



Functional thermal limits are determined by rate of warming during simulated marine heatwaves

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ABSTRACT: Marine heatwaves (MHWs) are increasing in both intensity and frequency against a backdrop of gradual warming associated with climate change. In the context of MHWs, animals are likely to experience sub-lethal rather than lethal effects, defining long-term limits to survival and/or impacting individual and population fitness. We investigated how functional sub-lethal limits track critical thresholds and how this relationship changes with warming rate. To this end, we monitored basic functioning, specifically the ability to right, feed and assimilate energy, as well as oxygen consumption rate in the common Antarctic sea urchin *Sterechinus neumayeri*. Water temperature in experimental systems was increased at rates of 1, 0.5 and 0.3°C d⁻¹, in line with the characteristics of MHW events previously experienced at the site where the study urchins were collected on the Antarctica Peninsula. Functioning was assessed during the simulation of MHWs, and sub-lethal limits were determined when the rate of functional degradation changed as temperature increased. Results suggest that thermal sensitivity varies between the key biological functions measured, with the ability to right having the highest thermal threshold. Functions deteriorated at lower temperatures when warming was more rapid (1°C d⁻¹), contrary to lethal critical thresholds, which were reached at lower temperatures when warming was slower (0.3°C d⁻¹). MHWs and their impacts extend far beyond Antarctica, and in this context, our analyses indicate that the onset rate of MHWs is critical in determining the ability of an organism to tolerate short-term elevated temperatures.

KEY WORDS: Extreme warming events · Sub-lethal limits · Thermal tolerance · Climate change · Polar · Segmented regression · Echinoderm · *Sterechinus neumayeri*

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1. INTRODUCTION

Historical temperature records have revealed positive temperature trends for the majority of the Earth's surface (Myrvoll-Nilsen et al. 2019), with the oceans being key to the regulation and capture of much of the excess heat present in the atmosphere (Marshall et al. 2015). As a result, marine environments are changing both physically and biochemically (Bopp et al. 2013). Included in these changes is the occurrence of marine heat waves (MHWs), which are increasing

in duration, magnitude and frequency, with alarming ecological consequences (Garrabou et al. 2009, Rubio-Portillo et al. 2016, Oliver et al. 2018).

Physiological flexibility of species is crucial to survival during MHW events (Peck 2011), and species at low latitudes may be able to acclimate and adapt across generations to altered environments (Donelson et al. 2012, Salinas & Munch 2012, Clark et al. 2019a). As a result, predicting effects of MHWs on lower-latitude species may need to consider shifting thermal ranges as these species adapt to climate change. It is

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unlikely that the same will apply to Antarctic species, since many are physiologically limited by their capacity to acclimate and adapt to new temperatures because of their long generation times and delayed reproductive maturity (Peck et al. 2014, Peck 2018). For example, several invertebrate species such as the Antarctic scallop *Adamussium colbecki*, the limpet *Nacella concinna* and the bivalves *Laternula elliptica* and *Adacnarca nitens* take 4–7 yr to mature. The Antarctic bivalve *Aequiyoldia eightsi* starts reproducing at around 12 yr (Peck & Bullough 1993), and the brachiopod *Liothyrella uva* can take up to 18 yr before brooding young (Peck 2005, 2018, Oliver et al. 2019).

Predicting species and ecosystem responses to MHWs is challenging, owing to the past infrequency and variability of each event (Oliver et al. 2018). However, if we can track the functional deterioration of organisms when temperatures exceed their typical thermal range, this can inform our understanding of the relationships between the sub-lethal and lethal limits likely to be encountered during MHW events.

For organisms with slow growth and development and long generation times, like many of those found in Antarctica, thermal stress caused by MHWs is likely to trigger other mechanisms for survival such as biochemical and cellular stress responses (e.g. Clark & Peck 2009, Payton et al. 2016). Biochemical and genetic mechanisms, including a range of chaperone proteins, provide a short-term buffer that allows functioning to continue temporarily at temperatures outside the thermal niche of an organism (Deschaseaux et al. 2010, Clark et al. 2019b). Once animals are no longer able to maintain basic functions by these mechanisms, the sub-lethal limit to survival is reached.

Data on the functional thermal limits of species and MHW characteristics (i.e. rate, magnitude and duration) at which these thresholds are reached are rare, especially in fluctuating environments (Janecki et al. 2010, Peck et al. 2014, Ardor Bellucci & Smith 2019). Little is known about functional deterioration as a species approaches its critical thermal limit, and in the context of MHWs, animals are likely to experience temperatures that cause sub-lethal rather than lethal effects, defining long-term limits to survival and/or inhibiting population health (Pörtner et al. 2007).

This study aims to understand how functional (sub-lethal) limits track critical (lethal) limits and how this relationship changes with warming rate during a simulated MHW. To this purpose, we monitored the ability to right, feed and assimilate energy, as well as the oxygen consumption rate, in the common Antarctic sea urchin *Sterechinus neumayeri*.

2. MATERIALS AND METHODS

2.1. Sample site and animal collections

Sterechinus neumayeri were sampled from South Cove, Rothera Point (67° 34' 09.1" S, 68° 07' 52.7" W), from sites near the British Antarctic Survey's Rothera Research Station on the Western Antarctic Peninsula (WAP) during December 2019 (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m685p183_supp.pdf). Adult urchins (test diameter range: 28–49 mm; n = 120) were collected by SCUBA divers at depths of 10–20 m and returned to the Rothera aquarium facility within 2 h of collection.

S. neumayeri is one of the most common and locally abundant members of the Antarctic marine shallow benthos, forming a significant component of the benthic community (Brockington 2001, Pierrat et al. 2012), with reported densities up to 600 m⁻² (Barnes & Brockington 2003). It is a major scavenger of dead organisms and occurs in iceberg scours on the shallow Antarctic seabed (Dunlop et al. 2014); it is also a significant grazer and bioturbator of sediments (Lenihan et al. 2018). Because of this, *S. neumayeri* is an important carbon transformer in Antarctic shallow seas. Moreover, because of its abundance and ease of maintenance in laboratory culture systems, *S. neumayeri* has been the subject of extensive study of its embryonic and larval development, which is highly extended, sometimes lasting >100 d (Bosch et al. 1987). This species has also been the subject of studies of the effects of temperature on embryonic and larval development (Stanwell-Smith & Peck 1998), and the impact of ocean acidification on reproduction (Suckling et al. 2014) and energy budgets (Morley et al. 2016). Furthermore, there are long-term cycles in its reproduction (De Leij et al. 2021). These factors all make *S. neumayeri* one of the most important members of the Antarctic shallow benthic ecosystem and key to investigating responses to MHWs.

2.2. Experimental set-up and warming system

A decade of temperature data (1997–2017) from Ryder Bay on the WAP (sourced from the Rothera Time-Series environmental monitoring programme; Clarke et al. 2008, Venables et al. 2013) was used in the R package 'heatwaveR' (Schlegel & Smit 2018), to detect past warming events (Fig. 1) (see details of warming event analysis methodology and characteristics summary in Text S1, Table S1 and Fig. S2).

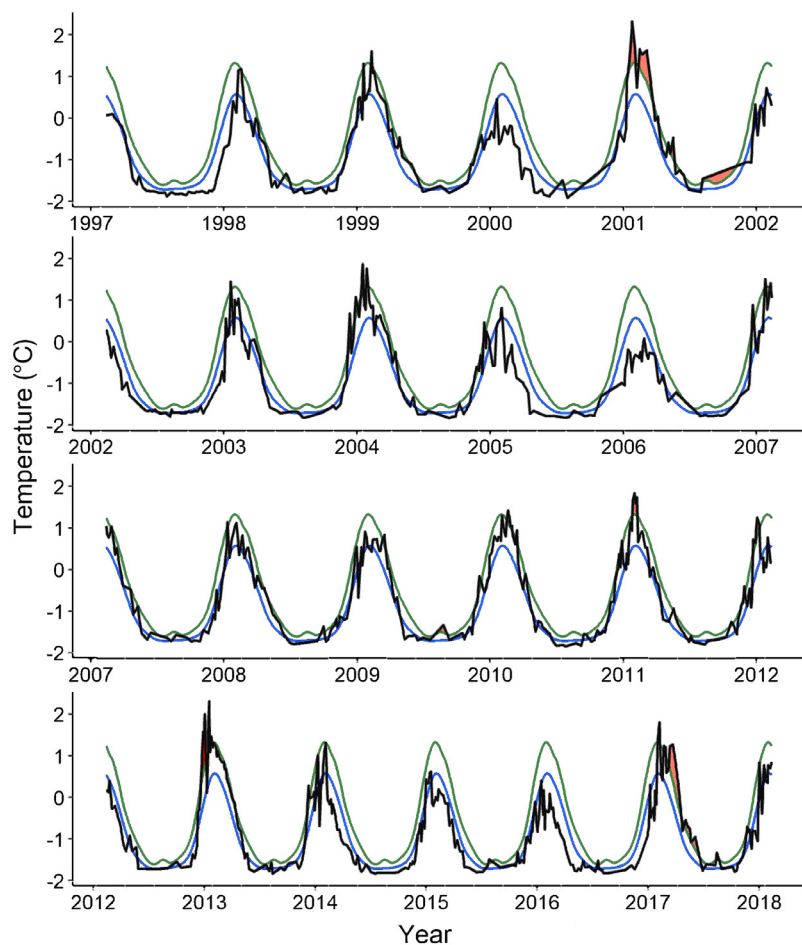


Fig. 1. Time-series of temperatures ($^{\circ}\text{C}$) experienced in Ryder Bay, Antarctica, at depths of 15 m, represented by the black lines. The data are split into panels to cover the entire span of the time-series, where the x-axis represents time in years. Blue lines represent the seasonal climatology of the region based on the full time-series of daily temperatures (1997–2018). Green lines represent the seasonally varying threshold for a marine heatwave (90th percentile). Temperatures exceeding the threshold for ≥ 5 d are highlighted in red and indicate the occurrence of a marine heatwave

Studying the characteristics of these past warming events, including onset rate and magnitude, allowed us to set realistic warming rates for the experimental systems.

Urchins were held in flow-through aquaria (170 l) at ambient temperatures typical for December and January (-1.5 to $+0.5^{\circ}\text{C}$) for 6 wk on a continuous light regime. During this time, animals were not fed to allow any ingested food to be processed and the production of faeces to cease. The cessation of faeces production is an indicator that metabolic rates have reached a ‘standard’ level at the start of the experiment. Previous research suggests that these urchins are able to sustain and experience natural periods of starvation for up to 6 mo during winter (Brockington

2001), and hence 6 wk without feeding was unlikely to be detrimental to the physiological metrics measured in this study. Previous studies of oxygen consumption in Antarctic marine invertebrates has demonstrated that standard levels are reached in less, and often significantly less, than this time in the brachiopod *Liothyrella uva* and the limpet *Nacella concinna* (Peck 1989), in the amphipod *Waldeckia obesa* (Chapelle et al. 1994), in the isopod *Glyptonotus antarcticus* (Robertson et al. 2001) and in the sea star *Odontaster validus* (Peck et al. 2008).

After urchins were maintained in the flow-through aquarium (170 l) at ambient temperatures, 30 urchins were distributed to 4 main aquarium tanks to represent each warming treatment and the ambient control treatment. Urchins were distributed at random. Replication within each of these treatments was achieved by floating 5 separate 6 l tanks, each containing 6 urchins in each main aquarium tank (170 l). Each main aquarium tank functioned as a temperature bath (Fig. S3; 30 urchins per treatment, 5 replicates per treatment where data from urchins in the same replicate floating tank were pooled). Temperature treatments were not replicated due to space restrictions. The same treatment conditions (i.e. temperature) was translated to all replicate urchins, and as such, temperature was closely monitored to note and control variability (Fig. S4).

The water in each floating tank was aerated using air stones and refreshed by 50% water change every other day. Water changes not only ensured that overall water quality was maintained, but also meant that any metabolic products, especially potentially toxic nitrogenous chemical species, were maintained at very low levels. Tank water samples were periodically analysed for pH (range: 7.5–8.0), NO_2 (0.05 – 0.1 mg l^{-1}), NO_3 (0.5 – 1.0 mg l^{-1}) and NH_4 (stable at 0.1 mg l^{-1}) to ensure good water quality. Throughout the experiment, concentrations of the aforementioned compounds remained within the ranges stated.

Urchins within each replicate tank were separated by aquaria egg crates and fine mesh partitions to ensure that individuals were isolated and any faeces

produced was retained within compartments (Fig. S3). During warming trials, experimental temperatures in the aquaria water baths were raised by 1, 0.5 or 0.3°C each evening, depending on treatment. Temperatures in the floating tanks increased more gradually than the water baths, allowing urchins to adjust slowly to each new temperature. Temperatures were checked every 30 min after each temperature change to ensure required temperatures were achieved and kept constant. Initially, temperatures fluctuated by up to $\pm 0.3^\circ\text{C}$ before stabilising after 1–2 h. Temperatures were subsequently monitored throughout the following day and held within $\pm 0.1^\circ\text{C}$ of the target experimental temperature (Fig. S4). For ambient controls, urchins were held in the aquarium with the set-up and light conditions identical to the warming treatment conditions. Temperatures were maintained at those experienced in Ryder Bay, which naturally fluctuated between 0.9 and 1.9°C.

2.3. Feeding trials

Urchins were fed pre-portioned amounts of food every 48 h. Previous studies fed *S. neumayeri* high-protein diets, such as fish fillets (*Polachius virens*) (Suckling et al. 2014, Morley et al. 2016). In the current study, urchins were fed the foot of the common Antarctic limpet *N. concinna*, which has a comparable protein content to that of *P. virens* muscle. Based on feeding protocols of Morley et al. (2016), urchins were fed ~4% of their mean body mass every 3 wk, but this was spread across 48 h feeding increments in order to keep feeding activity constant and reduce the variability in daily metabolic activity.

Limpets were chosen as a food source since nutrient content could be controlled and pre-portioned. A more representative diet would be a varied one with algal biofilm, animal tissues and/or detritus (McClintock 1994). However, administering a varied diet would make it difficult to assess the amount of food consumed per urchin at the same time as standardising the nutritional content. There is evidence that diet, especially protein levels, can affect development and gonad growth (Liu et al. 2007, Zupo et al. 2019) as well as ingestion and assimilation rates in sea urchins (Azad et al. 2011). As such, by feeding a diet of limpets, it is possible that body condition may be altered and the ability to tolerate stress may be improved as a result.

Feeding was initiated 2 d before the beginning of the experiment to start the digestion process. Each urchin was allowed to feed for 48 h before any

remaining food was removed and refreshed. After 48 h, each urchin was recorded as feeding or not feeding. Infrequently, urchins may have only partially consumed the food piece, which was recorded.

2.4. Faecal collection

Faecal production began 4 d into the experiment, 6 d after feeding was initiated. The presence of faeces was recorded for all urchins every 48 h. To measure faecal production, faeces were collected every 48 h by pipette and transferred to Falcon tubes from 10 urchins per treatment, where at least 1 sample was taken from each replicate tank within the treatment. The same urchins were targeted for faecal collection to minimise subconscious preferences towards urchins producing more faeces. This was not always possible since sometimes urchins did not produce any faeces or else the critical thermal limit (CT_{max}) was reached, and these urchins were removed. In these cases, a different urchin was chosen at random to sample from. For all other urchins, any remaining faecal matter was removed.

Collected faecal matter was centrifuged and the supernatant seawater decanted. Faeces were then rinsed with reverse osmosis purified water by agitating and centrifuging to remove any seawater salt. Washed faeces were pipetted into pre-ashed and pre-weighed foil boats and dried at 60°C for 24 h. Dry foil boats and faeces were placed in a desiccator to cool and then weighed (± 1 mg). Dry faeces were subsequently combusted in a muffle furnace at 475°C for 6 h. Foil boats and ashed faeces were cooled in a desiccator and weighed (± 1 mg). Dry mass and ash-free dry mass (AFDM) (i.e. organic content) were obtained by subtraction.

2.5. Respirometry

Oxygen consumption was recorded for 10 urchins per treatment, sampling 2 individuals from each replicate tank within each treatment. Oxygen consumption was recorded for the same urchins for every 2°C rise in temperature from ambient in each treatment. Methods for measuring oxygen consumption followed those described by Suckling et al. (2015), using 200–250 ml volume chambers. For each urchin, live wet mass (± 0.01 g) was recorded where O_2 consumption was measured. AFDM was determined from live wet mass vs. AFDM regressions determined from a subsample of urchins ($n = 40$) col-

lected from the same site. To obtain the ash mass of urchins, individuals were weighed live before freezing in liquid nitrogen and storing at -40°C . Frozen urchins were then placed in pre-ashed and pre-weighed ceramic crucibles and dried at 60°C until constant mass was obtained (± 0.01 g). Once dried, urchins were combusted in a muffle furnace at 475°C for 6 h and subsequently weighed to obtain ash mass after cooling in a desiccator (± 1 mg).

2.6. Righting

The time taken for urchins to right themselves was recorded for 10 urchins per treatment, sampling 2 urchins from each replicate tank within each treatment. The time taken to right was recorded for the same urchins every 2°C rise in temperature from ambient in each treatment. Ten individuals were removed from their experimental tanks and placed in individual containers. These containers were previously filled and floated in water already at the experimental target temperature. Urchins were immediately inverted following transfer from experimental tanks to the floating containers and timed until the individual was fully upright. Urchins could not reach the sides of containers to aid in righting. Once righted, urchins were returned to their experimental tanks.

2.7. Critical temperature limits (CT_{max})

The CT_{max} was recorded for all experimental urchins in the warming treatments, where the limit was defined as the point at which the individual was unable to right itself within 12 h and had stopped eating and producing faeces. When an urchin began to show signs of reaching the CT_{max} (not feeding or producing faeces), it was inverted in the tank and left for 12 h. If the urchin had not righted itself after this period, it was removed and weighed suspended in water to obtain its live wet volume (± 0.01 ml).

2.8. Statistical analysis

Where multiple urchins were sampled within the same floating tank, measurements of feeding, faecal production, righting and oxygen consumption were pooled so that $n = 5$, and the standard errors were calculated from these 5 replicate tanks.

To determine differences in functional responses between treatments, a 1-way repeat measures ANOVA

was carried out in R (v. 4.0.5). This analysis was considered appropriate for this experiment due to the related and non-independent groups at each temperature timepoint. For this analysis, treatment group variances were compared when treatments reached the same temperature increments. For ambient controls, temperature timepoints were aligned with measurements taken at similar dates to treatment sampling. Variances were compared between groups and within timepoints for righting and oxygen consumption rates, and the resultant p-value was adjusted using the Bonferroni correction method. Significant differences ($p < 0.05$) were followed up with a paired *t*-test, and again, p-values were adjusted using the Bonferroni correction method. Data were initially log transformed to ensure that the assumptions of a normal distribution were met.

Segmented linear regression models were fitted in the R package 'segmented' (Muggeo 2008) to identify breakpoints in the linear relationships between functional process and temperature. Breakpoints were identified where the gradient of the relationship changed (McWhorter et al. 2018). The change in gradient was used to define the functional threshold of the process measured. It was especially important to use a method such as segmented regression to identify breakpoints in process rates. Segmented regressions were used to model these relationships not necessarily for the purpose of fitting the simplest model, but rather to identify any change in the regressions gradient which then indicated that the functional response to temperature increase had changed. In some cases, a linear regression would be sufficient to explain the relationship; however, a linear model could mask the subtle change in the rate of degradation experienced when a species hits a thermal threshold. Alternatives would be to fit curves and identify changes in slope (e.g. Pörtner et al. 2006), but curves were not appropriate here. A Davies test was also conducted to determine significant ($p < 0.05$) differences in the gradients of the segmented slopes.

Size effects on functional response were explored through scatter plots. Where relationships were observed, the effect of size (test diameter) and temperature on the functional response was assessed with a linear mixed effects model using the package 'lme4' and the function 'lmer' in R (v. 4.0.5). Test diameter and temperature were added as interacting fixed terms, and replicate tank ID was added as a random effect. Prior to any modelling, function responses were transformed to achieve normality in the distribution.

3. RESULTS

3.1. Feeding and faecal egestion

On average (\pm SD), $80 \pm 19\%$ of animals fed in ambient conditions for the duration of the experiment. For the first 4 d of the experiment, in treatments where the temperature increase ($T\uparrow$) was 1°C d^{-1} , the proportion of animals feeding exceeded all other treatments ($97 \pm 4\%$), including ambient conditions ($87 \pm 10\%$). Fifty percent of animals stopped feeding in treatments when temperatures exceeded 7.2 , 8.2 and 9.2°C , where $T\uparrow$ by 1 , 0.5 and 0.3°C d^{-1} , respectively (Fig. 2).

A breakpoint (where the slope of the regression changed) for the percent of individuals feeding (% feeding) was identified at 4.0 and 6.2°C in treatments where $T\uparrow$ was 1 and 0.5°C d^{-1} , respectively (Table 1). However, changes in the segmented slope gradients were not significantly different from linear regressions for these 2 treatments (Davies $p = 0.329$ and 0.301 , respectively). A breakpoint for the % feeding in $T\uparrow 0.3^\circ\text{C d}^{-1}$ was identified at 8.2°C (Table 1), from which point the % feeding declined rapidly and the relationship between temperature and the % feeding became significant ($p < 0.001$). The mean temperature breakpoint for the function of % feeding was $6.1 \pm 1.2^\circ\text{C}$, averaged across all treatments.

The percentage of animals producing faeces tracked the % feeding after the first 4 d (Fig. 2). Following each breakpoint, the relationship between temperature and % producing faeces became significant (Table 1). For the fastest rate of warming where $T\uparrow$ was 1°C d^{-1} , a breakpoint was identified at 5.2°C , above which the % producing faeces rapidly declined from 100 to 10.3% within 6 d. Where $T\uparrow$ was 0.3 and 0.5°C d^{-1} , the regression breakpoint for faecal production was 8.3 and 4.5°C , respectively (Table 1). The mean temperature breakpoint for the function of % producing faeces was $6.0 \pm 2.0^\circ\text{C}$, averaged across all treatments.

The mean mass of faeces produced in treatments where $T\uparrow$ was 0.3°C d^{-1} was significantly greater than the faecal mass produced in ambient control conditions and treatments where $T\uparrow$ was 1°C d^{-1} , until temperatures exceeded 2.1°C ($t_4 = 8.74$, $p = 0.006$ and $t_4 = 5.02$, $p = 0.044$, respectively). At $T\uparrow$ of 0.5°C d^{-1} , the mass of faeces produced was significantly greater than at $T\uparrow$ of 1°C d^{-1} , until temperatures exceeded 2.1°C ($t_4 = 5.31$, $p = 0.036$). Despite this observation, no additional food was consumed in these treatments. There was no significant difference between the treatments or control as temperatures increased beyond 2.1°C (Fig. S5).

Breakpoints in regressions were identified at 5.0 and 3.1°C for treatments where $T\uparrow$ was 0.5 and 0.3°C d^{-1} , respectively (Table 1). The breakpoints for these regressions marked a reduction in the gradient of the second slope, whereby faeces produced $\text{d}^{-1} \text{mg}^{-1}$ AFDM as a function of temperature decreased at a slower rate as temperatures increased. The mean temperature breakpoint for faeces produced was $4.1 \pm 0.95^\circ\text{C}$, averaged across the slowest ($T\uparrow = 0.3^\circ\text{C d}^{-1}$) and intermediate ($T\uparrow = 0.5^\circ\text{C d}^{-1}$) rates of warming.

3.2. Righting

In treatments where $T\uparrow$ was 1.0°C d^{-1} , time taken to right became significantly longer than ambient controls when temperatures reached 9.2°C ($t_4 = 6.06$, $p < 0.022$). For treatments where $T\uparrow$ was 0.3°C d^{-1} , time taken to right only became significantly longer than ambient controls just before CT_{max} was reached, when temperatures reached 11.2°C ($t_4 = 6.04$, $p < 0.023$). For treatments where $T\uparrow$ was 0.5°C d^{-1} , time taken to right never exceeded ambient controls significantly; however, mean righting times were consistently higher than in control conditions throughout the warming period (Fig. S6).

A breakpoint in the linear regression was identified at 8.7°C in treatments where temperature was raised at 0.3°C d^{-1} (Table 1). The relationship between temperature and the time taken to right became significant above this breakpoint temperature ($p < 0.001$). For the other treatments, righting time increased linearly without a breakpoint in the regression.

The interactive effect of urchin size and temperature on the time taken to right was significant ($t_{204} = 2.11$, $p = 0.034$), where larger urchins took longer to right at higher temperatures (Fig. S7, Table S3).

3.3. Oxygen consumption

Oxygen consumption rates were significantly higher in heatwave treatments compared to ambient controls when temperatures reached 7.2°C for all treatments. However, oxygen consumption rates were significantly higher than ambient controls from lower temperatures of 3.2°C in treatments where $T\uparrow$ was 0.3°C d^{-1} ($t_4 = 5.62$, $p = 0.030$), and 5.2°C in treatments where $T\uparrow$ was 1.0°C d^{-1} ($t_4 = 4.98$, $p = 0.045$) (Fig. S8). Overall, there was a positive linear trend between oxygen consumption and temperature for all treatments. However, at $T\uparrow$ of 1°C d^{-1} , a drop in O_2 con-

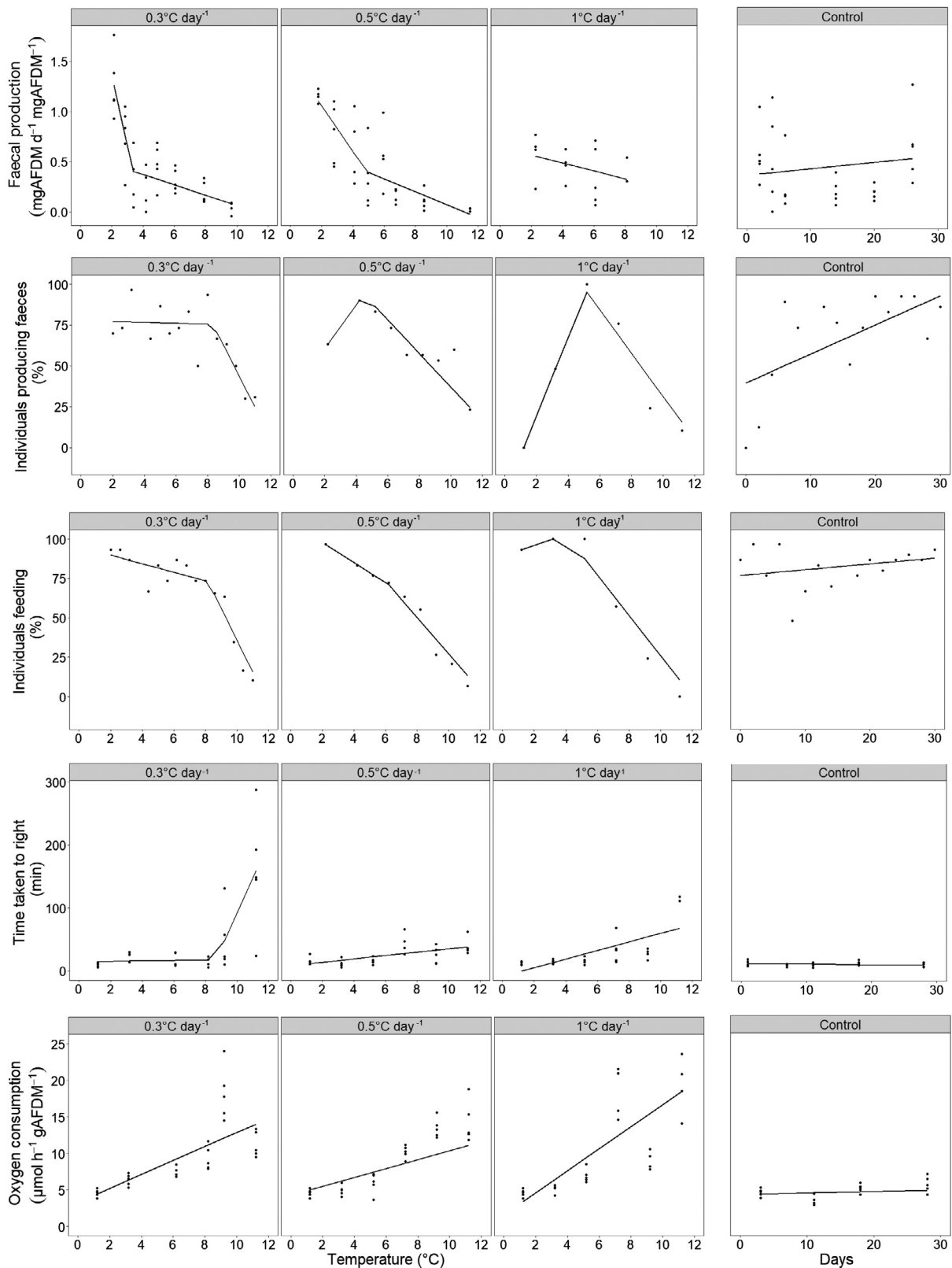


Fig. 2. Biological functions measured in *Stereochinus neumayeri* in experimental conditions where temperatures were increased daily by 0.3, 0.5 or 1°C. Functions in warming conditions are plotted against increasing temperature, and ambient control treatments are plotted against the number of days in the experiment. Data points represent the pooled data within replicate floating tanks ($n = 5$). Regressions are either segmented where appropriate for treatment conditions or linear for controls and treatment data where breakpoints were not identified. AFDM: ash-free dry mass

Table 1. Summary statistics for linear regression relationships between the measured functions of *Sterechinus neumayeri* and temperature. β : slope of the linear regression lines before (Slope_1) and after (Slope_2) the breakpoint (BP); SE_a: standard error for the intercept and slopes. **Bold** p-values indicate significant relationships ($p < 0.05$) between temperature and the variable measured, and **bold** Davies p-values represent a significant change ($p < 0.05$) in the gradient of the slope of segmented regressions. Values in the BP column indicate the localisation of the BP (NA indicates a single linear regression); SE_b and R² refer to the goodness of fit for the entire model

Function	β	SE _a	p	BP	SE _b	R ²	Davies p
Individuals feeding, 1°C d ⁻¹			df = 3	4.0	14.9	0.894	0.329
(Intercept)	89.0	25.4	0.039				
Slope_1	3.45	10.5	0.764				
Slope_2	-12.9	2.35	0.012				
Individuals feeding, 0.5°C d ⁻¹			df = 7	6.2	6.78	0.964	0.301
(Intercept)	110.3	12.7	<0.001				
Slope_1	-6.34	3.14	0.083				
Slope_2	-11.5	1.05	<0.001				
Individuals feeding, 0.3°C d ⁻¹			df = 12	8.2	8.48	0.922	0.001
(Intercept)	95.3	7.53	<0.001				
Slope_1	-2.73	1.38	0.071				
Slope_2	-20.3	2.92	<0.001				
Individuals producing faeces, 1°C d ⁻¹			df = 3	5.2	13.5	0.881	0.019
(Intercept)	-29.0	23.1	0.298				
Slope_1	24.1	9.54	0.085				
Slope_2	-13.3	2.13	0.008				
Individuals producing faeces, 0.5°C d ⁻¹			df = 7	4.5	12.1	0.844	0.039
(Intercept)	34.0	28.6	0.274				
Slope_1	13.3	8.54	0.162				
Slope_2	-10.3	8.68	<0.001				
Individuals producing faeces, 0.3°C d ⁻¹			df = 12	8.3	12.5	0.762	0.006
(Intercept)	77.9	11.1	<0.001				
Slope_1	-0.306	2.02	0.882				
Slope_2	-18.6	4.29	<0.001				
Faeces produced, 1°C d ⁻¹			df = 14	NA	0.216	0.071	0.858
(Intercept)	0.645	0.137	<0.001				
Slope_1	-0.040	0.027	0.165				
Faeces produced, 0.5°C d ⁻¹			df = 31	4.9	1.11	0.664	0.043
(Intercept)	1.52	0.214	<0.001				
Slope_1	-0.23	0.072	0.007				
Slope_2	-0.06	0.025	0.016				
Faeces produced, 0.3°C d ⁻¹			df = 34	3.3	0.294	0.729	<0.001
(Intercept)	3.54	0.509	<0.001				
Slope_1	-0.718	0.202	0.001				
Slope_2	-0.051	0.020	0.012				
Time taken to right, 1°C d ⁻¹			df = 26	NA	23.3	0.476	NA
(Intercept)	-8.60	9.04	0.350				
Slope_1 ^a	6.83	1.35	<0.001				
Time taken to right, 0.5°C d ⁻¹			df = 26	NA	13.1	0.302	NA
(Intercept)	8.88	5.03	0.089				
Slope_1 ^a	2.61	0.731	0.001				
Time taken to right, 0.3°C d ⁻¹			df = 25	8.7	0.556	0.588	<0.001
(Intercept)	14.6	20.1	0.237				
Slope_1	0.384	3.66	0.459				
Slope_2	55.7	13.8	<0.001				
Oxygen consumption, 1°C d ⁻¹			df = 28	NA	4.64	0.551	NA
(Intercept)	1.64	1.76	0.358				
Slope_1 ^a	1.50	0.248	<0.001				
Oxygen consumption, 0.5°C d ⁻¹			df = 33	NA	3.17	0.368	NA
(Intercept)	4.29	1.10	<0.001				
Slope_1 ^a	0.611	0.134	<0.001				
Oxygen consumption, 0.3°C d ⁻¹			df = 28	NA	3.49	0.471	NA
(Intercept)	3.30	1.36	0.022				
Slope_1 ^a	0.957	0.185	<0.001				

^aReporting only a single slope (Slope_1) indicates that no BP was detected in the regression, and statistics for a single linear regression model are reported for the data instead

sumption occurred at 9.2°C, and at $T\uparrow$ of 0.3°C d⁻¹, a drop occurred just before the CT_{max} at 11.2°C.

O₂ consumption increased at a faster rate per increase in temperature where warming rates were fastest at 1°C d⁻¹ (slope gradient = 1.50) and increased at the slowest rate when warming rates were slowest at 0.3°C d⁻¹ (slope gradient = 0.96) (Table 1). No breakpoint was identified in any treatment.

3.4. CT_{max}

The CT_{max} for urchins in treatments where $T\uparrow$ was 0.3, 0.5 and 1°C d⁻¹ had ranges of 10.6–13.8, 11.2–13.7 and 12.2–14.2°C, respectively. The effect of warming rate on the CT_{max} was significant ($F_{2,12} = 7.29$, $p = 0.008$), with post hoc analysis identifying that for treatments where temperature increased at the fastest rate (1°C d⁻¹), the CT_{max} was significantly higher compared to treatments where temperature increased at a slower rate (0.3°C d⁻¹) ($t_8 = -6.02$, $p = 0.001$).

Across all functions where breakpoints were identified, the slowest rate of warming (0.3°C d⁻¹) had a mean temperature breakpoint of 8.3 ± 1.3°C. In comparison, the mean temperature breakpoint was 5.4 ± 0.5°C, and 4.6 ± 0.6°C for intermediate (0.5°C d⁻¹) and fast (1°C d⁻¹) warming rates, respectively.

4. DISCUSSION

MHWs are predicted to increase in frequency, intensity and duration in the coming decades. Deterioration of basic animal functioning, critical for long-term survival, will likely be a more frequent consequence of the short-term warming (i.e. weeks to months) caused by MHWs, rather than mortality. However, little is known about functional impacts, especially thresholds and how these limits deteriorate with respect to CT_{max} . By understanding how key biological functions are affected by short-term temperature elevations and different warming rates, we can better understand how extreme climate events, typified by short-term warming, may impact individuals and populations, and hence communities.

In this study, we investigated the effect of warming rates typical of those expected during Antarctic MHW events on the functioning of the Antarctic sea urchin *Sterechinus neumayeri*. Functional thresholds were identified using segmented regressions, where a breakpoint indicated a gradient change in the response trend with temperature. The identification of regression breakpoints, or slope changes has been

used previously to define ecological thresholds, and is considered a more flexible and realistic approach when interpreting complex, often non-linear, ecological relationships (Piepho & Ogutu 2003, Ferrarini 2011, Morley et al. 2014).

Several studies have shown that faster warming rates result in higher CT_{max} in terrestrial (e.g. Terblanche et al. 2007, Allen et al. 2016) and marine (Peck et al. 2009) species. These observations, along with the CT_{max} data in this study, follow the failure rate model proposed by Kingsolver & Umbanhowar (2018), who showed that critical limits are reached at lower temperatures when warming accumulates over extended periods. However, our results for functional thermal limits follow the opposite trend to the CT_{max} , where functions are impacted negatively at lower temperatures when warming is rapid. Overall, in this study higher functional thresholds were reached when temperatures were raised slowly (thresholds averaging 8.3 ± 1.3°C). At the faster warming rates, functional thresholds were lower (5.4 ± 0.5°C or 4.6 ± 0.6°C). There was even evidence that some functions declined linearly, with significant deterioration from temperatures +2.8°C above ambient when warmed at the fastest rate. Thus, short-term exposure to more extreme temperatures has more impact on functioning than longer, chronic exposure to more slowly elevated temperatures.

Although metabolic acclimation is unlikely over such short time periods (apparent from the oxygen consumption data here, and also previous research on long-term acclimation of *S. neumayeri*; Peck et al. 2014, Suckling et al. 2015), short-term acclimation for some functions might be possible after an initial shock response when temperatures are increased slowly. In our study, the shock response did not appear to subside at faster rates of warming, and instead mean functional thresholds were lower as warming rate increased. These results suggest that functional and lethal limits are likely driven and determined by different mechanisms. Previous studies have shown that lethal limits are likely set by one or both of physiological processes or cellular and biochemical mechanisms. At very rapid rates of warming, such as 1°C h⁻¹ or 1°C d⁻¹, physiological mechanisms such as nervous and circulatory failure appear to be the limiting factors (Young et al. 2006, Pörtner et al. 2007, Bilyk & DeVries 2011). At slower rates of warming (1°C every 3 d to 1°C mo⁻¹), cellular and biochemical mechanisms such as accumulation of toxic products, e.g. protein carbonyls, enzyme tolerances or insufficiency of chaperone protein capacity appear to be limiting (Peck et al. 2009, Clark et al.

2017, 2018). The factors setting thermal limits and responses to warming were recently shown to be highly species specific (Clark et al. 2021, Collins et al. 2021).

Our results also indicate that thermal sensitivity varies among key biological functions. For example, the function of righting in urchins was similar between treatments and ambient control conditions until temperatures reached 9.2°C for the fastest rates of warming, and the highest breakpoint of 8.7°C was identified for the slowest rates of warming. However, lower thresholds were identified for the other functions related to digestion such as % feeding or % producing faeces. Variation between functional thresholds could be related to function complexity, where a function involving multiple processes would be more likely to fail (Pörtner et al. 2007, Stevens et al. 2010, Peck 2011). Another explanation could be related to the extent to which functions limit survival and fitness, where the energy reserves of an organism allow for short periods of negative energy balance. In Antarctic marine species, such periods of negative energy balance can be very long, extending to months or even years of low food supply or starvation, because of the extreme environmental seasonality and the very low metabolic energy use characteristic of this fauna (Brockington et al. 2001, Harper & Peck 2003, Obermüller et al. 2010). However, being able to right provides immediate protection from predation, equivalent to mechanisms such as the ability to stay attached to the substratum in limpets (Morley et al. 2012b) or reburial in infaunal clams when disturbed and removed from the sediment by, for example, iceberg scour (Peck et al. 2004). Finally, where a function has a higher metabolic energy demand, it is more likely to be limited by food availability and energy delivery capacity (van der Meer 2006, Morley et al. 2012a, Peck 2018).

The breakpoints identified for the mass of faeces produced might not indicate a functional threshold. Instead, the initial high faecal production in the slowest and intermediate warming rates is likely a result of the initial increase in temperature causing food to move faster through the urchin, as also seen in the Antarctic plunderfish *Harpagifer antarcticus* (Boyce et al. 2000). This elevation in faecal production was only observed when temperatures increased initially, after which faecal production reduced to rates similar to ambient control conditions. This effect was not observed in treatments with the fastest rates of warming since these slight increases in temperature of 1–2°C were likely not maintained long enough for gut passage rate to increase. Therefore, our results indicate that the breakpoints for faecal production

may not have any direct implications on functionality and instead give evidence for the relationship between temperature and gut evacuation rate.

In thermally stressed environments, animals usually increase their oxygen uptake in order to meet increasing demands of functional processes (Gillooly et al. 2001). However, when oxygen uptake is increased, yet functioning deteriorates, it is hypothesised that this indicates a threshold where uptake, transport and delivery of oxygen can no longer meet the functional demands of the animal. This theory has been termed the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (Pörtner et al. 2017). This theory focusses on the limitations set by the physiology of an animal. However, as temperature increases, the concentration of oxygen diminishes, further reducing the availability of oxygen to the animal and potentially amplifying the effects of OCLTT. Reducing the concentration of oxygen in the water can limit functioning (Peck et al. 2007, Pörtner et al. 2007), and as such, the functional thresholds identified in this study may not only indicate thermal limits but may also be influenced by the reduced oxygen content as temperatures increased. If oxygen concentration was controlled and elevated throughout warming, the functional thresholds identified would likely be higher (Pörtner et al. 2006). However, warmer oceans will be accompanied by lower oxygen concentrations (Oschlies et al. 2018, Spicer et al. 2019) and the functional thresholds determined in this study will be more representative of a natural system than if oxygen were controlled.

Food availability and quality can also be a significant factor in determining functional scope (Welch et al. 1998, Lemoine & Burkepile 2012, Cheng et al. 2018), whereby the nutritional status and condition of the animal could affect energy delivery capacity similarly to OCLTT. For example, feeding and digestive capacity limited the thermal tolerance of juvenile spiny lobsters *Sagmariasus verreauxi* (Fitzgibbon et al. 2017), and the digestive capacity and food intake of individuals at high temperatures was related to depressed mitochondrial respiratory capacity in brown trout *Salmo trutta* (Salin et al. 2016). The capacity to assimilate energy would also play a role in determining energy delivery to tissues and is determined by physiological processes including consumption rate, absorption of food and gut evacuation rate (Boyce et al. 2000, Angilletta 2001). Hence, assimilation itself is energetically demanding and may limit functional thermal thresholds (Sandersfeld et al. 2015, Salin et al. 2016).

Thus, OCLTT may be a possible mechanism for determining functional limits observed in our experi-

ments. However, there is no empirical support in our data for this theory. In both experiments and in natural MHWs, other factors are likely to be involved, and obtaining sufficient energy from food may be necessary for successful functioning. Impacts on animal condition from warming may be especially important in highly seasonal polar environments where warming in winter, when food supplies are scarce, would increase energy use with little or no opportunity to mitigate the cost (Peck 2018). Species such as *S. neumayeri* that have been shown to spend periods in winter up to 7 mo without feeding (Brockington 2001) may be particularly vulnerable to such impacts.

Our experiment included a period of 6 wk without feeding to allow metabolic activity to stabilise and be comparable between individuals. However, a caveat to this initial standardisation of conditions could influence the physiological response of the urchins to the warming in treatments. Nutritional status has been shown to affect the reproductive state of *S. neumayeri*, with a reduction in gonad index and maturation of gametes following 6 wk without food, compared to animals foraging naturally in the environment (De Leij 2021). Functional capacity has also been affected in other invertebrates under low food coupled with environmental stress; for example, blue mussels *Mytilus edulis* had a reduced ability to repair shells when high CO₂ was coupled with low food (Melzner et al. 2011), and green sea urchins *Strongylocentrotus droebachiensi* exhibited severe metabolic acidosis when exposed to elevated CO₂ with empty digestive tracts (Stumpp et al. 2012). Hence, we might consider that the elevated temperatures coupled with the suboptimal nutritional status at the start of the experiment may have impacted the thermal limits of certain functions. This would likely have resulted from a mismatch between a limited energy supply and stores, and an increased energy demand of the animal. However, the data in this study shows a reduction in the number of urchins feeding as temperatures increase, suggesting that food was not the limiting factor when this species approached its functional thermal limits.

From our analysis of the Rothera Time-Series environmental data, previous MHW events reached maximum temperatures of $2.3 \pm 0.36^\circ\text{C}$, with onset rates of 0.3°C d^{-1} . Days at heatwave status have extended up to 95 d, and cumulative intensities (a combination of temperature intensity and heatwave duration) have reached maxima of $54^\circ\text{C} \times \text{day}$ (Fig. S2). Mean climate temperatures are predicted to shift by $+2^\circ\text{C}$ by 2100, and with that, climate extremes such as MHWs will increase in magnitude relative to this

shift (IPCC 2014, 2019). Our results suggest that functions such as feeding and faecal egestion are likely to be affected by MHW events occurring in 2100, if not before, and this will include increased metabolic demands with consequent impacts on annual energy budgets.

For a long-lived (>40 yr; Brey et al. 1995) and slow to mature (8–9 yr; Peck 2018) species such as *S. neumayeri*, there will be less scope for phenotypic and genotypic adaptations to a warming climate as might be possible for short-lived and rapidly maturing species (Peck 2011, Donelson et al. 2012, Salinas & Munch 2012). However, there may still be opportunity for *S. neumayeri* to adapt to a warmer world. Within 80 yr (2020–2100), 8 generations of *S. neumayeri* will have succeeded the present population, and in the year 2100, the 5th, 6th and 7th generation could be present and reproducing in populations around Antarctica. If we consider the evidence of its capacity to acclimate, it may be possible for *S. neumayeri* to acclimate and adapt successfully to function in a $+2^\circ\text{C}$ warmer world (Morley et al. 2016). It is still uncertain, however, how this species will respond to acute warming, like that experienced during MHWs, in this warmer climate. The data in this study cannot be used to predict the implications of acclimation and adaptation on the subsequent tolerance to MHWs for *S. neumayeri*. Instead, the data provide insight into the effect of onset rate of acute warming, the thermal vulnerability of key biological functions and the difference between critical and functional thermal limits. Thus, according to our data, we could see reduced energy availability for *S. neumayeri* from changes in feeding and food processing rates during MHWs in warmer oceans, which would very likely reduce survival in marginal environments.

Following the results from this study, it would be important to explore recovery following MHW events. Our data indicate reduced functioning as temperatures are raised across all rates of warming. However, the ability and rate of *S. neumayeri* to resume 'normal' functioning if returned to ambient temperatures is uncertain. The marine snail *Littorina littorea* loses motility under thermal stress, but when temperatures are lowered again, this function returns (Hamby 1975). To resume a single function may not indicate full recovery, and our study shows that different biological functions have varying thermal tolerances. As such, performance of all functions, including metabolic activity, would need to return to baseline levels for an animal to recover completely (Walter et al. 2013). Developing our understanding of recovery following acute warming and even the effects of repeat

MHW events, could better predict the long-term implications of MHWs for this species.

It is important to note that the functional and critical limits measured in this study are likely an example of a 'best case scenario'. Experiments such as these can only predict the isolated effects of one variable. However, the additional energetic costs associated with physical factors such as salinity change and biological factors including varying food quality and quantity, species interactions, diseases and scavenging for food, need to be included before we can obtain dependable predictions for 'real world' scenarios that give information relevant to the variable conditions experienced across a species distribution range. What is limiting at the range margins for a species will differ from core areas (Kolzenburg et al. 2021).

Our data highlight that the deterioration of functioning when temperatures are raised, especially during MHWs, has implications for long-term survival and physiological functions. Therefore, functioning should be considered when determining organism thermal limits, rather than traditional critical thermal limits. Our findings show that fitness cannot be determined from a single function and instead functions vary in thermal sensitivity. A whole-organism approach to functional fitness is therefore necessary, considering functional complexity, importance and energetic demand. Our results suggest that contrary to the relationship between critical thermal limits and onset rate, functional degradation occurs at lower temperatures when exposed to rapid warming (1°C d^{-1}). Therefore, when investigating the impact of MHWs on organisms and populations, it is important to consider the key features of the heatwave event, including the onset rate, exposure duration and how these characteristics act together to determine functional thresholds.

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