, Table 2). The overall average P_{crit} for the total 31 animals tested was 25.44 ± 6.32 SD% (5.25 ± 1.30 kPa; 1.58 ± 0.39 mg L^{-1}). Hypoxia treatments in the subsequent experiment was ~35 % below the average P_{crit} at 16.50% (or 3.40 kPa; 1.01 mg L^{-1}).

How temperature impacts hypoxia survival

GLM analyses with time as a factor shows that there was a significant interaction between temperature and time on survival during the hypoxia exposure ($p < 0.001$). Only 55% of the animals survived 24 h of hypoxia at 32°C, while > 93% survived 24 h of hypoxia at 28°C (Figure 4; Table 3). Prior thermal exposure, either to MHW or control conditions, had no significant effect on survival during hypoxia exposure ($p = 0.560$).

As prior thermal exposure had no significant effect on survival, data were pooled by temperature to determine the time at which the two temperature populations (i.e., 28 or 32 °C under hypoxia) experience either 5% or 50% mortality, the LT_{05} or LT_{50} respectively. The LT_{05} of animals exposed to hypoxia and ambient temperature was 19.83 ± 1.28 h (mean ± SE), while the time for the population to reach 50% mortality (LT_{50}) was predicted to be more than twice as long as the 24 h experimental exposure, at 46.15 ± 5.51 h. In contrast, the LT_{05} and LT_{50} for animals experiencing hypoxia and extreme temperature was much shorter, with LT_{05} of 8.71 ± 0.79 h and LT_{50} just under 24 h (23.67 ± 0.58 h).

Heatwave and hypoxia occurrences on the reef

At least one MHW has occurred in Bahia Almirante every year for the last 19 years. From 2001 to 2019 there have been 60 MHWs (Figure 5), however the rarity of these events varies substantially depending on reef location. For example, the number of MHW days on the Cayo Roldan reef has been 10 times greater than the Cayo Agua reef. MHWs on the Cayo Roldan reef are 5.3 times more frequent (e.g., 3.2 vs. 0.6 occurrences) and 5.8 times longer (e.g., 21.3 vs. 3.7 d) on average than on the Cayo Agua reef. Site differences in mean temperature and variance reflect these MHW trends; Cayo Roldan reef is 0.61°C warmer and has ~10% more variation than the Cayo Agua reef (Figure 2, Table 1).

We find a positive correlation between the occurrence of MHWs and the manifestation of compound extreme conditions on shallow coral reefs. During the 9-month oxygen-temperature monitoring period on Obscura Reef, temperatures remained above the 90th temperature percentile for 5 or more days 8 distinct times (i.e., 8 MHWs). Compound extreme events consisting of temperatures above 32°C and oxygen levels below 4.5 kPa, occurred 4 times and always during a MWH event (Figure 4). Hypoxic conditions only ever lasted 10 h, while temperatures above 32°C were sustained for over 24 h. Compound extremes only ever occurred for a maximum of 3 h.

Discussion

Heating tolerance

All sea urchins exposed to five days of marine heatwave conditions survived, clearly demonstrating that these MHWs do not kill *E. lucunter*. Moreover, there was no effect of the 5-day MHW exposure on subsequent survival under extreme compound conditions of hypoxia and heating. This shows both a lack of acclimation, which would result in improved survival, and a lack of synergy, which would result in reduced survival, to MHW exposure. The lack of effect of MHW conditions most likely indicate *E. lucunter* has high thermal tolerances. This could be because MHW temperatures today are not as 'stressful' as they were in the past (i.e., shifting baselines in the definition of MHWs), or because these animals live near the surface where they naturally experience more temperature extremes. Unfortunately, there are few long-term data on the survival of *E. lucunter* at warmer temperatures. Short-term trials have demonstrated significant thermal tolerance to very extreme shorter exposures in *E. lucunter* from the Caribbean (Sherman, 2015; Collin *et al.*, 2018; Lucey *et al.*, 2021). Two-hour exposures of *E. lucunter* compromised survival at 37°C and above (Collin *et al.*, 2018), while preliminary trials of 12 h exposures of *E. lucunter* following the same protocols of Collin *et al.* (2018) and done during the same time period by the same researchers showed that all 9 individuals assayed at both 34°C and 36°C remained alive (i.e., none died) during the 12-hour exposure and for the 24-hour recovery period. However, some of those exposed to 36°C had sagging spines and other signs of stress after the 24-hour recovery period (Francesco Rendina and RC, STRI, unpublished data from 2016). Not only are *E. lucunter* able to survive high temperatures, but they also have high thermal performance limits. *Echinometra lucunter* from the Cayman Islands had thermal performance tolerances ranging from 32-33.6°C in the warmer summer conditions when temperatures were highest (~30.4°C) (Sherman, 2015). This strongly suggests *E. lucunter* has the capacity under normoxia to survive in much more intense short-term warming events than our extreme temperature exposures, and potentially any MHW documented so far in sites they are currently inhabit in Bocas del Toro.

Hypoxia tolerance

Just as thermal tolerance is sufficiently high to withstand current thermal extremes under normoxia, hypoxia tolerance is high at ambient temperatures. At oxygen levels 30% below average P_{crit} (3.8 kPa or 1.0 mg L^{-1}), it took almost 20 hours to reach the time at which 5% mortality occurs (LT_{05}). This is twice as long as the longest recorded time on the reef for oxygen to remain so low, i.e., 10 h (Figure 4). Therefore, hypoxia in the absence of extreme heating is unlikely to induce mass die-offs within a single day on the shallow reefs where these organisms occur.

*Echinometra lucunte*r is not the only sea urchin species that can survive surprisingly long times under hypoxia. Its sister species *Echinometra vanbrunti* on the Pacific coast of Panama, appears to endure cool hypoxic conditions driven by seasonal upwelling, however the durations for their hypoxia tolerance beyond 2 h remain untested (Lucey *et al.*, 2021). *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus* can tolerate similar oxygen concentrations of 1.0 mg L^{-1} for 36 hours at 14-15°C (~2.58 kPa) and have an LT_{50} of between 48 and 90 hours (Low and Micheli, 2018). Previous work with other strongylocentrotid sea urchins demonstrated they have effective anaerobic systems that can produce lactic acid which can be detected in the coelomic fluid, and that alanine can be metabolized under hypoxia (Drozdov and Drozdov, 2015). This suggest that *E. lucunter* and other sea urchins may use this non-specific adaptation to withstand extreme hypoxic events (Bookbinder and Shick, 1986; Drozdov and Drozdov, 2015).

Despite this evidence for low mortality under hypoxia at ambient temperatures on the order of a day or two, exposures of this duration may have negative effects on these animals. For example, in *Strongylocentrotus nudus* significant immune cell mortality was induced by 24-hour exposures to DO of 1.7 mg L^{-1}, and even to some extent 24-hour exposures to 4.7 mg L^{-1} (Suh *et al.*, 2014). These immune cells, also known as coelomocytes, help sea urchins respond to various types of environmental stressors like temperature and UV-radiation, and their reduction due to hypoxia is suggested to be a widespread phenomenon among sea urchins. The most hypoxic exposures in this species also resulted in suppression of gene expression across an array of genes, including RHN helicase and GATA-4/5/6, which are involved in cellular defense mechanisms that might help the individual cope with more moderate hypoxia (Suh *et al.*, 2014). Additionally, *Strongylocentrotus purpuratus* expressed significant sub-lethal effects on fitness related traits such as reduced calcification and grazing rates and more poorly developed gonads after longer-term exposures at 5.5 mg L^{-1} (Low and Micheli, 2018, 2020). This highlights how reduced oxygen levels could also negatively impact fitness (Vaquer-Sunyer and Duarte, 2008). If *E. lucunter* experiences similar sublethal effects, they may already be experiencing reduced performance with respect to these other functions despite having robust survival of current short-term extreme events.

Compound extreme events

Extreme heating and hypoxia act synergistically to significantly reduce survival, even though *E. lucunter* seems resistant to either factor alone. When oxygen conditions are below the critical oxygen limits of the animal (3.8 kPa) and temperatures are at the local maximum (32°C), survival declines sharply after 8 hours, reaching a LT_{05} in 9 hours, and LT_{50} in 24 hours.

Although we do not know the physiological mechanisms underpinning this synergistic response, we can confidently state that a mass die-off of sea urchins would occur if these extremes lasted for a 24-hour period. Two such die offs of reef fauna have been documented locally in Bocas del Toro (Altieri *et al.*, 2017; Johnson *et al.*, 2021), although they were not where *E. lucunter* normally live. The 2010 mortality event impacted reefs around STRI, while the 2017 event affected reefs near Cayo Roldan in the back bay (Figure 2). Mortality was primarily assessed for corals and during the 2017 event over 50% of the live coral was lost (Johnson *et al.* 2021), but many *Echinometra viridis,* a sister species of *E. lucunter,* were also found dead or dying (NML, pers. obs.). These devastating mass mortalities on coral reefs were associated with hypoxia and occurred during two of the longest MHWs on record, e.g., 38 day-long MHW in 2010 and 48 day-long MHW in 2017. Unfortunately, the oxygen conditions were not well-documented during these mortality events. Without high-resolution temporal data to determine how long low oxygen persisted during these MHWs, it is impossible to determine if the particularly long exposures to high temperatures, or the high temperatures in combination with hypoxia were primarily to blame for these mortality events.

The high-resolution temperature and oxygen dataset we present in this study, while not associated with a mass mortality event, does provide clues on how compound extremes arise on coral reefs. Hypoxic conditions often occurred on the days that temperature exceeded 32°C. The extremes primarily occurred within the same 24 h period, but not at the same time. This is because temperature and oxygen have inverse diel cycles (Nelson and Altieri, 2019; Lucey et al., 2020a). Water temperature is warmest in the early afternoon after solar radiation has warmed the shallow waters of the bay, while oxygen is lowest during the night when community respiration draws down oxygen in the absence of photosynthesis. Increasing temperature during the day are associated with increasing hypoxia at night. This highlights the importance of diurnal fluctuations in coastal systems and the need to measure and understand the associated temporal complexities, such as the stressor sequences, and the degree of stressor overlap associated with multi-stressor extreme events at highly resolved spatiotemporal scales (Grégoire *et al.*, 2021; Jackson *et al.*, 2021).

Despite the normal pattern of hypoxia at night and extreme temperatures during the day, we observed short durations of time when these stressors overlapped or co-occurred in the field (i.e., compound extremes). Interestingly, compound extremes only ever occurred on days coinciding with the occurrence of MHWs. This is best visualized in Figure 6 where every occurrence of compound extremes corresponds with a MHWs (i.e., blue shaded boxes represent days of sustained temperatures above 30.5°C). The longest duration of compound extremes we observed, 3 hours, is unlikely to cause mortality in *E. lucunter*. And while there seems to be a clear relationship between the occurrence of MHWs and compound events, the question remains: what exactly will increase the duration of compound extremes to the point at which mortality would be highly likely (e.g., length of MHWs, magnitude of daytime temperature)?

Physiology of extremes

During hot, hypoxic periods it is thought that deoxygenation reduces the supply of oxygen available to meet organisms' metabolic demands while warming simultaneously increases these demands (Pörtner and Knust, 2007). This mismatch between oxygen demand and supply could have serious, rapid impacts on the organism's ability to survive compound extreme events. This mechanism underlies a physiological breakdown at high temperatures. Examples of this mechanism in the context of hypoxia are scarce, particularly for marine sea urchins. One study provides evidence of temperature-mediated oxygen limitation in the tropical sea urchin *Colobocentratus atratus* Wilbur and Moran (2018). In this species, reduced performance due to increased temperature was improved by adding oxygen to high temperature treatments. Like most other studies examining temperature effects on sea urchin oxygen consumption (Ulbricht and Pritchard, 1972), we also found that sea urchin metabolic rates increase with warming. In the Australian sea urchin *Heliocidaris erythrogramma,* +3 °C temperature increase oxygen consumption by up to 35% (Harianto *et al.*, 2021). Delorme and Sewell (2016) attributed increased metabolic rates of the New Zealand sea urchin *Evechinus chloroticus* to warming in the field during the summer, indicating that these metabolic responses can be sustained for relatively long periods. However, these higher metabolic rates from warming can come at a cost, such as reduced gonad size in the tropical Pacific sea urchin *Echinometra sp.* (Uthicke *et al.*, 2014).

Metabolic rates are also expected to increase with animal size (Carey and Sigwart, 2014; Carey *et al.*, 2016) and larger animals are expected to be more oxygen limited at higher temperatures than small ones. One leading theory for this is that they have lower surface area to volume ratios (Atkinson *et al.*, 2006). This is likely true for the *E. lucunter* as we found that the increase in resting metabolic rates with increasing temperature was significant only in larger animals (Figure 3a). We also found larger sea urchins have higher absolute/whole animal metabolic rates than small animals across temperatures, with rates scaling with body mass by a factor of approximately 0.7 (i.e, *b*, slope of the regression, Figure 3b). This closely agrees with another warming study using the sea urchin *E. erythrogramma,* which found that metabolic rate scaled with body mass to the 0.70 - 0.72 power (Carey *et al.,* 2016). This highlights how the size of the animal can be a strong determinate for the magnitude of their response to temperature.

Together, these results suggest that large adults with the greatest reproduction potential will be both the most susceptible to oxygen limitation, and also demand the most oxygen during compound extreme events (Clark *et al.*, 2013).

The critical oxygen limits of organisms, i.e., P_{crit}, should also increase with warming. P_{crit} is temperature dependent in most marine ectotherms studied to date (Vaquer-Sunyer and Duarte, 2011; Deutsch *et al.*, 2015). P_{crit} should also decrease with size, with smaller animals expected to be more hypoxia tolerant than larger ones due to their lower O_2 demand. Surprisingly, the P_{crit} of *E. lucunter* did not increase significantly with temperature or body size over the range tested. It is possible that P_{crit} does depend on temperature, but that the intraspecific variation was too large, and the range of temperatures examined here too small to statistically detect this difference. Future efforts focusing on temperature and hypoxia tolerance traits together is needed to establish more meaningful biologically relevant definitions of environmental extremes, how how they scale with size, and vary across taxa.

Conclusion

We find compound events consisting of simultaneous hypoxia and extreme heating occur during marine heatwaves on shallow Caribbean coral reefs. Surprisingly there is no noticeable impact of prior exposure to these heatwaves on the survival of sea urchins when they are challenged with hypoxia, or with hypoxia and extreme heating. Hypoxia does not impact short-term survival on its own, either. However, when hypoxia is coupled with extreme heating, over half of the animals die within 24 hours. This illustrates how short durations of compound extreme events can drastically impact coral reef inhabitants. Because our short-term oxygen records show compound extreme events occur during MWHs, and long-term temperature records show there is a 50% chance of reefs experiencing at least one MHW per year, the frequency of such compound events is likely to be either underestimated and/or increasing. This agrees well with the small but growing body of research that unanimously emphasizes the severe biological and ecological impacts of compound extreme ocean events (Ummenhofer *et al.*, 2017; Gruber *et al.*, 2021; Wolf *et al.*, 2022). Increasing efforts to collect high-resolution oxygen and temperature data are necessary, as is knowledge of how multiple extreme stressors interact, how long extreme events last, as well as how their magnitude, intensity, and recurrences impact marine life. Understanding the basic anatomy of these compound events with their corresponding biological impacts is urgently needed to assess the risk they pose to coastal ecosystems globally.

Acknowledgements

We thank the staff of the Bocas del Toro research station for logistical support, the STRI environmental monitoring program for sharing their data, P. Calosi for use of respirometry equipment, and Panama's ministry of the environment (MiAmbiente) for issuing permits for this work. AH, NML and CA were supported by fellowships awarded by STRI. NML was supported by a grant from the National Science Foundation, BIO-OCE 2048955 during manuscript analysis and preparation.

Data availability

All data, code to generate results and figures will be accessible online upon publication.

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Figures

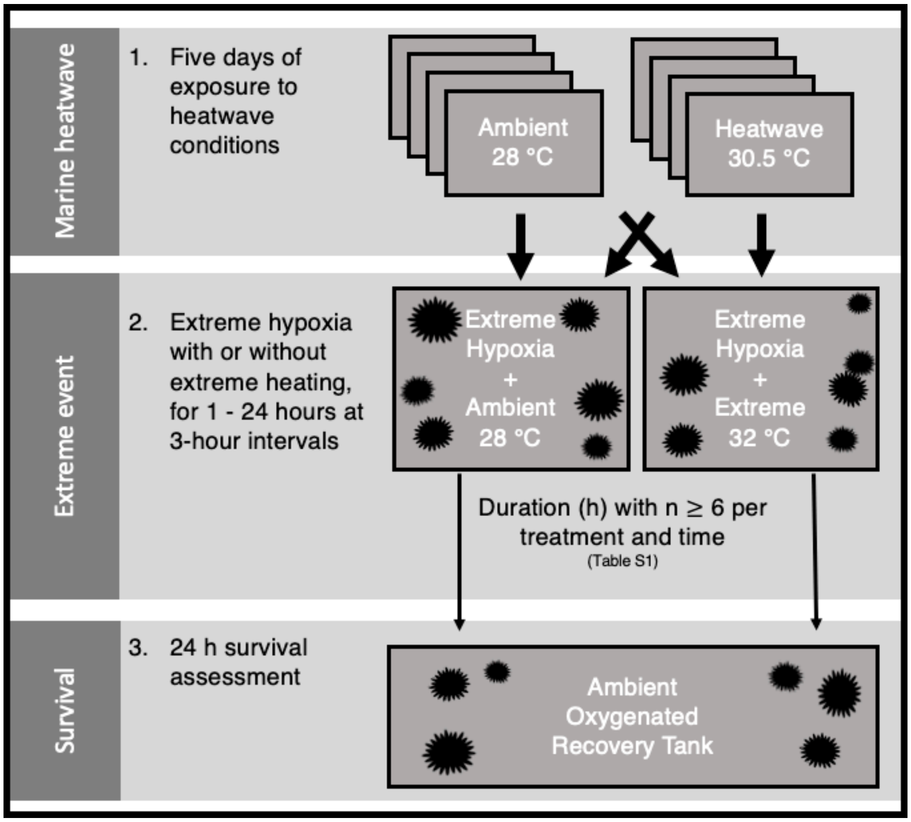

Figure 1: Schematic of the factorial experimental design using the rock-boring tropical sea urchin *Echinometra lucunter.* Animals were first exposed for 5 days to either control (28°C) or MHW (30.5°C) temperatures. They were then exposed to extreme hypoxia either with or without extreme heating (28 vs. 32°C) for durations ranging from 1 to 24 h (in 3 h intervals). This was followed by a 24 h recovery period, whereby survival was assessed.

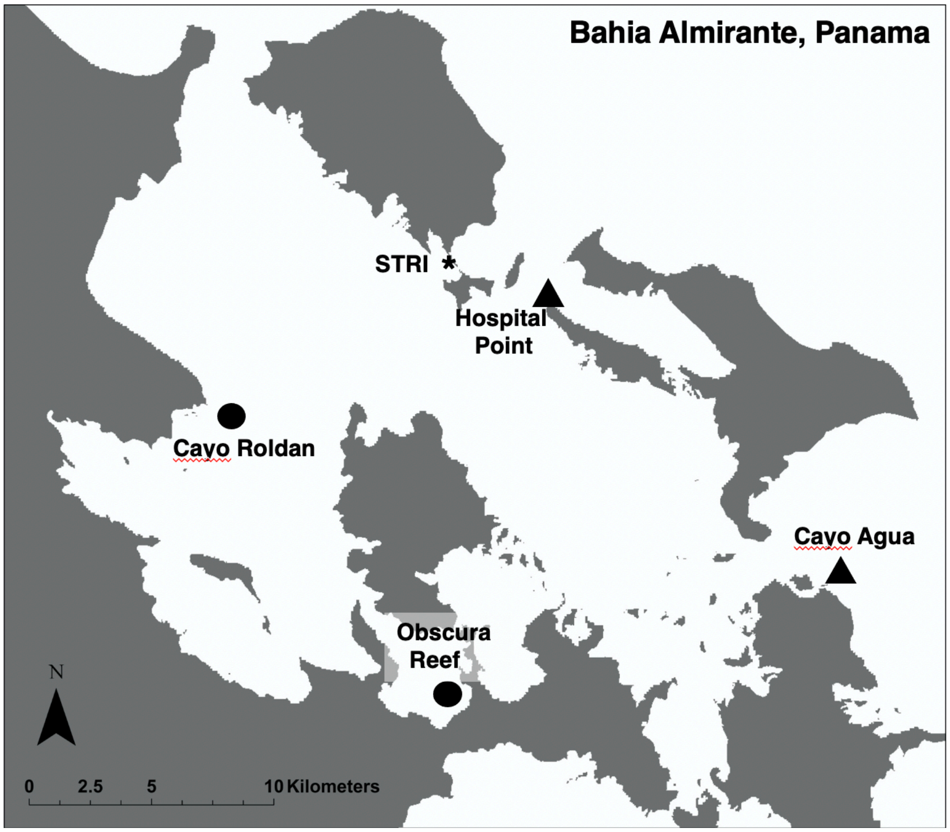

Figure 2. Map of Bahia Almirante, Bocas del Toro, Panama. All *Echinometra lucunter* sea urchins used in this study were collected from Hospital Point. 19-year temperature data was collected from loggers deployed on the reefs at 3 m in Cayo Roldan and Cayo Agua, while 9-month temperature and oxygen data were measured by loggers deployed on the Obscura reef at 3 m. Sites marked by circles represent reefs where the occurrence of *E. lucunt*er is rare, compared to sites marked by triangles which have higher abundances of this species (NML, pers. obs.). All laboratory work was conducted at the STRI Bocas Research Station.

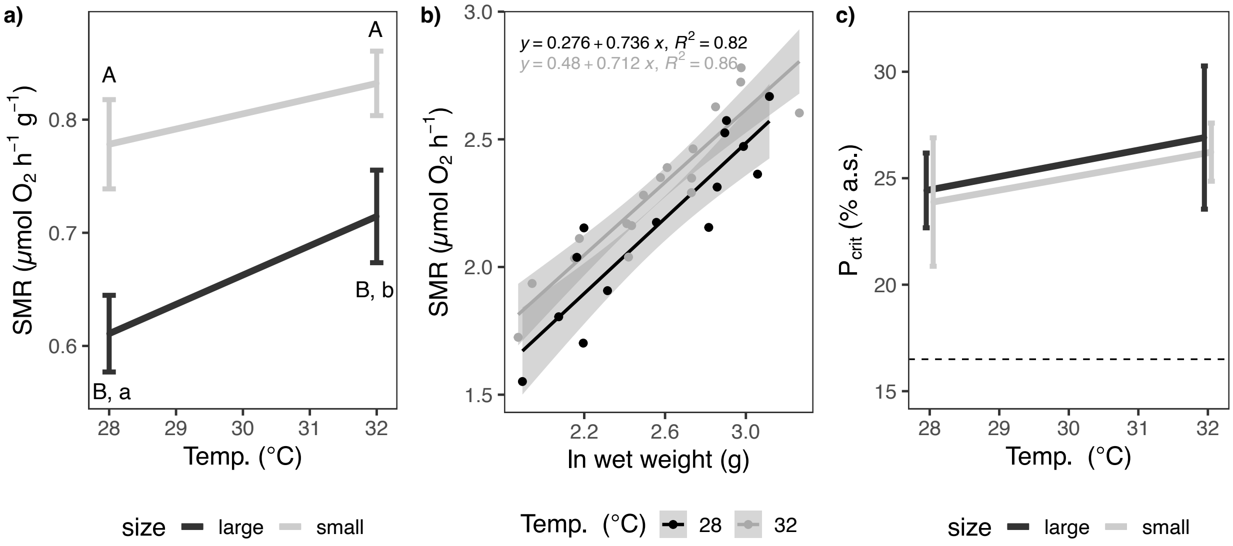

Figure 3. The standard metabolic rates (SMR) and critical oxygen limits (P_{crit}) of *E. lucunter*, considering temperature and body size [n=31]. a) Mass-specific SMR rates are calculated for each temperature and body size, with significantly different mean values ($p < 0.05$) in different temperature treatments for the same size class are indicated by lowercase letters, while significantly different mean values in the same temperature treatment among different sizes are indicated by uppercase letters. b) The linear relationship between absolute metabolic rate and body size (both log-transformed), with regression equations shown for each temperature treatment. c) The P_{crit} in percent air saturation for each treatment. The dashed line at 16.5% shows the oxygen level used in experimental hypoxia treatments. Error bars indicate ± SE.

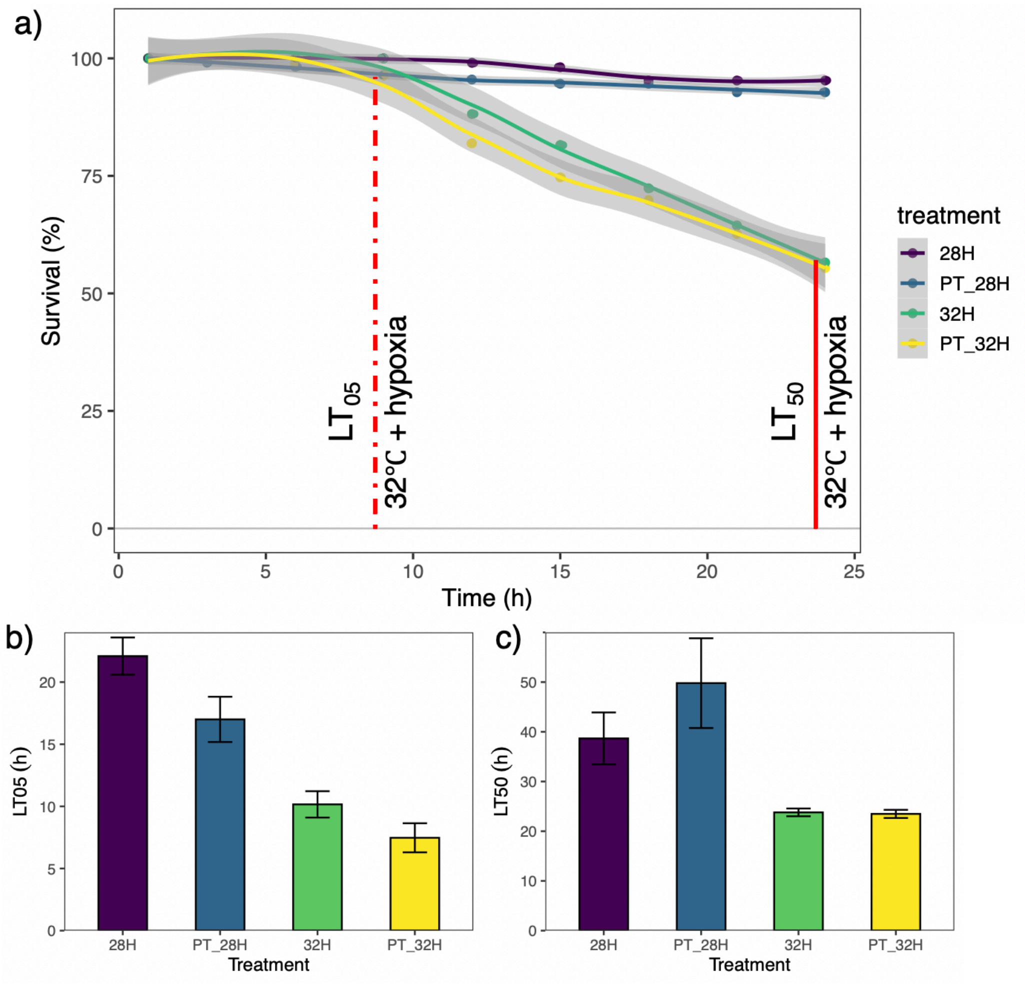

Figure 4: The duration in hours that *E. lucunter* survived extreme temperature and hypoxia. All animals were held at either ambient (28°C, blue and purple) or extreme temperature (32°C, yellow and green) under hypoxia (H) at 16.50% (~3.8 kPa; 1.01 mg L^{-1}). This was 35% below their critical oxygen limit (P_{crit}). Half of the animals were previously exposed to 5-days at either 28°C (blue and yellow) or 30.5°C (purple and green); prior thermal exposure, (PT). Survival was assayed in subsets of animals removed from the experiment every 3 h for a 24 h time-period. There was no effect of prior temperature exposure, so temperature treatments were pooled to show two important time points: the red dashed vertical line represents the time when 5% of the animals under both hypoxia and extreme temperatures died, LT_{05} (a). Likewise, the red solid line shows when 50% of the animals under both hypoxia and extreme temperatures died ($LT_{50} = 23.6$ h). Points represent average survival in each treatment at each sampled time point. Below, the LT_{05} (b) and LT_{50} (c) are given for treatments including prior acclimation exposures. Error bars note ± SE.

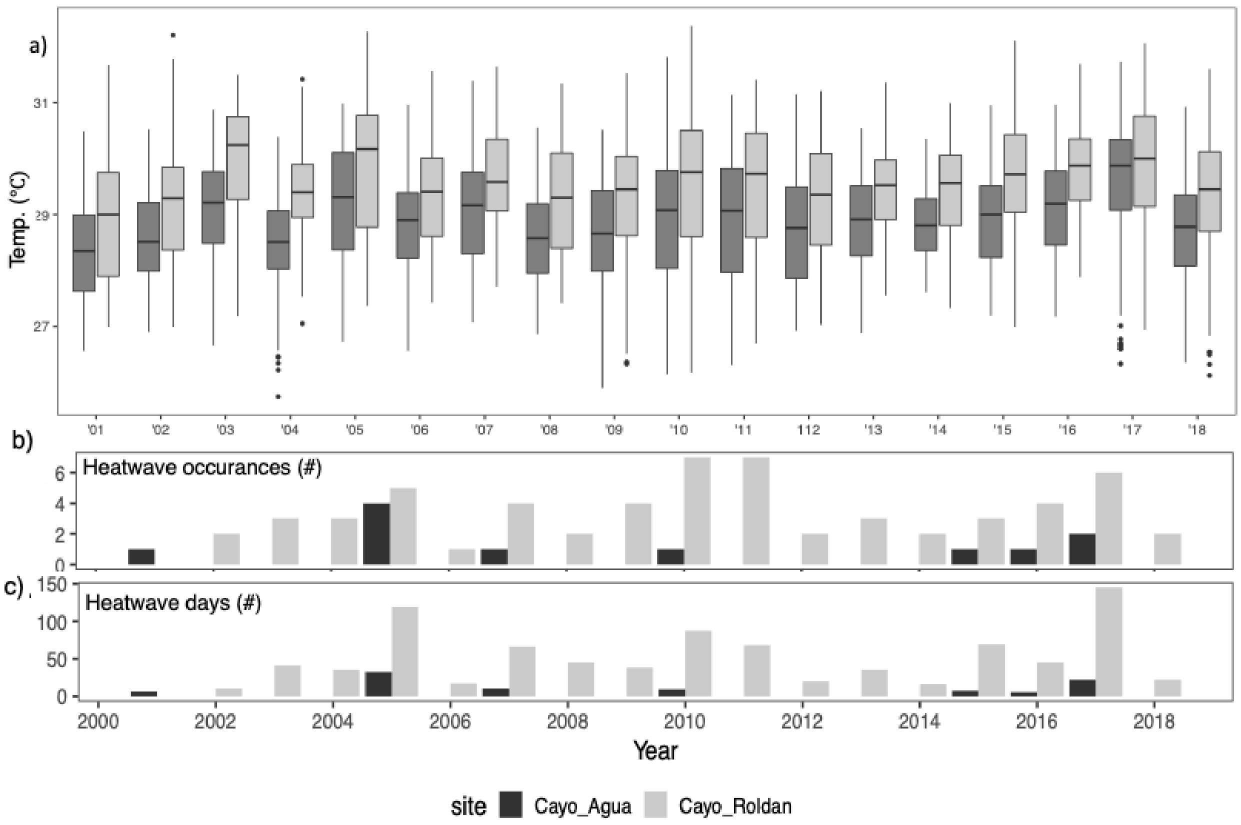

Figure 5. a) Range of seawater temperatures measured on two 3 m coral reefs near Cayo Roldan and Cayo Agua. Values are from measurements made each day at noon and only data spanning full years are included, i.e., 2001- 2018. b) The number of MHW occurring at each site every year, where temperatures were equal to or greater than 30.50°C for 5 or more consecutive days, and c) the total number of days per year MHWs occurred.

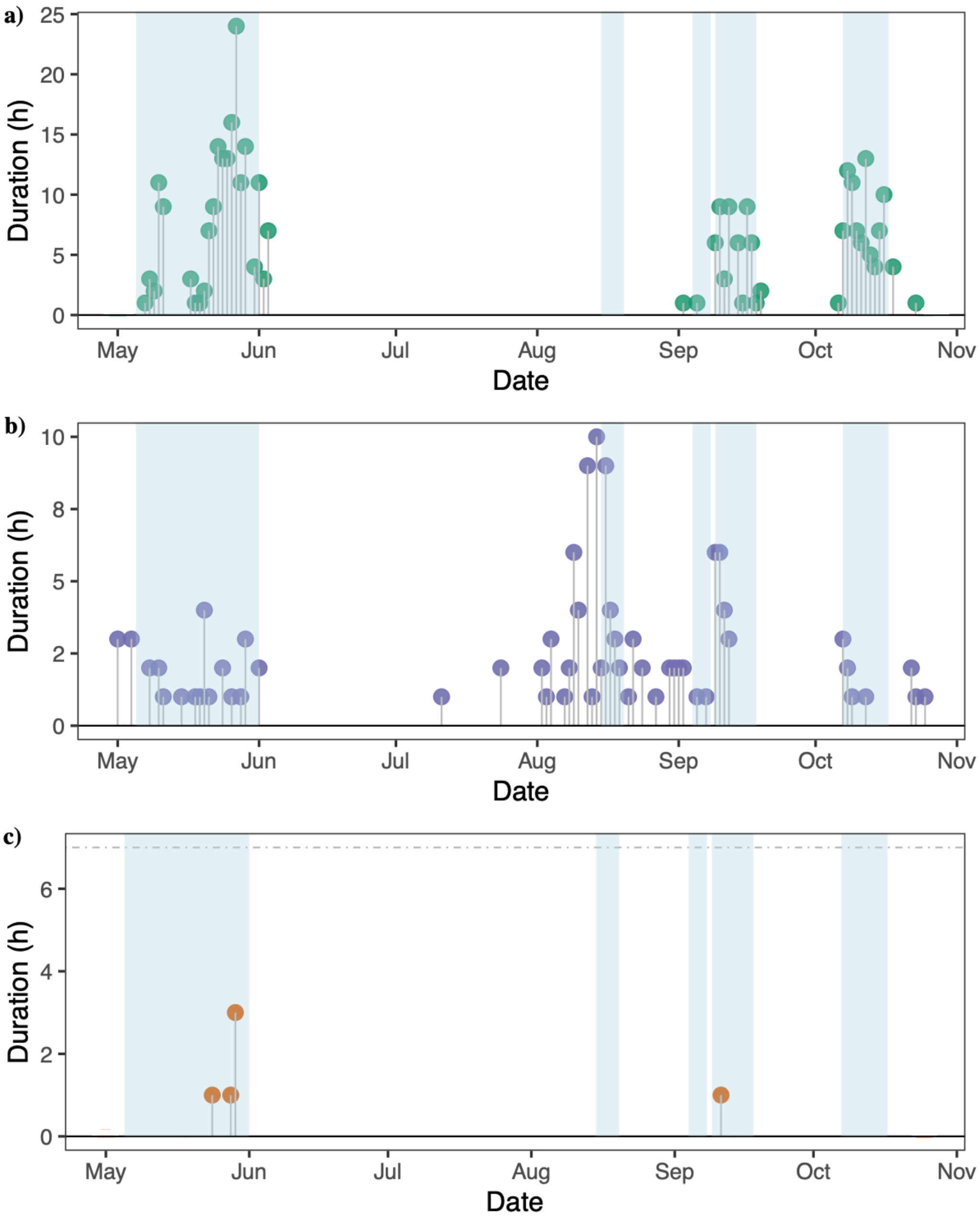

Figure 6. The duration of consecutive hours within a 24 h day in which (a) temperatures are equal to or above 32°C, (b) oxygen levels are equal to or below 16.5% (~4.5 kPa; 1.5 mg L^{-1}), or (c) both oxygen and temperature values are below (O_2) and above (temperature) these thresholds (compound extremes). Light blue shading represents time periods when the temperatures were consecutively above 30.5°C for five or more days (i.e., MHW occurrences). The time in hours when 5% of the *E. lucunter* test population dies (LT_{05}) from compound extreme exposure is shown by the dashed grey line in (c).

Table 1. Summary of environmental temperatures (°C) at 3 m on the two coral reefs, Cayo Roldan and Cayo Aqua. Bay averages are taken from their combination. Summary statistics include daily daytime data from the complete 19-year record. From bay averages, the 90% percentile temperature was used as the MHW experimental treatment (i.e., prior thermal exposure), and the maximum temperature was used for the 'extreme' temperature treatment.

Year	Cayo Roldan Mean ± SD (℃)	Cayo Agua Mean ± SD (℃)	Bay Average Mean ± SD (℃)
2000	28.08 ±0.43	27.76 ±0.27	27.92 ±0.39
2001	28.90 ±1.06	28.36 ±0.90	28.63 ±1.02
2002	29.18 ±1.00	28.67 ±0.76	28.90 ±0.91
2003	29.87 ±1.10	29.11 ±0.84	29.31 ±0.97
2004	29.43 ±0.77	28.55 ±0.79	28.99 ±0.89
2005	29.84 ±1.15	29.23 ±1.03	29.54 ±1.13
2006	29.33 ±0.89	28.84 ±078	29.09 ±0.87
2007	29.64 ±0.88	29.08 ±0.90	29.36 ±0.94
2008	29.31 ±1.00	28.61 ±0.79	28.97 ±0.97
2009	29.32 ±1.00	28.63 ±0.99	28.98 ±1.06
2010	29.58 ±1.24	28.94 ±1.14	29.26 ±1.23
2011	29.45 ±1.19	28.89 ±1.04	29.17 ±1.15
2012	29.27 ±0.98	28.71 ±0.92	28.99 ±0.99
2013	29.45 ±0.82	28.93 ±0.79	29.19 ±0.85
2014	29.46 ±0.78	28.83 ±0.59	29.15 ±0.76
2015	29.73 ±0.99	28.97 ±0.86	29.35 ± 1.00
2016	29.83 ±0.76	29.12 ±0.82	29.48 ±0.86
2017	29.94 ±1.07	29.60 ±1.03	29.77 ±1.06
2018	29.35 ±1.06	28.67 ±0.93	29.01 ±1.06
2019	29.16 ±0.72	28.56 ±0.53	28.86 ±0.70
Minimum temperature	26.12	25.74	25.74
Mean temperature	29.47 ± 1.02	28.86 ± 0.93	29.16 ± 1.02
Maximum temperature	32.37	31.82	**32.37**
90% Percentile	30.76	30.08	**30.50**

Table 2. Fixed-Effects ANOVA results of P_{crit} and mass-specific SMR, as a function of temperature (either 28 or 32 °C) and size class. P_{crit} was log transformed to meet assumptions of normality. Interactions between temperature and size were not significant in either case and removed from final models. Significant factors are in bold.

		Sum of Squares	*df*	Mean Square	*F*	*p*	partial η^2	partial η^2 90% CI [LL, UL]
SMR	Intercept	0.00	1	0.00	0.11	.743		
	temp	**0.04**	**1**	**0.04**	**4.80**	**.037**	**.13**	**[.00, .33]**
	size	**0.15**	**1**	**0.15**	**16.09**	**.000**	**.37**	**[.13, .53]**
	Error	0.26	28	0.01				
Log_{10} (P_{crit})	Intercept	0.02	1	0.02	1.80	.190		
	temp	0.01	1	0.01	0.85	.366	.03	[.00, .18]
	size	0.00	1	0.00	0.03	.869	.00	[.00, .06]
	Error	0.37	28	0.01				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

Table 3. Survival through time under hypoxia. Binomial GLM results indicate no effect of either collection date, sea urchin size, prior thermal exposure, or their interaction with time and/or extreme temperature. Only significant interactions were retained in this model.

	estimate	std error	z value	*P* (>|z|)
(Intercept)	-6.133	7.822	-0.784	0.433
Time	**2.481**	**0.614**	**4.041**	**0.000**
Temp.	0.350	0.265	1.322	0.186
Temp. x Time	**-0.091**	**0.021**	**-4.329**	**0.000**

Table S1: Experimental numbers of *E. lucunter* animals tested in each treatment at each time point during the period between August and November 2019.

time	PT-28H	PT-32H	28-H	32-H	total
1	9	9	5	8	31
3	17	10	16	9	52
6	16	11	15	9	51
9	18	11	16	10	55
12	22	18	22	15	77
15	11	6	12	6	35
18	6	6	6	7	25
21	6	6	7	6	25
24	6	6	7	6	25
total	111	83	106	76	376