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Survived but not safe: Marine heatwave hinders metabolism in two gastropod survivors

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2 **Survived but not safe: marine heatwave hinders metabolism in two gastropod survivors**

3

4 Deevesh A. Hemraj¹, Natasha C. Posnett², Jay J. Minuti¹, Louise B. Firth³, Bayden D. Russell^{1*}

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6 ¹The Swire Institute of Marine Science and School of Biological Sciences, The University of
7 Hong Kong, Pokfulam Road, Hong Kong SAR, China

8 ²School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake
9 Circus, Plymouth, PL4 8AA, UK

10 ³School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth,
11 PL4 8AA, UK

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13 *corresponding author: brussell@hku.hk

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15 Keywords: Marine heatwave; magnitude; mortality rate; metabolic regulation; thermal history

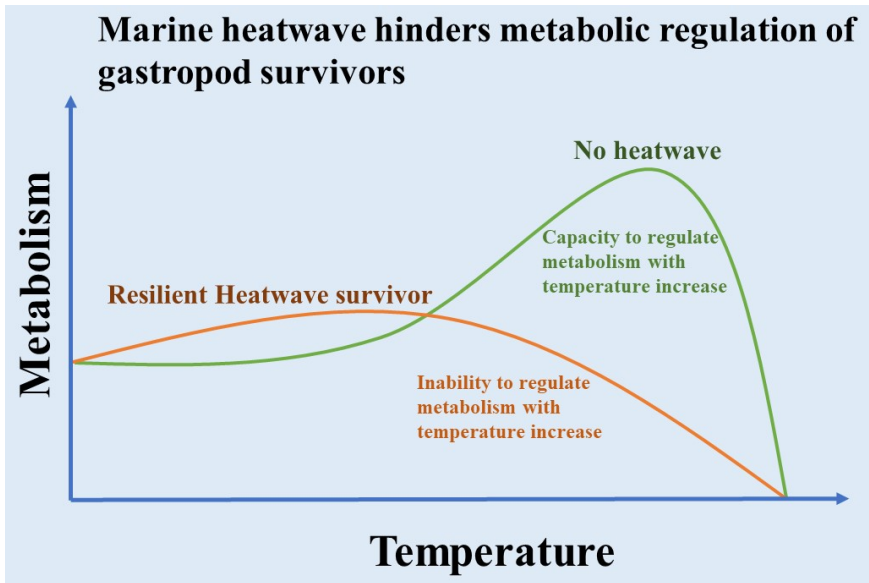
16 Highlights:

17

- 18 • Two marine gastropods (*T. sacellum* and *A. haematragum*) survived moderate marine
- 19 heatwave but perished under extreme heatwave conditions.
- 20 • Surviving heatwaves instigated ongoing physiological costs and non-adaptive
- 21 metabolic depression.
- 22 • Post-heatwave recovery will be key for species persistence under increasing heatwave
- 23 frequency.
- 24
- 25

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26 **Graphical abstract**



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40

41 **Abstract**

42 Marine heatwaves (MHWs) are an emerging threat to marine organisms that have increased in
43 frequency and magnitude in the past decade. These extreme heating events can have differential
44 impacts for organisms with some experiencing mortality while others survive. Here, we
45 experimentally exposed two species of subtidal gastropod (*Trochus sacellum* and *Astralium*
46 *haematragum*) to two realistic intensities of MHW to test the ability of different species to
47 physiologically cope with extreme heating events. Extreme MHW conditions caused 100%
48 mortality in both species within five days. In contrast, both species showed evidence of
49 nonadaptive metabolic depression under moderate MHW conditions. Both species
50 demonstrated an inability to upregulate their metabolic rates at the higher temperatures
51 following exposure to a MHW (i.e. reduced temperature of maximum metabolic rate; T_{MMR}),
52 suggesting a lack of molecular protective responses and ongoing physiological damage.
53 Therefore, the physiological damage endured by heatwave survivors may lessen their ability to
54 cope with subsequent stress until fully recovered. Repairing this damage may have serious
55 repercussions for the rate of recovery of these normally resilient species and their ability to
56 maintain their ecological functions post MHW, especially under the predicted increasing
57 frequency, duration and magnitude of MHWs.

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67 **1. Introduction**

68 Marine heatwaves (MHWs) are recognised as major threats to biodiversity because they drive
69 mass mortality of organisms (Garrabou et al., 2009; Le Nohaïc et al., 2017), changes in species
70 geographical distribution (Wernberg et al., 2016; Lonhart et al., 2019; Sanford et al., 2019),
71 and re-organisation of community composition (Arafteh-Dalmau et al., 2019; Brodeur et al.,
72 2019). The overarching effect of MHWs on biodiversity is dependent not only on the duration
73 and magnitude of thermal stress but also on the sensitivity of the organisms themselves
74 (Frölicher and Laufkötter, 2018; Leung et al., 2019). Since tolerance to thermal stress is
75 associated with the ability to regulate aerobic capacity and maintain physiological homeostasis
76 (Madeira, 2018 a), interspecific discrepancies in the effects of MHWs exist whereby some
77 species are resistant or resilient, while others are sensitive (Leung et al., 2019). Thus, some
78 species can be categorised as ‘winners’ for exhibiting adaptive phenotypic plasticity in
79 response to MHWs (Cavole et al., 2016; Watson, 2018) while others will be ‘losers’ when they
80 are unable to tolerate acute warming and die.

81

82 While different magnitudes of heat stress from MHWs can be lethal for some species, the
83 activation of physiological pathways associated with aerobic metabolism and cellular stress
84 response allow others to be resistant or resilient to thermal stress (Leung et al., 2019). Species-
85 specific regulation of physiological mechanisms to cope with stress under heatwaves are
86 present even among species from the same habitat (Bernal et al., 2020). Additionally, the
87 metabolic pathways that are activated to tolerate or survive thermal stress, such as cellular

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88 metabolism, cholesterol metabolism, or activation of the complement system (a component of
89 immune response), will differ among surviving species (Bernal et al., 2020). However, the
90 regulation of molecular mechanisms is energetically costly and, consequently, increases in
91 thermal tolerance come with the requirement of balancing responses with energy reserves and
92 enhancement in energy intake and assimilation following the heat stress (Giomi et al., 2016;
93 Leung et al., 2019).

94

95 ~~The capacity of an organism to physiologically adjust in response to fluctuating environmental~~
96 temperatures depends largely on their natural realised thermal windows, and whether they are
97 able to both display thermally compensated respiratory functions, as well as extend their lethal
98 temperature thresholds (Sandblom et al., 2016). Depending on the duration and magnitude of
99 a heatwave, the thermotolerance strategy employed by a species, the associated energetic costs,
100 and the ability of different species to regulate metabolism and energy assimilation after a MHW
101 will lead to different physiological consequences during and post-MHW. It is generally
102 accepted that sensitivity of an organism to environmental change and capacity for thermal
103 acclimation is determined by environmental selection pressure, which is often correlated with
104 latitude although a wider suite of selective pressures may concurrently be involved (Jupe et al.,
105 2020). Generally, species living in more stable tropical environments have greater capacity for
106 chronic thermal acclimation when the rate of onset is slower, whereas those which live in more
107 thermally variable environments are more equipped to deal with acute temperature change
108 through physiological plasticity given the range experienced in their natural environment
109 (Seebacher et al., 2015). Indeed, predicting the different responses of more tropical and
110 temperate-affiliated species in areas in which their ranges overlap is complex, due to variety in
111 thermotolerance mechanisms utilised by functionally similar species (Goldenberg et al., 2018).

Deleted: Given adequate time to acclimate, organisms subjected to high temperatures can be expected to achieve higher lethal temperature limits, but physiological trade-offs or adaptive metabolic depression may also impact other associated physiological processes (Harianto et al., 2018).

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117 An understanding of these responses is of increasing importance for predicting how warming
118 seas will drive species range shifts and novel biological communities (Donelson et al., 2019;
119 Sanford et al., 2019; Wernberg et al., 2016).

120

121 Here, we investigated the effect of different magnitudes of realistic MHW scenarios (moderate
122 and extreme; Hobday et al., 2018) on the metabolism and survival of two subtidal gastropods,
123 *Trochus sacellum* and *Astraliium haematragum*, which co-occur in a tropical-temperate
124 transition zone. *T. sacellum* is widely distributed in tropical and temperate waters between
125 Korea and the Philippines (Batomalaque et al., 2010; Noseworthy and Kwang-Sik, 2010) while
126 *A. haematragum* is distributed mainly around the cooler waters of Japan (Meyer et al., 2005)
127 and Korea (Noseworthy and Kwang-Sik, 2010), reaching its southern trailing edge near Hong
128 Kong. Both *T. sacellum* and *A. haematragum* are common subtidal grazers in Hong Kong,
129 where they are exposed to substantial seasonal fluctuations in water temperature. Here, we
130 investigate the capacity of these species with different geographic distributions, and therefore
131 evolutionary thermal histories, to regulate their metabolism during heatwave scenarios. We
132 then examine possible interspecific differences in the capacity to regulate metabolism post
133 MHW. We hypothesised that the magnitude of MHW will drive the metabolic rate and survival
134 of gastropods whereby the more tropical species (*T. sacellum*) will demonstrate greater survival
135 and ability to regulate metabolism under moderate heatwave conditions because of its
136 evolutionary history under warmer temperatures, but both species will have elevated mortality
137 under extreme heatwave conditions (i.e. inability to acclimate). Secondly, we hypothesised that
138 the capacity of gastropods to regulate their aerobic metabolism immediately following
139 heatwaves will be impaired because of the heatwave induced physiological damage. We predict
140 this reduced function will be more prominent in the more tropical species (*T. sacellum*) because

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141 while tropical species are thought to live closer to their upper thermal limits, the stable
142 conditions mean they have a smaller thermal safety margin and are more likely to endure high
143 physiological damage under subsequent stress, possibly leading to death. The physiological
144 state of these organisms post-MHW will have large repercussions for their ability to maintain
145 grazing, their key functional role in the ecosystem. Therefore, unravelling the consequences of
146 MHWs on their physiological state is key to understanding their ability for enduring
147 environmental stress, to recover, and to maintain their ecological function post MHW.

148

149 **2. Methods**

150 **2.1 Organism collection**

151 Individuals of *Trochus sacellum* and *Astrarium haematragum* were collected from the subtidal
152 waters of Bluff island, Hong Kong (22°19'18.0" N 114°21'18.2" E), by SCUBA diving, in
153 summer so that they were seasonally acclimated to warm summer conditions. Organisms were
154 brought to the laboratory and allowed to acclimate to laboratory conditions for a period of 24
155 hours at 28°C (ambient water temperature during collection) in aquaria fitted with an open
156 flow-through system providing clean filtered seawater to acclimate to laboratory conditions.

157

158 **2.2 Experiment 1: MHW simulation**

159 To start the heatwave treatment, replicate aquaria (12 L) were maintained at 28°C (ambient
160 control), or gradually increased to 31°C (moderate heatwave) or 34°C (extreme heatwave)
161 (n = 3 aquaria per treatment, n = 6 individuals per aquarium) over 24 hours using flow-through
162 systems to pump pre-heated water into experimental aquaria. These temperatures were chosen
163 to elucidate current summer conditions (control: Fig. 1) and predicted future heatwave
164 conditions under climate change. Temperatures were then maintained for seven days using

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165 flow-through heater/chiller units (Hailea, Model HC.2200BH) and water temperature was
166 monitored twice daily. Daily checks were made to record any mortality and dead individuals
167 were removed and not replaced. After the 7-day heatwave treatments, metabolic rates of
168 organisms were measured at treatment conditions (see “Metabolic rate” below) before
169 temperatures were gradually reduced back to 28°C over 24 hours. Following the 24-hour
170 recovery, all individuals were used in thermal ramps (see below). Gastropods were fed *ad*
171 *libitum* with filamentous turf-algae naturally growing on rocks collected from field for the
172 duration of the experiment.

173

174 **2.3 Metabolic rate**

175 Metabolic rates of individuals were measured both prior to exposure to (i.e. all at 28 °C) and
176 after the seven days of heatwave exposure (i.e., at 28°C, 31°C, or 34°C). Metabolism was
177 measured as change in oxygen concentration in sealed air-tight glass respirometry chambers
178 containing an oxygen sensor spot (PreSens SP-PSi3-YAU). Chambers were placed into a large
179 water bath at experimental temperature to maintain temperature constant throughout
180 measurements, with a magnetic stirrer in each chamber used to ensure water was thoroughly
181 mixed throughout measurements. One individual organism was placed into each chamber
182 which was sealed under water ensuring that no air bubbles were present. Organisms were
183 allowed to rest for five minutes, after which oxygen concentrations were measured every five
184 minutes for 30 minutes using a fibre optic meter (Firebox 4 Trace). For each temperature
185 treatment, one blank chamber with no gastropod was used to measure change in oxygen
186 concentration and to take into account any biological activity in the water. Ten individuals per
187 temperature treatment were selected randomly for the respiration measurements (n = 10). Final

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188 metabolic rate was calculated as the rate of oxygen concentration depletion in seawater as a
189 function of body weight and time ($\text{mgO}_2 \text{ L}^{-1} \text{ gfw}^{-1} \text{ h}^{-1}$).

190

191 **2.4 Experiment 2: Analysis of post MWH physiology through thermal ramps**

192 Following the 24-hour recovery after heatwave exposure, metabolic rates of six individuals
193 from each exposure type (control or heatwave) were measured ($n = 6$) on a thermal ramp
194 following the same procedure as for metabolic rate measurements. Prior to commencement of
195 the thermal ramp, individuals were housed in aquaria in which the temperature had been
196 reduced to 16°C over 24 hours. Thermal ramps had an initial temperature of 16°C (with a
197 ramping rate of 2°C every two hours until mortality, with metabolic rate measured at every 2°C
198 interval. Thermal ramps started at 16 °C because it represents the winter temperature
199 experienced by the organisms and was used to cover their full natural temperature range (Fig.
200 1). Mortality was used as the end point to assess whether acclimation to experimental
201 treatments allowed individuals to increase the temperature of mortality (upper lethal limit:
202 ULL). Prior to measuring oxygen consumption, the organisms were allowed to acclimate at
203 each temperature for one hour, after which the respirometry chambers were sealed and oxygen
204 concentration in the chambers were measured every five minutes for 20 minutes. Water
205 temperature was regulated using heater/chillers (Hailea, Model HC.2200BH).

206

207 **2.5 Data analysis**

208 To test for the effect of heatwaves (control vs. moderate heatwave) on respiration a one-way
209 permutational analysis of variance (PERMANOVA) was done using Euclidean distance
210 ($n = 10$) using Primer 7 + PERMANOVA. Exponentially Modified Gaussian Function (EMG)
211 models were fitted for temperature ramp data as per Angilletta (2006) to extract the temperature

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212 at maximum metabolic rate (T_{MMR}), upper lethal limit (ULL), and to model the change in
213 metabolic rate over the temperature ramp for both species pre- and post-heatwave exposure.
214 An EMG was performed for the thermal ramp dataset from each group independently, based
215 the mean and variances within each dataset. EMGs were modelled using RStudio Version
216 1.1.463.

217

218 **3. Results**

219 **3.1 Mortality**

220 Mortality of organisms exposed to the extreme heatwave (34°C) was higher than those from
221 the moderate heatwave (31°C; Fig. 2). Exposure to extreme heatwave conditions caused 100
222 % mortality after three days for *T. sacellum* and five days for *A. haematragum*. Moderate
223 heatwave conditions (31°C), caused the same level of mortality for both species, but mortality
224 was delayed for *A. haematragum*; 17 % of *T. sacellum* individuals died after four days followed
225 by no further deaths while 17 % of *A. haematragum* died after six of exposure. There was no
226 mortality at current summer temperatures (control; 28°C).

227

228 **3.2 Metabolic rates**

229 As extreme heatwave conditions caused 100 % mortality, metabolic rates were only obtained
230 for organisms exposed to moderate heatwave and control summer conditions. Contrary to
231 predictions, elevated temperature (31°C) did not cause a rise in metabolic rate of the individuals
232 which survived the moderate heatwave for both *T. sacellum* and *A. haematragum* (Fig. 3, all
233 $p > 0.1$).

234

235 **3.3 Thermal ramps**

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236 Exposure to moderate heatwave conditions caused a change in metabolic rates across thermal
237 ramps for both *T. sacellum* and *A. haematragum* compared to individuals kept at control
238 summer temperatures. Metabolism of *T. sacellum* exposed to control conditions (28°C)
239 remained low between 16°C to 28°C ($Q_{10} = 1.73$), then increased four-fold between 28°C and
240 34°C ($Q_{10} = 14.39$; Fig. 4a) before rapidly declining. In contrast, individuals previously
241 exposed to moderate heatwave conditions (31°C) displayed nonadaptive metabolic depression
242 (*sensu* Verberk et al., 2016) across the temperature ramp and did not have an increase in
243 metabolic rates at higher temperatures; metabolism remained low between 16°C to 28°C
244 ($Q_{10} = 1.77$), then decreased between 28°C and 34°C ($Q_{10} = 0.02$) (Fig. 4a). Metabolic rates of
245 *A. haematragum* followed a similar trend (16°C to 28°C: $Q_{10} = 1.3$ and 0.94 for control and
246 moderate heatwave, respectively; 28°C to 34°C: $Q_{10} = 21.06$ and 0.08 for control and moderate
247 heatwave, respectively). The T_{MMR} for gastropods exposed to control conditions were 32.6°C
248 and 35.5°C for *T. sacellum* and *A. haematragum*, respectively. Exposure to moderate heatwave
249 conditions changed the overall metabolic response to temperature across the thermal ramp,
250 reducing the metabolic rates of both species at temperatures above 30°C suggesting
251 nonadaptive metabolic depression (Fig. 4, Table 1). The T_{MMR} of both the temperate (*A.*
252 *haematragum*, -9.8°C) and the more tropical species (*T. sacellum*, -4.0°C) (Table 2) were also
253 reduced following exposure to the moderate heatwave, further suggesting nonadaptive
254 metabolic depression and stress. There was no change in the ULL of either species under the
255 different treatments.

256

257 **4. Discussion**

258 The impact of heatwaves on the physiology and survival of organisms is generally thought to
259 be dependent on their duration and magnitude, and, just as importantly, on the ability of the

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260 species to cope with thermal stress (Frölicher and Laufkötter 2018). Physiologically coping
261 with thermal stress is, in turn, dependent on factors including the ability to maintain
262 homeostasis of metabolic systems (Sokolova et al., 2012; Giomi et al., 2016; Leung et al.,
263 2019). Yet, the efficacy of coping mechanisms vary among even functionally similar species
264 within the same habitat (Bernal et al., 2020). Here, we show that the magnitude of MHW indeed
265 influenced the survival of two subtidal marine species with different distributions and
266 evolutionary histories, whereby neither species were able to survive under extreme heatwave
267 conditions (+ 6°C above summer temperatures) but only experienced 17 % mortality under the
268 moderate heatwave scenario (+ 3°C above summer temperatures). There was, however, a
269 difference in the onset of mortality between species with the more tropical *T. sacellum*
270 succumbing to thermal stress more rapidly in both extreme and moderate heatwave scenarios
271 than the more temperate *A. haematragum*. Theory suggests that thermal tolerance is linked to
272 species geographical distribution (Bozinovic et al., 2011; Seebacher et al., 2014), where more
273 tropical-affinity species experience more stable environments and have narrow thermal
274 windows, while temperate species possess the ability to maintain broader thermal windows
275 because they are adapted to greater environmental variation (Stillman and Somero, 2000). We
276 found that our tropical species experienced maximum metabolic rate at a lower temperature
277 (T_{MMR}) than the temperate species, which when surpassed, drove the more rapid onset of
278 mortality. Indeed, tropical species are generally thought to live close to their upper thermal
279 limits and have a reduced thermal safety margin (Bozinovic et al., 2011). Correspondingly, the
280 consistently higher mortality rate of *T. sacellum* observed across both heatwave magnitudes
281 suggests that it possesses lower capability to withstand high temperatures compared to *A.*
282 *haematragum*.

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284 Contrary to what is expected under metabolic theory, the metabolic rates of *T. sacellum* and
285 *A. haematragum* exposed to moderate MHW were not different to metabolic rates under
286 normal summer temperatures. While this could be interpreted as both species acclimating to
287 moderate MHW conditions (*sensu* Seebacher et al. 2015), metabolic acclimation is unlikely
288 over the 7-day heatwave. In contrast, the post-MHW thermal ramps revealed that exposure to
289 MHWs impeded the capacity of both species to maintain aerobic metabolism at higher
290 temperatures (i.e. nonadaptive metabolic depression), likely driven by thermal stress (Verberk
291 et al., 2016). The inability of both species to increase aerobic metabolism at temperatures above
292 30°C in line with individuals from the natural summer conditions (28°C), suggests that both
293 species were undergoing nonadaptive metabolic depression to reduce energetic costs (Jacobson
294 et al., 2016). Comparable effects have also been observed in the gastropod *Thalotia conica*
295 (Leung et al., 2017) and crustaceans *Rhynchocinetes durbanensis* and *Calcinus laevimanus*
296 (Madeira et al, 2018b) where although organisms survived thermal stress, they were unable to
297 maintain higher respiration at elevated temperatures and their physiological state was severely
298 impacted. Therefore, although *T. sacellum* and *A. haematragum* were able to survive the
299 moderate MHW over the shorter term, the physiological costs likely rendered them unable to
300 sustain additional thermal stress because of the increased energetic requirements.

301
302 The observed nonadaptive metabolic depression post-MHW likely results from one of two
303 possible mechanisms. Firstly, the immediate response of thermal stress involves the disruption
304 of systemic oxygen balance (Pörtner, 2001; Pörtner and Knust, 2007; Kassahn et al., 2009),
305 necessitating increased mitochondrial capacity, or activation of a complementary system to
306 restore normal function (Bernal et al., 2020; Kassahn et al., 2009; Sokolova et al, 2012). These
307 compensatory mechanisms increase energy demands which may lead to severe depletion in

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308 energy reserves (Ganser et al, 2015; Leung et al., 2017; Madeira et al, 2018a). Consequently,
309 following a MHW, surviving individuals may be physiologically impaired, including depleted
310 energy reserves and reduced cellular function (e.g. Madeira et al., 2018b). Such organisms
311 would have a drastically reduced capacity to regulate metabolic rates under further stress.
312 Secondly, at the edge of thermal tolerance, compensatory mechanisms may be insufficient to
313 maintain aerobic function and a switch to anaerobic function, reducing availability and supply
314 of cellular energy (Kassahn et al., 2009). Such metabolic switches cause cellular damage,
315 impaired mitochondrial function, and reduced oxygen delivery capacity (Dimitriadis et al.,
316 2012; Leung et al., 2017). While this strategy can limit metabolic costs and enhance short-term
317 survival, it is a maladaptive strategy and reduces the ability of organisms to maintain adequate
318 metabolic function under further stress, until partially or fully recovered.

319

320 We hypothesised that the capacity of the more tropical species (*T. sacellum*) to tolerate
321 heatwaves would be less than the temperate species given the history of stable conditions rather
322 than acute temperature change. A recent meta-analysis of the upper temperature limits of 34
323 tropical ectotherms found that under rapid warming the upper lethal temperature range for
324 subtidal organisms (more thermally stable, similar to *T. sacellum*) was between 37-41°C but
325 between 41-52°C for intertidal organisms (more thermally variable, similar to *A.*
326 *haematragum*) (Nguyen et al, 2011). As *T. sacellum* has a more tropical affinity, and therefore
327 evolved under more stable environmental conditions in its realised niche, it may have
328 physiological processes acclimated to tolerate the more stable tropical subtidal conditions and
329 hence experienced high mortality in response to rapid thermal stress. While less than 20% of
330 individuals died under the moderate heatwave scenario, longer, or repeated heatwaves could
331 reduce subsequent generations to the point where shifts in invertebrate assemblages occur

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332 (Smale et al, 2017), weakening or eliminating their ability to perform their ecological functions
333 and cascading to more drastic community- or ecosystem-wide changes (Brodeur et al., 2019;
334 Smale et al, 2017; Wernberg et al, 2013).

335 Even subtle environmental changes can cause vast shifts within communities, due to
336 intraspecific variation within different populations of a given species (Harley et al., 2017;
337 Wang et al., 2018). In our study, those individuals which did survive the moderate MHW (of
338 both species) were rendered incapable of regulating their metabolic rates at high temperatures.
339 Whilst their resting metabolic rate did not change, the maximum temperature threshold limits
340 showed little flexibility to increase (indeed, declined markedly), which ultimately reduced
341 survivability and resulted in a less successful thermal response strategy (Armstrong et al., 2019).
342 These species, therefore, demonstrate resilience rather than resistance to moderate MHWs
343 (Leung et al., 2019), meaning that they rely on recovering from thermal stress rather than
344 coping with it and maintaining function. Under such circumstances, the required recovery
345 period post MHW can play a crucial role in the future survival of both species, which would
346 also be dependent on the type of physiological damage incurred during the MHW. The inability
347 to physiologically resist acute heating events has caused extensive range contractions of
348 temperate species, even through singular acute thermal events. For example, the 2015/2016
349 heatwave in southeast Australia saw sea surface temperatures +3-4°C above climatological
350 averages, and observations of numerous fish and invertebrate species were found out of range
351 in Tasman waters (Oliver et al., 2017; Caputi., 2014). This heatwave caused a rapid climate
352 driven regime shift, losing the temperate defining kelp forests which were replaced by tropical
353 and subtropical associated species, a community wide tropicalisation event (Wernberg et al.,
354 2016). Therefore, the period of exposure to thermal stress, sustained physiological damage and
355 recovery period, and periodicity of recurring MHWs can determine the overlying impact on

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356 populations and the wider community (Arias-Ortiz et al., 2018; Madeira et al., 2018b). With
357 the number of MHW days expected to continue increasing over the coming decades (Oliver et
358 al., 2018), and with differences in the adaptive phenotypic plasticity of species with
359 overlapping distributions (Cavole et al., 2016; Watson, 2018), the success in fully recovering
360 from MHW exposure lies at the core of the ability of species to survive, recover, and resume
361 their ecological functions post-MHW.

362

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511 **Table 1.** The results of repeated measures PERMANOVAs testing for the effect of exposure
 512 to the moderate heatwave (Exposure) on metabolic rates at different temperatures
 513 (Temperature) across thermal ramps for both *Trochus sacellum* and *Astraliium haematragum*.
 514 Significant p-values are in **bold**.

515

	Factor	Df	F	p-value
<i>Trochus sacellum</i> Respiration rate	Exposure	1	3.2839	0.085
	Temperature	11	12.948	0.001
	Individual (Exposure)	10	3.8986	0.001
	Exposure x Temperature	11	14.046	0.001
<i>Astraliium haematragum</i> Respiration rate	Exposure	1	5.8452	0.03
	Temperature	11	4.5485	0.003
	Individual (Exposure)	9	2.2956	0.021
	Exposure x Temperature	11	6.0839	0.001

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517 **Table 2.** Temperature at maximum metabolic rate (T_{MMR}) as described by an Exponentially
518 Modified Gaussian Function (EMG) model for the more tropical *Trochus sacellum* and more
519 temperate *Astralium haematragum* after exposure to either summer water (28°C) or moderate
520 heatwave conditions (31°C). The difference in T_{MMR} in the heatwave compared to the control
521 treatment predicted by the EMG model are also shown.

522

		T_{MMR} (°C)	<i>Change</i>
<i>Trochus sacellum</i>	28 °C	32.6	/
	31 °C	28.6	-4.0
<i>Astralium haematragum</i>	28 °C	35.5	/
	31 °C	25.7	-9.8

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523 **Figure 1:** Mean monthly sea water temperature at Bluff Island (Mooring PM11) in Port
524 Shelter bay, Hong Kong from January 2015 to November 2018. Data downloaded from the
525 Environmental Protection Department, Hong Kong
526 ([https://www.epd.gov.hk/epd/english/environmentinhk/water/hkwqrc/waterquality/marine.ht](https://www.epd.gov.hk/epd/english/environmentinhk/water/hkwqrc/waterquality/marine.html)
527 [ml.](https://www.epd.gov.hk/epd/english/environmentinhk/water/hkwqrc/waterquality/marine.html)).

528 **Figure 2:** Cumulative mortality (%) of *Trochus sacellum* and *Astraliium haematragum*
529 exposed to heatwave of 31°C and 34°C. No mortality was recorded in the control treatment
530 (28°C).

531 **Figure 3:** Metabolic rate of *Trochus sacellum* and *Astraliium haematragum* at summer water
532 temperatures (control; 28°C) and following 7 days exposure to either control (28 °C) or
533 moderate heatwave conditions (31°C). Error bars: ± SE (n = 10).

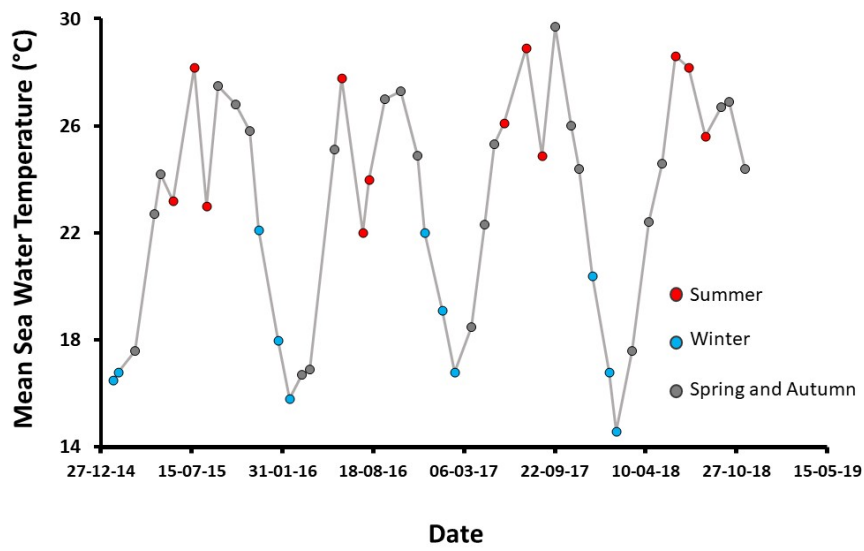
534 **Figure 4:** Respiration rates of (a) *Trochus sacellum* and (b) *Astraliium haematragum*
535 previously exposed to either control summer (28 °C) or moderate heatwave (31°C) across a
536 thermal ramp. Error bars ± SE (n = 6).

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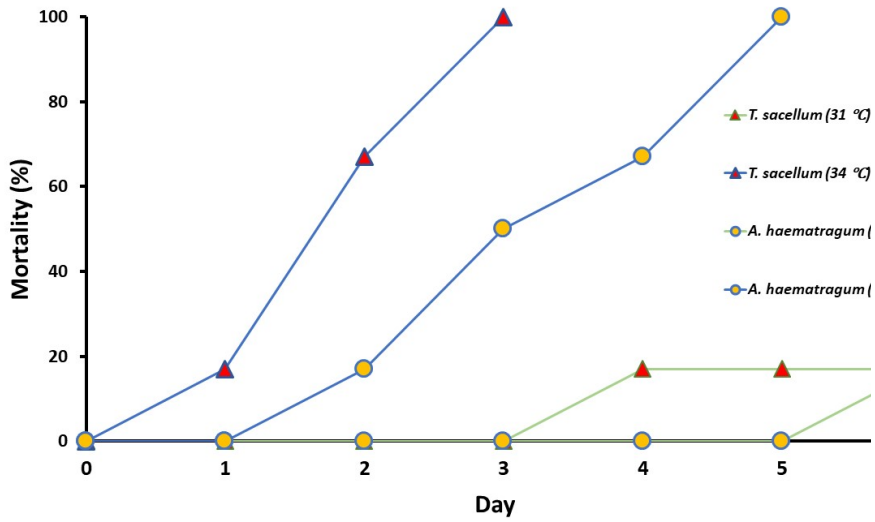
Figure 1



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560 Figure 2
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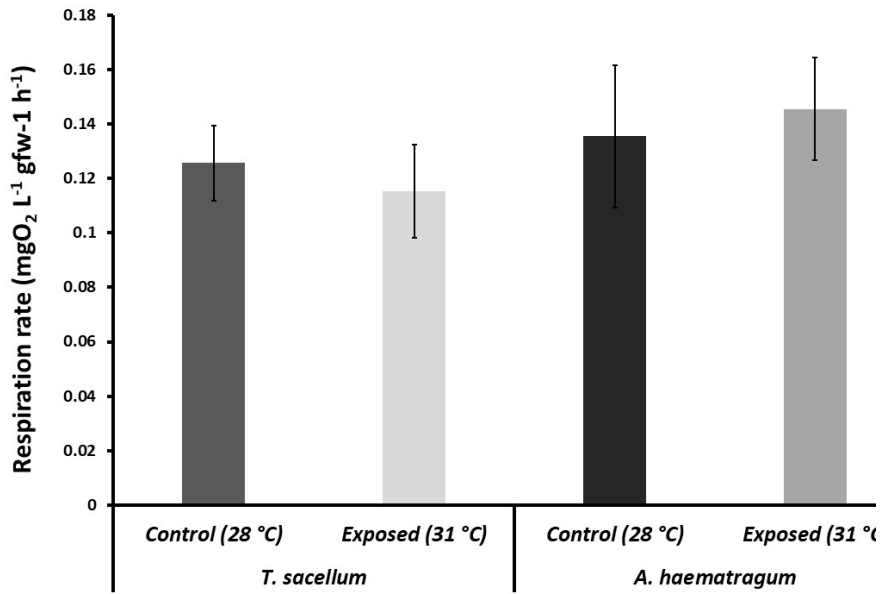
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568 Figure 3

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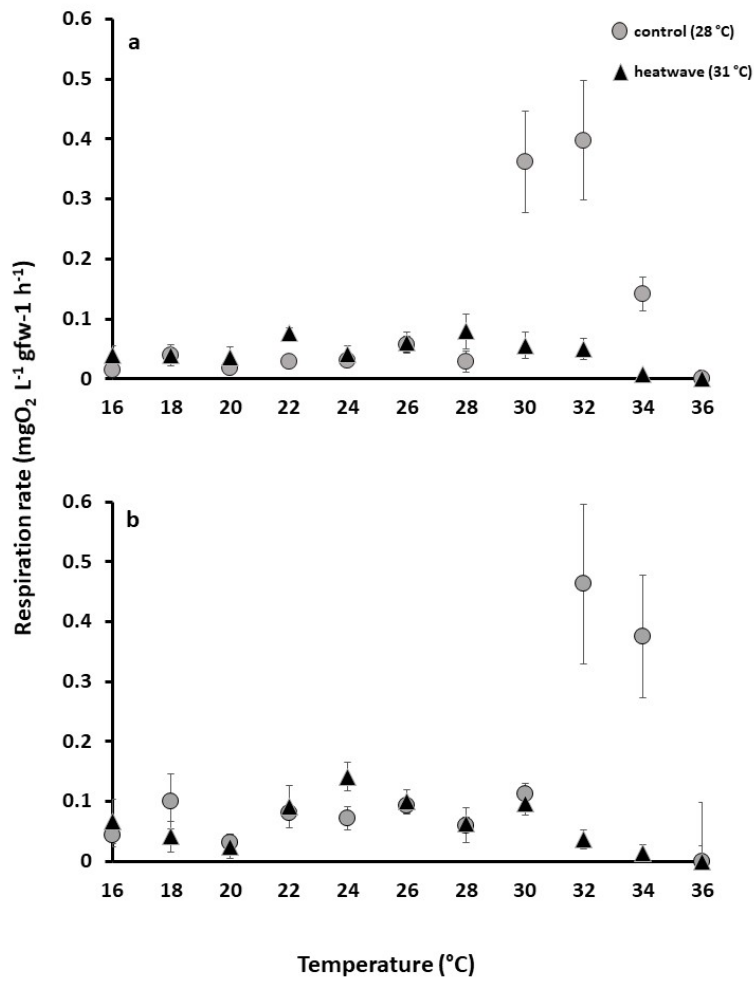
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575 Figure 4



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