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# Shifts in the Thermal Dependence of Locomotor Performance across an Altitudinal Gradient in Native Populations of *Xenopus laevis*

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1 **Shifts in the thermal dependence of locomotor performance across an altitudinal**  
2 **gradient in native populations of *Xenopus laevis*.**

3

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14

15 **Key words** *Xenopus laevis*, native populations, altitudes, temperature, locomotor  
16 performance, thermal performance curve.

17 **Summary statement** Few intraspecific studies exist on amphibians' performance depending  
18 on the elevation as few species are widely distributed along altitudes. Here, we characterized  
19 the locomotor performance of *Xenopus laevis* across a wide range of thermal environments  
20 along an altitudinal gradient and showed a decrease in the optimal temperature for exertion  
21 with altitude.

## 22 **Abstract**

23 Ectothermic species are dependent on temperature which drives many aspects of their  
24 physiology including locomotion. The distribution of the native populations of *Xenopus laevis*  
25 is characterized by an exceptional range in latitude and altitude. Along altitudinal gradients the  
26 thermal environments changes, and populations experience different temperatures. In this  
27 study, we compared critical thermal limits and the thermal performance curves of populations  
28 from the native range across an altitudinal gradient to test whether thermal optima for exertion  
29 differ depending on altitude. Data on exertion capacity were collected at six different  
30 temperatures (8, 12, 16, 19, 23, 27°C) for four population spanning an altitudinal gradient (60,  
31 1016, 1948 and 3197 m above sea level). Results show that the thermal performance optimum  
32 differs among populations. Populations from cold environments at high altitudes exhibit a lower  
33 optimal performance temperature than ones from warmer environments at lower altitudes. The  
34 ability of this species to change its thermal optimum for locomotor exertion across extremely  
35 different climatic environments within the native range may help explain its exceptional  
36 invasion potential. These results suggest that ectothermic species capable of adapting to broad  
37 altitudinal ranges may be particularly good at invading novel climatic areas given their ability  
38 to cope with a wide range of variation in environmental temperatures.

## 39 **Introduction**

40           Temperature is one of the dimensions of the ecological niche and consequently a  
41 characteristic of an animal's habitat (Magnuson *et al.*, 1979). Therefore, species distribution  
42 and abundance patterns are in large part driven by environmental temperature (Clarke 2003,  
43 Hoffmann *et al.*, 2003). The thermal environment varies depending on geological times,  
44 seasons and days, and consequently all living beings are submitted to this selective pressure  
45 (Brown *et al.*, 2004). The physiological processes of organisms are highly temperature  
46 dependent. Cellular responses to temperature are linked to the metabolism supplying ATP for  
47 cellular maintenance, membrane and protein synthesis (Hulbert and Else 2000; Gillooly 2001,  
48 Pörtner and Farrell 2008), and play a role in growth, reproduction, and locomotor  
49 performance. These processes have direct fitness consequences for living beings (Johnston  
50 and Temple 2002; Guderley 2004). Local or regional differences in temperature related to  
51 climate lead to a variation in the spatial distribution of species, moreover depending on  
52 altitude as temperature tends to decrease with altitude (Körner 2007; Montgomery 2006).

53           At high altitude the daily and seasonal variation between minimum and maximum  
54 mean environmental temperature can be extreme (Jacobsen and Dangles 2017). Previous  
55 studies have investigated the impact of altitude on amphibians, including patterns of  
56 distribution depending on elevational gradients, their metabolic and locomotor physiology,  
57 their thermal safety margin, lifespan, plasticity of metamorphic traits, and their environmental  
58 adaptations (Navas 2006; Navas 1996; Sunday *et al.*, 2014; Zhang and Lu 2012; Yu *et al.*,  
59 2016; Wang *et al.*, 2018; Wagener *et al.*, 2021).

60           Here, we study *Xenopus laevis*, an exceptional model for investigating the impact of  
61 altitude as it ranges from sea level to over 3000 m above sea level (a.s.l.), an unusually large  
62 range within a single species. *Xenopus laevis* is a largely aquatic frog from sub-Saharan  
63 Africa, and inhabits a wide range of geographical and thermal environments (Measey *et al.*,

64 2004; Furman *et al.*, 2015). In a previous study (Araspin *et al.*, 2020) it was demonstrated that  
65 optimal temperatures for locomotion differed between native (South Africa) and invasive  
66 (France) populations of *Xenopus laevis* associated with differences in the thermal  
67 environment experienced by these populations. In its native range, this species displays an  
68 important phenotypic diversity which may suggests a plastic physiology (Du Preez *et al.*,  
69 2009). Recent studies on tadpoles suggest both an adaptive and plastic underpinning of  
70 population-level differences in physiology (Wagener *et al.*, 2021; Kruger *et al.*, in press).  
71 *Xenopus laevis* is invasive on four continents (Measey *et al.*, 2012) and is a threat for local  
72 biota (Courant *et al.*, 2018; Kumschick *et al.*, 2017). Plasticity in its temperature tolerance  
73 may have been a key factor driving the invasive potential of this species. Furthermore,  
74 adaptation to new thermal environments may have facilitated survival in its invasive range.

75 The aim of the present study was to assess the thermal dependence of locomotor performance  
76 in adults from native populations across an altitudinal gradient and to identify potential  
77 differences among populations. We chose to measure exertion capacity (measured here as the  
78 distance or time swam until exhaustion) as it is fitness-relevant and a good proxy for dispersal  
79 capacity. We predict that populations from high altitudes and cold environments will perform  
80 relatively better at cold temperatures and conversely, populations from low altitude should  
81 perform better at warmer temperatures. We thus predict that the optimal temperatures for  
82 locomotor performance will decrease with increasing altitude. However, we do not expect  
83 changes in the upper thermal limit as it is determined by genetic and biochemical constraints  
84 and as behavioral thermoregulation is effective in avoiding critical maximal temperatures  
85 (Blackburn *et al.*, 2014; Grigg and Buckley 2013; von May *et al.*, 2017; Pintanel *et al.*, 2019).  
86 In contrast, the lower thermal limit is expected to decrease with altitude as observed in  
87 previous studies, at least partly as behavioral thermoregulation is less effective (Bodensteiner  
88 *et al.*, 2021). If so, then our results may have broader implications for our understanding of

89 the evolution of thermal performance limits in ectothermic organisms and the ability of  
90 species to cope with variation in their current or future thermal environment.

## 91 **Methods**

92         Adult *Xenopus laevis* individuals were caught in three different areas in Kwa-Zulu  
93 Natal, eastern South Africa. Populations were sampled at different altitudes; Hluhluwe (60 m  
94 a.s.l.), N = 26, 13 females and 13 males; Dalton (1016 m a.s.l.), N = 25, 13 females and 12  
95 males; near Phuthaditjhaba (1948 m a.s.l.), N = 26, 13 females and 13 males. Individuals from  
96 a fourth population were caught in Lesotho at 3197 m a.s.l., N = 28, 14 females and 14 males.  
97 Animals were captured at night in March 2021 using liver-baited funnel traps. Animals were  
98 transported to Stellenbosch University in South Africa. Upon arrival all individuals were pit-  
99 tagged allowing unique identification. Specimens were maintained in 72 L (60 x 30 x 40 cm)  
100 aquaria, 13 to 14 individuals per tank and separated by sex and the site of origin. Tanks  
101 contained rocks to provide hiding places. Animals were fed twice weekly with chicken heart.  
102 The temperature of the water was maintained at 18°C. All protocols and animal welfare  
103 conditions were in accordance with the Research Ethics Committee for Animal care and use,  
104 protocol number ACU-2021-19215.

### 105 *In situ temperature data collection*

106         Temperature data loggers (HOBO MX2201, Onset Computer Corporation) were  
107 placed in ponds at a depth of approximately 60 cm at the sites of collection and recorded the  
108 ambient temperature every 30 minutes. Data from March 2021 to May 2021 were recorded  
109 and downloaded via the HOBO application (HOBObconnect, version 1.2.4 (25775)).

### 110 *Morphometrics*

111 Body dimensions were measured following Herrel *et al.* (2012). The mass was  
112 measured with a digital scale (Ohaus, Brooklyn, NY, USA; precision  $\pm 0.1$  g) and snout-vent  
113 length (SVL) was measured using a pair of digital callipers (Mitutoya; precision  $\pm 0.01$  mm).

#### 114 *Critical temperatures*

115 Individuals were placed by three in containers (18 x 30 x 10 cm) with some water to  
116 prevent dehydration and placed in an incubator (Sanyo MIR 154 Incubator, -10 to +60°C).  
117 Experiments started at 18°C (i.e., the housing temperature) from which the temperature was  
118 gradually decreased or increased at an average rate of 2°C each 45 minutes. When reaching  
119 6°C the temperature was decreased at a rate of 1°C each 45 minutes for the determination of  
120 the critical minimum temperature. To determine the critical maximal temperature the  
121 temperature was increased at a rate of 1°C each 45 minutes from 27°C upwards. Animals  
122 were warmed up slowly as their thermal inertia was substantial and faster rates resulted in  
123 animals being cooler or warmer than the temperature of the incubator. Animals were  
124 inspected regularly and checked for lack of a righting response. As soon as animals were no  
125 longer able to turn over after placed on their back, we considered that temperature (measured  
126 with a thermocouple inserted into the cloaca) to be that animal's critical temperature.

#### 127 *Performance*

128 Aquatic exertion tests were performed at 8°C, 12°C, 16°C, 19°C, 23°C and 27°C.  
129 Individuals were placed by seven in containers (45x30x10 cm) with some water to prevent  
130 dehydration and left for 3 hours in an incubator set at the desired test temperature. The room  
131 was set at the test temperature. Before and after each performance trial body temperature was  
132 recorded using a K-type thermocouple. Measures of exertion were performed by chasing  
133 animals individually until exhaustion (defined as animals no longer being able to right  
134 themselves when put on their back) around a 4.43-meter-long circular track with an average

135 water depth of 20cm. The total distance and time to exhaustion at the end of the trial were  
136 recorded. After each trial animals were left to recover at an average temperature of 20°C and  
137 then returned to their tank (water at 18°C). Animals were fed and left to rest for at least three  
138 days. Trials were repeated twice per individual and the maximal distance was retained for  
139 further analyses.

#### 140 *Statistical analyses*

141 To fulfil assumptions of normality and homoscedasticity, all data were  $\log_{10}$ -  
142 transformed before analyses. Analyses were performed using R (version i386 4.1.2; R core  
143 team 2021). To test for significant differences in the different locomotor performance traits  
144 (i.e., distance and time to exhaustion) among populations, we used a mixed model, with  
145 temperature, population, and sex as fixed effects, SVL as covariate, and frog ID as a random  
146 effect. To test for differences in SVL and mass among populations, two-way ANOVAs were  
147 run. Within populations (i.e., Hluhluwe, Dalton, Phuthaditjhaba, and Lesotho) we further  
148 tested for effects of temperature and sex on performance as well as their interactions. We used  
149 mass and SVL as covariates. To do so, we used a repeated measures multivariate analysis of  
150 variance (MANOVAs). Next, pairwise multiple comparisons tests (pairwise.t.test, “Stats”  
151 package version 3.4.2) with Bonferroni-Holm adjusted  $P$ -values were performed to test which  
152 temperatures differed from one another (temperature range: 8, 12, 16, 19, 23 and 27°C).  
153 TukeyHSD tests were performed to test which populations differed from another depending  
154 on the tested temperature and to test for difference in critical temperatures among populations.  
155 For each individual thermal performance curves (Angilletta *et al.*, 2002) were built using the  
156 minimum convex polygon method (van Berkum, 1986). Optimal temperatures as well as  
157 temperature breadths  $T_{pb80}$  and  $T_{pb95}$  were measured and analysed using univariate ANOVAs  
158 and Welch ANOVAs to test for differences among populations in locomotor performance  
159 (i.e., aquatic exertion).

160 To further assess the relationship between temperature and exertion capacity for each  
161 population we fitted a generalized additive model (GAM). We used sex and population as  
162 fixed factors and applied a smoothing parameter to SVL and temperature. Because the same  
163 individuals were used, we added individual identity as a random term. The smoothing  
164 parameter (k) was optimized based on the model adjusted R-squared values and using the  
165 functions of the “mgcv” R package (v1.8-35; Wood 2011). To compare the thermal optimum  
166 of each population, a GAM with the same parameters was also fitted for each individual and  
167 an ANOVA with population as factor was conducted on the predicted thermal optima,  
168 followed by a Tuckey HSD test to inspect significant differences within populations.

169

## 170 **Results**

### 171 *Morphometric measurements*

172 We observed significant differences in SVL and in mass between sexes for each  
173 population (Hluhluwe:  $F_{1,24} = 19.27$ ,  $P < 0.001$ ; Dalton:  $F_{1,23} = 22.08$ ,  $P < 0.001$ ;  
174 Phuthaditjhaba:  $F_{1,24} = 29.63$ ,  $P < 0.001$ ; Lesotho:  $F_{1,26} = 106.7$ ;  $P < 0.001$ ), with females being  
175 larger than males. Mass and SVL of individuals from all populations were correlated ( $r = 0.94$   
176  $P < 0.001$ ). We found a significant differences in mass between the Lesotho and  
177 Phuthaditjhaba populations ( $P = 0.001$ ) and in SVL ( $P = 0.042$ ), with males from the high-  
178 altitude Lesotho population being substantially smaller than males from the Phuthaditjhaba  
179 population (Supplementary Table 1).

### 180 *In situ temperatures*

181 Average temperatures between March and May 2021 at each site show a dramatic  
182 decrease with altitude, with an average temperature of 23.03°C for Hluhluwe (min: 17.24°C;  
183 max: 32.34°C), 17.19°C for Dalton (min: 7.59°C; max: 30.84°C), 17.18°C for Phuthaditjhaba

184 (min: 9.39°C; max: 24.32°C) and 6.35°C for Lesotho (min: 2.96°C; 22.65°C). Minimum,  
185 maximum and average temperatures for each month between March and May are presented in  
186 Table 1.

### 187 *Exertion*

188 Temperature significantly affected the maximal distance swum in each population  
189 (Hluhluwe:  $F_{5,125} = 41.76$ ,  $P < 0.0003$ ; Dalton:  $F_{5,120} = 44.88$ ,  $P < 0.0001$ ; Phuthaditjhaba:  
190  $F_{5,125} = 68.18$ ,  $P < 0.0001$ ; Lesotho:  $F_{5,135} = 48.25$ ,  $P < 0.0001$ ). The GAM model explains  
191 87% of the variation in exertion and temperature has a non-linear significant effect on  
192 exertion within each population (Supplementary Figs. 1-4; Supplementary Table 2). SVL also  
193 had a significant effect on the maximum distance swum in populations (Hluhluwe:  $F_{1,23} =$   
194  $5.916$ ,  $P = 0.02$ ; Dalton:  $F_{1,22} = 18.29$ ,  $P = 0.0003$ ; Phuthaditjhaba:  $F_{1,23} = 15.94$ ,  $P = 0.0005$ ;  
195 Lesotho:  $F_{1,25} = 24.01$ ,  $P < 0.0001$ ). Maximal distance swum differed significantly among  
196 populations (Chisq = 16.97;  $P = 0.0007$ ), and the interaction between population and  
197 temperature was significant (Chisq = 114.67,  $P < 0.0001$ ) (Fig. 1).

198 Time to exhaustion was also significantly affected by temperature (Chisq = 240.09,  $P$   
199  $< 0.0001$ ) for each population (Fig. 2). Within populations, we also observed that temperature  
200 significantly affected the time to exhaustion (Hluhluwe:  $F_{5,125} = 26.73$ ,  $P < 0.0001$ ; Greytown:  
201  $F_{5,120} = 44.78$ ,  $P < 0.0001$ ; Phuthaditjhaba:  $F_{5,125} = 90.7$ ,  $P < 0.0001$ ; Lesotho:  $F_{5,135} = 80.92$ ;  $P$   
202  $< 0.0001$ ) (Fig. 2, Table 2). The GAM model explained 85.7% of the variation in the time to  
203 exhaustion and temperature has a non-linear significant effect for each population, using same  
204 factors as for the maximal distance (Supplementary Table 3). Time to exhaustion of the  
205 Lesotho and Phuthaditjhaba populations was significantly shorter than the Hluhluwe  
206 population at 23 and 27°C ( $P < 0.04$ ) and the Lesotho population exhibited a significantly  
207 shorter time to exhaustion than the Dalton population at 23 and 27°C (Table 2).

208 Post-hoc tests for maximal distance showed the significant differences depending on  
209 temperature within populations (Supplementary Tables 4-7). For maximal distance significant  
210 differences depending on temperature among populations were observed (Table 8). There  
211 were significant differences in performance at cold temperatures (8 to 16°C) between the  
212 Hluhluwe population from low-elevation and the others. No difference was observed in the  
213 maximal distance among populations at 19°C. Conversely, at hotter temperatures (23 and  
214 27°C), the performance of the Lesotho population from high elevation is significantly  
215 different from that of other populations. We further observed that the low-altitude Hluhluwe  
216 individuals were the least endurant at the lowest temperatures 8, 12 and 16°C. However, with  
217 increasing test temperatures (19, 23 and 27°C) the high-altitude Lesotho individuals swam  
218 shorter distances (Table 3).

#### 219 *Critical temperature, optimal temperature, and performance breadths*

220 The minimum critical temperature ( $CT_{min}$ ) differed significantly among populations  
221 ( $F_{3,36} = 11.26$ ,  $P < 0.0001$ ), particularly between Hluhluwe and the other populations ( $P <$   
222  $0.03$ ) and among the individuals from the Lesotho and Dalton populations ( $P = 0.04$ ). As  
223 predicted,  $CT_{min}$  decreased with the increase in altitude (see Table 4, 5 and Fig 1). The  
224 maximum critical temperature ( $CT_{max}$ ) did not differ, however, among populations ( $F_{3,36} =$   
225  $0.301$ ,  $P = 0.82$ ) (Table 4 and 6). Temperature optima ( $T_{opt}$ ) differed significantly among  
226 populations irrespective of whether the convex polygon method ( $F_{3,101} = 8.85$ ,  $P < 0.0001$ ) or  
227 the GAM predictions were used ( $F_{3,101} = 22.68$ ,  $P < 0.0001$ ). Differences were marked among  
228 Lesotho and Dalton ( $P = 0.008$ ) and Lesotho and Hluhluwe ( $P < 0.0001$ ), and tended to differ  
229 among individuals from Lesotho and Phuthaditjhaba ( $P = 0.059$ ). The high-altitude Lesotho  
230 population showed the lowest optimal temperature, followed by Phuthaditjhaba, Dalton, and  
231 the low-altitude Hluhluwe population (Table 4, Table 7, Fig. 1). There was no significant  
232 difference among populations in  $T_{pb80}$  ( $F_{3,101} = 2.11$ ,  $P = 0.09$ ) and  $T_{pb95}$  ( $F_{3,55.32} = 1.72$ ,  $P =$

233 0.18) (Table 4). However, there were significant differences in the upper and lower limits of  
234 the 80 and 95% performance breadth interval among populations (80% lower limit:  $F_{3,101} =$   
235 6.87,  $P < 0.0002$ ; 80% upper limit:  $F_{3,101} = 9.009$ ,  $P < 0.0001$ ; 95% lower limit:  $F_{3,101} = 8.43$ ,  $P$   
236  $< 0.0001$ ; 95% upper limit:  $F_{3,101} = 9.69$ ,  $P < 0.0001$ ), indicating shifts in the thermal  
237 performance curves (Table 4 and Fig. 1). We observed a left-shift in the thermal performance  
238 curve of the high-elevation Lesotho population towards colder temperatures and a right-shift  
239 in the thermal performance curve of the low-elevation Hluhluwe population towards warmer  
240 temperatures (Table 4).

## 241 **Discussion**

### 242 *Critical temperatures*

243 Ectotherms are dependent on temperature to maintain their physiological functions,  
244 yet many species can behaviourally thermoregulate to avoid temperature extremes or to select  
245 preferred temperature ranges (Sinclair *et al.*, 2016). However, behavioural thermoregulation  
246 and mitigation strategies to avoid temperature extremes may be more difficult for aquatic  
247 animals like *X. laevis* as shallow waterbodies are more homogenous in temperature. Variation  
248 in thermal sensitivity depends on metabolic processes which are effective across a specific  
249 range of temperatures, referred to as an organism's thermal tolerance. Outside this range  
250 organisms cannot maintain activity and locomotion, or even survive (Angilletta *et al.*, 2010,  
251 Gillooly 2001). In our study, the maximal thermal limit ( $CT_{max}$ ) did not differ significantly  
252 among populations from different altitudes, contrary to the minimal thermal limit ( $CT_{min}$ )  
253 (Table 5 and 6). It is known that the maximal thermal limit is more limited by genetic or  
254 biochemical constraints (Blackburn *et al.*, 2014; Grigg and Buckley 2013) and evolve less  
255 rapidly than the minimal thermal limit (von May *et al.*, 2017; Pintanel *et al.*, 2019).  
256 Furthermore, hot temperatures can also be more easily behaviourally avoided than cold ones  
257 (Bodensteiner *et al.*, 2021; Muñoz *et al.*, 2014, 2016), which could explain why this did not

258 differ significantly among populations. In contrast, with increasing altitude,  $CT_{min}$  decreased  
259 as observed in other studies on critical thermal limits depending on elevation (Catenazzi *et al.*,  
260 2014, Navas 2002). Our results show that adults from the populations examined conserved the  
261 same physiological upper thermal limit suggesting that part of the overall thermal ecology is  
262 conserved (Sinervo *et al.*, 2010). A study on tadpoles from parents collected at two of these  
263 sites (Phuthaditjhaba and Hluhluwe; Wagener *et al.*, 2021) shows that their  $CT_{min}$  and  $CT_{max}$   
264 vary in the same way, but they were lower (4.2 - 2.5°C) and higher (37.0 – 37.8°C),  
265 respectively, than for the adults measured here. This suggests that larvae have a wider thermal  
266 tolerance than adults in *X. laevis* (Heatwole *et al.*, 1968). Moreover, intraspecific variation in  
267  $CT_{max}$  tends to differ less than interspecific variation at the adult stage (Araújo *et al.*, 2013).  
268 The low-altitude Hluhluwe population exhibits the highest  $CT_{min}$  and shows significant  
269 differences in  $CT_{min}$  compared to the other populations (Table 4).

#### 270 *Exertion performance*

271 Thermal performance curves are expected to have the same general structure, with  
272 performance increasing with increasing temperature, reaching a maximum at the optimal  
273 temperature ( $T_{opt}$ ), and then quickly decreasing to reach zero performance are the critical  
274 minimal and maximal temperatures (Angilletta *et al.*, 2002, Schulte *et al.*, 2011). We  
275 observed a similar overall structure of thermal performance curves in the four populations  
276 (see Fig. 1). Temperature significantly affected locomotor performance as expected. In  
277 general, amphibians exhibit an increase of their resting or basal metabolism (oxygen  
278 consumption) with increasing temperature (Whitford 1973, Kreiman *et al.*, 2019). Endurance  
279 capacity relies strongly on the cardiovascular system and on oxygen transport, which is  
280 extremely dependent on temperature (Seymour 1972). Exertion, as measured here, is also  
281 dependent on the cardiovascular system in addition to being dependent on muscle contractile  
282 properties. Individuals from populations from different altitudes consequently exhibited

283 different optimal temperatures for locomotor performance (Table 4,  $T_{opt}$ ). The Lesotho  
284 population from the highest altitude (3197 m a.s.l.) has the lowest optimal temperature  
285 (16.64°C) and the Hlulhuwe population from the lowest altitude (60 m a.s.l.) has the highest  
286 optimal temperature (21.11°C). Temperature optima in populations from middle altitudes  
287 Dalton (1016 m a.s.l.) and Phuthaditjhaba (1948 m a.s.l.) are intermediate (19.44°C and  
288 18.8°C respectively). The optimal temperature for exertion performance of the populations  
289 tested here thus seems to be correlated with environmental temperatures depending on altitude  
290 with environmental temperatures decreasing with increasing altitude (Körner 2007; Table 1).  
291 Note that there is a mismatch between the mean environmental temperature and the optimal  
292 temperature for performance with frogs from Lesotho exhibiting a  $T_{opt}$  (16.64°C) that is  
293 higher than the mean environmental temperature (6.35°C). However, a decrease in  
294 environmental temperature did result in a decrease in the optimal temperature. These results  
295 are concordant with the prediction that optimal temperature for fitness-relevant functions is  
296 set at the most commonly encountered temperature in the environment (Huey and Kingsolver  
297 1993; Gilchrist 1995; Navas *et al.*, 2008).

### 298 *Performance breadths*

299         Similar to the differences in temperature optima among populations, we observed  
300 directional shifts in the 80% thermal performance curve for the populations from the two  
301 extreme altitudes (Lesotho: 3197 m and Hlulhuwe: 60 m a.s.l.). For example, the thermal  
302 performance curve of the Lesotho population shows a left-shift of about 3.7 degrees compared  
303 to the Hlulhuwe population (see Fig. 1, Table 4,  $T_{pb80}$  interval). Consequently, the population  
304 from the highest altitude, in Lesotho, appears better adapted to lower temperatures for aquatic  
305 exertion. It is known that adaptation to cold environment is predicted to entail the loss of  
306 performance in warmer environments (Bennett and Lenski 2007), as is effectively observed  
307 for the Lesotho population. On the other hand, the Hlulhuwe population from the lowest

308 altitude appears better adapted to higher temperatures. Overall, differences in thermal  
309 sensitivity of locomotor performance among populations tend to be mainly driven by two  
310 populations, Hluhluwe and Lesotho. However, the breadth of the 80% thermal performance  
311 interval did not differ among populations, showing that the overall shape of the performance  
312 curve did not evolve. These results can suggest a level of intrinsic constraint which appears to  
313 limit the ability in *X. laevis* adults to broaden the temperature performance curve.

314         Generally, species with broad distribution ranges express phenotypic differences  
315 among local populations, following predictable patterns along spatial and environmental  
316 gradients (Chevin and Lande 2011). Thus, thermal performance curves may shift to become  
317 locally adapted to environmental conditions such as temperature (Narum *et al.*, 2013).  
318 Environmental gradients exert a major effect on patterns of intraspecific variation (Mizera and  
319 Meszéna 2003) and coupled with natural selection in the long term may produce differences  
320 between populations resulting in local adaptation (Olsson and Uller 2003). Local adaptation is  
321 a process of natural selection whereby resident populations evolve higher relative fitness in  
322 their local habitat than populations originating elsewhere (Sexton *et al.*, 2017; Schmid and  
323 Guillaume 2017). The *Xenopus laevis* populations included in our study show different  
324 phenotypes in locomotor performance depending on temperature and altitude (Arnold, 1983).  
325 These may increase fitness in a given thermal environment as individuals and populations  
326 from cold environments and high altitudes perform relatively better at cold temperatures. The  
327 thermal performance curves from these populations thus show patterns in thermal physiology  
328 that covary with the thermal environment and the altitudinal gradient. Indeed, *in situ*  
329 temperature data from the collected sites show a thermal gradient in average temperature  
330 depending on the altitude ranging from 6.35°C to 23.03°C. The average temperatures from  
331 March to May are 23.03°C in Hluhluwe (60m a.s.l.), 17.19°C in Dalton (1016m a.s.l.),  
332 17.18°C in Phuthaditjhaba (1948m a.s.l.) and 6.35°C in Lesotho (3197m a.s.l.) (Table 1).

333 In order to understand the processes that underlie the observed differences in optimal  
334 temperatures for locomotion in *Xenopus laevis*, future studies may benefit from using  
335 common garden experiments to tease apart the role of phenotypic plasticity versus genetic  
336 adaptation. Even if the responses are plastic, this may promote future adaptation by allowing  
337 populations to perform well in novel environments (Crispo 2007; Ghalambor *et al.*, 2007) and  
338 may be one of the critical traits driving the invasion potential of the species. Moreover,  
339 genomic and transcriptomic approaches coupled to the study of phenotypic diversity would  
340 provide better insights into the processes underlying thermal adaptation in these organisms.  
341 The broad thermal tolerance and ability to adapt to different thermal environments may  
342 explain the invasive nature of this species and its presence on four continents (Furman *et al.*,  
343 2015; Measey *et al.*, 2012) comprising areas outside of its native climate envelope (Rödder *et*  
344 *al.*, 2017). Including physiological data on locomotor performance depending on temperature  
345 in species distribution models can provide better and more biologically informed insights into  
346 the potential future spread of this species under different scenarios of climate change (Coulin  
347 *et al.*, 2019; Gamliel *et al.*, 2020; Ginal *et al.*, in press). Finally, our results suggest that  
348 whereas optimal temperatures for locomotion show population-level differences, the shape of  
349 the performance curves did not. This may hint at a more general mechanism of the evolution  
350 of thermal dependence of locomotion, yet remains to be tested in other species.

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### 357 **Author contributions**

358 A.H., J.M. and L.A. conceived the study. J.M., C.W. and L.A. collected the animals in the  
359 field. L.A. collected the data. L.A. and A.H. and P.P. analysed the data. L.A. and A.H. wrote  
360 the article and all the authors contributed to and approved the final version.

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## Tables

**Table 1.** Average *in situ* temperatures on the sites of collection (March to May 2021).

	<b>Alti- tude (m. a.s.l.)</b>	<b>March to May (°C)</b>	<b>March to May Mini- mum (°C)</b>	<b>March to May Maxi- mum (°C)</b>	<b>March (°C)</b>	<b>April (°C)</b>	<b>May (°C)</b>
<b>Hluhluwe</b>	60	23.03 ± 0.04	17.24	32.34	25.73 ± 0.05	23.13 ± 0.04	20.24 ± 0.03
<b>(N=26)</b>							
<b>Dalton</b>	1016	17.19 ± 0.07	7.59	30.84	21.59 ± 0.06	17.39 ± 0.11	12.60 ± 0.07
<b>(N=25)</b>							
<b>Phuthaditjhaba</b>	1948	17.18 ± 0.06	9.39	24.32	20.95 ± 0.05	17.85 ± 0.04	12.77 ± 0.04
<b>(N=26)</b>							
<b>Lesotho</b>	3197	6.35 ± 0.04	2.96	22.65	7.66 ± 0.10	6.05 ± 0.04	5.44 ± 0.05
<b>(N=28)</b>							

Table entries are means ± standard errors.

**Table 2.** Time to exhaustion (in s) at different temperatures for the different populations.

	<b>Hluhluwe</b>	<b>Dalton</b>	<b>Phuthaditjhaba</b>	<b>Lesotho</b>
	<b>(60 m)</b>	<b>(1016 m)</b>	<b>(1948 m)</b>	<b>(3197 m)</b>
	<b>(N = 26)</b>	<b>(N = 25)</b>	<b>(N = 26)</b>	<b>(N = 28)</b>
<b>8°C</b>	205.48 ± 5.94	210.60 ± 5.52	193.27 ± 5.76	206.57 ± 7.25
<b>12°C</b>	221.63 ± 13.20	239.74 ± 11.97	224.28 ± 8.13	239.25 ± 9.02
<b>16°C</b>	232.59 ± 16.76	269.08 ± 15.19	244.78 ± 7.45	251.70 ± 13.34
<b>19°C</b>	273.48 ± 16.63	265.93 ± 16.22	239.19 ± 11.03	229.84 ± 13.09
<b>23°C</b>	271.96 ± 21.69	249.60 ± 16.72	210.35 ± 6.56	191.87 ± 12.03
<b>27°C</b>	149.35 ± 9.94	130.34 ± 6.39	110.31 ± 5.29	108.47 ± 6.34

Table entries are means ± standard errors.

**Table 3.** Aquatic exertion (distance covered, in cm) for the tested populations at different temperatures.

	<b>Hluhluwe</b>	<b>Dalton</b>	<b>Phuthaditjhaba</b>	<b>Lesotho</b>
	<b>(60 m)</b>	<b>(1016 m)</b>	<b>(1948 m)</b>	<b>(3197 m)</b>
	<b>(N = 26)</b>	<b>(N = 25)</b>	<b>(N = 26)</b>	<b>(N = 28)</b>
<b>8°C</b>	4104.69 ± 155.73	5135.16 ± 178.75	5023.11 ± 193.32	5185.28 ± 207.04
<b>12°C</b>	5445.23 ± 304.77	6621.48 ± 224.61	6614.42 ± 256.80	6616.86 ± 245.88
<b>16°C</b>	6709.92 ± 377.99	8076.68 ± 447.04	7118.57 ± 281.20	7293.61 ± 340.72
<b>19°C</b>	7943.57 ± 473.86	8548.12 ± 429.74	7948.65 ± 347.70	7190.25 ± 403.43
<b>23°C</b>	8468.46 ± 510.92	8110.12 ± 402.60	7228.08 ± 270.40	6250.54 ± 377.82
<b>27°C</b>	4951.11 ± 280.75	4797.76 ± 267.14	4534.23 ± 241.95	3665.39 ± 200.39

Table entries are means ± standard errors.

**Table 4.** Critical temperatures and optimal temperatures for the populations of *X. laevis* tested.

	<b>Altitude</b> (m a.s.l.)	<b>CTmin</b> (°C)	<b>CTmax</b> (°C)	<b>Topt ± SE</b> (°C)	<b>Topt</b> (GAM) (°C)	<b>Tpb80</b> (°C)	<b>Tpb80</b> interval (°C)	<b>Tpb95</b> (°C)	<b>Tpb95</b> interval (°C)
<b>Hluhluwe</b> (N = 26)	60	5.96 ± 0.30	29.54 ± 0.53	21.11 ± 0.30	21.67	9.46 ± 0.72	15.2 - 24.6	3.80 ± 0.48	18.8 - 22.6
<b>Dalton</b> (N = 25)	1016	5.08 ± 0.15	28.90 ± 0.63	19.44 ± 0.37	19.63	12 ± 0.81	12.5 - 24.2	5.65 ± 0.74	16 - 21.6
<b>Phutha- ditjhaba</b> (N = 26)	1948	4.71 ± 0.17	29.3 ± 0.27	18.80 ± 0.40	20.22	11.4 ± 0.66	12.3 - 23.8	3.83 ± 0.51	17.2 - 21
<b>Lesotho</b> (N = 28)	3197	4.26 ± 0.21	29.25 ± 0.44	16.64 ± 0.42	16.80	10.64 ± 0.79	11.5 - 22.2	4.51 ± 0.65	14.8 - 19.3

Critical minimum and maximum temperatures (CT<sub>min</sub> and CT<sub>max</sub>), optimal temperature (T<sub>opt</sub>), 80 and 95% thermal performance breadths (T<sub>pb80</sub> and T<sub>pb95</sub>) and their interval. Table entries are means ± standard errors.

**Table 5.** Results of Tukey post-hoc tests testing for differences in CT<sub>min</sub> among populations.

	<b>Lesotho</b> ( <i>N</i> = 28)	<b>Phutha- ditjhaba</b> ( <i>N</i> = 26)	<b>Dalton</b> ( <i>N</i> = 25)	<b>Hluhluwe</b> ( <i>N</i> = 26)
<b>Lesotho</b>	–	0.46	<b>0.04</b>	<b>0.000</b>
<b>Phuthaditjhaba</b>	–	–	0.62	<b>0.001</b>
<b>Dalton</b>	–	–	–	<b>0.03</b>

Bold values indicate significant differences.

**Table 6.** Results of Tukey post-hoc tests testing for differences in  $CT_{max}$  among populations.

	<b>Lesotho</b> ( <i>N</i> = 28)	<b>Phutha- ditjhaba</b> ( <i>N</i> = 26)	<b>Dalton</b> ( <i>N</i> = 25)	<b>Hluluwe</b> ( <i>N</i> = 26)
<b>Lesotho</b>	–	0.99	0.94	0.98
<b>Phuthaditjhaba</b>	–	–	0.91	0.99
<b>Dalton</b>	–	–	–	0.78

**Table 7.** Results of Tukey post-hoc tests testing for differences in  $T_{opt}$  (GAM) among populations.

	<b>Lesotho</b> ( <i>N</i> = 28)	<b>Phuthaditjhaba</b> ( <i>N</i> = 26)	<b>Dalton</b> ( <i>N</i> = 25)	<b>Hluluwe</b> ( <i>N</i> = 26)
<b>Lesotho</b>	–	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>
<b>Phuthaditjhaba</b>	–	–	0.86	<b>0.04</b>
<b>Dalton</b>	–	–	–	0.22

Bold values indicate significant differences.

**Table 8.** Results of Tuckey post-hoc tests testing for differences in maximal distance swum at each tested temperature among populations.

<b>Temperature (°C)</b>	<b>Populations</b>	<b>p-value</b>
<b>8</b>	Hluhluwe – Dalton	0.0003
	Hluhluwe – Lesotho	0.0005
	Hluhluwe – Phuthaditjhaba	0.002
<b>12</b>	Hluhluwe – Dalton	0.003
	Hluhluwe – Lesotho	0.009
	Hluhluwe – Phuthaditjhaba	0.004
<b>16</b>	Hluhluwe – Dalton	0.03
<b>19</b>	NS	
<b>23</b>	Lesotho – Dalton	0.001
	Lesotho – Hluhluwe	0.0001
<b>27</b>	Lesotho – Dalton	0.004
	Lesotho – Hluhluwe	0.001
	Lesotho – Phuthaditjhaba	0.03

## 1 **Figure legends**

2 **Fig.1 Temperature-performance curves illustrating the impact of temperature on**  
3 **maximum distance swum for *X. laevis* populations.** The population from Hluhluwe is  
4 represented by open circles, the Dalton population is represented by open squares, the  
5 Phuthaditjhaba population is represented by filled circles, and the Lesotho population is  
6 represented by open triangles. Indicated are the 80% thermal performance breadths for each  
7 population. Upper and lower ends of the curves are critical temperatures resulting in zero  
8 performance ( $CT_{max}$  and  $CT_{min}$ ). Symbols represent means  $\pm$  standard errors of the mean.  
9 Thermal optima ( $T_{opt}$ ) obtained from GAM model for each population are represented.

10

11 **Fig.2 Temperature-performance curves illustrating the impact of temperature on**  
12 **maximum time to exhaustion for *X. laevis* populations.** The population from Hluhluwe is  
13 represented by open circles, the Dalton population is represented by open squares, the  
14 Phuthaditjhaba population is represented by filled circles and the Lesotho population is  
15 represented by open triangles. Indicated are the 80% thermal performance breadths for each  
16 population. Upper and lower ends of the curves are critical temperatures resulting in zero  
17 performance ( $CT_{max}$  and  $CT_{min}$ ). Symbols represent means  $\pm$  standard errors of the mean.

Figure 1. Temperature-performance curves illustrating the impact of temperature on maximum distance swam for *X. laevis* populations.

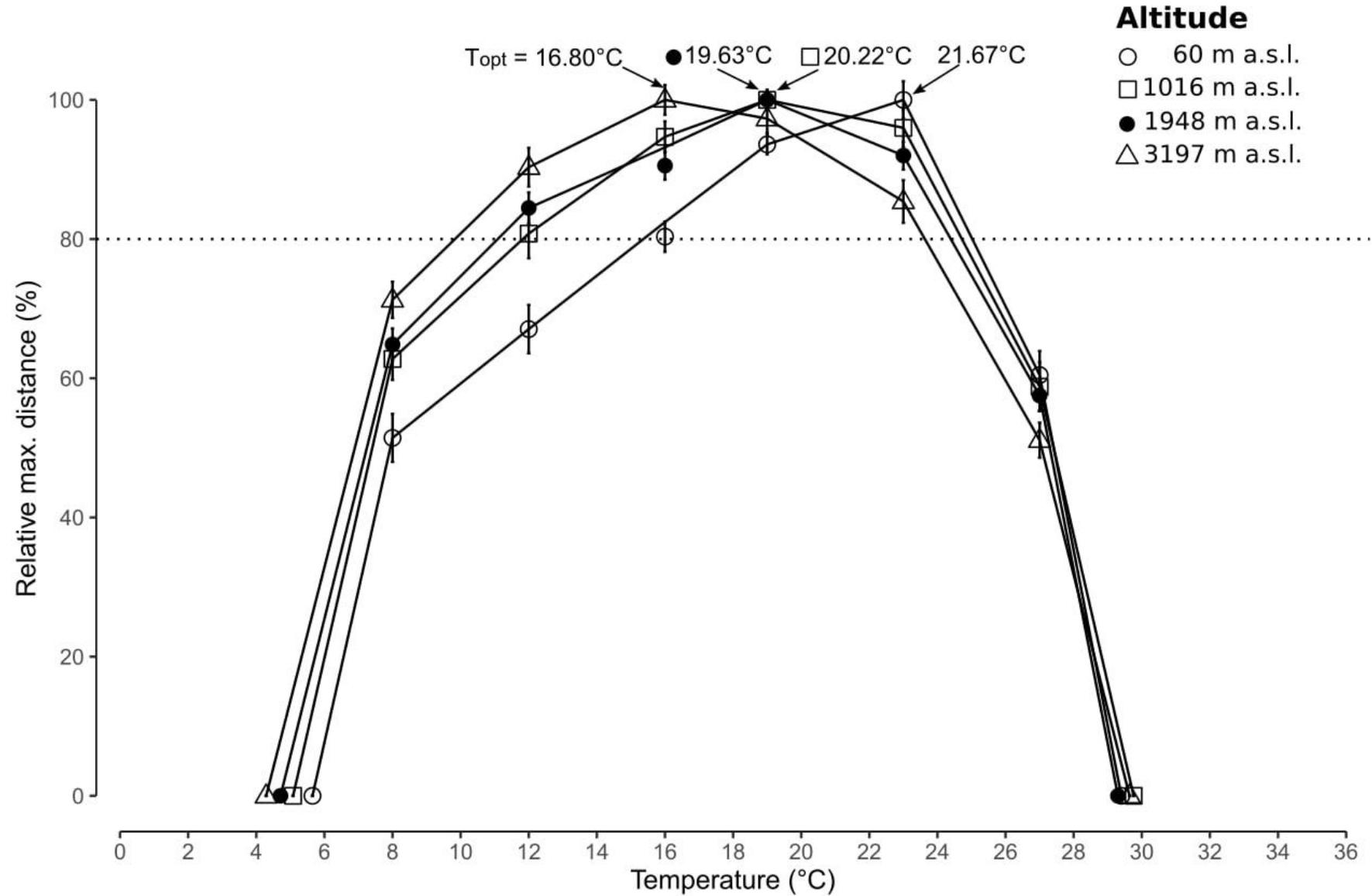
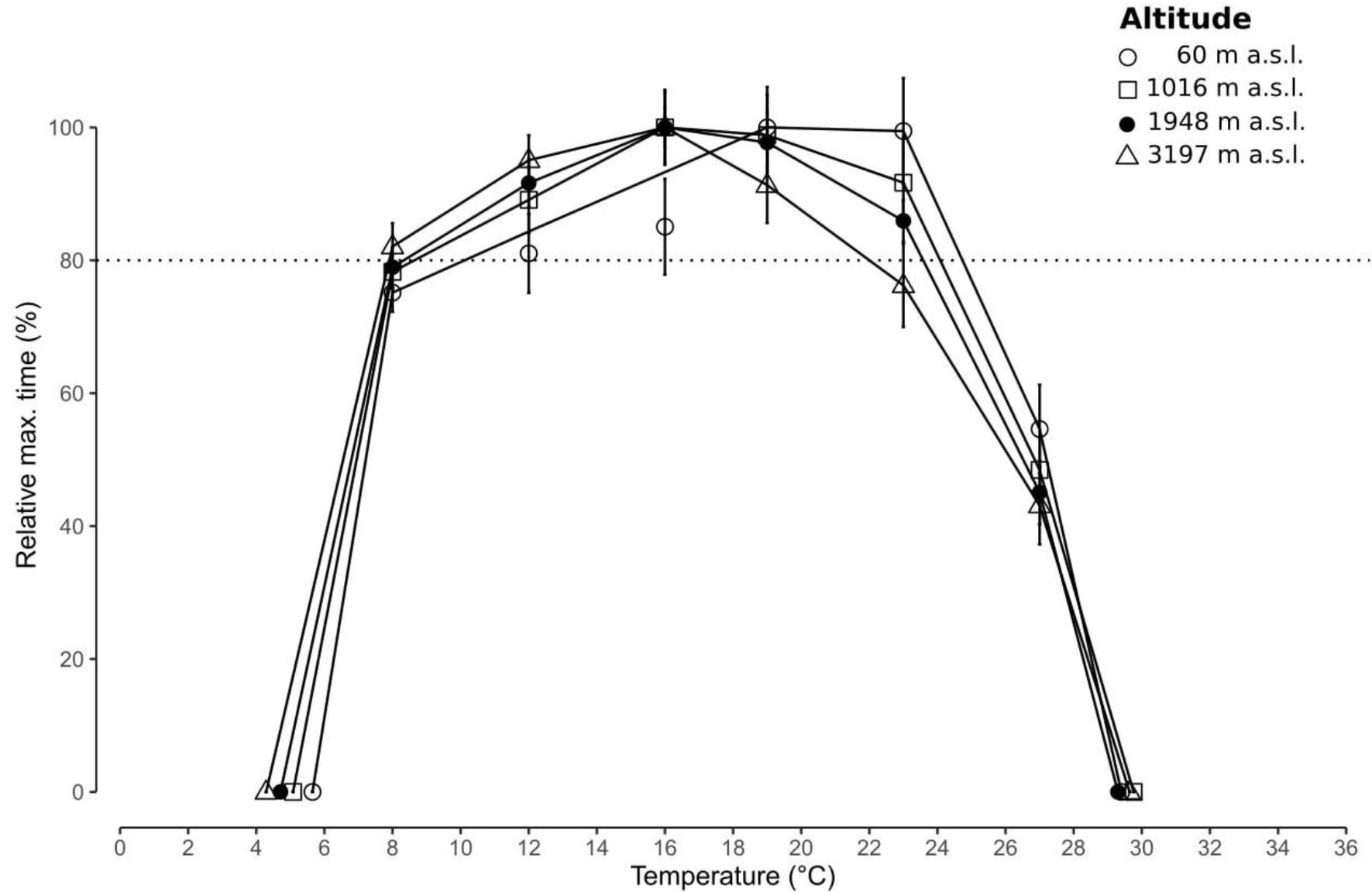


Figure 2. Temperature-performance curves illustrating the impact of temperature on maximum time to exhaustion for *X. laevis* populations.



## Supplementary Tables

**Supplementary Table 1.** Morphometric measurements and sample size for each population.

	SVL (mm)		Mass (g)	
	F	M	F	M
<b>Hluhluwe (60 m)</b> ( <i>N</i> = 26)	72.24 ± 0.70	62.12 ± 0.62	44.72 ± 1.51	28.33 ± 0.87
<b>Dalton (1016 m)</b> ( <i>N</i> = 25)	72.16 ± 0.73	60.86 ± 0.97	46.64 ± 1.54	29.5 ± 0.78
<b>Phuthaditjhaba (1948 m)</b> ( <i>N</i> = 26)	76.36 ± 0.90	61.60 ± 0.56	52.94 ± 1.71	31.01 ± 0.69
<b>Lesotho (3197 m)</b> ( <i>N</i> = 28)	74.63 ± 0.36	55.87 ± 0.56	45.87 ± 0.77	21.60 ± 0.57

Tables entries are means ± standard errors.

**Supplementary Table 2.** GAM model results testing for the effect of temperature on endurance distance for each population.

	<b>edf</b>	<b>F</b>	<b>p-value</b>
<b>Hluhluwe</b>	5.853	219.823	<0.0001
<b>Dalton</b>	5.865	240.925	<0.0001
<b>Phuthaditjhaba</b>	5.799	210.025	<0.0001
<b>Lesotho</b>	5.742	206.448	<0.0001

**Supplementary Table 3.** GAM model results testing for the effects of temperature on time to exhaustion for each population.

	<b>edf</b>	<b>F</b>	<b>p-value</b>
<b>Hluhluwe</b>	5.929	203.025	<0.0001
<b>Dalton</b>	5.855	204.026	<0.0001
<b>Phuthaditjhaba</b>	5.768	173.516	<0.0001
<b>Lesotho</b>	5.768	191.822	<0.0001

**Supplementary Table 4.** Results of post-hoc pairwise t-tests for differences in endurance distance depending on temperature for the Hluhluwe population (N = 26, alt = 60 m).

<b>Hluhluwe</b>	<b>8°C</b>	<b>12°C</b>	<b>16°C</b>	<b>19°C</b>	<b>23°C</b>	<b>27°C</b>
<b>8°C</b>	–	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.03</b>
<b>12°C</b>	–	–	<b>0.03</b>	<b>0.0002</b>	<b>&lt;0.0001</b>	0.22
<b>16°C</b>	–	–	–	<b>0.004</b>	<b>0.0002</b>	<b>0.002</b>
<b>19°C</b>	–	–	–	–	0.15	<b>&lt;0.0001</b>
<b>23°C</b>	–	–	–	–	–	<b>&lt;0.0001</b>
<b>27°C</b>	–	–	–	–	–	–

Bold values indicate significant differences.

**Supplementary Table 5.** Results of post-hoc pair-wise t-tests testing for differences in endurance distance depending on temperature for the Dalton population (N = 25, alt.= 1016 m).

<b>Dalton</b>	<b>8°C</b>	<b>12°C</b>	<b>16°C</b>	<b>19°C</b>	<b>23°C</b>	<b>27°C</b>
<b>8°C</b>	–	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.31
<b>12°C</b>	–	–	<b>0.01</b>	<b>0.0008</b>	<b>0.0006</b>	<b>&lt;0.0001</b>
<b>16°C</b>	–	–	–	0.31	0.85	<b>&lt;0.0001</b>
<b>19°C</b>	–	–	–	–	0.39	<b>&lt;0.0001</b>
<b>23°C</b>	–	–	–	–	–	<b>&lt;0.0001</b>
<b>27°C</b>	–	–	–	–	–	–

Bold values indicate significant differences.

**Supplementary Table 6.** Results of post-hoc pairwise t-tests testing for differences in endurance distance depending on temperature for the Phuthaditjhaba population (N = 26, alt = 1948 m) (Pairwise.t.test).

<b>Phuthaditjhaba</b>	<b>8°C</b>	<b>12°C</b>	<b>16°C</b>	<b>19°C</b>	<b>23°C</b>	<b>27°C</b>
<b>8°C</b>	–	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.069
<b>12°C</b>	–	–	0.062	<b>&lt;0.0001</b>	<b>0.049</b>	<b>&lt;0.0001</b>
<b>16°C</b>	–	–	–	<b>0.002</b>	0.62	<b>&lt;0.0001</b>
<b>19°C</b>	–	–	–	–	0.062	<b>&lt;0.0001</b>
<b>23°C</b>	–	–	–	–	–	<b>&lt;0.0001</b>
<b>27°C</b>	–	–	–	–	–	–

Bold values indicate significant differences.

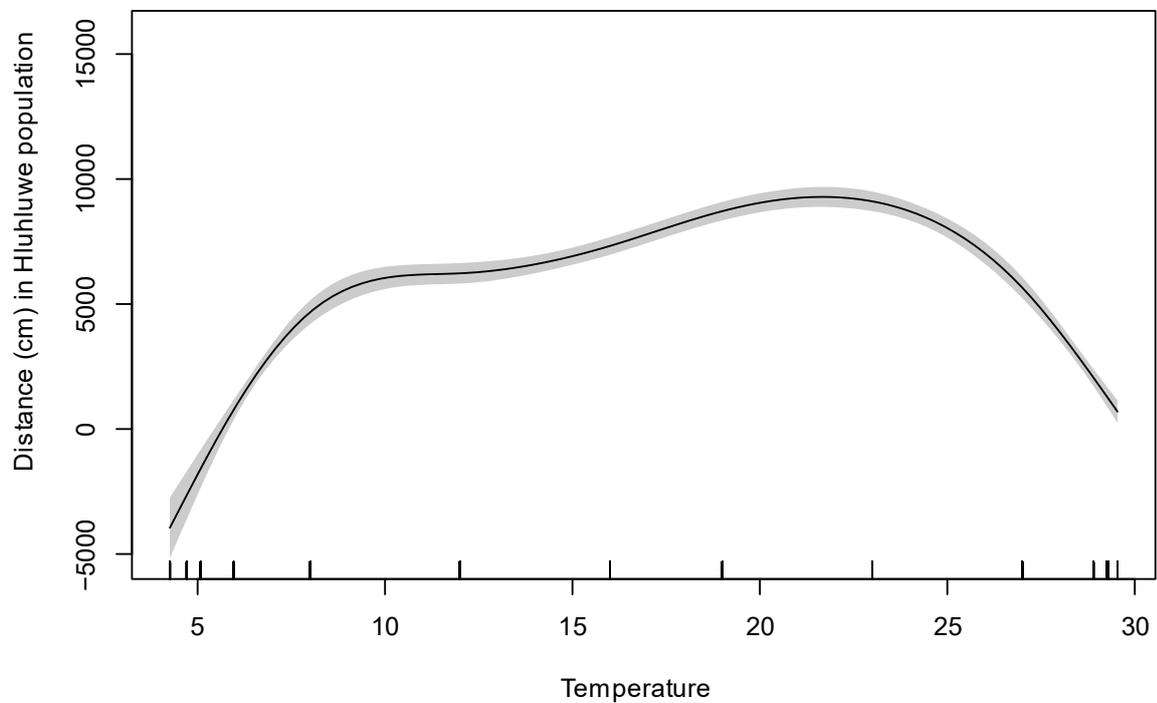
**Supplementary Table 7.** Results of post-hoc pair-wise t-tests testing for differences in endurance distance depending on temperature for the Lesotho population (N = 28, alt. = 3197 m).

<b>Lesotho</b>	<b>8°C</b>	<b>12°C</b>	<b>16°C</b>	<b>19°C</b>	<b>23°C</b>	<b>27°C</b>
<b>8°C</b>	–		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.018</b>	<b>&lt;0.0001</b>
<b>12°C</b>	–	–	0.62	0.81	0.81	<b>&lt;0.0001</b>
<b>16°C</b>	–	–	–	0.81	<b>0.02</b>	<b>&lt;0.0001</b>
<b>19°C</b>	–	–	–	–	<b>0.02</b>	<b>&lt;0.0001</b>
<b>23°C</b>	–	–	–	–	–	<b>&lt;0.0001</b>
<b>27°C</b>	–	–	–	–	–	–

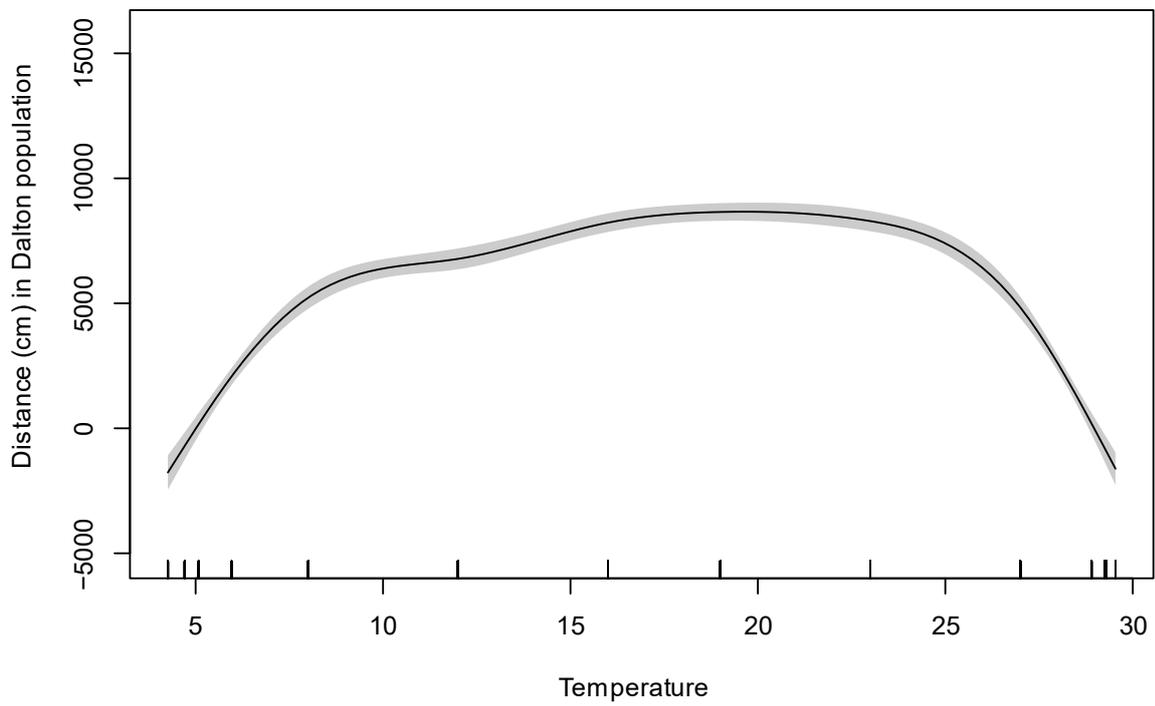
Bold values indicate significant differences.

## Supplementary figures

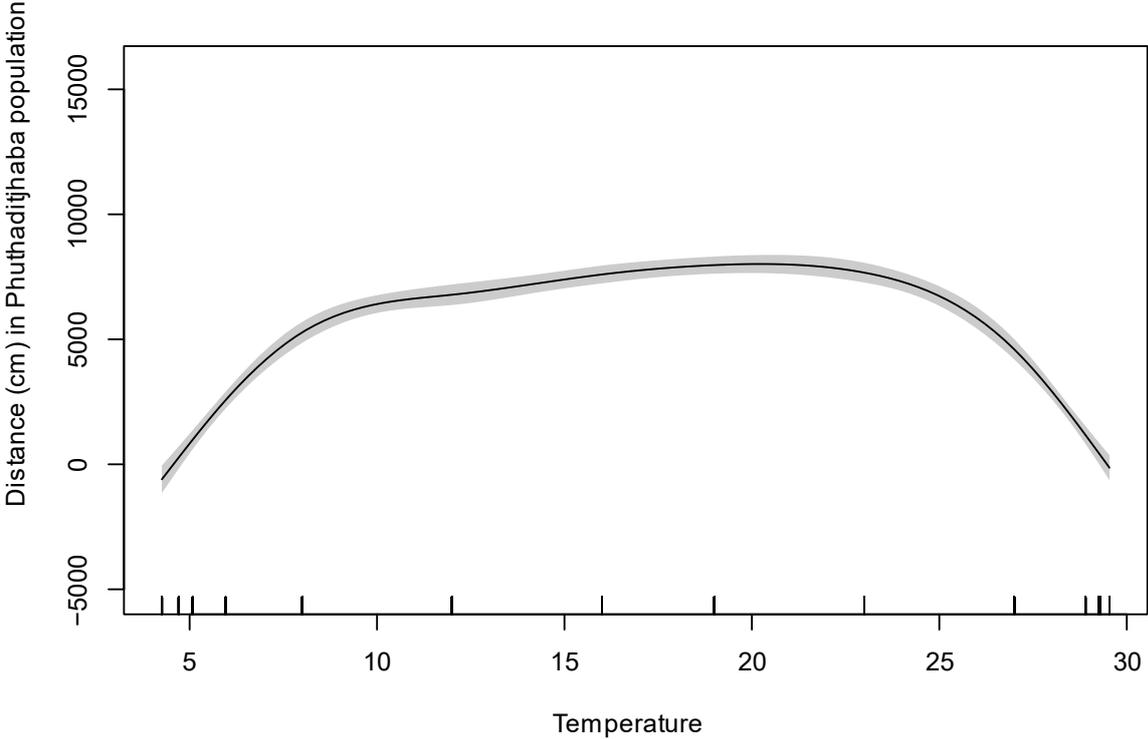
Suppl. Fig.1 **Temperature-performance curves predicted using GAM models illustrating the non-linear effect of temperature on aquatic endurance for the *X. laevis* population from Hluhluwe (60m a.s.l.).** Note that negative values can predicted values beyond the critical thermal limits, illustrating the limits of this approach beyond the estimate of the optimal temperature.



Suppl. Fig.2 **Temperature-performance curves predicted using GAM models illustrating the non-linear effect of temperature on aquatic endurance for the *X. laevis* population from Dalton (1016m a.s.l.).** Note that negative values can be predicted values beyond the critical thermal limits, illustrating the limits of this approach beyond the estimate of the optimal temperature.



Suppl. Fig.3 **Temperature-performance curves predicted using GAM models illustrating the non-linear effect of temperature on aquatic endurance for the *X. laevis* population from Phuthaditjhaba (1948m a.s.l.).** Note that negative values can be predicted values beyond the critical thermal limits, illustrating the limits of this approach beyond the estimate of the optimal temperature.



Suppl. Fig.4 **Temperature-performance curves predicted using GAM models illustrating the non-linear effect of temperature on aquatic endurance for the *X. laevis* population from Lesotho (3197m a.s.l.).** Note that negative values can be predicted values beyond the critical thermal limits, illustrating the limits of this approach beyond the estimate of the optimal temperature.

