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

Laurie Araspin[1,2,*]
Carla Wagener[2,3]
Pablo Padilla[1,4]
Anthony Herrel[1,†]
John Measey[2,5,†]
[1]Département Adaptations du Vivant, Bâtiment d'Anatomie Comparée, UMR 7179–CNRS, Muséum National d'Histoire Naturelle–Sorbonne Universités, 55 rue Buffon, 75005 Paris, France; [2]Centre for Invasion Biology, Stellenbosch University, Stellenbosch 7602, South Africa; [3]Department of Biology, University of Oxford, Oxford, United Kingdom; [4]Laboratory of Ecology and Conservation of Amphibians, Freshwater and Oceanic Science Unit of Research, University of Liège, Liège, Belgium; [5]Institute of Biodiversity, School of Ecology and Environmental Science, Yunnan University, Kunming, 650504, China

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ABSTRACT

Ectothermic species are dependent on temperature, which drives many aspects of their physiology, including locomotion. The distribution of the native populations of *Xenopus laevis* is characterized by an exceptional range in latitude and altitude. Along altitudinal gradients, thermal environments change, and populations experience different temperatures. In this study, we compared critical thermal limits and thermal performance curves of populations from the native range across an altitudinal gradient to test whether optimal temperatures for exertion differ depending on altitude. Data on exertion capacity were collected at six different temperatures (8°C, 12°C, 16°C, 19°C, 23°C, and 27°C) for four populations spanning an altitudinal gradient (60, 1,016, 1,948, and 3,197 m asl). Results show that the thermal performance optimum differs among populations. Populations from cold environments at high altitudes exhibit a lower optimal performance temperature than populations from warmer environments at lower altitudes. The ability of this species to change its optimal temperature for locomotor exertion across extremely different climatic environments within the native range may help explain its exceptional invasive potential. These results suggest that ectothermic species capable of adapting to broad altitudinal ranges may be particularly good at invading novel climatic areas, given their ability to cope with a wide range of variation in environmental temperatures.

Keywords: Xenopus laevis, native populations, altitudes, temperature, locomotor performance, thermal performance curve.

Introduction

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

At high altitude, the daily and seasonal variations between minimum and maximum mean environmental temperatures can be extreme (Jacobsen and Dangles 2017). Previous studies have investigated the effect of altitude on amphibians, including patterns of distribution depending on elevational gradients, metabolic and locomotor physiologies, thermal safety margin, lifespan, plasticity of metamorphic traits, and environmental adaptations (Navas 1996, 2006; Zhang and Lu 2012; Sunday et al. 2014; Yu et al. 2016; Wang et al. 2018; Wagener et al. 2021).

Here, we study *Xenopus laevis*, an exceptional model for investigating the impact of altitude because it ranges from sea level to over 3,000 m asl (an unusually large range within a single species). *Xenopus laevis* is a largely aquatic frog from southern Africa and inhabits a wide range of geographical and thermal environments (Measey 2004; Furman et al. 2015). In a previous study (Araspin et al. 2020), it was demonstrated that optimal

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*Corresponding author; email: laurie.araspin@mnhn.fr.
†These authors contributed equally to this work.

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temperatures (T_{opt}'s) for locomotion differed between native (South Africa) and invasive (France) populations of *X. laevis* and were associated with differences in the thermal environment experienced by these populations. In its native range, this species displays an important phenotypic diversity, which may suggest a plastic physiology (Du Preez et al. 2009). Recent studies on tadpoles suggest that there are adaptive and plastic underpinnings of population-level differences in physiology (Wagener et al. 2021; Kruger et al. 2022). *Xenopus laevis* is invasive on four continents (Measey et al. 2012) and a threat for local biota (Kumschick et al. 2017; Courant et al. 2018). Plasticity in its temperature tolerance may have been a key factor driving the invasive potential of this species. Furthermore, adaptation to new thermal environments may have facilitated survival in its invasive range.

The aim of the present study is to assess the thermal dependence of locomotor performance in adults from native populations across an altitudinal gradient and to identify potential differences among populations. We choose to measure exertion capacity (measured here as the distance or time swam until exhaustion), as it is fitness relevant and a good proxy for dispersal capacity. We predict that populations from high altitudes and cold environments will perform relatively better at cold temperatures and, conversely, that populations from low altitudes should perform better at warmer temperatures. We thus predict that the T_{opt}'s for locomotor performance will decrease with increasing altitude. However, we do not expect changes in the upper thermal limit, as it is determined by genetic and biochemical constraints and as behavioral thermoregulation is effective in avoiding critical maximum temperatures ($\mathrm{CT}_{\mathrm{max}}$'s; Grigg and Buckley 2013; Blackburn et al. 2014; von May et al. 2017; Pintanel et al. 2019). In contrast, the lower thermal limit is expected to decrease with altitude (as observed in previous studies), at least partly as behavioral thermoregulation is less effective at colder temperatures (Bodensteiner et al. 2021). If so, our results may have broader implications for our understanding of the evolution of thermal performance limits in ectothermic organisms and the ability of species to cope with variation in their current or future thermal environments.

Methods

Adult *Xenopus laevis* individuals were caught in three different areas in KwaZulu-Natal in eastern South Africa. Populations were sampled at the following different altitudes: Hluhluwe (60 m asl, $N = 26$, 13 females and 13 males), Dalton (1,016 m asl, $N = 25$, 13 females and 12 males), and near Phuthaditjhaba (1,948 m asl, $N = 26$, 13 females and 13 males). Individuals from a fourth population were caught in Lesotho (3,197 m asl, $N = 28$, 14 females and 14 males). The sites were chosen because all animals were known to belong to the same clade (Premachandra et al. 2023). Animals were captured at night during March 2021 using liver-baited funnel traps. Animals were transported to Stellenbosch University in South Africa. Upon arrival, all individuals were PIT tagged, allowing unique identification. Specimens were maintained in 72-L aquariums (60 cm × 30 cm × 40 cm, 13–14 individuals per tank) and separated by sex and site of origin. Tanks contained rocks to provide hiding places. Animals were fed chicken hearts twice weekly. The temperature of the water was maintained at 18°C. All protocols and animal welfare conditions were in accordance with the Research Ethics Committee for Animal Care and Use (protocol ACU-2021-19215).

In Situ Temperature Data Collection

Temperature data loggers (HOBO MX2201, Onset) were placed in ponds at a depth of approximately 60 cm at the sites of collection, and they recorded the ambient temperature every 30 min. Data from March to May 2021 were recorded and downloaded via the HOBO application (HOBOconnect, ver. 1.2.4, 25775).

Morphometrics

Body dimensions were measured following Herrel et al. (2012). The mass was measured with a digital scale (Ohaus, New York; precision: ±0.1 g), and snout-vent length (SVL) was measured with a pair of digital callipers (Mitutoyo; precision: ±0.01 mm).

Critical Temperatures

Individuals were placed in sets of three in containers (18 cm × 30 cm × 10 cm) with some water to prevent dehydration and placed in an incubator (MIR-154 Incubator, Sanyo; range: −10°C to 60°C). Experiments started at 18°C (i.e., the housing temperature) from which the temperature was gradually decreased or increased at an average rate of 2°C every 45 min. When 6°C was reached, the temperature was decreased at a rate of 1°C every 45 min for the determination of critical minimum temperature ($\mathrm{CT}_{\mathrm{min}}$). To determine $\mathrm{CT}_{\mathrm{max}}$, we increased the temperature at a rate of 1°C every 45 min from 27°C upward. Animals were warmed slowly, as their thermal inertia was substantial and faster rates resulted in animals being cooler or warmer than the temperature of the incubator. Animals were inspected regularly and checked for the lack of a righting response. As soon as an animal was no longer able to turn over after being placed on its back, we considered that temperature (measured with a thermocouple inserted into the cloaca) to be that animal's critical temperature.

Performance

Aquatic exertion tests were performed at 8°C, 12°C, 16°C, 19°C, 23°C, and 27°C. Individuals were placed in sets of seven in containers (45 cm × 30 cm × 10 cm) with some water to prevent dehydration and left for 3 h in an incubator that was set at the desired test temperature. The room was also set at the test temperature. Before and after each performance trial, body temperature was recorded using a K-type thermocouple. Measures of exertion were performed by chasing animals individually until exhaustion (defined as an animal no longer being able to right itself when put on its back) around a 4.43-m-long circular track with an average water depth of 20 cm. The total distance and time swam until exhaustion at the end of the trial were recorded. After each trial, animals were left to recover at an average temperature of 20°C and then were returned to their tank (water at 18°C). Animals were fed and left to rest for at least 3 d. Trials were repeated twice per individual, and the maximum distance was retained for further analyses.

Statistical Analyses

To fulfill assumptions of normality and homoscedasticity, we $\log_{10}$ transformed all data before analyses. Analyses were performed using R (ver. i386 4.1.2; R Core Team 2021). To test for significant differences in the different locomotor performance traits (i.e., distance and time to exhaustion) among populations, we used a mixed model with temperature, population, and sex as fixed effects; SVL as a covariate; and frog identity as a random effect. To test for differences in SVL and mass among populations, we ran two-way ANOVAs. Within populations (i.e., Hluhluwe, Dalton, Phuthaditjhaba, and Lesotho), we further tested for effects of temperature and sex on performance as well as for their interactions, with mass and SVL as covariates. To do so, we used a repeated-measures multivariate ANOVA. Next, pairwise multiple-comparisons tests (pairwise.t.test, stats package ver. 3.4.2) with Bonferroni-Holm adjusted P values were performed to test which temperatures differed from one another (temperature range: 8°C, 12°C, 16°C, 19°C, 23°C, and 27°C). Tukey's honestly significant difference (HSD) tests were performed to test which populations differed from one another depending on the tested temperature and to test for differences in critical temperatures among populations. For each individual, thermal performance curves (Angilletta et al. 2002) were built using the minimum convex polygon method (van Berkum 1986). T_{opt}'s, 80% thermal performance breadths (T_{pb80}'s), and 95% thermal performance breadths (T_{pb95}'s) were measured and analyzed using univariate ANOVAs and Welch ANOVAs to test for differences in locomotor performance (i.e., aquatic exercise) among populations.

To further assess the relationship between temperature and exertion capacity for each population, we fitted a generalized additive model (GAM). We used sex and population as fixed factors and applied a smoothing parameter (k) to both SVL and temperature. Because the same individuals were used, we added individual identity as a random term. We optimized k based on the model-adjusted R^2 values and using the functions of the mgcv R package (ver. 1.8-35; Wood 2011). To compare the T_{opt} of each population, we also fitted a GAM with the same parameters fitted for each individual and conducted an ANOVA with population as a factor on the predicted T_{opt}'s, followed by a Tukey's HSD test to inspect significant differences within populations.

Results

Morphometric Measurements

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

In Situ Temperatures

Average temperatures between March and May 2021 at each site show a dramatic decrease with an increase in altitude, with an average temperature of 23.03°C for Hluhluwe (minimum = 17.24°C, maximum = 32.34°C), 17.19°C for Dalton (7.59°C, 30.84°C), 17.18°C for Phuthaditjhaba (9.39°C, 24.32°C), and 6.35°C for Lesotho (2.96°C, 22.65°C). Minimum, maximum, and average temperatures for each month between March and May are presented in table 1.

Exertion

Temperature significantly affected the maximum distance swam in each population (Hluhluwe: $F_{5,125} = 41.76$, $P < 0.0003$; Dalton: $F_{5,120} = 44.88$, $P < 0.0001$; Phuthaditjhaba: $F_{5,125} = 68.18, P < 0.0001$; Lesotho: $F_{5,135} = 48.25, P < 0.0001$; table 2). The GAM explained 87% of the variation in exertion, and temperature had a nonlinear significant effect on exertion within each population (supplementary figs. 1–4; supplementary table 2). SVL also had a significant effect on the maximum distance swam

Table 1: Average in situ temperatures at the sites of collection (March–May 2021)

	March–May (°C)	March–May minimum (°C)	March–May maximum (°C)	March (°C)	April (°C)	May (°C)
Hluhluwe ($N = 26$)	23.03 ± .04	17.24	32.34	25.73 ± .05	23.13 ± .04	20.24 ± .03
Dalton ($N = 25$)	17.19 ± .07	7.59	30.84	21.59 ± .06	17.39 ± .11	12.60 ± .07
Phuthaditjhaba ($N = 26$)	17.18 ± .06	9.39	24.32	20.95 ± .05	17.85 ± .04	12.77 ± .04
Lesotho ($N = 28$)	6.35 ± .04	2.96	22.65	7.66 ± .10	6.05 ± .04	5.44 ± .05

Note. Table entries are mean ± SE. Altitudes for the populations are as follows: Hluhluwe (60 m), Dalton (1,106 m), Phuthaditjhaba (1,948 m), and Lesotho (3,197 m).

Table 2: Results of Tukey post hoc tests for differences in maximum distance swam at each tested temperature among populations

Temperature, populations	P
8°C:	
Hluhluwe − Dalton	.0003
Hluhluwe − Lesotho	.0005
Hluhluwe − Phuthaditjhaba	.002
12°C:	
Hluhluwe − Dalton	.003
Hluhluwe − Lesotho	.009
Hluhluwe − Phuthaditjhaba	.004
16°C:	
Hluhluwe − Dalton	.03
19°C:	
NS	...
23°C:	
Lesotho − Dalton	.001
Lesotho − Hluhluwe	.0001
27°C:	
Lesotho − Dalton	.004
Lesotho − Hluhluwe	.001
Lesotho − Phuthaditjhaba	.03

in each population (Hluhluwe: $F_{1,23} = 5.916, P = 0.02$; Dalton: $F_{1,22} = 18.29$, $P = 0.0003$; Phuthaditjhaba: $F_{1,23} = 15.94$, $P = 0.0005$; Lesotho: $F_{1,25} = 24.01$, $P < 0.0001$). The maximum distance swam differed significantly among populations ($\chi^2 = 16.97$, $P = 0.0007$), and the interaction between population and temperature was significant ($\chi^2 = 114.67$, $P < 0.0001$; fig. 1).

Time to exhaustion was also significantly affected by temperature for each population ($\chi^2 = 240.09, P < 0.0001$; fig. 2). Within populations, we also observed that temperature significantly affected time to exhaustion (Hluhluwe: $F_{5,125} = 26.73$, $P < 0.0001$; Dalton: $F_{5,120} = 44.78, P < 0.0001$; Phuthaditjhaba: $F_{5,125} = 90.7, P < 0.0001$; Lesotho: $F_{5,135} = 80.92, P < 0.0001$; fig. 2; table 3). The GAM explained 85.7% of the variation in time to exhaustion, and temperature had a nonlinear significant effect on time to exhaustion for each population, using the same factors as maximum distance (supplementary table 3). The Lesotho and Phuthaditjhaba populations exhibited significantly shorter times to exhaustion than the Hluhluwe population at 23°C and 27°C ($P < 0.04$), and the Lesotho population exhibited a significantly shorter time to exhaustion than the Dalton population at 23°C and 27°C (table 3).

Post hoc tests for maximum distance showed significant differences that depended on temperature within populations (supplementary tables 4–7). For maximum distance, significant differences depending on temperature among populations were observed (table 2). There were significant differences in performance at cold temperatures (8°C–16°C) between the Hluhluwe population from a low elevation and the other populations. No differences were observed in the maximum distances among populations at 19°C. Conversely, at hotter temperatures (23°C–27°C), the performance of the Lesotho population from a high elevation was significantly different from the performances of the other populations. We further observed that the low-altitude Hluhluwe individuals were the least endurant at the lowest temperatures (8°C, 12°C, and 16°C). However, with increasing test temperatures (19°C, 23°C, and 27°C), the high-altitude Lesotho individuals swam shorter distances (table 4).

Critical Temperature, Optimal Temperature, and Performance Breadths

CT_{min} differed significantly among populations ($F_{3,36} = 11.26$, $P < 0.0001$), particularly between the Hluhluwe population and the other populations ($P < 0.03$) and among the individuals from the Lesotho and Dalton populations ($P = 0.04$). As predicted, CT_{min} decreased with an increase in altitude (see tables 5, 6; fig. 1). CT_{max} did not differ, however, among populations ($F_{3,36} = 0.301, P = 0.82$; tables 5, 7). T_{opt} differed significantly among populations, irrespective of whether the convex polygon method ($F_{3,101} = 8.85$, $P < 0.0001$) or the GAM predictions ($F_{3,101} = 22.68$, $P < 0.0001$) were used. Differences in T_{opt} were marked among the Lesotho and Dalton populations ($P = 0.008$) and the Lesotho and Hluhluwe populations ($P < 0.0001$), and T_{opt} tended to differ among individuals from Lesotho and Phuthaditjhaba ($P = 0.059$). The high-altitude Lesotho population showed the lowest T_{opt}, followed by the Phuthaditjhaba, Dalton, and low-altitude Hluhluwe populations (tables 5, 8; fig. 1). There were no significant differences in T_{pb80} ($F_{3,101} = 2.11$, $P = 0.09$) and

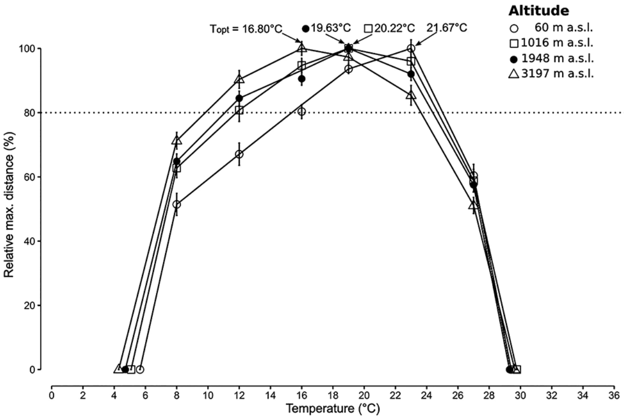

Figure 1. Temperature performance curves illustrating the impact of temperature on maximum distance swam for *Xenopus laevis* populations. The Hluhluwe population is represented by open circles, the Dalton population is represented by squares, the Phuthaditjhaba population is represented by filled circles, and the Lesotho population is represented by triangles. The 80% thermal performance breadths for each population are indicated. Upper and lower ends of the curves are critical temperatures resulting in zero performance (critical maximum temperature and critical minimum temperature). Symbols represent mean ± SEM. Optimal temperatures (T_{opt}'s) obtained from the generalized additive model for each population are represented.

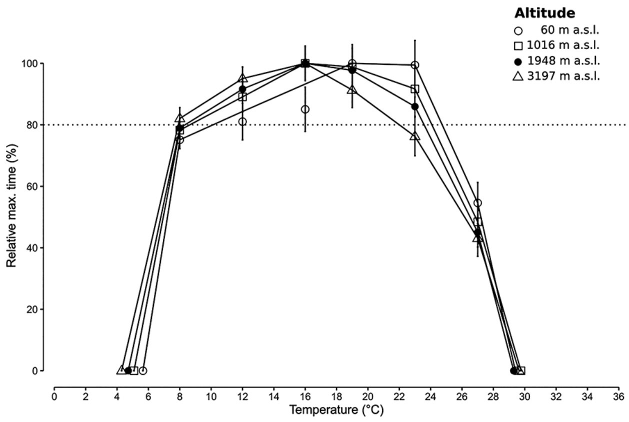

Figure 2. Temperature performance curves illustrating the impact of temperature on maximum time to exhaustion for *Xenopus laevis* populations. The Hluhluwe population is represented by open circles, the Dalton population is represented by squares, the Phuthaditjhaba population is represented by filled circles, and the Lesotho population is represented by triangles. The 80% thermal performance breadths for each population are indicated. Upper and lower ends of the curves are critical temperatures resulting in zero performance (critical maximum temperature and critical minimum temperature). Symbols represent mean ± SEM.

T_{pb95} ($F_{3,\,55.32} = 1.72$, $P = 0.18$; table 5) among populations. However, there were significant differences in the upper and lower limits of the T_{pb80} and T_{pb95} intervals among populations (80% lower limit: $F_{3,\,101} = 6.87$, $P < 0.0002$; 80% upper limit: $F_{3,\,101} = 9.009$, $P < 0.0001$; 95% lower limit: $F_{3,\,101} = 8.43$, $P < 0.0001$; 95% upper limit: $F_{3,\,101} = 9.69$, $P < 0.0001$), indicating shifts in the thermal performance curves (table 5; fig. 1). We observed a left shift in the thermal performance curve of the high-elevation Lesotho population toward colder temperatures and a right shift in the thermal performance curve of the low-elevation Hluhluwe population toward warmer temperatures (table 5).

Discussion

Critical Temperatures

Ectotherms are dependent on temperature to maintain their physiological functions, yet many species can behaviorally thermoregulate to avoid temperature extremes or to select preferred temperature ranges (Sinclair et al. 2016). However, behavioral thermoregulation and mitigation strategies to avoid temperature extremes may be more difficult for aquatic animals such as *Xenopus laevis*, as shallow bodies of water are more homogenous in temperature. Variation in thermal sensitivity depends on metabolic processes that are effective across a specific range of temperatures, which is referred to as an organism's thermal tolerance. Outside of this range, organisms cannot maintain activity and locomotion or even survive (Gillooly 2001; Angilletta et al. 2010). In our study, $\mathrm{CT_{max}}$ did not differ significantly among populations from different altitudes, which is contrary to $\mathrm{CT_{min}}$ (tables 6, 7). It is known that $\mathrm{CT_{max}}$ is more limited by genetic or biochemical constraints (Grigg and Buckley 2013; Blackburn et al. 2014) and evolves less rapidly than $\mathrm{CT_{min}}$ (von May et al. 2017; Pintanel et al. 2019). Furthermore, hot temperatures can also be more easily behaviorally avoided than cold temperatures (Muñoz et al. 2014, 2016; Bodensteiner et al. 2021), which could explain why $\mathrm{CT_{max}}$ did not differ significantly among populations. In contrast, with increasing altitude, $\mathrm{CT_{min}}$ decreased, as observed in other studies on critical thermal limits depending on elevation (Navas 2002; Catenazzi et al. 2014). Our results show that adults from the examined populations conserved the same physiological upper thermal limit, suggesting that part of the overall thermal ecology is conserved (Sinervo et al. 2010). A study on tadpoles from parents collected at two of these sites (Phuthaditjhaba and Hluhluwe; Wagener et al. 2021) showed that their $\mathrm{CT_{min}}$ and $\mathrm{CT_{max}}$ varied in the same way but were lower (4.2°C–2.5°C) and higher (37.0°C–37.8°C), respectively, than those of the adults measured here. This suggests that larvae have a wider thermal tolerance than adults in *X. laevis* (Heatwole et al. 1968). Moreover, intraspecific variation in $\mathrm{CT_{max}}$ tends to differ less than interspecific variation at the adult stage (Araújo et al. 2013). The low-altitude Hluhluwe population exhibited the highest $\mathrm{CT_{min}}$ and showed significant differences in $\mathrm{CT_{min}}$ compared to the other populations (table 5).

Exertion Performance

Thermal performance curves were expected to have the same general structure, with performance increasing with increasing temperature, reaching a maximum at T_{opt}, and then quickly decreasing to reach zero performance at $\mathrm{CT_{max}}$ and $\mathrm{CT_{min}}$ (Angilletta et al. 2002; Schulte et al. 2011). We observed a similar overall structure of thermal performance curves in the

Table 3: Time to exhaustion (s) at different temperatures for the different populations

	Hluhluwe ($N = 26$)	Dalton ($N = 25$)	Phuthaditjhaba ($N = 26$)	Lesotho ($N = 28$)
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

Note. Table entries are mean ± SE. Altitudes for the populations are as follows: Hluhluwe (60 m), Dalton (1,106 m), Phuthaditjhaba (1,948 m), and Lesotho (3,197 m).

Table 4: Aquatic exertion (distance covered in cm) at different temperatures for the tested populations

	Hluhluwe ($N = 26$)	Dalton ($N = 25$)	Phuthaditjhaba ($N = 26$)	Lesotho ($N = 28$)
8°C	4,104.69 ± 155.73	5,135.16 ± 178.75	5,023.11 ± 193.32	5,185.28 ± 207.04
12°C	5,445.23 ± 304.77	6,621.48 ± 224.61	6,614.42 ± 256.80	6,616.86 ± 245.88
16°C	6,709.92 ± 377.99	8,076.68 ± 447.04	7,118.57 ± 281.20	7,293.61 ± 340.72
19°C	7,943.57 ± 473.86	8,548.12 ± 429.74	7,948.65 ± 347.70	7,190.25 ± 403.43
23°C	8,468.46 ± 510.92	8,110.12 ± 402.60	7,228.08 ± 270.40	6,250.54 ± 377.82
27°C	4,951.11 ± 280.75	4,797.76 ± 267.14	4,534.23 ± 241.95	3,665.39 ± 200.39

Note. Table entries are mean ± SE. Altitudes for the populations are as follows: Hluhluwe (60 m), Dalton (1,106 m), Phuthaditjhaba (1,948 m), and Lesotho (3,197 m).

Table 5: Critical and optimal temperatures for the tested populations of *Xenopus laevis*

	Altitude (m asl)	CT_{min} (°C)	CT_{max} (°C)	T_{opt} (°C)	T_{opt} GAM (°C)	T_{pb80} (°C)	T_{pb80} interval (°C)	T_{pb95} (°C)	T_{pb95} interval (°C)
Hluhluwe ($N = 26$)	60	5.96 ± .30	29.54 ± .53	21.11 ± .30	21.67	9.46 ± .72	15.2–24.6	3.80 ± .48	18.8–22.6
Dalton ($N = 25$)	1,016	5.08 ± .15	28.90 ± .63	19.44 ± .37	19.63	12 ± .81	12.5–24.2	5.65 ± .74	16–21.6
Phuthaditjhaba ($N = 26$)	1,948	4.71 ± .17	29.3 ± .27	18.80 ± .40	20.22	11.4 ± .66	12.3–23.8	3.83 ± .51	17.2–21
Lesotho ($N = 28$)	3,197	4.26 ± .21	29.25 ± .44	16.64 ± .42	16.80	10.64 ± .79	11.5–22.2	4.51 ± .65	14.8–19.3

Note. Table entries are mean ± SE. CT_{max} = critical maximum temperature; CT_{min} = critical minimum temperature; GAM = generalized additive model; T_{opt} = optimal temperature; T_{pb80} = 80% thermal performance breadth; T_{pb95} = 95% thermal performance breadth.

Table 6: Results of Tukey post hoc tests for differences in critical minimum temperature among populations

	Lesotho ($N = 28$)	Phuthaditjhaba ($N = 26$)	Dalton ($N = 25$)	Hluhluwe ($N = 26$)
Lesotho	...	.46	**.04**	**.000**
Phuthaditjhaba	...	...	.62	**.001**
Dalton	...	...	...	**.03**

Note. Bold values indicate significant differences.

Table 7: Results of Tukey post hoc tests for differences in critical maximum temperature among populations

	Lesotho ($N = 28$)	Phuthaditjhaba ($N = 26$)	Dalton ($N = 25$)	Hluhluwe ($N = 26$)
Lesotho	...	.99	.94	.98
Phuthaditjhaba	...	...	.91	.99
Dalton	...	...	...	.78

Table 8: Results of Tukey post hoc tests for differences in optimal temperature (generalized additive model) among populations

	Lesotho ($N = 28$)	Phuthaditjhaba ($N = 26$)	Dalton ($N = 25$)	Hluhluwe ($N = 26$)
Lesotho	...	**<.0001**	**<.0001**	**<.0001**
Phuthaditjhaba	...	...	.86	**.04**
Dalton	...	...	...	.22

Note. Bold values indicate significant differences.

four populations (see fig. 1). Temperature significantly affected locomotor performance, as expected. In general, amphibians exhibited an increase of their resting or basal metabolism (oxygen consumption) with increasing temperature (Whitford 1973; Kreiman et al. 2019). Endurance capacity relies strongly on the cardiovascular system and the oxygen transport, which is extremely dependent on temperature (Seymour 1972). Exertion, as measured here, is also dependent on the cardiovascular system, in addition to being dependent on the muscle contractile properties. Individuals from populations from different altitudes consequently exhibited different T_{opt}'s for locomotor performance (table 5). The Lesotho population from the highest altitude (3,197 m asl) had the lowest T_{opt} (16.64°C), and the Hluhluwe population from the lowest altitude (60 m asl) had the highest T_{opt} (21.11°C). T_{opt}'s in populations from the middle altitudes (Dalton [1,016 m asl] and Phuthaditjhaba [1,948 m asl]) were intermediate (19.44°C and 18.8°C, respectively). The T_{opt} for exertion performance of the populations tested here thus seems to be correlated with environmental temperatures depending on altitude, with environmental temperatures decreasing with increasing altitude (Körner 2007; table 1). Note that there is a mismatch between the mean environmental temperature and the T_{opt} for performance, with frogs from the Lesotho population exhibiting a T_{opt} (16.64°C) that is higher than the mean environmental temperature (6.35°C). However, a decrease in environmental temperature did result in a decrease in T_{opt}. These results are concordant with the prediction that T_{opt} for fitness-relevant functions is set at the most commonly encountered temperature in the environment (Huey and Kingsolver 1993; Gilchrist 1995; Navas et al. 2008).

Performance Breadths

Similar to the differences in T_{opt}'s among populations, we observed directional shifts in the 80% thermal performance curve for the populations from the two extreme altitudes (Lesotho [3,197 m asl], Hluhluwe [60 m asl]). For example, the thermal performance curve of the Lesotho population shows a left shift of about 3.7°C compared to the Hluhluwe population (see fig. 1; table 5, T_{pb80} interval). Consequently, the Lesotho population from the highest altitude appears better adapted to lower temperatures for aquatic exertion. It is known that adaptation to a cold environment is predicted to entail the loss of performance in warmer environments (Bennett and Lenski 2007), which is effectively observed for the Lesotho population. However, the Hluhluwe population from the lowest altitude appears better adapted to higher temperatures. Overall, differences in thermal sensitivity of locomotor performance among populations tend to be mainly driven by two populations, the Hluhluwe and Lesotho populations. However, the T_{pb80} interval did not differ among populations, showing that the overall shape of the performance curve did not evolve. These results can suggest a level of intrinsic constraint, which appears to limit the ability of *X. laevis* adults to broaden the temperature performance curve.

Generally, species with broad distribution ranges express phenotypic differences among local populations, following predictable patterns along spatial and environmental gradients (Chevin and Lande 2011). Thus, thermal performance curves may shift to become locally adapted to environmental conditions such as temperature (Narum et al. 2013). Environmental gradients exert a major effect on patterns of intraspecific variation (Mizera and Meszéna 2003) and, coupled with natural selection, in the long term may produce differences between populations, resulting in local adaptation (Olsson and Uller 2003). Local adaptation is a process of natural selection whereby resident populations evolve higher relative fitness in their local habitat than populations originating elsewhere (Sexton et al. 2017; Schmid and Guillaume 2017). The *X. laevis* populations included in our study show different phenotypes in locomotor performance depending on temperature and altitude (Arnold 1983). These may increase fitness in a given thermal environment, as individuals and populations from cold environments and high altitudes perform relatively better at cold temperatures. The thermal performance curves from these populations thus show patterns in thermal physiology that covary with the thermal environment and the altitudinal gradient. Indeed, in situ temperature data from the collected sites show a thermal gradient in average temperature depending on the altitude, ranging from 6.35°C to 23.03°C. The average temperatures from March to May 2021 are 23.03°C in Hluhluwe (60 m asl), 17.19°C in Dalton (1,016 m asl), 17.18°C in Phuthaditjhaba (1,948 m asl), and 6.35°C in Lesotho (3,197 m asl; table 1).

To understand the processes that underlie the observed differences in T_{opt}'s for locomotion in *X. laevis*, we believe that future studies may benefit from using common garden experiments to tease apart the role of phenotypic plasticity versus genetic adaptation. Even if the responses are plastic, this may promote future adaptation by allowing populations to perform well in novel environments (Crispo 2007; Ghalambor et al. 2007) and may be one of the critical traits driving the invasive potential of the species. Moreover, genomic and transcriptomic approaches coupled to the study of phenotypic diversity would provide better insights into the processes underlying thermal adaptation in these organisms. The broad thermal tolerance and ability to adapt to different thermal environments may explain the invasive nature of this species and its presence on four continents (Measey et al. 2012; Furman et al. 2015), comprising areas outside of its native climate envelope (Rödder et al. 2017). Including physiological data on locomotor performance depending on temperature in species distribution models can provide better and more biologically informed insights into the potential future spread of this species under different scenarios of climate change (Coulin et al. 2019; Gamliel et al. 2020; Ginal et al. 2023). Finally, our results suggest that whereas T_{opt}'s for locomotion show population-level differences, the shape of the performance curves do not. This may hint at a more general mechanism of the evolution of thermal dependence of locomotion, yet it remains to be tested in other species.

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