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

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Dear Dr. Lauzon-Guay,

Thank you for the opportunity to submit a revised draft of the manuscript titled: 'Functional thermal limits are determined by rate of warming during simulated marine heatwaves' to Marine Ecology Progress Series. We appreciated the detailed and valuable feedback that was provided by the managing editor, Christine Paetzold, and all three referees. In particular, the suggestion from reviewer 1 to revise the statistical analysis has enabled an important error to be amended. In additional to this, we also value the comment by reviewer 3 to further explore relationship between size and thermal thresholds. This additional analysis has led to some interesting insight into the interactive effect of urchin size and temperature on the time taken to right. The authors have been able to incorporate most of the suggested changes in the manuscript, all of which have been highlighted as tracked changes in the pdf uploaded as 'Manuscript showing edits'. For comments that have not resulted in changes to the manuscript, we have provided further explanation in the responses below.

Managing Editor Christine Paetzold comments:

Please also address the following points in your revision:
(i) Please carefully check your manuscript for correct spelling (e.g. 'Absract').
This has been amended

(ii) P4 L22-25: are all these references essential to be listed? If possible, please avoid 'strings' of citations (more than 3 cites in a row). Such strings make the text very tedious to read and give the impression of a literature review rather than the selectivity expected in a scientific article. Try to be more selective and just list 3-4 references preceded by 'e.g.'.
This has been amended throughout

(iii) When you report the first result from a statistical test, please provide the name of the test. If the following results are from the same test, there is no need to repeat the name.
This has been amended

(iv) P15L20-22: if these results are from post-hoc tests, the p-value suffices. Otherwise, please change the df-value (after the t) to subscript font and remove the brackets. Please clarify what the d-value you report is (if this was not made clear previously).
This has been amended and clarified

(v) Table 1: is it intentional that for 'Faeces produced, 1o C day-1', there is no result for Slope_2?
Yes this is intentional. There was no breakpoint identified in this regression and hence no second slope. The statistics are therefore reported for Slope_1 only. Further explanation has been added as a footnote to the table.

(vi) Tables and figures: please only capitalize the first word in table headers and figure labels. E.g. "Individuals feeding (%)"
This has been amended

Reviewer 1 report:

The manuscript explores the impact of sublethal warming during heatwaves on the physiology of an Antarctic sea urchin. The authors performed an experiment involving temperature ramps with different rates of temperature increase. The authors focus on a combination of feeding physiology, activity levels and metabolism is an interesting a much needed approach to fully understand the impact of this events.
I think the manuscript deserves publication. However I have some comments that I think need to be taken care off.

- in the introduction and discussion, warming and heatwaves are often mixed, while they are related their impact will be different and while the experiments may apply well to current cases of heatwaves, we have seen already these experiments don't relate well to long term warming. Also since up the experiments were performed from just one base temperature, these results are difficult to discuss under the context of heatwaves on a warmer scenario.

This is a valid comment and we agree that the distinction between temperature increase from MHWs and temperature increase by gradual climate warming was not made clear. We also acknowledge that the terms were confused in the original manuscript and as such we have now made amendments to the text to adjust the focus on MHWs in the current climate with only cautious interpretation in the context of the future.

- the methods need to be clarified. ANOVA is not adequate for this type of data and experiment (repeated measurements). The use of cumulative degree days needs to be justified better, also the resulting scale is confusing, lower cumulative effect results in larger impact, there is nothing wrong there but the justification of their use (a common scale to compare time points) is a little bit weak. Other analyses may have been considered, such as nonlinear decaying models, survival models, etc that consider dosage and time and kinetics of processes which are specially affected by temperature.

Firstly, thank you for bringing this statistical error to our attention. Instead of a traditional one-way ANOVA, we have now re-analysed the differences between treatments during the experiment using a 'one-way repeated measures ANOVA'. This analysis accounts for related and non-independent groups as is appropriate for our data. Treatment group variance are compared when treatments reach the same temperature increments and are referred to as t1, t2, t3 etc. A figure of the results of this analysis is now included in the supplementary materials.

With regards to cumulative intensity, on revising the manuscript we have decided that this metric creates unnecessary confusion. Although cumulative intensity allows temperature and exposure to be translated into a metric directly comparable between treatments, we think this is achieved by reporting only temperature and observing the rate at which this temperature was reached (i.e. 0.3oC/day, 0.5oC/day or 1.0oC/day). We hope this change will also make the paper and the data easier to reference in other studies since most previous studies report on temperature limits.

I also have a series of minor comments in the attached doc file

These have all been carried out in the amended manuscript
In particular:

Comments in the introduction: the use of the term 'permanent heatwave' has been removed to avoid confusion and the wording and sentence structure has been changed where appropriate (page 4 and 5).

Comments on page 6, line 23 and page 7, line 4: Further information regarding rationale behind non-feeding period and standardisation of metabolic activity has been added on page 8, line 21-24 and page 9 lines 1-12.

Comment page 11, line 1: A repeated measures ANOVA has now been used and results have been amended throughout.

Comments on page 12: The use of cumulative intensity has now been replaced by temperature to avoid confusion.

Comment page 13, line 16: We were not necessarily looking for the simplest model, but rather we were looking for any change in the regressions gradient which then indicated that the functions response to temperature increase had changed. A linear regression may be sufficient to explain the relationship, however it may mask the subtle change in the rate of degradation experienced when a species hits a thermal threshold. We have therefore removed any reference to model fit (R^2) that was used to justify the segmented regression and have instead emphasised the reason behind using a segmented regression as explained above in the manuscript.

Comment page 19, line 9: This is an interesting point and something that had not been thought of when interpreting the results. As a result, we have added a section to the discussion relating to the faecal production rates and the reasons driving these on page 26, lines 22-25 and page 27, lines 1-10.

Comment on page 20: Discussion on the effects of nutritional status has been added on page 29, lines 12-25 and page 30, lines 1-6.

Comment page 21, line 4: This paragraph has been amended considering this comment.

The following linked document contains further information from this reviewer:
https://www.ManuscriptManager.net/sLib/v4/marked_docs/mm_meps~1997~ce9b5128585e~1~965.ReviewerMarkUp.docx

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Reviewer 2 report:

Functional thermal limits are determined by rate of warming

General comments
This is an important and excellent study, nicely executed and described. This is because while there are a number of studies examining the outcomes of warming in Antarctic species, the potential outcomes of marine heatwaves are less well understood in the regions ecosystems.

Research here is one of the few studies to directly address polar MHW, using the common Antarctic sea urchin Sterechinus neumayeri, to explore the outcomes of warming rate/time on key physiological processes. Treatments (rates of warming) are set at levels that a realistic based on observed temperatures in the Antarctic Peninsula level.

In line, with previous research on Antarctic invertebrates, the rate of warming is important in determining upper thermal limit and maintenance of the biological function. Such previous uni-directional experiments (temperature always increasing) have been used to understand the outcomes of long-term temperature increases, yet MHW are different in that they represent short-term warming periods (cyclic). These might need another experimental approach that mimics observed heat wave patterns (i.e. warming for a time then returning to ambient temperatures: as 0°C, up to +7°C for 10 days, then back to 10°C etc based on S2). Perhaps some discussion on the potential outcomes for animals on shorter term exposures and the responses that could be seen following returning animals back to ambient conditions. How might the present experiments be interpreted in terms of this type of cyclic treatment?

This is an interesting comment and something we have explored in a subsequent experiment (in prep). We have now added a section in the manuscript discussing the scope for recovery following MHWs (page 31, lines 17-25 and page 32, lines 1-4).

Minor comments
(1) Table 1 summarizes figures 2, 4, 6 and 8 so suggest these could go in the supplementary material.

After consideration, we have decided to only present the regression plots with the raw data within a single figure. This now includes the control data. It was apparent that the line plots did not provide any additional useful information and the raw data points plus the regression was enough to summarise the results. We also felt that the differing scales on the line plots and regressions (cumulative intensity vs temperature) was confusing and now therefore present temperature only.

(2) S1: Can you please include a plot over the 20 year period, indicating periods when the 90% percentile is exceeded. This would be more useful in addition to the table in understanding the magnitude and frequency of the HW in the region.

We have now presented a plot with this information in the manuscript (Figure 1).

(3) Page 19, line 8, and elsewhere: suggest changing ‘different functions’ to ‘key biological functions’.

This has been amended

(4) Is fig 12 needed?

The referee is correct that fig 12 is not needed. The CTmax values are mentioned in-text and figure 12 has now been removed.

Reviewer 3 report:

Overview
The impacts of climate change are being felt in every marine system, but understanding the impacts on some of the most at risk (i.e. Antarctic habitats) remains a priority. Whilst it may be possible for species to adapt to the gradual increases in base temperature, the effects of more frequent and more intensive extreme climate events are already being shown to be a major issue. Using S. neumayeri, a common (but keystone species), this study aims to understand the connection and relationship between functional sub-lethal limits and critical thresholds. Monitoring basic functions of the sea urchin under different warming regimes enables the authors to assess the ability of this species to tolerate elevated temperatures. I believe that the premise of this paper is valid and the scientific questions underpinning it are important and need addressing within a journal such as MEPS. However, it does require some revision before it can be accepted and I have highlighted the areas for improvement below.

Abstract
A lot more results are required. Currently, only lines 18-21 present the outputs from the experiments. I would recommend reducing the introduction and expanding the results accordingly.

This has been amended as suggested.

Page 2, Line 12. It would be better to specify that it was respiration (or even oxygen consumption) that was measured rather than metabolism. Metabolism means some substances are broken down to produce energy and some other substances are synthesised. In contrast, cellular respiration means oxidation of organic compounds in cytoplasm to produce energy in the form of ATP. This energy helps the cell to perform all metabolic activities.

This has been amended as suggested.

Introduction
The Introduction is a little long and should be shortened to reflect the focussed nature of the experimental procedure and outputs.

The introduction has now been reduced by 300 words

Page 4, line 3. A very long sentence that needs to be re-organised.
This sentence and the whole paragraph has now been re-worked.

Page 5, line 25. It is important to remain scientific with the writing so I would remove the reference to a ‘catholic diet’.

‘Catholic’ has now been replaced with ‘varied’

Page 6, line 1. Although the authors give a number of examples of slow maturity for Antarctic species, according to Pearse and Giese (1966) ‘….oocytes take from 18 to 24 months to reach maturity after beginning growth, and spawning occurs sometime in the winter or spring between May and December’ for S. neunmayeri. It is important to contextualise this species in the methods in more detail.

We have added a paragraph in the methods to contextualise the species as the reviewer suggests (page 7, lines 15-25 and page 8, lines 1-8).

Material and Methods

Page 6, line 10. Size ranges should be included and it would be important to look at using size as cofactor that may explain some of the variability in the results. Including survivorship and growth data would also underpin the 'quality' of the experimental system used.

Size ranges have now been included on page 7, line 12. We could not include size as a cofactor in the analysis since data were pooled within replicate tanks, so the relationship had to be explored separately. As suggested, scatter plots of size vs function have also been explored to identify whether this metric caused any variability in the results (Included in the supplementary materials, Figure S5 and Table S3). Both feeding and oxygen consumption were standardised by animal weight to account for size variability and as such, no relationship was found between size and these functions. For the function of righting, the data showed that the larger individuals had the longer righting times at high temperatures. Clearly there is an effect of size here and as such the analysis and results have now been included in the manuscript (page 16, lines 16-22 and page 21, lines 7-9)

There were no mortalities in the ambient control conditions and mortalities in treatment conditions only occurred following CTmax. Growth data was not collected in the experiment since the growth rate of *Sterechinus neumayeri* is slow, with individuals reaching a maximum diameter of 70mm after 40 years (Brey et al., 1995) and the size or weight increase across two months (experiment duration) would be lost within the variability encountered from measurement error.

Page 6, line 16. Here and elsewhere, the analysis of the timeseries data for heatwaves using the R package is an important part of the story and results. I would, therefore, like to see this as a more than just supplementary as it seems to be novel. For example, a figure showing the temperatures with the identified heatwaves could then be linked back to the results, especially re-purposing/replacing Figure 12. It would also be good to link to the cumulative intensity (e.g. Tables 2 and S2) so that this explicitly links this measure to the 'real world'.

We have now included a plot (Figure 1) covering the 20-year period which indicates periods when the 90th percentile is exceeded for ≥ 5 days (i.e. MHW). We have included reference to this figure throughout the manuscript. With regard to the cumulative intensity comment, on revising the manuscript we have decided that this metric creates unnecessary confusion. Although cumulative intensity allows temperature and exposure to be translated into a metric directly comparable between treatments, we think this is achieved by reporting only temperature and observing the rate at which this temperature was reached (i.e. 0.3oC/day, 0.5oC/day or 1.0oC/day). We hope this change will also make the paper and the data easier to reference in other studies since most previous studies report on temperature limits.

Page 6, line 22. I would change the replace 'starved' with 'not fed' as it is possible that the sea urchins were grazing the biofilms of the flow-through aquaria and gaining significant nutritional benefits during this time.

This has now been amended

Additional details of the these holding tanks e.g. capacity, light conditions, flow rates, physiochemical parameters are also needed.
Capacity and light regime are now included in-text. Reporting flow rate would not be useful for the experimental set-up. Urchin tanks were not operated on a flow-through system (urchin tanks were water changed every 48 hrs) but were instead floated in water baths which were flow-through. Flow rate in these tanks was variable and used for temperature maintenance.

Page 7, lines 6-16. The details of the experimental design (e.g. if the tank [water baths] were replicated themselves) need to be expanded. There is a hint of pseudoreplication, i.e. the temperature treatments were not replicated and this needs to be clarified and then considered for the statistical analyses performed. This also extends to the 6 urchins per tank, which are definitely pseudoreplicates and need to be accounted for in the data structure for the analyses (if not already).

Thank you for highlighting this error with regards to pseudo replication with floating tanks. We have now ensured that data are pooled prior to analysis and all results and data figures have been reconstructed considering this. With regards to potential pseudo replication within temperature treatments, we have addressed this comment in the manuscript and provided more detail on the experimental design (page 9, lines 13 – 24, page 10, lines 1-2).

Page 7, lines 12-14. I would contest that the parameters stated do not reflect good water quality conditions. Specifically, the large fluctuations in pH, and the presence of even low nitrite, (ammonia/ammonium needs to be clarified) and elevated nitrate are sub-optimum for invertebrates from pristine conditions. Physiochemical parameters: means, ranges etc should be presented in the supplementary. This will also be important as warming will have an impact on the level of dissolved oxygen (see Peck et al., 2007) and in addition to temperature will, therefore, influence the respiratory processes and the interpretation of the data in the discussion (page 20).

The ranges originally reported were those described on the water quality test kits, however the actual values/concentrations measured were much lower and we believe demonstrated good, if not, pristine conditions since they were comparable to the water pumped directly from Ryder Bay. Concentrations/values did not deviate outside the ranges stated in-text and the test kits used (JBL) did not provide further accuracy between these ranges. As such, we do not see the need to included additional information on these parameters in the supplementary.

We have, however, included a figure of daily temperature changes in each treatment in the supplementary information (Figure S4). Further to this we have added a section in the discussion regarding the relationship between temperature and dissolved oxygen, and the implications of this for our results (page 27, lines 16-25 and page28, lines 1-4).

Page 7, lines 21. It is not clear what was used as a control (no increase in temperature). In the figures ambient conditions are mentioned, but is this from urchins maintained in the same setup, but with ambient water/light levels etc. or is it from another system/field?

A sentence giving further explanation to the control system has been added on page 10, lines 24– 25 and page 11, lines 1-2) and the addition of the control data has now been included in the graphs (Figure 2)

Page 8, line 9. It is odd that this species was fed limpets when the authors highlight its 'Catholic' diet. Some discussion around the rationale and also the significant difference between an algal/biofilm grazing and essentially carnivorous diet is required as this will have an impact on the nutrient storage capacity (in the gonads) and ability to tolerate stress due to improved body condition.

It was understood prior to starting the experiment that a diet of limpets was not necessarily representative of a natural diet, however the rationale behind the decision has now been explained on page 11, lines 13-23. We also now state in this paragraph that body condition may be altered as a result with the inclusion of references which provide evidence of this. We originally considered in the discussion that food quality and quantity are factors which will likely have influence on temperature thresholds in the real world (page 32, lines 8-12).

Page 9, lines 3 and 16. Good examples of the potential replication issue (n = 5 rather than 10 as measurements of urchins from the same tank would have to be combined). Also, how were urchins selected and were they sampled more than once over the experimental period?

Further explanation on the sampling strategy has been added on page 12, lines 15-20 and again clarification for which urchins were sampled has been added on page 13, lines 8 and 24.

Page 10, line 4. Righting has been used as a biomarker for stress in many species, but it is known that some invertebrates can go in to a heat coma and then recover (see Sandison et al., 1966; Hamby, 1975; Watson et al., 2012). This also links in to other endpoints that were measured.

This is an interesting comment, especially in terms of how we defined CTmax for this species. However, if the individuals were to enter a heat coma from the experimental temperature, due to the continued ramping of temperature, we can expect that it would be unlikely that the animals would then recover (Sandison et al., 1966; Hamby, 1975). If temperatures resumed ambient levels, it would undoubtedly be possible that the urchins could recover, not only in righting ability but also the other key biological functions measured. Ability to recover following warming stress was not the aim of the experiment and to assess this would require further experimentation outside the scope of this paper. We have added a section on recovery post-MHWs in the manuscript (page 31, lines 17-25 and page 32, lines 1-4).

Page 11, line 11. The Wilcoxon test is a non-parametric equivalent of a T-test and so is not equivalent to multiple comparison tests (e.g. Tukey's). More detail is required for this.

The original analysis using ANOVA and equivalent non-parametric tests were not deemed appropriate for this type of experiment following reviewer 1 comments. As such, analyses have been replaced with a) a repeated measures ANOVA, transforming data initially to achieve normality of data distribution, followed by a post-hoc t-test, or b) a one-way ANOVA followed by a post-hoc Tukey test (as per ctmax data). All data

are now pooled by tank before any analysis.

Results
It is important to have the basic physiochemical, survivorship and control data presented. It should be in the supplementary but is essential and also needs to be discussed.

We have amended the graphs presented in the manuscript in light of some of the reviewer's other comments. These data figures also now include control data which we feel has improved the visual interpretation of the results.

Twelve figures/tables are excessive for this type of paper and the results presented. The authors need to reduce the number. I would have thought half this would be appropriate.

As mentioned, we have reduced the number of data figures and tables to two and one, respectively, which we feel more clearly summarise the data.

Discussion
Page 18, lines 3-12. I had difficulty interpreting this section. It reads as though the data presented follow the failure-rate model, but in line eight the authors state 'However, contrary to this…'. Some work on this ambiguity is required.

The CTmax data presented follow the failure rate model, however the functional thresholds do not. We have changed the wording slightly to try and make this message clearer.

Page 18, line 17. Figure 12 is not very informative and so it would be much better to include the link to the ambient conditions and heatwaves data as discussed above.

Figure 12 has been removed and data on ambient control conditions and heatwaves incorporated as suggested in Figure 2.

Page 19, lines1-6. The physiological processes stated as being important in lethal limits seem to be working at different scales. For example, nervous and circulatory failure are dependent on the higher functioning of an organisms, whilst enzyme tolerances and chaperone proteins are at the cellular/biochemical levels. These do not seem to sit together and, therefore, should be discussed separately.
This section has been amended so that these processes are discussed separately, and the distinction has been made clearer (page 25, lines 6–19).

Page 21, lines-1-15. This is a good example of where including the actual data from the environment would give added strength to the data presented. However, some discussion about the date 2100 and the number of generations that could enable phenotypic and genotypic adaptations would also be important.

We have added to the discussion (page 31 lines 1–22) to elaborate on the number of generations associated with 2100 for this species and the implications for adaptations. As mentioned before, we have now presented data on the MHWs from the environment in Figure 1 and Figure S2, and given reference to this in-text (page 8, line 14 and 16, and page 30, line 11).

Page 21, lines 16-20. This must link to the conditions provided in the experimental system, which I attest are suboptimum. The authors must also explore this in the context of the 'ambient control' and the potential food supply differences.
This comment has now been addressed through inclusion of temperature graphs, water quality ranges and discussion of diet and nutritional status. We have also included all ambient control data in the figures.

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Article to Marine Ecology Press Series

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

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Running head*:* Functional thermal limits during marine heatwaves

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. This study 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^{o}C day^{-1}, 0.5^{o}C day^{-1} and 0.3^{o}C day^{-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 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. Arguably, the most interesting result was that functions deteriorated at lower temperatures when warming was more rapid (1^{o}C day^{-1}), contrary to lethal critical thresholds, which were reached at lower temperatures when warming was slower (0.3^{o}C day^{-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 an organism's ability to tolerate short-term elevated temperatures.

Key words: Extreme warming events, sub-lethal limits, thermal tolerance, climate change, polar, segmented regression, echinoderm

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).

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 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 (Figure S1). 120 adult urchins (test diameter range, 28 mm – 49 mm) were SCUBA-diver collected at depths of 10-20 m and returned to the Rothera aquarium facility within two hours of collection.

Sterechinus 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 in iceberg scours on the shallow Antarctic seabed (Dunlop et al. 2014), and is 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. Further to this, due to 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, and up to in excess of 100 days (Bosch 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^{o}C to +0.5^{o}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

7.5 - 8.0), NO_2 (ranging 0.05 mg l^{-1} – 0.1 mg l^{-1}), NO_3 (ranging 0.5 mg l^{-1} - 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 individuals were isolated and any faeces produced was retained within compartments (Figure S3). During warming trials experimental temperatures in the aquaria water baths were raised by 1^{o}C, 0.5^{o}C or 0.3^{o}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 minutes after each temperature change to ensure required temperatures were achieved and kept constant. Initially, temperatures fluctuated by up to ± 0.3^{o}C before stabilising after 1-2 hrs. Temperatures were subsequently monitored throughout the following day and held within ± 0.1^{o}C of the target experimental temperature (Figure 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 ^{o}C and 1.9^{o}C.

2.3 Feeding trials

Urchins were fed pre-portioned amounts of food every 48 hrs. 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, *Nacella concinna,* which has a 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^{o}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^{o}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^{o}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) collected from the same site. To obtain the ash mass of urchins, individuals were weighed live before freezing in liquid nitrogen and storing at –40^{o}C. Frozen urchins were then placed in pre-ashed and pre-weighed ceramic crucibles and dried at 60^{o}C until constant mass was obtained (± 0.01 g). Once dried, urchins were ignited in a muffle furnace at 475^{o}C for 6 hrs 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 two urchins from each replicate tank within each treatment. The time taken to right was recorded for the same urchins every 2^{o}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 critical thermal limit (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 hrs, had stopped eating and producing faeces. When an urchin began to show signs of reaching the CT_{max} (not feeding or producing faeces), they were inverted in the tank and left for 12

hrs. If the urchin had not righted itself after this period, they were removed and weighed suspended in water to obtain live wet volumes (± 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 five replicate tanks.

To determine differences in functional responses between treatments, a one-way repeat measures analysis of variance (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 assumptions of 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 functions 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 (as 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, 80% ± 19% of animals fed in ambient conditions for the duration of the experiment. For the first four days of the experiment, in treatments where T↑ 1°C day^{-1}, the proportion of animals feeding exceeded all other treatments (97% ± 4%), including ambient conditions (87% ± 10%). Fifty percent of animals

stopped feeding in treatments when temperatures exceeded 7.2ºC, 8.2ºC, and 9.2ºC, where T↑ by 1ºC, 0.5ºC and 0.3ºC day^{-1}, respectively (Figure 1).

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

The percentage of animals producing faeces tracked the proportion of animals feeding after the first four days (Figure 1). Following each breakpoint, the relationship between temperature and % individuals producing faeces became significant (Table 1). For the fastest rate of warming where T↑ 1ºC day^{-1}, a breakpoint was identified at 5.2ºC, above which the % individuals producing faeces rapidly declined from 100% to 10.3% within 6 days. Where T↑ 0.3ºC day^{-1} and 0.5ºC day^{-1}, the regression breakpoint for faecal production was 8.3ºC and 4.5ºC respectively (Table 1). The mean temperature breakpoint for the function of % producing faeces was 6.0ºC ± 2.0ºC, averaged across all treatments.

The mean mass of faeces produced in treatments where T↑ 0.3ºC day^{-1}, was significantly greater than the faecal mass produced in ambient control

conditions and treatments where T↑ 1^{o} C day^{-1}, until temperatures exceeded 2.1^{o}C ($t_{(4)}$ = 8.74, p = 0.006 and $t_{(4)}$ = 5.02, p = 0.044, respectively). Where T↑ 0.5^{o}C day^{-1}, the mass of faeces produced was significantly greater than treatments where T↑ 1^{o} C day^{-1}, until temperatures exceeded 2.1^{o}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^{o}C .

Breakpoints in regressions were identified at 5.0^{o}C and 3.1^{o}C for treatments where T↑ 0.5^{o}C day^{-1} and 0.3^{o}C day^{-1}, respectively (Table 1). The breakpoints for these regressions marked a reduction in the gradient of the 2nd slope, whereby faeces produced day^{-1} mgAFDM^{-1} as a function of temperature decreased at a slower rate as temperatures increased. The mean temperature breakpoint for faeces produced was 4.1^{o}C ± 0.95^{o}C, averaged across the slowest (T↑ 0.3^{o}C day^{-1}) and intermediate (T↑ 0.5^{o}C day^{-1}) rates of warming.

3.2 Righting

In treatments where T↑ 1.0^{o}C day^{-1}, time taken to right became significantly longer than ambient controls when temperatures reached 9.2^{o}C ($t_{(4)}$ = 6.06, p < 0.022). For treatments where T↑ 0.3^{o}C day^{-1}, time taken to right only became significantly longer than ambient controls just before CT_{max} was reached, when temperatures reached 11.2^{o}C ($t_{(4)}$ = 6.04, p < 0.023). For treatments where T↑ 0.5^{o}C day^{-1}, time taken to right never exceeded ambient controls significantly, however mean righting times were consistently higher than control conditions throughout the warming period.

A breakpoint in the linear regression was identified at 8.7°C in treatments where temperature was raised at 0.3°C day^{-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 (Figure S5, 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↑ 0.3°C day^{-1} ($t_{(4)} = 5.62$, $p = 0.030$) and 5.2°C in treatments where T↑ 1.0°C day^{-1} ($t_{(4)} = 4.98$, $p = 0.045$). Overall, there was a positive linear trend between oxygen consumption and temperature for all treatments. However, where T↑ 1°C day^{-1}, a drop in O_2 consumption occurred at 9.2°C, and where T↑ 0.3°C day^{-1}, a drop occurred just before the CT_{max} at 11.2°C.

O_2 consumption increased at a faster rate per increase in temperature where warming rates were fastest at 1°C day^{-1} (slope gradient = 1.50) and increased at the slowest rate when warming rates were slowest at 0.3°C day^{-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↑ 0.3°C day^{-1}, T↑ 0.5°C day^{-1} and T↑ 1°C day^{-1} ranged from 10.6°C - 13.8°C, 11.2°C - 13.7°C, and 12.2°C - 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 (T↑ 1°C day^{-1}), the CT_{max} was significantly higher compared to treatments where temperature increased at a slower rate (T↑ 0.3°C day^{-1}) ($t_{(8)} = -6.02$, $p = 0.001$).

Across all functions where breakpoints were identified, the slowest rate of warming (T↑ 0.3°C day^{-1}) had a mean temperature breakpoint of 8.3°C ± 1.3°C. In comparison, the mean temperature breakpoint was 5.4°C ± 0.5°C, and 4.6°C ± 0.6°C for intermediate (T↑ 0.5°C day^{-1}) and fast (T↑ 1°C day^{-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-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, *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^{o}C ± 1.3^{o}C). At the faster warming rates functional thresholds were lower (5.4^{o}C ± 0.5^{o}C or 4.6^{o}C ± 0.6^{o}C). There was even evidence that some functions declined linearly, with significant deterioration from temperatures +2.8^{o}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 $1^{o}C\ h^{-1}$ or $1^{o}C\ day^{-1}$, 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 ($1^{o}C$ 3 $days^{-1}$ to $1^{o}C\ month^{-1}$) 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 $9.2^{o}C$ for the fastest rates of warming, and the highest breakpoint of $8.7^{o}C$ 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.

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 an organism's energy reserves 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 reburying 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^{o}C – 2^{o}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 (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 *Strongylocentrotus 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^{o}C ± 0.36^{o}C, with onset rates of 0.3^{o}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^{o}C x day (Figure S2). Mean climate temperatures are predicted to shift by +2^{o}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

increased metabolic demands with consequent impacts on annual energy budgets.

For a long-lived (>40 year (Brey et al. 1995)) and slow to mature (8-9 years (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 years (2020 - 2100), eight generations of *S. neumayeri* will have succeeded the present population, and in the year 2100, the 5^{th}, 6^{th} and 7^{th} generation could be present and reproducing in populations around Antarctica. If we consider the evidence of *S. neumayeri*'s capacity to acclimate, it may be possible for this species to acclimate and adapt successfully to function in a +2^{o}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 predict the implications of acclimation and adaptation on the subsequent tolerance to MHWs for *S. neumayeri*. Instead, the data provides insight into the effect of onset rate of acute warming, the thermal vulnerability of key biological functions, and the difference between critical thermal limits 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. It has been shown that the marine snail, *Littorina littorea*, loses motility under thermal stress, however if 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^{o}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

L.S.P and R.D conceived and designed the study. R.D carried out the practical work and data processing. R.D, L.J.G and L.S.P analysed the data, drafted the manuscript and approved its publication.

COMPETING INTERESTS

The authors declare no competing interests.

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TABLES

Table 1: Summary statistics for linear regression relationships between the measured functions of *Sterechinus neumayeri* and temperature. β indicates the slope of the linear regression lines before the breakpoint (Slope_1) and after the breakpoint (Slope_2); SE_a indicates standard error for the intercept and slopes; df = degrees of freedom; 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 column BP indicate the localisation of the breakpoint or else NA indicates a single linear regression; SE_b (standard 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

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

[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

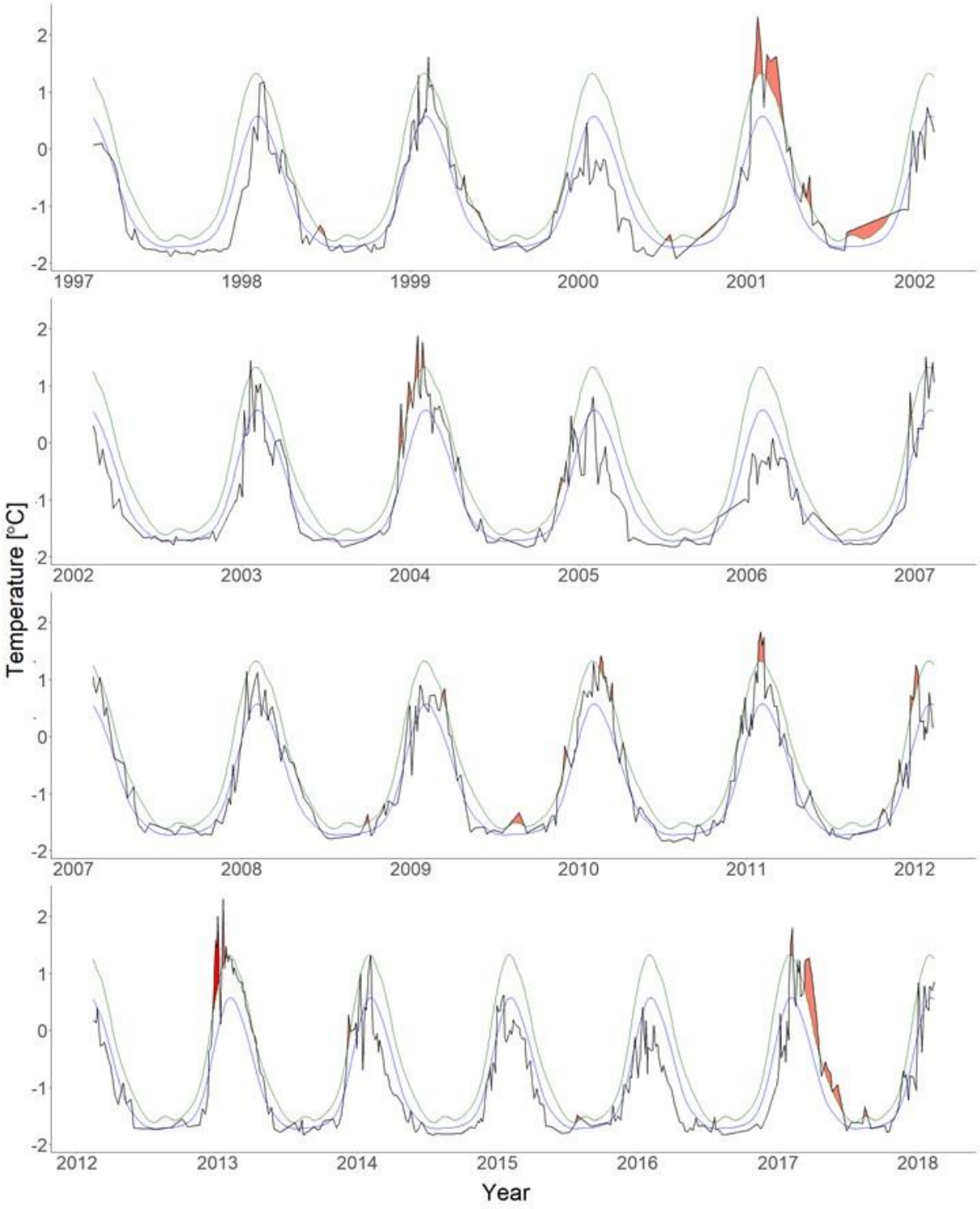
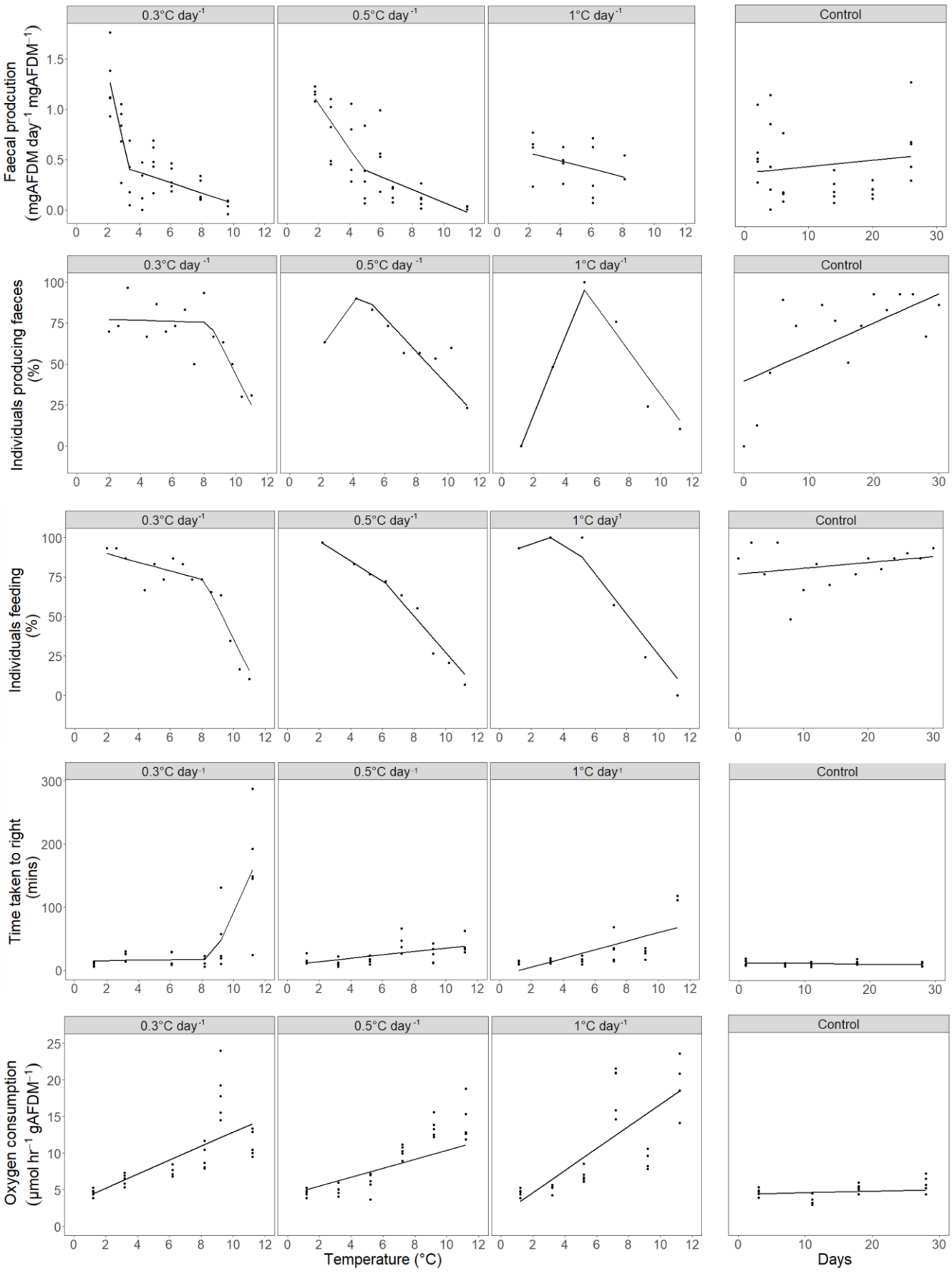


Figure 1: Times-series of temperatures (°C) experienced in Ryder Bay, Antarctica, at depths of 15 m, represented by the black lines. The data are split into panels to cover the entire span of the time-series, where the x-axis

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 days are highlighted in red and indicate the occurrence of a marine heatwave.



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

neumayeri in experimental conditions where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC. 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.

Article to Marine Ecology Press Series

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

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Running head: Functional thermal limits during marine heatwaves

ABSTRACT

Marine heatwaves (MHWs) are increasing in both intensity and frequency against a backdrop of gradual warming associated with climate change. ~~Temperatures experienced during these warming events are likely to cause sub-lethal effects to animals as organism functioning deteriorates. Little is known about functional deterioration as critical thermal limits are approached, and i~~In the context of ~~marine heat waves (~~MHWs~~)~~, animals are likely to experience ~~temperatures producing~~ sub-lethal, rather than lethal effects, defining long-term limits to survival and/or ~~inhibiting~~ impacting individual and population ~~health~~fitness. This study ~~aims to understand~~investigated how functional sub-lethal limits track critical thresholds and how this relationship changes with ~~the~~ warming rate. To this end~~purpose~~ we monitored basic functioning, specifically the ability to right, feed~~,~~ and assimilate energy, as well as oxygen consumption rate~~and metabolise energy,~~ in the common Antarctic sea urchin, *Sterechinus neumayeri.* Water temperature in experimental systems was increased at ~~warming~~ rates of 1°C day^{-1}, 0.5°C day^{-1} and 0.3°C day^{-1}, in line with the characteristics of ~~warming~~ MHW events previously experienced at the site where the study urchins were collected on the ~~in~~ Antarctica Peninsula. Functioning was assessed during the simulation of MHWs ~~warming~~ and sub-lethal limits determined when the rate of functional degradation changed as temperature increased. Results suggest that t~~he rate of functional degradation, and ultimately t~~hermal sensitivity~~,~~ varies ~~across warming rates and the specific~~between the key biological functions measured, with the ability to right having the highest thermal threshold. ~~-~~Arguably, the most interesting result was that functions deteriorated at lower temperatures when

warming was more rapid (1°C day^{-1}), contrary to lethal critical thresholds, which were reached at lower temperatures when warming was slower (0.3°C day^{-1}). MHWs and their impacts extend far beyond Antarctica. and i In this context, our analyses indicate that the onset rate of a warming MHWs is critical in determining an organism's ability to tolerate short-term elevated temperatures with global relevance.

Key words: Extreme warming events, sub-lethal limits, thermal tolerance, climate change, polar, segmented regression, echinoderm

1. INTRODUCTION

Paleoclimate records have identified the onset of industrial-era warming as early as the mid-19th century (Abram et al. 2016) and hHistorical temperature records have now detected positive temperature trends for the majority of the Earth's surface (Myrvoll-Nilsen et al. 2019). (Myrvoll-Nilsen et al. 2019), with Tthe oceans are being key to the regulation and capture of much of the excess heat present in the atmosphere (Marshall et al. 2015)., and asAs 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, frequency and intensity with alarming ecological consequences (Garrabou et al. 2009, Rubio-Portillo et al. 2016, Oliver et al. 2018). Not only are the duration, magnitude and frequency of MHW's expected to increase (Hobday et al. 2016, Oliver et al. 2018), but models also predict that by the end of the 21st century 50% of the world's oceans will be in a state of 'permanent MHW' (*sensu* Oliver et al. 2019) under RCP4.5 scenarios, and >90% under RCP8.5 scenarios (Oliver et al. 2019).

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 ~~through multiple~~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 ~~evolve~~adapt to new temperatures ~~Benthic marine invertebrates in Antarctica are characterised by a limited capacity to acclimate and evolve to new temperatures (Peck et al. 2014, Peck 2018). Therefore, the physiological flexibility of these species becomes crucial to their survival during extreme warming events (Peck 2011). The Antarctic has been subject to warming of 0.61 ± 0.34 °C per decade since 1990, more than three times the global average (Clem et al. 2020), with rapid warming in some marine areas such as the Western Antarctic Peninsula (Meredith & King 2005, Turner et al. 2014, Spence et al. 2017). Despite predictions that permanent heat wave status in Antarctica will be reached at a slower rate than lower latitudes (Oliver et al. 2019), the species that live in the Southern Ocean are less likely to be able to adapt quickly to changing temperatures. Species at lower latitudes may be able to acclimate and adapt through multiple generations to gradual warming. As a result, Ppredicting effects of MHWs on lower latitude species may need to consider a shift in species thermal thresholds over time.involve a shifting baseline as species adapt to warming 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).

~~. However, it is unlikely that the same will be possible for many Antarctic species owed to their long generation times and delayed reproductive maturity, with several invertebrate species, including the Antarctic scallop *Adamussium colbecki*, the limpet *Nacella concinna*, and the bivalves, *Laternula elliptica* and *Adacnarca nitens,* taking 4 – 7 years to mature, and others such as the brachiopod *Liothyrella uva*, taking 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). ~~a range of biological responses and ultimately survival mechanisms compared to those required for gradual warming (Somero 2010, Peck 2011). Where evolution or even acclimation is not possible due to the rate of temperature change, other~~

~~mechanisms for survival must come into play, such as biochemical and cellular stress responses (Clark & Peck 2009, Payton et al. 2016). Thus, if species are able to acclimate rapidly then a small temperature change might have little effect. However, for species that acclimate very slowly, increases in temperature not usually considered significant elsewhere, might put animals out of physiological balance with detrimental consequences.~~

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 ~~the warming~~ 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 ~~limits~~ (sub-lethal) limits track critical ~~limits~~ (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~~d,~~ assimilate energy as well as oxygen consumption rate~~and metabolise energy,~~, in the common Antarctic sea urchin, *Sterechinus neumayeri*.

~~*Sterechinus neumayeri* is an abundant Antarctic species that forms a significant component of the benthic community (Brockington 2001, Pierrat et al. 2012), with a circumpolar distribution (Kroh 2010). It is a grazing urchin with a catholic diet, and is a broadcast spawning species that releases gametes into the water column during the austral summer (Pearse & Giese 1966, Stanwell-Smith & Peck 1998, Brockington et al. 2007).~~

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 (Figure S1). 120 adult u~~U~~rchins (test diameter range, 28 mm – 49 mm) were SCUBA-diver collected at depths of 10-20 m and returned to the Rothera aquarium facility within two hours of collection.

Sterechinus 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 in iceberg scours on the shallow Antarctic seabed (Dunlop et al. 2014), and ~~it~~ is 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. Further to this, ~~because of~~due to 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, and up to in excess of 100 days (Bosch

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 ~~Overall~~ *S. neumayeri* ~~is~~ one of the most important members of the Antarctic shallow benthic ecosystem and key to investigating ~~e~~ 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 ~~starved~~ not fed to allow any ingested food to be processed and the production of faeces to cease. ~~Since feeding and faeces production was not occurring, it was assumed~~The cessation of ~~production of~~ faeces production is an indicator that metabolic rates ~~that~~ ~~This~~

step ensured all individuals were at the same had reached a "standard" metabolic state at thlevel at the start of the experiment. Previous research suggests that these urchins are able to sustain, and experience natural periods of starvation for up six6 months during winter (Brockington 2001), and hence six weeks starvation without feeding was unlikely to impact be detrimental to their physiological metrics measured in this studycapabilities.. SPrevious studies of oxygen consumption in other 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 starfishsea 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 (30 urchins per treatment) in each main aquarium tank (170 L),. Each main aquarium tank functioned which functioned as a temperature baths (Figure S3;) (30 urchins per treatment, 5 replicates per treatment where data from urchins in the same replicate floating tank were pooled). (Figure S2). Temperature treatments were not replicated due to space restrictions in the aquarium. It is acknowledged that tTreatment temperatureThe same treatment conditions (i.e., temperature) would bewas translated to all replicate urchins, and as such,

temperature was closely monitored to note and control ~~any unintentional~~ 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 (~~aim:~~ranging 7.~~9~~ 5 - 8.~~5~~0), NO_2 (~~aim:~~ranging ~~<0.2~~ 0.05 mg l^{-1} – 0.1 mg l^{-1}), NO_3 (~~aim: <20~~(ranging 0.5 mg l^{-1} ~~-~~ 1.0 mg l^{-1}) and NH_4 (~~aim:~~stable at ~~<1.2~~0.1 mg l^{-1}) to ensure good water quality. Throughout the experiment, concentrations of the aforementioned compounds remained within ~~the advised~~ the ranges stated. ~~.~~

Urchins within each replicate tank were separated by aquaria egg crates and fine mesh partitions to ensure individuals were isolated and any faeces produced was retained within compartments (Figure ~~S2~~S3). During warming trials~~, we aimed to increase~~ experimental temperatures ~~-~~ in the aquaria water baths were raised by 1ºC, 0.5ºC or 0.3ºC each evening, depending on treatment. Temperatures in the floating tanks increased more gradually than the water baths, allowing urchins to adjust ~~more~~ slowly to ~~the~~ each new temperature. Temperatures were checked every 30 minutes after each temperature change to ensure required temperatures were achieved and kept constant. Initially, temperatures fluctuated by up to ± 0.3ºC before stabilising after 1-2 hrs. Temperatures were subsequently monitored throughout the following day and held within ± 0.1ºC of the target experimental temperature (Figure 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 ºC and 1.9ºC.

2.3 Feeding trials

Urchins were fed pre-portioned amounts of food every 48 hrs. 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, *Nacella concinna,* which has a 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). ~~H~~however, ~~with these sources it~~administering a varied diet would ~~be very difficult to assess~~ ~~the~~make it difficult to assess the amount of food consumed per urchin ~~as well as~~at the same time as standardisinge 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 ~~should be acknowledged~~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 ~~start~~ beginning of the experiment to start the digestion process. ~~Urchins were offered food by placing it directly onto the test. This technique had been used previously in experimental trials and allows the urchin to detect the food and move it to the mouth without disturbing the animal (references needed).~~ 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 ~~not~~ only partially consumed ~~all the~~the ~~entire~~ 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, ~~and~~ where at least one sample was taken from each replicate tank within the treatment. The same urchins were targeted for faecal collection ~~in order to~~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~~ For all other urchins, any remaining faecal matter was removed ~~and disposed of~~.

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^{o}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 475ºC 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 2ºC 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) collected from the same site. ~~For the purpose of~~To obtain~~ing~~ the ash mass of urchins, individuals were weighed live before freezing in liquid nitrogen and storing at –40ºC. Frozen urchins were then placed in pre-ashed and pre-weighed~~,~~ ceramic crucibles~~,~~ and dried at 60ºC until constant mass was obtained (± 0.01 g). Once dried, urchins were ~~then~~ ignited in a muffle furnace at 475ºC for 6 hrs and subsequently weighed to obtain ash mass ~~once~~ after cooling in a desiccator~~ed~~ (± 1 mg).

2.6 Righting

The time taken for urchins to right themselves was recorded for 10 urchins per treatment, sampling two urchins from each replicate tank within each treatment. The time taken to right was recorded for the same urchins every 2ºC 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 critical thermal limit (CT_{max}) was recorded for ~~each urchin~~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 hrs, had stopped eating and ~~had stopped~~ producing faeces. When an urchin began to show signs of reaching the CT_{max} (not feeding or producing faeces), they were inverted in the tank and left for 12 hrs. If the urchin had not righted itself after this period, they were removed and weighed suspended in water to obtain live wet volumes (± 0.01 mL).

2.8 Statistical Analysis

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

To determine differences in functional responses between treatments, a one-way repeat measures ~~n~~ analysis of variance (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 assumptions of normal distribution were met.

~~When a significant difference was observed ($p < 0.05$), a post-hoc Tukey test was undertaken to test all pairwise comparisons among means. A Shapiro-Wilk and Levene's test were run on the residuals of the ANOVA for normality of distribution, and to test for homogeneity of variance, respectively. Where the results of these tests indicated either a non-normal distribution of residuals or unequal variances between treatment groups, the response data were logarithmically transformed and all tests repeated. Where normality and/ or homogeneity of variance could not be achieved by transforming the data, the non-parametric Kruskal-Wallis and pair-wise Wilcoxon tests were used to determine differences in functional response between treatments.~~

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 ~~points~~ 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 functions 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. ~~Where the R^2 and standard error of the segmented regression model was improved compared to~~better than ~~a linear regression, a segmented regression was used to model the data. It was especially important to use a method such as segmented regression to identify breakpoints in process rates.~~ 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 (as 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.

For time points to be comparable between treatments, the mean temperature and time exposed to temperatures above ambient were combined to estimate cumulative intensity (°C x day). Other variations on cumulative temperature have been used to combine temperature intensity and duration in heatwave studies (Perkins-Kirkpatrick & Lewis 2020, Domínguez et al. 2021), however cumulative intensity was used to detect past warming events in the "heatwaveR" package (Text S1), and so this metric was used to facilitate comparison between our experimental conditions and 'real world' events. (Comparisons between cumulative temperature and cumulative intensity is provided in the Supplementary Materials, Table S2).

The following equation was used to calculate cumulative intensity for a temperature in each treatment:

$$Exp(\bar{T} - Amb\ \bar{T}) = Cumulative\ intensity\ (^{o}C\ x\ day)$$

Where: Exp = Exposure in days, above ambient temperatures; $\bar{T}$ = Mean temperature experienced °C; Amb $\bar{T}$ = Mean ambient temperature.

Warming rate was not consistent across treatments averaging 0.32 ± 0.13 °C day^{-1} for the slowest warming rate, 0.49 ± 0.17 °C day^{-1} for intermediate warming rates, and 0.97 ± 0.31 °C day^{-1} for the fastest warming rate. Therefore, it was not possible to calculate cumulative intensity directly using the equation. Instead, polynomial regressions that took account of the varying rate of temperature increase were obtained for each treatment and used to estimate cumulative intensity (Figure S3) (T↑ = temperature increase):

T↑ of 1°C day^{-1} : $(0.49T^2) - (0.99T)$ = Cumulative intensity (°C x day)

T↑ of 0.5°C day^{-1} : $(0.96T^2) - (1.52T)$ = Cumulative intensity (°C x day)

~~T↑ of 0.3°C day^{-1}: $(1.47T^2) - 2.22T$) = Cumulative intensity (°C x day)~~

~~Cumulative intensity as an explanatory variable was used for comparing functional responses between treatments (Table 2).~~

3. RESULTS

3.1 Feeding and faecal egestion

On average, 80% ± 19% of animals fed in ambient conditions for the duration of the experiment. For the first four days of the experiment, in treatments where T↑ 1°C day^{-1}, the proportion of animals feeding exceeded all other treatments (97% ± 4%), including ambient conditions (87% ± 10%). ~~50%~~Fifty percent of animals stopped feeding in treatments when ~~cumulative intensity~~temperatures exceeded ~~18°C x day~~7.2°C, ~~52°C x day~~8.2°C, and ~~104°C x day~~9.2°C, where T↑ by 1°C, 0.5°C and 0.3°C day^{-1}, respectively (Figure 1).

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

The percentage of animals producing faeces tracked the proportion of animals feeding after the first four days (Figure ~~3~~1). Following each breakpoint, the relationship between temperature and % individuals producing faeces became significant (Table 1). For the fastest rate of warming where T↑ 1ºC day^{-1}, a breakpoint was identified at 5.2ºC, ~~whereby~~ above which the % individuals producing faeces rapidly declined from 100% to 10.3% ~~of individuals,~~ within 6 days ~~(Figure 4A)~~. Where T↑ 0.3ºC day^{-1} and 0.5ºC day^{-1}, the regression breakpoint for faecal production was 8.3ºC and 4.5ºC respectively (Table 1). The mean temperature breakpoint for the function of % producing faeces was 6.0ºC ± 2.0ºC, averaged across all treatments.

The mean mass of faeces produced ~~per day~~ in treatments where T↑ ~~0.5ºC day^{-1} and~~ 0.3ºC day^{-1}, was significantly greater than~~exceeded~~ the faecal mass produced in ambient ~~ambient~~ control conditions ~~(mean = 2.11 mg day^{-1} ± 0.23 mg day^{-1})~~ and ~~treatments also in treatments~~ where T↑ 1º C day^{-1}, until ~~cumulative intensity~~temperatures exceeded ~~2.1ºC~~ ($t_{(4)}$ = 8.74, p = 0.006 and $t_{(4)}$ = 5.02, p = 0.044, respectively)~~reached 6ºC x day, and 7ºC x day, respectively (Figure 5)~~. Where T↑ 0.5ºC day^{-1}, the mass of faeces produced was significantly greater than treatments where T↑ 1º C day^{-1}, until temperatures exceeded 2.1ºC ($t_{(4)}$ = 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 2.1ºC ~~urchins in those in. 5~~

Breakpoints in regressions were identified at 5.0~~6.5~~ºC and 3.1~~3~~ºC for treatments where T↑ 0.5ºC day^{-1} and 0.3ºC day^{-1}, respectively (~~Figure 6B & 6C,~~ Table 1). The breakpoints for these regressions marked a reduction in the

gradient of the 2nd slope, whereby faeces produced day^{-1} mgAFDM^{-1} as a function of temperature decreased at a slower rate as temperatures increased~~(Table 1)~~. The mean temperature breakpoint for ~~the function of~~ faeces produced was 4.~~1~~9ºC ± ~~1.6~~0.95ºC, averaged across the slowest (T↑ 0.3ºC day^{-1}) and intermediate (T↑ 0.5ºC day^{-1}) rates of warming.

3.2 Righting

~~After 6 days, righting time was significantly longer in treatments where T↑ 0.3ºC day^{-1} compared to ambient conditions (W = 396, p = .003). However, beyond 6 days, righting time reduced as cumulative intensity increased until 194ºC x days. From here, righting time increased linearly until CT_{max} was reached (Figure 7). In treatments where T↑ 0.5ºC day^{-1},~~ In treatments where T↑ 1.0ºC day^{-1}, time taken to right became significantly longer than ambient controls when temperatures reached 9.2ºC~~low~~ ($t_{(4)}$ = 6.06, p < 0.022). ~~6~~For treatments where T↑ 0.3ºC day^{-1}, time taken to right only became significantly longer than ambient controls just before CT_{max} was reached, when temperatures reached 11.2ºC ($t_{(4)}$ = 6.04, p < 0.023). For treatments where T↑ 0.5ºC day^{-1}, time taken to right never exceeded ambient controls significantly, however mean righting times were consistently higher than control conditions throughout the warming period.

~~and T↑ 1ºC day^{-1}, righting time was significantly greater than in ambient conditions when exposed for 39ºC x day (W = 440, p = 0.003) and 18ºC x day (W = 357, p = 0.003), respectively. From here, the time taken to right fluctuated, but was significantly longer than ambient values until CT_{max} was reached.~~

A breakpoint in the linear regression was identified at ~~6.8°C and~~ 8.7°C in treatments where temperature was raised at ~~T↑ 1°C day^{-1} and~~ 0.3°C day^{-1}~~,~~ ~~respectively~~ (~~Figure 8A & 8C,~~ Table 1). ~~-~~The relationship between temperature and the time taken to right became significant above ~~these~~ this breakpoint temperature~~s~~ (~~6.8°C; p = 0.001 and 8.7°C;~~ p <0.001). For the other treatments righting time increased linearly without a breakpoint in the regression. ~~The mean temperature breakpoint for the function of righting was 7.8°C ± 1.0°C, averaged across the fastest (T↑ 1°C day^{-1}) and slowest (T↑ 0.3°C day^{-1}) rates of warming.~~

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 (Figure S5, Table S3).

3.3 Oxygen consumption

Oxygen consumption rates were significantly higher in heatwave treatments compared to ambient controls ~~after cumulative intensities of 2°C x day~~ when temperatures reached 7.2°C for all treatments~~.~~. H~~,~~ ~~h~~owever, oxygen consumption rates were significantly higher than ambient controls from lower temperatures of 3.2°C in treatments where T↑ 0.3°C day^{-1} ($t_{(4)}$ = 5.62, p = 0.030) and 5.2°C in treatments where T↑ 1.0°C day^{-1} ($t_{(4)}$ = 4.98, p = 0.045). ~~where T↑ 0.3°C day^{-1} ($t_{(57)}$ = 4.69, p < 0.001, d = 1.63), of 18°C x day, where T↑ 0.5°C day^{-1} ($t_{(56)}$ = 3.79, p < 0.001, d = 1.38), and of 8°C x day, where T↑ 1°C day^{-1} ($t_{(57)}$ = 6.28, p < 0.001, d = 2.18) (Figure 9).~~ Overall, there was a positive linear trend between oxygen consumption and temperature for all treatments. However, where T↑ 1°C day^{-1}, a drop in O_2 consumption occurred

at ~~32°C x day~~9.2°C, and where T↑ 0.3°C day^{-1}, a ~~peak~~ drop occurred just before the CT_{max} at ~~104°C x day~~11.2°C.~~.~~

O_2 consumption increased ~~more per cumulative intensity~~at a faster rate per increase in temperature where warming rates were fastest at 1°C day^{-1} (slope gradient = 1.50) and increased at the slowest rate when warming rates were slowest at 0.3°C day^{-1} (slope gradient = 0.96) (Ta~~, compared to the other two slower warming rates (Figure~~ble 1~~9~~). No breakpoint was identified in any treatment~~To this effect, the linear relationship between O_2 consumption rate and cumulative intensity was significantly different for treatments where T↑ 1°C day^{-1}, compared to 0.3°C day^{-1} and 0.5°C day^{-1} ($F_{(2,143)}$ = 16.86, p < 0.001). Owed to the variability observed in treatments where T↑ 1.0 day^{-1} and 0.3°C day^{-1}, a breakpoint could only be identified in the treatment where T↑ 0.5°C day^{-1} (Figure 10B). This breakpoint occurred at 7.0°C, after which the relationship between temperature and O_2 consumption became significant (p < 0.001), increasing at a faster rate as the temperature was increased, However, the gradient of the two slopes was not significantly different (Davies p-value = 0.260).~~

3.4 CT_{max}

The CT_{max} for urchins in treatments where T↑ 0.3°C day^{-1}, T↑ 0.5°C day^{-1} and T↑ 1°C day^{-1} ranged from 10.6°C - 13.8°C, 11.2°C - 13.7°C, and 12.2°C - 14.2°C, respectively. ~~(Figure 11).~~ The effect of warming rate on the CT_{max} was significant ($F_{(2, 12)}$ = 7.29, p = 0.008~~chi square = 16.9, p = < 0.001, df = 2~~), with post-hoc analysis identifying that for treatments where temperature increased at the fastest rate (T↑ 1°C day^{-1}), the CT_{max} was significantly higher compared

to treatments where temperature increased at a slower rate ~~T↑ 0.5°C day^{-1} (Wilcoxon rank sum test, p = 0.020) and where~~ (T↑ 0.3°C day^{-1}) (~~Wilcoxon rank sum test, p < 0.001~~$t_{(8)}$ = -6.02, p = 0.001).

Across all functions where breakpoints were identified, the slowest rate of warming (T↑ 0.3°C day^{-1}) had a mean temperature breakpoint of ~~7~~8.~~3~~1°C ± 1.3°C. In comparison, the mean temperature breakpoint was ~~6.1~~5.4°C ± 0.5°C, and 4.6~~5.3~~°C ± ~~1.4~~0.6°C for intermediate (T↑ 0.~~3~~5°C day^{-1}) and fast (T↑ 1°C day^{-1}) warming rates, respectively.

4. DISCUSSION

~~Marine heat waves~~MHWs are predicted to increase in frequency, ~~intensity~~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-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 ~~different functions~~key biological functions are affected by ~~increasing~~ short term temperature elevations and different warming rates, we can better ~~predict~~ 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~~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 findings here~~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, ~~contrary to this,~~ our results ~~indicate that~~for functional thermal limits follow the opposite trend to the CT_{max}, where functions are impacted ~~detrimentally~~ 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~~7~~.3~~1~~°C ± 1.3°C). ~~,~~ At t~~compared to t~~he faster warming rates~~, where~~ functional thresholds were ~~either~~ lower (5.4~~4.9~~°C ± 0.5°C or 4.~~5.3~~6°C ± ~~1.4~~0.6°C). There was even evidence that ~~or else~~ some functions declined linearly, with significant ~~functional~~ deterioration from temperatures ~~from~~ +2.8~~1~~°C above ambient when warmed at the fastest rate~~ing~~. Thus, short-term exposure to more extreme temperatures has more impact on functioning than longer, chronic exposure to more slowly elevated temperatures. ~~(Figure 12).~~

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. ~~Our data suggest, with~~limits ~~to functionsing are likely restricted related to by energy availability (as seen in this study) and .~~ Previous studies have shown that lethal limits are likely ~~restricted~~ 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 day^{-1}, physiological mechanisms such as nervous ~~or~~ and circulatory failure appear to ~~to~~ be the limiting factors (Young et al. 2006, Pörtner et al. 2007, Bilyk & DeVries 2011). At slower rates of warming (1°C ~~per~~ 3 $days^{-1}$ to 1°C $month^{-1}$) ~~or~~, ~~or~~ ~~c~~cellular and biochemical mechanisms such ~~as~~as accumulation of toxic products, e.g. protein carbonyls, enzyme tolerances or insufficiency of chaperone protein~~s~~ 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~~ (Clark et al. 2021, Collins et al. 2021).

Our results also indicate that thermal sensitivity varies among ~~different functions~~key biological functions ~~have different thermal sensitivities~~. ~~Mean thresholds were lowest for faecal production (mg day^{-1}) (4.9°C ± 1.6°C), and highest for righting (7.8oC ± 1.0°C).~~ For example, ~~for~~ the function of righting in urchins ~~, this function~~ was ~~maintained~~ similar between treatments and~~to~~

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 in the slowest rates of warming ~~for this function~~. ~~Comparatively~~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 ~~fundamental~~ functions limit survival and fitness, where an organism's energy reserves 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 reburying in infaunal clams when disturbed and removed from the sediment by ~~e.g~~, 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 ~~comparative~~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°C – 2°C were likely not maintained long enough for gut passage rate to increase ~~as a result~~. 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~~transport, and delivery of ~~O_2~~ 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. H~~, h~~owever, 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 ~~may~~would likely be higher (Pörtner et al. 2006). However, warmer oceans will be ~~conjusive to~~accompanied by lower~~d~~ oxygen concentrations (Oschlies et al. 2018, Spicer et al. 2019) and as such the functional thresholds determined in this study ~~maybe~~will be more representative of a natural system than if oxygen were controlled.

~~However, this theory is the subject of much debate and there are concerns over its use (Clark et al. 2013b, Clark & Mark 2017, Jutfelt et al. 2018). In particular, the theory makes the assumption that functioning is limited by energy derived from respiration only and does not consider effects from the variation in the energetic value of food~~is the subject of much debate and there are concerns over its use (Clark et al. 2013b, Clark & Mark 2017, Jutfelt et al. 2018).~~.~~ ~~Evidence suggests that f~~Food availability and quality can ~~be~~ 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 ~~effect~~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 ~~the~~ 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 ~~rapid warming~~ both experiments and in natural ~~heatwaves~~ 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 7 months without feeding (Brockington 2001) may be particularly vulnerable to such impacts.

Our experiment included a period of 6 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 6 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 ~~Rothera environmental monitor (~~RaTS~~)~~ environmental data, previous MHW events reached maximum temperatures of 2.3^{o}C ± 0.36^{o}C, with onset rates of 0.3^{o}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^{o}C x day (Figure S2). ~~If we consider the latest climate change predictions (IPCC 2014, 2019)~~ Mean climate temperatures are predicted to shift by +2^{o}C by 2100, and with that, climate extremes such as MHWs will increase in magnitude relative to this (IPCC 2014, 2019). ~~of the most likely scenario of +2^{o}C ocean warming, then o~~Our results suggest that functions such as feeding and faecal egestion are likely to be affected by MHW events ~~by~~ occurring in 2100, if not before, and this will include increased metabolic demands with consequent impacts on annual energy budgets. ~~Thus we would predict reduced energy availability for *S. neumayeri* from reduced feeding rates and food processing rates in warmer oceans, which is very likely to reduce survival in marginal environments. In our experiments, functional limits were reached within the cumulative intensities already experienced in the environment. However, this observation was limited to the most rapid warming rate of 1^{o}C day^{-1}, a rate not yet reported from the Southern Ocean.~~

For a long-lived (>40 year (Brey et al. 1995)) and slow to mature (8-9 years (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 ~~acclimate~~adapt to a warmer world. Within 80 years (2020 - 2100), eight 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 *S. neumayeri*'s capacity to acclimate, it may be possible for this species to acclimate and adapt successfully to ~~and~~ function ~~at~~in a +2^{o}C warmer world (Morley et al. 2016).~~,~~ ~~however i~~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 predict the implications of acclimation and adaptation ~~and~~on the subsequent tolerance to MHWs for *S. neumayeri*. Instead, the data provides insight into the effect of onset rate of acute warming, the thermal vulnerability of key biological functions, and the difference between critical thermal limits and functional thermal limits. Thus, according to our data we c~~w~~ould ~~predict~~see reduced energy availability for *S. neumayeri* ~~from reduced~~from changes in feeding ~~rates~~ and food processing rates during MHWs in warmer oceans, which ~~is~~ would very likely ~~to~~ 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. H~~, h~~owever, the ability and rate of *S.*

neumayeri to resume 'normal' functioning if returned to ambient temperatures is uncertain. It has been shown that the marine snail, *Littorina littorea*, loses motility under thermal stress, however if 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 ~~acknowledge~~ 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., ~~H~~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, ~~must~~ need to be ~~considered~~ included before we can obtain dependable predictions for 'real world' scenarios that give information relevant to the ~~wide range of~~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). ~~The results reported here are best-case scenarios for *this species*~~*S. neumayeri*~~, with a high protein food supply, which likely resulted in functional limits at the high end for this species.~~

~~This study~~Our data highlight~~s~~ that the deterioration of functioning ~~with warming~~when temperatures are raised, especially ~~heat waves~~during MHWs,

has implications for long term survival, and physiological functions. Therefore, functioning ~~al processes~~ 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~~importance, and energetic demand. Our results suggest ~~that,~~that contrary to the relationship between critical thermal limits and onset rate, functional degradation occurs at lower temperatures when exposed to rapid warming (1^{o}C day^{-1}). Therefore, when investigating the impact of ~~marine heatwaves~~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

L.S.P and R.D conceived and designed the study. R.D carried out the practical work and data processing. R.D, L.J.G and L.S.P analysed the data, drafted the manuscript and approved its publication.

COMPETING INTERESTS

The authors declare no competing interests.

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TABLES

Table 1: Summary statistics for linear regression relationships between the measured functions of *Sterechinus neumayeri* and temperature. β indicates the slope of the linear regression lines before the breakpoint (Slope_1) and after the breakpoint (Slope_2); SE_a indicates standard error for the intercept and slopes; df = degrees of freedom; 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 column BP indicate the localisation of the breakpoint or else NA indicates a single linear regression; SE_b (standard error) and R^2 refers to the goodness of fit for the entire model.

Function ~~Function~~	β~~β~~	SE_a ~~S E$_a$~~	p-value ~~p-value~~	BP ~~B P~~	SE_b ~~S E$_b$~~	R^2 ~~R^2~~	Davies

							p-value~~Davies p-value~~
Individuals feeding, 1°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals feeding, 1°C day^{-1} (Intercept) Slope_1 Slope_2~~	89.0 3.45 -12.9 ~~89.0 3.45 -12.9~~	25.4 10.5 2.35 ~~25.4 10.5 2.35~~	df=3 0.039 0.764 **0.012**~~df=3 0.039 0.764 **0.012**~~	4.04~~.0~~	14.91~~4.9~~	0.894 ~~0.894~~	0.329~~0.329~~
Individuals feeding, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals feeding, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2~~	110.3 -6.34 -11.5 ~~110.3 -6.34 -11.5~~	12.7 3.14 1.05 ~~12.7 3.14 1.05~~	df=7 <0.001 0.083 **<0.001**~~df=7 <0.001 0.083 **<0.001**~~	6.26~~.2~~	6.78~~.78~~	0.964 ~~0.964~~	0.301~~0.301~~
Individuals feeding, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals feeding, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2~~	95.3 -2.73 -20.3 ~~95.3 -2.73 -20.3~~	7.53 1.38 2.92 ~~7.53 1.38 2.92~~	df=12 <0.001 0.071 **<0.001**~~df=12 <0.001 0.071 **<0.001**~~	8.28~~.2~~	8.488~~.48~~	0.922 ~~0.922~~	**0.001**~~**0.001**~~
Individuals producing faeces, 1°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals producing faeces, 1°C day^{-1} (Intercept) Slope_1 Slope_2~~	-29.0 24.1 -13.3 ~~-29.0 24.1 -13.3~~	23.1 9.54 2.13 ~~23.0 9.54 2.13~~	df=3 0.298 0.085 **0.008**~~df=3 0.298 0.085 **0.008**~~	5.25~~.2~~	13.51~~3.5~~	0.881 ~~0.882~~	**0.019**~~**0.019**~~
Individuals producing faeces, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals producing faeces, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2~~	34.0 13.3 -10.3 ~~34.0 13.3 -10.3~~	28.6 8.54 8.68 ~~28.6 8.54 8.68~~	df=7 0.274 0.162 **<0.001**~~df=7 0.274 0.162 **<0.001**~~	4.54~~.5~~	12.11~~2.1~~	0.844 ~~0.844~~	**0.039**~~**0.039**~~
Individuals producing faeces, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2~~Individuals producing faeces, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2~~	77.9 -0.306 -18.6 ~~77.9 -0.306 -19.0~~	11.1 2.02 4.29 ~~11.1 2.02 4.29~~	df=12 <0.001 0.882 **<0.001**~~df=12 <0.001 0.882 **<0.001**~~	8.38~~.3~~	12.51~~2.5~~	0.762 ~~0.762~~	**0.006**~~**0.006**~~
Faeces produced, 1°C day^{-1} (Intercept)	0.645 -0.040	0.137 0.027	df=14 <0.001	NA~~NA~~	0.216 ~~1.35~~	0.071 ~~0.364~~	0.858~~NA~~

Slope_1[1]Faeces produced, 1°C day^{-1} (Intercept) Slope_1	3.63 0.31	0.422 0.057	0.165 df=47 <0.001 <0.001				
Faeces produced, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2Faeces produced, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2	1.52 -0.23 -0.06 9.53 -1.33 -0.118	0.214 0.072 0.025 0.771 0.184 0.185	df=31 <0.001 0.007 0.016 df=74 <0.001 <0.001 0.526	4.6.59	1.111.92	0.6640.611	0.043<0.001
Faeces produced, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2Faeces produced, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2	3.54 -0.718 -0.051 18.4 -4.86 -0.232	0.509 0.202 0.020 2.54 1.01 0.065	df=34 <0.001 0.001 0.012 df=87 <0.001 <0.001 0.001	3.33.8	0.2941.57	0.7290.673	<0.001<0.001
Time taken to right, 1°C day^{-1} (Intercept) Slope_1[1]Time taken to right, 1°C day^{-1} (Intercept) Slope_1 Slope_2	-8.60 6.83 11.7 0.897 15.2	9.04 1.35 7.33 2.03 2.86	df=26 0.350 <0.001df=44 0.119 0.661 <0.001	NA6.8	23.318.19	0.4760.553	NA<0.001
Time taken to right, 0.5°C day^{-1} (Intercept) Slope_1[1]Time taken to right, 0.5°C day^{-1} (Intercept) Slope_1	8.88 2.61 9.54 2.42	5.03 0.731 4.46 0.649	df=26 0.089 0.001df=51 0.037 <0.001	NANA	13.116.2	0.3020.198	NANA
Time taken to right, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2Time taken to right, 0.3°C day^{-1} (Intercept) Slope_1 Slope_2	14.6 0.384 55.7 14.2 0.537 64.1	20.1 3.66 13.8 15.0 2.77 10.9	df=25 0.237 0.459 <0.001 df=51 0.350 0.847 <0.001	8.78.7	0.55645.8	0.5880.589	<0.001<0.001
Oxygen consumption, 1°C day^{-1} (Intercept) Slope_1[1]Oxygen consumption, 1°C day^{-1} (Intercept) Slope_1	1.64 1.50 -0.042 0.177	1.76 0.248 0.227 0.030	df=28 0.358 <0.001df=45 0.856 <0.001	NANA	4.640.581	0.5510.425	NANA
Oxygen consumption, 0.5°C day^{-1} (Intercept) Slope_1[1]	4.29 0.611	1.10 0.134	df=33 <0.001 <0.001 df=52	NA7.0	3.170.169	0.3680.804	NA0.260

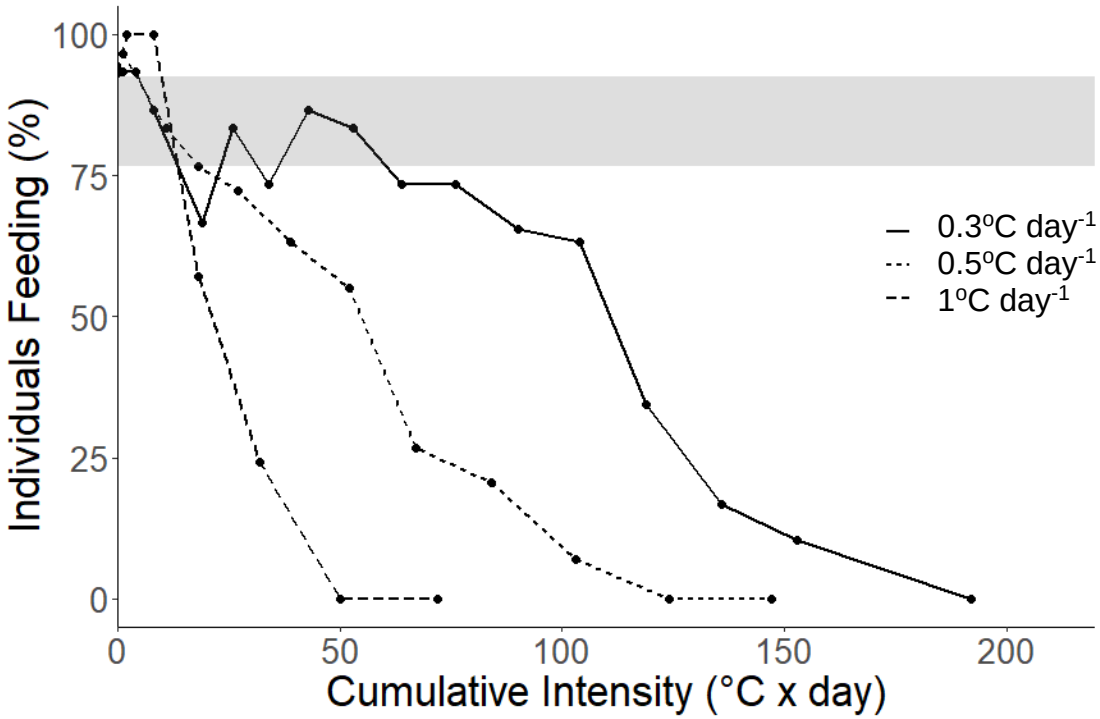
[1] Reporting only a single slope (Slope_1) indicates that no breakpoint was detected in the regression and statistics for a single linear regression model is reported for the data instead.

Oxygen consumption, 0.5°C day^{-1} (Intercept) Slope_1 Slope_2	0.261 0.069 0.120	0.172 0.040 0.013	0.135 0.088 **<0.001**				
Oxygen consumption, 0.3°C day^{-1} (Intercept) Slope_1Oxygen consumption, 0.3°C day^{-1} (Intercept) Slope_1	3.30 0.957 0.327 0.100	1.36 0.185 0.155 0.020	df=28 **0.022** **<0.001** df=44 0.040 **<0.001**	NANA	3.490.391	0.471 0.339	NANA

Table 2: Cumulative intensity (°C x day) in relation to temperature for each treatment. Colours represent cumulative intensity magnitude, where green indicates low magnitude and red indicates high magnitude, relative to those experienced in the experiment. Cumulative intensity calculated from following regressions: 1°Cday^{-1}: $(0.49T^2) - (0.99T)$, 0.5°C day^{-1}: $(0.96T^2) - (1.52T)$ and 0.3°C day^{-1}: $(1.47T^2) - (2.22T)$, where T=Temperature (Supplementary Materials, Figure S3).

Temperature (°C) / Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Cumulative Intensity (°C x day)														
1°C day^{-1}	1	3	6	10	15	21	28	36	45	55	66	78	91	105	120
0.5°C day^{-1}	2	7	13	21	31	43	57	72	90	110	131	155	180	207	237
0.3°C day^{-1}	3	9	18	30	45	63	84	108	135	165	198	234	273	315	361

1 **FIGURES**


Individuals Feeding (%)
100
75
50
25
0
0
50
100
150
200
Cumulative Intensity (°C x day)
0.3ºC day-1
0.5ºC day-1
1ºC day-1

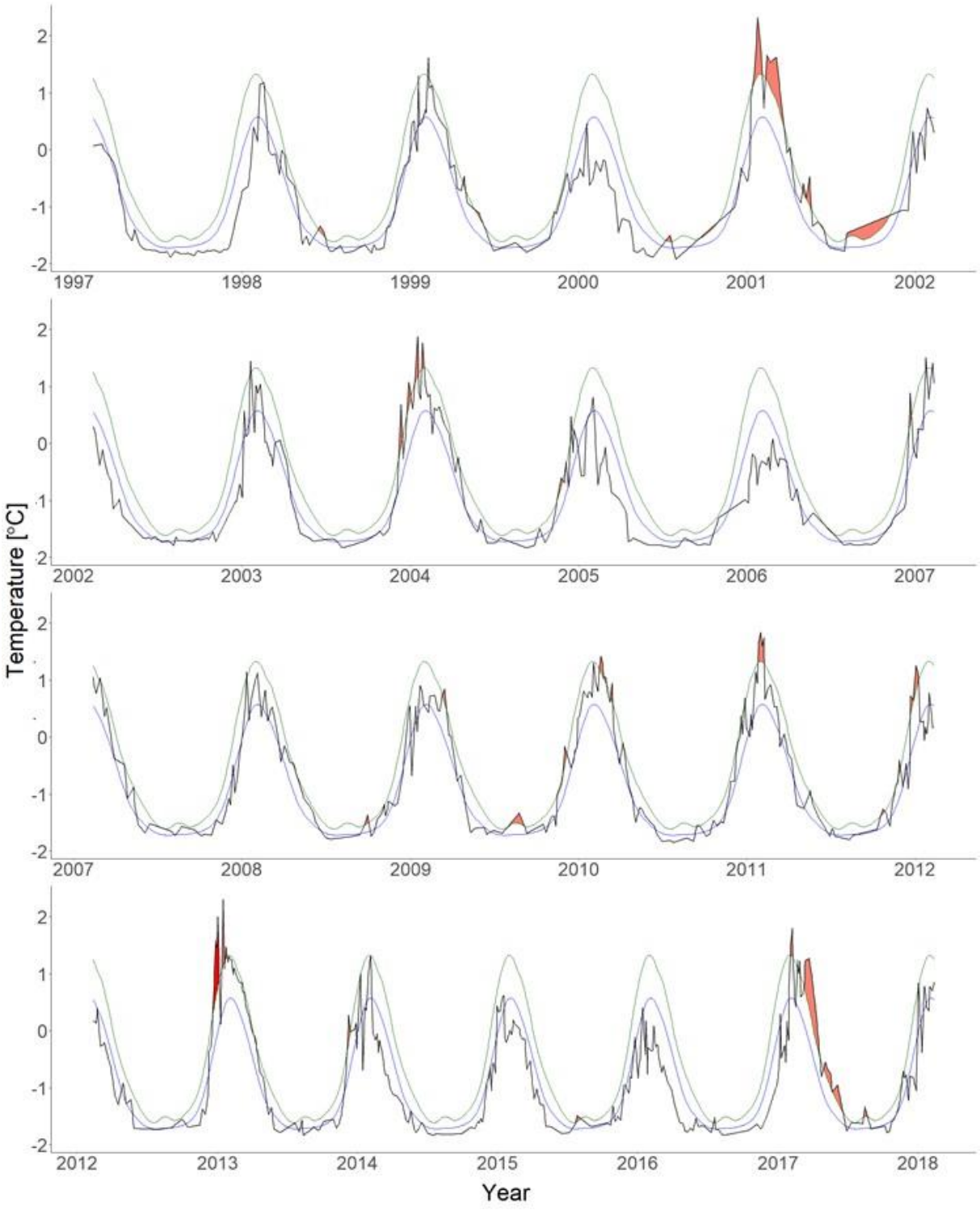
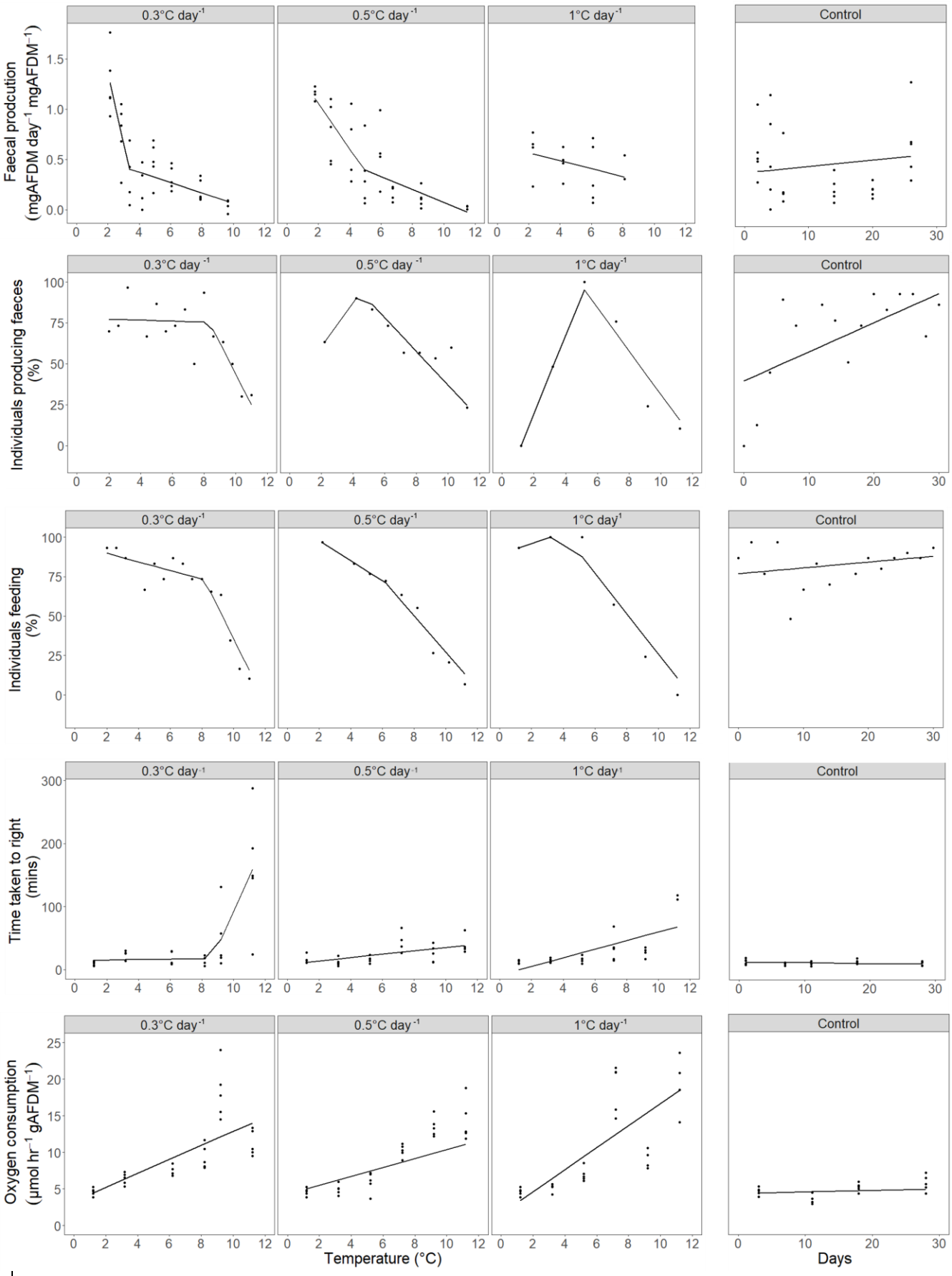


Figure 1: Times-series of temperatures (°C) experienced in Ryder Bay, Antarctica, at depths of 15 m, represented by the black lines. The data are split into panels to cover the entire span of the time-series, where the x-axis represents time in years. Blue lines represent the seasonal climatology of 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 days are highlighted in red and indicate the occurrence of a marine heatwave.



1 Figure ~~1~~2: *Sterechinus neumayeri.* ~~Percentage of individuals recorded as~~

~~feeding in experimental treatments (n=30)~~ Biological functions measured in *Sterechinus neumayeri* in experimental conditions ~~-~~where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC. Functions in warming conditions are plotted against ~~, relative to the~~ increasing ~~e in cumulative intensity~~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. ~~Grey area represents interquartile range of % individuals feeding in ambient conditions throughout the experiment.~~

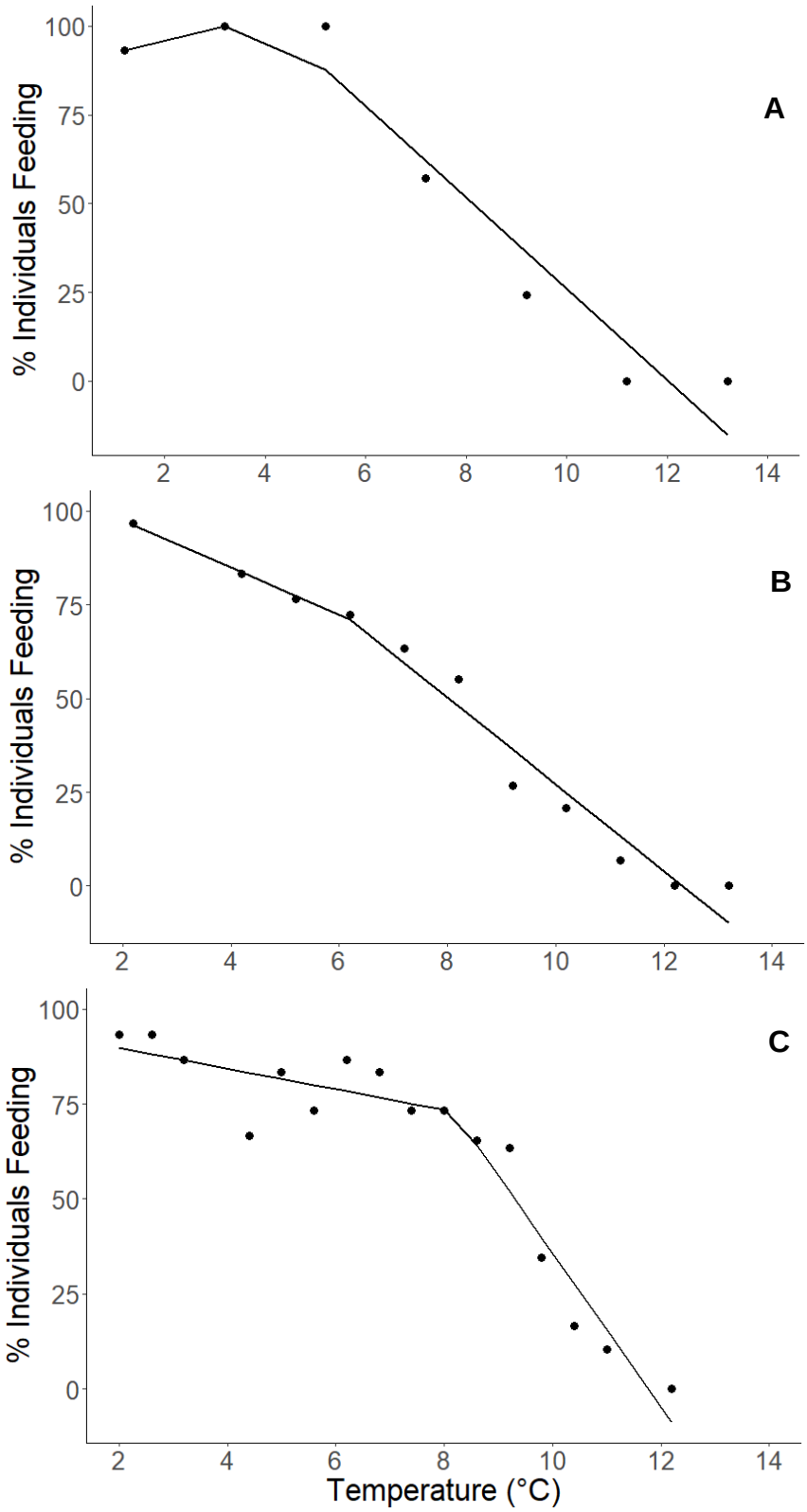


Figure 2: *Sterechinus neumayeri.* Segmented linear regression models for the percentage of animals feeding in experimental treatments (n=30) where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC. A)

Temperatures increased by 1°C day^{-1}, breakpoint identified at 4°C (R^2 = 0.894). B) Temperatures increased by 0.5°C day^{-1}, breakpoint identified at 6.2°C (R^2 = 0.964). Temperatures increased by 0.3°C day^{-1}, breakpoint identified at 8.2°C (R^2 = 0.922).

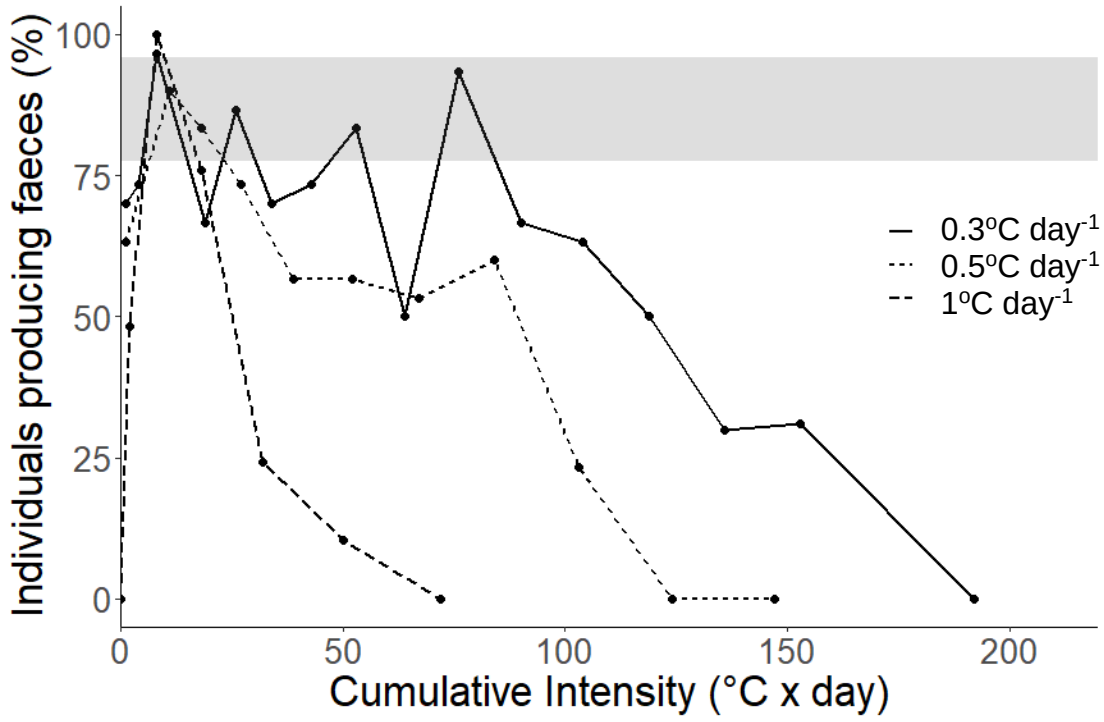
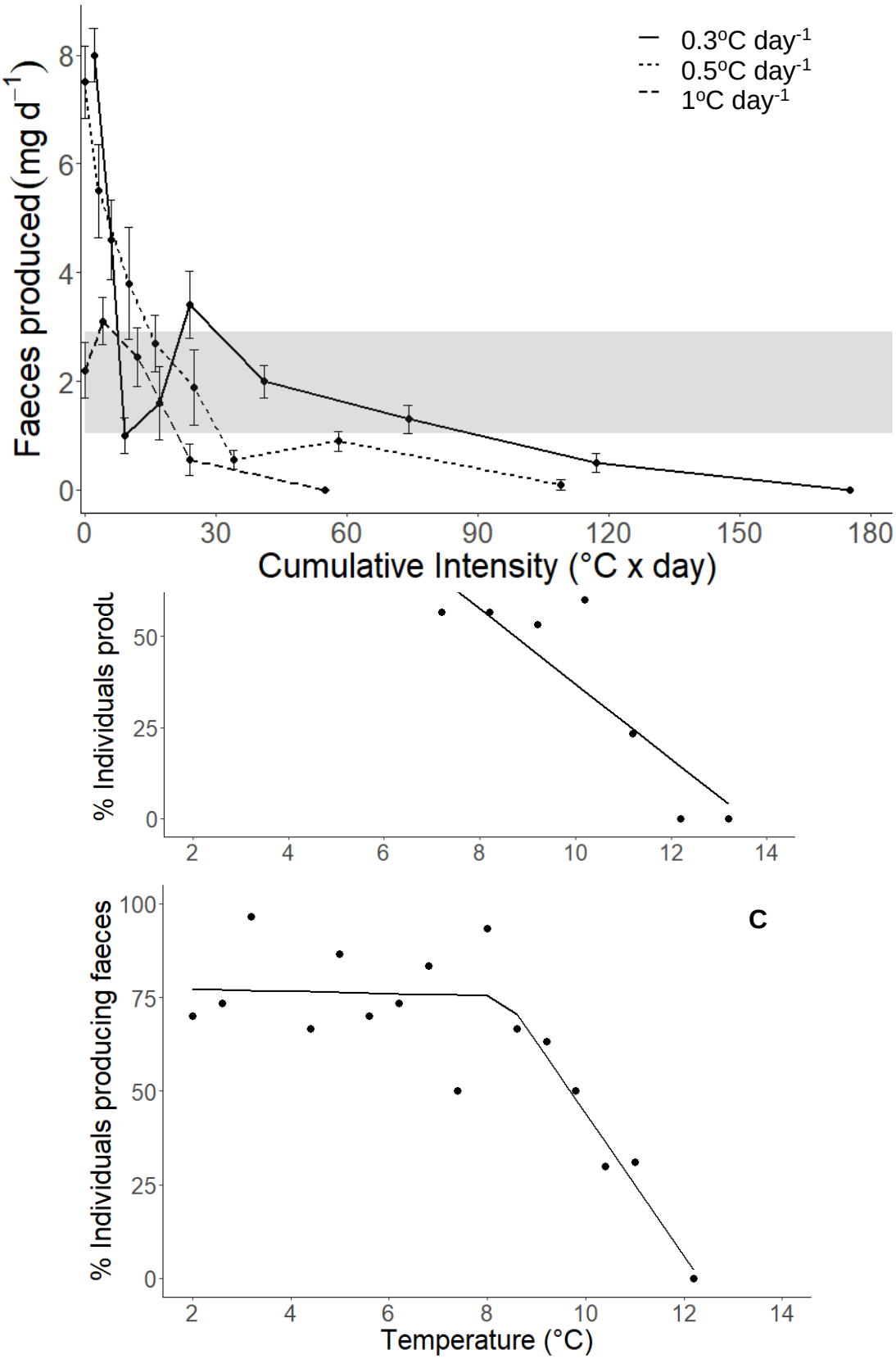


Figure 3: *Sterechinus neumayeri*. Percentage of individuals recorded as producing faeces in experimental treatments (n=30) where temperatures were increased daily by 0.3°C, 0.5°C and 1°C, relative to the increase in cumulative intensity. Grey area represents interquartile range of % individuals producing faeces in ambient conditions throughout the experiment.

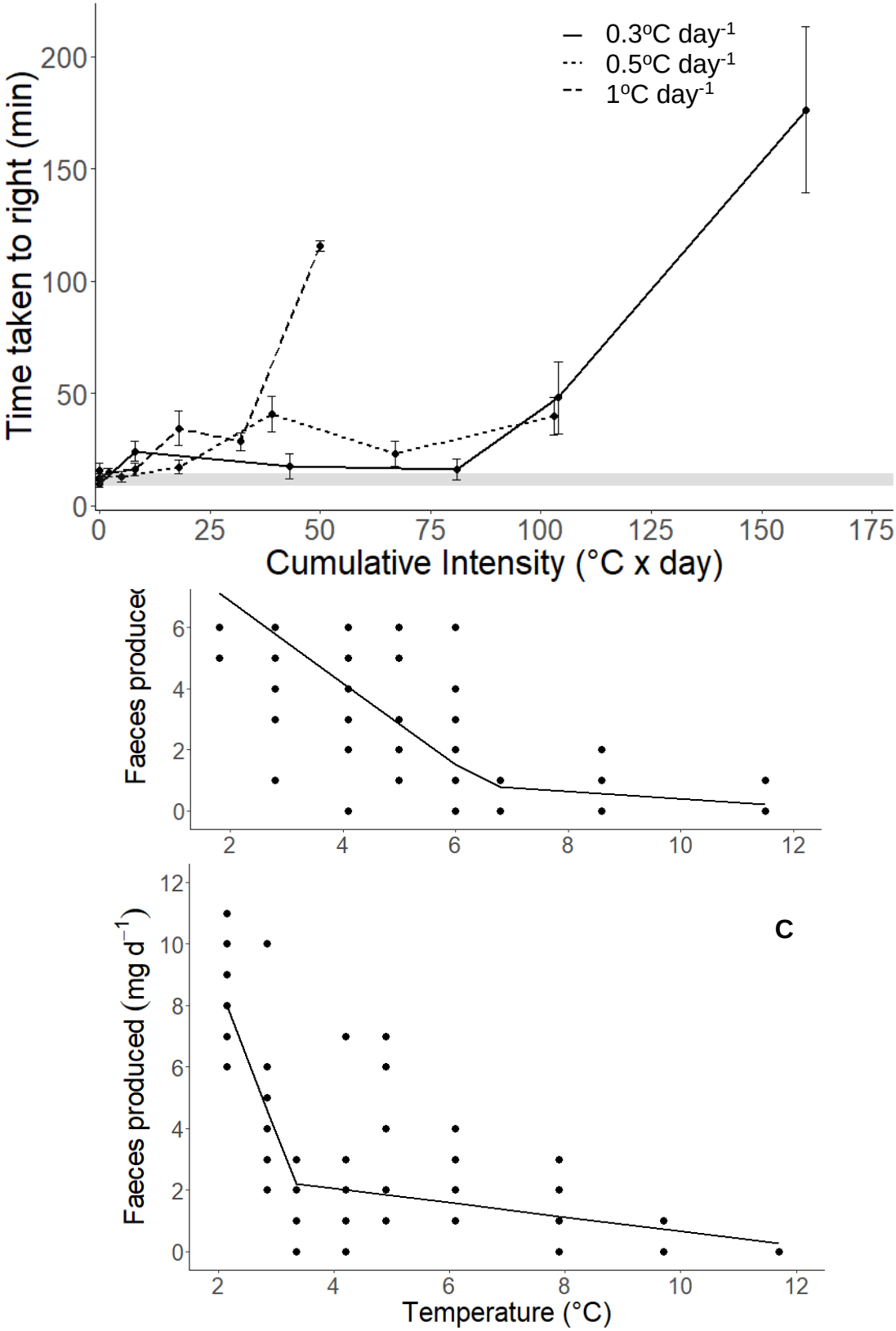


1 ~~Figure 4: *Sterechinus neumayeri.* Segmented linear regression models for the~~

2 ~~percentage of animals producing faeces in experimental treatments (n=10)~~

where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC. A) Temperatures increased by 1ºC day^{-1}, breakpoint identified at 5.2ºC (R^2 = 0.882). B) Temperatures increased by 0.5ºC day^{-1}, breakpoint identified at 4.5ºC (R^2 = 0.844). Temperatures increased by 0.3ºC day^{-1}, breakpoint identified at 8.3ºC (R^2 = 0.762).

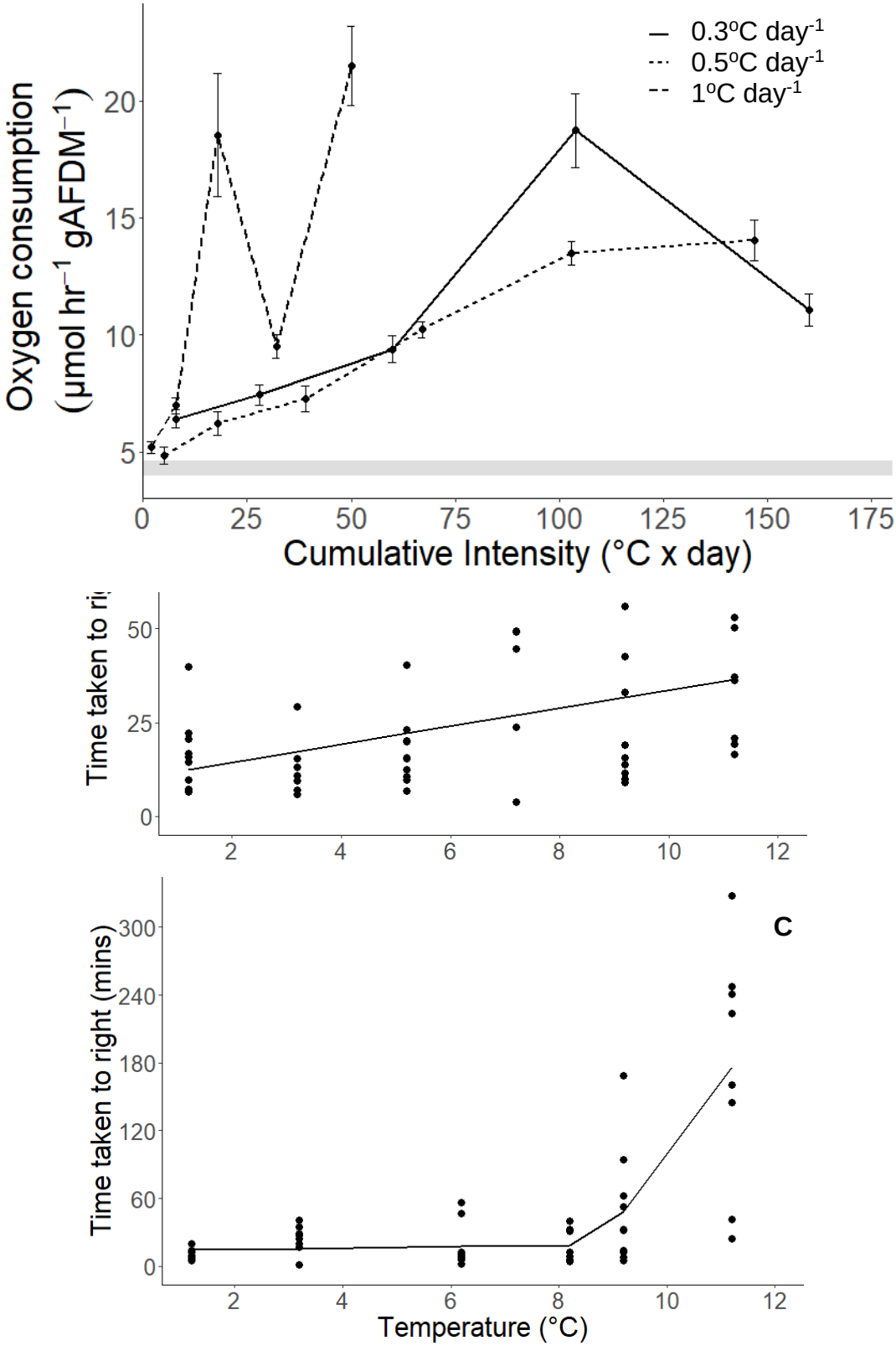
Figure 5: *Sterechinus neumayeri.* Faeces produced (mg day^{-1}) in experimental treatments (n=10) where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC, relative to the increase in cumulative intensity. Error bars = +/- standard error. Grey area represents interquartile range of faeces produced (mg day^{-1}) in ambient conditions throughout the experiment.



~~Figure 6: *Sterechinus neumayeri*. Linear and segmented regression models for the rate of faecal production in experimental treatments (n=10) where temperatures were increased daily by 0.3ºC, 0.5ºC and 1ºC. A)~~

~~Temperatures increased by 1°C day^{-1}, no breakpoint identified (R^2 = 0.364). B) Temperatures increased by 0.5°C day^{-1}, breakpoint identified at 6.5°C (R^2 = 0.611). Temperatures increased by 0.3°C day^{-1}, breakpoint identified at 3.3°C (R^2 = 0.553).~~

~~Figure 7: *Sterechinus neumayeri.* Time taken to right in experimental treatments (n=10) where temperatures were increased daily by 0.3°C, 0.5°C and 1°C, relative to the increase in cumulative intensity. Error bars = +/- standard error. Grey area represents interquartile range of time taken to right in ambient conditions throughout the experiment.~~



1 ~~Figure 8: *Sterechinus neumayeri.* Linear and segmented regression models for~~

2 ~~the time taken to right in the experimental treatments (n=10) where~~

3 ~~temperatures were increased daily by 0.3°C, 0.5°C and 1°C. A) Temperatures~~

~~increased by 1.0°C day^{-1}, breakpoint identified at 6.8°C (R^2 = 0.553). B) Temperatures increased by 0.5°C day^{-1}, no breakpoint identified (R^2 = 0.198). Temperatures increased by 0.3°C day^{-1}, breakpoint identified at 8.7°C (R^2 = 0.589).~~

~~Figure 9: *Sterechinus neumayeri*. Oxygen consumption rate of urchins in experimental treatments (n=10) where temperatures were increased daily by 0.3°C, 0.5°C and 1°C, relative to the increase in cumulative intensity. Error bars = +/- standard error. Grey area represents interquartile range of oxygen consumption rates in ambient conditions throughout the experiment.~~

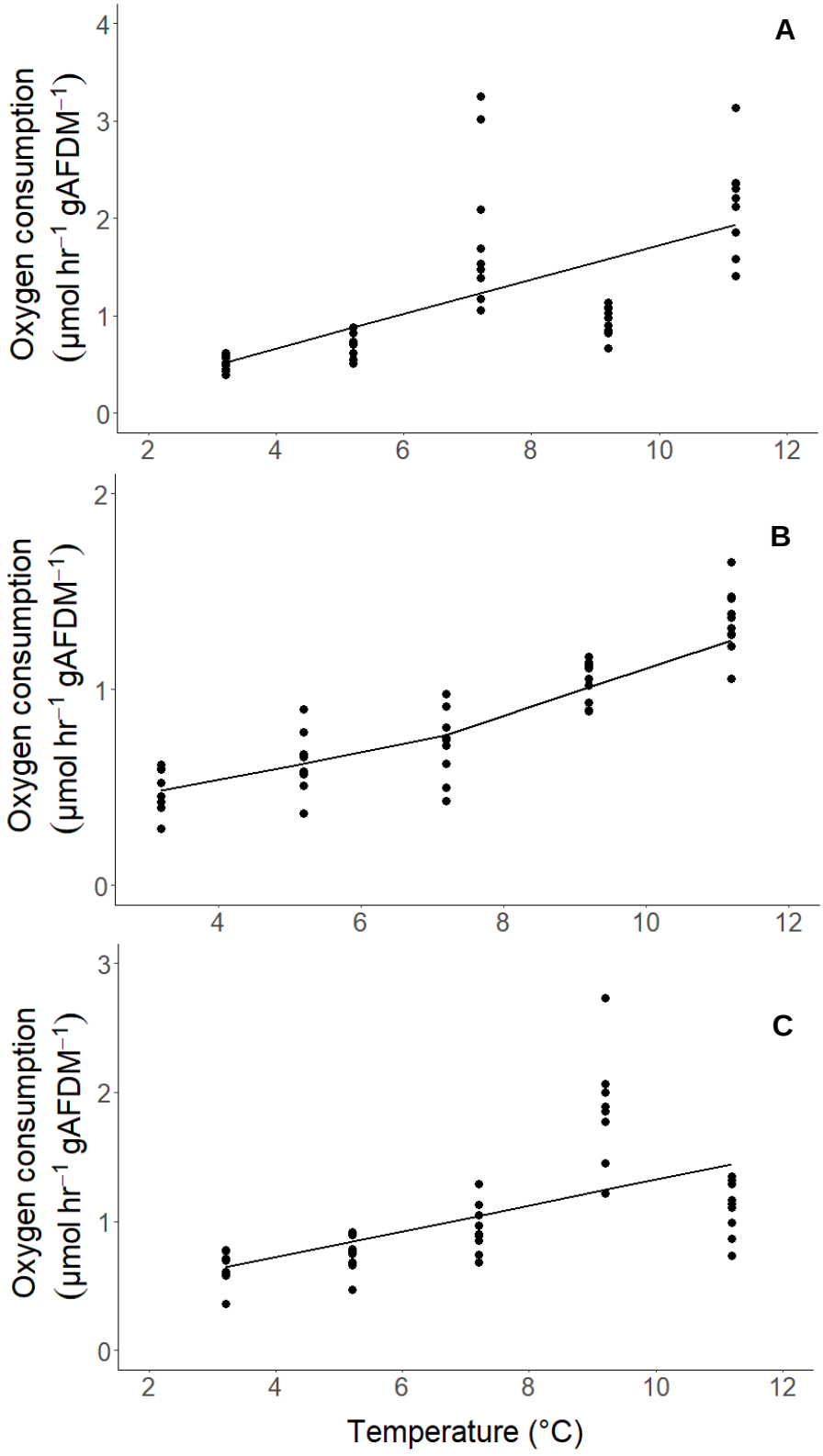
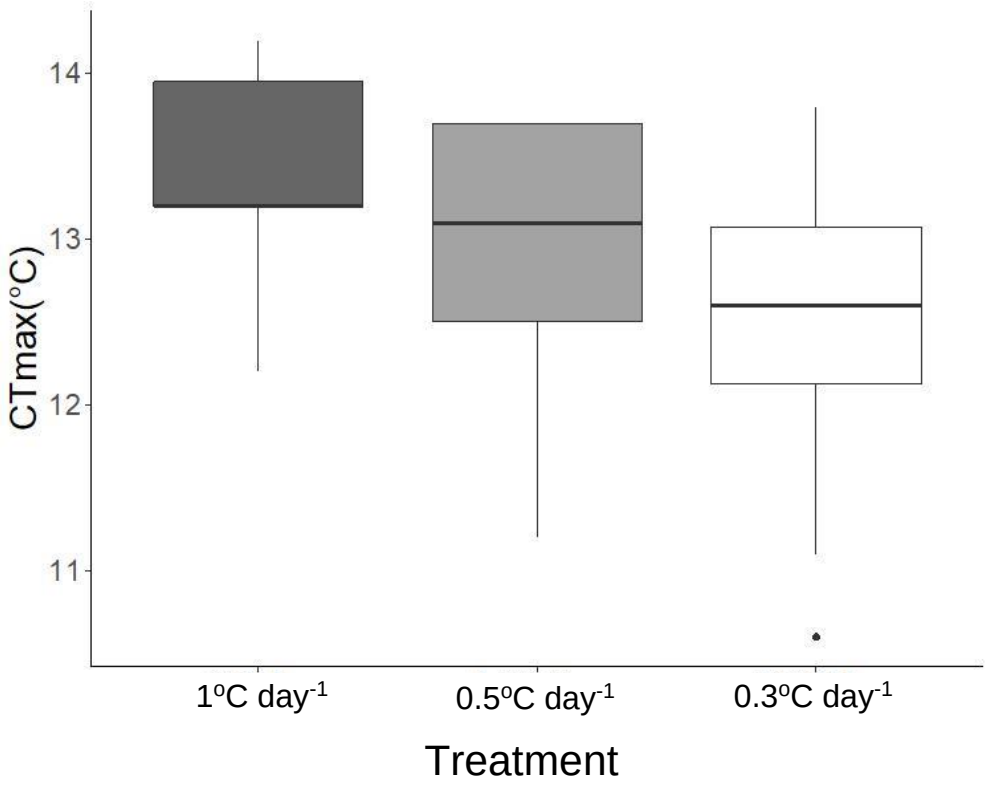
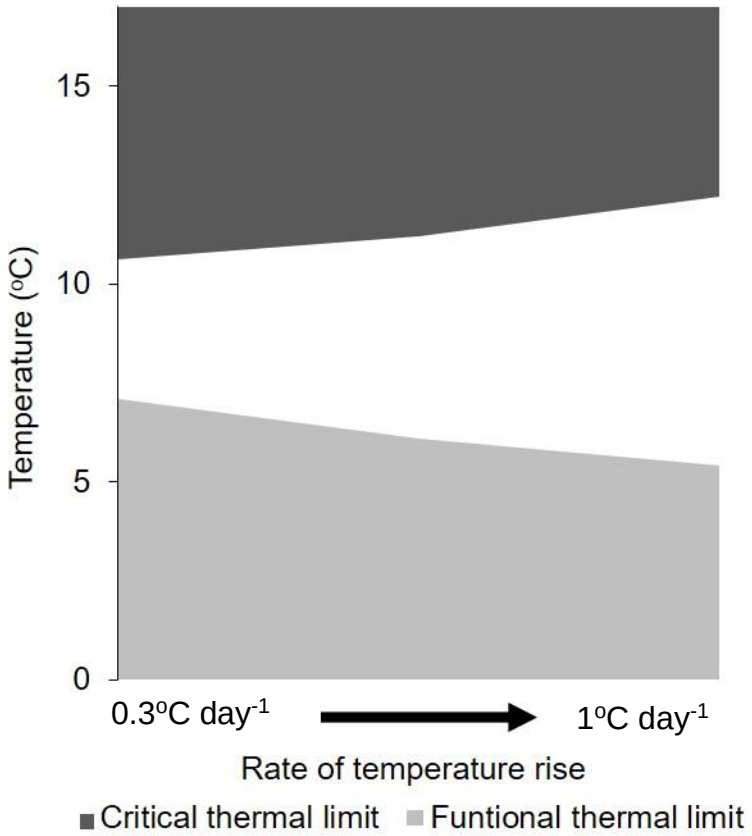


Figure 10: *Sterechinus neumayeri.* Segmented and linear regression models for the rate of oxygen consumption in the experimental treatment (n=10) where temperatures were increased daily by 0.5ºC. A) Temperatures increased by

~~1°C day^{-1}, no breakpoint identified (R^2 = 425). B) Temperatures increased by 0.5°C day^{-1}, breakpoint identified at 7.0°C (R^2 = 0.804). Temperatures increased by 0.3°C day^{-1}, no breakpoint identified (R^2 = 0.339).~~



~~Figure 11: Critical thermal maxium (CT_{max}) recorded in the experimental treatments (n=30) where temperatures were increased daily by 0.3°C, 0.5°C and 1°C. Data are displayed as box plots with the central line in the boxes representing the median value, the upper and lower hinges representing the 25th and 75th percentiles, and the upper/lower whiskers representing the largest/smallest value, no further than 1.5 times the interquartile range from the hinge. Data outside these ranges are plotted as points.~~



~~Figure 12: Schematic representation of the relationship between functional thermal limits and critical thermal limits for *Sterechinus neumayeri* as the rate of warming is increased from 0.3°C day^{-1} to 1°C day^{-1}.~~