

1           **Thermal Tolerance Plasticity and Dynamics of Thermal Tolerance in *Eublepharis***  
2                           ***macularius*: Implications for Future Climate-Driven Heat Stress**

3 Emma White<sup>1</sup>, Solyip Kim<sup>1</sup>, Garrett Wegh<sup>1</sup>, Ylenia Chiari<sup>1,\*</sup>

4 1 George Mason University, Department of Biology, Fairfax, VA, USA.

5 YC ORCID #0000-0003-2338-8602

6

7

8 \*Author(s) for correspondence:

9 Ylenia Chiari

10 Dept. of Biology,

11 George Mason University,

12 Fairfax 22030, VA, USA

13 Email: [ychiari@gmu.edu](mailto:ychiari@gmu.edu)

14

15

16

17

18

19

20

21

22

23

24 **Abstract**

25 The intensity and duration of heat waves, as well as average global temperatures, are expected to  
26 increase due to climate change. Heat waves can cause physiological stress and reduce fitness in  
27 animals. Species can reduce overheating risk through phenotypic plasticity, which allows them to  
28 raise their thermal tolerance limits over time. This mechanism could be important for ectotherms  
29 whose body temperatures are directly influenced by available environmental temperatures.

30 Geckos are a large, diverse group of ectotherms that vary in their thermal habitats and times of  
31 daily activity, which could affect how they physiologically adjust to heat waves. Data on thermal  
32 physiology are scarce for reptiles, with only one study in geckos. Understanding thermal  
33 tolerance and plasticity, and their relationship, is essential for understanding how some species  
34 are able to adjust or adapt to changing temperatures. In this study, we estimated thermal  
35 tolerance and plasticity, and their interaction, in the crepuscular gecko, *Eublepharis macularius*,  
36 a species that is emerging as a model for reptile biology. After estimating basal thermal tolerance  
37 for 28 geckos, thermal tolerance was measured for each individual a second time at several  
38 timepoints (3, 6, or 24 h) to determine thermal tolerance plasticity. We found that thermal  
39 tolerance plasticity (1) does not depend on the basal thermal tolerance of the organism, (2) was  
40 highest after 6 hours from initial heat shock, and (3) was negatively influenced by individual  
41 body mass. Our findings contribute to the increasing body of work focused on understanding the  
42 influence of biological and environmental factors on thermal tolerance plasticity in organisms  
43 and provide phenotypic data to further investigate the molecular basis of thermal tolerance  
44 plasticity in organisms.

45 **Key-words:** Critical thermal maximum, Ectotherms, Geckos, Heat hardening, Heat waves,  
46 Reptiles, Thermal physiology, Trade-off hypothesis (TOH).

47

## 48 **1. Introduction**

49 Anthropogenic climate change has led to the rise of extreme heat events caused by higher  
50 average temperatures across the year (Stillman et al., 2019). Extreme heat events such as heat  
51 waves have become more prevalent within the summer months and are expected to increase in  
52 frequency, duration, and severity in the coming century (Stillman et al., 2019; Angélil et al.,  
53 2017; Guo et al., 2018; Pachauri et al., 2014). Heat waves can induce physiological stress on  
54 organisms and can reduce fitness (Stillman et al., 2019; Coffel et al., 2017; Froelicher et al.,  
55 2019). This is especially worrisome for species living in warm, aseasonal environments, since  
56 these species typically have narrow thermal tolerance ranges compared to species living in more  
57 variable, heterogeneous environments (Stillman et al., 2019, Williams et al., 2016; Tewksbury et  
58 al., 2008; Hoffmann et al., 2012; Ruibal, 1961; Janzen, 1967). Organisms can respond in many  
59 ways to high and potentially lethal temperatures; for example, behaviorally by seeking cooler  
60 microclimates within their local environment to prevent overheating (Stillman et al., 2019). At  
61 the same time, organisms may also plastically adjust the upper thermal tolerance of their internal  
62 body temperature when exposed to thermal extremes. This process of physiological plasticity is  
63 mediated by relatively rapid gene expression and cellular response changes (Kregel, 2002; Hu et  
64 al., 2014; Hamdoun et al., 2002; Horowitz, 2001; Buckley & Hofmann, 2002; Stillman et al.,  
65 2019, Angilletta, 2009), so that organisms plastically adjusting the upper limit of thermal  
66 tolerance can revert back to the original state after the heat stress has occurred, for example in  
67 response to short thermal perturbations (Sørensen et al., 2019).

68 Rapid reversible thermal tolerance plasticity could happen through heat hardening. Heat  
69 hardening (Bowler, 2005) occurs when an organism is exposed to its thermal extreme (either hot  
70 or cold) over a short period of time (minutes to hours), triggering a stress response in the

71 organism with a consequent production of an abundance of heat-shock proteins (HSPs) or cold-  
72 shock proteins (CSPs) (Horowitz, 2001; Sørensen et al., 2003; Gangloff & Telemeco, 2018).  
73 HSPs act as molecular chaperones, protecting other cellular proteins from denaturing at higher  
74 temperatures and allowing the organism to increase its thermal tolerance over time (Kregel,  
75 2002; Daugaard & Jäättelä, 2007; Sørensen et al. 2003; Gangloff & Telemeco, 2018). HSP  
76 expression has been shown to correlate with thermal tolerance across ectotherm species, where  
77 species adapted to warm environments (i.e., desert) have higher HSP concentrations and  
78 maintain the production of HSPs at higher temperatures compared to species adapted to cooler  
79 environments (i.e., forest) (Ulmasov et al., 1992; Zatespina et al., 2000; Madeira et al., 2012;  
80 Madeira et al., 2014; Qin et al., 2003; Newman et al., 2005; Dehghani et al., 2011). Heat  
81 hardening capacity varies across ectotherm species and populations (Phillips et al., 2016; Deery  
82 et al., 2021; Gilbert & Miles, 2019; Lapwong et al., 2021; Mottola et al., 2022; Morgan et al.,  
83 2018; van Heerwaarden et al., 2014). The heterogeneity of the thermal conditions during the  
84 developmental stages of an organism and of the habitat in which the species occurs (i.e., seasonal  
85 thermal variation and differences across the distribution range of the species), for example, are  
86 known to often influence heat hardening: the more variable the thermal environment is, the more  
87 plastic the organism will be in its response to thermal variation (Manenti et al., 2014; Phillips et  
88 al., 2016; Deery et al., 2021; Gilbert & Miles, 2019; Sasaki & Dam, 2019; but see Gunderson &  
89 Stillman, 2015; van Heerwaarden et al., 2014). Heat hardening is also strongly influenced by the  
90 biology of the species (Gunderson et al., 2017; Deery et al., 2021). For example, in ectotherms,  
91 the internal body temperatures, and thus the physiology and behavior of these organisms, are  
92 directly affected by temperatures available within their respective environments (Huey, 1991;  
93 Dayananda et al., 2016). As such, ectotherms can be particularly vulnerable to thermal extremes

94 and thus thermal tolerance plasticity through heat hardening has the potential to reduce  
95 overheating risks.

96 In addition to the influence of thermal environmental heterogeneity, the “trade-off”  
97 hypothesis (TOH) has been proposed as another mechanism to explain variation in heat  
98 hardening across and within species (van Heerwaarden & Kellermann, 2020). This hypothesis  
99 suggests that species already adapted to high temperatures, thus having high thermal tolerance,  
100 have limited potential to improve their thermal tolerance via phenotypic plasticity. The  
101 consequence of this hypothesis is that species cannot evolve both high thermal tolerance and  
102 high plasticity in response to extreme temperatures (van Heerwaarden & Kellermann, 2020). The  
103 TOH has recently been debated to be supported across and within different lizard species  
104 because not all species show a negative correlation between heat hardening capacity and basal  
105 upper thermal tolerance (Gunderson, 2023), which suggests that other factors may be influencing  
106 variation in thermal tolerance plasticity. Data on heat hardening are available for an extremely  
107 limited number of lizard species (only six species out of the 7,641 species described, Uetz et al.,  
108 2023) (Phillips et al., 2016; Deery et al., 2021; Gilbert & Miles, 2019; Lapwong et al., 2021).

109 Among lizards, geckos - a clade including 1,935 species (Uetz et al., 2020) - are  
110 tremendously understudied with data so far available for only one species (Lapwong et al.,  
111 2021). However, geckos have a wide range of variation for traits that can influence their  
112 thermophysiology and potentially their thermal tolerance, including time of activity, habitat,  
113 distribution, locomotion, morphology, and body size (Meiri, 2019; Gamble et al., 2015; Grismer  
114 et al., 2015; Heinicke et al., 2017; Oliver et al., 2019). As nighttime air temperatures have been  
115 increasing at a faster rate than daytime air temperatures due to climate change (Karl et al., 1991;  
116 Easterling et al., 1997; Vose et al., 2005; Alexander et al., 2006), understanding how species

117 active during the crepuscular hours or at night can respond to increasing temperatures –  
118 something largely unknown across squamate reptiles – will allow predictions on the influence of  
119 climate change on the fitness of species active in dim light (Rutschmann et al., 2023).

120 In this study, we established the critical thermal maximum (basal upper thermal  
121 tolerance), inferred the thermal tolerance plasticity and assessed their relationship in the gecko,  
122 *Eublepharis macularius*. *E. macularius* is becoming a model species for reptile studies in various  
123 fields of research (e.g., Hastings et al., 2023, Pinto et al., 2023, Katlein et al., 2022, Glimm et al.,  
124 2021, Kiskowski et al., 2021) and has well-documented molecular and genomic data compared  
125 to most other lizard species. *E. macularius* is typically active during dusk (Khan 2009; Gamble  
126 et al., 2015) and native to parts of northeast Iran and northwest India, where it inhabits deserts,  
127 shrublands, grasslands and rocky cliffs (Papenfuss et al., 2021). In the coming century, the native  
128 habitat of *E. macularius* is expected to experience higher frequency and duration of heat waves  
129 (Rohini et al., 2019; Khan et al., 2020), which could increase this species' vulnerability to heat  
130 stress (Stillman et al., 2019).

131 In ectotherms, thermal tolerance plasticity through heat hardening has been measured as  
132 the change in the critical thermal maximum ( $CT_{max}$ ) over time.  $CT_{max}$  corresponds to the upper  
133 thermal tolerance of an organism, which is the internal body temperature at which the organism  
134 loses physiological function and cannot tolerate any higher temperatures (Angilletta, 2009).  
135 Measurements of  $CT_{max}$  can be influenced by heating rates (i.e., the rate at which individuals  
136 approach their upper thermal tolerance) (Ribeiro et al., 2012; Terblanche et al., 2007), which  
137 have been reported in previously published heat hardening studies; however, validation of  
138 consistent heating rates using statistical analyses is generally missing from methodologies in  
139 these papers. As such, in addition to measuring heat hardening capacity and thermal tolerance

140 within *E. macularius*, in this work we also investigate the influence of individual body mass,  
141 body size, and sex on heating rates across  $CT_{max}$  experiments. We hypothesized that: (1) in  
142 support of the TOH, individuals with lower basal thermal tolerance have greater heat hardening  
143 capacity compared to individuals with higher basal thermal tolerance; (2) overtime, change in  
144 thermal tolerance will be highest at 6 hours after initial heat shock, following what has been  
145 shown in other lizard species (Phillips et al., 2016, Lapwong et al., 2021; Gilbert & Miles, 2019);  
146 and (3) individuals with larger body size will have greater heat hardening capacity compared to  
147 smaller individuals since acclimation capacity has been shown to increase with body size across  
148 ectotherms (Rohr et al., 2018).

149       Ultimately, the costs and benefits of heat hardening for organisms will be dependent upon  
150 the thermal extremes (either hot or cold) that an organism is exposed to within its native  
151 environment (Stillman et al., 2019; Noer et al., 2022). Although, heat hardening alone may not  
152 allow organisms to fully compensate for the effects of climate warming in the future (Gunderson  
153 et al., 2017), it has been estimated to reduce overheating risk in species to some degree  
154 (Gunderson et al., 2017; Deery et al., 2021). As such, understanding the breadth of thermal  
155 tolerance plasticity for an organism gives context for how the organism may respond to future  
156 temperature extremes in their native environment and whether the organism can physiologically  
157 adjust quickly enough to the thermophysiological stress associated with these higher  
158 environmental temperatures.

159

## 160 **2. Materials and Methods**

161       All capture, handling, and experimental protocols were approved by George Mason  
162 University IACUC committee (Permit number #1901361). Experiments were carried out to

163 minimize stress and disturbance to the animals and in accordance with relevant guidelines and  
164 regulations.

## 165 **2.1 Study species and captivity conditions**

166 A total of 28 adult individuals (14 females and 14 males) were used for this study. Only  
167 adult individuals of *E. macularius* were tested, as using individuals of different life-stages may  
168 affect measurements of critical thermal maximum ( $CT_{max}$ ) (Telemeco & Gangloff, 2021;  
169 Camacho & Rusch, 2017). All geckos were part of a captive colony housed at George Mason  
170 University for several years. In their captive conditions, geckos were housed in plastic cages  
171 (Sterilite 28 quart/27 liter clear storage box) with two small plastic bowls of which one is for  
172 food and one for water, humid and dry hide boxes, and heating pads at one end of the cage for  
173 thermoregulation. In their captive environment, geckos were fed every other day with a mixed  
174 diet of mealworms, crickets, and waxworms dusted with calcium powder and had access to water  
175 *ad libitum*. Gecko cages were housed in a temperature-controlled room operating on a 12h:12h  
176 light-dark cycle with daily room temperatures ranging from 24°C to 26°C and relative humidity  
177 ranging from 20-50%. All individuals were obtained from different captive breeders and held in  
178 these laboratory conditions for at least two months before the start of  $CT_{max}$  experiments.

## 179 **2.2 Experimental set up**

180 Prior to conducting the experiments, geckos were fasted for at least three days to prevent  
181 any influence of feeding on  $CT_{max}$  measurements. Before the start of each experiment (both basal  
182 and final  $CT_{max}$  for each individual), the mass (g) of each individual was measured using a digital  
183 scale. In addition, each individual's snout-vent-length (SVL) was measured for all geckos. Final  
184  $CT_{max}$  corresponds to the  $CT_{max}$  obtained to estimate heat hardening and measured at 3h, 6h, or  
185 24h.



186  $CT_{max}$  experiments were performed at George Mason University in an empty  
187 temperature-controlled room set to 28°C, which enabled individuals to acclimate to an internal  
188 body temperature of 28°C prior to starting  $CT_{max}$  experiments. For  $CT_{max}$  experiments,  
189 individuals were placed in a plastic holding container (Hefty 6.5 quart) placed on a lab bench in  
190 the temperature-controlled room with the 150W heat emitter domed lamp (ReptiZoo, 120V,  
191 60Hz) placed 23 cm above the base of the container using a lamp stand. All sides of the plastic  
192 holding container were covered with Teflon, which was secured to the bottom of the plastic  
193 holding container using black electrical tape to ensure that the plastic holding container did not  
194 overheat during  $CT_{max}$  experiments. An iButton data-logger (DS1921G Thermochron, Maxim  
195 Integrated Products, precision = +/- 0.5°C) was placed both inside and outside the plastic holding  
196 container during  $CT_{max}$  experiments to monitor ambient temperatures (Supp. Materials Figure S1  
197 for images of the experimental setup).

198 Geckos were tested one at the time. Unless a gecko was tested, it was in its housing room  
199 and cage. For all measurements of  $CT_{max}$ , it is important that all individuals start at the same  
200 internal body temperature, as starting temperatures have been shown to influence  $CT_{max}$  values  
201 (Terblanche, et al., 2007; Camacho and Rusch, 2019). Prior to the start of  $CT_{max}$  experiments, a  
202 T-type 36-gauge wire thermocouple (Evolution Sensors and Controls, LLC) was inserted  
203 approximately 3 mm into the cloaca of the gecko to measure internal body temperature using a  
204 digital thermometer (Evolution Sensors and Controls, LLC; resolution = 0.1t < 1000°; 1.0t ≥  
205 1000°; accuracy = ±0.1% + 0.6°C). If an individual's cloacal temperature was approximately  
206 28°C (± 0.9°C),  $CT_{max}$  experiments would be started. If an individual's cloacal temperature was  
207 lower or higher than 28°C, the individual would either be placed in a water bath until the  
208 individual's body temperature was lowered to 28°C, or in the plastic holding container to allow

209 its internal body temperature to acclimate to 28°C as the room temperature. The small plastic  
210 container filled with water was used to allow the organism to recover after measurements of  
211  $CT_{max}$ .

### 212 **2.3 Data collection**

213 Basal  $CT_{max}$  - corresponding to the thermal tolerance of the individual at 0 hours - was  
214 measured for each of the 28 adult geckos as the internal body temperature at which each  
215 individual lost righting response for 10 sec. after being flipped over the individual's dorsal side.  
216 This approach has been commonly used as a method of measuring  $CT_{max}$  in lizards (Deery et al.,  
217 2021, Phillips et al., 2016; Lapwong et al., 2021), as it is related to the internal body temperature  
218 at which the organism loses proper physiological function and cannot tolerate any higher  
219 temperatures.

220 To measure heat hardening (thermal tolerance plasticity) as the change in  $CT_{max}$  over  
221 time, the 28 individuals were randomly assigned a time interval to measure  $CT_{max}$  again after the  
222 initial measurement of basal  $CT_{max}$ . Between measurements of basal and final  $CT_{max}$ , individuals  
223 were taken from the testing room and placed in their respective housing enclosures to recover,  
224 since allowing the individual to remain at its basal  $CT_{max}$  would be lethal to the organism. Time  
225 intervals for measuring  $CT_{max}$  a second time (final  $CT_{max}$ ) ranged from 3 hours, 6 hours, or 24  
226 hours after initial basal  $CT_{max}$  measurements. All individuals were initially tested at 0h for their  
227 basal  $CT_{max}$  and then at either 3h, 6h, or 24h after 0h (final  $CT_{max}$ ), depending on their assigned  
228 time-interval treatment group. These time intervals were chosen based on previously published  
229 studies using similar time intervals across 24 hours to measure  $CT_{max}$ . These studies showed that  
230 heat hardening occurs as a short-term plastic response over 24 hours with the highest change in  
231  $CT_{max}$  for most species occurring at 6 hours after initial heat shock (Deery et al., 2021; Lapwong

232 et al., 2021; Phillips et al., 2016; Gilbert and Miles, 2019). The 3h time-interval group included 4  
233 male and 5 female individuals, the 6h time-interval group included 5 male and 4 female  
234 individuals, and the 24h time-interval group included an equal ratio of 5 male and 5 female  
235 individuals.

236 Although *E. macularius* is a crepuscular gecko species, individuals would be expected to  
237 experience higher environmental temperatures during the day in this species' native environment  
238 (Supp. Materials Figure S2 A and B). Thus, all  $CT_{max}$  experiments were conducted during  
239 daytime hours. Basal  $CT_{max}$  experiments were conducted from approximately 10:00 am to 12:00  
240 pm, and final  $CT_{max}$  experiments were conducted 3 hours, 6 hours, and 24 hours after the start of  
241 basal  $CT_{max}$  experiments.

242 To obtain data on basal and final  $CT_{max}$ , the internal body temperature of each individual  
243 for all  $CT_{max}$  experiments was raised at approximately  $1^{\circ}C/min$  (mean basal heating rate =  $0.976$   
244  $^{\circ}C/min$ ; mean final heating rate =  $0.911^{\circ}C/min$ ) using a 150W heat emitter domed lamp placed  
245 above the experimental plastic container (Supp. Materials Figure S1) until each individual lost  
246 righting response for 10 seconds. Different heating rates can affect  $CT_{max}$  values, which are  
247 mainly affected by differences in individual body size (Ribeiro et al., 2012; Terblanche et al.,  
248 2007; Rezende et al., 2011). Because of this, a heating rate of  $1^{\circ}C/min$  – similar to what used in  
249 previous studies (Phillips et al., 2016; Gilbert & Miles, 2019; Lapwong et al., 2021) – was  
250 targeted for all individuals. To measure  $CT_{max}$  for each individual (basal and final  $CT_{max}$ ),  
251 individuals were flipped on their dorsal side inside the plastic holding container to check for  
252 righting response and cloacal temperature was measured immediately by quickly (within 10  
253 seconds max) lifting the gecko from the plastic holding container and inserting 3 mm into the  
254 cloaca a T-type 36-gauge wire thermocouple attached to a digital thermometer (Evolution

255 Sensors and Controls, LLC). Previous heat hardening experiments conducted on different lizard  
256 species left the thermocouple inside the cloaca during the entire  $CT_{max}$  experiment by securing it  
257 with a piece of medical tape (Deery et al., 2021). However, prior to conducting our trial  
258 experiments with individuals not used in this study, leopard geckos were able to remove the  
259 medical tape and the attached thermocouple from their cloaca and we could therefore not use this  
260 approach in our study. After measuring  $CT_{max}$  (both basal and final), the gecko was placed in a  
261 small container filled with water to allow the organism to recover.

## 262 **2.4 Native microclimate temperatures for *E. macularius***

263 Seasonal variation of native environmental temperatures has been shown to influence heat  
264 hardening capacity in lizard species (Phillips et al., 2016). Therefore, in addition to measuring  
265 thermal tolerance and heat hardening capacity of *E. macularius*, both daily and seasonal temperature  
266 ranges of native microclimates were estimated for this species. To obtain an overall estimation of  
267 the native microclimatic environmental temperatures for *E. macularius*, occurrences for this species  
268 were downloaded from the Global Biodiversity Information Facility (GBIF, September 2023) and  
269 imported into Rstudio (v. 4.3.0, R Core Team 2023) using the “occ\_download\_get” function from  
270 the *rgbif* package (Chamberlain et al., 2017). Species occurrences for *E. macularius* were filtered for  
271 species’ scientific name mismatches as well as NA values for latitude, longitude, species’ scientific  
272 names, and country codes. Species occurrences were also cleaned and cross-checked for coordinate  
273 validity using the “clean\_coordinates” function from the package, *Coordinate Cleaner* (Zizka et al.,  
274 2019). Species occurrences which resulted in at least one flagged test labeled as, “FALSE,” were  
275 removed from the dataset. From a total of 23 unique occurrences for *E. macularius*, microclimate  
276 temperatures were estimated using the “micro\_global” function from the package *NiceMapR*  
277 (Kearney & Porter, 2017). Microclimate temperatures for each coordinate were estimated every 60  
278 min across 365 days for 10 years using the NicheMapR microclimate model. Local height for the

279 model was set to 1.5 cm, which is the measured midpoint height of captive *E. macularius*. In  
280 addition, the maximum shade parameter was set to 0% and 100% to estimate microclimate  
281 temperatures in full sun and full shade, respectively. Native microclimate temperatures estimated for  
282 *E. macularius* are shown in the Supplementary Materials (Supp. Materials Figure S2).

## 283 **2.5 Statistical analyses**

284 All statistical analyses were performed using the software Rstudio (v. 4.3.0, R Core Team  
285 2023). All the tested 28 individuals were used for all analyses. A paired, two-sample t-test was  
286 first used to determine if the mass measured before basal CT<sub>max</sub> and final CT<sub>max</sub> experiments for  
287 all individuals significantly differed using the “t.test” function from the *stats* package (R Core  
288 Team 2023). We found a significant difference between mass measured before basal CT<sub>max</sub>  
289 experiments and before final CT<sub>max</sub> experiments across all individuals ( $t = 5.3729$ ,  $df = 26$ ,  $p$ -  
290  $value = 1.259 \times 10^{-5}$ , mean difference = 0.911, 95% CI = (0.562, 1.259); as such, analyses were  
291 repeated taking into account the mass measured before basal CT<sub>max</sub> or the mass taken before final  
292 CT<sub>max</sub> experiments and tested independently. One-way ANOVAs were performed using the  
293 “aov” function from the *stats* package to determine if SVL or mass were influenced by the sex  
294 (male or females) of the individuals using separate models for each variable. One-way ANOVAs  
295 were also used to determine if SVL or mass significantly differed across time-interval treatment  
296 groups.

297 Individual heating rates during CT<sub>max</sub> experiments were calculated as the slope  
298 corresponding to the change in internal body temperature over time measured every minute at  
299 time 0 (basal heating rate) and at the time of treatment (3, 6, or 24 hours). As such, each  
300 individual had a single basal and a single final heating rate value. The basal heating rate for each  
301 individual was calculated from the slope obtained by plotting the internal body temperatures  
302 obtained every minute during measurements of CT<sub>max</sub> experiments at 0 hours on the time at

303 which each measurement was obtained. Similarly, the final heating rate for each individual was  
304 calculated from internal body temperatures measured during  $CT_{max}$  experiments for either the 3h,  
305 6h, or 24h time-interval treatment group. Paired, two-sample t-tests were performed to compare  
306 basal heating rates and final heating rates for all individuals within (e.g., for all the geckos tested  
307 after 3 hours) and across time-interval treatment groups (e.g., geckos tested at 3 hours, 6 hours,  
308 and 24 hours grouped together independently of the treatment time). The assumptions for each  
309 paired, two-sample t-test were checked using Shapiro-Wilks and Levene's tests to examine the  
310 normality and homogeneity of variance for the differences between basal and final heating rates  
311 (Supp. Materials for details). In addition, one-way ANOVAs were performed to determine if  
312 basal heating rates for all individuals were influenced by time-interval treatment group, sex,  
313 mass measured before basal  $CT_{max}$  experiments, and SVL by testing each variable separately.  
314 Two-way ANOVAs were performed to test for the influence of sex, mass measured before basal  
315  $CT_{max}$  and final  $CT_{max}$  experiments, and SVL separately on final heating rates with time-interval  
316 treatment group as a covariate for each model. To measure the potential influence of each  
317 individual on the fitted response values for each linear model as described above for basal and  
318 final heating rates, a Cook's Distance test (Cook, 1977) was performed using the  
319 "cooks.distance" function on each model separately with a threshold of 1 (individuals with a  
320 Cook's Distance > 1 are influential)

321       According to the TOH, individuals with low basal thermal tolerance are expected to have  
322 greater heat hardening and vice versa (van Heerwaarden & Kellermann, 2020). To test for a  
323 trade-off between basal thermal tolerance (basal  $CT_{max}$  for each individual) and heat hardening  
324 capacity (difference between basal  $CT_{max}$  and final  $CT_{max}$  for each individual), a Pearson's  
325 product-moment correlation analysis was performed on the 3h, 6h, and 24h time-interval

326 treatment groups using the “cor.test” function from the *stats* package. If the relationship between  
327 basal  $CT_{max}$  and change in  $CT_{max}$  (i.e., final  $CT_{max}$  at 3h, 6h, or 24h - basal  $CT_{max}$  at 0h) values  
328 (“unadjusted”) for each time-interval treatment group showed a significant (p-value < 0.05)  
329 negative relationship, then change in  $CT_{max}$  values were adjusted to remove the effect of  
330 regression to mean following the correction method by Kelly and Price (Kelly and Price, 2005).  
331 Change in  $CT_{max}$  values were only adjusted for the 3h time-interval treatment group and not for  
332 the 6h or 24h time-interval treatment group, since basal  $CT_{max}$  and change in  $CT_{max}$  showed a  
333 significant negative relationship (t-value = -4.8348, df = 7, p-value = 0.001889, r = -0.8772416,  
334 95% CI = (-0.973, -0.510)) for the 3h time-interval treatment group. Following adjustment of  
335 change in  $CT_{max}$  values, the Pearson’s product-moment correlation analysis was then performed  
336 on the basal  $CT_{max}$  and adjusted change in  $CT_{max}$  values for the 3h time-interval treatment group.

337       The mean and standard deviation for  $CT_{max}$  values measured at either 0, 3, 6, or 24 hours  
338 were calculated using the “mean” (base R) and “sd” (*stats* package) functions, respectively.  
339 Standard error of measurement (SEM) was calculated for basal  $CT_{max}$  values measured at 0 hours  
340 or final  $CT_{max}$  values measured at either 3, 6, or 24 hours by dividing the standard deviation for  
341 each set of  $CT_{max}$  values by the square root of the sample size for each set of  $CT_{max}$  values.  
342 Generalized linear models (GLMs) were used to determine the effects of treatment groups on  
343 basal  $CT_{max}$  (basal  $CT_{max}$  at 0h) using sex, SVL, and mass as covariates in the models. GLMs for  
344 final  $CT_{max}$  (final  $CT_{max}$  at 3h, 6h, and 24h) and change in  $CT_{max}$  (final  $CT_{max}$  at either 3h, 6h, or  
345 24h - basal  $CT_{max}$  at 0h) were also run using sex, SVL, mass, and the interaction between time-  
346 interval treatment group and basal  $CT_{max}$  as covariates in each of these models. The same GLMs  
347 for final  $CT_{max}$  and change in  $CT_{max}$  were also performed with the mass measured for each  
348 individual before final  $CT_{max}$  experiments instead of using the mass of each individual measured

349 before basal  $CT_{max}$  experiments. Each GLM for basal  $CT_{max}$ , final  $CT_{max}$ , and change in  $CT_{max}$   
350 was then performed a second time with only significant variables included within each model.  
351 GLMs were performed using the “glm” function from the *stats* package. Change in  $CT_{max}$   
352 included adjusted values for the 3h time-interval treatment group (see section above) and  
353 unadjusted values for the 6h and 24h time-interval treatment groups. The assumptions for each  
354 GLM were checked using Shapiro-Wilks and Levene’s tests to examine the normality and  
355 homogeneity of variance for basal  $CT_{max}$ , final  $CT_{max}$ , and change in  $CT_{max}$  values as well as the  
356 residual values for each GLM model (Supp. Materials for details). Linear regressions were used  
357 to determine the influence of basal  $CT_{max}$  on final  $CT_{max}$  separately within each time-interval  
358 treatment group (3h, 6h, and 24h) using the “lm” function from the *stats* package. To measure  
359 the influence of each individual on the fitted response values for all of the GLM models for basal  
360  $CT_{max}$ , final  $CT_{max}$ , and change in  $CT_{max}$ , a Cook’s Distance test was performed using the  
361 “cooks.distance” function on each model separately with a threshold of 1 (individuals with a  
362 Cook’s Distance > 1 are influential).

### 363 **3. Results**

#### 364 **3.1 Time-interval treatment group and sex differences**

365 We found no significant differences in body mass measured before basal  $CT_{max}$   
366 experiments (df = 1, sum of squares = 128.1, mean square = 128.14, F-value = 2.991, p-value =  
367 0.0956) or final  $CT_{max}$  experiments (df = 1, sum of squares = 135.8, mean square = 135.83, F-  
368 value = 3.029, p-value = 0.0941) between male and female individuals; however, SVL (df = 1,  
369 sum of squares = 1.800, mean square = 1.8004, F-value = 5.426, p-value = 0.0279) significantly  
370 differed between sex with males being larger than females (male mean SVL = 12.06 cm; female  
371 mean SVL = 11.55 cm). Neither SVL (df = 2, sum of squares = 0.20, mean square = 0.0998, F-



372 value = 0.244, p-value = 0.785), mass measure before basal  $CT_{max}$  experiments (df = 2, sum of  
373 squares = 35.2, mean of squares = 17.58, F-value = 0.364, p-value = 0.698) or mass measured  
374 before final  $CT_{max}$  experiments (df = 2, sum of squares = 47.4, mean square = 23.71, F-value =  
375 0.47, p-value = 0.63) significantly differed across time-interval treatment groups.

### 376 **3.2 Heating rates across and within time-interval treatment groups**

377 Paired, two-sample t-tests found no significant differences between basal and final  
378 heating rates for individuals across all time-interval treatment groups (t = 1.7754, df = 27, p-  
379 value = 0.08711, mean difference = 0.0646, 95% CI = (-0.010, 0.139)) (Supp. Materials Figure  
380 S3), within the 3h (t = 0.36466, df = 8, p-value = 0.7248, mean difference = 0.0281, 95% CI = (-  
381 0.149, 0.205)), and 6h (t = 0.64225, df = 8, p-value = 0.5387, mean difference = 0.0478, 95% CI  
382 = (-0.124, 0.219)) time-interval treatment groups. However, the 24h time-interval treatment  
383 group showed a significant difference between basal and final heating rates for individuals (t =  
384 2.8825, df = 9, p-value = 0.0181, mean difference = 0.1127, 95% CI = (0.024, 0.201) with final  
385 heating rates (mean = 0.87 °C/min) on average being higher than basal heating rates (mean =  
386 0.98 °C/min).

387 One-way ANOVAs found no significant influence of sex, mass, SVL, or time-interval  
388 treatment group on basal heating rates across all time-interval treatment groups (p-values > 0.05)  
389 (Table 1). After considering time-interval treatment group, sex, mass measured before basal  
390  $CT_{max}$  (or final  $CT_{max}$ ) experiments, and SVL showed no significant effects on final heating  
391 rates (all p-values > 0.05) except for the interaction between time-interval treatment group and  
392 mass (for mass measured before basal  $CT_{max}$  p-value = 0.0423 and for mass measured before  
393 final  $CT_{max}$  experiments p-value = 0.0216) (Table 1). Cook's Distance tests indicated that no  
394 individuals had a Cook's Distance > 1.

395  
396  
397  
398  
399  
400  
401  
402

**Table 1:** Influence of sex, mass, SVL, and time-interval treatment group on basal and final heating rates. Sex, mass measured before basal  $CT_{max}$  experiments, SVL, and time-interval group were tested separately on basal heating rates. Mass measured before basal and final  $CT_{max}$  experiments, SVL, and sex were tested separately with time-interval group as a covariate on final heating rates. P-values < 0.05 are in bold to represent statistical significance (df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square).

Source	Df	Sum Sq	Mean Sq	F-value	p-value
<b>Basal Heating Rate</b>					
Sex	1	0.0221	0.02206	1.601	0.217
Mass	1	0.0394	0.03937	3.001	0.095
SVL	1	0.0001	$8.4 \times 10^{-5}$	0.006	0.94
Treatment Group	2	0.0027	0.001349	0.089	0.915
<b>Final Heating Rate</b>					
Sex	1	0.0010	0.00097	0.030	0.864
Treatment Group	2	0.0228	0.01139	0.354	0.706
Treatment Group:Sex	2	0.0238	0.01188	0.369	0.695
Mass (before basal $CT_{max}$ )	1	0.0324	0.03238	1.356	0.2567
Treatment Group	2	0.0228	0.01139	0.477	0.6271
Treatment Group:Mass	2	0.1750	0.08752	3.665	<b>0.0423</b>
Mass (before final $CT_{max}$ )	2	0.0282	0.01408	0.753	0.4834
Treatment Group	1	0.0425	0.04251	2.273	0.1466
Treatment Group:Mass	2	0.1732	0.08658	4.629	<b>0.0216</b>
SVL	1	0.0022	0.00220	0.073	0.789
Treatment Group	2	0.0228	0.03438	1.143	0.337
Treatment Group:SVL	2	0.0688	0.01138	0.388	0.682

403

### 404 3.3 Thermal tolerance and heat hardening capacity

405 Average basal  $CT_{max}$  across all individuals for all time-interval treatment groups was

406 41.07 °C (Table 2). Final  $CT_{max}$  averaged across individuals was highest for the 3h time-interval

407 treatment group (mean = 41.71 °C) compared to the averages across individuals for the 6h (mean  
408 = 41.20 °C) and 24h treatment groups (mean = 40.87 °C) (Table 3; Figure 1A).

409 In comparison to native microclimate temperatures, basal  $CT_{max}$  averaged across  
410 individuals of *E. macularius* (mean = 41.07 °C) was found to be higher than native shade  
411 microclimate temperatures across the day and season (mean = 19.22 °C) (Supp. Materials Figure  
412 S2). Basal  $CT_{max}$  for *E. macularius* was also higher than the native sun microclimate  
413 temperatures across the day and season (mean = 22.14 °C) (Supp. Materials Figure S2).  
414 Maximum temperatures in the species' native habitat reach 59°C and 43°C across the year in the  
415 sun and shade, respectively (Supp. Materials Figure S2).

416

417 **Table 2:** Mean and standard deviation of  $CT_{max}$  values with standard error of measurement  
418 (SEM) in parentheses of *E. macularius* before (basal  $CT_{max}$ , treatment group 0h) and after heat  
419 hardening (final  $CT_{max}$ , 3h, 6h, or 24h) for each time-interval treatment group.  $n$  = number of  
420 individuals.

Treatment Group	$CT_{max}$ (°C)	$n$
0h	41.07±1.99 (0.37)	28
3h	41.71±1.48 (0.49)	9
6h	41.20±1.68 (0.56)	9
24h	40.87±2.09 (0.69)	10

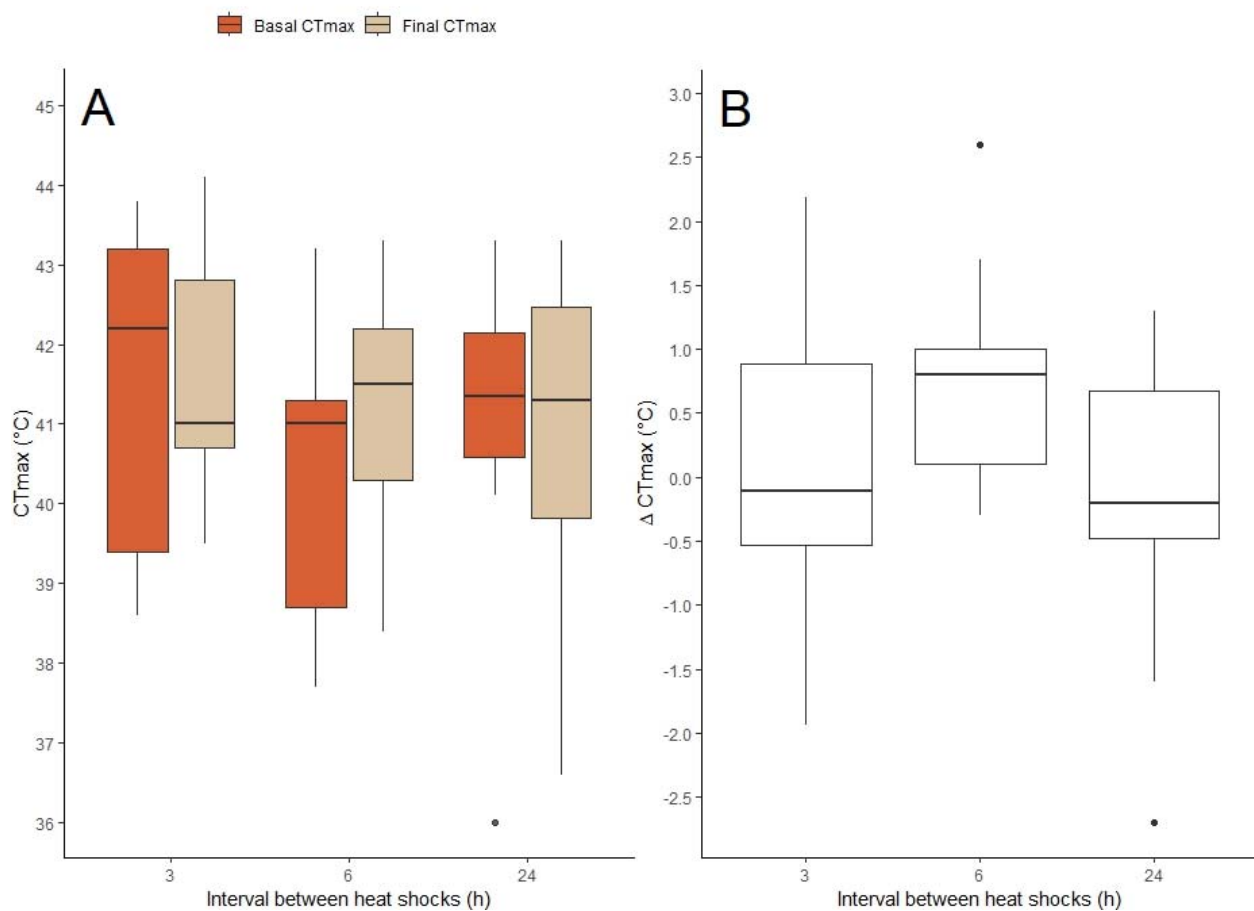
421 -

422 Pearson's product-moment analyses found no significant correlations between basal  
423  $CT_{max}$  and change in  $CT_{max}$  (final – basal) for the 3h, 6h, or 24h time-interval treatment groups  
424 ( $p$ -values > 0.05) (Table 3). Average change in  $CT_{max}$  was found to be highest for individuals  
425 within the 6h time-interval treatment group (mean = 0.76 °C) compared to the average change in  
426  $CT_{max}$  for individuals within the 3h (mean = 0.03 °C) and 24h (mean = -0.23 °C) treatment groups  
427 (Figure 1B).

428 **Table 3:** Summary of Pearson's product-moment correlation analysis to test for the relationship  
429 between basal  $CT_{max}$  and change in  $CT_{max}$  within time-interval treatment groups (df = degrees of  
430 freedom, CI = confidence interval).

Treatment Group	t-value	df	p-value	r	95% CI
3h	0.297	7	0.775	0.111	-0.596, 0.772
6h	-1.209	7	0.265	-0.415	-0.846, 0.343
24h	-0.804	8	0.444	-0.273	-0.770, 0.430

431



432

433 **Figure 1:** Boxplots showing (A) basal  $CT_{max}$  and final  $CT_{max}$  for all individuals of each  
434 time-interval treatment group and (B) heat hardening (change in  $CT_{max}$  between basal  
435  $CT_{max}$  and final  $CT_{max}$ ) for all individuals of each time-interval treatment group.

436

437 The initial general linear model (GLM) found no effect of time interval treatment group,

438 sex, or mass on basal  $CT_{max}$  (p-values > 0.05) (Supp. Materials Table S1a); however, SVL did

439 show a significant effect on basal  $CT_{max}$  (p-value = 0.00359) (Supp. Materials Table S1a). This  
440 result was confirmed for the GLM with only SVL used as a variable in the model (p-value =  
441 0.0297) (Table 4). For both sexes, individuals with higher SVL tended to have lower basal  
442  $CT_{max}$ .

443 On the other hand, the initial GLM including all the variables indicated that final  $CT_{max}$   
444 was significantly affected by time-interval treatment group (p-value = 0.000375), basal  $CT_{max}$  (p-  
445 value = 0.000434), and the interaction between time-interval treatment group and basal  $CT_{max}$  (p-  
446 value = 0.000440) (Supp. Materials Table S1b). These results were confirmed when the GLM  
447 was re-run using a simpler model including only significant variables based on the initial more  
448 complex model (data not shown).

449 Finally, change in  $CT_{max}$  was not affected by sex, mass measured before basal  $CT_{max}$   
450 experiments, SVL, basal  $CT_{max}$ , time-interval treatment group, or the interaction between time-  
451 interval treatment group and basal  $CT_{max}$  (p-values > 0.05) (Supp. Materials Table S1c).

452 Analyses repeated using the mass of all individuals measured before final  $CT_{max}$   
453 experiments generally confirmed the results obtained using the mass measured before basal  
454  $CT_{max}$ . The only difference found was that in the case of the mass measured before final  $CT_{max}$ ,  
455 the mass of the individuals had an influence on final  $CT_{max}$  and on the change in  $CT_{max}$  (p-value  
456 = 0.03 in both cases, data not shown). Furthermore, we found that individuals with higher body  
457 mass exhibited lower final  $CT_{max}$  and lower change in  $CT_{max}$  (plasticity) compared to individuals  
458 with lower body mass (p-value = 0.012 and 0.010, respectively) (Table 4). Cook's Distance tests  
459 identified no individuals with Cook's Distance > 1. When analyses were re-run using simpler  
460 models including only significant variables (Supp. Materials Table S1), we found that across  
461 time-interval treatment groups, there is a significant positive relationship between basal  $CT_{max}$

462 and final  $CT_{max}$  (p-value = 0.00007) (Table 4). Linear regression models reveal that for the 6h ( $r^2$   
 463 = 0.7435; F-statistic = 20.29 on 1 and 7 df; p-value = 0.00278; slope = 0.7883; standard error =  
 464 0.1750; t-value = 4.504) and 24h ( $r^2$  = 0.6978; F-statistic = 18.47 on 1 and 8 df; p-value =  
 465 0.00262; slope = 0.8424; standard error = 0.190; t-value = 4.298) treatment groups, individuals  
 466 with high basal  $CT_{max}$  tended to also have high final  $CT_{max}$ . However, for the 3h treatment group,  
 467 there was no significant relationship between basal  $CT_{max}$  and final  $CT_{max}$  ( $r^2$  = 0.1097; F-statistic  
 468 = 0.8626 on 1 and 7 df; p-value = 0.38393; slope = -0.2378; standard error = 0.2560; t-value = -  
 469 0.929).

470 **Table 4:** Summary of general linear models (GLMs) to test for the influence of significant  
 471 variables based on the initial more complex GLM models (Supp. Table S1) on basal  $CT_{max}$ , final  
 472  $CT_{max}$ , and change in  $CT_{max}$ . GLMs include mass measured before final  $CT_{max}$  experiments for  
 473 final  $CT_{max}$  and change in  $CT_{max}$  models. Statistically significant p-values are shown in bold.  
 474 SVL = snout-vent length (measured in cm). Mass measured in grams.

Coefficients	Estimate	Std. Error	t-value	p-value
<i>(a) glm(Basal <math>CT_{max}</math> ~ SVL, family = "gaussian") AIC: 117.82</i>				
(Intercept)	56.6367	6.7742	8.361	<b>7.66 x 10<sup>-9</sup></b>
SVL (cm)	-1.3179	0.5728	-2.301	<b>0.0297</b>
Null deviance: 107.057 on 27 df; Residual deviance: 88.947 on 26 df				
<i>(b) glm(Final <math>CT_{max}</math> ~ Group*Basal <math>CT_{max}</math> + Mass, family = "gaussian")</i>				
<b>AIC: 87.621</b>				
(Intercept)	10.283	7.141	1.440	0.165
Group (3h)	44.562	10.311	4.322	<b>0.000331</b>
Group (6h)	4.781	12.745	0.375	0.711
Basal $CT_{max}$	0.847	0.169	4.991	<b>7.01 x 10<sup>-5</sup></b>
Mass (g)	-0.089	0.0325	-2.734	<b>0.012790</b>
Group(3h):Basal $CT_{max}$	-1.057	0.248	-4.253	<b>0.000390</b>
Group(6h):Basal $CT_{max}$	-0.103	0.312	-0.333	0.742
Null deviance: 82.241 on 26 df, Residual deviance: 22.435 on 20 df				
<i>(c) glm(Change in <math>CT_{max}</math> ~ Mass, family = "gaussian") AIC = 82.106</i>				

(Intercept)	3.919	1.396	2.806	<b>0.00957</b>
Mass (g)	-0.080	0.029	-2.773	<b>0.01034</b>

Null deviance: 34.637 on 26 df, Residual deviance: 26.488 on 25 df

---

475  
476  
477

#### 4. Discussion

478 Short-term heat hardening (thermal tolerance plasticity) has the potential to buffer the  
479 effects of increased warming caused by heatwaves on organisms, especially for species which  
480 already occupy harsh xeric habitats. In this study, we determined the basal thermal tolerance  
481 (basal  $CT_{max}$ ) and heat hardening capacity (change in  $CT_{max}$  between basal and final  $CT_{max}$ ) in  
482 one such species, the crepuscular gecko, *Eublepharis macularius*. Consistent with the known  
483 male-biased sexual size dimorphism in native populations of *E. macularius* (Kratochvíl &  
484 Frynta, 2002), sexual-size dimorphism was shown between males and females studied in our  
485 work, with males having larger SVL on average than females.

486 To our knowledge, in this study, we estimated for the first time the influence of variables  
487 on heating rates, the rates at which the organism increases its body temperature per minute, in  
488 heat hardening studies. While other studies have looked at the influence of heating rates for  
489  $CT_{max}$  studies, this approach is not normally included in studies on heat hardening. We found  
490 that basal and final heating rates for  $CT_{max}$  experiments did not differ across and within time-  
491 interval treatment groups, except for the 24h time-interval treatment group, which showed a  
492 mean difference = 0.1127 °C/min between basal and final heating rates. We also found that sex,  
493 mass, and SVL did not influence basal and final heating rates except for an interaction between  
494 mass and time-interval group for final heating rates. To note that our data indicate that the  
495 individual mass does not differ among the different treatment groups. These results support the  
496 comparison of basal and final  $CT_{max}$  data to study heat hardening in this species as they validate

497 the consistency of heating rates for measuring  $CT_{max}$  regardless of the sex, mass, SVL and time-  
498 interval treatment group. We suggest that future research incorporate analyses of the heating  
499 rates used for measurements of  $CT_{max}$ , in order to take into account potential variation in basal  
500 and final  $CT_{max}$  due to different heating rates.

501 We found that heat hardening capacity - the aptitude of an organism to plastically adjust  
502 its thermal limit - in *E. macularius* was not affected by the basal  $CT_{max}$  of the individual. Thus,  
503 we reject the TOH - according to which heat hardening is higher in organisms with lower basal  
504 thermal tolerance and vice-versa - for *E. macularius*. In fact, we found that individuals with  
505 relatively low basal  $CT_{max}$  did not exhibit greater change in  $CT_{max}$  and vice versa across the 3h  
506 and 6h time-interval treatment groups. Although comparative studies of heat hardening capacity  
507 across squamates are lacking, heat hardening capacity in different lizard species have generally  
508 rejected the TOH with the exception of two tropical species, *Lampropholis coggeri* (Phillips et  
509 al., 2016; Gunderson, 2023) and *Hemidactylus frenatus* (Lapwong et al., 2021). Furthermore, the  
510 TOH also vary in its support across other taxa of ectotherms. For example, one species of  
511 crustaceans has shown strong support for the TOH across multiple studies, whereas two fish  
512 species have shown no support for the TOH (Gunderson, 2023). Differences in the trade-off  
513 between thermal tolerance and plasticity across and within species could be caused by different  
514 mechanisms of selection (i.e., correlational, opposing) for these two traits based on the  
515 organism's native environment (van Heerwaarden & Kellermann, 2020). For example, high or  
516 low thermal tolerance together with high or low plasticity may be adaptive in some  
517 environments, whereas only high plasticity or only high thermal tolerance may be adaptive in  
518 others (van Heerwaarden & Kellermann, 2020) because of differences in the species thermal



519 environment. Uncovering how widespread TOH may be and which factors may influence it will  
520 require more research, since heat hardening has been studied in a limited number of species.

521 Basal  $CT_{max}$  measured in individuals of *E. macularius* was found to be relatively lower  
522 (mean = 41.07 °C) compared to other species within the family *Eublepharidae* (mean across  
523 species = 42.3 °C; Clusella-Trullas & Chown, 2014); however, thermal tolerance has been  
524 scarcely studied within *Eublepharidae*. The two species that have been studied, *Coleonyx brevis*  
525 ( $CT_{max}$  = 41.6 °C; SVL = 5.1 cm) and *Coleonyx variegatus* ( $CT_{max}$  = 43.0 °C; SVL = 6.5 cm)  
526 (Dial & Grismer, 1992), have much smaller body size than *E. macularius*, which could explain  
527 the higher thermal tolerance exhibited within these two closely related species. To further  
528 corroborate the relationship between body size and thermal tolerance observed in ectotherms  
529 (Peralta-Maraver & Rezende, 2021), our data indicate that in our dataset, independently of the  
530 sex of the individuals, individuals with smaller body size (SVL) were found to have higher basal  
531  $CT_{max}$  compared to larger individuals. Intraspecific differences in body size have been shown to  
532 influence  $CT_{max}$  measurements in other lizard species (Brusch IV, 2016; Claunch, 2021). Larger  
533 individuals tend to have lower  $CT_{max}$  in four *Sceloporus* species, which has been hypothesized to  
534 be affected by thermal inertia, metabolic/oxygen delivery constraints, or a combination of both  
535 (Claunch et al, 2021). Smaller ectotherms have also been shown to have higher thermal tolerance  
536 in response to acute heat stress compared to larger ectotherms (Peralta-Maraver & Rezende,  
537 2021). The higher basal thermal tolerance of *E. macularius* in our study (mean ~ 41°C) than the  
538 mean microclimate temperatures observed in its native environment (mean sun ~ 22°C; mean  
539 shade ~ 19 °C) suggests that although this species is primarily active at dawn and dusk and  
540 hibernates during the winter months of the year (Rawat et al., 2019), it has adapted its upper  
541 thermal tolerance to the highest possible temperatures experienced in its native environment

542 across the day and season. Although, maximum temperatures in the species' native habitat reach  
543 59°C and 43°C across the year in the sun and shade, respectively, the mean temperature is close  
544 to 40°C during the mid-day hours in the sun across the year and above 25°C for several months  
545 during the year (Supp. Materials Figure S2).

546 Final  $CT_{max}$  – the maximum temperature that the organism could tolerate after 3, 6, or 24  
547 hours - was found to be influenced by both basal  $CT_{max}$  and time-interval treatment group for  
548 individuals of *E. macularius*. Individuals within the 6h and 24h treatment groups showed a  
549 significant positive relationship between basal and final  $CT_{max}$  in which individuals with  
550 relatively low or high basal  $CT_{max}$  also showed relatively low or high final  $CT_{max}$  after 6 hours or  
551 24 hours from initial heat shock, respectively. In the only other species of geckos studied so far,  
552 *Hemidactylus frenatus*, heat hardening experiments also found that basal  $CT_{max}$  also significantly  
553 affected final  $CT_{max}$  (Lapwong et al., 2021). To note that in our study, individuals within the 3h  
554 time-interval group did not show a significant relationship between basal and final  $CT_{max}$ . These  
555 results suggest that individuals of *E. macularius* may require a certain amount of time in between  
556 heat shocks (i.e, greater than 3 hours) to physiologically recover from initial heat stress and  
557 increase their thermal tolerance through the upregulation of heat shock proteins at the cellular  
558 level in order to prepare for the next heat stress event (Angilletta et al., 2009; Gangloff &  
559 Telemeco 2018). Furthermore, individuals of *E. macularius*, regardless of sex, showed a  
560 significant negative relationship between body mass and final  $CT_{max}$  where individuals with  
561 higher body mass exhibited a lower final  $CT_{max}$ , similar to what observed for basal  $CT_{max}$  and  
562 body size (SVL) in our experiments. Thus, both body mass and size can impact thermal tolerance  
563 for individuals of *E. macularius*, which is consistent with the previously discussed trend found

564 not only across lizard species (Brusch IV, 2016; Claunch, 2021), but also across ectotherms  
565 (Peralta-Maraver & Rezende, 2021).

566 Our results also support our hypothesis that changes in  $CT_{max}$  (heat hardening capacity)  
567 would be highest after 6 hours since initial heat shock for individuals of *E. macularius*, in  
568 agreement with what observed in other lizards (Phillips et al., 2016; Lapwong et al., 2021;  
569 Gilbert & Miles, 2019). In addition, the time course of heat hardening response across 24 hours  
570 for individuals of *E. macularius* is very similar to what found in other lizard species, where the  
571 magnitude of plasticity (i.e., change in thermal tolerance) increases to a set point and then  
572 decreases close to zero across 24 hours. To note that although this trend has been described for  
573 some diurnal species (e.g., *Anolis carolinensis* and *Lampropholis coggeri*; Deery et al., 2021,  
574 Phillips et al., 2016), it was not observed in the diurnal lizard *Anolis sagrei* (Deery et al., 2021)  
575 or in the crepuscular gecko *Hemidactylus frenatus* (Lapwong et al., 2021), in which thermal  
576 tolerance plasticity did not increase over time. Thus, the ability to respond quickly to heat stress  
577 by plasticly adjusting thermal tolerance may vary across and within species independently of the  
578 time of activity and may be instead more related to the seasonal variation and predictability of  
579 thermal variation for native environmental temperatures (e.g., Phillips et al., 2016; Deery et al.,  
580 2021). To highlight that the individuals of *E. macularius* used in this study are of captive origin  
581 and as such thermal tolerance plasticity may be lower than what it would be observed on wild  
582 individuals, since the native thermal environment of this species varies considerably across the  
583 day and season (Supp. Materials Figure S2).

584 Finally, although we found an influence of SVL and body mass on basal and final  $CT_{max}$ ,  
585 respectively, we found that changes in  $CT_{max}$  (heat hardening capacity) were not influenced by  
586 body size. On the other hand, changes in  $CT_{max}$  were influenced by body mass in the studied

587 species, with individuals with lower body mass experiencing greater change in  $CT_{max}$  compared  
588 to individuals with higher body mass. Acclimation capacity has been shown to be positively  
589 influenced by organism body size and mass across ectotherm species (Rohr et al., 2018; Brown  
590 et al., 2004; Kingsolver & Huey, 2008; Pörtner et al., 2017). Larger individuals (or species) have  
591 been found to have greater acclimation capacity (i.e., change in the upper thermal tolerance  
592 relative to change in mean temperature, Claussen, 1977), although smaller organisms have a  
593 faster acclimation rate (Rohr et al., 2018). As a consequence, during short-term acclimation,  
594 smaller organisms may show greater plasticity due to their faster acclimation (Rohr et al., 2018).  
595 Thus, although our results where smaller individuals have higher change in  $CT_{max}$  (heat  
596 hardening/plasticity) seem to not support the general relationship between body mass and  
597 acclimation capacity (heat hardening) as observed in other species, this may be due to  
598 individuals being exposed to short-term instead of long-term thermal stress. In addition, smaller  
599 organisms have higher mass-specific metabolic rates and lower oxygen demand and delivery  
600 constraints compared to organisms with greater body mass, which has been theorized to aid in  
601 the faster acclimation responses of smaller organisms (Brown et al., 2004; Kingsolver & Huey,  
602 2008; Pörtner et al., 2017)

## 603 **5. Conclusions**

604 Our results suggest that basal thermal tolerance does not influence heat hardening  
605 capacity in *E. macularius*, indicating that individuals with lower basal thermal tolerance do not  
606 compensate for this by possessing a greater capacity to plasticly adjust their upper thermal limit.  
607 Although this may be the results of an adaptation to captivity conditions, where the  
608 developmental environment may be more controlled and homogeneous than in the wild, the lack  
609 of trade-off between basal thermal tolerance and plasticity seems to largely vary within and

610 across different species. To further understand the interplay between basal thermal tolerance and  
611 plasticity in organisms, future studies should focus not only on collecting similar data across  
612 species and populations of ectotherms, but also on investigating the mechanisms underlying  
613 differences in selection influencing this trade-off. Additionally, our data support a strong  
614 similitude in heat hardening capacity between the studied captive population of *E. macularius*  
615 and what observed in other squamates sampled from the wild. These results suggest that  
616 although plasticity of thermal tolerance via heat hardening may be somehow influenced due to  
617 captivity, the species' biology, physiology, and evolutionary history may have a stronger impact.  
618 Our work, together with the work done so far on thermal tolerance plasticity in ectotherms,  
619 highlights the need of carrying out comparative studies across species and collecting data across  
620 different levels of complexity, from molecular to ecological and physiological, to uncover the  
621 abiotic factors influencing thermal tolerance plasticity and the molecular mechanisms underlying  
622 the observed variation.

623 Finally, heat stress caused by climate change has been predicted to negatively impact  
624 ectothermic species, especially for tropical and mid-latitude organisms (Kingsolver et al., 2013).  
625 This study increases our understanding of how quickly organisms can respond to daily thermal  
626 extremes. To note that the studies available so far on this topic do not address responses across  
627 multiple or prolonged heat stress events. As heat waves and heat stress are predicted to increase  
628 in length and frequency in the future (Stillman et al., 2019; Angéilil et al., 2017; Guo et al., 2018;  
629 Pachauri et al., 2014), to properly assess how organisms will be impacted, it will be necessary for  
630 future studies to also investigate variation in thermal tolerance plasticity across days or weeks.

631 Taken all together, our study contributes to the limited data available so far on thermal  
632 tolerance plasticity in squamates, focusing on an extremely understudied group for this topic

633 (geckos), a species mostly active during crepuscular/early night hours, and a population that has  
634 been captive bred for several (unknown) generations. Furthermore, our study also provides  
635 methodological guidelines on checking potential variation (in heating rates) that may influence  
636 results and their interpretation of studies on thermal tolerance plasticity, independently of the  
637 studied species.

638

### 639 **Acknowledgements**

640 We are thankful to Alex Gunderson, Vincent Farallo, Miguel Carretero, Catarina Rato, and Eric  
641 Gangloff for helpful advice with designing and setting up experiments, and to Patrick Gillevet  
642 for technical help with the temperature-controlled room used for the experiments. We are  
643 thankful to Patrick Gillevet and Rebecca Forkner for providing helpful comments on an earlier  
644 version of this manuscript.

645

### 646 **Author Contributions**

647 Conceptualization: E.W., Y.C.; Methodology: E.W.; Y.C.; Formal Analysis: E.W.; Resources:  
648 Y.C.; Investigation: E.W., S.K., G.W.; Writing - original draft: E.W., Y.C.; Writing - review &  
649 editing: S.K., G.W.; Supervision: Y.C.; Project administration: Y.C.

650

### 651 **Declaration of competing interest**

652 The authors declare no competing or financial interests.

653

### 654 **Data accessibility**

655 Full dataset and Rscript used for analyses will be available on Dryad *after manuscript*  
656 *acceptance*.

657

## 658 **References**

- 659 Alexander, L. V., Zhang, X., Peterson, T. C., Caesar, J., Gleason, B., Klein Tank, A. M. G., ... &  
660 Vazquez-Aguirre, J. L. (2006). Global observed changes in daily climate extremes of  
661 temperature and precipitation. *Journal of Geophysical Research: Atmospheres*, *111*(D5).
- 662 Angéllil, O., Stone, D., Wehner, M., Paciorek, C. J., Krishnan, H., & Collins, W. (2017). An  
663 independent assessment of anthropogenic attribution statements for recent extreme  
664 temperature and rainfall events. *Journal of Climate*, *30*(1), 5-16.
- 665 Angilletta, M. J. (2009). Thermal adaptation: a theoretical and empirical synthesis. Oxford  
666 University Press.
- 667 Bilyk, K. T., Evans, C. W., & DeVries, A. L. (2012). Heat hardening in Antarctic notothenioid  
668 fishes. *Polar biology*, *35*(9), 1447-1451.
- 669 Bowler, K. (2005). Acclimation, heat shock and hardening. *Journal of Thermal Biology*, *30*(2),  
670 125-130.
- 671 Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a  
672 metabolic theory of ecology. *Ecology*, *85*(7), 1771-1789.
- 673 Bruschi, G. A., Taylor, E. N., & Whitfield, S. M. (2016). Turn up the heat: thermal tolerances  
674 of lizards at La Selva, Costa Rica. *Oecologia*, *180*(2), 325-334.
- 675 Buckley, B. A., & Hofmann, G. E. (2002). Thermal acclimation changes DNA-binding activity  
676 of heat shock factor 1 (HSF1) in the goby *Gillichthys mirabilis*: implications for plasticity

677 in the heat-shock response in natural populations. *Journal of Experimental Biology*,  
678 205(20), 3231-3240.

679 Camacho, A., & Rusch, T. W. (2017). Methods and pitfalls of measuring thermal preference and  
680 tolerance in lizards. *Journal of Thermal Biology*, 68, 63-72.

681 Chamberlain, S., Ram, K., Barve, V., Mcglinn, D., & Chamberlain, M. S. (2017). Package  
682 'rgbif'. *Interface to the Global Biodiversity Information Facility 'API*, 5(0.9).

683 Claunch, N. M., Nix, E., Royal, A. E., Burgos, L. P., Corn, M., DuBois, P. M., ... & Taylor, E. N.  
684 (2021). Body size impacts critical thermal maximum measurements in lizards. *Journal of*  
685 *Experimental Zoology Part A: Ecological and Integrative Physiology*, 335(1), 96-107.

686 Claussen, D. L. (1977). Thermal acclimation in ambystomatid salamanders. *Comparative*  
687 *Biochemistry and Physiology A*, 58, 333-340.

688 Clusella-Trullas, S., & Chown, S. L. (2014).  
689 Lizard thermal trait variation at multiple scales: a review. *Journal of Comparative*  
*Physiology B*, 184, 5-21.

690 Coffel, E. D., Horton, R. M., & De Sherbinin, A. (2017). Temperature and humidity based  
691 projections of a rapid rise in global heat stress exposure during the 21st century.  
692 *Environmental Research Letters*, 13(1), 014001.

693 Cook, R. D. (1977). Detection of influential observation in linear  
694 regression. *Technometrics*, 19(1), 15-18.

695 Daugaard, M., Rohde, M., & Jäätelä, M. (2007). The heat shock protein 70 family: Highly  
696 homologous proteins with overlapping and distinct functions. *FEBS letters*, 581(19),  
697 3702-3710.



- 698 Dayananda, B., Gray, S., Pike, D., & Webb, J. K. (2016). Communal nesting under climate  
699 change: fitness consequences of higher incubation temperatures for a nocturnal lizard.  
700 *Global Change Biology*, 22(7), 2405-2414.
- 701 Deery, Rej, J. E., Haro, D., & Gunderson, A. R. (2021). Heat hardening in a pair of Anolis  
702 lizards: constraints, dynamics and ecological consequences. *Journal of Experimental*  
703 *Biology*, 224(Pt 7).
- 704 Dial, B. E., & Grismer, L. L. (1992). A phylogenetic analysis of physiological-ecological  
705 character evolution in the lizard genus *Coleonyx* and its implications for historical  
706 biogeographic reconstruction. *Systematic Biology*, 41(2), 178-195.
- 707 Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., ... &  
708 Folland, C. K. (1997). Maximum and minimum temperature trends for the  
709 globe. *Science*, 277(5324), 364-367.
- 710 Froelicher, T., Fischer, E. M., Gruber, N., Striegel, S., & Laufkötter, C. (2019, January). Marine  
711 Heat Waves under Global Warming (Invited Presentation). In *99th American*  
712 *Meteorological Society Annual Meeting*. AMS.
- 713 Gamble, T., Greenbaum, E., Jackman, T. R., & Bauer, A. M. (2015). Into the light: diurnality has  
714 evolved multiple times in geckos. *Biological Journal of the Linnean Society*, 115(4), 896-  
715 910.
- 716 Gangloff, E. J., & Telemeco, R. S. (2018). High temperature, oxygen, and performance: Insights  
717 from reptiles and amphibians. *Integrative and Comparative Biology*, 58(1), 9-24.
- 718 Gilbert, A. L., & Miles, D. B. (2019). Antagonistic responses of exposure to sublethal  
719 temperatures: adaptive phenotypic plasticity coincides with a reduction in organismal  
720 performance. *The American Naturalist*, 194(3), 344-355.

- 721 Glimm, T., Kiskowski, M., Moreno, N., & Chiari, Y. (2021). Capturing and analyzing pattern  
722 diversity: an example using the melanistic spotted patterns of leopard geckos. *PeerJ*, 9,  
723 e11829.
- 724 Grismer, L. L., Wood Jr, P. L., Ngo, V. T., & Murdoch, M. L. (2015). The systematics and  
725 independent evolution of cave ecomorphology in distantly related clades of Bent-toed  
726 Geckos (Genus *Cyrtodactylus* Gray, 1827) from the Mekong Delta and islands in the  
727 Gulf of Thailand. *Zootaxa*, 3980(1), 106-126.
- 728 Gunderson, A. R. (2023). Trade-offs between baseline thermal tolerance and thermal tolerance  
729 plasticity are much less common than it appears. *Global Change Biology*.
- 730 Gunderson, A. R., Dillon, M. E., & Stillman, J. H. (2017). Estimating the benefits of plasticity in  
731 ectotherm heat tolerance under natural thermal variability. *Functional Ecology*, 31(8),  
732 1529-1539.
- 733 Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential  
734 to buffer ectotherms from global warming. *Proceedings of the Royal Society B:  
735 Biological Sciences*, 282(1808), 20150401.
- 736 Guo, Y., Gasparrini, A., Li, S., Sera, F., Vicedo-Cabrera, A. M., de Sousa Zanotti Stagliorio  
737 Coelho, M., ... & Tong, S. (2018). Quantifying excess deaths related to heatwaves under  
738 climate change scenarios: A multicountry time series modelling study. *PLoS medicine*,  
739 15(7), e1002629.
- 740 Hamdoun, A. M., Cheney, D. P., & Cherr, G. N. (2003). Phenotypic plasticity of HSP70 and  
741 HSP70 gene expression in the Pacific oyster (*Crassostrea gigas*): implications for thermal  
742 limits and induction of thermal tolerance. *The Biological Bulletin*, 205(2), 160-169.

- 743 Hastings, B. T., Melnyk, A., Ghyabi, M., White, E., Barroso, F. M., Carretero, M. A., ... &  
744 Chiari, Y. (2023). On the role of melanistic coloration on thermoregulation in the  
745 crepuscular gecko *Eublepharis macularius*. *bioRxiv*, 2023-05.
- 746 Heinicke, M. P., Jackman, T. R., & Bauer, A. M. (2017). The measure of success: geographic  
747 isolation promotes diversification in *Pachydactylus* geckos. *BMC Evolutionary Biology*,  
748 *17*(1), 1-17.
- 749 Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2013). Upper thermal limits in  
750 terrestrial ectotherms: how constrained are they?. *Functional Ecology*, *27*(4), 934-949.
- 751 Horowitz, M. (2001). Heat acclimation: phenotypic plasticity and cues to the underlying  
752 molecular mechanisms. *Journal of Thermal Biology*, *26*(4-5), 357-363.
- 753 Hu, J. T., Chen, B., & Li, Z. H. (2014). Thermal plasticity is related to the hardening response of  
754 heat shock protein expression in two *Bactrocera* fruit flies. *Journal of Insect Physiology*,  
755 *67*, 105-113.
- 756 Huey, R. B. (1991). Physiological consequences of habitat selection. *The American Naturalist*,  
757 *137*, S91-S115.
- 758 Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American*  
759 *Naturalist*, *101*(919), 233-249.
- 760 Karl, T. R., Kukla, G., Razuvayev, V. N., Changery, M. J., Quayle, R. G., Heim Jr, R. R., ... &  
761 Fu, C. B. (1991). Global warming: Evidence for asymmetric diurnal temperature  
762 change. *Geophysical Research Letters*, *18*(12), 2253-2256.
- 763 Katlein, N., Ray, M., Wilkinson, A., Claude, J., Kiskowski, M., Wang, B., Glaberman, S. &  
764 Chiari, Y. (2022). Does colour impact responses to images in geckos?. *Journal of*  
765 *Zoology*, *317*(2), 138-146.

- 766 Kearney, M. R., & Porter, W. P. (2017). NicheMapR - an R package for biophysical modeling:  
767 The microclimate model. *Ecography*, 40(5), 664–674.
- 768 Kelly, C., & Price, T. D. (2005). Correcting for regression to the mean in behavior and ecology.  
769 *The American Naturalist*, 166(6), 700-707.
- 770 Khan, M. S. (2009). Natural history and biology of hobbyist choice leopard gecko *Eublepharis*  
771 *macularius*. *Talim ul Islam College, Rabwah, Pakistan*.
- 772 Khan, N., Shahid, S., Ahmed, K., Wang, X., Ali, R., Ismail, T., & Nawaz, N. (2020). Selection  
773 of GCMs for the projection of spatial distribution of heat waves in Pakistan. *Atmospheric*  
774 *Research*, 233, 104688.
- 775 Kingsolver, J., & Huey, R. (2008). Size, temperature, and fitness: three rules. *Evolutionary*  
776 *Ecology Research*, 10(2), 251-268.
- 777 Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness  
778 consequences of climate change for terrestrial ectotherms. *Functional Ecology*, 27(6),  
779 1415-1423.
- 780 Kiskowski, M., Glimm, T., Moreno, N., Gamble, T., & Chiari, Y. (2019). Isolating and  
781 quantifying the role of developmental noise in generating phenotypic variation. *PLOS*  
782 *Computational Biology*, 15(4), e1006943.
- 783 Kratochvíl, L., & Frynta, D. (2002). Body size, male combat and the evolution of sexual  
784 dimorphism in eublepharid geckos (Squamata: Eublepharidae). *Biological Journal of the*  
785 *Linnean Society*, 76(2), 303-314.
- 786 Kregel, K. C. (2002). Invited review: heat shock proteins: modifying factors in physiological  
787 stress responses and acquired thermotolerance. *Journal of Applied Physiology*, 92(5),  
788 2177-2186.

- 789 Lapwong, Y., Dejtardol, A., & Webb, J. K. (2021). Plasticity in thermal hardening of the  
790 invasive Asian house gecko. *Evolutionary Ecology*, 35(4), 631-641.
- 791 Madeira, D., Narciso, L., Cabral, H. N., & Vinagre, C. (2012). Thermal tolerance and potential  
792 impacts of climate change on coastal and estuarine organisms. *Journal of Sea*  
793 *Research*, 70, 32-41.
- 794 Madeira, D., Narciso, L., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2014). Role of thermal  
795 niche in the cellular response to thermal stress: Lipid peroxidation and HSP70 expression  
796 in coastal crabs. *Ecological indicators*, 36, 601-606.
- 797 Manenti, T., Sørensen, J. G., Moghadam, N. N., & Loeschcke, V. (2014). Predictability rather  
798 than amplitude of temperature fluctuations determines stress resistance in a natural  
799 population of *Drosophila simulans*. *Journal of Evolutionary Biology*, 27(10), 2113-2122.
- 800 Meiri, S. (2019). What geckos are—an ecological-biogeographic perspective. *Israel Journal of*  
801 *Ecology and Evolution*, 66(3-4), 253-263.
- 802 Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CTmax is repeatable and doesn't reduce  
803 growth in zebrafish. *Scientific reports*, 8(1), 7099.
- 804 Mottola, G., Lopez, M. E., Vasemägi, A., Nikinmaa, M., & Anttila, K. (2022). Are you ready for  
805 the heat? Phenotypic plasticity versus adaptation of heat tolerance in three-spined  
806 stickleback. *Ecosphere*, 13(4), e4015.
- 807 Noer, N. K., Ørsted, M., Schiffer, M., Hoffmann, A. A., Bahrndorff, S., & Kristensen, T. N.  
808 (2022). Into the wild—a field study on the evolutionary and ecological importance of  
809 thermal plasticity in ectotherms across temperate and tropical regions. *Philosophical*  
810 *Transactions of the Royal Society B*, 377(1846), 20210004.

- 811 Oliver, P. M., Ashman, L. G., Bank, S., Laver, R. J., Pratt, R. C., Tedeschi, L. G., & Moritz, C.  
812 C. (2019). On and off the rocks: persistence and ecological diversification in a tropical  
813 Australian lizard radiation. *BMC Evolutionary Biology*, *19*, 1-15.
- 814 Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., ... & van  
815 Ypserle, J. P. (2014). *Climate change 2014: Synthesis report. Contribution of Working*  
816 *Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on*  
817 *Climate Change* (p. 151). IPCC.
- 818 Papenfuss, T., Shafiei Bafti, S. & Sharifi, M. 2021. *Eublepharis macularius*. *The IUCN Red List*  
819 *of Threatened Species* 2021:  
820 e.T164745A1072324. [https://dx.doi.org/10.2305/IUCN.UK.2021-](https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T164745A1072324.en)  
821 [3.RLTS.T164745A1072324.en](https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T164745A1072324.en). Accessed on 19 April 2022.
- 822 Peralta-Maraver, I., & Rezende, E. L. (2021). Heat tolerance in ectotherms scales predictably  
823 with body size. *Nature Climate Change*, *11*(1), 58-63.
- 824 Phillips, Muñoz, M. M., Hatcher, A., Macdonald, S. L., Llewelyn, J., Lucy, V., & Moritz, C.  
825 (2016). Heat hardening in a tropical lizard: geographic variation explained by the  
826 predictability and variance in environmental temperatures. *Functional Ecology*, *30*(7),  
827 1161–1168.
- 828 Pinto, B. J., Gamble, T., Smith, C. H., Keating, S. E., Havird, J. C., & Chiari, Y. (2023). The  
829 revised reference genome of the leopard gecko (*Eublepharis macularius*) provides insight  
830 into the considerations of genome phasing and assembly. *bioRxiv*, 2023-01.
- 831 Pörtner, H. O., Bock, C., & Mark, F. C. (2017). Oxygen-and capacity-limited thermal tolerance:  
832 bridging ecology and physiology. *Journal of Experimental Biology*, *220*(15), 2685-2696.

- 833 Rawat, Y. B., Thapa, K. B., Bhattarai, S., & Shah, K. B. (2019). First Records of the Common  
834 Leopard Gecko, *Eublepharis macularius* (Blyth 1854) (Eublepharidae), in Nepal. *Reptiles*  
835 *& Amphibians*, 26(1), 58-61.
- 836 Rezende, E. L., Tejedo, M., & Santos, M. (2011). Estimating the adaptive potential of critical  
837 thermal limits: methodological problems and evolutionary implications. *Functional*  
838 *Ecology*, 25(1), 111-121.
- 839 Ribeiro, P. L., Camacho, A., & Navas, C. A. (2012). Considerations for assessing maximum  
840 critical temperatures in small ectothermic animals: insights from leaf-cutting ants. *PLoS*  
841 *One*, 7(2), e32083.
- 842 Rohini, P., Rajeevan, M., & Mukhopadhyay, P. (2019). Future projections of heat waves over  
843 India from CMIP5 models. *Climate Dynamics*, 53(1), 975-988.
- 844 Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The  
845 complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*,  
846 21(9), 1425-1439.
- 847 Ruibal, R. (1961). Thermal relations of five species of tropical lizards. *Evolution*, 15(1), 98-111.
- 848 Sørensen, J. G., Kristensen, T. N., & Loeschcke, V. (2003). The evolutionary and ecological role  
849 of heat shock proteins. *Ecology Letters*, 6(11), 1025-1037.
- 850 Rutschmann, A., Perry, C., Le Galliard, J. F., Dupoué, A., Lourdais, O., Guillon, M., Bruschi, G.,  
851 4th, Cote, J., Richard, M., Clobert, J., & Miles, D. B. (2023). Ecological responses of  
852 squamate reptiles to nocturnal warming. *Biological reviews of the Cambridge*  
853 *Philosophical Society*, 10.1111/brv.13037.

- 854 Sasaki, M. C., & Dam, H. G. (2019). Integrating patterns of thermal tolerance and phenotypic  
855 plasticity with population genetics to improve understanding of vulnerability to warming  
856 in a widespread copepod. *Global change biology*, 25(12), 4147-4164.
- 857 Sørensen, M. H., Kristensen, T. N., Lauritzen, J. M. S., Noer, N. K., Høye, T. T., & Bahrndorff,  
858 S. (2019). Rapid induction of the heat hardening response in an Arctic insect. *Biology*  
859 *Letters*, 15(10), 20190613.
- 860 Stillman, J. H. (2019). Heat waves, the new normal: summertime temperature extremes will  
861 impact animals, ecosystems, and human communities. *Physiology*, 34(2), 86-100.
- 862 Telemeco, R. S., & Gangloff, E. J. (2021). Introduction to the special issue—Beyond CTMAX  
863 and CTMIN: Advances in studying the thermal limits of reptiles and amphibians. *Journal*  
864 *of Experimental Zoology Part A: Ecological and Integrative Physiology*, 335(1), 5-12.
- 865 Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical  
866 thermal limits depend on methodological context. *Proceedings of the Royal Society B:*  
867 *Biological Sciences*, 274(1628), 2935-2943.
- 868 Tewksbury, J. J., Huey, R. B., & Deutsch, C. A. (2008). Putting the heat on tropical animals.  
869 *Science*, 320(5881), 1296-1297.
- 870 Uetz, P., Slavenko, A., Meiri, S., & Heinicke, M. (2020). Gecko diversity: a history of global  
871 discovery. *Israel Journal of Ecology and Evolution*, 66(3-4), 117-125.
- 872 Uetz, P., Freed, P., Aguilar, R., & Hošek, J. (2023). The Reptile Database. <http://www.reptile->  
873 [database.org](http://www.reptile-database.org), accessed 2023-12-08.
- 874 Ulmasov, K. A., Shammakov, S., Karaev, K., & Evgen'ev, M. B. (1992). Heat shock proteins  
875 and thermoresistance in lizards. *Proceedings of the National Academy of Sciences*, 89(5),  
876 1666-1670.



- 877 van Heerwaarden, B., Lee, R. F. H., Overgaard, J., & Sgrò, C. M. (2014). No patterns in thermal  
878 plasticity along a latitudinal gradient in *Drosophila simulans* from eastern  
879 Australia. *Journal of Evolutionary Biology*, 27(11), 2541-2553.
- 880 van Heerwaarden, B., & Kellermann, V. (2020). Does plasticity trade off with basal heat  
881 tolerance?. *Trends in Ecology & Evolution*, 35(10), 874-885.
- 882 Vose, R. S., Easterling, D. R., & Gleason, B. (2005). Maximum and minimum temperature  
883 trends for the globe: An update through 2004. *Geophysical Research Letters*, 32(23).
- 884 Williams, C. M., Buckley, L. B., Sheldon, K. S., Vickers, M., Pörtner, H. O., Dowd, W. W., ... &  
885 Stillman, J. H. (2016). Biological impacts of thermal extremes: mechanisms and costs of  
886 functional responses matter. *Integrative and Comparative Biology*, 56(1), 73-84.
- 887 Zatsepina, O. G., Ulmasov, K. A., Beresten, S. F., Molodtsov, V. B., Rybtsov, S. A., &  
888 Evgen'Ev, M. B. (2000). Thermotolerant desert lizards characteristically differ in terms of  
889 heat-shock system regulation. *Journal of Experimental Biology*, 203(6), 1017-1025.
- 890 Zizka, A., Silvestro, D., Andermann, T., Azevedo, J., Duarte Ritter, C., Edler, D., ... & Antonelli,  
891 A. (2019). CoordinateCleaner: Standardized cleaning of occurrence records from  
892 biological collection databases. *Methods in Ecology and Evolution*, 10(5), 744-751.
- 893