

1 **Intraspecific variation in thermal tolerance differs between tropical and**
2 **temperate fishes**

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15 **Abstract:**

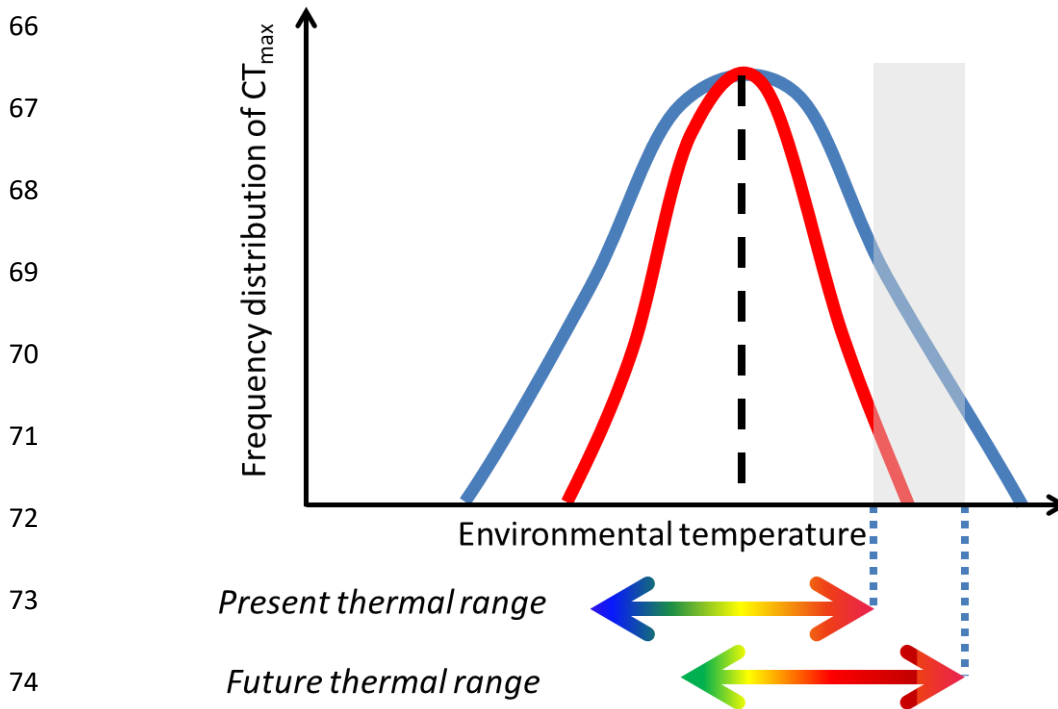
16 How ectothermic animals will cope with global warming, especially more frequent
17 and intense heatwaves, is a critical determinant of the ecological impacts of climate
18 change. There has been extensive study of upper thermal tolerance limits among
19 fish species but how intraspecific variation in tolerance may be affected by habitat
20 characteristics and evolutionary history has not been considered. Intraspecific
21 variation is a primary determinant of species vulnerability to climate change, with
22 implications for global patterns of impacts of ongoing warming. Using published
23 critical thermal maximum (CT_{max}) data on 203 marine and freshwater fish species,
24 we found that intraspecific variation in upper thermal tolerance varies according to a
25 species' latitude and evolutionary history. Notably, freshwater tropical species have
26 lower variation in tolerance than temperate species in the northern hemisphere,
27 which implies increased vulnerability to impacts of thermal stress. The extent of
28 variation in CT_{max} among fish species has a strong phylogenetic signal, which may
29 indicate a constraint on evolvability to rising temperatures in tropical fishes. That is,
30 in addition to living closer to their upper thermal limits, tropical species may have
31 higher sensitivity and lower adaptability to global warming compared to temperate

32 counterparts. This is evidence that tropical fish communities, worldwide, are
33 especially vulnerable to ongoing climate change.

34 The capacity of ectothermic species to cope with ongoing global warming, especially
35 the increasing frequency, intensity and duration of extreme heatwaves, will be
36 influenced by their upper thermal tolerance limits¹⁻³. Tolerance of acute warming,
37 measured as the critical thermal maximum (CT_{max}), varies among fish species
38 according to thermal conditions in their habitat⁴. Tropical species live in warm,
39 relatively thermally stable habitats; they have narrow thermal tolerance ranges but
40 higher CT_{max} than species at temperate latitudes. Their warm habitat temperatures
41 are also, however, closer to their limits of upper thermal tolerance, so they have a
42 limited thermal safety margin (defined as the difference between upper thermal
43 tolerance limit CT_{max} of adult life stage and the maximum habitat temperature during
44 summer⁵) and consequently are considered to be especially vulnerable to global
45 warming⁶⁻⁹. Temperate species have lower absolute thresholds for tolerance of
46 warming, but they have broader tolerance ranges, presumably because they
47 encounter a wide range of habitat temperatures, both seasonally and spatially. This
48 is linked to wider thermal safety margins than in tropical species^{4,10}. These patterns
49 of vulnerability to global warming, among species at a geographic scale, are major
50 issues in projecting impacts of warming. They have a strong phylogenetic basis,
51 which is believed to reflect local adaptation to common ancestral thermal regimes in
52 related species¹¹.

53 Studies of broadscale geographic patterns in vulnerability have, to date, focused
54 upon average values for CT_{max} among fish species. The significance of intraspecific
55 variation in tolerance remains to be explored. The extent of variation in functional
56 traits within species, particularly of physiological tolerances, is expected to have a
57 profound influence on their vulnerability to global change¹²⁻¹⁵. Possessing a broad
58 range of tolerance phenotypes in populations can reduce sensitivity to impacts of
59 environmental stressors, through various proximate ecological mechanisms¹²⁻¹⁴. If
60 phenotypic variation is linked to underlying genetic diversity in the species, this can
61 provide scope for adaptability and evolvability, by yielding genotypes for selection in
62 changing environments¹²⁻¹⁴. When fish species are challenged by thermal stressors
63 such as increased seasonal temperatures and extreme heatwaves, the population

64 sensitivity and adaptability will be major determinants of their relative vulnerability ¹³⁻
65 ¹⁶ (Figure 1).



75 **Figure 1| Theoretical representation of different frequency distribution curves of CT_{max} .** The
76 curves of two species have the same mean CT_{max} (dashed line) but different standard deviations
77 (S.D.). With ongoing climate change, represented by the shift in the thermal range (double-pointed
78 arrows), individuals of the species with the narrower S.D. CT_{max} (red curve) are less likely to survive
79 compared to individuals of the species with the wider S.D. CT_{max} (blue curve), since maximum
80 environmental temperatures will include values (grey area) outside their thermal tolerance range.

81 Fish species show intraspecific variation in CT_{max} , which has a component of both
82 phenotypic plasticity and heritable genetic variation ^{15, 17-19}. The CT_{max} varies among
83 populations of fish species, due to local adaptation ²⁰⁻²², indicating that the trait
84 evolves in response to prevailing thermal regimes. Given the broader thermal range
85 experienced by temperate fish species, within generations and over evolutionary
86 time, we hypothesized that they would exhibit greater intraspecific variation in their
87 thermal tolerance, measured as CT_{max} , than tropical species. We expected that the
88 extent of variation might be linked to the magnitude of the thermal safety margin,
89 because a small margin might constrain scope to express variation ¹⁰. We also
90 expected the extent of variation in CT_{max} to have a phylogenetic basis, indicating that
91 it reflected evolutionary processes of adaptation.

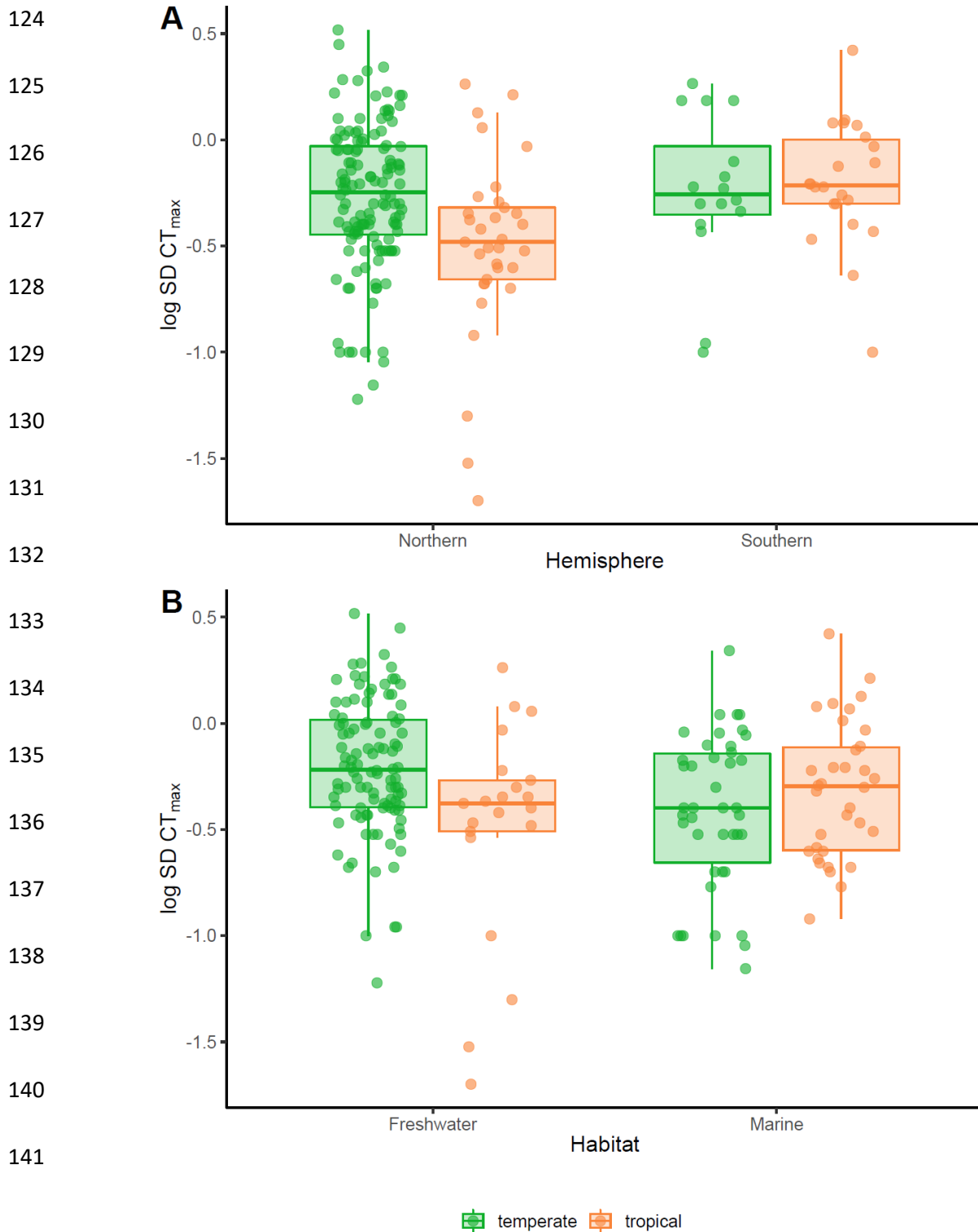
92 We used published data ⁴ and, after a data selection process (see Methods), we
93 estimated the extent of intraspecific variation in CT_{max} of 203 species of ray-finned

94 (actinopterygian) fish ($n = 127$ freshwater, $n = 76$ marine), based on the standard
95 deviation of the mean. We were well aware that the selected studies in the dataset
96 did not have the same protocol procedures. They did not use the same heating rate
97 ($0.0017\text{-}1^\circ\text{C}/\text{min}$) and fish size, both of which can influence the outcoming CT_{max} and
98 standard deviation of the mean. We choose to not include these variables in our
99 analysis because of the high variation of heating rate used and for fish size most
100 studies did not report the size. We then compared two latitudinal groups, temperate
101 to tropical species, considering the boundary to be 23° latitude. We also evaluated if
102 variation in CT_{max} depended on whether species were from northern or southern
103 hemisphere or whether species were marine or freshwater. Finally, we used the
104 magnitude of the difference between acclimation temperature (T_a) and CT_{max} , which
105 we denoted delta temperature ($\Delta T = \text{CT}_{\text{max}} - T_a$), as an indication of the capacity to
106 increase CT_{max} depending on the acclimation temperature, and evaluated if it was
107 linked to intraspecific variation in CT_{max} . All of the results were based on a
108 phylogenetically informed analysis (phylogenetic least squares regression, PGLS,
109 see Methods), to establish how patterns in the extent of variation were linked to
110 evolutionary thermal history of the species.

111 There were significant differences in intraspecific variation in thermal tolerance in the
112 two latitudinal groups (Figure 2). Freshwater tropical species showed lower
113 intraspecific variation in CT_{max} (\log_{10} S.D. CT_{max}) than temperate (tropical species:
114 PGLS, $t = -2.054$, $p = 0.041$, Figure 2). Species from northern hemisphere species
115 had significantly lower variation in \log_{10} S.D. CT_{max} than southern ones (PGLS, $t =$
116 2.318 , $p = 0.022$; Figure 2A). Marine species did not differ from freshwater species
117 (Figure 2B, PGLS, $t = -1.683$, $p = 0.094$). The ΔT had no significant association with
118 \log_{10} S.D. CT_{max} (PGLS, $t = 1.972$, $p = 0.05$; Figure S1.). There was no interaction
119 between latitude and ΔT on \log_{10} S.D. CT_{max} . Phylogenetic relatedness among
120 species contributed strongly to observed variation in \log_{10} S.D. CT_{max} (PGLS, $\lambda =$
121 0.553 , $F_{6,195} = 4.397$, $p < 0.001$, $R^2 = 11.92$; Figure S3).

122

123



142 **Figure 2| Intraspecific variation in CT_{max} (\log_{10} transformed standard deviation**
143 **CT_{max}) divided into either temperate (148 species) or tropical (55 species). (A)**
144 **Separated by hemisphere, Northern (132 temperate, 33 tropical species) or**
145 **Southern (16 temperate and 22 tropical species). (B) Separated into freshwater (106**
146 **temperate, 21 tropical species) and marine (42 temperate, 34 tropical species).**

147 These results show that freshwater tropical species have reduced within-species
148 variation in thermal tolerance compared to temperate species. If this reflects a
149 reduced capacity for phenotypic plasticity, this will increase their sensitivity to
150 warming in the short term. If it reflects diminished heritable genetic variation, this will
151 decrease adaptability and evolvability to a warmer and more thermally stressful
152 future, over generational timescales. That is, the lower intraspecific variation in CT_{max}
153 in freshwater tropical as compared to temperate species (Figure 2) renders the
154 former especially vulnerable to future warming, in particular to extreme events ^{24,25}
155 (Figure 1). This will compound the vulnerability of tropical species that derives from
156 living near their upper thermal limits ^{4,6,7,26}.

157 The fact that variation in thermal tolerance was more pronounced and variable in the
158 northern compared to southern hemisphere could be the result of two phenomena:
159 1) greater thermal variability in the northern hemisphere ^{4,6}; or 2) a relative paucity of
160 data for the southern hemisphere ²⁷. Nevertheless, the effect of hemisphere had a
161 positive influence on intraspecific variation in CT_{max} . Therefore, local thermal
162 conditions experienced by species are determinant in setting the natural individual
163 variation within populations.

164 The strong phylogenetic signal for the extent of intraspecific variation in CT_{max} is
165 presumably because many families contain species with a relatively common history
166 of thermal adaptation (see Figure S3). That is, they have occupied similar thermal
167 regimes within temperate or tropical habitats. In particular, there is a latitudinal effect
168 on family distributions, with some families only being present in temperate (e.g.
169 Gadidae) or tropical (e.g. Apogonidae) habitats, although some cosmopolitan
170 families have species in both (e.g. Gobiidea, Blennidae) (Figure S2). In addition to
171 the geographic collinearity that may be occurring with some families, the
172 phylogenetically based differences in intraspecific variation among species may
173 cause evolutionary constraints on evolvability in the face of ongoing warming and
174 exposure to extreme events. The extent of such constraints is not clear and would
175 depend on the exact genes affecting thermal tolerance and how these are
176 represented within each family ¹¹. Further highlighting how temperature regime may
177 shape evolutionary trajectories within closely related species or those with a
178 common ancestor, with potential consequences for their vulnerability to thermal
179 stress.

180 This evidence for higher vulnerability of tropical species to climate variability and
181 extreme marine warming events²⁸ may have numerous ecological implications
182 beyond simple tolerance thresholds. Tropical species may be obliged to seek
183 thermal refugia in colder areas if these are available, potentially changing community
184 structures^{9,29}; such distribution shifts could have major ecological consequences
185^{30,31}. Overall, the extent of intraspecific variation in CT_{max} must be considered in
186 models that project impacts of warming on fishes. Intraspecific variation for
187 tolerance in other environmental conditions such as hypoxia and acidification would
188 be the next step for future research. Further research should focus on the
189 mechanisms that underly latitudinal variation in CT_{max} and whether these reflect
190 universal principles across all species.

191 **Methods:**

192 **Dataset and data selection process.** We used the data on CT_{max} in marine,
193 brackish and freshwater fish species (2722 observations unimputed data set)
194 published by⁴. We performed a three-step selection procedure to identify the
195 species for this study. First, we excluded data where CT_{max} was measured using
196 death as an endpoint (1256 observations) as these do not correspond to the
197 accepted definition of CT_{max} (loss of equilibrium but not death)³², so the
198 temperatures recorded will have exceeded the critical threshold. Second, we
199 excluded polar species because of the sample size (n = 5) and discarded brackish
200 water species because no indication was given about the nature of the brackish
201 habitat (e.g. lagoon, estuary or others). Third, several species were tested at
202 different acclimation temperatures resulting in multiple CT_{max} measures for the same
203 species. We therefore took CT_{max} values measured at the lowest or mid-point tested
204 acclimation temperature with the largest sample size of individuals used. This data
205 selection procedure produced a dataset of 203 fish species for which we have S.D.
206 of their CT_{max} (standard deviation).

207 **Calculation of delta temperature.** We calculated the ΔT

$$208 \quad \Delta T = T_{CTmax} - T_a$$

209 The ΔT defines the distance from thermal acclimation to thermal tolerance limit,
210 providing an index of vulnerability to acute heating¹⁰. This accounts for the fact that
211 acclimation temperature is often asymptotically linked to CT_{max} ^{15,24}.

212 **Data analysis.** Analyses and models were made in R (3.4.4, R Foundation for
213 Statistical Computing) using the phylogenetic generalized least squared method^{33,34}
214 (PGLS, with caper package³⁵. Model selection was completed by AIC values using
215 the AIC function estimating the best model fit (see Suppl. Table 1). The phylogeny of
216 203 fish species was found and generated from the comprehension tree of life
217 (Suppl. Figure S3)³⁶ using the “rotl” package³⁷. A measure of phylogenetic
218 correlation, λ , the degree to which this trait evolution deviates from Brownian motion
219³⁸, was evaluated by fitting PGLS models with different values of λ to find that which
220 maximized the log-likelihood of the best-fitted model. The level of statistical
221 significance was set at alpha = 0.05.

222 **Phylogenetic analysis.** This was performed by PGLS on the 203 species’ specific
223 geographical location, habitat, ΔT and number of individuals measured. As fishes’
224 physiology is dependent on the environmental thermal conditions, hemisphere was
225 incorporated into the model because of the significant differences in thermal
226 variability between the two hemispheres⁶, with the north having higher thermal
227 variation than the south²⁷. Due to the effects of local thermal variation on fish
228 thermal physiology, we included an interaction term between latitudinal groups
229 (tropical versus temperate) and the ΔT . We also conducted general linear model
230 (GLM) analysis to exclude the effect of phylogeny on the outcome of the observed
231 variation in $\log_{10} S.D.CT_{max}$, testing the individual effects of our variables in the
232 model (suppl. Table 3).

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336 **Authors contributions**

337 J.J.H. Nati, M.B.S. Svendsen, S. Marras, S.S. Killen, J.F. Steffensen, D.J. McKenzie,
338 P. Domenici designed the study. J.J.H. Nati and S.S. Killen performed the statistical
339 analyses. J.J.H. Nati wrote the manuscript. J.J.H. Nati, M.B.S. Svendsen, S. Marras,
340 S.S. Killen, J.F. Steffensen, D.J. McKenzie, P. Domenici revised the manuscript.

341 **Competing financial interests**

342 The authors declare to have no financial interests