**RESEARCH ARTICLE**

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# From performance curves to performance surfaces: Interactive effects of temperature and oxygen availability on aerobic and anaerobic performance in the common wall lizard

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## Abstract

1. Accurately predicting the responses of organisms to novel or changing environments requires the development of ecologically-appropriate experimental methodology and process-based models.

2. For ectotherms, thermal performance curves (TPCs) have provided a useful framework to describe how organismal performance is dependent on temperature. However, this approach often lacks a mechanistic underpinning, which limits our ability to use TPCs predictively. Furthermore, thermal dependence varies across traits, and performance is also limited by additional abiotic factors, such as oxygen availability.

3. We test a central prediction of our recent Hierarchical Mechanisms of Thermal Limitation (HMTL) Hypothesis which proposes that natural hypoxia exposure will reduce maximal performance and cause the TPC for whole-organism performance to become more symmetrical.

4. We quantified TPCs for two traits often used as fitness proxies, sprint speed and aerobic scope, in lizards under conditions of normoxia and high-elevation hypoxia.

5. In line with the predictions of HMTL, anaerobically fuelled sprint speed was unaffected by acute hypoxia while the TPC for aerobic scope became shorter and more symmetrical. This change in TPC shape resulted from both the maximum aerobic scope and the optimal temperature for aerobic scope being reduced in hypoxia as predicted.

6. Following these results, we present a mathematical framework, which we call Temperature–Oxygen Performance Surfaces, to quantify the interactive effects of temperature and oxygen on whole-organism performance in line with the HMTL hypothesis. This framework is transferrable across traits and levels of organization to allow predictions for how ectotherms will respond to novel

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combinations of temperature and other abiotic factors, providing a useful tool in a time of rapidly changing environmental conditions.

# 1 | INTRODUCTION

Abiotic factors, such as temperature and oxygen availability, influence the vital rates of organisms both directly and through their interaction (Angilletta, 2009; Ern, 2019; Gangloff & Telemeco, 2018; Rezende et al., 2014). Understanding the relationship between abiotic factors and organismal performance has long been an aim of biologists, with the global climate and biodiversity crises adding urgency to this goal. Perhaps the best-developed paradigm describing the relationship between organismal performance and an abiotic factor is the thermal performance curve (TPC; Angilletta, 2009; Huey & Stevenson, 1979; Taylor et al., 2021). Primarily applicable to ectothermic organisms, TPCs quantify the performance or functionality of a physiological process or integrated performance measure across the range of temperatures at which these processes can occur. Importantly, such curves are applicable across levels of biological organization, from subcellular processes (Licht, 1964; Somero, 2020) to metabolism (Clark et al., 2013; Schulte, 2015) to whole-organism performance (Gilbert & Miles, 2019; Stevenson et al., 1985) to population growth (Luhring & DeLong, 2016; Ratkowsky et al., 2005). From these curves, parameters describing organismal function can be extracted, including the maximal performance, optimal temperature for performance, upper and lower thermal limits, and performance breadth (Angilletta, 2009; Taylor et al., 2021). These parameters, as well as their variation across traits, individuals, and populations, are used in myriad applications, including modelling responses to novel or changing environments (Deutsch et al., 2008; Kearney et al., 2008; Levy et al., 2015).

While undoubtedly useful, TPCs have limitations. For example, TPCs are descriptive representations of the thermal dependence of traits measured in a specific time and place (typically a controlled laboratory environment), and their context dependence makes them difficult to extrapolate to diverse natural conditions (Bodensteiner, Agudelo-Cantero, et al., 2021; Gangloff & Telemeco, 2018; Kingsolver & Buckley, 2020; Rezende et al., 2014; Schulte et al., 2011; Woods et al., 2018). Furthermore, TPCs are univariate models, despite physiological traits responding to numerous interacting abiotic factors in nature. In this study, we explore the link between aerobic capacity and thermal performance (Ern, 2019; Gangloff & Telemeco, 2018; Pörtner et al., 2017; Pörtner & Knust, 2007; Schulte, 2015). The oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT) proposes that whole-organism thermal performance is constrained by an organism's metabolic demands and oxygen supply capacity, with organismal thermal limits set when oxygen demands at extreme temperatures exceed the organism's capacity to acquire and circulate

oxygen (Pörtner, 2001; Pörtner, 2002). The OCLTT hypothesis has received mixed support in diverse taxa (Ern et al., 2016; Gangloff & Telemeco, 2018; Pörtner & Knust, 2007; Verberk et al., 2016) and induced heated debate about its ecological relevance and universality (Clark et al., 2013; Jutfelt et al., 2018; Pörtner et al., 2018).

More recently, the Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis was developed to reconcile observations that support and refute OCLTT in ectothermic animals (Box 1; Gangloff & Telemeco, 2018). Similar to OCLTT, the HMTL hypothesis proposes that whole-organism performance is driven by aerobic capacity across suitable temperatures. However, HMTL explicitly recognizes that critical thermal limits can be set by multiple mechanisms, including lack of aerobic capacity and failure of subcellar components such as proteins or membranes. Whether limits on aerobic capacity or subcellular functionality are reached first proximally sets thermal limits, and this hierarchy can change predictably with life-history stage and environmental context. In addition to mechanistically explaining the asymmetrical shape of the TPC characteristic of many ectotherms, HMTL leads to specific predictions about how TPC shape is affected by oxygen environment and capacity (Box 1; for full details, see Gangloff & Telemeco, 2018). Principally, HMTL predicts that peak performance and the optimal temperature for performance are reduced when the organism's ability to obtain or utilize oxygen becomes limited, even when thermal limits, such as the critical thermal maximum ($CT_{MAX}$), are unaffected. Invasion of high elevation, breath-holding, intense activity, pregnancy, disease and ontogenetic stage are common circumstances where reduced aerobic capacity could meaningfully affect TPC shape in ectotherms. Thus, the HMTL framework could provide an essential tool in predicting organismal responses to novel or changing environments. Such quantitative predictions about both the sublethal and lethal effects of extreme temperatures across life-history stages or environments that differ in oxygen capacity or availability are especially prescient in ecological contexts presented by the global climate emergency, including in the context of warming waters or upslope migration (Ern et al., 2016; Jacobsen, 2020; Rodgers et al., 2021; Storz, 2021).

A further challenge to utilizing TPCs as predictive tools is the selection of relevant traits as proxies for whole-organism performance or fitness. TPCs can vary among traits (Clark et al., 2013; Kellermann et al., 2019; Stevenson et al., 1985) and respond differently to the interaction of temperature with factors such as oxygen. Thus, if a performance measure is to be used as a fitness proxy, the trait and context in which it is measured must be carefully considered. Locomotor performance, specifically sprint speed in lizards, is an easily and widely measured trait relevant to multiple

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**BOX 1  Hierarchical mechanisms of thermal limitation**

The Hierarchical Mechanisms of Thermal Limitation hypothesis (HMTL; Gangloff & Telemeco, 2018) combines ideas from the Oxygen-and Capacity-Limited Thermal Tolerance hypothesis (OCLTT; Pörtner, 2001, 2002; Pörtner et al., 2017), Marginal Stability hypothesis (Hochachka & Somero, 2002) and Thermal Performance Curve paradigm (TPC; Huey & Stevenson, 1979) to mechanistically describe how temperature and oxygen availability interact to affect whole-organism performance in ectothermic animals. Figure B1 provides a schematic of the HMTL hypothesis. As with OCLTT, HMTL proposes that whole-organism performance is proportional to aerobic scope, the difference between resting metabolic demand and maximum metabolic capacity. As temperature increases above the critical thermal minimum ($CT_{MIN}$), aerobic scope increases before plateauing when maximum metabolic rate (MMR) equals the maximum aerobic capacity of the organism (horizontal dashed lines in the top panels of Figure B1). As temperature increases above the thermal optimum for performance ($T_{OPT}$), MMR remains fixed but resting metabolic rate (RMR) continues to increase due to thermal effects on metabolic kinetics, resulting in reduced aerobic scope. This slow drop in aerobic performance will continue until RMR equals MMR and aerobic scope equals zero (Figure B1). We call this point the 'aerobic critical temperature' (aerobic $T_{CRIT}$). By contrast, we call the temperature where subcellular components critically lose function, such as when membranes and enzymes become too flexible or unstable to properly function (Marginal Stability Hypothesis; Hochachka & Somero, 2002), the 'subcellular $T_{CRIT}$'. While the breakdown of subcellular processes can (and likely will) lead to aerobic capacity failure, this process is distinct from organismal performance limits being determined directly by aerobic capacity, as defined at aerobic $T_{CRIT}$. HMTL proposes that $CT_{MAX}$ is driven by whichever $T_{CRIT}$ is lower (hence '*hierarchical mechanisms*' in HMTL). Under HMTL, any change in aerobic capacity that reduces aerobic $T_{CRIT}$ could induce a shift from subcellular to aerobic mechanisms driving $CT_{MAX}$. This applies even in species typically limited by subcellular $T_{CRIT}$, as illustrated for the 'Extremely reduced aerobic capacity' condition in Figure B1c. The primary conceptual advance of HMTL is its explicit acknowledgement that either oxygen limitation or marginal stability can underlie lost performance at high temperatures. Furthermore, this mechanism can transition plastically and predictably.

HMTL makes multiple novel and testable predictions for the shape of the TPC when either aerobic or subcellular $T_{CRIT}$ proximally set high-temperature limits, and for how TPC shape will change in response to variation in oxygen environment or aerobic capacity. When aerobic $T_{CRIT}$ underlies $CT_{MAX}$, HMTL predicts that the TPC will be symmetrical (Figure B1c). This symmetry results from aerobic scope decreasing with temperature above $T_{OPT}$ at the same rate that aerobic scope increased with temperature below $T_{OPT}$. However, when subcellular $T_{CRIT}$ underlies $CT_{MAX}$, HMTL predicts that the TPC will be left skewed, as is typical of most ectotherms (Figure B1a,b). Moreover, HMTL predicts that this skew will increase with the distance between the aerobic $T_{CRIT}$ and subcellular $T_{CRIT}$. This asymmetry results from subcellular $T_{CRIT}$ inducing rapid performance losses at temperatures below those that would induce major aerobic limitation due to a mismatch between demand and supply capacity. HMTL provides a framework that can be used to make mechanistic predictions about both critical thermal limits and how TPC shape is affected by the oxygen environment across ectothermic taxa and ecological contexts.

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ecological contexts including predator escape, foraging and conflicts between conspecifics (Gilbert & Miles, 2017; Miles, 2004). However, there are important considerations when utilizing this measure as a fitness proxy (Irschick, 2003). For example, sprint speed over short distances is almost entirely anaerobically fuelled in lizards (Bennett & Licht, 1972; Gleeson, 1991). As a result, lizards can only maintain sprinting performance for seconds-to-minutes before they must rest and use aerobic respiration to regain homeostasis (Gleeson, 1982; Gleeson & Hancock, 2002). Any application of anaerobic TPCs to predict organismal responses to long-term changes in temperature, such as result from climate change, implicitly assumes that aerobic performance capacity, which powers the majority of functions such as digestion, growth and reproduction, is at least as high as anaerobic performance across temperatures. If this assumption is not met, then anaerobic traits such as sprint speed will be poor proxies for whole-organism performance and more-aerobic traits should be preferred. Recent

work emphasizes the ecological relevance of aerobic scope as an integrated measure of whole-organism performance. Aerobic scope is the difference between resting metabolic demand and maximum metabolic capacity, which represents the capacity for animals to power aerobic processes (Gangloff & Telemeco, 2018; Pörtner et al., 2017; Schulte, 2015; Seibel & Deutsch, 2020). TPCs for aerobic scope are useful in bridging laboratory measurements of individual animals to broader biogeographical patterns and predictions (Bozinovic & Pörtner, 2015; Clark et al., 2013; Pörtner, 2021). While both sprint speed and aerobic scope exhibit temperature dependence, they differ in how they are fuelled and thus likely differ in how they will respond to variation in the oxygen environment. Because sprint speed is almost entirely fuelled by anaerobic respiration (Bennett & Licht, 1972), it is not expected to be limited by acute reductions in oxygen availability, whereas maximal aerobic capacity, and therefore aerobic scope, will be constrained (Storz, 2021).
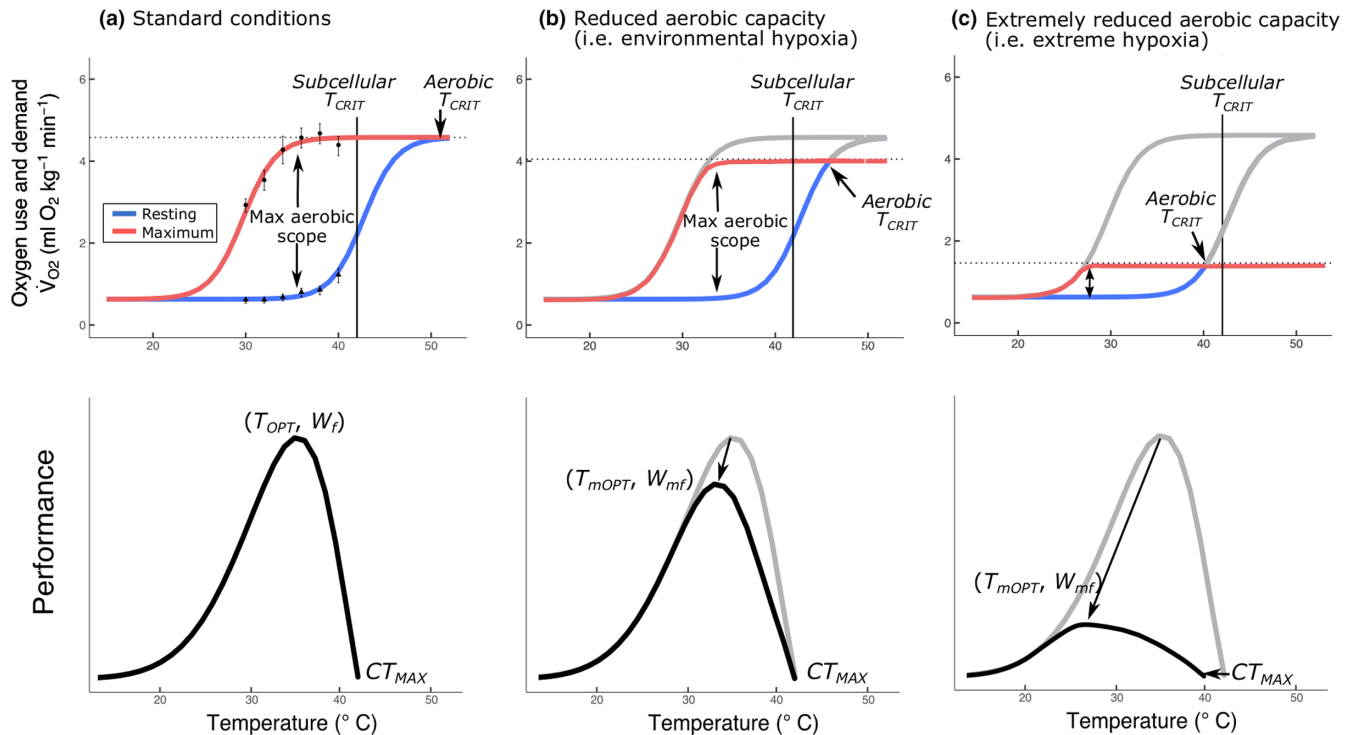
**FIGURE B1** Schematic representation of the Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis displaying the effects of three oxygen levels on aerobic scope, *subcellular* $T_{CRIT}$ and *aerobic* $T_{CRIT}$ (top panels), and the resulting thermal performance curves (TPCs; bottom panels). Conditions from left to right are (a) standard (i.e. healthy, non-reproductive adult measured at sea level in normoxia), (b) reduced aerobic capacity, (c) extremely reduced aerobic capacity. Under standard conditions, the resting and maximum metabolic rate lines were fit to data for ball pythons *Python regius* from Fobian et al. (2014). In the reduced-capacity plots, grey lines depict resting and active metabolic rate under standard conditions for visual reference, whereas the coloured lines display predicted consequences of reduced aerobic capacity. The horizontal dashed line illustrates maximum aerobic capacity under each condition. $T_{OPT}$, $W_f$, $T_{mOPT}$ and $W_{mf}$ are parameters from the beta equation describing TPCs and the modified beta equation describing temperature–oxygen performance surfaces (TOPS), respectively (see Equations 1–3 in this study). Figure adapted from Gangloff and Telemeco (2018).

With this study, we test and support predictions of the HMTL hypothesis by measuring individual TPCs for both sprint speed and aerobic scope within the context of upslope-migrating common wall lizards *Podarcis muralis*. This widespread species was historically limited to elevations up to 2,000 m above sea level (ASL), but in recent years has been observed colonizing higher elevation habitat (routinely observed up to 2,300 m ASL; Pottier, 2020, F. Aubret, pers. obs.). Transplant experiments demonstrate that adult *P. muralis* lizards are oxygen limited at elevations above their current range limit, suffering from reduced aerobic scope and performance (Bodensteiner, Gangloff, et al., 2021; Gangloff et al., 2019). We hypothesize that TPCs for sprint speed and aerobic scope will exhibit broadly similar patterns when measured at low elevations, but that TPCs for aerobic scope will be affected by hypoxia at high elevation while sprint speed will remain unaffected. Specifically, as predicted by the HMTL hypothesis (Gangloff & Telemeco, 2018), we expect that both maximum aerobic scope and the optimal temperature for aerobic scope will be reduced at high elevation. These predictions arise because reduced oxygen availability causes the TPC for aerobic performance to become more symmetrical and flattened in shape, with maximum performance greatly reduced and the optimal temperature for peak performance reduced

(Box 1, Figure B1b, Figure 1). Based on our empirical observations and HMTL, we then develop a mathematical framework for both describing and predicting the dual effects of temperature and environmental oxygen availability on organismal performance, Temperature-Oxygen Performance Surfaces (TOPS). Finally, we leverage this approach to make predictions about the potential for *P. muralis* to colonize high-elevation habitats.

## 2 | MATERIALS AND METHODS

### 2.1 | Animal collection and experimental design

We measured 37 adult male common wall lizards *Podarcis muralis* collected from sites in the Vallée du Lez (Ariège, France, elevation range 425–475 m ASL) 14–29 May 2019 (Table S1). Animal collection, intake and husbandry protocols were as described in detail elsewhere (Gangloff et al., 2019; Kouyoumdjian et al., 2019). In brief, lizards were housed in groups of 3–6 lizards within plastic enclosures (26×38×23 cm) with wood mulch bedding and shelters that also served as basking platforms (15×5×3.5 cm). Ambient light was provided for 14 hr/day and heat lamps provided a temperature
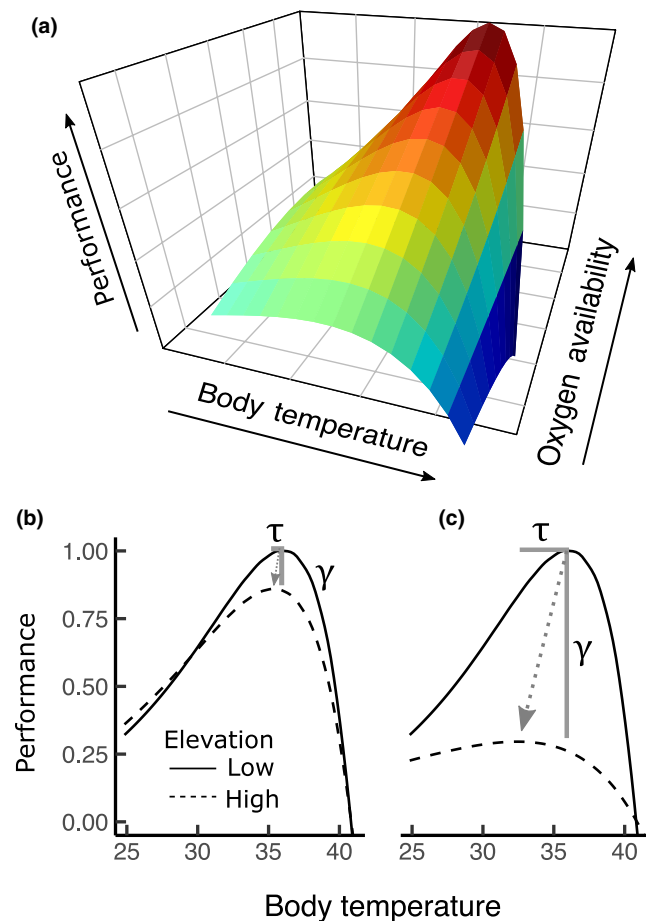
**FIGURE 1** Temperature–oxygen performance surfaces (TOPS). (a) Three-dimensional TOPS diagram illustrating the interaction between temperature and oxygen environment on organismal performance predicted by the Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis. Reduced oxygen availability is predicted to alter the peak of this surface (dark red region), causing declines in both maximum performance ($W_f$) and the temperature where maximum performance occurs ($T_{OPT}$). (b) and (c) depict predicted effects of temperature and elevation on performance for traits with low or high oxygen-sensitivity TOPS, respectively. The arrows depict the primary axes of change in oxygen-dependent performance when animals are moved from low to high elevation. These changes result from declines in both $T_{OPT}$ and $W_f$. The sensitivity of $T_{OPT}$ and $W_f$ to reductions in oxygen availability are described by the oxygen-sensitivity coefficients, $\tau$ and $\gamma$, respectively. See Equations 1–3 for further mathematical description of TOPS.

gradient of *c*. 25–40°C for 6 hr/day. Water was provided ad libitum via a small water bowl, cages were misted 3–4 times per week and lizards fed meal worms (*Tenebrio* spp. larvae) 3–4 times/week. Animals were randomly assigned to two treatment groups, with measures taking place at low elevation (Station d'Ecologie Théorique et Expérimentale du CNRS à Moulis: 42°57′26.8″N 1°05′08.3″E; 436 m ASL; $PO_2$ ~ 20.1 kPa) or high elevation (Pic du Midi de Bigorre: 42°56′11.0″N 0°08′32.9″E; 2,877 m ASL; $PO_2$: ~15.3 kPa). The low-elevation laboratory is at the elevation of capture, while the high-elevation laboratory is 600 m above the local elevational range limit

(Pottier, 2020). All animals were left to adjust to captive conditions for 4–6 days before measurement. The day prior to first measures, we removed lizards from their home cages, placed them in small sacks and either transported them to the high-elevation laboratory or transported them locally in identical conditions to simulate transport to high elevation. Lizards were then re-housed in clean boxes containing fresh substrate. We withheld food beginning 48 hr before the first measurement and throughout the experiment to ensure an empty gut (Van Damme et al., 1991).

To calculate TPCs for aerobic scope and sprint speed, we measured performance at low elevation and high elevation at seven temperatures (26, 29, 32, 34, 36, 38, 40°C; see Table S1 for sample sizes). Different individual animals were measured at each elevation to control for time in captivity and limit effects of prior experimental exposure. Lizards were randomly assigned to three measurement groups in the low-elevation treatment and four groups in the high-elevation treatment, with the order of temperature treatments randomized for each group. Experimental temperatures ranged from sub-optimal but active temperatures through those just below the critical thermal maximum (Bodensteiner, Gangloff, et al., 2021; Trochet et al., 2018). We measured aerobic scope in animals (see below) at up to two temperatures per day for four consecutive days. For each animal used to measure aerobic scope, we also placed a size-matched animal in our incubators to serve as a temperature model, allowing us to estimate the body temperature of lizards more precisely for all measurements. We measured sprint performance on both temperature-model and aerobic-scope lizards, which is why sample sizes are greater for sprint performance than aerobic scope. All measurements were made during daylight hours when lizards are naturally active. We measured mass to the nearest 0.01 g with a precision balance (Digital Mini Scale, AMIR) and snout–vent length (SVL) with digital callipers (CD-6, Mitutoyo, Japan) to the nearest 0.01 mm at the time of capture and on the first and last measurement days. Treatment groups did not differ in mass or SVL at the time of capture, nor did the subset of animals for which we measured aerobic scope differ in mass or SVL ($p \geq 0.17$ for all comparisons, Table S2).

## 2.2 | Metabolic rate measurements

To measure aerobic scope, we developed a methodology allowing measurement of both resting and active metabolic rates of animals in a single assay. Lizards were transferred from their home cages to custom-made 750-ml plastic metabolic chambers. These chambers were placed in the incubator and lizards were left undisturbed for a minimum of 30 min, during which time we monitored the body temperature of temperature-model lizards to confirm lizard body temperature equilibrated with the incubator (see temperature logging protocols below). We then measured gas exchange for 20 min to quantify RMR (Andrews & Pough, 1985; Kouyoumdjian et al., 2019). After 20 min, we briefly opened the metabolic chamber and

placed inside a remote-controlled robot 'insect' (70 mm L × 55 mm W × 30 mm H; 28.1 g, Fire Ant HEXBUG; Innovation First Labs, Inc.), then re-sealed the chamber and allowed temperature to equilibrate for a minimum of 10 min. We then measured gas exchange while operating the robotic insect by infrared remote control to perturb the lizard. The robotic insect moves via the rotation of six spindle-like wheels with processes that resemble insect legs. During perturbation, lizards typically attempted to escape, attack the robot or remained motionless while visibly breathing heavily. This procedure never induced visible injury, nor did it affect sprint performance immediately following trials. Moreover, the protocol elicits an aerobic metabolic response 2× greater than that of the maximum post-exercise metabolic response in similarly sized lizards (*Sceloporus occidentalis* measured at $T_{OPT}$; Leibold et al., 2022). To quantify MMR, we measured gas exchange while the lizard was perturbed for a total of 6 min. This was sufficient to observe metabolic rate rise and plateau in all lizards (see Figure S1).

We measured carbon dioxide ($\dot{V}_{CO2}$) production and oxygen ($\dot{V}_{O2}$) consumption corrected for barometric pressure via push-mode flow-through respirometry (Foxbox-C Field $O_2$ and $CO_2$ Analysis System or FMS-3 Field Metabolic System, Sable Systems, Inc.). Air was pumped at a rate of 450 ml/min through the metabolic chamber, dried of water vapour with Drierite, then measured for both $CO_2$ and $O_2$ content. We processed the data using ExpeData software (version 1.7.30, Sable Systems, Inc.). We extracted the average of the lowest values of gas exchange over a 10-min interval to estimate RMR, which allows the elimination of elevated metabolic rates due to activity during the measurement period (Kouyoumdjian et al., 2019; Vicenzi et al., 2021). For MMR, we extracted the highest average rate of gas exchange over a 15-s interval after the robotic insect was activated (Gangloff et al., 2019). For analyses, we used $\dot{V}_{CO2}$ rather than $\dot{V}_{O2}$ measurements because $\dot{V}_{CO2}$ traces were more stable giving us greater confidence in estimates derived from these traces. We calculated absolute aerobic scope (hereafter, aerobic scope) as MMR-RMR for each trial (Figure S1).

## 2.3 | Sprint speed measurements

Following metabolic rate measurements, we ran both aerobic-scope and temperature-model lizards on an open-ended, 1-m, level racetrack of artificial turf. Infrared sensors placed at 25-cm intervals recorded data to the nearest 0.001 s via computer interface (Trackmate Racing). Lizards were removed from incubators and run within 10 s. We ran each lizard three times at each test temperature, after allowing several minutes of rest in the incubator between trials for body temperature to re-equilibrate. For analyses, we extracted the fastest 50-cm sprint interval for a lizard at a given temperature (Gangloff et al., 2019). Sensor malfunction resulted in the loss of data from some sprint trials, resulting in $N = 206$ observations of $N = 37$ individuals for analysis (minimum of 3 and median of 6 temperatures per individual; see full details in Table S3).

## 2.4 | Temperature logging

We measured body temperature of temperature-model lizards that were size-matched to the lizards for which we measured aerobic scope. Each temperature model was treated identically to its paired metabolic rate animal: they were removed from their home cage at the same time and placed in identical containers with air flow on the same shelf in the incubator. Using elastic bandage material and tape, we affixed a thermistor probe (PT907; Pace Scientific) to the venter of the lizard on the abdomen, and we recorded body temperature every 10 s with a data logger (XR5-SE-20mV; Pace Scientific, USA). We associated these time-stamped data with the time of metabolic rate measurements to provide precise body temperatures for analysis rather than relying on the incubator's set temperature. This approach allowed us to closely monitor the temperature that lizards experienced during trials, thereby accounting for variation in incubator temperature across treatments and experimental days. For each trial, we used the average body temperature of the model lizard during the period when we measured metabolic rate on test lizards as the temperature value for TPC analyses.

We kept animals for several days after experimental measures to feed and monitor health. All animals were then released at their point of capture. Field sampling and experimental protocols were conducted under permit provided by the Direction régionale de l'environement, de l'aménagement et du logement (DREAL) Midi-Pyrénées (Arrêté Préfectoral no. 2017-s-02 30 March 2017), under current ethical committee approval (APAFIS#16359-201808011445465 v4), and in accordance with Directive 2010/63/EU on protection of animals used for scientific purposes.

## 2.5 | Statistical methods

### 2.5.1 | Thermal performance curves

All analyses were conducted in the Programming Language R (R Core Team, 2022) and data plots created using the GGPLOT2 and PLOT3D packages (Soetaert, 2019; Wickham et al., 2019). To characterize TPCs for aerobic scope and sprint speed, we used the 'beta function', which was originally developed to describe the effects of temperature on plant growth (Yan & Hunt, 1999) and provides the best fit to most observations in ectothermic animals, especially lizards (Tomlinson, 2019). The beta function also benefits from having parameters with straightforward biological interpretation:

$$Performance = W_f \times \left( \frac{CT_{MAX} - T_b}{CT_{MAX} - T_{OPT}} \right) \times \left( \frac{T_b}{T_{OPT}} \right)^{\frac{T_{OPT}}{CT_{MAX} - T_{OPT}}} \quad (1)$$

Where $T_b$ is the body temperature, $W_f$ is the peak performance, $T_{OPT}$ is the temperature at $W_f$, and $CT_{MAX}$ is the high temperature where performance equals zero (all temperatures in Kelvin; Yan & Hunt, 1999; Tomlinson, 2019). We fit performance curves using the *nls* function in R for each individual, using the body temperature of the temperature

model as the value for $T_b$ (see above) and using an empirical measure of $CT_{MAX}$ of 41.3°C (Bodensteiner, Gangloff, et al., 2021). This empirical $CT_{MAX}$ was estimated using the same source population as the present study and was measured at the same low- and high-elevation sites. As expected by HMTL and observations from other lizards, elevation did not affect $CT_{MAX}$ in *P. muralis*. We assessed the sensitivity of our conclusions to the assumption of a fixed $CT_{MAX}$ by (a) repeating all analyses with individual $CT_{MAX}$ extrapolated from our performance data using *nls* and (b) simulating 1,000 TPCs for each individual and trait while allowing $CT_{MAX}$ to be drawn from a normal distribution matching that empirically documented by Bodensteiner, Gangloff, et al. (2021). These sensitivity analyses are detailed in Supporting Information and demonstrate that all conclusions were robust to the assumption of a fixed $CT_{MAX}$ value.

Once TPCs were fit to each individual and trait, we extracted estimates of $W_f$ and $T_{OPT}$ from the fit models. We also assessed model fit by calculating the root mean square error (RMSE) for each performance curve (aerobic scope mean $\pm$ SD RMSE = 0.035 $\pm$ 0.014 ml $CO_2$/min; sprint speed mean $\pm$ SD RMSE = 0.324 $\pm$ 0.15 m/s). RMSE did not differ between lizards measured at low or high elevation for either performance trait, although we detected a trend for lower RMSE (i.e. better model fits) for aerobic scope measured at high elevation than at low elevation (aerobic scope RMSE: $t_{18.05} = 2.05$, $p = 0.06$; sprint speed RMSE: $t_{34.0} = -0.61$, $p = 0.55$). We then compared estimates of $W_f$ and $T_{OPT}$ between treatment groups using one-tailed *t*-tests for aerobic scope (based on the prediction that aerobic scope is reduced under conditions of reduced oxygen availability sensu HMTL, Box 1; Gangloff & Telemeco, 2018) and two-tailed *t*-tests for sprint speed. We also compared the optimal temperature for performance between traits at low and high elevation using two-tailed *t*-tests. We found no effect of temperature exposure order on $W_f$ or $T_{OPT}$ for aerobic scope or sprint speed within each treatment group (assessed with MANOVA, all $p > 0.10$; See Table S4). Because we found no evidence that exposure to the robot-perturbation treatment affected sprint performance (no difference in sprint speed between lizards on which we measured metabolic rate and those serving as temperature models; $F_{1,32.6} = 2.32$; $p = 0.14$), we analysed sprint speed data of all animals together. Finally, to understand how RMR and MMR contributed to observed differences in aerobic scope, we used linear mixed models implemented in R via the LME4 package (Bates et al., 2015). These models included either RMR or MMR as the dependent variable, elevation treatment, mass and their interaction as independent variables, and lizard ID as a random intercept to account for repeated measurements.

## 2.5.2 | Temperature–oxygen performance surfaces

To model HMTL and estimate parameters that explicitly quantify the interactive effect of temperature and oxygen environment on performance, we created TOPS for both aerobic scope and sprint speed. We estimated each TOPS using data pooled across all animals because we did not measure the same individuals at high and low

elevation. The estimated performance surfaces therefore represent the average effects of body temperature and oxygen availability on performance for the entire population, unlike our TPCs which were estimated for each individual. We modelled TOPS by replacing $T_{OPT}$ and $W_f$ in the beta function (Equation 1) with the following equations:

$$T_{mOPT} = T_{OPT} - \tau(1 - O_f)T_{OPT} \quad (2)$$

$$W_{mf} = W_f - \gamma(1 - O_f)W_f \quad (3)$$

$T_{mOPT}$ and $W_{mf}$ are the optimal temperature and peak performance after accounting for aerobic capacity, respectively, whereas $T_{OPT}$ and $W_f$ are optimal temperature and peak performance measured under reference conditions (here, oxygen availability at sea level). $O_f$ is the fractional oxygen capacity, or the ratio of oxygen capacity at the condition of interest to the reference condition. We used $O_f = 0.95$ for low-elevation measurements and $O_f = 0.72$ for high-elevation measurements because oxygen partial pressures were 95% and 72% of sea level at the respective sites. The parameters $\gamma$ and $\tau$ are oxygen-sensitivity coefficients describing the effects of $O_f$ on $W_{mf}$ and $T_{mOPT}$. The HMTL hypothesis predicts linear effects of $O_f$ on $W_{mf}$ and $T_{mOPT}$ under normoxic and hypoxic conditions, and thus $\gamma$ and $\tau$ are simple coefficients that can be compared directly across traits, individuals or populations (see Figures 1 and B1). As with TPCs, we used a fixed $CT_{MAX}$ of 41.3°C (Bodensteiner, Gangloff, et al., 2021) in the TOPS model because theory and data agree that $CT_{MAX}$ is not affected by ecologically relevant $O_f$ (Gangloff & Telemeco, 2018; Bodensteiner, Gangloff, et al., 2021; Leibold et al., 2022). We fit TOPS models for aerobic scope and sprint speed using the *nls* function for measurements pooled across all animals, and we assessed model fit via RMSE (aerobic scope RMSE = 0.055 ml $CO_2$/min, sprint speed RMSE = 0.542 m/s). As with TPC estimates, body temperatures came from lizard models paired with each respiration measurement.

## 2.5.3 | Within- and among-individual correlations

We further assessed the within- and among-individual relationship of sprint speed and aerobic scope across temperatures using bivariate mixed linear models, creating separate models for the two treatment groups. We implemented models in a Bayesian framework using the MCMCGLMM package (Hadfield, 2010) to estimate within- and among-individual trait variances, covariances and correlations (Dingemanse & Dochtermann, 2012). We included the fixed effect of temperature (the target temperature for each measurement) to account for the co-dependence of both dependent variables on temperature. We specified uninformative parameter-expanded priors, used a Gaussian distribution and ran models for $5 \times 10^6$ iterations with a burn-in phase of $5 \times 10^3$ and a thinning interval of 500. These specifications led to models with low autocorrelation, MCMC chains mixing well and an adequate effective sample size ($N = 9,990$). We report results as point estimates (mode of the posterior mean) with 95% credible intervals.

# 3 | RESULTS

## 3.1 | Thermal performance curves

We modelled TPCs for aerobic scope and sprint speed for individuals at low and high elevation (Figure 2; Figure S2). Lizards transplanted to high elevation reduced maximal aerobic scope by 50.1% ($W_f : t_{18.9} = 4.57$, $p = 0.0001$) and reduced the optimal temperature for aerobic performance from 35.8°C at low elevation to 34.1°C at high elevation (1.7°C difference in $T_{OPT} : t_{11.5} = 1.83$, $p = 0.047$; Figure 2a,b). Differences in aerobic scope were driven by hypoxia-induced reductions in MMR with a trend for a greater effect of hypoxia on the MMR of larger animals (Interaction: $F_{1,16.9} = 3.23$, $p = 0.09$; Elevation: $F_{1,16.9} = 4.04$, $p = 0.061$; Mass: $F_{1,16.9} = 5.48$, $p = 0.032$). By contrast RMR was unaffected by elevation treatment, mass variation or their interaction ($p > 0.43$ for all). The average maximum sprint speed was 1.79 m/s and this occurred at an average temperature of 33.7°C with no differences between treatment groups ($W_f : t_{31.4} = 0.43$, $p = 0.67$; $T_{OPT} : t_{19.8} = 0.35$, $p = 0.73$; Figure 3c,d). Optimal temperature for aerobic scope was 1.96°C higher than optimal temperature for sprint performance at low elevation ($t_{29.7} = -3.42$, $p = 0.0018$), but $T_{OPT}$ for performance measures did not differ at high elevation ($t_{21.2} = -0.496$, $p = 0.63$).

## 3.2 | Temperature–oxygen performance surfaces

TOPS for sprint speed and aerobic scope are depicted in Figure 4 while the oxygen sensitivity coefficients ($\tau$ and $\gamma$) estimated from these TOPS are shown in Figure 3 and Table S5. The oxygen sensitivity of both $T_{OPT}$ ($\tau$) and $W_f$ ($\gamma$) were greater for aerobic scope than for sprint speed (Figures 3 and 4).

## 3.3 | Within- and among-individual correlations

We found no evidence of within- or among-individual correlations between sprint speed and aerobic scope (Table S6).

# 4 | DISCUSSION

A mechanistic framework for describing the temperature dependence of performance- and fitness-related traits is essential to predict the impact of novel or changing environments (Levy et al., 2015; Taylor et al., 2021) yet has remained challenging due to the complex interactions of factors that can drive variation in these response curves (Bodensteiner, Agudelo-Cantero, et al., 2021; Kingsolver & Buckley, 2020; Rezende et al., 2014; Woods et al., 2018). With this study, we present TPCs for aerobically and anaerobically powered performance traits in ectothermic lizards, under conditions of normoxia and high-elevation hypoxia. Results closely match predictions based on the HMTL hypothesis with TPC shape for aerobic
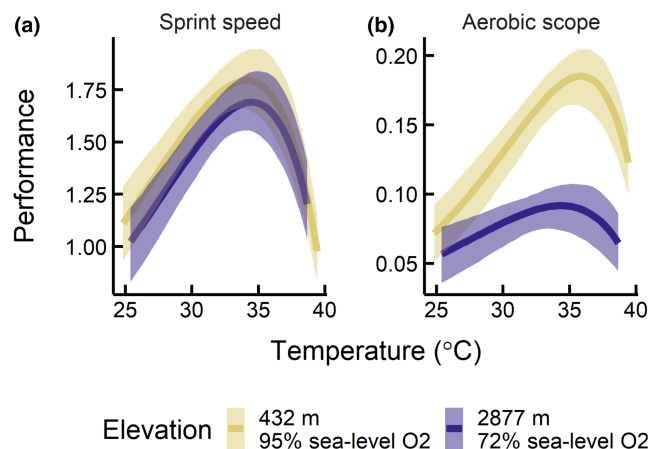


**FIGURE 2** Thermal performance curves (TPCs) for (a) sprint speed (m/s) and (b) aerobic scope (maximum metabolic rate-resting metabolic rate [MMR-RMR] in ml$CO_2$/min) for adult male *Podarcis muralis* lizards measured at low- and high-elevation match predictions of the Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis. Curves are beta-function TPCs fit to data from each location via nonlinear least-squares regression with shaded regions representing 95% CI estimated via bootstrapping (see text for statistical details). Notice similarity to idealized, hypothetical curves in Figure 1b,c.

performance predictably changing with elevation (left-shifted, reduced and more symmetrical). This level of hypoxia halved aerobic scope across a broad temperature range and reduced the optimal temperature for aerobic scope by 1.7°C. By contrast, TPC shape for anaerobically fuelled sprint performance was unaffected (Box 1; Gangloff & Telemeco, 2018). Given the direct relationship between aerobic scope and an organism's ability to fuel long-term functions underlying fitness (e.g. digestion, growth, reproduction; Clark et al., 2013; Fobian et al., 2014; Pörtner, 2001; Seibel & Deutsch, 2020), we emphasize the importance of understanding how aerobic scope is affected by both temperature and its interaction with other relevant environmental gradients, such as oxygen availability.

Conclusions from studies predicting the long-term implications of novel environmental temperatures vary greatly depending on which trait is selected as a proxy for organismal fitness (Beissinger & Riddell, 2021; Green et al., 2022; Huey, 1982; Sinclair et al., 2016). Our results clearly demonstrate that aerobic scope is greatly reduced under conditions of moderate hypoxia. Consistent with the prediction that aerobic scope is the mechanism that underlies long-term physiological processes, *P. muralis* artificially housed at this elevation (~2,500 m above their origin and 600 m above the species' elevational range limit) for several weeks declined in body condition despite ad libitum food availability (Gangloff et al., 2019). While sprint speed is ecologically relevant to fitness in a variety of contexts (Husak et al., 2006; Miles, 2004) and is likely important for lizards in this specific ecological context, basing predictions of population establishment on TPCs for sprint performance alone would be misleading in indicating no decline in performance at high elevation. Because most physiological and behavioural processes directly
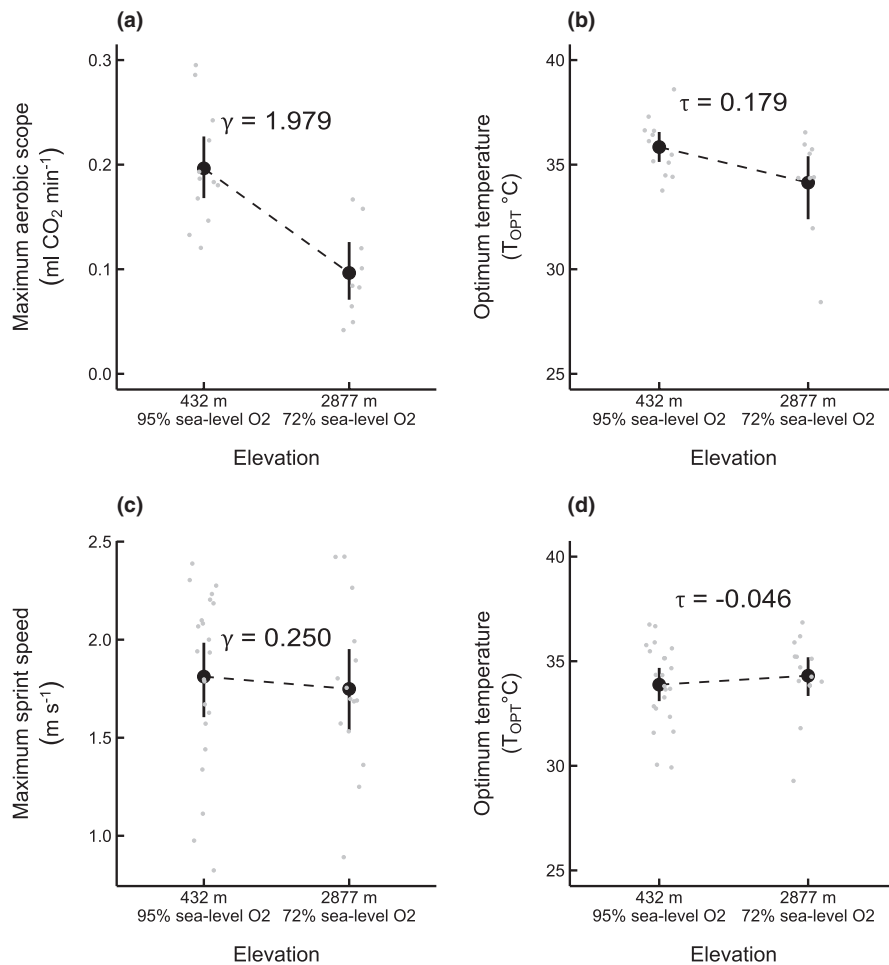
**FIGURE 3** Peak performance ($W_f$) for aerobic scope (a) and optimum temperature ($T_{OPT}$) for aerobic scope (b) are reduced at high elevation, whereas peak performance ($W_f$) for sprint speed (c) and optimum temperature ($T_{OPT}$) for sprint speed (d) are unaffected by translocation to high elevation in adult male *Podarcis muralis* lizards. Large points are means ±95% CI and grey points are estimates for individuals jittered on the *x*-axis to avoid overlap. $\gamma$ is the oxygen-sensitivity coefficient describing the effect of the oxygen environment on $W_f$ and $\tau$ is the oxygen-sensitivity coefficient describing the effect of the oxygen environment on $T_{OPT}$. Values for $\tau$ and $\gamma$ were derived from temperature-oxygen performance surface (TOPS) models fit to pooled data for each performance trait.

linked to fitness in vertebrate animals are aerobically powered (e.g. most movement, food digestion, growth, reproduction), aerobic scope may offer a more appropriate fitness proxy when assessing the long-term consequences of environmental changes for organisms. This is especially important to consider across gradients of environmental parameters that may not impact short-term performance measures, as we demonstrate here for oxygen partial pressure across an elevation gradient.

In response to ongoing climate warming, the geographical ranges of many organisms are shifting to track environmental conditions, specifically expanding both upslope and poleward (Freeman et al., 2018; Pauchard et al., 2016; Pinsky et al., 2013). This movement will have profound effects on ecosystems by altering community composition, promoting new interspecific interactions, increasing exposure to novel pathogens and exposing organisms to new abiotic constraints (HilleRisLambers et al., 2013; Schweiger et al., 2012). However, not all organisms are moving at the same rate. Variation in climate velocity, the speed and direction with which isotherms shift across space in response to climate change, can explain some of this variation, especially in marine systems (Burrows et al., 2011; Pinsky et al., 2013; Williams & Blois, 2018). However, even after accounting for variation in climate velocity and dispersal ability, substantial variation exists in observed range shifts suggesting additional important biotic or abiotic factors must be involved

(Beissinger & Riddell, 2021; HilleRisLambers et al., 2013; Williams & Blois, 2018). For example, within a five-species lizard community occurring in a montane region of southern California, USA, three species have substantially increased their elevational range in response to recent climate change, whereas two species have not (Barrows & Fisher, 2014). Each of these lizard species has similar dispersal ability and are experiencing similar climate–velocity pressure. Such range shift variation, especially in montane regions, could be driven by species-specific variation in oxygen sensitivity, potentially representing an adaptive response in species or populations which experience variation in oxygen availability across their geographical range. Similarly, our results suggest that rapidly transplanted *P. muralis* would face physiological challenges in establishing at our high-elevation site because of their limited oxygen capacity, but other lizards such as the Pyrenean rock lizard *Iberolacerta bonnali* thrive at this elevation. Furthermore, in some cases, phenotypic plasticity in response to extended exposure to low-oxygen environments (i.e. acclimation) may provide a mechanism for organisms to successfully respond to such novel limitations. In this case, however, *P. muralis* suffers from reduced performance and physiological capacity, with physiological acclimation unable to fully compensate for performance decrements after weeks of exposure to low-oxygen environments (Bodensteiner, Gangloff, et al., 2021; Gangloff et al., 2019). Additional research comparing the sensitivity of performance to

**FIGURE 4** Temperature–oxygen performance surfaces (TOPS) estimated using aerobic scope (a–c) and sprint speed (d–f) in *Podarcis muralis*. The *y*-axes are absolute aerobic scope or sprint speed scaled to a maximum value of 1. The *x*- and *z*-axes are fractional oxygen availability ($O_f$) and body temperature ($T_b$). Each column of panels (i.e. a–c and d–f) represent the same TOPS viewed from three angles, whereas the rows of panels (i.e. a and d, b and e, c and f) represent TOPS for different traits viewed from the same angles for direct comparison. Warmer colours indicate higher values for the performance trait and are included to aid visualization.
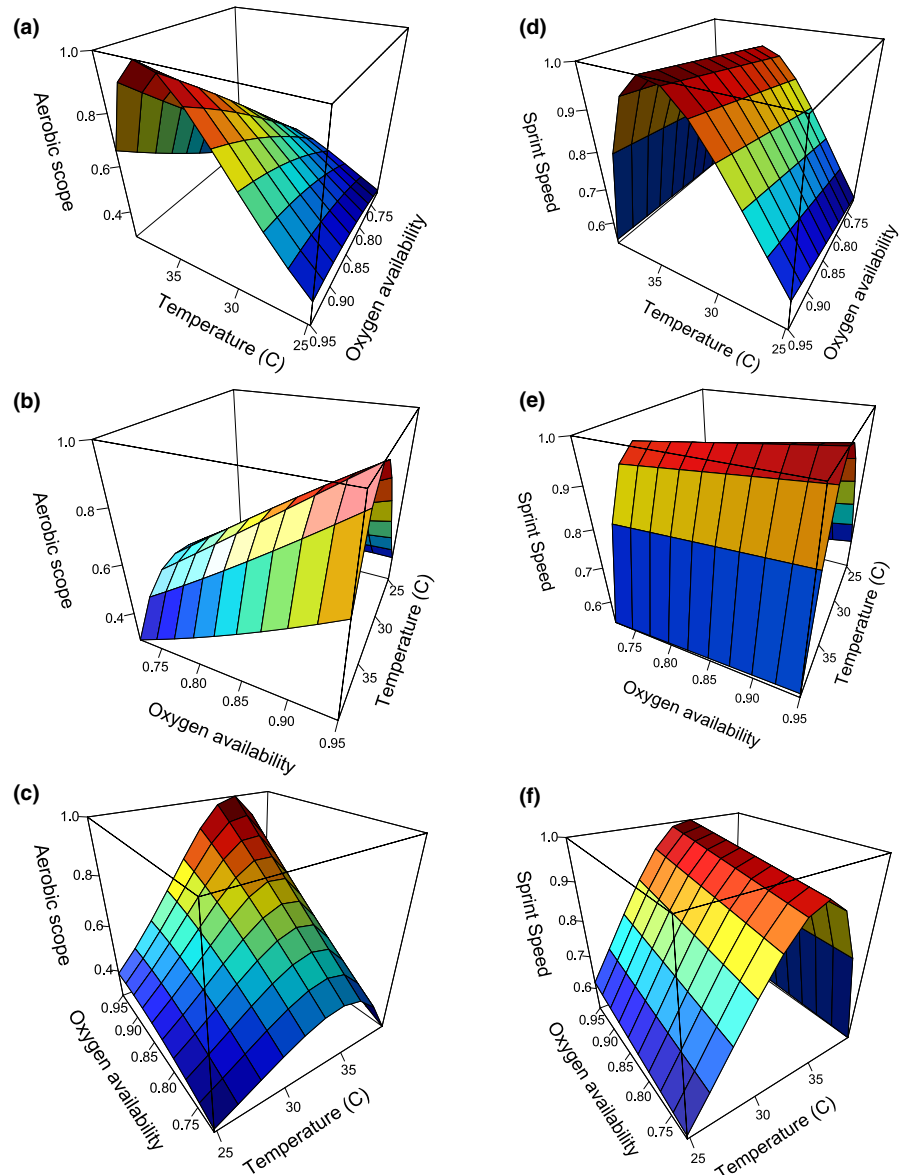
oxygen variation of different species or populations, particularly those separated by elevation, are needed to assess the general importance of this physiological mechanism in setting biogeographical limits, as well as the potential for plasticity or local adaptation to shape these responses (Catullo et al., 2019).

To predict and model how TPCs are affected by variation in oxygen availability following HMTL, we provide an empirical mathematical framework, TOPS, which describe the oxygen sensitivity of maximum performance and optimal temperature for performance, and thus the shape of the TPC. This framework can be extended to quantify the sensitivity of TPC shape to additional axes of variation (e.g. water availability, nutritional status, toxins) and additional traits (e.g. growth rates, fecundity) in a variety of useful applications, although HMTL only provides predictions for the functional response of TPCs to variation in the oxygen environment. TOPS models provide simple parameters that quantify the sensitivity of TPC shape, which can be directly compared across traits, species and contexts. In the TOPS framework presented here, $\gamma$ and $\tau$ are oxygen-sensitivity

coefficients describing the effects of oxygen availability on two essential parameters estimated by TPCs: maximum performance and optimal temperature for performance, respectively. The sensitivity to oxygen availability has been characterized extensively for water-breathing organisms (Ern et al., 2016; Pörtner et al., 2017; Seibel et al., 2021), but the importance of this abiotic gradient is often overlooked in the context of terrestrial organisms. Recent work proposes a protocol for calculating the oxygen dependence of metabolic rates in aquatic organisms (the 'alpha-method', Seibel et al., 2021), building upon previous work in fishes (but see Farrell et al., 2021). While the physiological importance of such sensitivity holds across aquatic and terrestrial taxa, our approach builds directly upon traditional methods of TPC estimation and thus offers a straightforward framework to construct predictive mathematical models. While there are clear parallels in models developed for aquatic taxa and the TOPS model presented here, the approach in aquatic organisms is generally directed towards identifying the oxygen partial pressure in water at which an organism cannot sustain its metabolic rate.

Identifying this critical limit of oxygen availability is useful when animals are likely to encounter such critical oxygen partial pressures in their experienced environment. However, for terrestrial ectotherms, critical limits will be less important than the interactive effects of temperature and oxygen on performance within suitable ranges for both, whose effects can be evident in a variety of ecological contexts, including diving behaviours, reproduction or in subterranean nests (reviewed in Gangloff & Telemeco, 2018; Hall & Warner, 2021; Rodgers & Franklin, 2021; Virens & Cree, 2019). Thus, our TOPS approach focuses on predicting performance variation across accessible environmental variation and quantifying the sensitivity of TPC shape to variation in oxygen capacity. Much recent work in ectotherm thermal ecology and distribution modelling is directed towards characterizing the temperature dependence of performance across a relevant range of temperatures, not just the endpoints of TPCs (reviewed in Telemeco & Gangloff, 2021). The TOPS framework extends this approach to other limiting abiotic factors in addition to temperature.

## 5 | CONCLUSIONS

TPCs continue to be a useful tool in characterizing the thermal dependence of traits across scales of biological organization. Mechanistic approaches towards describing how TPCs for whole-organism performance change under novel conditions are essential if these tools are to be useful in a predictive context (Gangloff & Telemeco, 2018; Rezende et al., 2014). By creating TPCs for two ecologically relevant and commonly measured traits, sprint performance and aerobic scope, at native and high elevation, we quantified the sensitivity of these traits to oxygen availability and potential suitability as fitness proxies. As predicted, anaerobically fuelled sprint performance was insensitive to relative hypoxia, demonstrating that this trait may have limited application when predicting long-term consequences in novel environments, especially in the context of upslope migration. Aerobic scope was dramatically impacted by relative hypoxia, with both maximum capacity and optimum temperature decreasing as predicted by the HMTL hypothesis. Future work will be directed towards characterizing oxygen sensitivity across a broader range of oxygen levels, including estimating sensitivity parameters within individuals, across traits and across taxa. When estimated for multiple individuals, TOPS parameters can be directly compared statistically, similar to any other continuous trait. These parameters can also be compared across time, to assess the potential for acclimation to buffer organisms against novel abiotic limitations. Importantly, our model describes the effects of oxygen restriction on whole-organism performance, but its predictions for organisms that have developed or evolved in low-oxygen conditions (e.g. at high elevation) when exposed to relative hyperoxia may not hold (e.g. Gangloff et al., 2021). Using TOPS to account for interactive effects of oxygen and temperature on aerobic traits could greatly increase our power to predict whole-organism performance in ectotherms across diverse environments.

## AUTHORS CONTRIBUTIONS

Rory S. Telemeco and Eric J. Gangloff conceived the ideas and designed the methodology; All authors collected the data; Rory S. Telemeco and Eric J. Gangloff analysed the data; Rory S. Telemeco and Eric J. Gangloff led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.73n5tb30p (Telemeco et al., 2022).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.