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

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6 Rebecca De Leij[12], Laura J. Grange[2], Lloyd S. Peck[3]

7 [1]University of Southampton, Waterfront Campus, European Way,

8 Southampton, SO14 3ZH

9 [2]School of Ocean Sciences, Bangor University, Bangor, Gwynedd, North

10 Wales, LL57 2DG

11 [3]British Antarctic Survey, High Cross, Madingley Rd, Cambridge, CB3 0ET

12 Correspondence: Email: ridl1n17@soton.ac.uk; Phone: +44(0)7544553603

13 Running head*:* Functional thermal limits during marine heatwaves

1 **ABSTRACT**

2 Marine heatwaves (MHWs) are increasing in both intensity and frequency

3 against a backdrop of gradual warming associated with climate change. In the

4 context of MHWs, animals are likely to experience sub-lethal, rather than lethal

5 effects, defining long-term limits to survival and/or impacting individual and

6 population fitness. This study investigated how functional sub-lethal limits track

7 critical thresholds and how this relationship changes with warming rate. To this

8 end we monitored basic functioning, specifically the ability to right, feed and

9 assimilate energy, as well as oxygen consumption rate in the common Antarctic

10 sea urchin, *Sterechinus neumayeri.* Water temperature in experimental

11 systems was increased at rates of $1^{\circ}C\ day^{-1}$, $0.5^{\circ}C\ day^{-1}$ and $0.3^{\circ}C\ day^{-1}$, in

12 line with the characteristics of MHW events previously experienced at the site

13 where the study urchins were collected on the Antarctica Peninsula.

14 Functioning was assessed during the simulation of MHWs and sub-lethal limits

15 determined when the rate of functional degradation changed as temperature

16 increased. Results suggest that thermal sensitivity varies between the key

17 biological functions measured, with the ability to right having the highest

18 thermal threshold. Arguably, the most interesting result was that functions

19 deteriorated at lower temperatures when warming was more rapid ($1^{\circ}C\ day^{-1}$),

20 contrary to lethal critical thresholds, which were reached at lower temperatures

21 when warming was slower ($0.3^{\circ}C\ day^{-1}$). MHWs and their impacts extend far

22 beyond Antarctica and in this context, our analyses indicate that the onset rate

23 of MHWs is critical in determining an organism's ability to tolerate short-term

24 elevated temperatures.

1. INTRODUCTION

Historical temperature records have now detected 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 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 years to mature. The Antarctic bivalve, *Aequiyoldia eightsi*, starts reproducing at around 12 years (Peck &

Bullough 1993) and the brachiopod *Liothyrella uva*, can take up to 18 years before brooding young (Peck 2005, 2018, Oliver et al. 2019).

Predicting species and ecosystem responses to MHWs is challenging, owed 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 allow functioning to continue temporarily at temperatures outside an organism's thermal niche (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).

1   This study aims to understand how functional (sub-lethal) limits track critical

2   (lethal) limits and how this relationship changes with warming rate during a

3   simulated MHW. To this purpose, we monitored the ability to right, feed and

4   assimilate energy as well as oxygen consumption rate, in the common Antarctic

5   sea urchin, *Sterechinus neumayeri*.

6   **2. MATERIALS AND METHODS**

7   2.1 Sample site and animal collections

8   *Sterechinus neumayeri* were sampled from South Cove, Rothera Point

9   (67°34'09.1"S 68°07'52.7"W), from sites near the British Antarctic Survey's

10 Rothera Research Station on the Western Antarctic Peninsula (WAP) during

11 December 2019 (Figure S1). 120 adult urchins (test diameter range, 28 mm –

12 49 mm) were SCUBA-diver collected at depths of 10-20 m and returned to the

13 Rothera aquarium facility within two hours of collection.

14 *Sterechinus neumayeri* is one of the most common and locally abundant

15 members of the Antarctic marine shallow benthos, forming a significant

16 component of the benthic community (Brockington 2001, Pierrat et al. 2012),

17 with reported densities up to 600 m^2 (Barnes & Brockington 2003). It is a major

18 scavenger of dead organisms and in iceberg scours on the shallow Antarctic

19 seabed (Dunlop et al. 2014), and is a significant grazer and bioturbator of

20 sediments (Lenihan et al. 2018). Because of this *S. neumayeri* is an important

21 carbon transformer in Antarctic shallow seas. Further to this, due to its

22 abundance and ease of maintenance in laboratory culture systems, *S.*

23 *neumayeri* has been the subject of extensive study of its embryonic and larval

24 development, which is highly extended, and up to in excess of 100 days (Bosch

25 et al. 1987). It has also been the subject of studies of the effects of temperature

on embryonic and larval development (Stanwell-Smith & Peck 1998), the

impact of ocean acidification on reproduction (Suckling et al. 2014) and energy

budgets (Morley et al. 2016). Furthermore, it has been shown that 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 (RaTS) 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

(Figure 1) (see details of warming event analysis methodology and

characteristics summary in the Supplementary Materials, Text S1, Table S1,

Figure S2). 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°C to +0.5°C) for six weeks 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 had 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 six months during winter (Brockington 2001), and hence six

weeks 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 four main aquarium tanks to represent each warming treatment as well as the ambient control treatment. Urchins were distributed at random. Replication within each of these treatments was achieved by floating five separate 6-litre tanks, each containing six urchins in each main aquarium tank (170 L). Each main aquarium tank functioned as a temperature bath (Figure 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 (Figure 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 any metabolic products, especially potentially toxic nitrogenous chemical species, were maintained at very low levels. Tank water samples were periodically analysed for pH (ranging

1   7.5 - 8.0), NO_2 (ranging 0.05 mg I^{-1} – 0.1 mg I^{-1}), NO_3 (ranging 0.5 mg I^{-1} - 1.0

2   mg I^{-1}) and NH_4 (stable at 0.1 mg I^{-1}) to ensure good water quality. Throughout

3   the experiment, concentrations of the aforementioned compounds remained

4   within the ranges stated.

5   Urchins within each replicate tank were separated by aquaria egg crates and

6   fine mesh partitions to ensure individuals were isolated and any faeces

7   produced was retained within compartments (Figure S3). During warming trials

8   experimental temperatures in the aquaria water baths were raised by 1°C,

9   0.5°C or 0.3°C each evening, depending on treatment. Temperatures in the

10   floating tanks increased more gradually than the water baths, allowing urchins

11   to adjust slowly to each new temperature. Temperatures were checked every

12   30 minutes after each temperature change to ensure required temperatures

13   were achieved and kept constant. Initially, temperatures fluctuated by up to ±

14   0.3°C before stabilising after 1-2 hrs. Temperatures were subsequently

15   monitored throughout the following day and held within ± 0.1°C of the target

16   experimental temperature (Figure S4). For ambient controls, urchins were held

17   in the aquarium with the set-up and light conditions identical to the warming

18   treatment conditions. Temperatures were maintained at those experienced in

19   Ryder Bay which naturally fluctuated between 0.9 °C and 1.9°C.

20   2.3 Feeding trials

21   Urchins were fed pre-portioned amounts of food every 48 hrs. Previous studies

22   fed *S. neumayeri* high protein diets, such as fish fillets, *Polachius virens*

23   (Suckling et al. 2014, Morley et al. 2016). In the current study, urchins were fed

24   the foot of the common Antarctic limpet, *Nacella concinna,* which has a

25   comparable protein content to that of *P. virens* muscle. Based on feeding

protocols in Morley et al. (2016b), urchins were fed ~4% of their mean body mass every three weeks, but this was spread across 48 hr 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 two days before the beginning of the experiment to start the digestion process. Each urchin was allowed to feed for 48 hrs before any remaining food was removed and refreshed. After 48 hrs, 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 four days into the experiment, 6-days after feeding was initiated. The presence of faeces was recorded for all urchins every 48 hrs. To measure faecal production, faeces were collected every 48 hrs by pipette and transferred to falcon tubes from 10 urchins per treatment, where at least

one 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 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 RO (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 hrs. Dry foil boats and faeces were placed in a desiccator to cool and then weighed (± 1 mg). Dry faeces were subsequently ignited in a muffle furnace at 475°C for 6 hrs. Foil boats and ashed faeces were cooled in a desiccator and weighed (± 1 mg). Dry mass (DM) 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 two 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

1 (n = 40) collected from the same site. To obtain the ash mass of urchins,

2 individuals were weighed live before freezing in liquid nitrogen and storing at –

3 40°C. Frozen urchins were then placed in pre-ashed and pre-weighed ceramic

4 crucibles and dried at 60°C until constant mass was obtained (± 0.01 g). Once

5 dried, urchins were ignited in a muffle furnace at 475°C for 6 hrs and

6 subsequently weighed to obtain ash mass after cooling in a desiccator (± 1 mg).

7 2.6 Righting

8 The time taken for urchins to right themselves was recorded for 10 urchins per

9 treatment, sampling two urchins from each replicate tank within each treatment.

10 The time taken to right was recorded for the same urchins every 2°C rise in

11 temperature from ambient in each treatment. Ten individuals were removed

12 from their experimental tanks and placed in individual containers. These

13 containers were previously filled and floated in water already at the

14 experimental target temperature. Urchins were immediately inverted following

15 transfer from experimental tanks to the floating containers and timed until the

16 individual was fully upright. Urchins could not reach the sides of containers to

17 aid in righting. Once righted, urchins were returned to their experimental tanks.

18 2.7 Critical temperature limits (CT_{max})

19 The critical thermal limit (CT_{max}) was recorded for all experimental urchins in

20 the warming treatments, where the limit was defined as the point at which the

21 individual was unable to right itself within 12 hrs, had stopped eating and

22 producing faeces. When an urchin began to show signs of reaching the CT_{max}

23 (not feeding or producing faeces), they were inverted in the tank and left for 12

1   hrs. If the urchin had not righted itself after this period, they were removed and

2   weighed suspended in water to obtain live wet volumes (± 0.01 mL).

3   2.8 Statistical Analysis

4   Where multiple urchins were sampled within the same floating tank,

5   measurements of feeding, faecal production, righting, and oxygen consumption

6   were pooled so that n = 5, and the standard errors were calculated from these

7   five replicate tanks.

8   To determine differences in functional responses between treatments, a one-

9   way repeat measures analysis of variance (ANOVA) was carried out in R (v.

10   4.0.5). This analysis was considered appropriate for this experiment due to the

11   related and non-independent groups at each temperature timepoint. For this

12   analysis, treatment group variances were compared when treatments reached

13   the same temperature increments. For ambient controls, temperature

14   timepoints were aligned with measurements taken at similar dates to treatment

15   sampling. Variances were compared between groups and within timepoints for

16   righting and oxygen consumption rates and the resultant p-value was adjusted

17   using the Bonferroni correction method. Significant differences ($p < 0.05$) were

18   followed up with a paired t-test and again, p-values were adjusted using the

19   Bonferroni correction method. Data were initially log transformed to ensure

20   assumptions of normal distribution were met.

21   Segmented linear regression models were fitted in the R package 'segmented'

22   (Muggeo 2008) to identify breakpoints in the linear relationships between

23   functional process and temperature. Breakpoints were identified where the

24   gradient of the relationship changed (McWhorter et al. 2018). The change in

1   gradient was used to define the functional threshold of the process measured.

2   It was especially important to use a method such as segmented regression to

3   identify breakpoints in process rates. Segmented regressions were used to

4   model these relationships not necessarily for the purpose of fitting the simplest

5   model, but rather to identify any change in the regressions gradient which then

6   indicated that the functions response to temperature increase had changed. In

7   some cases, a linear regression would be sufficient to explain the relationship,

8   however a linear model could mask the subtle change in the rate of degradation

9   experienced when a species hits a thermal threshold. Alternatives would be to

10 fit curves and identify changes in slope (e.g. Pörtner et al. 2006), but curves

11 were not appropriate here. A Davies test was also conducted to determine

12 significant ($p < 0.05$) differences in the gradients of the segmented slopes.

13 Size effects on functional response were explored through scatter plots. Where

14 relationships were observed, the effect of size (as test diameter) and

15 temperature on the functional response, was assessed with a linear mixed

16 effects model using the package 'lme4' and the function 'lmer' in R (v. 4.0.5).

17 Test diameter and temperature were added as interacting fixed terms and

18 replicate tank ID was added as a random effect. Prior to any modelling, function

19 responses were transformed to achieve normality in the distribution.

20 **3. RESULTS**

21 3.1 Feeding and faecal egestion

22 On average, 80% ± 19% of animals fed in ambient conditions for the duration

23 of the experiment. For the first four days of the experiment, in treatments where

24 T↑ 1°C day^{-1}, the proportion of animals feeding exceeded all other treatments

25 (97% ± 4%), including ambient conditions (87% ± 10%). Fifty percent of animals

1   stopped feeding in treatments when temperatures exceeded 7.2°C, 8.2°C, and

2   9.2°C, where T↑ by 1°C, 0.5°C and 0.3°C day^{-1}, respectively (Figure 1).

3   A breakpoint (where the slope of the regression changed) for the % individuals

4   feeding was identified at 4.0°C and 6.2°C in treatments where T↑ 1°C day^{-1}

5   and 0.5°C day^{-1}, respectively (Table 1). However, changes in the segmented

6   slope gradients were not significantly different from linear regressions for these

7   two treatments (Davies p-value = 0.329 and 0.301, respectively). A breakpoint

8   for the % feeding in T↑ 0.3°C day^{-1} was identified at 8.2°C (Table 1), from which

9   point the % individuals feeding declined rapidly and the relationship between

10   temperature and the proportion of individuals feeding became significant (p

11   <0.001). The mean temperature breakpoint for the function of % feeding was

12   6.1°C ± 1.2°C, averaged across all treatments.

13   The percentage of animals producing faeces tracked the proportion of animals

14   feeding after the first four days (Figure 1). Following each breakpoint, the

15   relationship between temperature and % individuals producing faeces became

16   significant (Table 1). For the fastest rate of warming where T↑ 1°C day^{-1}, a

17   breakpoint was identified at 5.2°C, above which the % individuals producing

18   faeces rapidly declined from 100% to 10.3% within 6 days. Where T↑ 0.3°C

19   day^{-1} and 0.5°C day^{-1}, the regression breakpoint for faecal production was

20   8.3°C and 4.5°C respectively (Table 1). The mean temperature breakpoint for

21   the function of % producing faeces was 6.0°C ± 2.0°C, averaged across all

22   treatments.

23   The mean mass of faeces produced in treatments where T↑ 0.3°C day^{-1}, was

24   significantly greater than the faecal mass produced in ambient control

1 conditions and treatments where T↑ 1°C day^{-1}, until temperatures exceeded

2 2.1°C (t$_{(4)}$ = 8.74, p = 0.006 and t$_{(4)}$ = 5.02, p = 0.044, respectively). Where T↑

3 0.5°C day^{-1}, the mass of faeces produced was significantly greater than

4 treatments where T↑ 1°C day^{-1}, until temperatures exceeded 2.1°C (t$_{(4)}$ = 5.31,

5 p = 0.036). Despite this observation, no additional food was consumed in these

6 treatments. There was no significant difference between the treatments or

7 control as temperatures increased beyond 2.1°C .

8 Breakpoints in regressions were identified at 5.0°C and 3.1°C for treatments

9 where T↑ 0.5°C day^{-1} and 0.3°C day^{-1}, respectively (Table 1). The breakpoints

10 for these regressions marked a reduction in the gradient of the 2nd slope,

11 whereby faeces produced day^{-1} mgAFDM^{-1} as a function of temperature

12 decreased at a slower rate as temperatures increased. The mean temperature

13 breakpoint for faeces produced was 4.1°C ± 0.95°C, averaged across the

14 slowest (T↑ 0.3°C day^{-1}) and intermediate (T↑ 0.5°C day^{-1}) rates of warming.

15 3.2 Righting

16 In treatments where T↑ 1.0°C day^{-1}, time taken to right became significantly

17 longer than ambient controls when temperatures reached 9.2°C (t$_{(4)}$ = 6.06, p <

18 0.022). For treatments where T↑ 0.3°C day^{-1}, time taken to right only became

19 significantly longer than ambient controls just before CT$_{max}$ was reached, when

20 temperatures reached 11.2°C (t$_{(4)}$ = 6.04, p < 0.023). For treatments where T↑

21 0.5°C day^{-1}, time taken to right never exceeded ambient controls significantly,

22 however mean righting times were consistently higher than control conditions

23 throughout the warming period.

1 A breakpoint in the linear regression was identified at 8.7°C in treatments where

2 temperature was raised at 0.3°C day^{-1} (Table 1). The relationship between

3 temperature and the time taken to right became significant above this

4 breakpoint temperature (p <0.001). For the other treatments righting time

5 increased linearly without a breakpoint in the regression.

6 The interactive effect of urchin size and temperature on the time taken to right

7 was significant ($t_{(204)}$ = 2.11, p = 0.034), where larger urchins took longer to right

8 at higher temperatures (Figure S5, Table S3).

9 3.3 Oxygen consumption

10 Oxygen consumption rates were significantly higher in heatwave treatments

11 compared to ambient controls when temperatures reached 7.2°C for all

12 treatments. However, oxygen consumption rates were significantly higher than

13 ambient controls from lower temperatures of 3.2°C in treatments where T↑

14 0.3°C day^{-1} ($t_{(4)}$ = 5.62, p = 0.030) and 5.2°C in treatments where T↑ 1.0°C

15 day^{-1} ($t_{(4)}$ = 4.98, p = 0.045). Overall, there was a positive linear trend between

16 oxygen consumption and temperature for all treatments. However, where T↑

17 1°C day^{-1}, a drop in O_2 consumption occurred at 9.2°C, and where T↑ 0.3°C

18 day^{-1}, a drop occurred just before the CT_{max} at 11.2°C.

19 O_2 consumption increased at a faster rate per increase in temperature where

20 warming rates were fastest at 1°C day^{-1} (slope gradient = 1.50) and increased

21 at the slowest rate when warming rates were slowest at 0.3°C day^{-1} (slope

22 gradient = 0.96) (Table 1). No breakpoint was identified in any treatment.

23 3.4 CT_{max}

1   The CT_{max} for urchins in treatments where T↑ 0.3°C day⁻¹, T↑ 0.5°C day⁻¹ and

2   T↑ 1°C day⁻¹ ranged from 10.6°C - 13.8°C, 11.2°C - 13.7°C, and 12.2°C -

3   14.2°C, respectively. The effect of warming rate on the CT_{max} was significant

4   ($F_{(2, 12)}$ = 7.29, p = 0.008), with post-hoc analysis identifying that for treatments

5   where temperature increased at the fastest rate (T↑ 1°C day⁻¹), the CT_{max} was

6   significantly higher compared to treatments where temperature increased at a

7   slower rate (T↑ 0.3°C day⁻¹) ($t_{(8)}$ = -6.02, p = 0.001).

8   Across all functions where breakpoints were identified, the slowest rate of

9   warming (T↑ 0.3°C day⁻¹) had a mean temperature breakpoint of 8.3°C ± 1.3°C.

10   In comparison, the mean temperature breakpoint was 5.4°C ± 0.5°C, and 4.6°C

11   ± 0.6°C for intermediate (T↑ 0.5°C day⁻¹) and fast (T↑ 1°C day⁻¹) warming

12   rates, respectively.

13   **4. DISCUSSION**

14   MHWs are predicted to increase in frequency, intensity, and duration in the

15   coming decades. Deterioration of basic animal functioning, critical for long-term

16   survival, will likely be a more frequent consequence of the short-term warming

17   (i.e., weeks-months) caused by MHWs, rather than mortality. However, little is

18   known about functional impacts, especially thresholds and how these limits

19   deteriorate with respect to CT_{max}. By understanding how key biological

20   functions are affected by short term temperature elevations and different

21   warming rates, we can better understand how extreme climate events, typified

22   by short-term warming, may impact individuals and populations, and hence

23   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, *S. 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°C ± 1.3°C). At the faster warming rates functional thresholds were lower (5.4°C ± 0.5°C or 4.6°C ± 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^oC h^{-1} or 1^oC day^{-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^oC 3 $days^{-1}$ to 1^oC $month^{-1}$) 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). Recently the factors setting thermal limits and responses to warming have been 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^oC for the fastest rates of warming, and the highest breakpoint of 8.7^oC was identified in the slowest rates of warming. However, lower thresholds were identified for the other functions related to digestion such as % feeding or producing faeces.

1 Variation between functional thresholds could be related to function complexity,

2 where a function involving multiple processes would be more likely to fail

3 (Pörtner et al. 2007, Stevens et al. 2010, Peck 2011). Another explanation could

4 be related to the extent to which functions limit survival and fitness, where an

5 organism's energy reserves allow for short periods of negative energy balance.

6 In Antarctic marine species such periods of negative energy balance can be

7 very long, extending to months or even years of low food supply or starvation,

8 because of the extreme environmental seasonality and the very low metabolic

9 energy use characteristic of this fauna (Brockington et al. 2001, Harper & Peck

10 2003, Obermüller et al. 2010). However, being able to right provides immediate

11 protection from predation, equivalent to mechanisms such as the ability to stay

12 attached to the substratum in limpets (Morley et al. 2012b) or reburying in

13 infaunal clams when disturbed and removed from the sediment by, for example,

14 iceberg scour (Peck et al. 2004). Finally, where a function has a higher

15 metabolic energy demand, it is more likely to be limited by food availability and

16 energy delivery capacity (van der Meer 2006, Morley et al. 2012a, Peck 2018).

17 The breakpoints identified for the mass of faeces produced might not indicate

18 a functional threshold. Instead, the initial high faecal production in the slowest

19 and intermediate warming rates is likely a result of the initial increase in

20 temperature causing food to move faster through the urchin, as also seen in

21 the Antarctic plunderfish *Harpagifer antarcticus* (Boyce et al. 2000). This

22 elevation in faecal production was only observed when temperatures increased

23 initially, after which faecal production reduced to rates similar to ambient control

24 conditions. This effect was not observed in treatments with the fastest rates of

25 warming since these slight increases in temperature of $1^oC - 2^oC$ 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 (GER).

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 animal's functional demands. This theory has been termed the oxygen and capacity limited thermal tolerance hypothesis (OCLTT) (Pörtner et al. 2017). This theory focuses on the limitations set by the animal's physiology. 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 as such 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 digestive capacity and food intake of individuals at high temperatures 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 GER (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 experiments. 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 important, and obtaining sufficient energy from food may be important 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 seven months without feeding (Brockington 2001) may be particularly vulnerable to such impacts.

Our experiment included a period of six weeks without feeding to allow metabolic activity to stabilise and be comparable between individuals. However, a caveat to this initial standardisation of condition could influence the urchin's physiological response 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 six weeks without food, comparative 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 the blue mussel *Mytilus edulis* had a reduced ability to repair shells when high CO_2 was coupled with low food (Melzner et al. 2011) and the green sea urchin *Strongylo-centrotus droebachiensis*, exhibited severe metabolic acidosis when exposed to elevated CO_2 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 RaTS environmental data, previous MHW events reached maximum temperatures of 2.3°C ± 0.36°C, with onset rates of 0.3°C day^{-1}. Days at heatwave status have extended up to 95 days, and cumulative intensities (a combination of temperature intensity and heatwave duration) have reached maxima of 54°C x day (Figure S2). Mean climate temperatures are predicted to shift by +2°C by 2100, and with that, climate extremes such as MHWs will increase in magnitude relative to this (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

1 increased metabolic demands with consequent impacts on annual energy

2 budgets.

3 For a long-lived (>40 year (Brey et al. 1995)) and slow to mature (8-9 years

4 (Peck 2018)) species such as *S. neumayeri,* there will be less scope for

5 phenotypic and genotypic adaptations to a warming climate as might be

6 possible for short-lived and rapidly maturing species (Peck 2011, Donelson et

7 al. 2012, Salinas & Munch 2012). However, there may still be opportunity for *S.*

8 *neumayeri* to adapt to a warmer world. Within 80 years (2020 - 2100), eight

9 generations of *S. neumayeri* will have succeeded the present population, and

10 in the year 2100, the 5th, 6th and 7th generation could be present and

11 reproducing in populations around Antarctica. If we consider the evidence of *S.*

12 *neumayeri*'s capacity to acclimate, it may be possible for this species to

13 acclimate and adapt successfully to function in a +2°C warmer world (Morley et

14 al. 2016). It is still uncertain, however, how this species will respond to acute

15 warming, like that experienced during MHWs, in this warmer climate. The data

16 in this study cannot predict the implications of acclimation and adaptation on

17 the subsequent tolerance to MHWs for *S. neumayeri*. Instead, the data provides

18 insight into the effect of onset rate of acute warming, the thermal vulnerability

19 of key biological functions, and the difference between critical thermal limits and

20 functional thermal limits. Thus, according to our data we could see reduced

21 energy availability for *S. neumayeri* from changes in feeding and food

22 processing rates during MHWs in warmer oceans, which would very likely

23 reduce survival in marginal environments.

1 Following the results from this study, it would be important to explore recovery

2 following MHW events. Our data indicate reduced functioning as temperatures

3 are raised across all rates of warming. However, the ability and rate of *S.*

4 *neumayeri* to resume 'normal' functioning if returned to ambient temperatures

5 is uncertain. It has been shown that the marine snail, *Littorina littorea*, loses

6 motility under thermal stress, however if temperatures are lowered again, this

7 function returns (Hamby 1975). To resume a single function may not indicate

8 full recovery, and our study shows that different biological functions have

9 varying thermal tolerances. As such, performance of all functions, including

10 metabolic activity, would need to return to baseline levels for an animal to

11 recover completely (Walter et al. 2013). Developing our understanding of

12 recovery following acute warming and even the effects of repeat MHW events,

13 could better predict the long-term implications of MHWs for this species.

14 It is important to note that the functional and critical limits measured in this study

15 are likely an example of a 'best case scenario'. Experiments such as these can

16 only predict the isolated effects of one variable. However, the additional

17 energetic costs associated with physical factors such as salinity change and

18 biological factors including varying food quality and quantity, species

19 interactions, diseases and scavenging for food, need to be included before we

20 can obtain dependable predictions for 'real world' scenarios that give

21 information relevant to the variable conditions experienced across a species

22 distribution range. What is limiting at the range margins for a species will differ

23 from core areas (Kolzenburg et al. 2021).

24 Our data highlight that the deterioration of functioning when temperatures are

25 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 day^{-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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AUTHOR CONTRIBUTIONS

1   L.S.P and R.D conceived and designed the study. R.D carried out the

2   practical work and data processing. R.D, L.J.G and L.S.P analysed the data,

3   drafted the manuscript and approved its publication.

4   **COMPETING INTERESTS**

5   The authors declare no competing interests.

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15

16   **TABLES**

17   Table 1: Summary statistics for linear regression relationships between the

18   measured functions of *Sterechinus neumayeri* and temperature. β indicates the

19   slope of the linear regression lines before the breakpoint (Slope_1) and after

20   the breakpoint (Slope_2); SE_a indicates standard error for the intercept and

21   slopes; df = degrees of freedom; bold p-values indicate significant relationships

22   (p < 0.05) between temperature and the variable measured and bold Davies p-

23   values represent a significant change (p < 0.05) in the gradient of the slope of

24   segmented regressions. Values in the column BP indicate the localisation of

25   the breakpoint or else NA indicates a single linear regression; SE_b (standard

26   error) and R^2 refers to the goodness of fit for the entire model.

Function	β	SE_a	p-value	BP	SE_b	R^2	Davies p-value

	Estimate	Std. Error	p				
Individuals feeding, 1°C day^{-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^{o}C day^{-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^{o}C day^{-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 day^{-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^{o}C day^{-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^{o}C day^{-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 day^{-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^{o}C day^{-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^{o}C day^{-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 day^{-1}			df=26	NA	23.3	0.476	NA
(Intercept)	-8.60	9.04	0.350				
Slope_1[1]	6.83	1.35	<0.001				
Time taken to right, 0.5^{o}C day^{-1}			df=26	NA	13.1	0.302	NA
(Intercept)	8.88	5.03	0.089				
Slope_1[1]	2.61	0.731	**0.001**				
Time taken to right, 0.3^{o}C day^{-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 day^{-1}			df=28	NA	4.64	0.551	NA
(Intercept)	1.64	1.76	0.358				
Slope_1[1]	1.50	0.248	**<0.001**				
Oxygen consumption, 0.5^{o}C day^{-1}			df=33	NA	3.17	0.368	NA
(Intercept)	4.29	1.10	<0.001				
Slope_1[1]	0.611	0.134	<0.001				

Oxygen consumption, 0.3^{o}C day^{-1} (Intercept) Slope_1[1]	3.30 0.957	1.36 0.185	df=28 **0.022** **<0.001**	NA	3.49	0.471	NA

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[1] Reporting only a single slope (Slope_1) indicates that no breakpoint was detected in the regression and statistics for a single linear regression model is reported for the data instead.
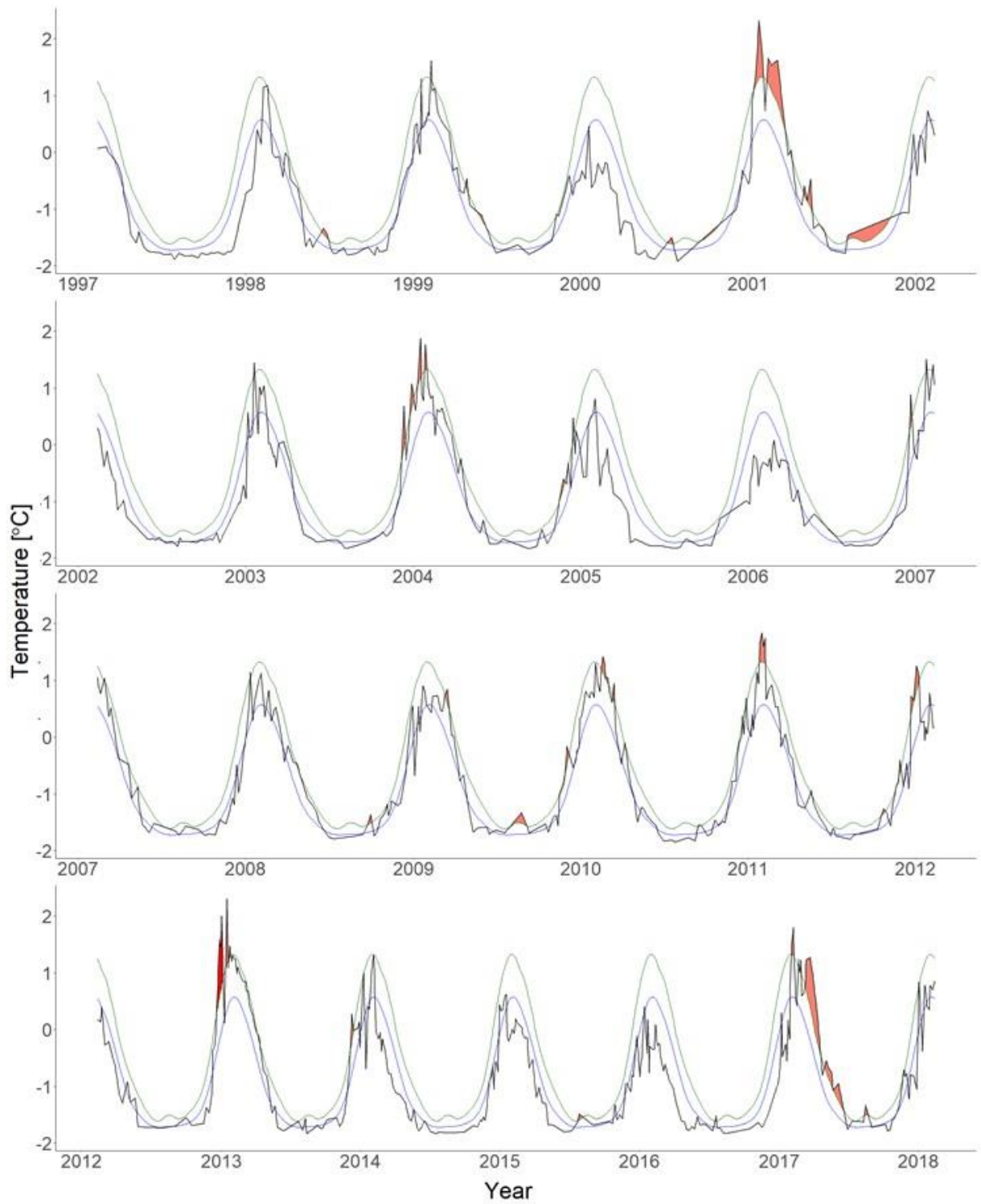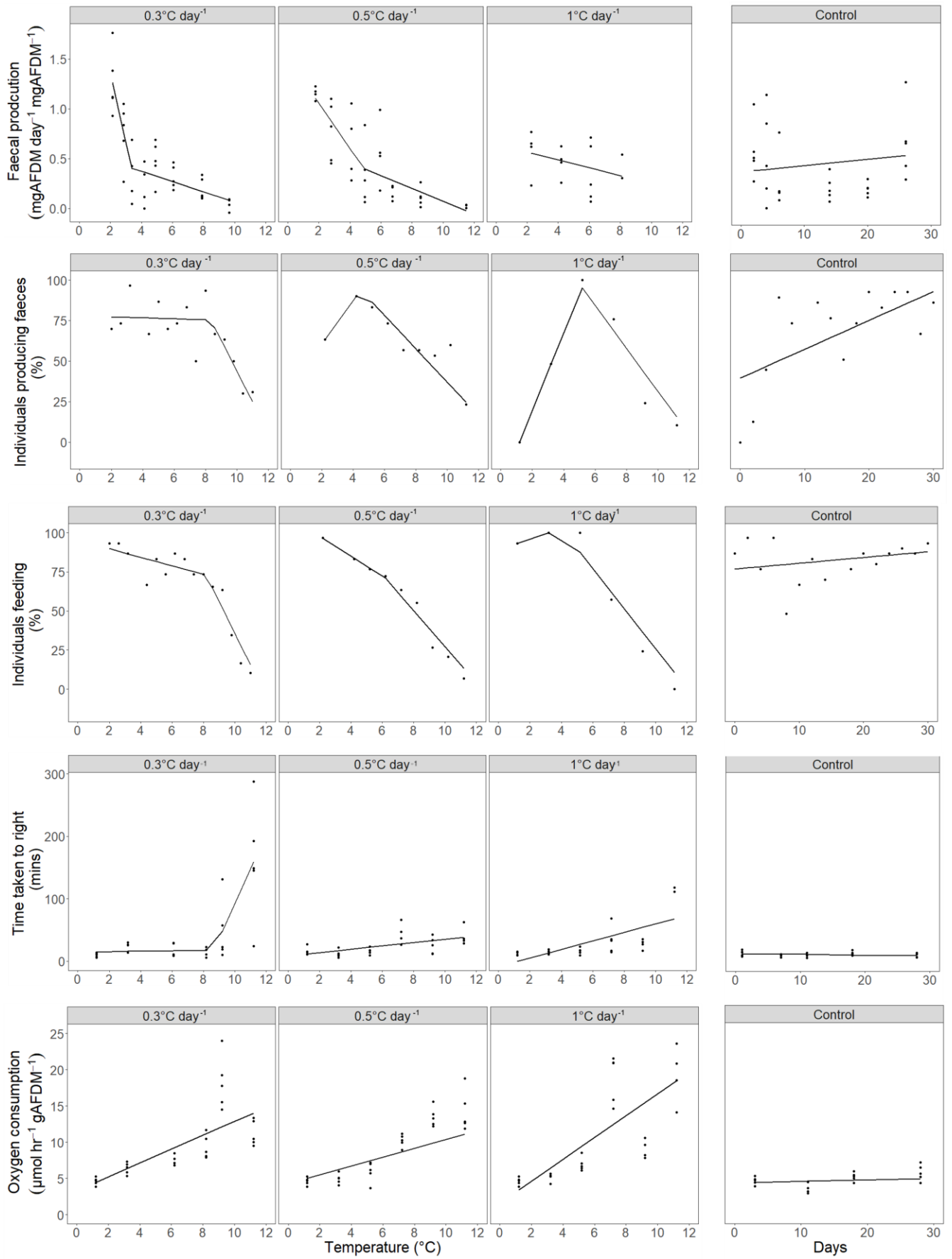
FIGURES

3   Figure 1: Times-series of temperatures (^oC) experienced in Ryder Bay,

4   Antarctica, at depths of 15 m, represented by the black lines. The data are split

5   into panels to cover the entire span of the time-series, where the x-axis

1   represents time in years. Blue lines represent the seasonal climatology of the

2   region based on the full time-series of daily temperatures (1997 – 2018). Green

3   lines represent the seasonally varying threshold for a marine heatwave (90th

4   percentile). Temperatures exceeding the threshold for ≥ 5 days are highlighted

5   in red and indicate the occurrence of a marine heatwave.

1   Figure 2: *Sterechinus neumayeri.* Biological functions measured in *Sterechinus*

1 *neumayeri* in experimental conditions where temperatures were increased daily

2 by 0.3ºC, 0.5ºC and 1ºC. Functions in warming conditions are plotted against

3 increasing temperature and ambient control treatments are plotted against the

4 number of days in the experiment. Data points represent the pooled data within

5 replicate floating tanks (n=5). Regressions are either segmented where

6 appropriate for treatment conditions or linear for controls and treatment data

7 where breakpoints were not identified.