Lizards at the Peak: Physiological Plasticity Does Not Maintain Performance in Lizards Transplanted to High Altitude

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ABSTRACT

Warming climates are facilitating the range expansion of many taxa to habitats that were formerly thermally inhospitable, including to higher latitudes and elevations. The potential for such colonization, however, varies widely among taxa. Because environmental factors may interact to affect colonization potential, an understanding of underlying physiological and behavioral mechanisms is necessary to predict how species will respond to potentially suitable habitats. For example, temperature and oxygen availability will interact to shape physiological and performance traits. Our model species, the wall lizard, *Podarcis muralis*, is a widely distributed ectotherm that continues to expand its range in Europe despite being limited by cold temperatures at high elevations and latitudes. To test the potential for organisms to expand to warming high-altitude environments, we conducted a transplant experiment to quantify the within-individual effects of high-altitude hypoxia on physiological and performance traits. Transplanted lizards maintained individual differences in physiological traits related to oxygen capacity and metabolism (hemoglobin concentration, hematocrit, and peak postexhaustion metabolic rate), as well as performance traits tied to fitness (sprint speed and running endurance). Although lizards altered blood biochemistry to increase oxygen-carrying capacity, their performance was reduced at high altitude. Furthermore, lizards at high altitude suffered a rapid loss of body condition over the 6-wk experiment, suggesting an energetic cost to hypoxia. Taken together, this demonstrates a limited potential for within-individual plasticity to facilitate colonization of novel high-altitude environments.

Keywords: climate change, colonization, high-altitude hypoxia, metabolic rate, performance, physiological plasticity, *Podarcis muralis*, range expansion.

Introduction

Formerly inhospitable habitats at high elevations have warmed to become thermally suitable for some low-altitude species (Parmesan 2006; Sinervo et al. 2010; Pauchard et al. 2015). This has resulted in range expansion to higher elevations in some cases, but species vary widely in their response to new potentially suitable habitat (Walther et al. 2002; Chen et al. 2011). In mountainous regions, such upward migration is dependent not only on warming thermal environments but also on the ability of colonizing individuals to cope with lower atmospheric pressure at altitude and thus reduced oxygen availability (hereafter, high-altitude hypoxia). In terrestrial ectothermic vertebrates, thermal performance curves and, potentially, thermal limits are closely tied to the capacity for an individual to obtain oxygen from the environment and deliver it to tissues in sufficient quantities (reviewed in Jackson 2007; Gangloff and Telemeco 2018). Given the interdependence of physiological systems on temperature regimes and oxygen availability, an understanding of the physiological mechanisms by which ectotherms respond to high-altitude hypoxia is necessary to characterize the ability of individuals, and by extension populations, to move up in elevation (Storz et al. 2010).

Acute exposure to hypoxia will limit aerobically dependent performance and recovery, while physiological acclimation can serve to alleviate potentially negative repercussions. However, short-term responses to hypoxia may compensate for only some consequences of hypoxia, may only partially compensate, or may bear energetic or trade-off costs (Storz et al. 2010). Thus, measuring traits related to aerobic capacity and oxygen transport, as well as performance traits, is necessary to examine the short-term response, consequences, and costs of oxygen limitation. Compensatory mechanisms generally include increased oxygen diffusion and transport capacity, reduced metabolic demand for oxygen, and a shift toward greater dependence on anaerobiosis (reviewed in Hochachka et al. 1996; Gangloff and Telemeco 2018). Thus, we expect that individuals demonstrating greater physiological plasticity will also be able to maintain performance traits in hypoxic conditions. In general, within-individual plas-

ticity is expected to be a primary mechanism by which many taxa respond to novel environments (Hoffmann and Sgrò 2011; Urban et al. 2014; Llewelyn et al. 2018). Therefore, an integrative whole-organism approach is required to describe the complex relationships among physiological parameters, and their plasticity, with outcomes and fitness proxies (Forsman 2015).

The goal of this study is to assess the physiological response of adult lizards exposed to acute hypoxia beyond what is experienced in natural populations. We test the hypothesis that altitude-induced hypoxia will promote plastic physiological responses to maintain oxygen delivery and compensate for lower O_2 partial pressure (P_{O_2}). Specifically, we predict that lizards transplanted to high elevations will experience performance limitations in endurance capacity and reduced peak postexhaustion metabolic rates compared to lizards kept in normoxic conditions. Over several weeks at altitude, we expect that blood chemistry will shift to favor greater oxygen-carrying capacity, including increases in hematocrit and hemoglobin concentration. This physiological compensation will then result in at least a partial recovery of performance traits relative to lizards maintained at low altitude. Because burst speed in lizards is generally dependent on anaerobic respiration (Bennett and Licht 1972; Gleeson 1982, 1991), we do not predict differences in absolute sprint speed between lizards at low and high altitudes, but we do predict that hypoxia will result in a performance decrement over short-term repeated trials, as well as a reduction in running endurance. Sprint speed and running endurance are well-established performance traits in small lizard species, with direct links to survival, reproduction, dispersal, and, ultimately, fitness (Huey and Bennett 1987; Irschick and Garland 2001; Miles 2004; Hoskins et al. 2017). Finally, we predict that potential energetic trade-offs involved with physiological remodeling will result in reduced body condition over time in hypoxia. Taken together, we test the within-individual plastic response to high-altitude hypoxia in a widespread vertebrate currently expanding its range into warming alpine environments.

Methods

Source Populations and Husbandry

The common wall lizard, *Podarcis muralis*, is cosmopolitan and often conspicuous in a variety of habitats across its broad geographic range (Speybroeck et al. 2016). Importantly, its geographic distribution is restricted by thermal environment, since recruitment is reduced at lower temperatures because of an inability of embryos to complete development (Strijbosch et al. 1980; Van Damme et al. 1992; While et al. 2015). However, as a result of the acceleration of climate warming, wall lizards have recently been observed colonizing higher parts of the mountainous environment beyond their previous range, extending up to ~2,600 m asl (in southern France, notably; Pottier 2012). We sampled lizards from six populations in the area around Moulis, France (421–522 m asl; for sampling details and habitat description, see table A1), between August 13 and August 30, 2017. Adult male lizards (all snout-vent length [SVL] > 50 mm; $N = 82$) were collected with looped thread attached to a

telescopic fishing pole (McDiarmid 2012). Four lizards that were initially included dropped their tails during the experiment and are therefore excluded from analyses. On the day of capture, we measured SVL to the nearest 0.01 mm using digital calipers (mean ± SD: 62.88 ± 4.13 mm) and weighed lizards to the nearest 0.01 g using a digital scale (mean ± SD: 6.63 ± 1.18 g). We maintained groups of three to six lizards from a single population in plastic enclosures (26 cm × 38 cm × 23 cm) containing wood mulch bedding and two plastic shelters (15 cm × 5 cm × 3.5 cm) that also served as basking platforms. Ambient room temperature fluctuated between approximately 15°C (night) and 20°C (day), ambient light was provided with fluorescent bulbs for 14 h/d, and heat lamps provided a temperature gradient of ca. 25°–40°C for 6 h/d in 1-h intervals. We provided water ad lib. via a small water bowl, and cages were misted three or four times per week. Lizards were fed mealworms (*Tenebrio* spp. larvae) ad lib., with fresh worms added three or four times per week, with the exception that food was withheld for 48 h before performance measures to ensure a postabsorptive state (Van Damme et al. 1991; Angilletta 2001).

After an acclimation period (mean: 9.1 d; range: 7–12 d), lizards were either transferred to identical housing at Observatoire Midi-Pyrénées in Pic du Midi de Bigorre (42°56′11.0″N, 0°08′32.9″E; 2,877 m asl; P_{O_2}: ~15.3 kPa) or moved into a new enclosure at the Station d'Ecologie Théorique et Expérimentale du Centre National de la Recherche Scientifique à Moulis (42°57′26.8″N, 1°05′08.3″E; 436 m asl; P_{O_2}: ~20.1 kPa). The difference in atmospheric pressure between locations results in approximately 25% reduced oxygen availability at the Pic du Midi lab (Bouverot 1985; Cordero et al. 2017). After an overnight acclimation period, we began the experiment with performance trials the next day and blood collection the day after. We maintained animals under uniform conditions, conducting performance trials and collecting blood samples on consecutive days at two additional time points, after 3 wk and 6 wk. Lizards were weighed and measured as above after each performance trial (see table A3). We calculated lizard body condition as the residual of the linear regression of $\log_{10}$ mass on $\log_{10}$ SVL, a common metric of relative energy stores used in numerous taxa, including lizards. Although this provides a measure of mass corrected for size, it does not describe the component driving variation in relative mass (e.g., muscle, fat stores, water; Warner et al. 2016). Because of logistical constraints and occasional mistrials, sample size varied slightly among measures at each time point, with measures of metabolic rate conducted on a subset of lizards (for sample size details, see tables 2, A2).

Performance and Postexhaustion Metabolic Rate

At three time points (1 d, 3 wk, and 6 wk), we employed a novel assay to efficiently quantify multiple aspects of lizard locomotor performance (sprint speed, speed decrement, and running endurance), as well as peak postexhaustion metabolic rate. This approach allowed us to streamline repeated measures of these traits while minimizing handling stress on the animals. Trials were conducted on a 1-m level racetrack of artificial grass with lines drawn at intervals of 25 cm. Racetracks were constructed to identical

specifications with the same materials at both laboratories. All performance trials were conducted during daylight active hours (between 0800 and 1930 hours). Before each trial, we placed lizards in a shelter identical to that in their enclosures, modified with secure flaps. This was placed in an incubator set to 31°C, the average body temperature of active lizards during the summer (F. Aubret, unpublished data; Osojnik et al. 2013), for 30 min to allow body temperature to equilibrate (Zajitschek et al. 2012). This provided an ecologically relevant temperature at which we could test performance traits, while also being below the maximum at which temperature effects would be compounded with reduced oxygen availability in hypoxia (Gangloff and Telemeco 2018). Lizards were placed at one end of the racetrack and raced 12 times with exactly 30 s rest between each run. This allowed us to measure both maximum sprint performance and performance decrement over repeated trials. If lizards were reluctant to sprint, we gently chased them with a soft paintbrush. To maintain consistency, the same researcher (E.J.G.) acted as lizard motivator for all trials. Room temperature was maintained at 25°C with electric space heaters (ambient temperature mean $\pm$ SD: 25.70° $\pm$ 0.90°C). Furthermore, we provided lighting at either end of the racetrack with portable LED and halogen light stands, positioned identically at the same height for each track. Immediately after the final trial, we recorded lizard body temperature using a cloacal thermometer (model T-6000, Miller and Weber, Ridgewood, NY; body temperature mean $\pm$ SD: 29.69°$\pm$0.69°C). We video recorded the trials and used Solomon Coder software (Péter 2017) and the programming language R (R Core Team 2017) to extract the fastest speed attained over a 50-cm interval as the top velocity for each trial, a distance within the range of *P. muralis* movements in the field (Braña 2003) and that has been used in similar-sized lizards (Huey and Bennett 1987; Van Damme et al. 1989). In >90% of trials, lizards achieved their top speed before reaching the end of the track, indicating that the track length was sufficient for lizards to achieve top performance. Corruption of data files and inconsistencies in protocols precluded the inclusion of some sprint trials, resulting in a total of $N = 2,393$ sprint speed values for analysis. A single author (M.S.) scored all videos, with high intra-rater reliability on a randomly selected subset of trials scored twice (Kendall's $W = 0.98$, $F_{136, 136} = 47.92$, $P < 0.0001$).

After the twelfth sprint for each lizard, one researcher (E.J.G.) continually chased the lizard with a soft paintbrush back and forth without stopping on the racetrack. We quantified endurance as the time that lizards continually moved until exhaustion. Lizards were considered exhausted when further stimulation with the paintbrush elicited no locomotor response for 5 s and the lizard then did not further attempt to flee (Gleeson and Dalessio 1989). As a measure of the maximum postexhaustion rate of oxidative metabolism in lizards, we quantified the peak postexhaustion rate of carbon dioxide production ($\dot{V}CO_{2peak}$) in a subset of lizards (for sample size for each measure, see table A2). Immediately after being run to exhaustion (within approximately 10 s), we placed lizards in a modified shelter with secure flaps (as above) and with many holes drilled along the length to ensure gas circulation. This shelter was placed in a cylindrical acrylic metabolic chamber (700 mL), which was then placed in the incubator set to 31°C. We
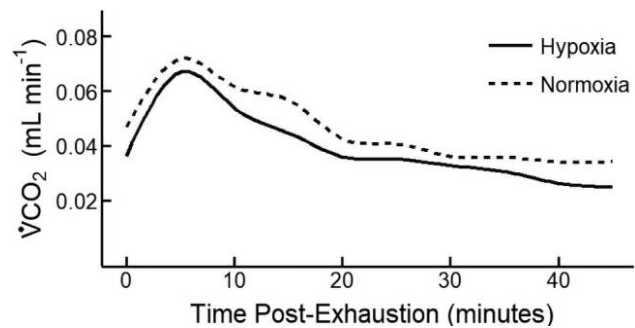

Figure 1. Postexhaustion metabolic response of adult male *Podarcis muralis* lizards. Curves are general additive models fitted to raw data from lizards in hypoxia and normoxia treatments. $\dot{V}CO_2$, rate of carbon dioxide production.

utilized pull-mode respirometry to quantify gas concentrations (Foxbox-C Field O_2 and CO_2 Analysis System, Sable Systems, Las Vegas, NV; Lighton 2008). Air was circulated through the metabolic chamber at a rate of 500 mL/min, dried of water vapor with Drierite, and then measured for both CO_2 and O_2 content, corrected for barometric pressure. Data were analyzed with ExpeData software (ver. 1.7.30, Sable Systems) to calculate the rates of carbon dioxide production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$). We report results for $\dot{V}CO_2$ because O_2 sensor drift precluded reliable extraction of data and resulted in the loss of $\dot{V}O_2$ from many trials. Both $\dot{V}O_2$ and $\dot{V}CO_2$ are elevated simultaneously after exercise, with measures of postexhaustion $\dot{V}CO_2$ representing an important physiological parameter that comprises both the oxidative capacity to replenish energy substrates and the maintenance of acid-base balance, including lactate processing (Gleeson and Bennett 1982; Hailey et al. 1987; Jackson et al. 2015). Metabolic rates peaked for lizards in both treatment groups within the first several minutes of measurement (fig. 1; Hailey et al. 1987). We measured each lizard continuously for 45 min after exhaustion and extracted the highest average rate of carbon dioxide production over a 15-s interval as our measure of $\dot{V}CO_{2peak}$.

Blood Sampling and Measures

On the day after performance measures, we collected blood samples from the retro-orbital sinus with a heparinized glass capillary tube (MacLean et al. 1973; Meylan et al. 2003). Lizards were removed from their enclosures under normal husbandry conditions and blood was collected generally within 3 min (bleed time mean $\pm$ SD: 1.85 $\pm$ 1.22 min). We collected 25–40 μL of whole blood and stored blood on ice until processing (generally 4–6 h). To measure hematocrit, we spun 10–15 μL of whole blood in a capillary tube (capacity: 19 μL) for 5 min at 5,000 g and then measured the volume of packed red blood cells relative to total volume (%Hct). Hemoglobin concentration was measured in 5 μL whole blood utilizing the colorimetric cynamethemoglobin method, following manufacturer's instructions (Drabkin's reagent, Sigma-Aldrich, St. Louis, MO).

Lizards were kept over winter and released the next spring at the site of capture. Field sampling and experimental protocols were

conducted under current permit (Arrêté Préfectoral 2017-s-02). All procedures are in accordance with Directive 2010/63/EU on Protection of Animals Used for Scientific Purposes (http://ec.europa.eu /environment/chemicals/lab_animals/pdf/guidance/directive/en .pdf) and the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research (http://www.asih.org/sites /default/files/documents/resources/guidelinesherpsresearch2004 .pdf).

Statistics

We used linear mixed models with proc mixed in SAS 9.4 (SAS Institute, Cary, NC) to assess repeatability of physiological and performance measures, as well as the relative influences of treatment (normoxia/hypoxia) and acclimation over time. To better meet assumptions of normality, measures of $\dot{V}_{CO_{2peak}}$ and time to exhaustion were $\log_{10}$ transformed before analysis. We first estimated repeatability of measures within each treatment group. Because the mean values of our measured traits changed over time, we included time as a factor in our repeatability models and report constancy repeatability (R_c), which provides a measure of among-individual variation after accounting for changes in such mean values over time (Biro and Stamps 2015). We created models with the fixed effect of time point and the random effect of individual, modeled with a compound symmetric covariance structure, and calculated constancy repeatability as the ratio of variance explained by individual to total variance. We assessed significance with a likelihood ratio test, using the difference in log likelihoods between models with and without individual as a χ^2 statistic with 1 and 0 df (mathematically equivalent to halving the P value obtained from a χ^2 test with 1 df; $\chi^2_{0:1}$ in "Results"), which accounts for the fact that variance component estimates are bounded by 0 (Stram and Lee 1994; Bolker et al. 2009; Snijders and Bosker 2012). For measures of sprint speed, we assessed constancy repeatability at two timescales: across measurement time points (as with other traits) and within time point (using the 12 repeated sprints from each lizard at each time point).

To assess the impact of hypoxia treatment on traits across time, we specified models with the fixed effects of treatment (hypoxia/ normoxia), time point (1 d, 3 wk, 6 wk), and their interaction. To account for heterogeneity among sampled populations, we also included population of origin as a fixed effect. Except for analyses of body mass and body condition, initial models contained lizard size (SVL) as a covariate. This effect did not approach significance in any model and was removed from further analysis (all $P > 0.35$), with the exception of sprint speed, in which it was retained because of the known effect of size on sprint speed (e.g., Zajitschek et al. 2012). Sprint speed was analyzed using data from all trials (12 trials at each time point); therefore, models also included the fixed effect of trial as well as all two-way interactions between trial, time point, and treatment. Because of its positive influence on sprint speed, body temperature was retained (taken immediately after sprinting trials) in models of sprint speed. Models of $\dot{V}_{CO_{2peak}}$ included log-transformed mass as a covariate; initial models also included body temperature, but this was removed because it did not influence metabolic rate ($P > 0.93$). For all mixed models, we estimated

denominator degrees of freedom for F-tests using the Kenward-Roger degrees of freedom approximation (Kenward and Roger 1997).

To further describe the within-individual relationships among physiology and performance, we utilized a path analysis with the *lavaan* package (Rosseel 2012) in the programming language R (R Core Team 2017). Path analyses describe the magnitude and significance of potential causal relationships among variables within an a priori specified model. Our model includes the influence of hematocrit on hemoglobin concentration, the influence hemoglobin on $\dot{V}_{CO_{2peak}}$, running endurance and sprint speed, and, finally, the influence of $\dot{V}_{CO_{2peak}}$ on running endurance and sprint speed (fig. 3). We specified the model in this way, assuming that increased hematocrit is the primary driver of increased hemoglobin, which in turn would permit greater oxygen-carrying capacity and thus greater maximum aerobic metabolic rate. Further, we tested whether this increase in aerobic capacity would improve sprint speed over repeated trials, which relies on aerobic respiration to replenish ATP stores once anaerobic capacity is expended (Gleeson 1991), and running endurance, which is linked to activity metabolism (Garland and Else 1987; Clemente et al. 2009). Because of its effect on blood viscosity, hematocrit may also directly influence physiological and performance measures, independent of the effect on oxygen-carrying capacity. However, preliminary models demonstrated that hematocrit was not a significant predictor of either $\dot{V}_{CO_{2peak}}$ or running endurance (all $P > 0.11$); therefore, we excluded this relationship from subsequent models. Path analysis requires that all variables are measured for an individual at a given time point, resulting in a reduction in the number of observations that were available to analyze (because time constraints precluded the measurement of metabolic rates for all animals; $N = 84$ total observations included in path analysis). Because we combined data across all three time points, this analysis does not account for the correlation of repeated measures within individuals. Nonetheless, our goal is to examine the relationship among these physiological and performance traits over time; thus, including all available observations allows us to quantify within-individual relationships of these parameters for lizards in both treatment groups.

Results

Repeatability

Constancy repeatability of physiological and performance traits ranged from 0.168 to 0.949, with all estimates except for hemoglobin under hypoxia statistically significant (table 1). Sprint speed was highly repeatable in both experimental treatments, both across the 6 wk of the experiment and within each measurement day, indicating among-individual variation in sprint performance across multiple timescales.

Treatment and Acclimation

All seven physiological and performance measures were influenced either by treatment (normoxia/hypoxia) or by the interaction of treatment and time point (fig. 2; table 2). Peak

Table 1: Constancy repeatabilities of physiological and performance traits in adult male *Podarcis muralis* lizards in normoxia and hypoxia

Trait	Normoxia			Hypoxia		
	R_c	$\chi^2_{0:1}$	P	R_c	$\chi^2_{0:1}$	P
[Hemoglobin]	**.181**	3.2	.037	.178	2.1	.074
Hematocrit	**.265**	6.7	.0048	**.168**	3.0	.042
$\dot{V}_{CO_{2peak}}$	**.577**	7.7	.0028	**.292**	2.8	.047
Running endurance	**.691**	30.5	<.0001	**.313**	5.2	.011
Body mass	**.949**	337.2	<.0001	**.946**	353.2	<.0001
Body condition	**.690**	53.9	<.0001	**.709**	64.8	<.0001
Overall	**.403**	549.9	<.0001	**.413**	620.8	<.0001
Sprint 1 d	**.487**	195.9	<.0001	**.539**	269.4	<.0001
Speed 3 wk	**.535**	223.6	<.0001	**.516**	243.6	<.0001
6 wk	**.505**	210.6	<.0001	**.500**	214.7	<.0001

Note. Traits were measured in lizards at three time points over the 6 wk of the experiment. Additionally, sprint speed was assessed in 12 consecutive trials at each time point. Significant estimates ($P < 0.05$) are shown in bold. R_c, constancy repeatability; $\dot{V}_{CO_{2peak}}$, peak postexhaustion rate of carbon dioxide production.

postexhaustion rate of carbon dioxide production ($\dot{V}_{CO_{2peak}}$) decreased by 10% and running endurance was reduced by 21% in hypoxic conditions across all time points (effect of treatment: $P = 0.04$ and $P = 0.0035$, respectively), though overall $\dot{V}_{CO_{2peak}}$ decreased and running endurance increased across the time of the experiment (effect of time point: $P = 0.0093$ and $P < 0.0001$, respectively). Models of maximal postexhaustion metabolic rate include mass and thus account for changes in individual mass over the course of the experiment; as expected, mass exerted a positive effect on $\dot{V}_{CO_{2peak}}$ (scaling exponent ± SE: 0.60 ± 0.12). Lizards in the normoxic treatment began with higher values of hemoglobin concentration and hematocrit, but this pattern was reversed after 3 wk, and then values converged after 6 wk (treatment × time point interaction: $P = 0.041$). Body mass was identical between treatments to start and then increased after the first measurement in the normoxia treatment and remained elevated compared to starting values (effect of time and treatment × time point interaction: both $P < 0.0001$). Similarly, body condition was not different to start but diverged across the duration of the experiment (significant treatment × time point interaction: $P = 0.0037$). Sprint speed decreased with short-term repetition (within the 12 consecutive trials run by a lizard in a day) but increased over the duration of the experiment (effect of trial and time point: both $P < 0.0001$). Performance decrement over the repeated sprints was not affected by treatment (treatment × trial number interaction: $P = 0.32$), though improvement over the time of the experiment was more pronounced in lizards in the normoxia treatment (treatment × time point interaction: $P = 0.027$). Finally, we found significant among-population heterogeneity in running endurance ($P = 0.0049$), largely driven by lizards from one population running for longer times before exhaustion (Engomer; see table A1).

Path Analysis

The path analysis demonstrates that for lizards in both normoxic and hypoxic conditions, hemoglobin concentration is largely de-termined by hematocrit. In turn, hemoglobin positively influenced $\dot{V}_{CO_{2peak}}$ in normoxia but not in hypoxia. Hemoglobin also positively influenced sprint speed in normoxia and running endurance in hypoxia. Interestingly, $\dot{V}_{CO_{2peak}}$ exerted a negative influence on sprint speed in both treatments, though the effect was significant only in hypoxia. Counter to our expectations, $\dot{V}_{CO_{2peak}}$ did not influence running endurance in either treatment group.

Discussion

Our results demonstrate a limited capacity of adult male *Podarcis muralis* lizards to acclimate to high-altitude hypoxia over a time frame of several weeks. Although hypoxia induced shifts in blood biochemistry to increase oxygen-carrying capacity, measures of sprint speed and running endurance were not maintained by lizards in hypoxia relative to lizards at low altitudes. Further, we found evidence that exposure to hypoxia over this amount of time results in a rapid decrease in body condition and reduced mass gain, likely due to negative consequences for energy balance. We also found strong evidence that lizards maintain individual differences over time in physiological and performance traits, both in normoxia and under conditions of high-altitude hypoxia.

Our repeatability estimates are within the reported range for physiological traits in wild vertebrates generally and for lizard performance specifically (e.g., Huey and Dunham 1987; Tsuji et al. 1989; Zajitschek et al. 2012; Goulet et al. 2017; Hoskins et al. 2017). All physiological traits were significantly repeatable, except for hemoglobin concentration in hypoxia, with estimates indicating moderate to high levels of among-individual variation. Among-individual differences in performance were also present in both treatments, corroborating previous studies of both running endurance and sprint speed in this and other lizard species (e.g., Garland and Else 1987; Huey and Dunham 1987; Sorci et al. 1995; Zajitschek et al. 2012). Furthermore, sprint speed was repeatable across multiple timescales, indicating significant among-individual variation in this trait both under rapid repeated trials and across the
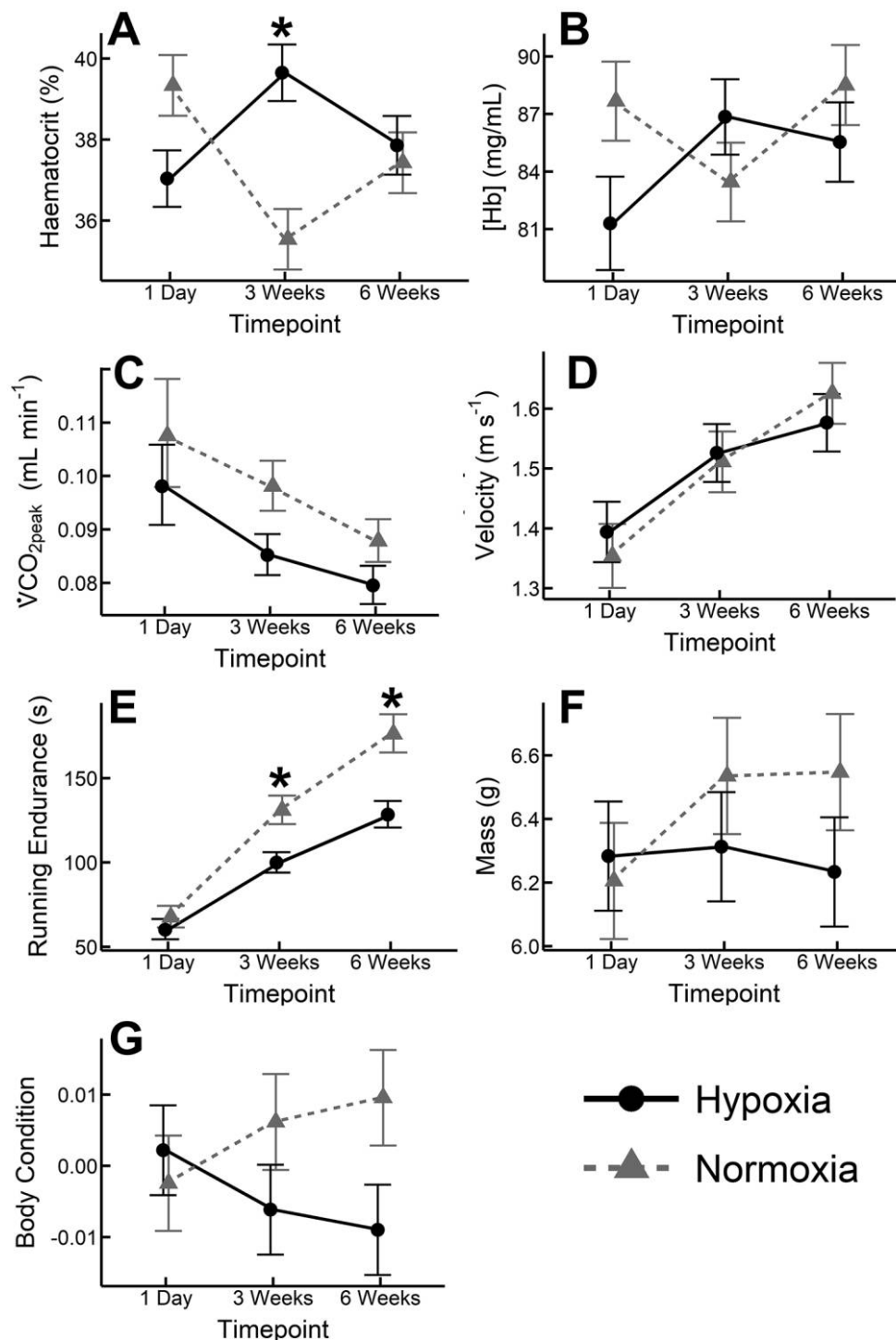
Figure 2. Effects of high-altitude hypoxia and time on physiological and performance traits in adult male *Podarcis muralis* lizards. *A*, Hematocrit (%). *B*, Hemoglobin concentration (mg/mL). *C*, Peak postexhaustion rate of carbon dioxide production ($\dot{V}CO_{2peak}$; mL/min). *D*, Sprint velocity (m/s). *E*, Running endurance (s). *F*, Body mass (g). *G*, Body condition. Data are least squares means from linear mixed models $\pm$ SE. Significantly different estimates between treatments at a given time point are indicated with asterisk (adjusted $P < 0.05$). [Hb], hemoglobin concentration.

several weeks of the experiment. Sprint speed is commonly tested in lizard species because of its established ecological and fitness importance (see above), but seldom is the stability of this trait tested over multiple timescales. Our results further support the use of

sprint speed as proxy for individual performance capacity in this lizard species, as has been established in some fish species (e.g., Oufiero and Garland 2009). To our knowledge, this is the first study to establish the repeatability of sprint speed under hypoxic con-

Table 2: Results of linear mixed-model analyses of physiological and performance measures in adult male *Podarcis muralis* lizards measured at three time points over the 6-wk experiment

Source of variation	[Hb] (N = 207)	Hct (N = 228)	$\dot{V}_{CO_2peak}$ (N = 91)	Endurance (N = 172)	Body mass (N = 232)	Body condition (N = 232)	Sprint speed (N = 226)
Treatment	$1.03_{1,69.3}$, .313	$1.14_{1,70.5}$, .288	$4.48_{1,42.1}$, **.0402**	$9.02_{1,83.8}$, **.0035**	$.38_{1,71}$, .534	$1.12_{1,71.1}$, .295	$.1_{1,92.9}$, .752
Time point	$.89_{2,134}$, .412	$.52_{2,148}$, .598	$5.14_{2,49.7}$, **.0093**	$74.87_{2,95}$, **<.0001**	$11.46_{2,150}$, **<.0001**	$.01_{2,150}$, .993	$24.93_{2,235}$, **<.0001**
Treatment × time point	$3.26_{2,134}$, **.0413**	$12.2_{2,148}$, **<.0001**	$.14_{2,57}$, .869	$1.06_{2,103}$, .351	$13.23_{2,150}$, **<.0001**	$5.81_{2,150}$, **.0037**	$3.61_{2,232}$, **.0273**
Population	$1.24_{5,70.7}$, .302	$2.23_{5,70.6}$, .0612	$.92_{5,37.7}$, .479	$3.69_{5,74.3}$, **.0049**	$.65_{5,71}$, .663	$.61_{5,71.1}$, .695	$1.03_{5,71.5}$, .406
Body size:							
$\log_{10}$(mass) for $\dot{V}_{CO_2peak}$, SVL for sprint speed			$22.96_{1,32.4}$, **<.0001**				$.51_{1,484}$, .474
Trial number	…	…	…	…	…	…	$85.98_{1,2.305}$, **<.0001**
Treatment × trial number	…	…	…	…	…	…	$.98_{2,305}$, .323
Time point × trial number	…	…	…	…	…	…	$2.75_{2,305}$, .0643
Body temperature	…	…	…	…	…	…	$8.43_{1,375}$, **.0037**

Note. Values shown are $F_{df1, df2}$, Pr > F. Significant effects are shown in bold. See table A2 for additional details. [Hb], hemoglobin concentration; Hct, hematocrit; $\dot{V}_{CO_2peak}$, postexhaustion rate of carbon dioxide production.
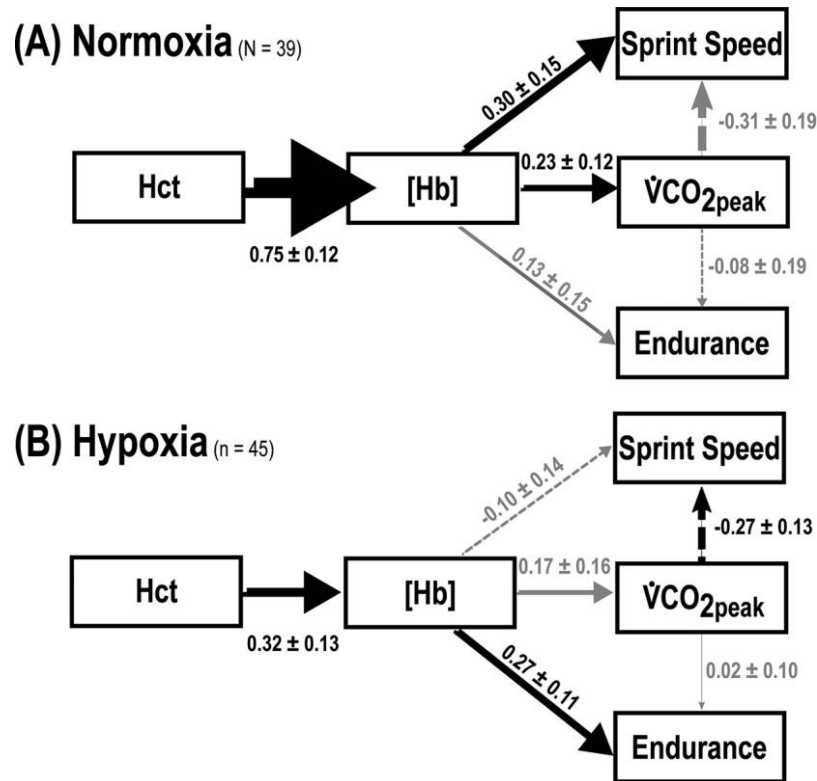
Figure 3. Path analysis results of relationships among physiological and performance measures in adult male *Podarcis muralis* lizards in normoxia and hypoxia. Coefficient estimates are shown $\pm$ SE. Path thickness is proportional to the estimated effect, with statistically significant paths ($P < 0.05$) shown in black and nonsignificant paths in gray. Positive relationships are shown with solid lines and negative relationships with dashed lines. Hct, hematocrit; [Hb], hemoglobin concentration; $\dot{V}_{CO_{2peak}}$, peak postexhaustion rate of carbon dioxide production.

ditions in lizards, corroborating a recent study in striped bass (*Morone saxatilis*) that found individual consistency in swimming speed under hypoxic conditions (Kraskura and Nelson 2018).

As we predicted based on previous work in a variety of taxa, lizards adjusted blood chemistry in response to high-altitude hypoxia (Storz 2007; Storz et al. 2010). Two important parameters related to blood-oxygen capacity—hematocrit and hemoglobin concentration—were correlated and increased within the first 3 wk of the experiment (increases of 12% and 4%, respectively). This response is concordant with numerous studies demonstrating that lizards from high-altitude populations have higher hemoglobin concentration and hematocrit compared to lowland populations (Vinegar and Hillyard 1972; Weathers and White 1972; Newlin and Ballinger 1976; González-Morales et al. 2015; Lu et al. 2015). Lowland lizards acclimated to simulated high-altitude hypoxia increased hemoglobin concentration and hematocrit and changed other parameters related to oxygen transport and use, but these responses were not identical to those in lizards native to high-altitude populations (Weathers and McGrath 1972; He et al. 2013). As demonstrated in mammals and birds, the acute plastic response may differ from changes resulting from natural selection and may, in fact, be maladaptive (reviewed in Storz et al. 2010). After 6 wk, the values of hemoglobin concentration and hematocrit converged between the treatment groups. This could be due to the potential

energetic costs of maintaining high levels of these parameters. For example, increased red blood cell density can increase blood viscosity and the energetic cost of circulation (Hedrick et al. 1986; Dunlap 2006), which could negatively impact an individual's energy balance. Correspondingly, there are several potential nonexclusive reasons for the observed decline in body condition and failure to regain mass of lizards in hypoxia. These could include an energetic cost associated with such physiological shifts, reduced efficiency of overall energy processing, or reduced food intake. Future work will benefit from examining hypoxia-induced shifts in other aspects of blood biochemistry, such as hemoglobin oxygen-binding affinity and hemoglobin isoforms, as well as their energetic consequences (Storz 2007, 2016; Lu et al. 2015).

The plasticity in parameters important for oxygen-carrying capacity in blood impacted postexhaustion metabolic rate only in normoxia (fig. 3). After exhaustion, CO_2 production is elevated as a result of both increased oxidative metabolism and the maintenance of acid-base balance in response to elevated lactic acid concentrations (Gleeson and Bennett 1982). This implies that the postexhaustion increase in metabolism in hypoxia may be more strongly driven by the need to regulate acid-base balance due to anaerobiosis during exercise rather than oxidative capacity after exhaustion. Previous work in lizards describes a weak but significant relationship between hematocrit and maximum metabolic rates

(Garland et al. 1987), though intermediate levels of blood parameters related to oxygen capacity are optimal in mammals (Villafuerte et al. 2004; Schuler et al. 2010). Furthermore, we found a negative relationship between postexhaustion metabolic rate and sprint speed under hypoxic conditions, suggesting that individuals that had the greatest capacity for burst speed were less rapidly able to resupply energetic capacity via oxidative metabolism. Given the known dependence of lizard sprinting on anaerobiosis (Bennett and Licht 1972; Gleeson 1991), this relationship suggests that hypoxic conditions evoke a trade-off between anaerobic and aerobic capacity. In our study, this trade-off is further evidenced by the effect of acclimation in both treatment groups: during their time in captivity, lizards decreased maximum metabolic rates while increasing running endurance. Additionally, lizards in normoxia increased their sprint speed during the course of the experiment to a greater extent than hypoxic lizards. That time in captivity has such a strong effect on these measures is surprising, though consistent with previous studies in the lizard *Amphibolurus nuchalis* kept in captivity over similar time spans (Garland et al. 1987). It may be that the strong effect of time on performance is due not to physiological changes but to habituation effects after exposure to repeated test stimuli (e.g., Rodríguez-Prieto et al. 2010). These results collectively suggest a great acclimation potential for plasticity in the physiological and biochemical traits underlying performance, though not sufficient to compensate for low oxygen availability. Future studies should examine the biochemical basis for differences in oxidative and glycolytic capacity of muscle, as these pathways likely determine variation in running endurance and trade-offs between aerobic and anaerobic capacity (Garland et al. 1987).

We note that our experimental treatment represents an extreme situation: individual lizards are unlikely to naturally migrate up 2,500 m in elevation. However, *P. muralis* is an exemplar species to illustrate the potential for human-assisted transport to facilitate range expansion. For example, this species is well established in areas in both the United States and England, likely because of isolated incidents whereby small numbers of individuals were relocated (Deichsel and Gist 2001; Michaelides et al. 2015). Though human-assisted movement to high-elevation sites is plausible, our results indicate that relocation to 2,877 m asl is beyond the upper limit of acclimation capacity for adult male lizards. Although this does not preclude the possibility that this lizard species is capable of colonizing habitat at higher altitudes, it does suggest that other processes—such as developmental plasticity triggered during earlier life-history stages or local adaptation—will play a role in facilitating such range expansion. Three non–mutually exclusive mechanisms may, individually or concurrently, permit the colonization of high-altitude environments: within-individual reversible plasticity (or flexibility), developmental plasticity in response to early-life environments and/or resulting from maternal effects, or local adaptation (selection on currently existing genetically determined variation within populations). Our previous work in this system indicates that embryos can adjust their physiology to compensate for high-altitude hypoxia, thus pointing to an important role for developmental plasticity in the colonization of habitats at higher elevation (Cordero et al. 2017).

Despite the observed short-term shifts in hematocrit and hemoglobin concentration observed in this study, adult lizards in high-altitude hypoxia exhibited lower maximum metabolic rates across all time points and impaired sprint speed and running endurance over the duration of the experiment. This suggests that within-individual plasticity of physiology is not sufficient to maintain important performance traits in adult male lizards. Furthermore, this level of hypoxia appears to bear important long-term health consequences for lizards, demonstrated by the rapid decrease in body condition after just 6 wk at altitude. Thus, we hypothesize that early-life exposure to trigger developmental plasticity may be necessary for lizards to respond successfully to high-altitude environments. We are currently conducting studies directed toward assessing the relative roles of local adaptation, maternal effects, and long-term developmental plasticity in facilitating range expansion of this widespread lizard under continually increasing temperature regimes (Bravo et al. 2008; IPCC 2014).

APPENDIX

Table A1: Habitat description and sample sizes from sampled populations of *Podarcis muralis*

Name	Latitude, longitude	Altitude (m asl)	Habitat type	N hypoxia, N normoxia
Alas	42°56′58″N, 001°2′47″E	472	Cemetery	6, 8
Astien	42°56′20″N, 001°3′53″E	522	Rock wall, stone buildings	7, 6
Aubert	42°57′52″N, 001°6′10″E	425	Rock wall and bridge	8, 6
Engomer	42°56′46″N, 001°3′20″E	473	Cemetery	7, 8
Lambège	42°58′41″N, 001°7′18″E	425	Rock wall	7, 4
Luzenac[a]	42°57′22″N, 001°5′1″E	443	Cemetery	6, 5

[a]This population is also studied in Calsbeek et al. (2010).

Table A2: Sample sizes for each physiological and performance measure

	[Hb]	Hct	$\dot{V}_{CO_2peak}$	Endurance	Body mass	Condition	Sprint speed
1 d:							
Normoxia	36	36	5	14	37	37	36
Hypoxia	26	41	8	12	41	41	41
3 wk:							
Normoxia	36	36	17	34	36	36	35
Hypoxia	39	41	21	39	41	41	40
6 wk:							
Normoxia	35	36	20	35	37	37	36
Hypoxia	35	38	20	38	40	40	38

Note. [Hb], hemoglobin concentration; Hct, hematocrit; $\dot{V}_{CO_2peak}$, peak postexhaustion rate of carbon dioxide production.

Table A3: Mean ± SD for body mass at time of capture (feeding status unknown) and at three experiment time points (fasted) for experimental lizards (*Podarcis muralis*)

	Capture	1 d	3 wk	6 wk
Normoxia	6.74 ± 1.32	6.20 ± 1.13	6.52 ± 1.11	6.54 ± 1.17
Hypoxia	6.53 ± 1.06	6.28 ± 1.06	6.31 ± 1.05	6.23 ± 1.02

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