Received: 8 February 2017 | Accepted: 22 July 2017

DOI: 10.1111/1365-2656.12738

RESEARCH ARTICLE

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

Physiology at near-critical temperatures, but not critical limits, varies between two lizard species that partition the thermal environment

Rory S. Telemeco[1,2] | Eric J. Gangloff[1] | Gerardo A. Cordero[1,3] | Rebecca L. Polich[1] | Anne M. Bronikowski[1] | Fredric J. Janzen[1]

[1]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA

[2]Department of Biology, University of Washington, Seattle, WA, USA

[3]Department of Biology, Lund University, Lund, Sweden

Correspondence
Rory S. Telemeco
E-mail: telemeco@csufresno.edu

Present address
Rory S. Telemeco, Department of Biology, California State University, Fresno, CA, USA

Funding information
National Science Foundation, Directorate for Biological Sciences, Grant/Award Number: DEB-0640932, DEB-1242510 and EF-1065638; Chicago Herpetological Society; Ecology, Evolution, and Organismal Biology Department at Iowa State University (ISU); Sigma Xi; U.S. Environmental Protection Agency Science to Achieve Results Fellowship, Grant/Award Number: FP-91723101; Office of Biotechnology at ISU

Handling Editor: Dehua Wang

Abstract

1. The mechanisms that mediate the interaction between the thermal environment and species ranges are generally uncertain. Thermal environments may directly restrict species when environments exceed tolerance limits (i.e. the fundamental niche). However, thermal environments might also differentially affect relative performance among species prior to fundamental tolerances being met (i.e. the realized niche).
2. We examined stress physiology (plasma glucose and corticosterone), mitochondrial performance and the muscle metabolome of congeneric lizards that naturally partition the thermal niche, *Elgaria multicarinata* (southern alligator lizards; SALs) and *Elgaria coerulea* (northern alligator lizards; NALs), in response to a thermal challenge to quantify variation in physiological performance and tolerance.
3. Both NAL and SAL displayed physiological stress in response to high temperature, but neither showed signs of irreversible damage. NAL displayed a higher baseline mitochondrial respiration rate than SAL. Moreover, NAL substantially adjusted their physiology in response to thermal challenge, whereas SAL did not. For example, the metabolite profile of NAL shifted with changes in key energetic molecules, whereas these were unaffected in SAL.
4. Our results indicate that near-critical high temperatures should incur greater energetic cost in NAL than SAL via an elevated metabolic rate and changes to the metabolome. Thus, SAL displace NAL in warm environments that are within NAL's fundamental thermal niche, but relatively costly.
5. Our results suggest that subcritical thermal events can contribute to biogeographic patterns via physiological differences that alter the relative costs of living in warm or cool environments.

KEYWORDS
corticosterone, OCLTT, pejus temperatures, reactive oxygen species, state III respiration

1 | INTRODUCTION

While temperature has long been recognized as an important component of the fundamental niche that affects macro-ecological patterns (Addo-Bediako, Chown, & Gaston, 2000; Angilletta, 2009; Cruz, Fitzgerald, Espinoza, & Schulte, 2005; Pörtner, 2002), the need to predict the impact of climate change on species distributions has reinvigorated interest in understanding the mechanisms underlying temperature's influence on organisms (e.g. Deutsch et al., 2008; Kearney & Porter, 2009; Levy et al., 2015; Sunday, Bates, & Dulvy, 2012). Only

with an understanding of these mechanisms can we extrapolate beyond simple correlations to predict the impacts of novel thermal environments on species (Deutsch et al., 2008; Levy et al., 2015; Sunday, Bates, & Dulvy, 2011; Telemeco et al., 2016). Even so, the mechanisms that translate the thermal environment into fitness are poorly understood (reviewed in Angilletta, 2009; Dowd, King, & Denny, 2015).

Perhaps, the best example of our limited understanding of the mechanisms underlying thermal tolerance is the fact that the proximate mechanism responsible for setting high-temperature limits is not known for most animals. Classically, animals were thought to die at high temperatures because their proteins or membranes lost structural integrity ("denaturation hypothesis" hereafter, Fields, 2001; Pörtner, 2002; Schulte, 2015; Tansey & Brock, 1972). However, most animals die at temperatures below those that melt proteins or membranes (Hochachka & Somero, 2002; Pörtner, 2002). More conservatively, loss of organismal function might result from reversible loss of subcellular function that could be restored if the animal survived to be cooled (Hochachka & Somero, 2002; Schulte, 2015; Somero, 1995). Alternatively, organisms might lose function at high temperatures because of organ-system failures (Pörtner, 2002; Schulte, 2015). For example, the oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis proposes that circulatory and respiratory systems are unable to deliver sufficient oxygen to meet the elevated demand of tissues at high temperature, resulting in system failure and death (Pörtner, 2001, 2002; Pörtner et al., 2006).

In addition to our limited understanding of the mechanisms setting thermal limits, the relative importance of such limits for determining geographic distributions is uncertain (Addo-Bediako et al., 2000; Sunday et al., 2012). Importantly, critical limits describe but one component of how temperature affects performance and responses to subcritical temperatures might have greater effects on species distributions. The effect of temperature on performance is classically depicted as a left-skewed, hump-shaped curve (i.e. thermal performance curve, Huey & Stevenson, 1979), with the range between optimal (T_{OPT}) and critical thermal maximum (CT_{MAX}) temperatures referred to as the upper pejus (getting worse) range (Frederich & Pörtner, 2000; Pörtner, 2002). The relative fitness consequences of pejus temperatures should be especially important if the outcomes of species interactions are altered (Dunson & Travis, 1991; Gilbert et al., 2014). For example, asymmetric responses among competing species to temperature can cause species to transition from locally superior to inferior competitors even when temperatures are within critical limits (Carmona-Catot, Magellan, & Garcia-Berthou, 2013; Finstad et al., 2011; Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Liles, Cecala, Ennen, & Davenport, 2017; Olsen, Topper, Skarpaas, Vandvik, & Klanderud, 2016). Thus, variable responses to pejus temperatures could lead to local extinctions via increased competitor colonization and competitive exclusion (Gilman et al., 2010). Knowing both the thermal limits and relative consequences of subcritical temperature exposure are necessary for understanding how the thermal environment affects species biogeography.

We examined mechanisms underlying thermal tolerance and the physiological consequences of subcritical temperature exposure in congeneric, sympatric lizard species that occupy different thermal habitats to illuminate the potential importance of these physiological parameters for biogeography. We examined northern alligator lizards (*Elgaria coerulea*; Wiegmann 1828; NAL) and southern alligator lizards (*Elgaria multicarinata*; de Blainville, 1835; SAL). Although these species diverged *c*. 6.6 mya (Macey, Shulte, Larson, & Tuniyev, 1999), they display similar morphology, generalist diet and habitat choice (Beck, 2009a, 2009b; Cunningham, 1956). Both species occur along the Pacific coast of North America, but SAL has a more southern range than NAL (Stebbins, 2003, see Figure S1). In regions where the coarse geographic range overlaps, these lizards are rarely syntopic with NAL occurring at higher elevations (Beck, 2009a, 2009b, and personal observation). Given their ecological similarity but divergent distributions, thermal niche partitioning is hypothesized to underlie their biogeography. However, these lizards have similar thermal activity ranges (mean active temperature of *c*. 24–25°C with an observed range of 10–35°C for both species; Brattstrom, 1965; Cunningham, 1966; Kingsbury, 1994; Sheen, 2001; Stewart, 1984; Table 1), and both thermoregulate to *c*. 28°C in the laboratory (Dawson & Templeton, 1966; Telemeco & Addis, 2014). Moreover, NAL and SAL display remarkably similar CT_{MAX} (estimates range from 38.2 to 41.4°C with no discernable difference between species, Brattstrom, 1965; Cunningham, 1966; Dawson & Templeton, 1966; Licht, 1964a; Table 1). Thus, despite living in different thermal habitats, classic descriptors of thermal preference, performance and tolerance fail to distinguish NAL and SAL (summarized in Table 1). Notably, these animals differ in reproductive mode (NAL is viviparous and SAL is oviparous), which may explain NAL occurring in cold habitats unsuitable for SAL (Blackburn, 1982; Shine, 2002; Stewart, 1984; Telemeco, 2014). Nonetheless, both SAL and NAL can successfully reproduce in warm environments. Thus, adult thermal performance within fundamental limits could underlie SAL occurring in warmer environments than NAL.

We hypothesize that variation in the physiological response to high temperatures, particularly those in the pejus range, contribute to biogeographic differences of NAL and SAL. We first tested the hypothesis that SAL naturally occupies warmer environments than NAL using occurrence data. Next, we exposed lizards to control and near-critical hot treatments and quantified physiological stress (plasma glucose and corticosteroid concentrations), mitochondrial function, and the skeletal muscle metabolome. We examined subcellular performance because whole-organism performance rapidly fails near-critical limits making precise quantification difficult (Angilletta, 2009; Huey, 1982) and coarse whole-organism data are already available for comparison (Table 1). Using these physiological data, we first attempted to elucidate the mechanism responsible for setting fundamental thermal limits in these species and assessed variation in tolerance. If fundamental limits result from protein or membrane denaturation, we predicted that near-critical temperature exposure would damage mitochondria, resulting in lasting functional reduction and increased reactive oxygen species (ROS) production. By contrast, if fundamental limits result from oxygen limitation, we predicted near-critical temperature exposure would increase anaerobic metabolites and decrease aerobic metabolites in the muscle metabolome (Verberk, Sommer, Davidson, & Viant,

TABLE 1 Literature summary of alligator lizard (*Elgaria multicarinata* [SAL] and *E. coerulea* [NAL]) thermal tolerance. Data are presented as "mean [range]," with superscripts referencing methodology, and sample size (*N*) in parentheses. When available, subspecies and collection location are also provided. Although historically recognized subspecies are no longer accepted for SAL and questionable for NAL, they provide general location information. Hyphens denote unavailable data

Species/subspecies	Location	Active T_b (°C)	T_{Pejus} (°C)	CT_{MAX} (°C)	T_{Lethal} (°C)	Citation
Elgaria multicarinata [SAL]						
E. m. ignavus	—	22.4 [16.4–22.4][a] (*N* = 5)	—	—	—	Zweifel (1958)
E. m. multicarinatus	Riverside Co, CA	—	33[c]	39.5 [—][g]	—	Licht (1964b)
E. m. multicarinatus	—	—	32[d]	39 [—][h] (*N* = 6)	—	Licht (1964a)
E. m. webbi	—	21.2 [11.0–33.2][a] (*N* > 15)	—	40.3 [40.0–40.5][i] (*N* > 15)	—	Brattstrom (1965)
E. m. webbi	Los Angeles Co, CA San Bernadino Co, CA Ventura Co, CA	21.1 [4.9–35.7][a] (*N* = 102)	—	41.4 [—][i] (*N* = 5)	43.6–43.8, *N* = 2	Cunningham (1966)
E. m. webbi	Los Angeles Co, CA	—	33[e]	39.5 [39–40][j] (*N* = 12)	—	Dawson and Templeton (1966)
—	San Diego Co, CA	23.8 [9.5–33.8][b] (*N* = 16)	33[f]	—	—	Kingsbury (1994)
Elgaria coerulea [NAL]						
E. c. principis	—	15.8 [11.0–19.0][a] (*N* = 5)	—	38.2 [—] (*N* = 1)	—	Brattstrom (1965)
E. c. principis	Whatcom Co, WA	24.9 [20–30][a] (*N* = 27)	—	—	—	Vitt (1973)
E. c. shastensis	—	19.8 [—][a] (*N* = 1)	—	40.1 [38.5–41.2][i] (*N* = 3)	—	Brattstrom (1965)
—	Monterrey Co, CA	25.2 [13.6–34][a] (*N* = 73)	—	—	—	Stewart (1984)
—	Manchester Co, CA Mendocino Co, CA	24.4 [11.8–31.2][a] (*N* = 91)	—	—	—	Stewart (1984)
—	San Mateo, Co, CA	25.9 [16–31] (*N* = 86)	—	—	—	Levin (1967)
—	King Co, CA Klickitat Co, CA Whatcom Co, CA	25.5 [20–31] (*N* = 35)	—	—	—	Vitt (1974)

[a]Body temperature at capture.
[b]Radio transmitting data loggers.
[c]Maximal ATPase activity.
[d]Reduction in muscle contraction tension.
[e]Breathing rate and evaporative water loss.
[f]Behavioural avoidance.
[g]ATPase 20% denatured.
[h]Muscle irreversibly damaged.
[i]Loss of righting ability.
[j]Oxygen consumption and heart rate.

2013; Verberk et al., 2016). In addition, if variation in the fundamental thermal niche underlies NAL and SAL biogeography (i.e. SAL tolerate higher temperatures than NAL), we predicted increased mortality under prolonged exposure to acutely near-critical temperatures, a loss of mitochondrial function, or a shift to anaerobic metabolism in NAL but not SAL. Finally, we tested the hypothesis that SAL and NAL differ in their physiological response to pejus temperatures. If so, we predicted no mitochondrial damage from exposure to hot temperatures in either species, but (1) greater energy demand in NAL than SAL resulting from elevated mitochondrial metabolic rate as a consequence of metabolic compensation to cold environments, and (2) greater physiological stress or defence in NAL than in SAL, as demonstrated by shifts in the metabolome. When considered with prior whole-organism data (Table 1), our subcellular performance measures illuminate the mechanisms responsible for limiting function at high temperatures and suggest subtle differences in physiological performance, rather than fundamental tolerances, contribute to the biogeography of alligator lizards.

2 | MATERIALS AND METHODS

2.1 | Thermal environments of NAL and SAL

We leveraged the large number of occurrence records available for NAL and SAL to confirm that NAL generally occupy cooler environments than SAL. We downloaded all occurrence records for each species from the VertNet data portal (version 2016-09-29, www.vertnet.org) and cleaned these records by removing obvious inaccuracies and eggs. This left 3,996 NAL and 4,306 SAL observations (Figure S1). Next, we downloaded BIOCLIM data at 30 arc-second (*c.* 1 km) resolution from the WorldClim database (www.worldclim.org, Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). The BIOCLIM data are composed of 19 biologically relevant climate variables derived from monthly temperature and precipitation data, although we focused on the seven that describe the thermal environment (Table 2). For each NAL and SAL occurrence, we extracted the BIOCLIM variables for the corresponding location. All geographic information system analyses were performed using the SP and RASTER packages in R (Bivand, Pebesma, & Gomez-Rubio, 2013; Hijmans, 2016).

2.2 | Animal collection and laboratory maintenance

We collected adult NAL (N = 19) and SAL (N = 20) during April–July of 2010–2012 from California and transported them to a captive colony at Iowa State University (Table S1; Figure S1a). Our sampling allowed us to examine responses to high temperature where the species overlap as well as where animals might naturally experience pejus temperatures. NALs were collected near the southernmost portion of their range where they overlap with SAL, and SALs were collected where they overlap NAL and farther south (Figure S1a). All SALs derive from the "southern" SAL clade, as described by Feldman and Spicer (2006, mitochondrial DNA evidence suggests that SALs are divided into "southern" and "northern" clades) and represent a single broadly defined population. Given how conserved CT_{MAX}, preferred body temperature and pejus temperatures are for these animals (Table 1), and how little high-temperature tolerance varies among ectotherms in general (Addo-Bediako et al., 2000; Sunday et al., 2011), we expect minimal local adaptation. Animals were housed in the laboratory following standard husbandry protocols (see Telemeco & Addis, 2014 for details). At the onset of our thermal experiments, lizards were in captivity between 7.5 and 29 months (Table S1). Including year of capture in our statistical models did not alter any of our conclusions and thus was not included in final models.

2.3 | Heat stress experiments

We exposed each lizard to either a control or heat stress treatment (24 or 38°C, respectively) for 2.5 hr prior to euthanasia and tissue collection. These temperatures were chosen because 24°C approximates the average activity temperature for both species, and 38°C is well above the pejus threshold but just below CT_{MAX} (Table 1). We assigned lizards of each species uniformly to temperature treatments. To minimize potential external stressors, we kept contact between lizards and researchers to a minimum. We last weighed lizards on 25 September 2012 and provided their last meal on 10 December 2012 before the experiments began on 17 December 2012. Because of the logistical constraints associated with assaying live tissue, we arrayed the experiments over 5 days with lizards randomly assigned to an experiment

TABLE 2 Summary of the thermal environment occupied by *Elgaria coerulea* (NAL) and *E. multicarinata* (SAL). For each BIOCLIM variable examined, $M \pm SD$ for each species, and t, df, and p are presented

BIOCLIM variable (description)	NAL	SAL	t	df	p
BIO1 (annual mean temperature, °C)	10.6 ± 2.5	15.0 ± 2.5	75.7	7,501.8	<.0001
BIO4 (temperature seasonality [*SD*], °C)	4.5 ± 1.7	4.2 ± 1.5	−8.7	7,287.4	<.0001
BIO5 (maximum temperature of warmest month, °C)	24.7 ± 3.7	28.4 ± 4.2	41.4	7,682.7	<.0001
BIO6 (minimum temperature of coldest month, °C)	0.1 ± 4.2	3.8 ± 3.5	41.8	6,950.9	<.0001
BIO7 (annual temperature range, °C)	24.6 ± 6.7	24.6 ± 6.2	−0.3	7,386.4	.8912
BIO10 (mean temperature of warmest quarter, °C)	16.6 ± 2.2	20.6 ± 2.8	69.7	7,568.1	<.0001
BIO11 (mean temperature of coldest quarter, °C)	5.3 ± 3.8	9.9 ± 3.4	56.2	7,176.0	<.0001

day (6–10 lizards daily). During experiments, lizards were housed individually in plastic test chambers (15.6 × 15.6 × 5.7 cm) with air holes. Within their test chambers, we moved lizards to a dark incubation chamber set at 24°C the day prior to their experiment to allow 18 hr acclimation. Lizards and test chambers were then moved to large incubators pre-set to the treatment temperature and illuminated with LED lights. Lizards were not disturbed during treatment and could not be observed. However, we staggered animals in and out of the thermal treatments at 10-min intervals to allow time for post-treatment processing which resulted in brief periods of disturbance at the beginning or end of treatment. We confirmed all chamber temperatures using iButton thermal data loggers (Model DS1921-F5, factory accuracy ±1°C; Maxim Integrated). After 2.5 hr in their treatment, lizards were immediately euthanized by decapitation, pithed and exsanguinated (death within seconds of removal). We first collected whole blood and placed it on ice, then collected liver tissue and placed it in STE buffer solution (250 mM sucrose, 5 mM Tris, 2 mM EGTA, pH 7.4) on ice, and finally collected muscle tissue from the right-rear leg and snap-froze it in liquid nitrogen for storage at −80°C. For all animals, <10 min passed between the end of treatment and collection of all tissues.

2.4 | Plasma physiology

To assess physiological stress at the time of euthanasia, we quantified plasma circulating glucose and corticosterone (CORT; the primary glucocorticoid in reptiles). Immediately following euthanasia and collection of whole blood, we centrifuged the samples (4,700 × *g* for 10 min) and aliquoted plasma for storage at −80°C. We measured glucose from 1.5 μl of the thawed plasma using a FreeStyle Lite Glucometer (Abbott Diabetes Care). CORT was assayed as in Telemeco and Addis (2014) using the Immun-Chem Double Antibody Corticosterone I-125 RIA kit (Cat #07-120103; MP Biomedical). We also examined CORT from 11 additional SAL, housed under identical conditions and exposed to 24°C. These animals were excluded from other experiments (mitochondria, metabolomics) because of time constraints. Their inclusion did not qualitatively alter our CORT results.

2.5 | Mitochondrial physiology

We examined respiration rates and ROS production of fresh mitochondria isolated from liver tissue. We chose liver mitochondria because of the high metabolic activity and large size of livers, which allowed isolation of sufficient mitochondria from individual lizards. All mitochondrial assays were completed within 8 hr of death. To isolate fresh mitochondria, we homogenized the liver tissue (Robert, Brunet-Rossinni & Bronikowski, 2007) and used differential centrifugation to separate mitochondria from the cellular debris (Palloti & Lenaz, 2001; Pon & Schon, 2007). We estimated the concentration of mitochondria within our isolates via Bradford protein determination and used this estimate to standardize our assays (Robert et al., 2007).

We estimated mitochondrial respiration rates by measuring oxygen consumption within an airtight chamber using a Clark-type oxygen electrode (Hansatech) according to established protocols (Brand, Harper, & Taylor, 1993; Herrero & Barja, 1997; Trounce, Kim, Jun, & Wallace, 1996) optimized for reptiles (Robert et al., 2007). Using these data, we calculated State III respiration (oxidative phosphorylation), State IV respiration (proton leak) and respiratory control ratio (RCR) for each individual using the equations of Robert and Bronikowski (2010). We included analyses of RCR to ensure functional mitochondria in all groups. All mitochondrial respiration rates were assayed at 24°C, regardless of whole-animal thermal treatment. Thus, any effects of thermal treatment on mitochondrial respiration indicate plasticity (e.g. acclimation) or damage rather than the direct effect of temperature on metabolism. As an indicator of ROS production by the mitochondria, we measured mitochondrial H_2O_2 production using the Amplex® red hydrogen peroxide/peroxidase assay kit (Invitrogen Molecular Probes) according to the manufacturer's instructions for a 96-well microplate as optimized for reptiles (Schwartz & Bronikowski, 2013). For analyses, we calculated the rate of mitochondrial H_2O_2 production (μmol H_2O_2 min^{-1} mg^{-1}) as the difference between the average 30- and 5-min measurements for each individual, divided by 25 min. For all mitochondrial assays, we assayed samples from each individual in duplicate, and averaged measurements for analyses. See Appendix S1 for further details on the mitochondrial assays.

2.6 | Muscle metabolomics

We examined the muscle metabolome to test for a global shift in metabolic physiology of NAL vs. SAL when exposed to near-critical temperatures and to test the OCLTT hypothesis which predicts an increase in anaerobic metabolism at high temperatures. Metabolite extraction and quantification took place at the W. M. Keck Metabolomics Research Laboratory at Iowa State University (http://www.biotech.iastate.edu/biotechnology-service-facilities/w-m-keck-metabolomics-research-laboratory/). We prepared skeletal muscle from the right-rear limb using two-step methane/water/chloroform extraction (A. J. et al., 2005; Wu, Southam, Hines, & Viant, 2008). Skeletal muscle has high energetic needs and thus may be more rapidly affected by temperature-induced oxygen limitation than other tissues. Polar and non-polar extracts from each sample were analysed using an Agilent 6890 Gas Chromatograph/Mass Spectrometer (GC/MS; Agilent Technologies). Peak areas were transformed to concentration values using internal standards and tissue dry mass using ChemStation software (v. 2.0; Agilent Technologies). Additional details on metabolite extraction and quantification are provided in Appendix S1.

We first filtered the raw GC/MS data to exclude putative metabolites that were not represented in ≥90% of the samples from at least one species by treatment grouping. We identified remaining metabolites using AMDIS software (version 2.71; NIST) to compare peaks to the National Institutes of Standards and Technology database and a proprietary reference library maintained by the W.M. Keck Metabolomics Research Laboratory. We then consolidated the data for metabolites present in both the polar and non-polar fractions, which yielded 190 metabolites for analysis. Finally, we assigned metabolites to one or more functional categories for analyses (amino acids, anaerobic metabolites, antioxidants, endocrine metabolites,

fatty acids, hydrocarbons, organic acids, steroids, sugars, sugar alcohols, TCA metabolites and vitamins) using online databases: Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/KEGG) and PubChem (https://pubchem.ncbi.nlm.nih.gov).

2.7 | Statistical analyses

We performed statistical analyses and data visualization using R statistical software (version 3.1.0, R Core Team, 2016). Prior to analyses, we graphically assessed each univariate dataset for normality and outliers using boxplots, histograms and q–q plots (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We natural-log transformed the CORT, State III, and RCR data, and square-root transformed the H_2O_2 data. We identified no outliers in the plasma glucose or CORT datasets, but identified 2–5 outliers from each mitochondrial dataset, and these points were removed. For all ANOVAs, we began with a full model and used backwards selection to reduce the model using AIC_c and *p*-values (Zuur et al., 2009). We calculated AIC_c using the "aictab" function in the AICcmodavg package (Mazerolle, 2017). We assessed significance using type-III sum of squares with the ANOVA function from the car package (Fox & Weisberg, 2011). To explore statistically significant interactions, we employed Tukey-corrected pairwise tests using the "lsmeans" function in the lsmeans package (Lenth, 2016).

We analysed metabolome data using Euclidean distance-based, nonparametric multivariate analyses of variance (NP-MANOVA; also known as permutational MANOVA; see Anderson, 2001 for details) via the "adonis" function in the vegan package (statistical significance assessed from 1,000 permutations, Oksanen et al., 2017). We used NP-MANOVA because, unlike traditional MANOVA, this method allows analysis when variables outnumber samples (i.e. dependent-variable matrix has more columns than rows, Anderson, 2001). Prior to metabolite analyses, we used scatterplot matrices to graphically examine the multivariate distributions and natural-log-transformed functional groupings as necessary to bring them qualitatively close to multivariate normal. Significant effects revealed by NP-MANOVA might result from either variation in the quantity or relative composition of metabolites. To distinguish between these two possibilities, any time NP-MANOVA suggested statistically significant effects, we also summed the concentrations of all metabolites tested and reanalysed the summed data using ANOVA.

We report actual (i.e. uncorrected) *p*-values because each analysis is effectively the result of an independent experiment (Cabin & Mitchell, 2000; Moran, 2003; Perneger, 1998). Multiple-comparison tests, such as sequential Bonferroni, control study-wide type I error at the expense of type II error; however, both forms of error are equally undesirable for our study. Therefore, rather than employing study-wide, multiple-comparison corrections, we conceptually combat statistical error by basing conclusions on the bulk of our analyses rather than on individual tests. General agreement across independent statistical tests is extremely unlikely to occur by chance alone (Moran, 2003).

3 | RESULTS

3.1 | Thermal environments of NAL and SAL

As predicted, NAL occupy cooler environments than SAL: annual mean temperatures for NAL are 4.4°C cooler than for SAL, on average (Welch two-sample *t* test, Table 2). Each thermal BIOCLIM variable suggests a similar *c*. 4°C difference (Table 2). In addition, NAL occupy slightly more seasonal environments than SAL (annual thermal *SD* 0.3°C greater for NAL, Table 2).

3.2 | Plasma physiology

All animals survived thermal treatment. Behavioural responses varied, but lizards exposed to 38°C generally appeared lethargic compared with animals exposed to 24°C at the time of euthanasia. Temperature treatment and species interacted to affect plasma glucose levels (ANOVA, $F_{1,34} = 13.83$, $p = .0007$, Figure 1a, Table S2). Plasma glucose did not differ between the species at 24°C (Tukey-corrected comparison, $t_{34} = 1.11$, $p = .2755$), but SAL had higher plasma glucose than NAL at 38°C (Tukey-corrected comparison, $t_{34} = -4.15$, $p = .0002$, Figure 1a). Plasma glucose was higher in lizards exposed to 38°C than 24°C in both species (Tukey-corrected comparison, NAL: $t_{34} = -4.614$, $p = .0001$; SAL: $t_{34} = -10.267$, $p < .0001$, Figure 1a). CORT tended to be higher in NAL than in SAL (ANOVA, $F_{1,49} = 3.70$, $p = .0601$), while temperature treatment (ANOVA, $F_{1,49} = 0.08$, $p = .7805$) and its interaction with species (ANOVA, $F_{1,49} = 1.32$, $p = .2559$, Table S2, Figure 1b) were unimportant.

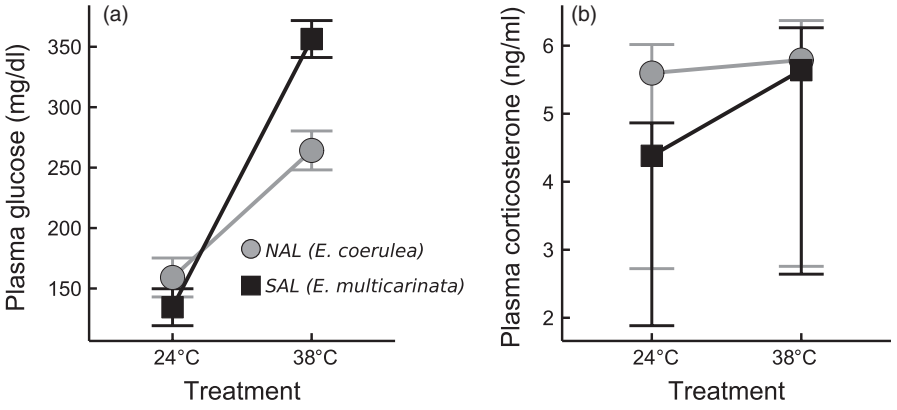

FIGURE 1 Exposure to near-critical high temperature for 2.5-hr elevated plasma glucose (a, $p < .01$) but not plasma corticosterone (b, $p = .78$) in both northern (NAL; *Elgaria coerulea*) and southern (SAL; *E. multicarinata*) alligator lizards. Data are back-transformed least-squares means ± *SEM*

3.3 | Mitochondrial physiology

Neither treatment (ANOVA, $F_{1,34} = 0.10$, $p = .7509$) nor species (ANOVA, $F_{1,34} = 0.56$, $p = .4583$) affected the RCR, and there was no interaction (ANOVA, $F_{1,33} = 0.18$, $p = .6744$, Table S2). The mean (±*SEM*) RCR across all samples was 2.6 ± 0.1, similar to estimates previously reported for reptiles, indicating functional mitochondria (Cassuto, 1971; Robert & Bronikowski, 2010; Robert et al., 2007).

State III respiration (oxidative phosphorylation, ADP present) was higher in NAL than in SAL (ANOVA, $F_{1,34} = 16.04$, $p = .0003$, Figure 2a) and was unaffected by temperature treatment (ANOVA, $F_{1,34} = 1.47$, $p = .2329$) or its interaction with species (ANOVA, $F_{1,33} = 0.34$, $p = .5612$, Table S2). Conversely, mitochondrial H_2O_2 production rate was higher in SAL than in NAL (ANOVA, $F_{1,35} = 5.65$, $p = .0231$), while neither temperature treatment (ANOVA, $F_{1,35} = 1.46$, $p = .2347$) nor the interaction between temperature treatment and species (ANOVA, $F_{1,35} = 1.86$, $p = .1815$) was important (Figure 2b, Table S2).

3.4 | Muscle metabolomics

We calculated mean (±*SEM*) concentration within each treatment by species level for the 190 metabolites that met our criteria for analysis, assigned those metabolites to functional groups, and analysed each functional group (Tables S3–S4). Temperature treatment and species interacted (NP-MANOVA, $F_{1,33} = 2.49$, $p = .013$) to affect metabolite profiles when all metabolites were analysed together (Figure 3, Table S3). This interaction was the result of temperature treatment affecting the metabolite profile of NAL (NP-MANOVA, $F_{1,16} = 2.92$, $p = .022$), but not SAL (NP-MANOVA, $F_{1,19} = 0.854$, $p = .548$). This general pattern of temperature affecting NAL metabolites but not SAL metabolites was evident for numerous functional groups. Of the 12 functional metabolite groupings that we examined, four were affected by temperature treatment in NAL (amino acids, sugars, endocrine metabolites and organic acids), and an additional five (antioxidants, fatty acids, vitamins, sugar alcohols and steroids) trended similarly ($.05 < p < .10$, Table S3). By contrast, temperature treatment never affected metabolite profiles in SAL (all $p > .15$; Table S3). In all but two cases (endocrine metabolites and sugar alcohols), statistically significant effects resulted from metabolite composition rather than metabolite quantity (Table S4).

In both species, heat stress did not affect the overall profile of anaerobic-indicator metabolites (lactic acid, succinate and alanine (Verberk et al., 2013), NP-MANOVA: Treatment: $F_{1,33} = 0.28$, $p = .794$; Species: $F_{1,33} = 1.50$, $p = .222$; Interaction: $F_{1,33} = 2.02$, $p = .132$, Figure 4a) or Krebs cycle metabolites (phosphoric acid, fumeric acid, succinic acid and oxalacetic acid (Verberk et al., 2013), NP-MANOVA: Treatment: $F_{1,33} = 0.12$, $p = .791$; Species: $F_{1,33} = 0.81$, $p = .405$; Interaction: $F_{1,33} = 1.19$, $p = .300$, Figure 4b). To confirm that high temperature failed to elicit a shift to anaerobic metabolism, we examined lactic acid and succinic acid in isolation, similar to more classic approaches (Bennett & Licht, 1972). Neither treatment (ANOVA; lactic acid: $F_{1,31} = 1.16$, $p = .303$; succinic acid: $F_{1,33} = 0.23$, $p = .634$), species (ANOVA; lactic acid: $F_{1,34} = 0.11$, $p = .732$; succinic acid: $F_{1,33} = 0.33$, $p = .570$), nor their interaction (ANOVA; lactic acid: $F_{1,31} = 0.42$, $p = .521$; succinic acid: $F_{1,33} = 0.04$, $p = .849$) affected either of these primary metabolites in skeletal muscle (Figure 4c, d).

4 | DISCUSSION

We used an integrative approach to assess relative physiological performance at high temperatures of congeneric lizards that naturally partition the thermal environment. We predicted that, because SAL occupy warmer habitats, SAL would display increased temperature tolerance (i.e. higher-temperature fundamental niche)

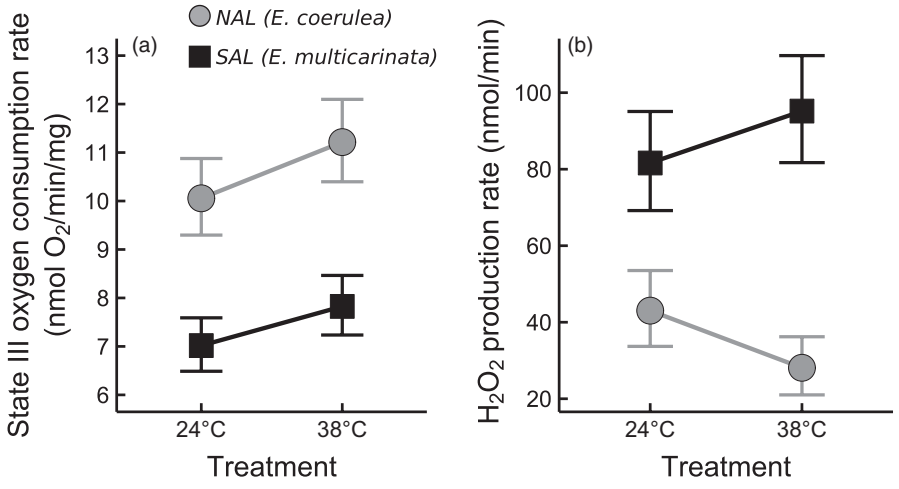

FIGURE 2 Although species differed, temperature treatment (2.5-hr exposure to control or near-critical maximum) had no lasting effect on mitochondrial physiology in alligator lizards. (a) State III respiration (oxidative phosphorylation, ADP present) was higher in northern (NAL; *Elgaria coerulea*) than in southern (SAL; *E. multicarinata*) alligator lizards ($p < .01$), but unaffected by temperature treatment ($p = .23$); (b) H_2O_2 production was higher in SAL than NAL ($p < .01$), but unaffected by temperature treatment ($p = .74$). Data presented are back-transformed least-squares means ± *SEM*. Measurements were made within 8 hr of treatment

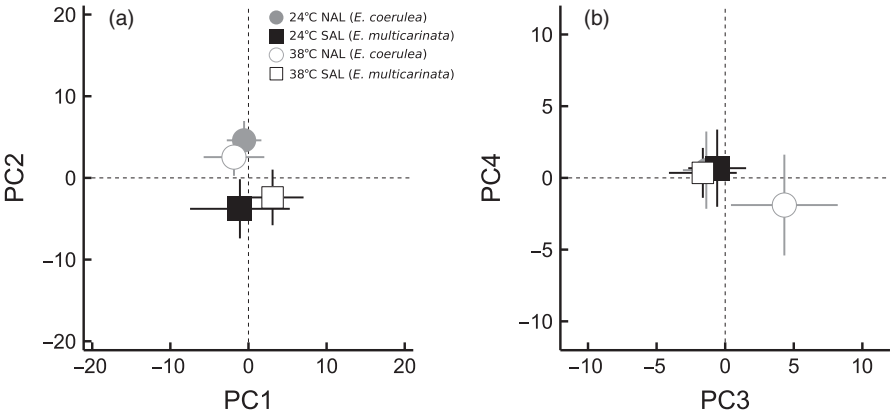

FIGURE 3 Exposure to near-critical high temperature induced a change in the metabolome of northern (NAL; *Elgaria coerulea*, $p = .02$) but not southern (SAL; *E. multicarinata*, $p = .55$) alligator lizards. Plots are principal components plots of metabolites ($N = 190$) from the muscle of lizards exposed to 24°C (control) and 38°C. Points represent the mean for each group ± 95% CI. In b, the mean for 24°C NAL is hidden behind the SAL points

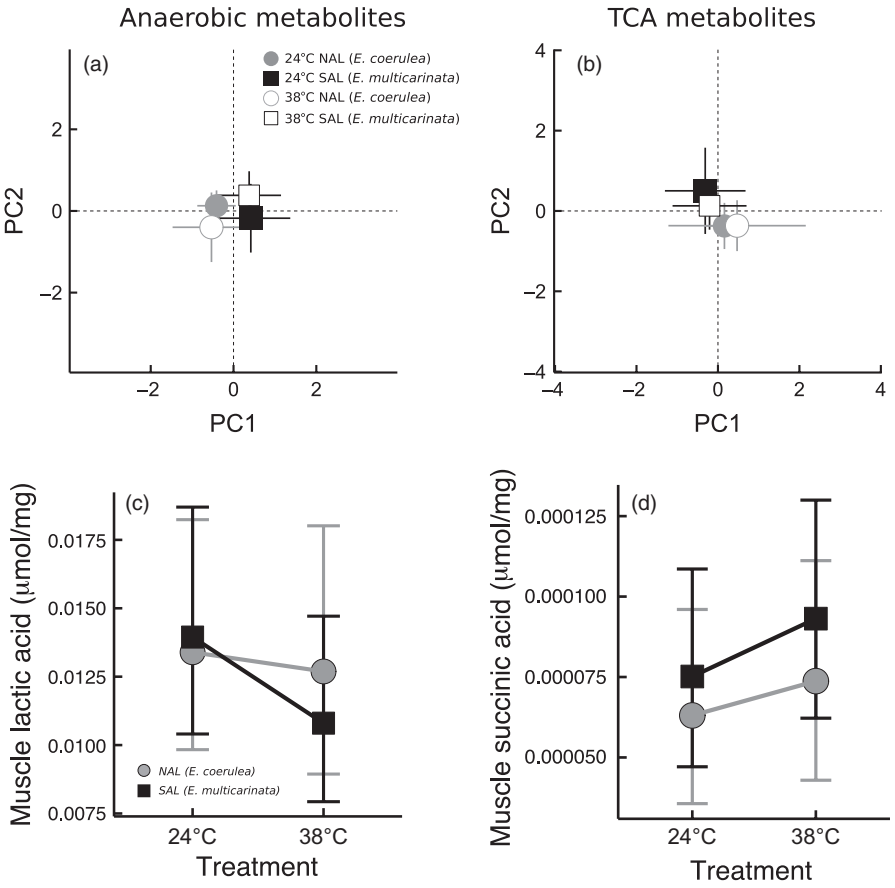

FIGURE 4 Exposure to near-critical maximum temperature (38°C) for 2.5 hr did not alter the profile of anaerobic ($N = 3$) or Krebs cycle (TCA) metabolites ($N = 4$) in the muscle of either northern (NAL; *Elgaria coerulea*) or southern (SAL; *E. multicarinata*) alligator lizards when compared with control lizards (24°C, $P > .1$ for all). a and b are principal components plots for the anaerobic and TCA metabolites, respectively. Points represent mean values ± 95% CI for each group. c and d display the effects of temperature treatment on two key primary metabolites: lactic acid and succinic acid. Data are back-transformed least-squares means ± 95% CI

or greater physiological performance at near-critical temperatures relative to NAL (i.e. potential for temperature to alter the outcome of biotic interactions and thus the realized niche). Our results confirm prior observations that the absolute heat tolerances of SAL and NAL are similar despite a *c*. 4°C difference in thermal environment. Even so, SAL appears more robust to high temperatures: SAL maintained function through prolonged exposure to near-critical temperatures without making the physiological changes apparently required for NAL to maintain function. The mitochondrial metabolic rate of NAL was also higher than SAL suggesting that high temperatures incur a greater energetic cost in NAL. Such differences in robustness and energetics could affect the outcome of competitive interactions (either direct or indirect) between these ecologically similar species.

Our results demonstrate that focusing on thermal limits may obscure physiological adaptations underlying biogeographic patterns. Performance will be compromised by high-temperature exposure below critical thermal limits. Although such reductions in performance will not cause dramatic and rapid die-offs, they could lower the intrinsic rate of increase below one or cause a species to become an inferior competitor: either of which would cause local extinction (Carmona-Catot et al., 2013; Finstad et al., 2011; Pörtner & Knust, 2007; Roff, 1992). Thus, any traits affecting the rate of performance reduction at upper pejus temperatures should be under strong selection as animals

are exposed to thermal stress near their low-latitude and low-elevation range boundaries, or as a consequence of global climate change. Identifying traits that underlie responses to pejus temperatures, such as mitochondrial respiration rate and metabolome homeostasis in alligator lizards, is necessary to predict high-temperature effects on ecological interactions, evolution by natural selection, and population persistence in response to warming environments.

Our observation that lizards of both species in the 38°C treatment displayed elevated plasma glucose concentrations indicate that they were in the midst of a stress response at the time of euthanasia (Bradshaw, 2003; Broom & Johnson, 1993). In addition, we qualitatively observed behavioural variation among the lizards consistent with thermal stress. Immediately following perception of a stressor, vertebrate animals elevate production of catecholamine and glucocorticoid hormones, which act to elevate plasma glucose for use in emergency action (Norris, 2007; Stevenson, Coulson, & Hernandez, 1957). Catecholamines are fast acting: elevating within 1–2 s in birds and mammals and within minutes in reptiles (Akbar, Afroz, & Ali, 1978; Matt, Moore, Knapp, & Moore, 1997; McCarty, 1983; Palme, Rettenbacher, Touma, El-Bahr, & Möstl, 2005). Somewhat surprisingly, we did not observe elevated plasma CORT in lizards exposed to 38°C, despite observing elevated glucose concentrations. This result may indicate that CORT had not yet elevated and plasma glucose was only responding to catecholamine production, or that CORT had already elevated and returned to baseline despite continued heat exposure. Glucocorticoid hormones require longer to elevate than do catecholamines (Akbar et al., 1978; Norris, 2007; Palme et al., 2005), potentially requiring hours to reach peak levels in reptiles (Anderson, Cree, Towns, & Nelson, 2014; Gangloff et al., 2017; Palacios, Sparkman, & Bronikowski, 2012). In alligator lizards, elevated CORT is detectable after 5-hr exposure to 28°C, but not 35°C (Telemeco & Addis, 2014, same individuals as the present study in some cases). These data confirm that CORT responds to temperature in alligator lizards, but suggest temperature may affect the rate of the CORT response. Even so, our results indicate that the lizards exposed to 38°C were acutely stressed at the time of euthanasia, with catecholamines and possibly CORT having acted to elevate glucose.

We were unable to detect signs of either subcellular damage or reduced aerobic respiration in animals of either species exposed to the 38°C treatment, suggesting that critical limits are not set by denaturation or by OCLTT. In addition, surviving exposure to 38°C for 2.5 hr without discernable damage corroborates prior work suggesting that the high-temperature critical limits of NAL and SAL are similar (Brattstrom, 1965; Dawson & Templeton, 1966; Licht, 1964a). If protein or membrane denaturation were responsible for critical limits in alligator lizards, we expect the mitochondria to be compromised. However, our measurements of State III respiration (oxidative phosphorylation), RCR, and H_2O_2 production suggest no lasting damage to proteins or mitochondrial membranes. On the other hand, if critical thermal limits resulted from a limited capacity to deliver oxygen to tissues at high temperatures (OCLTT hypothesis), we would expect animals to increase anaerobic respiration to accommodate reduced aerobic capacity (Fobian, Overgaard, & Wang, 2014; Pörtner & Knust, 2007; Verberk et al., 2013, 2016). For example, in response to limited oxygen during exercise, lizards rapidly increase anaerobic respiration, which is detectable by examining metabolites such as lactic acid (Bennett & Licht, 1972). We did not detect an effect of temperature treatment on the anaerobic metabolites of either species, indicating that lizards were not oxygen limited at near-critical temperatures. This observation adds to growing evidence that the OCLTT mechanism is not important for setting critical thermal limits in adult reptiles (DuBois, Shea, Claunch, & Taylor, 2017; Fobian et al., 2014; Gangloff, Holden, Telemeco, Baumgard, & Bronikowski, 2016). Given our observations, the most parsimonious explanation for the mechanism setting critical limits is that protein or membrane functions underlying key cellular processes are reversibly disrupted at high temperatures (Hochachka & Somero, 2002). Further work is needed to directly test this hypothesis.

Even though exposure to near-critical temperatures did not cause mitochondrial damage or reduce aerobic respiration, the NAL metabolome shifted in response to heat challenge, whereas the SAL metabolome did not. Such physiological changes likely represent compensatory mechanisms that allowed NAL to avoid damage and survive high-temperature exposure. This effect was apparent in the entire skeletal muscle metabolome, as well as for energetically important subgroupings, such as sugars and amino acids. Similarly, the metabolome of *Drosophila melanogaster* flies artificially selected for rapid recovery from cold-induced coma is relatively unaffected by cold exposure, whereas control lines display marked metabolomic responses to cold (Williams et al., 2014). A robust metabolome might generally underlie improved thermal performance within the pejus ranges of animals, possibly indicating greater capacity to maintain homeostasis. However, further work is needed to assess the generality of such metabolomic robustness for allowing animals to maintain high performance at extreme temperatures.

SAL also had higher rates of ROS production by the mitochondria than did NAL. Thus, SAL should incur greater cellular damage as a consequence of respiration at a given temperature than NAL (Finkel & Holbrook, 2000; Schwartz & Bronikowski, 2013), even though NALs have a higher State III respiration rate. Given that NALs and SALs are active across similar body temperatures in nature (Brattstrom, 1965; Cunningham, 1966; Kingsbury, 1994; Stewart, 1984), we predict that reduced ROS production by NALs will allow them to senesce more slowly and have longer life spans than SALs, as observed in other taxa (Dowling & Simmons, 2009; Finkel & Holbrook, 2000; Schwartz & Bronikowski, 2013). Unfortunately, data on the longevity of these lizards are not available.

NAL and SAL differentially responded to stressful high temperatures consistent with their biogeography, even though their critical limits and optima are indistinguishable (Brattstrom, 1965; Cunningham, 1966; Kingsbury, 1994; Sheen, 2001; Stewart, 1984). These physiological differences may contribute to NAL and SAL biogeography by influencing the outcome of competitive interactions. Even so, other aspects of the biology of these species will also affect realized distributions. For example, oviparous reproduction allows SAL to

produce 2–4 times more offspring than NAL are able to produce via viviparity (Goldberg, 1972; Stewart, 1979; Vitt, 1973). This life-history difference could allow SAL to rapidly outnumber and potentially outcompete NAL in warm environments well within the NAL fundamental thermal niche (Pincheira-Donoso, Tregenza, Witt, & Hodgson, 2013). The differences in thermal physiology at upper pejus temperatures that we observed between NAL and SAL should exacerbate this difference in reproductive rate. Thus, differences in the thermal physiology of adults may facilitate biogeographic differences more proximally driven by life history and competition.

Our results highlight the importance of considering all aspects of an organism's biology when attempting to understand the mechanisms underlying biogeographic differences, even when distributions appear straightforwardly governed by an abiotic factor such as temperature. Although the specific physiological patterns and mechanisms that we observed in NAL and SAL will not be universal to other systems, they illustrate how a simple exploration of thermal tolerance can fail to capture physiological adaptation. Moreover, NAL and SAL illustrate how effects of the abiotic environment on organismal function that appear minor in isolation (e.g. high mitochondrial metabolic demand and shifts in the metabolite profile of NAL at high temperature) might have broad consequences when ecological interactions are considered, potentially altering competitive landscapes. As abiotic conditions such as temperature shift as a result of climate change, the impact of novel conditions on relative performance among interacting species within critical limits might frequently drive natural selection and determine the ultimate viability of populations.

ACKNOWLEDGEMENTS

We thank M. Westphal and the U.S. Bureau of Land Management for access to Ft. Ord National Monument and the San Lorenzo Valley Water District for access to Zayante Quarry. For assistance collecting lizards, we thank numerous volunteers including T. Breitman, S. Deering, L. Erickson, C. Feldman, J. Homen, J. Lucas, T. Marino, K. Mondragon, P. Moravcsik, J. Richmond, R. Seymore, M. Telemeco, M. Westphal, K. Wiseman and S. Young. For assistance with the metabolomics analyses, we thank A. Perera and Z. Song of the W.M. Keck Metabolomics Research Laboratory. We also thank B. Nikolau for allowing us access to his laboratory for extracting metabolites. For assistance in the laboratory, we thank M. Barazowski, B. Bodensteiner, A. Brouillette, E. Hernandez, K. Holden, J. Reneker, M. Telemeco and D. Warner. The research was conducted under approved animal care protocols (IACUC #4106893J and #4106894J) and a California Department of Fish and Game permit (SC-11085). The research was supported by grants from the Chicago Herpetological Society, the Ecology, Evolution, and Organismal Biology Department at Iowa State University (ISU) and Sigma Xi to R.S.T, and National Science Foundation grants DEB-0640932 and DEB-1242510 to F.J.J. Further support was received from an U.S. Environmental Protection Agency Science to Achieve Results Fellowship (FP-91723101) and a National Science Foundation GK-12 Fellowship to R.S.T. In addition, R.S.T. was partially supported by the National Science Foundation grant to L.B. Buckley (EF-1065638) and E.J.G. was partially supported by a fellowship from the Office of Biotechnology at ISU.

AUTHORS' CONTRIBUTIONS

R.S.T., A.M.B. and F.J.J. conceived and designed the experiment. The thermal challenge treatment and mitochondrial physiology assays were performed by R.S.T., A.M.B., R.L.P. and G.A.C. Metabolomics data collection and processing was performed by R.S.T. and E.J.G. R.S.T. performed statistical analyses and wrote the initial draft. All authors contributed to interpreting the results and to subsequent drafts.

DATA ACCESSIBILITY

All data, including plasma and mitochondrial physiology, and muscle metabolome data, are available in the Dryad Digital Repository https://doi.org/10.5061/dryad.b5n38 (Telemeco et al., 2017).

ORCID


Rory S. Telemeco http://orcid.org/0000-0002-0767-8565

REFERENCES

A. J., Trygg, J., Gullberg, J., Johansson, A. I., Jonsson, P., Antti, H., … Moritz, T. (2005). Extraction and GC/MS analysis of the human blood plasma metabolome. *Analytical Chemistry*, *77*, 8086–8094.

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B*, *267*, 739–745.

Akbar, H. H., Afroz, H., & Ali, S. S. (1978). Effects of adrenaline on blood sugar and plasma lipid levels of a hibernating lizard during active and hibernation period. *Comparative Biochemistry and Physiology*, *60C*, 205–209.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*, 32–46.

Anderson, L., Cree, A., Towns, D., & Nelson, N. (2014). Modulation of corticosterone secretion in tuatara (*Sphenodon punctatus*): Evidence of a dampened stress response in gravid females. *General and Comparative Endocrinology*, *201*, 45–52.

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. New York, NY: Oxford University Press.

Beck, D. D. (2009a). Northern alligator lizard. In L. L. C. Jones, & R. E. Lovich (Eds.), *Lizards of the American Southwest: A photographic field guide* (pp. 476–479). Tucson, AZ: Rio Nuevo Publishers.

Beck, D. D. (2009b). Southern alligator lizard. In L. L. C. Jones, & R. E. Lovich (Eds.), *Lizards of the American southwest: A photographic field guide* (pp. 484–487). Tucson, AZ: Rio Nuevo Publishers.

Bennett, A. F., & Licht, P. (1972). Anaerobic metabolism during activity in lizards. *Journal of Comparative Physiology*, *81*, 277–288.

Bivand, R., Pebesma, E., & Gomez-Rubio, V. (2013). *Applied spatial data analysis with R* (2nd edn). New York, NY: Springer.

Blackburn, D. G. (1982). Evolutionary origins of viviparity in the Reptilia. I. Sauria. *Amphibia-Reptilia*, *3*, 185–205.

Bradshaw, D. (2003). *Vertebrate ecophysiology: An introduction to its principles and applications*. Cambridge, UK: Cambridge University Press.

Brand, M. D., Harper, M.-E., & Taylor, H. C. (1993). Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes. *Biochemical Journal*, *291*, 739–748.

Brattstrom, B. H. (1965). Body temperatures of reptiles. *American Midland Naturalist*, *73*, 376–422.

Broom, D. M., & Johnson, K. G. (1993). *Stress and animal welfare*. London, UK: Chapman & Hall.

Cabin, R. J., & Mitchell, R. J. (2000). To Bonferroni or not to Bonferroni: When and how are the questions. *Bulletin of the Ecological Society of America*, *81*, 246–248.

Carmona-Catot, G., Magellan, K., & Garcia-Berthou, E. (2013). Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS ONE*, *8*, e54734.

Cassuto, Y. (1971). Oxidative activities of liver mitochondria from mammals, birds, reptiles, and amphibia as a function of temperature. *Comparative Biochemistry and Physiology*, *39B*, 919–923.

Cruz, F. B., Fitzgerald, L. A., Espinoza, R. E., & Schulte, J. A. (2005). The importance of phylogenetic scale in tests of Bergmann's and Rapoport's rules: Lessons from a clade of South American lizards. *Journal of Evolutionary Biology*, *18*, 1559–1574.

Cunningham, J. D. (1956). Food habits of the San Diego alligator lizard. *Herpetologica*, *12*, 225–230.

Cunningham, J. D. (1966). Thermal relations of the alligator lizard *Gerrhonotus multicarinatus webbi*. *Herpetologica*, *22*, 1–7.

Dawson, W. R., & Templeton, J. R. (1966). Physiological responses to temperature in the alligator lizard, *Gerrhonotus multicarinatus*. *Ecology*, *47*, 759–765.

de Blainville, M. H. D. (1835). *Elgaria multicarinata*. *Nouvelles Annales du Muséum D'Histoire Naturelle*, *Paris*, *4*, 298.

Deutsch, C. A., Tewksbury, J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 6668–6672.

Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *Journal of Experimental Biology*, *218*, 1956–1967.

Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of the Royal Society B*, *276*, 1737–1745.

DuBois, P. M., Shea, T. K., Claunch, N. M., & Taylor, E. N. (2017). Effects of oxygen on responses to heating in two lizard species sampled along an elevational gradient. *Journal of Thermal Biology*, *68*, 170–176.

Dunson, W. A., & Travis, J. (1991). The role of abiotic factors in community organization. *American Naturalist*, *138*, 1067–1091.

Feldman, C., & Spicer, G. (2006). Comparative phylogeography of woodland reptiles in California: Repeated patterns of cladogenesis and population expansion. *Molecular Ecology*, *15*, 2201–2222.

Fields, P. A. (2001). Review: Protein function at thermal extremes: Balancing stability and flexibility. *Comparative Biochemistry and Physiology A*, *129*, 417–431.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*, 239–247.

Finstad, A. G., Forseth, T., Jonsson, B., Bellier, E., Hesthagen, T., Jensen, A. J., … Foldvik, A. (2011). Competitive exclusion along climate gradients: Energy efficiency influences the distribution of two salmonid fishes. *Global Change Biology*, *17*, 1703–1711.

Fobian, D., Overgaard, J., & Wang, T. (2014). Oxygen transport is not compromised at high temperature in pythons. *Journal of Experimental Biology*, *217*, 3958–3961.

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd edn). Thousand Oaks, CA: Sage.

Frederich, M., & Pörtner, H.-O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *American Journal of Physiology*, *279*, R1531–R1538.

Gangloff, E. J., Holden, K. G., Telemeco, R. S., Baumgard, L. H., & Bronikowski, A. M. (2016). Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. *Journal of Experimental Biology*, *219*, 2944–2954.

Gangloff, E. J., Sparkman, A. M., Holden, K. G., Corwin, C. J., Topf, M., & Bronikowski, A. M. (2017). Georgaphic variation and within-individual correlations of physiological stress markers in a widespread reptile, the common garter snake (*Thamnophis sirtalis*). *Comparative Biochemistry and Physiology A*, *205*, 68–76.

Gilbert, B., Tunney, T. D., McCann, K. S., DeLong, J. P., Vasseur, D. A., Savage, V., … O'Connor, M. I. (2014). A bioenergetic framework for the temperature dependence of trophic interactions. *Ecology Letters*, *17*, 902–914.

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology and Evolution*, *25*, 325–331.

Goldberg, S. R. (1972). Reproduction in the southern alligator lizard *Gerrhonotus multicarinatus*. *Herpetologica*, *28*, 267–273.

Herrero, A., & Barja, G. (1997). Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon. *Mechanisms of Ageing and Development*, *98*, 95–111.

Hijmans, R. J. (2016). *raster: Geographic data analysis and modelling*. R package version 2.5-8. CRAN. Retrieved from https://CRAN.R-project.org/package=raster

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978.

Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. Oxford, UK: Oxford University Press.

Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In C. Gans, & F. H. Pough (Eds.), *Biology of the Reptilia* (pp. 25–74). New York, NY: Academic Press.

Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: Discussion of approaches. *American Zoologist*, *19*, 357–366.

Kearney, M., & Porter, W. P. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, *12*, 334–350.

Kingsbury, B. A. (1994). Thermal constraints and eurythermy in the lizard *Elgaria multicarinata*. *Herpetologica*, *50*, 266–273.

Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, *69*, 1–33.

Levin, P. H. (1967). *Temperature relations of the northern alligator lizard, Gerrhonotus coeruleus, on Ano Nuevo Island*. Biol Invest Ano Nuevo State Res Ann Rep. Santa Cruz, CA: University of California.

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S., & Angilletta, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. *Proceedings of the Royal Society B*, *282*, 20150837.

Licht, P. (1964a). A comparative study of the thermal dependence of contractility in saurian skeletal muscle. *Comparative Biochemistry and Physiology*, *13*, 27–34.

Licht, P. (1964b). The temperature dependence of myosin-adenosinetriphosphatase and alkaline phosphatase in lizards. *Comparative Biochemistry and Physiology*, *12*, 331–340.

Liles, L. A., Cecala, K. K., Ennen, J. R., & Davenport, J. M. (2017). Elevated temperatures alter competitive outcomes and body condition in southern Appalachian salamanders. *Animal Conservation*. https://doi.org/10.1111/acv.12342 [Epub ahead of print].

Macey, J. R., Shulte, J. A. II, Larson, A., & Tuniyev, B. S. (1999). Molecular phylogenetics, tRNA evolution, and historical biogeography in Anguid lizards and related taxonomic families. *Molecular Phylogenetics and Evolution*, *12*, 250–272.

Matt, K. S., Moore, M. C., Knapp, R., & Moore, I. T. (1997). Sympathetic mediation of stress and aggressive competition: Plasma catecholamines in free-living male tree lizards. *Physiology and Behavior*, *61*, 639–947.

Mazerolle, M. J. (2017). *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)*. R package version 2.1-1. CRAN. Retrieved from https://cran.r-project.org/package=AICcmodavg

McCarty, R. (1983). Stress, behaviour, and experimental hypertension. *Neuroscience and Behavioural Reviews*, *7*, 493–502.

Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, *100*, 403–405.

Norris, D. O. (2007). *Vertebrate endocrinology* (4th ed.). Burlington, MA: Elsevier Academic Press.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). *vegan: Community ecology package*. R package version 2.4-3. CRAN. Retrieved from https://CRAN.R-project.org/package=vegan

Olsen, S. L., Topper, J. P., Skarpaas, O., Vandvik, V., & Klanderud, K. (2016). From facilitation to competition: Temperature-driven shift in dominant plant interactions affects population dynamics in seminatural grasslands. *Global Change Biology*, *22*, 1915–1926.

Palacios, M. G., Sparkman, A. M., & Bronikowski, A. M. (2012). Corticosterone and pace of life in two life-history ecotypes of the garter snake *Thamnophis elegans*. *General and Comparative Endocrinology*, *175*, 443–448.

Palloti, F., & Lenaz, G. (2001). Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. In L. A. Pon, & E. A. Schon (Eds.), *Mitochondria* (pp. 1–35). San Diego, CA: Academic Press.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., & Möstl, E. (2005). Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences*, *1040*, 162–171.

Perneger, T. V. (1998). What's wrong with Bonferroni adjustments. *British Medical Journal*, *316*, 1236–1238.

Pincheira-Donoso, D., Tregenza, T., Witt, M. J., & Hodgson, D. J. (2013). The evolution of viviparity opens opportunities for lizard radiation but drives it into a climate cul-de-sac. *Global Ecology and Biogeography*, *2013*, 1–11.

Pon, L. A., & Schon, E. A. (2007). *Mitochondria* (2nd edn). San Diego, CA: Academic Press.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, *88*, 137–146.

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology A*, *132*, 739–761.

Pörtner, H. O., Bennett, A. F., Bozinovic, F., Clarke, A., Lardies, M. A., Lucassen, M., … Stillman, J. H. (2006). Trade-offs in thermal adaptation: The need for a molecular to ecological integration. *Physiological and Biochemical Zoology*, *79*, 295–313.

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*, 95–97.

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Robert, K. A., & Bronikowski, A. M. (2010). Evolution of senescence in nature: Physiological evolution in populations of garter snake with divergent life histories. *American Naturalist*, *175*, 147–159.

Robert, K. A., Brunet-Rossinni, K. A., & Bronikowski, A. M. (2007). Testing the 'free radical theory of aging' hypothesis: Physiological differences in long-lived and short-lived colubrid snakes. *Aging Cell*, *6*, 395–404.

Roff, D. A. (1992). *The evolution of life-histories*. New York, NY: Chapman and Hall.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*, 1856–1866.

Schwartz, T. S., & Bronikowski, A. M. (2013). Dissecting molecular stress networks: Identifying nodes of divergence between life-history phenotypes. *Molecular Ecology*, *22*, 739–756.

Sheen, J. P.-Y. (2001) *Reproductive cost in the Northern and Southern Alligator Lizards (Elgaria coerulea and E. multicarinata)*. Doctor of Philosophy, University of California, Berkeley, CA.

Shine, R. (2002). Reconstructing an adaptationist scenario: What selective forces favor the evolution of viviparity in montane reptiles. *American Naturalist*, *160*, 582–593.

Somero, G. N. (1995). Proteins and temperature. *Annual Review of Physiology*, *57*, 43–68.

Stebbins, R. C. (2003). *A field guide to western reptiles and amphibians* (3rd edn). New York, NY: Houghton Mifflin.

Stevenson, O. R., Coulson, R. A., & Hernandez, T. (1957). Effects of hormones on carbohydrate metabolism in the alligator. *American Journal of Physiology*, *191*, 95–102.

Stewart, J. R. (1979). The balance between number and size of young in the live bearing lizard *Gerrhonotus coeruleus*. *Herpetologica*, *35*, 342–350.

Stewart, J. R. (1984). Thermal biology of the live-bearing lizard *Gerrhonotus coeruleus*. *Herpetologica*, *40*, 349–355.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B*, *278*, 1823–1830.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, *2*, 686–690.

Tansey, M. R., & Brock, T. D. (1972). The upper temperature limit for eukaryotic organisms. *Proceedings of the National Academy of Sciences of the United States of America*, *69*, 2426–2428.

Telemeco, R. S. (2014). Immobile and mobile life-history stages have different thermal physiologies in a lizard. *Physiological and Biochemical Zoology*, *87*, 203–215.

Telemeco, R. S., & Addis, E. A. (2014). Temperature has species-specific effects on corticosterone in alligator lizards. *General and Comparative Endocrinology*, *206*, 184–192.

Telemeco, R. S., Fletcher, B., Levy, O., Riley, A., Rodriguez-Sanchez, Y., Smith, C., … Buckley, L. B. (2016). Lizards fail to plastically adjust nesting behavior or thermal tolerance as needed to buffer populations from climate warming. *Global Change Biology*, *23*, 1075–1084.

Telemeco, R., Gangloff, E., Cordero, G., Polich, R., Bronikowski, A., & Janzen, F. (2017). Data from: Physiology at near-critical temperatures, but not critical limits, varies between two lizard species that partition the thermal environment. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.b5n38

Trounce, I. A., Kim, Y. L., Jun, A. S., & Wallace, D. C. (1996). Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines. *Methods in Enzymology*, *264*, 484–509.

Verberk, W. C. E. P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., & Terblanche, J. S. (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comparative Biochemistry and Physiology A*, *192*, 64–78.

Verberk, W. C. E. P., Sommer, U., Davidson, R. L., & Viant, M. R. (2013). Anaerobic metabolism at thermal extremes: A metabolomic test of the oxygen limitation hypothesis in an aquatic insect. *Integrative and Comparative Biology*, *53*, 609–619.

Vitt, L. J. (1973). Reproduction biology of the anguid lizard, *Gerrhonotus coeruleus principis*. *Herpetologica*, *29*, 176–184.

Vitt, L. J. (1974). Body temperatures of high latitude reptiles. *Copeia*, *1974*, 255–256.

Wiegmann, A. F. A. (1828). Beyträge zur Amphibien Kunde. *Isis von Oken*, *21*, 364–383.

Williams, C. M., Watanabe, M., Guarracino, M. R., Ferraro, M. B., Edison, A. S., Morgan, T. J., … Hahn, D. A. (2014). Cold adaptation shapes the robustness of metabolic networks in *Drosophila melanogaster*. *Evolution*, *68*, 3505–3523.

Wu, H., Southam, A. D., Hines, A., & Viant, M. R. (2008). High-throughput tissue extraction protocol for NMR and MS-based metabolomics. *Analytical Biochemistry*, *372*, 204–212.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer Science+Business Media.

Zweifel, R. G. (1958). Notes on reptiles and amphibians from the Pacific coastal islands of Baja California. *American Museum Novitates*, *1895*, 17.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Telemeco RS, Gangloff EJ, Cordero GA, Polich RL, Bronikowski AM, Janzen FJ. Physiology at near-critical temperatures, but not critical limits, varies between two lizard species that partition the thermal environment. *J Anim Ecol*. 2017;86:1510–1522. https://doi.org/10.1111/1365-2656.12738