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1 **Title:** Adapt, move, or die – how will tropical coral reef fishes cope with ocean warming?
2

3 **Running head:** Thermal preference of coral reef fishes

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21 **Key words:** acclimation, aerobic scope, behavioural thermoregulation, critical thermal limits,
22 global warming, temperature preference

23

24

25 **Abstract**

26 Previous studies hailed thermal tolerance and the capacity for organisms to acclimate and
27 adapt as the primary pathways for species survival under climate change. Here we challenge
28 this theory. Over the past decade more than 365 tropical stenothermal fish species have been
29 documented moving pole-ward, away from ocean warming hotspots where temperatures 2-3
30 °C above long-term annual means can compromise critical physiological processes. We
31 examined the capacity of a model species - a thermally-sensitive coral reef fish, *Chromis*
32 *viridis* (Pomacentridae) – to use preference behaviour to regulate its body temperature.
33 Movement could potentially circumvent the physiological stress response associated with
34 elevated temperatures and may be a strategy relied upon before genetic adaptation can be
35 effectuated. Individuals were maintained at one of six temperatures (23, 25, 27, 29, 31 and 33
36 °C) for at least six weeks. We compared the relative importance of acclimation temperature to
37 changes in upper critical thermal limits, aerobic metabolic scope, and thermal preference.
38 While acclimation temperature positively affected the upper critical thermal limit, neither
39 aerobic metabolic scope nor thermal preference exhibited such plasticity. Importantly, when
40 given the choice to stay in a habitat reflecting their acclimation temperatures or relocate, fish
41 acclimated to end-of-century predicted temperatures (i.e., 31 or 33 °C) preferentially sought
42 out cooler temperatures, those equivalent to long-term summer averages in their natural
43 habitats (~29 °C). This was also the temperature providing the greatest aerobic metabolic
44 scope and body condition across all treatments. Consequently, acclimation can confer
45 plasticity in some performance traits, but may be an unreliable indicator of the ultimate
46 survival and distribution of mobile stenothermal species under global warming. Conversely,
47 thermal preference can arise long before, and remain long after, the harmful effects of
48 elevated ocean temperatures take hold and may be the primary driver of the escalating pole-
49 ward migration of species.

50 **Introduction**

51 Over the evolutionary history of vertebrates, adaptation to thermal changes has occurred
52 at a rate of approximately 1 °C per million years (Quintero & Weins, 2013). As current ocean
53 temperatures and rates of warming exceed those over the past 420,000 years (Hoegh-
54 Guldberg *et al.*, 2007; Collins *et al.*, 2013), the fundamental biogeographical principle of
55 “adapt, move, or die” has perhaps never been more relevant. Global climate change is not
56 only predicted to increase average sea surface temperatures (SSTs) by 2.0-4.8 °C by the end
57 of the 21st century but also increase the intensity and frequency of transient thermal
58 fluctuations (Ficke *et al.*, 2007; Ganachaud *et al.*, 2011; Wernberg *et al.*, 2012; Collins *et al.*,
59 2013; IPCC 2013). Organisms living at extreme latitudes (i.e., near the equator or poles) are
60 expected to be particularly sensitive to predicted thermal changes because these species are
61 thought to have evolved within narrow and stable temperature ranges (Huey & Kingsolver,
62 1993; Tewksbury *et al.*, 2008; Gardiner *et al.*, 2010; Lough, 2012). Consequently, these
63 species may also be less capable of acclimating and/or adapting to contemporary temperature
64 changes, particularly over the short time scales predicted with global warming (Pörtner, 2002;
65 Stillman, 2003; Somero, 2010; Neuheimer *et al.*, 2011; Nguyen *et al.*, 2011; Rummer *et al.*,
66 2014), highlighting a serious threat to long-term fitness and survival of countless species
67 unless relocation to more favourable thermal habitats is possible.

68 Reversible thermal acclimation, which occurs over days to months, usually in response
69 to daily or seasonal changes, can allow organisms to cope with changes in temperatures by
70 enhancing important performance traits (Kinne, 1962; Angilletta *et al.*, 2006; Sandblom *et al.*,
71 2014). Most species examined appear to adopt this strategy. For example, the Columbia River
72 redband trout (*Oncorhynchus mykiss gairdneri*) significantly reduces its heat shock response
73 to repeated thermal stress following six weeks of acclimation (Narum *et al.*, 2013). However,
74 while acclimation is recognized as a primary driving force for adaptive phenotypic changes

75 (Gienapp *et al.*, 2008; Teplitsky *et al.*, 2008; Hoffmann & Sgró, 2011; Culumber & Monks,
76 2014), acclimation may come at a cost, i.e. may involve detrimental energetic trade-offs
77 (Angilletta, 2009; Donelson *et al.*, 2011, 2014) or not happen fast enough to keep pace with
78 the rate of environmental changes (Angilletta, 2009; Chown *et al.*, 2010; Quintero & Weins
79 2013; Sandblom *et al.*, 2014).

80 Capacity for acclimation may be extremely limited for stenothermal tropical coral reef
81 fishes; studies have already shown that temperatures just 2-3 °C above annual summer
82 maxima can compromise a wide range of life-history traits including swimming, growth,
83 activity, and reproduction (Munday *et al.*, 2008; Johansen & Jones, 2011; Donelson *et al.*,
84 2012b; Zarco-Perello *et al.*, 2012; Johansen *et al.*, 2013, 2015; Rummer *et al.*, 2014). These
85 important traits are supported by the animal's aerobic metabolic scope (AMS), which is
86 essentially the capacity to direct energy toward critical tasks beyond that required for basic
87 maintenance. In principle, AMS is defined as the difference in oxygen consumption rates
88 ($\dot{M}O_2$) between resting and maximal performance (Fry & Hart, 1948; Pörtner & Farrell, 2008)
89 and is thought to follow a right skewed, bell-shaped curve with temperature (Pörtner & Knust,
90 2007). A stenothermal species will typically have the greatest AMS at a particular
91 temperature (T_{opt}), beyond which AMS declines, perhaps due to cardiorespiratory limitations
92 that reduce O_2 supply to the tissues (Pörtner & Knust, 2007). Transient heating events – for
93 example, those associated with El Niño – can elevate temperatures beyond the thermal
94 tolerance of a species and consequently reduce AMS to near zero, causing imminent death as
95 fish lose the ability to maintain bodily functions (Beitinger *et al.*, 2000; Ospína & Mora,
96 2004; Pörtner & Knust, 2007).

97 While an overall loss of AMS is detrimental to a species (Pörtner, 2001, 2002; Pörtner
98 & Knust, 2007), some physiological processes are likely to be more thermally sensitive than
99 others and may consequently be impacted at temperatures closer to optimum than those

100 tolerated from a whole-animal perspective (see Steinhausen *et al.*, 2008; Casselman *et al.*,
101 2012; Iftikar & Hickey, 2013; Iftikar *et al.*, 2014). This phenomenon is sometimes interpreted
102 as the “multiple performances – multiple optima” theorem (see, e.g., Clark *et al.*, 2013). For
103 instance, the common coral reef damselfish (*Acanthochromis polyacanthus*) exhibits a
104 reduction in aerobic scope when reared at 1.5 °C above their summer average temperatures,
105 but still maintains reproductive output at this temperature, suggesting a mismatch between the
106 thermal optimum for metabolic enzymes and reproductive hormones (Donelson *et al.*, 2014).
107 Thus, many populations may be sensitive to relatively small increases in SSTs at some aspect
108 of their performance, which can jeopardize biological fitness and population sustainability
109 (Pörtner & Farrell, 2008; Pörtner & Peck, 2010).

110 Numerous studies have examined the short- and long-term physiological impacts of
111 elevated SSTs on tropical coral reef fishes, but many species – if not most – are also capable
112 of behavioural thermoregulation (Casterlin & Reynolds, 1980; Reynolds & Casterlin, 1981;
113 Angilletta *et al.*, 2006). An organism can behaviourally thermoregulate by moving out of a
114 challenging thermal habitat (e.g., across a thermocline) and into a different thermal habitat
115 where temperatures are more optimal (T_{opt}) (Reynolds & Casterlin 1979; Johnson & Kelsch,
116 1998; Khan & Herbert, 2012). Given that AMS and the associated capacity for movement,
117 swimming, etc. are maximized at a specific range of body temperatures, individuals that seek
118 out habitats where this body temperature can be maintained will, in theory, maximize fitness
119 (Kelsch & Neill, 1990; Johnson & Kelsch, 1998; Angilletta *et al.*, 2002).

120 Physiological thermal sensitivity and thermoregulatory behaviour appear to be co-
121 adapted (Huey & Bennett, 1987; Angilletta *et al.*, 2002, 2006) as the thermal history that
122 defines a species' T_{opt} often determines its preferred temperature range (Kelsch & Neil, 1990;
123 Johnson & Kelsch, 1998). Therefore, in nature, most species are likely to preferentially
124 pursue temperatures that coincide with their T_{opt} during a given life stage (Brett, 1971;

125 Beitinger & Fitzpatrick, 1979; Jobling, 1981; Pörtner & Knust, 2007; Pörtner & Farrell, 2008;
126 Payne *et al.*, 2016). Critically, for behavioural thermoregulation to help mitigate the effects of
127 rapid climate change, evolutionary changes in T_{opt} should also provide a strong selective
128 pressure for changes in T_{pref} (Kelsch & Neill, 1990; Angilletta *et al.*, 2002). This inherent
129 relationship between T_{opt} and T_{pref} has not previously been examined in thermally-sensitive
130 species such as coral reef fishes, and it remains uncertain whether phenotypic shifts in
131 temperature sensitivity of aerobic performance (e.g., see Donelson *et al.*, 2011, 2012a) also
132 lead to changes in T_{pref} .

133 It is critically important to understand the relative roles of acclimation and behavioural
134 thermoregulation in order to predict how warming ocean temperatures will ultimately impact
135 fitness and distribution of individual species. As oceans warm, cooler conditions more closely
136 aligned with a species T_{opt} may be found in deeper aquatic habitats or at higher latitudes,
137 suggesting that species distribution ranges will shift accordingly (Perry *et al.*, 2005; Booth *et*
138 *al.*, 2007; Figueira & Booth, 2010; Wu *et al.*, 2012; Jones *et al.*, 2014). Indeed, more than 365
139 tropical reef fish species have already been identified to be expanding their latitudinal ranges
140 pole-ward at a rate of up to 26 km per decade (Figueira & Booth, 2010; Feary *et al.*, 2013;
141 Nakamura *et al.*, 2013). However, for the numerous other coral reef species that are either
142 partly or completely reliant on coral reefs for survival, higher latitudes may not provide
143 suitable habitats. Such species will have to follow the distribution changes of coral and other
144 benthic flora and fauna that are less mobile than fish species and expected to relocate at a
145 slower pace, if at all. The survival of such habitat-dependent species may consequently be
146 predicted by their capacity to acclimate their T_{opt} and T_{pref} to projected temperatures.

147 Focussing on a model species – a thermally-sensitive coral reef fish, *Chromis viridis*
148 (Pomacentridae) – we examined the coordinated adjustments of T_{opt} and T_{pref} to elevated
149 temperatures expected within generational timescales and the propensity of individuals to

150 remain or evade elevated temperatures. We tested three hypotheses: (i) the preferred
151 temperature (T_{pref}) of a tropical stenothermal coral reef fish will coincide with their optimal
152 temperature (T_{opt}) for aerobic performance (specifically aerobic scope); (ii) prolonged thermal
153 acclimation will improve some, but not all aspects of metabolic performance at elevated
154 temperatures; and (iii) the thermal preference of this stenothermal coral reef fish will be fixed
155 and a stronger behavioural driver than phenotypic acclimation, causing individuals to move
156 toward established optimal temperatures. These hypotheses are thus focusing on short
157 term/localized movements, yet the results could provide explanations for whole animal
158 responses, including range shifts.

159

160 Materials and methods

161 *Study species, collection, and holding conditions*

162 The model species – a thermally sensitive coral reef fish, the blue-green damselfish,
163 *Chromis viridis* – is a representative of one of the most species-rich families (Pomacentridae,
164 >360 spp.) of coral reef fishes and are known to occupy an array of habitats in both tropical
165 and subtropical environments around the world. This species is highly abundant and widely
166 distributed on coral reefs (~27°N to 27°S) at depths of 12m or shallower (Randall *et al.*,
167 1997). This species is highly site-attached, and at all post-larval life stages, is closely
168 associated with corals (typically *Acropora* spp.) that provide shelter from predators and a
169 nocturnal retreat (Fishelson *et al.*, 1974).

170 In January 2014, a total of 72 individuals exhibiting adult coloration and of equal body
171 mass ($4.63 \pm 1.23\text{g}$; mean \pm SD), were collected using monofilament barrier nets from reef
172 crest sites around Lizard Island (14° 40' 08"S, 145° 27' 34"E) in the northern part of the
173 Great Barrier Reef, Australia under Marine Parks Permit #G10/33239.1. The annual sea

174 surface temperatures (SST) of this area range from 23.5 to 30.0 °C (data averaged monthly
175 since 1982, summarized in Rummer *et al.*, 2014 and Johansen *et al.*, 2015).

176 All individuals were transported to the Marine Aquaculture Research Facilities Unit
177 (MARFU) at James Cook University (JCU) in Townsville, Queensland, Australia and
178 randomly distributed between six 100 L cylindrical tanks (65 x 40 cm, height x diameter) with
179 12 individuals per tank. Tanks were maintained under a 12:12 light:dark photoperiod and
180 continuously supplied with recirculated, filtered, aerated, and UV-sterilized sea water (34 ppt,
181 28 °C). Fish were fed to satiation twice daily with commercial pellets and hatched *Artemia*
182 spp. (NRD 500-800 µM, INVE Aquaculture®, Salt Lake City, USA) and provided with
183 multi-sized shelters in which to hide. Tanks were cleaned on a daily basis. Then, at least one
184 week prior to experimentation, each fish was tagged with visible elastomer implanted into the
185 epaxial muscle or caudal region (Northwest Marine Technology®, Inc., Shaw Island, USA),
186 which made it possible to identify individuals for repeated measures experimentation.
187 Throughout the duration of the project, fish were maintained under James Cook University
188 Animal Ethics Committee regulations (permit: #A2089, approved for this study)

189

190 *Temperature treatment*

191 One week following tagging, all tanks were randomly subdivided across six temperature
192 treatments representing the annual temperature range that this population of *C. viridis*
193 experiences near Lizard Island (23, 25, 27 or 29 °C) in addition to two higher temperatures
194 (31 or 33 °C) corresponding to the 2.1-4.0 °C projected increase in SST by 2100 (Collins *et*
195 *al.*, 2013). Temperatures were reduced or increased by 0.5 °C day⁻¹ until target temperatures
196 were reached. Water for the 29, 31, and 33 °C treatments was heated using 300 W
197 submersible heaters (JEBO®, Guangdong, China) that were controlled by automated
198 temperature controllers (N323, NOVUS Automation®, Porto Alegre, Brazil). Water for the

199 23, 25, and 27 °C treatments was cooled using external water chillers (HC-130A Hailea®,
200 Guangdong, China). Temperatures were manually checked three times daily and always
201 maintained within a range of ± 0.2 °C.

202 Fish were maintained at each target temperature for a minimum of six weeks prior to
203 experimentation so that acclimation at the level of metabolism, if any, would be presumed
204 complete by this time (Nilsson *et al.*, 2010). To determine growth trajectories, all fish were
205 weighed at the initial point of tagging, following each experiment, and at the conclusion of
206 the 27-week experimental period. Prior to all experimental trials, each individual was starved
207 for 24h to ensure a post-absorptive state that maximized energy available for performance
208 (Niimi & Beamish, 1974; J.L. Rummer, unpublished data).

209

210 *Metabolic rates and the scope for aerobic metabolism*

211 Intermittent flow respirometry was used to estimate maximal metabolic rates (MMR),
212 standard metabolic rates (SMR) and aerobic metabolic scopes (AMR) of eight randomly
213 selected individuals from each temperature treatment. All protocols followed Steffensen
214 (1989) and Rummer *et al.* (2016). The MMR was estimated from the maximal oxygen
215 consumption rate measured immediately after a fish was exercised by chasing. The SMR was
216 estimated from the oxygen consumption rate of a fish at rest, and AMS was calculated as the
217 difference between MMR and SMR (see also Clark *et al.*, 2012, 2013; Roche *et al.*, 2013;
218 Rummer *et al.*, 2016 for details on these protocols).

219 Metabolic rates were estimated using well established methodologies and techniques
220 that all aim at ensuring low levels of systemic interference. In short, each fish was first chased
221 continuously by hand for 3 min in a 100L circular (65cm x 40cm: height x diameter)
222 aquarium containing well-aerated and temperature-controlled seawater maintained at the
223 fish's treatment temperature. During this time, the experimenter would only touch the tail of

224 the fish if it slowed down or stopped swimming. Individuals were considered exhausted when
225 they became unresponsive to chasing, which always occurred before the end of the 3-min
226 chase period. The fish was then scooped into a rubber mesh net and maintained out of the
227 water for 1 min to necessitate anaerobic metabolism (Clark *et al.*, 2013; Roche *et al.*, 2013;
228 Rummer *et al.*, 2016). Following air exposure, individual fish were immediately placed into
229 one of eight custom-built cylindrical acrylic respirometry chambers (each ~360 ml in total
230 volume, 21.6 x 4.6 cm; length x diameter) submerged in a temperature-controlled bath (5000
231 W heaters, Control Distributions®, Carlton, Australia or HC-1000A chillers, Hailea®,
232 Guangdong, China, via sump). Each respirometry chamber was sealed within 5 seconds
233 where after, reductions in O₂ concentration in the respirometry chambers were measured for
234 the following 7-min. The maximal oxygen consumption rate (i.e., MMR) was calculated from
235 the steepest 1-min slope during this 7-min interval. Then, the respirometry chamber was
236 flushed with fully oxygenated seawater for 7-min before a new 7-min measuring cycle was
237 initiated. The fish was maintained in the respirometry chamber for 22-24 h until O₂
238 consumption rates had stabilized and no longer decreased (Rummer *et al.*, 2016). During this
239 time, each repeat 14-min flush-measuring cycle was controlled by a digital relay timer
240 (MFRT-1 Multi Function Recycling Timer, Xiamen SUPERPRO Technology Co., Ltd.,
241 Xiamen, Fujian, China) connected to submersible flush pumps (Eheim®, Germany, 200L h⁻¹).
242 The measurement period was short enough to ensure that O₂ within the respirometry chamber
243 always remained above 80% air saturation, which is important to avoid metabolic changes
244 associated with hypoxia (Hughes, 1973, Tetens & Lykkeboe, 1985, Boutilier *et al.*, 1988).
245 The flush period was long enough to ensure oxygen levels returned to 100% air saturation.
246 Each of the eight respirometry chambers was also connected to its own in-line pump
247 (Aquapro® AP200LV, 200 L h⁻¹) to continuously recirculate water within the chamber at all
248 times regardless of flushing cycle, thus ensuring complete mixing and homogenous water

249 PO_2 . All water used for experiments was first sterilized using a 36 W UV filter (Blagdon Pro
250 UVC 16200®, China) to minimize bacterial growth within the respirometry chambers, but in
251 addition, all chambers, tubing, and pumps were flushed with chlorinated (10% bleach)
252 freshwater and sundried for at least 8h every 24-48h. Bacterial respiration within the
253 chambers (i.e., the drop in O_2 concentration within an empty chamber) was quantified in each
254 chamber prior to and after each trial but, because of the regular cleaning, never exceeded 10%
255 of $\dot{M}O_{2\text{ (rest)}}$ of the fish. During all trials, each respirometry chamber was partly covered using
256 a cylinder of black plastic with a 2 x 12 cm window. This setup allowed light to enter the
257 chamber but prevented fish from being disturbed via external visual stimuli.

258 The O_2 concentrations (temperature and barometric pressure compensated) in each
259 respirometry chamber were measured using a Firesting Optical Oxygen Meter (two four-
260 channel units, Pyro Science e. K.®, Aachen, Germany) and associated manufacturer's
261 software (via PC computer) at a rate of 0.5 Hz and saved as text files. After each trial, raw
262 Firesting text files were imported into Lab Chart version 6.1.3 (AD Instruments®, Colorado
263 Springs, CO, USA) for analysis. The $\dot{M}O_2$ in $mg\ O_2\ kg^{-1}\ h^{-1}$ at each time point over the 22-
264 24h trial was calculated based on the volume of the respirometry chamber, volume of fish,
265 and mass of fish following equations of Bushnell *et al.*, (1994) and Schurmann and Steffensen
266 (1997). Specifically, maximal metabolic rate estimated from the first measuring period as
267 describe above. The remaining ~94 measuring periods were used to estimate SMR based on
268 the “mean of the leftmost normal distribution” method (MLND) of Chabot *et al.*, (2016),
269 which is understood to be a rigorous and accurate way to estimate SMR (see Svendsen *et al.*,
270 2016). In short, this method detects the bimodal normal distribution of oxygen consumption
271 rates over the 22-24h recovery period and assigns the peak of the leftmost distribution curve
272 as SMR.

273 Only slopes with $R^2 \geq 0.90$ were used in order to reduce the impact of systemic
274 variations in O_2 measurements (Svendsen *et al.*, 2016). The proportional background O_2
275 consumption rate (measured as O_2 depletion in the empty respirometry chambers before and
276 after each trial, assumed linear) was subtracted from each $\dot{M}O_2$ measurement. Temperature
277 quotients (Q_{10}) were calculated according to Clarke and Johnston (1999) to evaluate the level
278 of temperature dependence of SMR at the higher end of the thermal range.

279

280 *Critical thermal maxima*

281 Upper critical thermal limits (i.e., CT_{Max}) were determined for 40 randomly selected fish
282 – specifically, 8 fish from the 23 °C treatment, 8 from 25 °C, 7 from 27 °C, 6 from 29 °C, 7
283 from 31 °C, and 4 from 33 °C – using critical thermal methodology (CTM; Beitinger *et al.*,
284 2000). The low sample size at 33 °C was due to low survival rates at this temperature (see
285 discussion). Experiments were performed using four 3-L containers (each holding one
286 individual) suspended within a 100-L water bath. Containers were pierced on all sides,
287 allowing seawater to flow through readily, and each container also contained an air stone to
288 ensure sufficient aeration. The water bath was provided with a continuous supply of clean,
289 aerated, and UV-filtered seawater, which was heated (5000 W, Control Distributions®,
290 Australia) or chilled (HC-1000A, Hailea®, China) to the fish's respective treatment
291 temperature prior to commencing experimentation. To ensure sufficient mixing, two pumps
292 (WH-500, Weipro®, Guangdong, China) were placed on the bottom of the water bath. Pilot
293 trials confirmed that temperatures remained the same between all four 3-L containers
294 throughout experimentation. After being placed in their respective containers, the four fish
295 were left to habituate to the containers for approximately 15 min., after which time, water
296 temperature was increased by 0.28 ± 0.03 °C min^{-1} until fish lost equilibrium. Loss of
297 equilibrium (LOE) has been previously defined as when a fish loses its inability to right itself

298 (see Beitinger *et al.*, 2000). Upon LOE, each fish was quickly removed from its respective
299 container using a mesh net and placed into a separate aquarium to recover at its original
300 treatment temperature. The temperature at which each fish reached LOE was recorded in each
301 container using a digital thermometer (Type T C26 (temperature resolution: 0.1 °C),
302 Comark®, Norfolk, United Kingdom).

303

304 *Temperature preference*

305 The preferred temperature (T_{pref}) of 48 individual fish from each temperature treatment
306 (i.e., 23, 25, 27, 29, 31 and 33 °C) was determined using a custom-designed two-chamber
307 shuttlebox system (developed by J.F. Steffensen and described in detail in Nay *et al.*, 2015,
308 but see also Schurmann *et al.*, 1991; Schurmann & Steffensen, 1994; Petersen & Steffensen,
309 2003). This setup allows a fish to use movement to control the temperature of its water and
310 therefore its body temperature (i.e., preferred temperature; T_{pref}). In brief, the system
311 consisted of a “hot” chamber and a “cold” chamber, each made from white PVC walls and a
312 transparent Plexiglas bottom (ø35 cm). A 10 cm x 5 cm opening in the wall joined the two
313 cylindrical chambers (i.e., like a figure-eight), and the fish was able to swim freely between
314 the two chambers. The temperature differential between the two chambers was always
315 maintained at 1.5-2 °C (using two submersible pumps, WH-500, Weipro®, Yongcheng
316 Aquarium, China), which ensured that the fish could detect a temperature difference when
317 selecting between the two chambers (J.F. Steffensen, personal observation). When the fish
318 entered the “hot” chamber, the temperature of both chambers would increase at a rate of 6 °C
319 h⁻¹ until the fish moved to the “cold” chamber, which would be 1.5-2 °C cooler. While the
320 fish was in the “cold” chamber, the temperature of both chambers would cool at a rate of 6 °C
321 h⁻¹ until the fish moved back to the “hot” chamber, which was 1.5-2 °C warmer. By

322 swimming back and forth between the two chambers, the fish could effectively control the
323 temperature of its environment and thus its own body temperature.

324 The temperatures for each cylindrical chamber were maintained by pumping in water
325 (480 L h^{-1} , pump HX-6510, Guangdong, China) from corresponding external buffer tanks
326 ($\varnothing 10 \text{ cm}$), which then passively returned from each chamber to its corresponding buffer tank.
327 Inlets and outlets in each chamber were constructed to ensure water flowed in a clockwise
328 direction in one chamber and a counter-clockwise direction in the other chamber, which
329 prevented unwanted mixing of water between the “hot” and the “cold” chambers in the
330 connecting wall opening. The temperatures of the buffer tanks were controlled by pumping
331 water at a rate of 420 L h^{-1} (WH-500, Weipro®, Yongcheng Aquarium, Guangdong, China)
332 through stainless steel spirals from the buffer tanks into cooling and heating reservoirs, and
333 back. The reservoirs were either heated to $45 \text{ }^\circ\text{C}$ (5000W heater, Control Distributions®,
334 Carlton, Australia) or externally chilled to $5 \text{ }^\circ\text{C}$ (HC-130A, HC-1000A, Hailea®, Guangdong,
335 China and 1/3 hp Aqua One Arctic, Aqua One®, Southampton, UK). The water level in all
336 compartments of the system was $\sim 20 \text{ cm}$ (total system volume of $\sim 42 \text{ L}$), and the entire setup
337 was shielded with black plastic sheeting to protect fish from external visual stimuli while still
338 maintaining 12:12 light:dark photoperiod.

339 During each trial, temperatures within the “hot” and the “cold” chambers were
340 continuously recorded (5 Hz) using inline thermocouple sensors mounted along the inside
341 chamber walls. The temperature sensors were connected to temperature readers (PR-5714, PR
342 Electronics®, Rønne, Denmark) and a computerized software system. Above the shuttlebox
343 setup, a mirror angled downward at 45° allowed video monitoring of fish movements via a
344 video camera (SONY® HDR-XR100E) mounted on the opposite wall (4.75 m distance).
345 Infrared lights mounted below the transparent Plexiglas bottoms were used to illuminate the
346 chambers from below to create a detectable contrast between the fish and its surroundings. A

347 PC video frame-grabber (USB 2.0 DVD maker®) transmitted the video signal from the digital
348 video camera to a laptop. Then, by using position analyzer software (LoliTrack, Loligo
349 Systems®, Tjele, Denmark), the position of the fish was continuously tracked, thus allowing
350 the custom-designed software (Labtech Notebook) to automatically heat or cool the two
351 chambers based on the real-time movement of the fish (for further details, see Schurmann *et*
352 *al.*, 1991; Schurmann & Steffensen, 1994). During the night, a small lamp provided a level of
353 light similar to full moon on the reef, which ensured that fish could continue to navigate
354 between the two chambers.

355 The length of time necessary for fish to learn the shuttlebox system and select T_{pref} was
356 determined via a series of 48 h pilot trials using fish from two of the temperature treatment
357 groups (25 and 31°C). As a result, a 24 h experimental period was chosen because T_{pref}
358 stabilized within 24 h in all pilot trials and did not change with longer exposure time (paired t-
359 test; $P_{25^{\circ}\text{C}} = 0.253$, $P_{31^{\circ}\text{C}} = 0.742$).

360 Following the 24 h fasting period, individual fish were placed into the shuttlebox
361 system set to their respective treatment temperature for 1.5 h to habituate to the aquaria
362 without experiencing any change in water temperature. Movement-controlled heating/cooling
363 patterns commenced at 1830 each evening and continued until 1630 the following day. To
364 preclude the potential effects of specific dynamic action on T_{pref} (Wallman & Bennett, 2006)
365 no food was provided 24h prior to or during experimentation. For the night time periods
366 (1830 until 0630 the following day), reservoir temperatures were set so that the shuttlebox
367 would not continue to heat or cool the fish past lethal limits (A. Habary, personal
368 observation). This meant that if the fish did not move appropriately between the two
369 chambers at night, the shuttlebox system would not inadvertently kill the fish while the
370 computer waited for the fish to move. During daylight hours (from 0630 – 1630), however,
371 when the observer was able to regularly monitor the fish, reservoir temperatures could exceed

372 the fish's lethal thermal limits. Following each trial, all tanks were rinsed with clean seawater
373 to eliminate olfactory cues from the previous fish. After half of all trials were completed for
374 each treatment temperature, the "hot" and "cold" chambers were switched to eliminate spatial
375 bias.

376 The T_{pref} data were analyzed by averaging the selected temperature range for every 10
377 minutes during the last 5 h of each trial for each fish. Means were then compared between the
378 fish from each treatment temperature (similar to the methodology described by Killen 2014).
379 If the tracking software was unable to detect the fish movement for more than one minute
380 (e.g., if the fish was positioned too close to the chamber wall), then the missing time period
381 was excluded from analysis in addition to the same amount of data following the missing time
382 period (Nay *et al.*, 2015). During the time period when the tracking software could not detect
383 the fish, the system would continue to increase or decrease the temperature. Removing these
384 missing data as well as the following time period allowed for the system to stabilize back to
385 the original point where the fish was 'lost' and to compensate for any change in temperature
386 caused by the system's inability to track the fish.

387

388 *Data analyses*

389 The SMR, MMR, AMS and the effect of body mass were all analyzed using general
390 linear models (GLM) with SMR, MMR, and AMS as dependent variables, treatment as a
391 categorical predictor, and body mass as a continuous predictor. The CT_{Max} data were analyzed
392 using a one-way ANOVA followed by the Tukey HSD test, while mean body mass values
393 within and between temperature treatments were compared using a one-way ANOVA and,
394 when necessary, Holm-Sidak *post hoc* tests. The final T_{pref} values between groups were
395 analyzed using a nonparametric Kruskal Wallis test, and differences between treatments were
396 compared using the Tukey HSD test. Growth data (mass loss/gain over the 27-week project)

397 was analyzed using a one-way ANOVA and, when necessary, Holm-Sidak *post hoc* tests.
398 Linearity, normality and homoscedasticity were verified for all data sets using residual-fit
399 plots. When necessary, variables (SMR, MMR and AMS) were \log_{10} transformed for use in
400 models. The level of significance was $\alpha = 0.05$ for all tests, and all statistical analyses were
401 performed using Statistica v. 12 (Statsoft Inc., Dell®, Tulsa, Okla., USA).

402 **Results**

403 *Metabolic rates and aerobic metabolic scope*

404 Treatment temperature did not significantly influence aerobic metabolic scope (AMS)
405 (Fig. 1b; GLM, $F_{5,47} = 2.25$, $P = 0.06$) despite a 31.2% and 30.2% reduction in AMS between
406 fish maintained at 33 °C versus fish maintained at 27 or 29 °C, respectively.

407 By contrast, treatment temperature did significantly influence estimates of both standard
408 metabolic rate (SMR) (GLM, $F_{5,47} = 5.89$, $P < 0.01$) and maximum metabolic rate (MMR)
409 (Fig. 1a; GLM, $F_{5,47} = 2.85$, $P = 0.02$). Here, SMR increased by ~86% from 23 °C to 31 °C
410 before decreasing again at 33 °C (Fig. 1a), and MMR increased by up to 36.4% between 23
411 and 29 °C. Yet temperature only had an overall effect on MMR as there were no statistically
412 significant differences between individual treatments (Fig. 1a). The temperature quotient
413 (Q_{10}) calculated for SMR between 29 and 31 °C was 2.06 but fell to 0.97 for SMR estimates
414 between 29 and 33 °C and 0.46 between 31 and 33 °C.

415

416 *Critical thermal maxima*

417 There was a significant positive effect of treatment temperature on CT_{Max} ($F = 42.55$, P
418 < 0.01). Specifically, CT_{Max} increased by approximately 0.5 °C for every 1 °C increase in
419 treatment temperature (Fig. 2a, regression equation: $CT_{Max} = 0.45x + 25.2$, $x = \text{°C}$, $R^2 = 0.97$)
420 with average CT_{Max} values ranging from 35.4 ± 0.2 °C (mean \pm SEM) for fish maintained at
421 23 °C to 39.2 ± 0.2 °C (mean \pm SEM) for fish maintained at 31 °C (Fig. 2a).

422 Importantly, fish from the 33 °C treatment reached a mean CT_{Max} of 40.5 °C, but were
423 unable to recover from this exposure and died within 24 h following experimentation. One of
424 the criteria, i.e., the ability of an animal to escape from conditions that will lead to its death,
425 for CTM (see discussion in Beitinger *et al.*, 2000) was therefore not met, and consequently
426 these data were excluded from analyses however still depicted in Fig. 2a for reference.

427

428 *Temperature preference*

429 Treatment temperature had a significant effect on the average T_{pref} of *C. viridis* when
430 compared across all treatments (mean T_{pref} = 28.9 °C, Kruskal-Wallis, H = 15.7, *P* < 0.01;
431 Fig. 2b). However, only the T_{pref} of the 23 °C and 33 °C treatment groups differed from one
432 another (T_{pref} = 26.1 °C ± 0.96 and 30.4 °C ± 0.37, means ± SEM, respectively, *P* = 0.04; Fig.
433 2b), and neither the T_{pref} of fish at 23 °C nor the T_{pref} of fish at 33 °C were statistically distinct
434 from the T_{pref} of fish from 25, 27, 29, or 31 °C (*P* = 0.31). The overall mean T_{pref} for all
435 treatment groups was 28.9 °C, which coincided with the optimum temperature (T_{opt}) for AMS
436 (27-29 °C) (Figs. 1b and 2b).

437

438 *Growth*

439 Temperature treatment had a significant overall effect on body mass (Fig. 3, *P* < 0.01).
440 Specifically, fish that were maintained at temperatures they would normally experience in the
441 wild (i.e., 23, 25, 27, or 29 °C) had increased their body mass by 15.4, 16.7, 19.2 and 15.7%,
442 respectively after 27 weeks (*P* = 0.04). By comparison, fish maintained at 31 °C showed no
443 change in body mass (*P* < 0.05), while fish maintained at 33 °C lost, on average, 30% of their
444 body mass (*P* = 0.02).

445

446

447 **Discussion**

448 In a time of rapid global warming, the capacity for acclimation has been hailed as the
449 primary pathway for species survival. Here, we highlight another mechanism by
450 demonstrating that a model species – a thermally-sensitive coral reef fish, *Chromis viridis*
451 (Pomacentridae) – appears to lack the ability to acclimate at the level of metabolic rate, but
452 instead maintains a behavioural preference and actively seeks out habitat temperatures that
453 maximize metabolic performance and growth, even after prolonged exposure to water
454 temperatures outside of this range. This is the first time thermal preference for lower ambient
455 temperatures has been shown to persist after prolonged acclimation to elevated temperatures,
456 thus providing one explanation for the rapid pole-ward expansion of thermally sensitive
457 species worldwide (Feary *et al.* 2013).

458 Modifying behaviour to select preferred temperatures can be one of the initial responses
459 an organism has to elevated temperatures (Olla *et al.*, 1978; Sloman & McNeil, 2012).
460 Multiple factors can potentially modify temperature preferences (e.g., sex and individual
461 variation, see Wallman & Bennett, 2006; Podrabsky *et al.*, 2008; Biro *et al.*, 2010; Killen,
462 2014), and here we show that a narrow thermal range for optimal metabolic performance
463 confers an equally narrow range of preferred temperature (see also Angilletta *et al.*, 2006).
464 Despite a minimum of six weeks of acclimation to one of six temperatures spanning the total
465 yearly temperature range experienced by these individuals in the wild as well as global
466 warming projections, most individuals maintained a preference for the exact same
467 temperature range found in their natural habitat during the summer months. This is likely the
468 result of strong evolutionary selection for and adaptation to a narrow and stable thermal range
469 (Johnson & Kelsch, 1998; Angilletta *et al.*, 2002, 2006). The thermal range for which species
470 are adapted indicates the temperature range that maximizes fitness and thus promotes optimal
471 growth, reproduction, swimming, etc. (Jobling, 1981; Kelsch & Neil, 1990). Here, the mean

472 preferred temperature across all treatments (28.9 °C) coincided with the optimal thermal
473 range for aerobic metabolic scope (27-29 °C) and growth (29 °C) for fish used in this study,
474 and the temperature range that has been reported to optimize growth in other tropical coral
475 reef fishes (Zarco-Perello *et al.*, 2012, see also Payne *et al.*, 2016).

476 While a combination of performance metrics, such as aerobic scope, CT_{Max}, and thermal
477 preference may be used to explain broader fitness consequences and population movement
478 patterns, taken alone, each individual metric may not provide enough insight into how an
479 organism will cope with long-term warming. Similar to previous studies, we found a
480 significant increase in CT_{Max} following long-term exposure to elevated temperatures.
481 However, despite the prolonged exposure to 33 °C, *C. viridis* was not able to tolerate rapid or
482 transient heating events. That is, those individuals were not able to recover from the CT_{Max}
483 trials, indicating that long-term exposure to temperatures projected for the near future (33 °C)
484 could prove detrimental, as compensatory mechanisms associated with recovery from heat
485 stress may be exhausted. Consequently, the increase in CT_{Max} only provided a short-term
486 advantage but no clear benefit to the long-term thermal tolerance of individuals. Likewise, the
487 lack of significant change in AMS in fish maintained between 27 °C and 33 °C could lead to
488 the erroneous conclusion that this species is highly thermally tolerant to a 3 °C increase in
489 temperatures, even though critical changes to whole animal fitness may occur in the wild.
490 Specifically, the relatively minor, non-significant, reductions in aerobic scope seen here, may
491 pose a serious physiological threat through the cascading effects of thermal stress on
492 thermoregulatory behaviour (Kearney *et al.*, 2009; Sinervo *et al.*, 2010; *sensu* Du Plessis *et*
493 *al.*, 2012). That is, while some ectotherms buffer the impacts of elevated temperature by
494 temporarily sheltering in thermal refugia, this requires time and energetic expenses that could
495 have been used for foraging and other important metabolic functions such as growth (Kearney
496 *et al.*, 2009; Sinervo *et al.*, 2010). Consequently, there may be severe fitness consequences for

497 individuals despite a lack of impact on a single physiological process or metabolic
498 performance measure (Clark *et al.*, 2013; Donelson *et al.*, 2014).

499 Although most tropical reef fishes are thought to have some capacity for acclimation
500 and adaptation (Donelson *et al.*, 2011, 2014, Grenchik *et al.*, 2013), any insufficiencies could
501 render many tropical stenotherms vulnerable to temperature changes projected to occur within
502 the next 50-100 years. Several studies have shown improvements in some metabolic
503 performance traits following prolonged acclimation, but this was accompanied by reductions
504 in other fitness attributes, such as reproductive output (Angilletta, 2009; Donelson *et al.*,
505 2011, 2014). In this study, the critical thermal maxima of *C. viridis* increased proportionally
506 with acclimation temperature, but the associated SMR plateaued at 33 °C. A 10 °C increase in
507 body temperature typically requires 2-3 times more energy ($Q_{10} = 2-3$, Clarke & Johnston,
508 1999) in order to maintain standard metabolic rate functions (SMR), and the Q_{10} of 0.97 at 33
509 °C for *C. viridis* indicates that these fish were unable to fully compensate for the increase in
510 temperature, presumably at a cost to basic maintenance. Accordingly, the body mass of
511 individuals maintained at 33 °C decreased by a staggering 30% during the 27 week trial.
512 Energetic requirements of different organs and tissues vary according to their mass and
513 metabolic requirements (Crnokrak & Roff, 2002; Darveau *et al.*, 2002; Crispin & White,
514 2013) causing tissue-specific physiological processes to contribute differently to the animal's
515 total energetic requirements. For example, 35% of the variation in basal metabolic expenses
516 in some ectotherms can be attributed to differences in heart and liver masses (Garland, 1984).
517 Consequently, even though some tissues/organs keep consuming more energy, denaturation or
518 suppression (Nilsson & Renshaw, 2004; Richards, 2011) of highly O₂-consuming organs
519 and/or tissue may still lead to a lower mass-adjusted O₂ consumption rate and hence SMR, as
520 observed here. This would be considered a strong sign of unsustainable thermal effects on
521 physiological processes and overall fitness. As a result, the reduction in growth concurrent

522 with inadequate increases in SMR at elevated temperatures may have serious impacts on the
523 overall fitness of this species and be a strong motivation for relocating to more favourable
524 thermal environments.

525 Ultimately, the potential for successful relocation of coral reef fish species such as *C.*
526 *viridis*, representing the versatile Pomacentridae family, may depend on their level of coral
527 dependence. Relocation is facilitated or constrained not only by physiologically-mediated
528 thermal preferences, but also by the species' versatility in life history characteristics (Wilson
529 *et al.*, 2010; Figueira & Booth, 2010; Nakamura *et al.*, 2013) such as inter- and intra-specific
530 competition, resource and habitat requirements, and dispersal and/or recruitment potentials
531 (Feary *et al.*, 2013; Harborne, 2013; Nakamura *et al.*, 2013; Seth *et al.*, 2013). For the 90 % of
532 coral reef fish species (and the majority of pomacentrid species) that are only partly
533 dependent of coral reefs (Jones *et al.*, 2004), relocation ultimately depends on each species'
534 level of coral dependence in terms of, for example, food and refugia (Nakamura *et al.*, 2013).
535 Among the pomacentrids, only 16 % of the species are obliged to coral reefs (Jones *et al.*,
536 2004; Cole *et al.*, 2008; Coker *et al.*, 2014) in terms of habitat (Coker *et al.*, 2014) or feeding
537 (e.g., corallivory; Cole *et al.*, 2008). The remaining pomacentrids are capable of multi-species
538 cohabitation (McCormick & Makey; 1997; Nadler *et al.*, 2016), suggesting redistribution may
539 not only be plausible but also a viable option for these species. Theoretically, obligate coral
540 associated species may be more limited in their capacity for rapid relocation pole-ward (Ben-
541 Tzvi *et al.*, 2008; Feary *et al.*, 2013). However, recent studies have determined that many
542 reef-forming coral species and associated coral reef fishes are also moving toward cooler
543 regions (Yamano *et al.*, 2011; Feary *et al.*, 2013) at rates of up to 14 km year⁻¹, perhaps
544 establishing the foundation for less vagrant species.

545 The pomacentrid, *C. viridis*, shows little or no capacity for acclimation at the level of
546 aerobic metabolic performance or growth when maintained for extended periods of time to

547 temperatures projected for tropical coral reefs by 2100. Rather, we determined that this
548 species can use movement to behaviourally seek out lower, potentially less thermally-stressful
549 temperatures, those that coincide with present day temperatures from where this fish was
550 collected. Temperature-mediated movement has not previously been evaluated as a primary
551 pathway for coping with rapid ocean warming but is likely to have serious implications for
552 the long-term sustainability of tropical fish populations in their current home ranges,
553 particularly when thermal adaptation is not possible. Critically, the impact of behavioural
554 thermoregulation is likely to be rapidly growing as more and more species are found shifting
555 their distribution ranges toward areas with more hospitable temperatures. Indeed, current
556 evidence suggests that 365 different species across 55 families of tropical fishes are either on
557 the move or have already undergone bio-geographical redistributions or range shifts as a
558 result of climate change and more specifically ocean warming (Figueira & Booth, 2010; Feary
559 *et al.*, 2013; Nakamura *et al.*, 2013). If our findings for a model coral reef fish species from
560 the species-rich family, Pomacentridae, hold true for other coral reef fishes, then increases in
561 ocean temperatures relative to the thermal preference of species may become the primary
562 driving factor for changing coral reef biodiversity. Tropical coral reef fishes are of global
563 ecological and economical importance (Depczynski *et al.*, 2007; Hopkins *et al.*, 2011; Cinner,
564 2014) and require effective management and conservation strategies. However, our capacity
565 to implement these strategies will hinge on our understanding of temperature-mediated
566 changes in physiological performance, resilience, diversity, and distribution over the long-
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568

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909 Figure 1

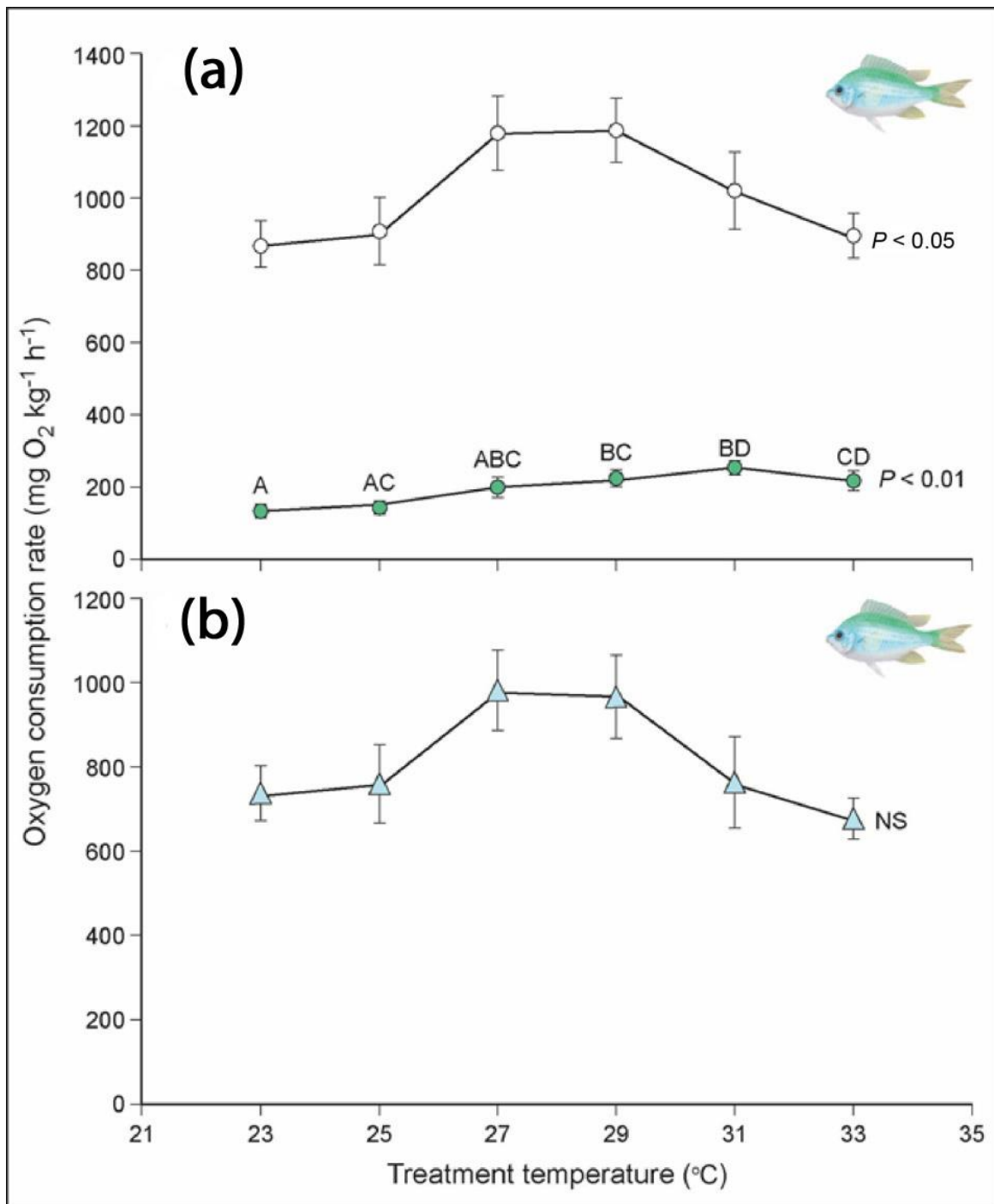
910 The effect of treatment temperature on O₂ consumption rates as estimates of standard
911 metabolic rates (SMR, green circles; panel **(a)**), maximum metabolic rates (MMR, white
912 circles; panel **(a)**), and aerobic metabolic scope (AMS = MMR-SMR, blue triangles; panel
913 **(b)**) on *C. viridis*. Values are means \pm SEM. Letters demarcate statistically significant
914 differences between fish from the different temperature treatments. NS indicates no
915 significant differences.

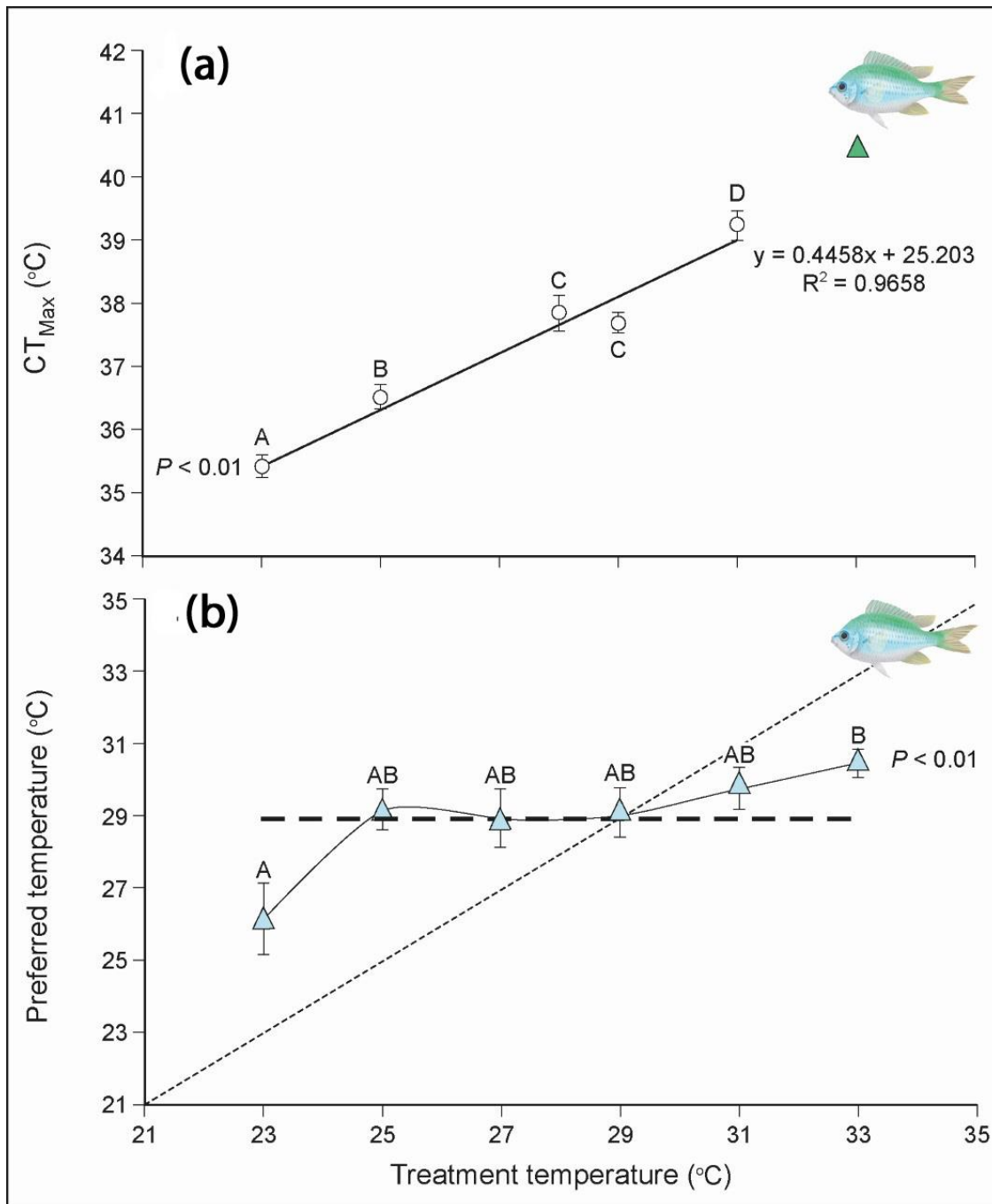
916 Figure 2

917 **(a)** The effect of treatment temperature on the upper critical thermal limits (CTMax) of *C.*
918 *viridis*. The solid triangle indicates the CTMax for fish from the 33 °C treatment temperature;
919 however, no fish from this group survived longer than 24 h following the experiment and
920 were thus excluded from statistical analyses. Values are means \pm SEM. Letters demarcate
921 statistically significant differences between fish from the different temperature treatments. **(b)**
922 The effect of treatment temperature on the preferred temperature (T_{pref}) of *C. viridis*. The
923 dashed, bold line represents the mean T_{pref} across all treatments (28.9 °C). The line of
924 equality (dotted line) represents the T_{pref} if it were to exactly match the treatment
925 temperature. Values are means \pm SEM. Letters demarcate statistically significant differences
926 between fish from the different temperature treatments.

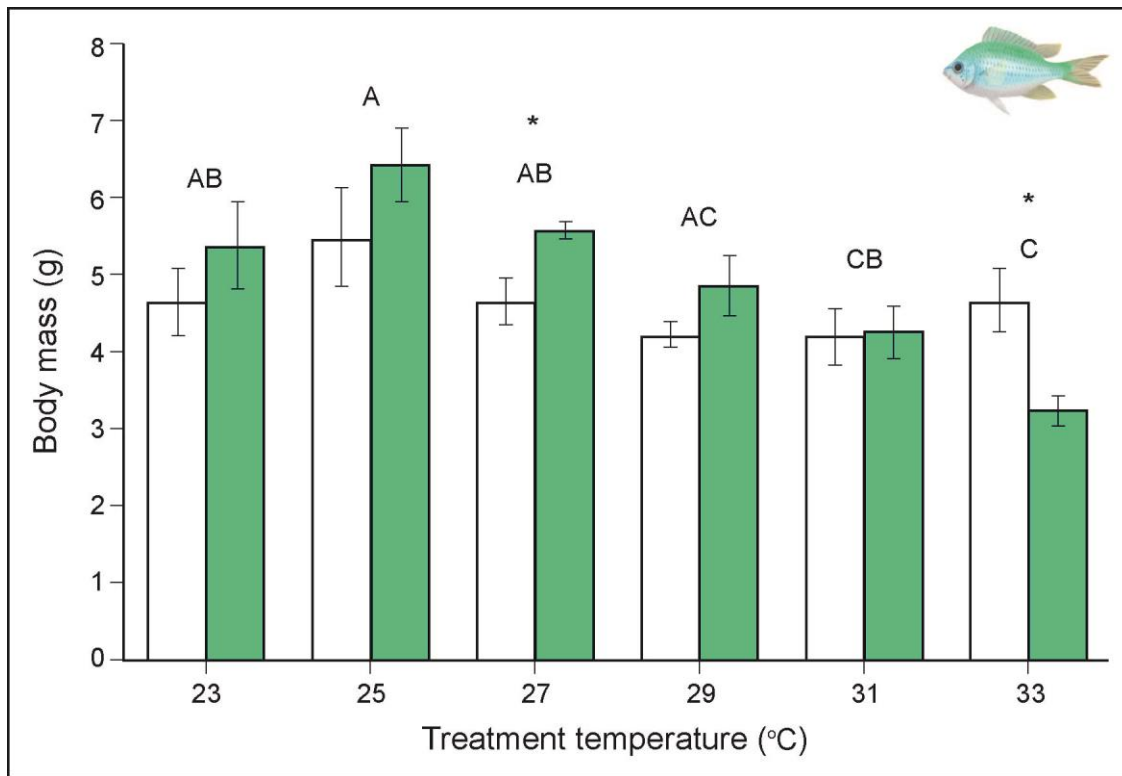
927 Figure 3

928 Body mass of *C. viridis* across all temperature treatments prior to experimentation (white
929 bars) and following 27 weeks exposure to treatment temperatures (shaded bars). Values are
930 means \pm SEM. Letters demarcate statistically significant differences between fish from the
931 different temperature treatments. Asterisks demarcate differences between body mass at the
932 start of the study and after 27 weeks within each treatment temperature.





937 Figure 3



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