1 **Does heat tolerance actually predict animals’ geographic thermal limits?**

2 ***Agustín Camacho¹, Miguel Trefaut Rodrigues², Refat Jayyusi³, Mohamed**
3 **Harun⁴, Marco Geraci^{5,6}, Catarina Vinagre^{7,8}, Miguel Carretero^{9,10}, Miguel**
4 **Tejedo¹.**

5 1 Departamento de Ecología Evolutiva, Estación Biológica de Doñana, CSIC, Av.
6 Américo Vespucio s/n, 41092, Sevilla, Spain.

7 2 Laboratório de Herpetologia, Departamento de Zoologia. Instituto de Biociências,
8 USP. Rua do Matão, trav. 14, nº 321, Cidade Universitária, São Paulo - SP, CEP:
9 05508-090, Brasil.

10 3 School of Life Sciences, Arizona State University, USA.

11 4 Administração Nacional das Áreas de Conservação. Ministério da Terra, Ambiente e
12 Desenvolvimento Rural. Av. Francisco Orlando Magumbwe. 2HHV+66 Maputo,
13 Mozambique.

14 5 MEMOTEF, School of Economics, Sapienza – University of Rome, Italy.

15 6 Department of Epidemiology and Biostatistics, Arnold School of Public Health,
16 University of South Carolina, US

17 7 CCMAR - Centre of Marine Sciences, University of Algarve, Faro, Portugal

18 8 MARE - Centro de Ciências do Mar e do Ambiente, Faculdade de Ciências,
19 Universidade de Lisboa, 1749-016 Lisboa, Portugal

20 9 CIBIO-InBIO, Universidade do Porto, Campus de Vairão, Rua Padre Armando
21 Quintas, 4485-661 Vairão, Portugal

10 Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do
Campo Alegre, 4169-007 Porto, Portugal

Keywords: Climatic vulnerability, Geographic thermal limits, Heat tolerance, Animals,
Endotherms, Ectotherms.

Abstract

To understand species’ climatic vulnerability, our measures of species’ thermal
tolerance should predict their geographic thermal limits. Yet, this assumption is
ungranted. We tested if animals’ heat tolerance restrict the warmest temperatures they
can live at (Tmax), distinguishing among species differently challenged by their
thermal environment. For that, we compiled 2350 measurements of species’ heat
tolerance indexes and corresponding Tmax, measured at different microhabitats. We
show that reptiles, a flagship for climatic vulnerability studies, are particularly
unbounded by their heat tolerance. Contrarily, tolerance restricted marine fish’
geography in a non-linear fashion which contrasts with terrestrial taxa. Behavioral
tolerance indexes, widely used to predict vulnerability, predicted Tmax inconsistently
across Tmax indexes, or were inversely related to it. Animals’ heat tolerance restricts
geographic limits more strongly for more thermally challenged species. In turn, factors
uncoupling heat tolerance and Tmax (plasticity, thermoregulation, adaptation) should
be more important for less thermally challenged species.

43 **Significance Statement**

44 To identify climatic vulnerability, heat tolerance indices need to predict species’
45 thermal limits to geographic distribution. Yet, we show that heat tolerance predicts
46 geographic limits quite heterogeneously, depending on taxa, the type of measure of
47 heat tolerance and how challenged are species at their hottest known location.
48 Particularly, reptiles, a flagship of vulnerability studies, were less bounded by tolerance
49 than taxa regarded as more capable to evade high temperatures, due to their capacity to
50 evaporate water, find refuge, or migrate (Ex. amphibians, arthropods, birds and
51 mammals). Measures of species’ behavioral heat tolerance may still need to improve.
52 Factors thought to uncouple thermal tolerance and geographic limits should be stronger
53 for less thermally challenged species.

54

55 **Main**

56 A reliable identification of the climatic vulnerability of populations is crucial to
57 correctly allocate the huge investments necessary to preserve biodiversity from climate
58 warming¹. Previous evaluations have used different thermal tolerance indexes,
59 searching for global patterns in species’ vulnerability^{2,3,4,5,6,7}, that rely on a key
60 assumption.

61 This is that heat tolerance restricts the sites supporting individual’s survival,
62 dispersal, or population’s growth, to the level of establishing the warm edges of species’
63 geographic distribution⁸. This assumption, is however ungranted for most animal
64 groups, since they readily decouple their body temperatures from exceedingly hot
65 temperatures across their ranges^{6,9,10}, potentially buffering the evolution of heat
66 tolerance itself^{11,12}. Several other factors may also unlink species’ geographic limits
67 from their known measures of heat tolerance. For example, local adaptation at species’
68 warm edges, due to exposure to relatively extreme temperatures¹³, plasticity and
69 adaptation in heat tolerance traits^{14,15}, species interactions blocking dispersal^{16,17},
70 incorrect estimation of heat tolerance^{18,19} and environmental temperatures²⁰, or the
71 wallacean shortfall.

72 Correctly predicting temperatures that limit species’ distribution is imperative to
73 identify the conditions that set species’ geographic warm edges, where further climatic
74 warming should make species’ populations’ dwindle^{21,22}. However, previous global
75 patterns of geographic variation in thermal tolerance and climatic risk have compared
76 species’ heat tolerance with the thermal environment of the sampling sites of the
77 measured individuals^{6,21,23}. This conservative approach helps avoiding the outlined
78 decoupling factors. However, it means we still do not know whether heat tolerance,

79 insofar measured in animals, widely predicts the environmental temperatures that
80 restrict species' geographic distribution, or how it does so for most groups.

81 We tested whether species' thermal tolerance predicts maximum environmental
82 temperatures at each species' known warm edges during the hottest time of the year
83 (hereafter Tmax, see methods). Both, tolerance and Tmax have been estimated using
84 many indexes, often associated to specific taxa (see methods). Different tolerance
85 indexes describe different levels of thermal stress for organisms (ex. acute to
86 chronic²⁴). Thus, we assorted these indexes within three main groups that induce
87 declining levels of thermal stress (Thermal limits, upper limits to optimal physiological
88 temperatures, and indexes of behavioral tolerance, see methods), and taxa. We used
89 quantile mixed models to separate species according to how much Tmax would
90 challenge their heat tolerance. Thus, higher quantiles represent species that reach higher
91 Tmax at their geographic warm edges, given their heat tolerance levels.

92 Our first group of heat tolerance indexes, the physiological thermal limits
93 (hereafter CTmax, from Critical Thermal Maximum), represent temperatures that can
94 kill during acute exposures (i.e. minutes, See methods). The CTmax does restrict the
95 highest Tmax values (i.e., significantly predicts its 90th percentile) across marine fish,
96 and terrestrial ectotherms, with the exception of reptiles (Fig.1, Figs. S2: A B C, Table
97 1). While results for Marine fish are robust across Tmax indexes (Ocean's surface and
98 mid depth temperatures), the relationships of terrestrial ectotherms varied more across
99 Tmaxexp, Tmaxprot and Tmaxair, estimated at exposed and protected microhabitats,
100 and in the air, respectively (Tables S1: A, B, C; Figs S2: A, B, C, See methods). Thus,
101 CTmax can be used to spot species' climatic limits for the distribution of ectotherms
102 from different realms, but with special caution for terrestrial species as Tmax may be
103 unrelated to CTmax. This is particularly true for reptiles, a flagship group for the

104 climatic vulnerability problem, whose CTmax was less strongly or not related to Tmax
105 (Table 1).

106 Studies comparing species' heat tolerance and the climate of collection sites
107 have portrayed arthropods and amphibians as especially able to decouple their body
108 temperatures from high environmental temperatures (ex. hiding in small cracks or
109 evaporating body water^{6,21}). Yet, their heat tolerance predicts their geographic thermal
110 limits more strongly than reptiles' heat tolerance. Compared to the former, reptiles
111 exhibit similar CTmax acclimation capacities²⁵, and their geographic distribution seems
112 not contrastingly solved. Also, studied arthropods show less constrained CTmax
113 evolution than Squamates²⁶, being able to change their CTmax more than two degrees
114 in three generations. In turn, reptiles do have lower body water loss rates²⁷ and often
115 bigger size, compared to amphibians and arthropods, respectively. The large variation
116 shown in water loss by this group²⁸ should help identifying the importance of this trait
117 for CTmax-distribution relationships.

118 Biotic interactions may limit range more strongly at warmer edges, compared to
119 cold edges⁴⁴. We did not account for biotic interactions in our analysis and thus it is
120 either possible that 1) biotic interactions contribute to blur tolerance-Tmax
121 relationships at less challenging warmer edges, 2) intensify the importance of a
122 constitutively high thermal tolerance at more challenging warmer edges, or 3) there is
123 no interaction between these factors¹⁷.

124 Accordingly, there should be a feedback between Tmax at warm edges and
125 thermal tolerance, where encountering more challenging Tmax may select for higher
126 heat tolerance, and it in turn may help the expansion of warm distribution edges. In a
127 context of climatic warming, warm edges can be either the trailing fronts of thermally
128 challenged species or the advance fronts for not so challenged ones. Species more

challenged by the new Tmax conditions may need to evolve higher heat tolerance. In turn, at less challenging ones, a plastic heat tolerance might be more useful.

The strong restriction of CTmax on geographic thermal limits of marine fish (Fig.1, Table 1) agrees with their capability to full fill their thermal niches across latitudinal ranges. The CTmax of warm-adapted fish (CTmax > 35 °C) surpasses sea surface's thermal maxima registered in geographic datasets (~35 °C, Sbrocco & Barber 2013 29). This and the very low CTmax of polar fish³⁰, strongly restricting Tmax, leads to strong and non-linear relationships between CTmax and Tmax for this group (Fig. 1, Supporting figures, Supporting tables set 1). CTmax levels over maximum sea Tmax should facilitate fish dispersal across equatorial coasts, where the sea is warmest²¹. This physiological liberation helps explaining why fish species distributed through the equator often have larger geographic ranges³¹. The highest CTmax among marine fish likely have evolved in response to selective pressures existing at globally hyperthermic microhabitats (ex. coastal rock pools exceeding 41 °C³²).

Our second group of tolerance indexes identify thermal levels over which physiological maintenance costs become especially high. Long exposures to higher temperatures undermine sustained performance, energy assimilation and hydration and can be ultimately lethal (see methods). Among endotherms, these levels have been more often measured as the upper limit of the thermoneutral zone (UTNZ, see methods), while in lizards, they have been often represented by the body temperatures for fastest sprint speed (Topt, See methods). In agreement with Khaliq et al⁴ endotherms' warm edges are generally predicted by their UTNZ. Yet, again, squamates appear again as less, or no geographically restricted by their optimal temperatures (see Figs. 1, and S2 A, B, C, D, and Tables S1). Apart from the factors mentioned in the introduction, endotherms might be more powerful dispersers than lizards, but still

blocked by too hot temperatures. Also, hiding from exceedingly high temperatures (ex. aestivating³³) is less costly for lizards than for endotherms, suggesting they rely more on heat tolerance for extending their ranges than lizards, this seems to be particularly true for birds, less able to use underground refuges.

Behavioral indexes, estimated for different ranges of temperatures voluntarily tolerated by animals, could provide less stressful, more comparable, and readily measurable indexes of heat tolerance for endotherms and ectotherms (ex. Panting³⁴). We made a compilation of these indexes for reptile species (mostly lizards), where they have been most extensively used to assess climatic vulnerability^{3,9}. Specifically, we obtained data on species' panting temperatures, voluntary thermal maxima, maximum preferred temperatures, maximum field temperatures, and both, the upper set point and the mean of body temperatures recorded for active individuals (see methods). However, behavioral traits predicted Tmax quite heterogeneously among tolerance and Tmax indexes (See below, and Tables S1 A, B, C, D). The weakest predictors were panting and the maximum of preferred temperatures. Panting costs much body water, thus chronic use of this behavior can lead to progressive decrement in body condition³⁵. Even drought-adapted species are reluctant to pant when dehydrated³⁴. Thus, this behavior might only help to avoid instant heat shock, rather than to survive stressfully hot, and often dry, periods at warm edges. Instead, measures of maximal preferred temperatures traditionally carry over many methodological pitfalls¹⁸, affecting their use for this end.

Instead, the most robust predictors of Tmax where the maximum field body temperatures and the mean preferred body temperatures. The latter shows a blurry relationship with Tmax (Fig. 1J) and small slopes compared to other behavioral indexes (Fig. 3). Thus, the significant correlations are likely favored by the much larger data

179 availability. Still, the former might provide particularly useful data. Lizards adjust their
180 activity levels and active body temperatures with respect to limiting factors, such like
181 hydration state³⁶. Thus, if collected in a controlled fashion (for animals under
182 environmental distress), maximum field body temperatures might integrate these
183 factors and show which temperatures actually limit species' activity and geographic
184 warm edges. The case of the upper set point is also interesting. It negatively correlates
185 with Tmax (Fig. 1, Table 1, and Tables S1, A, B, C, D) which makes it less useful to
186 predict climatic vulnerability. Since mean body temperatures showed blurry but always
187 positive relations with Tmax, lizards living at particularly cold distribution edges might
188 raise their upper set point. Doing this should boost their growth rates³⁷ and locomotor
189 function³⁸ within still tolerable thermal levels. Our results highlight the need of field
190 evaluations of restrictions predicted by behavioral indexes, which are so far rare^{3,39}.

191 Among thermal tolerance indexes, Tmaxexp exhibited stronger correlations
192 with thermal tolerance, than Tmaxprot and Tmaxair. Steeper slopes are expected for
193 Tmaxexp, given that its range of variation is much wider than Tmaxprot and Tmaxair
194 (Ranges: Texp: 53.8, 26.2-80; Tprot: 31.8, 6.4-38.2 Tair: 40.8, 6.4-47.2). These
195 temperatures may vary differently with latitude, with Tmax prot being usually higher at
196 tropical regions⁶. However, when sampled across species' warm edges, they are
197 linearly and strongly correlated with each other across the warm edges of terrestrial
198 species (Fig. 3 A, Tables S2 A, B, C). Such covariation translates into varied sources of
199 thermal stress for terrestrial species at their geographic limits. For example, the more
200 environmental temperatures at exposed sites depart from thermal tolerance, the more
201 body heating rates should speed up⁴⁰. This should increase overheating risks for small
202 ectotherms when crossing exposed microhabitats during their daily activities⁴¹.
203 Likewise, at geographic warm edges of terrestrial species, Tmaxair, measured in the

shade, often reach levels close to CTmax (particularly for species of the 90th percentile). This means that individuals will likely experience sublethal temperatures for some periods even in the shade. In such situations, longer resistance to sublethal thermal levels should be particularly adaptive¹⁹. Finally, since Tmaxprot, measured at underground shelters lay well below the thermal tolerance of organisms (Fig. S2C; Table S2C), we propose that these shelters are often unavailable (Ex. For birds) or ineffective to extend species' ranges. Thus, shelters might be less useful as previously thought to protect them from warming-induced range contractions. When hidden for long periods, some species might decrease energy intake and mating rates³, limiting the utility of shelters for population persistence at warm edges. Differently from land, at the sea, Tmaxsurf and Tmaxmid correlate with great heteroscedasticity (Fig. 2B). Vertical thermoclines are smallest at the hottest and at the coldest sites (tropical and polar coasts), and largest at deeper seas. This situation hampers thermoregulation in these regions, making it more necessary to evolve a suitable CTmax to cross-by. In turn, at regions with steeper thermoclines in maximum temperatures, warm-adapted fish could extend their geographic warm edges via superficial and warmer waters, while cold adapted ones should use deeper layers to disperse.

Our global evidence (Fig.3, Table S2C) supports simulations predicting that CTmax might evolve more readily when species' physiology are more strongly hit by thermal extremes¹³. When the necessary data becomes available to account for plasticity, our discovered relationships might somewhat change, as the relationships between thermal tolerance and latitudinal ranges²³. However, such changes should be minor for the dispersal or persistence of species at their geographic warm edges, or otherwise it would have been unlikely to find so pervasive effects for the more challenged species. These results are further supported by the fact that warm-tolerant

animals living at high environmental temperatures show less plastic CT_{max}⁴². Despite heat tolerance is widely viewed as relatively insensitive to thermal gradients⁴³, it can covary strongly with challenging thermal extremes (Table 1, supporting tables set 1). In these situations, the strong interspecific covariation likely means that local adaptation is less capable of inducing geographic variation in heat traits (ex. between measured populations and populations at warm edges). Likewise, other restrictors to the evolvability of heat tolerance²⁶ should be less important for more heat challenged species, too. Particularly, behaviors that buffer exposures to thermal extremes (The Bogert Effect¹²).

Behavior and plasticity (i.e. acclimation) make use of spatiotemporal heterogeneity in temperatures²³, that may be relatively small at the hottest sites (ex. Fig. 2B; Sunday et al⁶). Specially given that temperatures are strongly correlated across microhabitats (Fig. 2B) at these edges. Thus, these traits might be less able to affect T_{max}-Heat tolerance relationships at challenging warm edges. In agreement, heat tolerance-T_{max} correlations were almost always weaker for species less challenged at their warm edges. The exceptions were two: First, the lizards' Upper set point showed stronger correlations at colder sites (Fig. 3), further supporting the idea of behavioral adjustments being more important at relatively cold sites (see above). The second exception was for T_{max} at Ocean's mid depths, which shows no clear pattern (Fig. 3). T_{max} indexes coming from high resolution climatic datasets (CHELSA and MARSPEC) behaved in parallel with T_{max} indexes coming from 5 min datasets (Microclim and Biooracle, Fig. 3). This indicates that our findings are robust to the scale of the climatic datasets.

References

- 253 1. Wintle, B. A. *et al.* Ecological–economic optimization of biodiversity
254 conservation under climate change. *Nat. Clim. Chang.* **1**, 355–359 (2011)
- 255 2. Deutsch, C. A. *et al.* Impacts of climate warming on terrestrial ectotherms
256 across latitude. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6668–6672 (2008)
- 257 3. Sinervo, B. *et al.* Erosion of lizard diversity by climate change and altered
258 thermal niches. *Science* **328**, 894–899 (2010)
- 259 4. Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K. & Pfenninger, M. Global
260 variation in thermal tolerances and vulnerability of endotherms to climate
261 change. *Proc. Biol. Sci.* **281**, 20141097 (2014)
- 262 5. Kingsolver, J. G., Diamond, S. E. & Buckley, L. B. Heat stress and the fitness
263 consequences of climate change for terrestrial ectotherms. *Funct. Ecol.* **27**,
264 1415–1423 (2013)
- 265 6. Sunday, J. M. *et al.* Thermal-safety margins and the necessity of
266 thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci.*
267 *U. S. A.* **111**, 5610–5615 (2014)
- 268 7. Comte, L. & Olden, J. D. Climatic vulnerability of the world’s freshwater and
269 marine fishes. *Nat. Clim. Chang.* **7**, 718–722 (2017)
- 270 8. Khaliq, I., Böhning-Gaese, K., Prinzinger, R., Pfenninger, M. & Hof, C. The
271 influence of thermal tolerances on geographical ranges of endotherms. *Glob.*
272 *Ecol. Biogeogr.* **26**, 650–668 (2017)
- 273 9. Meiri, S. *et al.* *Are Lizards Feeling the Heat?: A Tale of Ecology and Evolution*
274 *Under Two Temperatures.* (Universiti Malaysia Sarawak, 2013)
- 275 10. Buckley, L. B., Miller, E. F. & Kingsolver, J. G. Ectotherm thermal stress and
276 specialization across altitude and latitude. *Integr. Comp. Biol.* **53**, 571–581
277 (2013)

- 278 11. Huey, R. B., Hertz, P. E. & Sinervo, B. Behavioral drive versus behavioral
279 inertia in evolution: a null model approach. *Am. Nat.* **161**, 357–366 (2003)
- 280 12. Muñoz, M. M. & Bodensteiner, B. L. Janzen’s Hypothesis Meets the Bogert
281 Effect: Connecting Climate Variation, Thermoregulatory Behavior, and Rates
282 of Physiological Evolution. *Integr Org Biol* **1**, oby002 (2019)
- 283 13. Buckley, L. B. & Huey, R. B. How Extreme Temperatures Impact Organisms
284 and the Evolution of their Thermal Tolerance. *Integr. Comp. Biol.* **56**, 98–109
285 (2016)
- 286 14. Geerts, A. N. *et al.* Rapid evolution of thermal tolerance in the water flea
287 *Daphnia*. *Nat. Clim. Chang.* **5**, 665–668 (2015)
- 288 15. Herrando-Pérez, S. *et al.* Heat tolerance is more variable than cold tolerance
289 across species of Iberian lizards after controlling for intraspecific variation.
290 *Funct. Ecol.* **34**, 631–645 (2020)
- 291 16. Amundrud, S. L. & Srivastava, D. S. Thermal tolerances and species
292 interactions determine the elevational distributions of insects. *Glob. Ecol.*
293 *Biogeogr.* **29**, 1315–1327 (2020)
- 294 17. Cahill, A. E. *et al.* Causes of warm-edge range limits: systematic review,
295 proximate factors and implications for climate change. *Journal of Biogeography*
296 vol. 41 429–442 (2014)
- 297 18. Camacho, A. & Rusch, T. W. Methods and pitfalls of measuring thermal
298 preference and tolerance in lizards. *J. Therm. Biol.* **68**, 63–72 (2017)
- 299 19. Rezende, E. L., Bozinovic, F., Szilágyi, A. & Santos, M. Predicting temperature
300 mortality and selection in natural *Drosophila* populations. *Science* **369**, 1242–
301 1245 (2020)

20. Garcia, R. A., Allen, J. L. & Clusella-Trullas, S. Rethinking the scale and formulation of indices assessing organism vulnerability to warmer habitats. *Ecography* **42**, 1024–1036 (2019)
21. Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L. & Sunday, J. M. Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* vol. 569 108–111 (2019)
22. Wiens, J. J. Climate-Related Local Extinctions Are Already Widespread among Plant and Animal Species. *PLoS Biol.* **14**, e2001104 (2016)
23. Sunday, J. *et al.* Thermal tolerance patterns across latitude and elevation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20190036 (2019)
24. Hochachka, P. W. & Somero, G. N. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. (Oxford University Press, 2002).
25. Gunderson, A. R. & Stillman, J. H. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. Biol. Sci.* **282**, 20150401 (2015)
26. Hoffmann, A. A., Chown, S. L. & Clusella-Trullas, S. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* **27**, 934–949 (2013)
27. Ladd Prosser, C. *Comparative Animal Physiology, Environmental and Metabolic Animal Physiology*. (John Wiley & Sons, 1991).
28. Galliard, J. L. *et al.* A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Global Ecology and Biogeography* (2021) doi:10.1111/geb.13355
29. Sbrocco, E. J. & Barber, P. H. MARSPEC: ocean climate layers for marine spatial ecology. *Ecology* **94**, 979–979 (2013)

327 30. Somero, G. N. & DeVries, A. L. Temperature Tolerance of Some Antarctic
328 Fishes. *Science* vol. 156 257–258 (1967)

329 31. Stuart-Smith, R. D., Edgar, G. J. & Bates, A. E. Thermal limits to the
330 geographic distributions of shallow-water marine species. *Nat Ecol Evol* **1**,
331 1846–1852 (2017)

332 32. Vinagre, C. *et al.* Upper thermal limits and warming safety margins of coastal
333 marine species--Indicator baseline for future reference. *Ecol. Indic.* **102**, 644–
334 649 (2019)

335 33. Navas, C. A. & Carvalho, J. E. *Aestivation: Molecular and Physiological*
336 *Aspects*. (Springer Science & Business Media, 2010).

337 34. Parmenter, C. J. & Heatwole, H. Panting thresholds of lizards. IV. The effect of
338 dehydration on the panting threshold of *Amphibolurus barbatus* and
339 *Amphibolurus muricatus*. *J. Exp. Zool.* **191**, 327–332 (1975)

340 35. Conradie, S. R., Woodborne, S. M., Cunningham, S. J. & McKechnie, A. E.
341 Chronic, sublethal effects of high temperatures will cause severe declines in
342 southern African arid-zone birds during the 21st century. *Proc. Natl. Acad. Sci.*
343 *U. S. A.* **116**, 14065–14070 (2019)

344 36. Sannolo, M. & Carretero, M. A. Dehydration constrains thermoregulation and
345 space use in lizards. *PLoS One* **14**, e0220384 (2019)

346 37. Autumn, K. & De Nardo, D. F. Behavioral Thermoregulation Increases Growth
347 Rate in a Nocturnal Lizard. *J. Herpetol.* **29**, 157–162 (1995)

348 38. Bonino, M. F. *et al.* Running in cold weather: morphology, thermal biology,
349 and performance in the southernmost lizard clade in the world (*Liolaemus*
350 *lineomaculatus* section: *Liolaemini*: Iguania). *Journal of Experimental Zoology*
351 *Part A: Ecological Genetics and Physiology* vol. 315A 495–503 (2011)

352 39. Recoder, R. S., Magalhães-Júnior, A. & Rodrigues, J. Thermal constraints
353 explain the distribution of the climate relict lizard *Colobosauroides carvalhoi*
354 (*Gymnophthalmidae*) in the semiarid caatinga. *South Am. J. Herpetol.* (2018)
355 40. Bakken, G. S. Measurement and application of operative and standard operative
356 temperatures in ecology. *Am. Zool.* **32**, 194–216 (1992)
357 41. Camacho, A. *et al.* Interaction of morphology, thermal physiology and
358 burrowing performance during the evolution of fossoriality in
359 *Gymnophthalmini* lizards. *Funct. Ecol.* **29**, 515–521 (2015)
360 42. van Heerwaarden, B. & Kellermann, V. Does Plasticity Trade Off With Basal
361 Heat Tolerance? *Trends Ecol. Evol.* **35**, 874–885 (2020)
362 43. Araújo, M. B. *et al.* Heat freezes niche evolution. *Ecology Letters* vol. 16 1206–
363 1219 (2013)
364 44. Paquette, A. & Hargreaves, A. L. Biotic interactions are more often important at
365 species’ warm versus cool range edges. *Ecology Letters* (2021)
366 doi:10.1111/ele.13864

367 **Tables**

Group	Par.	10th percentile			50th percentile			90th percentile			N
		Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	
Thermal limits (CTmax)											
Fish	As.	31.13	0.33	<0.01	31.42	0.30	<0.01	33.334	1.12	<0.01	184
Fish	Inf.	27.59	1.01	<0.01	22.75	1.70	<0.01	15.195	1.96	<0.01	184
Fish	Sca.	3.38	0.44	<0.01	3.58	0.92	<0.01	6.195	1.73	<0.01	184
Arthropods	Slo.	0.34	0.14	0.02	0.49	0.14	<0.01	0.697	0.13	<0.01	193
Amphibians	Slo.	0.74	0.22	<0.01	0.86	0.23	<0.01	1.094	0.23	<0.01	167
Reptiles	Slo.	<0.01	0.24	0.99	0.22	0.24	0.34	0.385	0.25	0.130	248
Thermal limits for optimal performance (UTNZ for birds and mammals; Topt for lizards)											
Birds	Slo.	0.47	0.15	<0.01	0.697	0.15	<0.01	0.930	0.15	<0.01	103
Mammals	Slo.	0.11	0.15	0.48	0.282	0.15	0.07	0.514	0.15	<0.01	227
Lizards	Slo.	0.40	0.34	0.24	0.624	0.36	0.08	0.821	0.38	0.03	84
Indexes of behavioral thermal tolerance (lizards only)											
Panting T	Slo.	-0.13	0.50	0.784	0.164	0.59	0.781	0.372	0.62	0.555	63
VTM	Slo.	0.69	0.17	<0.01	0.833	0.18	<0.01	0.970	0.19	<0.01	285
Field max	Slo.	0.29	0.17	0.08	0.460	0.20	0.03	0.657	0.22	>0.01	36
PBT max	Slo.	0.47	0.63	0.46	0.727	0.57	0.20	1.059	0.57	0.067	63
USP	Slo.	-1.87	0.48	<0.01	-1.406	0.44	<0.01	-1.260	0.42	<0.01	68
Mean T	Slo.	0.06	0.09	0.46	0.261	0.08	<0.01	0.526	0.09	<0.01	884

368

369 **Table 1. Relationships between Tmax and Heat tolerance across animals.**

370 **Describes parameters, standard errors (SE) and p-values from nonlinear (marine**

371 **fish) and linear (other animal groups) quantile mixed models for the 10th, 50th, and**

372 **90th percentiles of Tmax conditional on indices of thermal tolerance. CTmax,**

373 **critical thermal maximum; UTNZ: upper thermoneutral zone limit; Topt: optimal**

374 **temperature for sprint speed; Panting T: body temperature that induces panting;**
375 **VTM: voluntary thermal maximum, body temperature that induces retreat; Field**
376 **max: maximum temperature observed in the field; PBT max: maximum body**
377 **temperature measured in a laboratory thermal gradient; USP: Upper Set Point**
378 **75th percentile of preferred temperatures, ; Mean T: mean body temperature of**
379 **active individuals; Par.=Parameter, As.=Asymptote, Inf.=Inflection point,**
380 **Sca.=Scale, Slo.=Slope, <N=species number per analysis. See methods for**
381 **definitions of tolerance indexes. Correlations' intercepts can be observed in Table**
382 **S1. Colors relate to quantiles in Fig. 1. Tmaxexp source: Microclim for terrestrial**
383 **spp./Bio-oracle for marine ones.**

384 **Figures**

385 **Figure 1. Upper thermal tolerance indexes capable of predicting Geographic**
386 **thermal limits (T_{max} : Maximum temperature available) among different animal**
387 **groups. A-C) Thermal limits as measured in marine fish, terrestrial arthropods,**
388 **amphibians, and terrestrial reptiles. E-G) Upper limits of physiologically optimal**
389 **temperatures measured in birds, mammals, and lizards. H-K) Indexes of**
390 **behavioral thermal tolerance measured in reptiles (see definitions in Table 1 and**
391 **methods). Dotted line represents a 1:1 relationship between thermal tolerance and**

392 **the geographic thermal limit. Solid lines represent the slopes of phylogenetically**
393 **detrended predictions of Tmax based on thermal physiology. Red, orange and**
394 **blue lines represent predicted 90th, 50th, and 10th conditional percentiles of Tmax**
395 **on thermal tolerance, respectively. Slashed lines represent non-significant**
396 **associations. See graphs for all heat tolerance and Tmax indexes in Fig. S2.**

397

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

Figure 2. Relationships among Tmax measured at different microhabitats of species' geographic warm edges. A panel shows relationships for terrestrial animals. B panel shows relationships for Marine fish. Whereas relationships for terrestrial species are strongly linear, in the sea, differences between mid and surface Tmax change across increasingly hot geographic thermal limits.

Figure 3. Effect sizes of relationships between thermal tolerance on the geographic thermal limit for species differently challenged by temperatures at their geographic thermal limit. Except for marine fish and the upper set point of lizards, thermal tolerance is more strongly linked to the geographic thermal limit of species reaching places with higher environmental temperatures with respect to their thermal tolerance (species at the 90th percentile, see methods). See Table 1 legend for definition of indexes, with three different measures of maximal

temperatures (Tmax protected, Tmax exposed and Tmax air). In the case of marine fish, instead of Tmaxair, we included a different estimate of Tmax exposed, see methods). See supporting tables set 2 for tests' results.

Methods

Estimation of Tmax for each species

Our dependent variable, Tmax, represents the maximum environmental temperature that each species will face off across its geographic distribution. In nature, Tmax may depend on each species' ecology and ability to thermoregulate¹. Because it is unknown which temperature most affects each species, we repeated the analyses over different Tmax estimates. For land species, we used: 1) Tmaxair, the temperature of shaded air at around 2m off the ground, as widely used in previous studies^{2,3}, 2) Tmax protected (Tmaxpro), estimated for 20 cm underground under fully shaded soil, and 3) Tmaxexp, describing the temperature of the surface of soil fully exposed to sun rays. For marine species, Tmaxsurf represent temperatures measured at the sea surface, and Tmax mid depth represents the temperature at the middle of the water column. For each species, we estimated Tmax from global raster layers describing current climate. Tmax is the mean of maximum temperatures of the hottest month, measured across a period of 20 recently past years. This method for summarizing extreme temperatures is known as bioclim 5⁴. Estimates of climatic vulnerability may vary with the resolution of the climatic layer used⁵. Therefore, we used the highest resolution available for each type of Tmax estimation. Pursuing this aim, for on land Tmax, we extracted Tmaxair from the CHELSA dataset (30 sec resolution). In turn, Tmaxpro and Tmaxexp were extracted from the microclim dataset (soil substrate, 5 min resolution⁶). For marine Tmax, we extracted sea surface from the MARSPEC dataset (30 sec resolution, MARSPEC²⁹, and also, Tmax from sea surface and at mid depth from the

Bioracle dataset (5 min resolution⁷). Since we are using datasets with different scales, different trends would be expected for their associated correlations. However, as we discuss in the main text, we found quite the opposite: datasets with different scale behaved similarly.

To estimate the Tmax of each species' hottest location, we extracted these data for each location of each species and kept the maximum value. This temperature is hereafter used as the geographic thermal limit, and the location defined as the warm edge (AKA: trailing edge^{8,9,10}). We obtained all known locations for each species with thermal tolerance data from the Global Biodiversity Information Facility (GIBF). We only used locations associated to specimens deposited in scientific collections. These records were cleaned for likely captivity sites (ex. zoos), outliers, lon/lat zeroed records, mirrored records, records in the sea for terrestrial species, and vice versa. We then obtained the maximum Tmax across all records of each species and its corresponding Tmax value (see supporting material-DATA). In the main text, we show results for Tmaxexp and Tmaxsurf (temperatures for exposed soil and sea surface), following recommendations by Sunday et al¹¹, p4, 2nd paragraph), but results for other indices can be found in the supporting tables set 1 and in the supporting figures file. Besides, we discuss, in the main text, the robustness of our findings to using different Tmax indexes.

Indexes of thermal tolerance

Our compiled thermal tolerance indexes represent a continuous gradient of thermal stress intensity, namely, from an acute extreme to a chronic medium heat stress, (¹², pp: 331). Thus, we grouped thermal tolerance indexes accordingly, in three groups. Our first group represents physiological thermal limits. These are temperatures that kill almost immediately. For terrestrial ectotherms, they have been measured as the

temperature that blocks individuals' locomotion (CTMAX, from Critical Thermal Maximum¹³). We obtained them for 200 terrestrial arthropods, 151 adult amphibians, and 300 reptiles (Squamates and tortoises). For marine fish, we obtained data on the loss of equilibrium (LOE, 121 species), and of the lethal limit (63 species), namely, the mean of the first temperature that causes death in a fish during experimental heating¹⁴.

Our second group of indexes coarsely represent the upper thermal limits for optimal physiological performance. In lizards, these limits have been often measured as the body temperature that maximizes sprint speed (Topt³, 85 species in our dataset). Since, many physiological processes exhibit lower thermal optima, lizards tend to select temperatures below Topt, and even their sprint speed sharply decreases above that temperature¹⁵. In turn, among endotherms, these limits correspond to the Upper Thermoneutral Zone limits (UTNZ), an environmental temperature over which metabolic and water loss costs sharply increase¹⁶. We used measures for 105 birds and 232 mammals¹⁷.

Our third group of indexes describes body temperatures voluntarily tolerated by lizards, a widely studied organism in thermal physiology. For lizards, upper voluntary thermal limits are often represented by either the display of panting behavior¹⁸ (herein called Panting temperatures, 63 species), or the maximum body temperature measured in active individuals of a species. This latter index has been either measured in the field (herein called Field max, 285 species^{19,20}, in laboratory thermal gradients (PBT max, for maximum preferred body temperatures, 36 species), or in heating chambers (Voluntary Thermal Maximum (VTM), 63 species). Others have used either the 75th percentile (68 species^{21,22}), or the mean (884 species²³) of body temperatures, measured on active lizards. Also, to avoid misrepresentation of voluntary limits in field maximum

temperatures, we excerpted field maximum temperatures measured in less than 10 individuals per species.

Most of these datasets use previous compilations for adult animals by various studies^{3,11,14,17,18,20,22,23,24,25}. Further, we added new data whenever possible^{26,27,28} (more on the supporting material-data).

We also added data from a field trip to Mozambique that includes new data for the VTmax of 28 lizard species (see supporting methods file for details). Creating a fully comprehensive and up-to-date dataset is not the aim of this study. That would be very difficult because new data appear frequently and each species follows a long process for gathering and checking the locality records, obtain the three different Tmax estimates, and then re-run the analyses. Raw data and sources can be accessed at the supporting Data-tables. All these indexes have been measured in sites that may strongly differ in climatic conditions from each species' warm edges. Precisely, the purpose of this study is to evaluate whether these indexes, in the way they have been measured, can still predict the climatic conditions at species' warm edges.

Previous studies have tried to correct tolerance levels for effects of acclimation temperature, ramping rates or exposure duration²⁹. Geographical patterns in thermal limits appears to be not strongly affected by covariates of acclimation temperature and ramping rate or exposure duration²⁹. Besides, estimates for these effects are very rare and species' specific^{30,31}, which limits the effectivity of corrections for whole groups from data on a few species. Since acclimation is one of the multiple confusion factors discussed in the main text, the cases in which heat tolerance predicted Tmax less successfully (ex. among reptiles, or for lower quantiles), might indicate where future corrections like the these might provide more advance in the future.

Analyses

Quantile correlations between Tmax and heat tolerance indexes

The relationship between the 10th, 50th, and 90th percentiles of the geographic thermal limit distribution and thermal tolerance was modelled via linear quantile mixed models (LQMM) [1] separately for all animal groups, except for fishes. For fishes only, a logistic nonlinear quantile mixed model (NLQMM) [2] was used instead. (Results in Table 1 and Figure 1).

The random effects specifications for LQMM, NLQMM, and AQMM were at the genus level. Standard errors and 95% confidence intervals were obtained via bootstrap with 199 replications. The analysis was conducted in R version 4.0.0 [4] using the lqmm [5], nlqmm [6], and aqmm [7] packages.

Correlations among Tmax indices

We used the same specifications to correlate the different Tmax indices among them. For terrestrial species, this included the following correlations: Tmaxexp X Tmax prot, Tmaxexp X Tmaxair, Tmaxprot X Tmaxair. For marine fish, we correlated Tmaxfsurf with Tmaxmid depth, both obtained from the Bio-Oracle dataset. Results are shown in the supporting tables set 2 and in Figure 2B.

Test of differences in effect sizes across quantile correlations of Tmax-Heat tolerance.

To test if tolerance impacts Tmax more strongly when species are more challenged by environmental temperatures, we tested whether the effect size of the quantile correlations would change across quantiles. For mixed models, the effect size can be represented by the slope of linear models³², and we used the scale of the non-linear mixed models for fish. For that, we fitted another mixed model (lmer function from lme4 package³³). Here, quantile was the fixed effect (categorical, three levels), and the grouping variables were: dataset (five levels) and group (thirteen levels). In this fashion, we directed all the available degrees of freedom (from 117 effect size estimates) to test for a general effect of the fixed effect (i.e across taxonomic groups, indices, and datasets), keeping comparisons of effect sizes within the same dataset and

565 taxonomic group. For example, the effect size for the 10th quantile correlation obtained
566 for fish’s Tmax surf-BIORACLE dataset was compared only with the correlations for
567 the 50th and 90th of the same dataset and animal group. Finally, we calculated statistical
568 significance for the fixed effects only, using the Kenward-Roger method for p-value
569 calculation (LmerTest³⁴; and pbkrtest³⁵, packages). Full results are given in the
570 supporting tables 2 (2D).

571 Scripts for all analyses are available under request.

572 **Methods References**

573 1. Sunday, J. M., Bates, A. E. & Dulvy, N. K. Thermal tolerance and the global
574 redistribution of animals. *Nat. Clim. Chang.* **2**, 686–690 (2012)

575 2. Deutsch, C. A. *et al.* Impacts of climate warming on terrestrial ectotherms
576 across latitude. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6668–6672 (2008)

577 3. Huey, R. B. *et al.* Why tropical forest lizards are vulnerable to climate warming.
578 *Proc. Biol. Sci.* **276**, 1939–1948 (2009)

579 4. Fick, S. E. & Hijmans, R. J. WorldClim 2: new 1 □ km spatial resolution climate
580 surfaces for global land areas. *Int. J. Climatol.* **37**, 4302–4315 (2017)

581 5. Garcia, R. A., Allen, J. L. & Clusella-Trullas, S. Rethinking the scale and
582 formulation of indices assessing organism vulnerability to warmer habitats.
583 *Ecography* **42**, 1024–1036 (2019)

584 6. Kearney, M. R., Isaac, A. P. & Porter, W. P. Microclim: Global estimates of
585 hourly microclimate based on long-term monthly climate averages. *Sci Data* **1**,
586 140006 (2014)

587 7. Assis, J. *et al.* Bio □ ORACLE v2.0: Extending marine data layers for
588 bioclimatic modelling. *Global Ecology and Biogeography* vol. 27 277–284
589 (2018)

- 590 8. Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L. & Sunday, J. M.
591 Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*
592 vol. 569 108–111 (2019)
- 593 9. Donelson, J. M. *et al.* Understanding interactions between plasticity, adaptation
594 and range shifts in response to marine environmental change. *Philos. Trans. R.*
595 *Soc. Lond. B Biol. Sci.* **374**, 20180186 (2019)
- 596 10. Stuart-Smith, R. D., Edgar, G. J. & Bates, A. E. Thermal limits to the
597 geographic distributions of shallow-water marine species. *Nat Ecol Evol* **1**,
598 1846–1852 (2017)
- 599 11. Sunday, J. M. *et al.* Thermal-safety margins and the necessity of
600 thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci.*
601 *U. S. A.* **111**, 5610–5615 (2014)
- 602 12. Hochachka, P. W. & Somero, G. N. *Biochemical Adaptation: Mechanism and*
603 *Process in Physiological Evolution.* (Oxford University Press, 2002).
- 604 13. Lutterschmidt, W. I. & Hutchison, V. H. The critical thermal maximum: history
605 and critique. *Canadian Journal of Zoology* vol. 75 1561–1574 (1997)
- 606 14. Comte, L. & Olden, J. D. Climatic vulnerability of the world’s freshwater and
607 marine fishes. *Nat. Clim. Chang.* **7**, 718–722 (2017)
- 608 15. Martin, T. L. & Huey, R. B. Why ‘suboptimal’ is optimal: Jensen’s inequality
609 and ectotherm thermal preferences. *Am. Nat.* **171**, E102–18 (2008)
- 610 16. McNab, B. K. & Morrison, P. Body Temperature and Metabolism in Subspecies
611 of *Peromyscus* from Arid and Mesic Environments. *Ecol. Monogr.* **33**, 63–82
612 (1963)
- 613 17. Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K. & Pfenninger, M. Global
614 variation in thermal tolerances and vulnerability of endotherms to climate
615 change. *Proc. Biol. Sci.* **281**, 20141097 (2014)

18. Heatwole, H., Firth, B. T. & Webb, G. J. W. Panting thresholds of lizards—I. Some methodological and internal influences on the panting threshold of an agamid, *Amphibolurus muricatus*. *Comparative Biochemistry and Physiology Part A: Physiology* vol. 46 799–826 (1973)
19. Cowles, R. B. & Bogert, C. M. *A Preliminary Study of the Thermal Requirements of Desert Reptiles*. (American Museum of Natural History, 1944).
20. Brattstrom, B. H. Body Temperatures of Reptiles. *American Midland Naturalist* vol. 73 376 (1965)
21. Hertz, P. E., Huey, R. B. & Stevenson, R. D. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**, 796–818 (1993)
22. Sinervo, B. *et al.* Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894–899 (2010)
23. Meiri, S. *et al.* *Are Lizards Feeling the Heat?: A Tale of Ecology and Evolution Under Two Temperatures*. (Universiti Malaysia Sarawak, 2013)
24. Curry-Lindahl, K. Thermal ecology of the tree agama (*Agama atricollis*) in Zaire with a review of heat tolerance in reptiles. *J. Zool.* (1979)
25. Diele-Viegas, L. M. *et al.* Thermal physiology of Amazonian lizards (Reptilia: Squamata). *PLOS ONE* vol. 13 e0192834 (2018)
26. Garcia-Robledo, C., Chuquillanqui, H., Kuprewicz, E. K. & Escobar-Sarria, F. Lower thermal tolerance in nocturnal than in diurnal ants: a challenge for nocturnal ectotherms facing global warming. *Ecological Entomology* vol. 43 162–167 (2018)
27. Fanguie, N. A. *et al.* Temperature and hypoxia tolerance of selected fishes from a hyperthermal rockpool in the Dry Tortugas, with notes on diversity and behavior. *Caribb. J. Sci.* **37**, 81–87 (2001)

642

28. Vinagre, C. *et al.* Upper thermal limits and warming safety margins of coastal

643

marine species--Indicator baseline for future reference. *Ecol. Indic.* **102**, 644–

644

649 (2019)

645

29. Sunday, J. *et al.* Thermal tolerance patterns across latitude and elevation.

646

Philos. Trans. R. Soc. Lond. B Biol. Sci. **374**, 20190036 (2019)

647

30. Clusella-Trullas, S. & Chown, S. L. Lizard thermal trait variation at multiple

648

scales: a review. *Journal of Comparative Physiology B* vol. 184 5–21 (2014)

649

31. Camacho, A. & Rusch, T. W. Methods and pitfalls of measuring thermal

650

preference and tolerance in lizards. *J. Therm. Biol.* **68**, 63–72 (2017)

651

32. Geraci, M. & Bottai, M. Linear quantile mixed models. *Statistics and*

652

Computing vol. 24 461–479 (2014)

653

33. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects

654

Models Usinglme4. *Journal of Statistical Software* vol. 67 (2015)

655

34. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package:

656

Tests in Linear Mixed Effects Models. *Journal of Statistical Software* vol. 82

657

(2017)

658

35. Halekoh, U. & Højsgaard, S. A Kenward-Roger Approximation and Parametric

659

Bootstrap Methods for Tests in Linear Mixed Models - TheRPackagepbkrtest.

660

Journal of Statistical Software vol. 59 (2014)

661

662

Acknowledgements

663

Supporting fellowships for ACG: (PNPD-CNPq, process: 0001; Fapesp-BEPE, 015-

664

003, MSCAH2020: 897901), UIDB/04292/2020; UIDB/04326/2020), FCT/FEDER

665

(28014 02/SAICT/2017); MTR (FAPESP 2011/50146-6 and CNPq)

666

667

668 **Author contributions**

669 Conception: ACG/MTM, data access and fieldwork: ACG/RJ/MTR/MH, Analyses:
670 ACG/MG, Writing: ACG/MTM, Review: ACG/MTR/MG/CV/MC.

671 **Competing interest declaration**

672 The authors declare no competing interests.

673 **Additional information**

674 **Supplementary Information** is available for this paper.

675 Correspondence and requests for materials should be addressed to AC.

676 **Extended data figure/table legends.**