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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 (HOBOconnect, 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 ± 0.1 g) and snout-vent
113 length (SVL) was measured using a pair of digital callipers (Mitutoya; precision ± 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.

Literature cited

Angilletta M. J., P.H. Niewiarowski, and C.A. Navas. 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27:249–268.

Angilletta M. J., R.B. Huey, and M.R. Frazier. 2010. Thermodynamic effects on organismal performance: Is hotter better? *Physiological and Biochemical Zoology* 83:197–206.

Araspin L., S.A. Martinez, C. Wagener, J. Courant, V. Louppe, P. Padilla, J. Measey, and A. Herrel. 2020. Rapid shifts in the temperature dependence of locomotor performance in an invasive frog, *Xenopus laevis*, implications for conservation. *Integrative and Comparative Biology* 60:456–466.

Araújo M. B., F. Ferri-Yáñez, F. Bozinovic, P.A. Marquet, F. Valladares, and S.L. Chown. 2013. Heat freezes niche evolution. *Ecology Letters* 16:1206–1219.

Arnold S. J. 1983. Morphology, Performance and Fitness. *Am Zool* 23:347–361.

Bennett A. F. and R.E. Lenski. 2007. An experimental test of evolutionary trade-offs during temperature adaptation. *Proceedings of the National Academy of Sciences, U.S.A.* 104:8649–8654.

Blackburn S., V. Kellermann, B. van Heerwaarden, and C. Sgro. 2014. Evolutionary capacity of upper thermal limits: Beyond single trait assessments. *The Journal of experimental biology* 217:1918–1924.

Bodensteiner B. L., G.A. Agudelo-Cantero, A.Z.A. Arietta, A.R. Gunderson, M.M. Muñoz, J.M. Refsnider, and E.J. Gangloff. 2021. Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 335:173–194.

- Brown J. H., J.F. Gillooly, A.P. Allen, V.M. Savage, and G.B. West. 2004. Toward a metabolic theory of ecology. *Ecology* 85:1771–1789.
- Catenazzi A., E. Lehr, and V.T. Vredenburg. 2014. Thermal physiology, disease, and amphibian declines on the Eastern slopes of the Andes. *Conservation Biology* 28:509–517.
- Chevin L.M. and R. Lande. 2011. Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *Journal of Evolutionary Biology* 24:1462–1476.
- Clarke A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology & Evolution* 18:573–581.
- Coulin C., G.J. de la Vega, L. Chifflet, L.A. Calcaterra, and P.E. Schilman. 2019. Linking thermo-tolerances of the highly invasive ant, *Wasmannia auropunctata*, to its current and potential distribution. *Biological Invasions* 21:3491–3504.
- Courant J., J. Secondi, J. Vollette, A. Herrel, and J-M. Thirion. 2018. Assessing the impacts of the invasive frog, *Xenopus laevis*, on amphibians in western France. *Amphibia-Reptilia* 39:219–227.
- Crispo E. 2007. The Baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* 61:2469–2479.
- Du Preez L. H., N. Kunene, R. Hanner., J.P. Giesy, K.R. Solomon, A. Hosmer, and G.J. Van Der Kraak. 2009. Population-specific incidence of testicular ovarian follicles in *Xenopus laevis* from South Africa: A potential issue in endocrine testing. *Aquatic Toxicology* 95:10–16.
- Furman B.L.S., A.J. Bewick, T.L. Harrison, E. Greenbaum, V. Gvoždík, C. Kusamba, and B.J. Evans. 2015. Pan-African phylogeography of a model organism, the African clawed frog ‘*Xenopus laevis*.’ *Molecular Ecology* 24:909–925.

- Gamliel I., Y. Buba, T. Guy-Haim, T. Garval, D. Willette, G. Rilov, and J. Belmaker. 2020. Incorporating physiology into species distribution models moderates the projected impact of warming on selected Mediterranean marine species. *Ecography* 43:1090–1106.
- Ghalambor C. K., J.K. McKay, S.P. Carroll, and D.N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21:394–407.
- Gilchrist G.W. 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *The American Naturalist* 146:252–270.
- Gillooly, J. F. 2001. Effects of size and temperature on metabolic rate. *Science* 293:2248–2251.
- Ginal, P., N. Kruger, L. Araspin, M. Mokhatla, J. Secondi, A. Herrel, J. Measey and D. Rödder. in press. More time for aliens? Performance shifts lead to increased activity time budgets propelling invasion success. *Biological Invasions* Doi: 10.1007/s10530-022-02903-6
- Grigg J. W. and L.B. Buckley. 2013. Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. *Biology Letters* 9:20121056.
- Guderley H. 2004. Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 139:371–382.
- Heatwole H., S.B. De Austin, and R. Herrero. 1968. Heat tolerances of tadpoles of two species of tropical anurans. *Comparative Biochemistry and Physiology* 27:807–815.
- Herrel A., L.N. Gonwouo, E.B. Fokam, W.I. Ngundu, and C. Bonneaud. 2012. Intersexual differences in body shape and locomotor performance in the aquatic frog, *Xenopus tropicalis*:

Sexual dimorphism in morphology and performance in *Xenopus*. *The Journal of Zoology* 287:311–316.

Hoffmann A.A., J.G. Sørensen, and V. Loeschcke. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* 28:175–216.

Huey R.B. and J.G. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. *The American Naturalist* 142:S21–S46.

Hulbert A.J. and P.L. Else. 2000. Mechanisms underlying the cost of living in animals. *Annual Reviews Physiology* 62:207–235.

Jacobsen D. and O. Dangles. 2017. *Ecology of high altitude waters*. Oxford University Press.

Johnston I. A. and G.K. Temple. 2002. Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *Journal of Experimental Biology* 205:2305–2322.

Körner C. 2007. The use of ‘altitude’ in ecological research. *Trends in Ecology & Evolution* 22:569–574.

Kreiman L.E., J.J. Solano-Iguaran, L.D Bacigalupe, and D.E. Naya. 2019. Testing the metabolic homeostasis hypothesis in amphibians. *Philosophical Transactions of the Royal Society B* 374:20180544.

Kumschick, S., G. Vimercati, F.A. de Villiers, M.M. Mokhatla, S.J. Davies, C.J. Thorp, A.D. Rebelo, and G.J. Measey. 2017. Impact assessment with different scoring tools: How well do alien amphibian assessments match? *NeoBiota* 33:53–66.

- Magnuson, J.J., L.B. Crowder, and P.A. Medvick. 1979. Temperature as an ecological resource. *American Zoologist* 19:331–343.
- Measey G.J., L.R. Minter, M. Burger, J.A Harrison, H.H. Braack, & P.J. Bishop. 2004. Species account: *Xenopus laevis* (Daudin 1802). *Atlas and red data book of the frogs of South Africa, Lesotho and Swaziland*, 266-267.
- Measey, G. J., D. Rödder, S.L Green, R. Kobayashi, F. Lillo, G. Lobos, R. Rebelo, and J-M. Thirion. 2012. Ongoing invasions of the African clawed frog, *Xenopus laevis*: a global review. *Biological Invasions* 14:2255–2270.
- Mizera, F. and G. Meszéna. 2003. Spatial niche packing, character displacement and adaptive speciation along an environmental gradient. *Evolutionary Ecology Research* 5:1–20.
- Montgomery, K. 2006. Variation in temperature with altitude and latitude. *Journal of Geography* 105:133–135.
- Muñoz, M.M., M.A. Stimola, A.C. Algar, A. Conover, A.J. Rodriguez, M.A. Landestoy, G.S. Bakken, and J.B. Losos. 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society B*. 281:20132433.
- Muñoz, M.M., G.M. Langham, M.C. Brandley, D.F. Rosauer, S.E. Williams, and C. Moritz. 2016. Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards. *Evolution* 70:2537–2549.
- Narum S.R., N.R. Campbell, K.A. Meyer, M.R. Miller, and R.W. Hardy. 2013. Thermal adaptation and acclimation of ectotherms from differing aquatic climates. *Molecular Ecology* 22:3090–3097.
- Navas C.A. 1996. Implications of microhabitat selection and patterns of activity on the thermal ecology of high elevation Neotropical anurans. *Oecologia* 108:617–626.

- Navas C.A. 2002. Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 133:469–485.
- Navas C.A. 2006. Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology* 46:82–91.
- Navas C.A., F.R. Gomes, and J.E. Carvalho. 2008. Thermal relationships and exercise physiology in anuran amphibians: Integration and evolutionary implications. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 151:344–362.
- Olsson M. and T. Uller. 2003. Thermal environment, survival and local adaptation in the common frog, *Rana temporaria*. *Evolutionary ecology research* 5:431–437.
- Pintanel P., M. Tejedo, S.R. Ron, G.A. Llorente, and A. Merino-Viteri. 2019. Elevational and microclimatic drivers of thermal tolerance in Andean *Pristimantis* frogs. *Journal of Biogeography* 46:1664–1675.
- Pörtner H.O. and A.P. Farrell. 2008. Physiology and climate change. *Science* 322:690–692.
- Rödger D., F. Ihlow, J. Courant, J. Secondi, A. Herrel, R. Rebelo, G.J. Measey, F. Lillo, F.A.D. Villiers, C.D. Busschere, et al. 2017. Global realized niche divergence in the African clawed frog *Xenopus laevis*. *Ecology and Evolution* 7:4044–4058.
- Schmid M. and F. Guillaume. 2017. The role of phenotypic plasticity on population differentiation. *Heredity* 119:214–225.
- Schulte P.M., T.M. Healy, and N.A. Fanguie. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology* 51:691–702.

- Sexton J.P., J. Montiel, J.E. Shay, M.R. Stephens, and R.A. Slatyer. 2017. Evolution of ecological niche breadth. *Annual Reviews in Ecology Evolution and Systematics* 48:183–206.
- Seymour R.S. 1972. Behavioral thermoregulation by juvenile Green Toads, *Bufo debilis*. *Copeia* 1972(3):572–575.
- Sinclair B.J., K.E. Marshall, M.A. Sewell, D.L. Levesque, C.S. Willett, S. Slotsbo, Y. Dong, C.D.G. Harley, D.J. Marshall, B.S. Helmuth, et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* 19 :1372–1385.
- Sinervo B., F. Mendez-de-la-Cruz, D.B. Miles, B. Heulin, E. Bastiaans, M. Villagran-Santa Cruz, R. Lara-Resendiz, N. Martinez-Mendez, M.L. Calderon-Espinosa, R.N. Meza-Lazaro, et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328:894–899.
- Sunday J.M., A.E. Bates, M.R. Kearney, R.K. Colwell, N.K. Dulvy, J.T. Longino and R.B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences U.S.A.* 111:5610–5615.
- van Berkum F. H. 1986. Evolutionary patterns of the thermal sensitivity of sprint speed in *anolis* lizards. *Evolution* 40:594–604.
- von May R., A. Catenazzi, A. Corl, R. Santa-Cruz, A.C. Carnaval, and C. Moritz. 2017. Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution* 7:3257–3267.
- Wagener C., N. Kruger, and J. Measey. 2021. Progeny of *Xenopus laevis* from altitudinal extremes display adaptive physiological performance. *Journal of Experimental Biology*

224(7):jeb233031.

Wang G.D., B.L. Zhang, W.W. Zhou, Y.X. Li, J.Q. Jin, Y. Shao, H. Yang, Y.H. Liu, F. Yan, H.M. Chen, et al. 2018. Selection and environmental adaptation along a path to speciation in the Tibetan frog *Nanorana parkeri*. *Proceedings of the National Academy of Sciences U.S.A.* 115:E5056–E5065.

Whitford W. G. 1973. The effects of temperature on respiration in the Amphibia. *American Zoologist* 13:505–512.

Yu T.L., M. Busam, D.L. Wang, and K. Chen. 2016. Plasticity of metamorphic traits in a high-altitude toad: interactive effects of food level and temperature. *Amphibia-Reptilia* 37:33–43.

Zhang L. and X. Lu. 2012. Amphibians live longer at higher altitudes but not at higher latitudes. *Biological Journal of the Linnean Society* 106:623–632.

Tables

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

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

Table entries are means ± standard errors.

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

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

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

	Altitude (m a.s.l.)	CTmin (°C)	CTmax (°C)	Topt ± SE (°C)	Topt (GAM) (°C)	Tpb80 (°C)	Tpb80 interval (°C)	Tpb95 (°C)	Tpb95 interval (°C)
Hluhluwe (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
Dalton (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
Phutha- ditjhaba (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
Lesotho (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_{\min} and CT_{\max}), optimal temperature (T_{opt}), 80 and 95% thermal performance breadths ($T_{\text{pb}80}$ and $T_{\text{pb}95}$) and their interval. Table entries are means ± standard errors.

Table 5. Results of Tukey post-hoc tests testing for differences in CT_{min} among populations.

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

Bold values indicate significant differences.

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

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

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

	Lesotho (<i>N</i> = 28)	Phuthaditjhaba (<i>N</i> = 26)	Dalton (<i>N</i> = 25)	Hluluwe (<i>N</i> = 26)
Lesotho	–	< 0.0001	< 0.0001	< 0.0001
Phuthaditjhaba	–	–	0.86	0.04
Dalton	–	–	–	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.

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

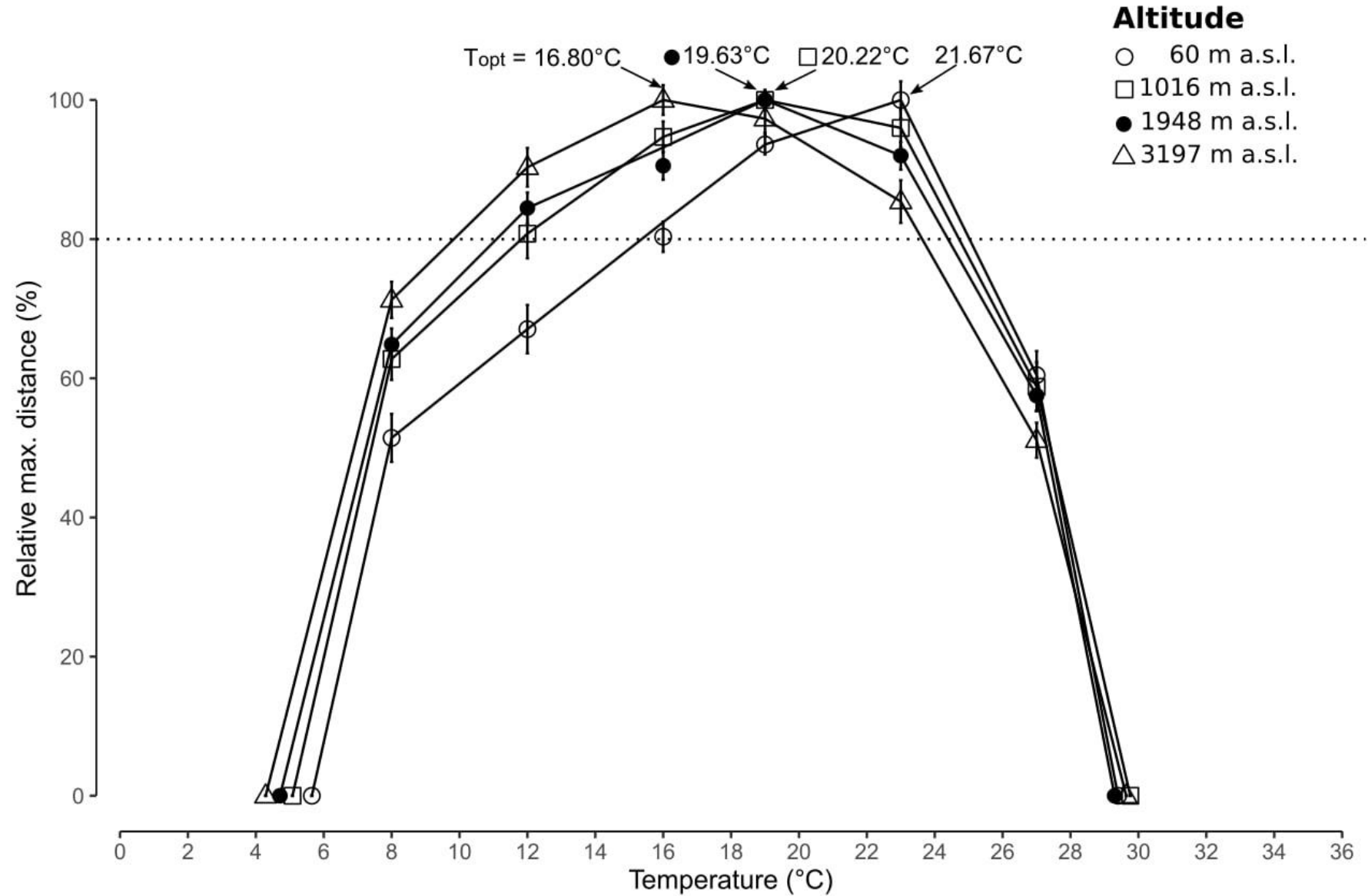
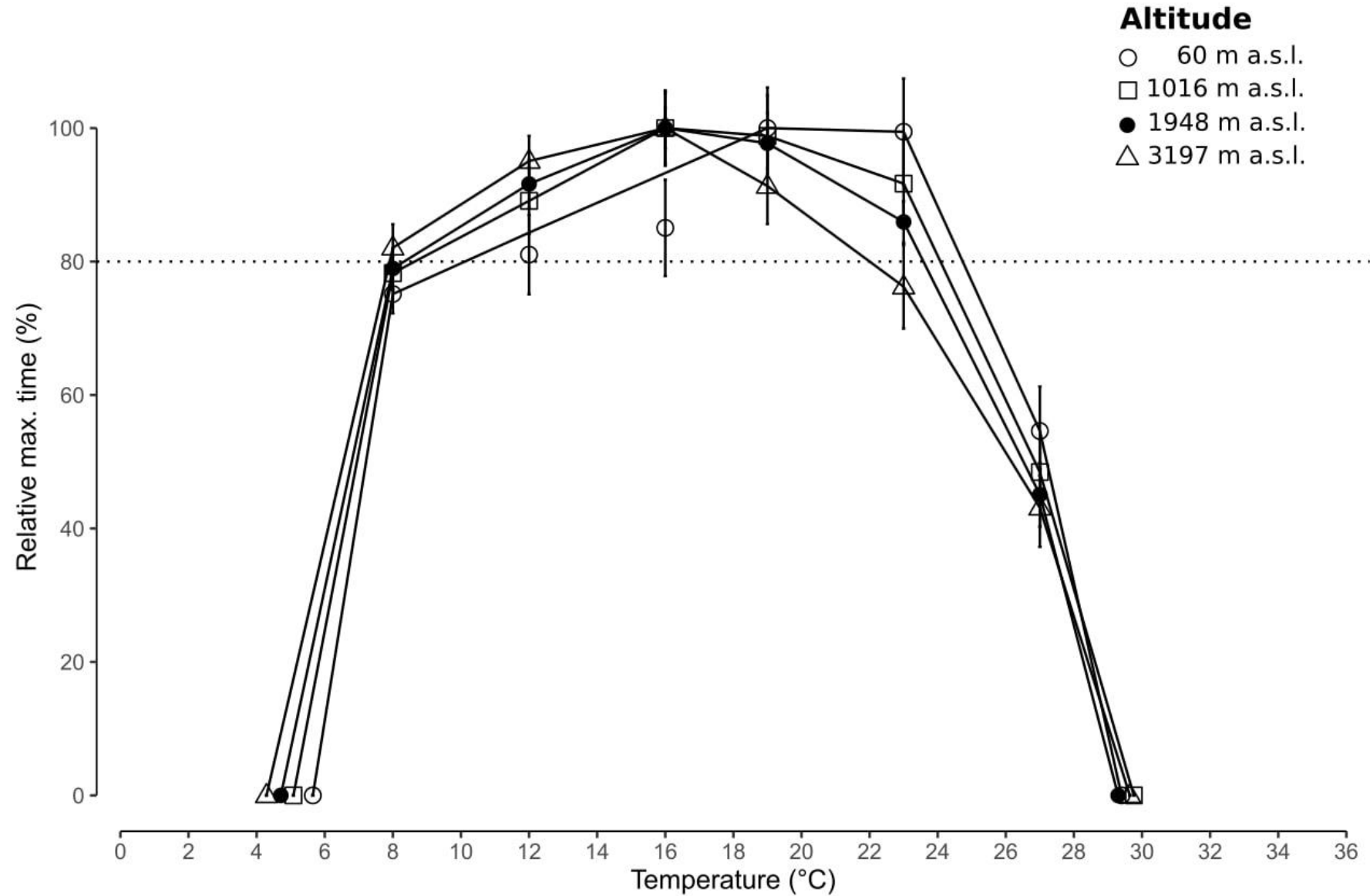


Figure 2. Temperature-performance curves illustrating the impact of temperature on maximum time to exhaustion for *X. laevis* populations.



Supplementary Tables

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

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

	edf	F	p-value
Hluhluwe	5.853	219.823	<0.0001
Dalton	5.865	240.925	<0.0001
Phuthaditjhaba	5.799	210.025	<0.0001
Lesotho	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.

	edf	F	p-value
Hluhluwe	5.929	203.025	<0.0001
Dalton	5.855	204.026	<0.0001
Phuthaditjhaba	5.768	173.516	<0.0001
Lesotho	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).

Hluhluwe	8°C	12°C	16°C	19°C	23°C	27°C
8°C	–	<0.0001	<0.0001	<0.0001	<0.0001	0.03
12°C	–	–	0.03	0.0002	<0.0001	0.22
16°C	–	–	–	0.004	0.0002	0.002
19°C	–	–	–	–	0.15	<0.0001
23°C	–	–	–	–	–	<0.0001
27°C	–	–	–	–	–	–

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).

Dalton	8°C	12°C	16°C	19°C	23°C	27°C
8°C	–	<0.0001	<0.0001	<0.0001	<0.0001	0.31
12°C	–	–	0.01	0.0008	0.0006	<0.0001
16°C	–	–	–	0.31	0.85	<0.0001
19°C	–	–	–	–	0.39	<0.0001
23°C	–	–	–	–	–	<0.0001
27°C	–	–	–	–	–	–

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).

Phuthaditjhaba	8°C	12°C	16°C	19°C	23°C	27°C
8°C	–	<0.0001	<0.0001	<0.0001	<0.0001	0.069
12°C	–	–	0.062	<0.0001	0.049	<0.0001
16°C	–	–	–	0.002	0.62	<0.0001
19°C	–	–	–	–	0.062	<0.0001
23°C	–	–	–	–	–	<0.0001
27°C	–	–	–	–	–	–

Bold values indicate significant differences.

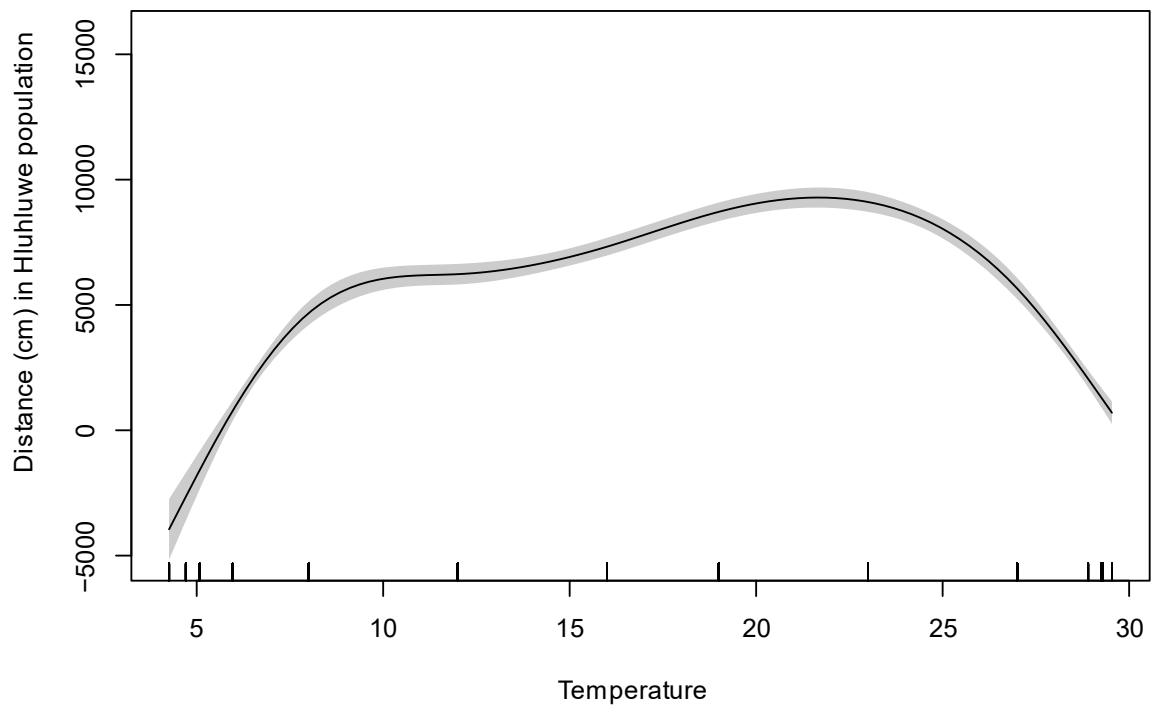
Supplementary Table 7. Results of post-hoc pair-wise t-tests testing for differences in endurance distance depending on temperature for the Lesotho population (N = 28, alt. = 3197 m).

Lesotho	8°C	12°C	16°C	19°C	23°C	27°C
8°C	–		<0.0001	<0.0001	0.018	<0.0001
12°C	–	–	0.62	0.81	0.81	<0.0001
16°C	–	–	–	0.81	0.02	<0.0001
19°C	–	–	–	–	0.02	<0.0001
23°C	–	–	–	–	–	<0.0001
27°C	–	–	–	–	–	–

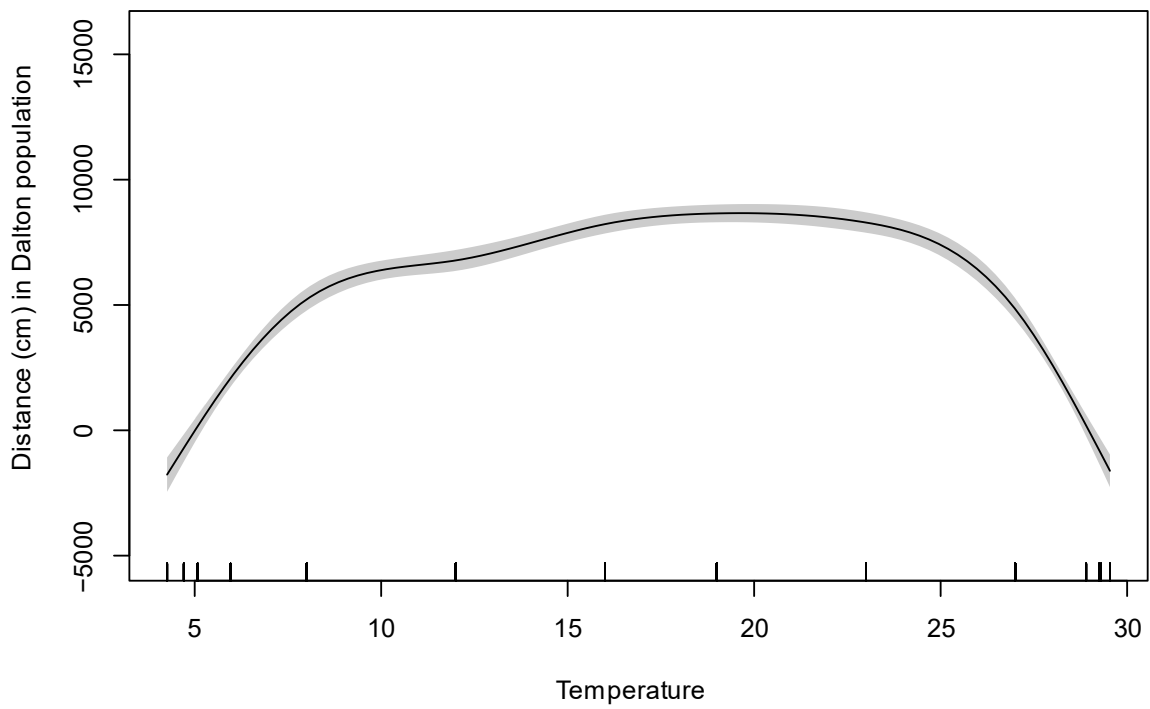
Bold values indicate significant differences.

Supplementary figures

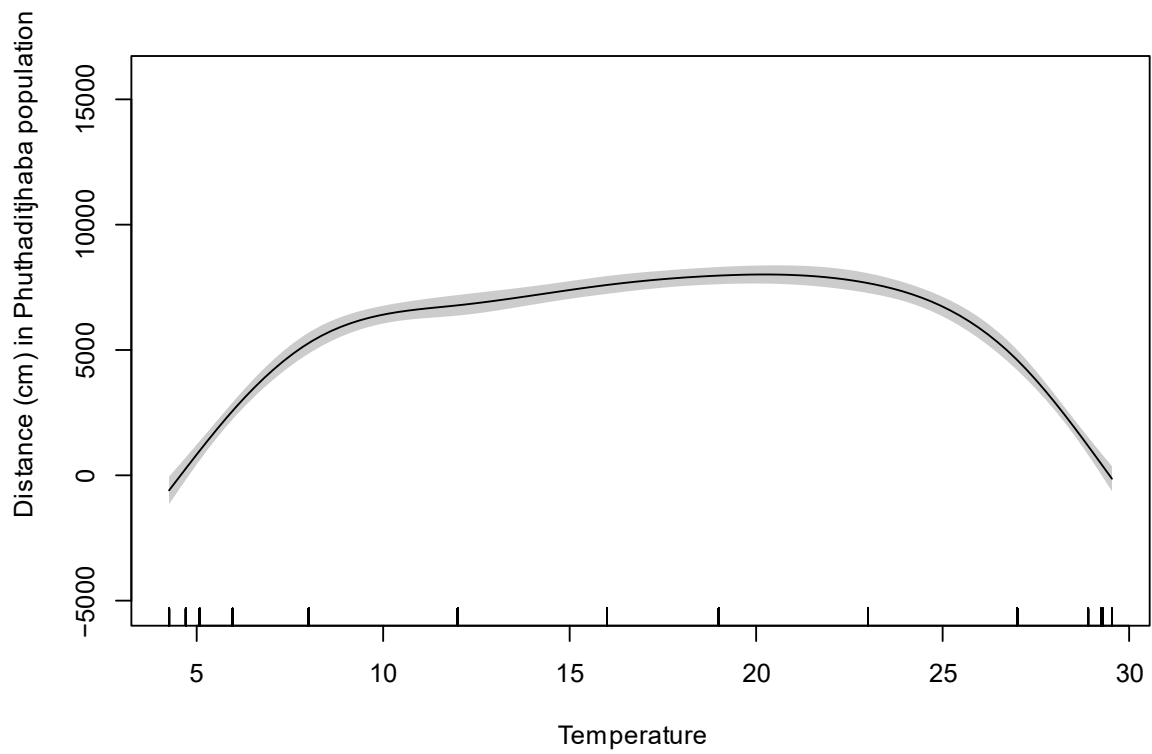
Suppl. Fig.1 **Temperature-performance curves predicted using GAM models illustrating the non-linear effect of temperature on aquatic endurance for the *X. laevis* population from Hluhluwe (60m a.s.l.).** Note that negative values can predicted values beyond the critical thermal limits, illustrating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

