USDA Wildlife Services - Staff Publications

U.S. Department of Agriculture: Animal and Plant Health Inspection Service

2023

Invasive frogs show persistent physiological differences to elevation and acclimate to colder temperatures

Jack R. Marchetti

Karen H. Beard

Emily E. Virgin

Erin L. Lewis

Steven C. Hess

See next page for additional authors

Authors

Jack R. Marchetti, Karen H. Beard, Emily E. Virgin, Erin L. Lewis, Steven C. Hess, Kwanho C. Ki, Layne O. Sermersheim, Adriana P. Furtado, and Susannah S. French

Invasive frogs show persistent physiological differences to elevation and acclimate to colder temperatures

Jack R. Marchetti [a], Karen H. Beard [b,*], Emily E. Virgin [a,1], Erin L. Lewis [a], Steven C. Hess [c], Kwanho C. Ki [a], Layne O. Sermersheim [a], Adriana P. Furtado [d], Susannah S. French [a]

[a] Department of Biology and the Ecology Center, Utah State University, Logan, UT, 84322, USA
[b] Department of Wildland Resources, Utah State University, Logan, UT, 84322, USA
[c] USDA APHIS-WS National Wildlife Research Center, Hawaii Field Station, Hilo, HI, 96720, USA
[d] Departamento de Ciências Animais, Universidade de Brasília, Distrito Federal, 70910900, Brazil

ABSTRACT

The coqui frog (*Eleutherodactylus coqui*) was introduced to the island of Hawai'i in the 1980s and has spread across much of the island. Concern remains that this frog will continue to expand its range and invade higher elevation habitats where much of the island's endemic species are found. We determined whether coqui thermal tolerance and physiology change along Hawai'i's elevational gradients. We measured physiological responses using a short-term experiment to determine baseline tolerance and physiology by elevation, and a long-term experiment to determine the coqui's ability to acclimate to different temperatures. We collected frogs from low, medium, and high elevations. After both the short and long-term experiments, we measured critical thermal minimum (CTmin), blood glucose, oxidative stress, and corticosterone levels. CTmin was lower in high elevation frogs than low elevation frogs after the short acclimation experiment, signifying that they acclimate to local conditions. After the extended acclimation, CTmin was lower in frogs acclimated to cold temperatures compared to warm-acclimated frogs and no longer varied by elevation. Blood glucose levels were positively correlated with elevation even after the extended acclimation, suggesting glucose may also be related to lower temperatures. Oxidative stress was higher in females than males, and corticosterone was not significantly related to any predictor variables. The extended acclimation experiment showed that coquis can adjust their thermal tolerance to different temperatures over a 3-week period, suggesting the expansion of coqui into higher elevation habitats may still be possible, and they may not be as restricted by cold temperatures as previously thought.

1. Introduction

Changing climatic conditions along elevational gradients are thought to contribute to limiting species distributions (von Humboldt, 1849; Janzen, 1967). For example, thermal environments change significantly with elevation, with temperatures dropping on average 6.5 °C with every 1000 m increase in elevation (Briggs et al., 1997). Differing temperatures across elevations can act as strong selective forces for ectotherms in particular due to their sensitivity to thermal environments (Wang et al., 2022). Previous research supports this idea and shows the thermal physiology (e.g., critical thermal limits, body temperature) of ectotherms often differs along elevational gradients and in response to the elevation at which they are found (Navas, 2006; Navas et al., 2013).

Furthermore, many studies show a strong decline in critical thermal minimum (CTmin) with elevation suggesting that ectotherms adapt to deal with temperature barriers (Muñoz et al., 2014; Pintanel et al., 2019; von May et al., 2017).

Thermal tolerance of invasive amphibians is of particular interest because of their potential negative impacts to native ecosystems and their potential to spread into undisturbed areas, often beyond hypothesized limits (Cortes et al., 2016). For example, in both Australia (McCann et al., 2014; Winwood-Smith et al., 2015) and Florida, USA (Mittan and Zamudio, 2019), the thermal tolerance of non-native cane toads (*Rhinella marina*) collected at different latitudes and elevations has been studied. Cane toads are thought to have evolved a narrow thermal tolerance in their native tropical region and have a limited ability to

function in cold conditions (Angilletta et al., 2003). Yet, the cane toad's thermal tolerance in its invasive range has a surprising amount of plasticity and acclimation potential (McCann et al., 2014; Mittan and Zamudio, 2019; Winwood-Smith et al., 2015). These types of studies are important in determining the thermal limits of an invasive species, and the extent to which they can invade habitats outside their native thermal environment. Yet critical thermal limits are just one of many traits which undergo changes as ectotherms are exposed to new climates.

Other physiological metrics, such as those related to energy metabolites, are often overlooked when examining an invasive species' response to novel temperatures. For example, circulating glucose levels have been shown to be higher in frogs found at high elevation relative to low elevation congeners (Carvajalino-Fernández et al., 2021). Here, glucose is likely acting as a cryoprotectant against extracellular ice formation from freezing temperatures at high elevations, similar to high latitudes (Costanzo et al., 2015; Storey, 1987, 1997). Glucose levels have also been shown to be elevated in autumn months in anurans, presumably to allow them to remain active during cooler conditions and in anticipation of freezing winter conditions (Farrar and Frye, 1977; Rocha and Branco, 1998). Therefore, invasive anurans may similarly increase glucose levels as they spread into habitats with colder temperatures. Although, some studies show that amphibians acclimated to colder temperatures have lower blood glucose than the warm treatments (Hermansen and Jørgensen 1969; Jungreis 1970; Jungreis and Hooper 1970). Therefore, changes in glucose levels in response to temperature is not straightforward.

Likewise, physiological metrics related to an individual's the body condition and stress response can change as a result of novel temperatures. Some studies have found oxidative stress, a measure of cellular damage from reactive oxygen metabolites produced during metabolism (Andersson et al., 2018; Frisard and Ravussin, 2006), and antioxidant defenses decrease with lower temperatures (Reguera et al., 2014; Souri and Rastegar-Pouyani, 2018), while another study found it increased (Bury et al., 2018). Studies of corticosterone, the predominate glucocorticoid and energy mobilizing hormone in many ectotherms, and temperature have also found varying responses. Different studies have found temperature increases (Hudson et al., 2020a,b; Narayan et al., 2012), decreases (Dupoué et al., 2013, 2018), and does not influence corticosterone levels (Jessop et al., 2018). While the relationships between oxidative status, corticosterone, and temperature are complex, both are critical to understand because they elucidate whether an invasive species can effectively manage stress when faced with novel climatic conditions. Studying these metrics in combination with energy metabolites such as glucose can highlight the physiological costs associated with spreading into new climates.

The coqui frog is a nocturnal, terrestrial (i.e., they do not require bodies of water to breed), direct-developing frog endemic to Puerto Rico, but is invasive on the island of Hawai'i. First introduced in the late 1980s (Kraus et al., 1999), coqui have been shown to influence native and non-native species populations in ways that can have detrimental impacts on native Hawaiian species and habitats. These include increasing non-native mongoose abundance (Hill et al., 2019), serving as a food source for non-native birds (Smith et al., 2018), altering invertebrate communities (Choi and Beard, 2012), and increasing available nutrients which favors non-native plant growth (Sin et al., 2008). The coqui has spread across much of the island, although its distribution was historically limited mainly to low elevation habitats on the eastern side of the island. It is thought that its distribution was mainly restricted by temperature, as is seen in other invasive ectotherms (Lin et al., 2019) because while it occupies the highest peaks in its native range, which reach 1100 m, the highest peaks in Hawai'i exceed 4000 m where temperatures regularly drop below freezing (Zhang et al., 2017). Additionally, coqui introduced to Hawai'i were from the areas around San Juan, Puerto Rico (Peacock et al., 2009; Velo-Antón et al., 2007), suggesting their thermal tolerance was adapted to warm, lowland temperatures. Previous research has looked into the thermal tolerance of coqui frogs in both their native range (Christian et al., 1988; Delgado-Suazo and Burrowes, 2022) and in Hawai'i (Haggerty, 2016), but have not found elevational differences in CTmin. However, Haggerty (2016) demonstrated flexibility in cold tolerance among populations and acclimation to cold temperatures. This, along with the coqui frog's current presence in high elevation environments (e.g., Hawai'i Volcanoes National Park, ~1200 m), suggests temperature may not be as strong a barrier as previously thought.

The current study tested the effects of an elevational – and resultant climatic – gradient on the thermal physiology of the invasive coqui, and whether temperature at higher elevations may act as a barrier to further expansion. We measured the CTmin of coqui frogs from low, medium, and high elevations shortly after collection in the field (3-days) to test for basal differences in CTmin among elevations. We then conducted an extended acclimation experiment (3-weeks) where we exposed frogs to a high or low temperature treatment meant to represent ambient temperatures at low and high elevations, respectively, to test whether CTmin has acclimated to local temperature conditions at each elevation – evidence of phenotypic change – or if CTmin is plastic in response to temperature changes over time. We chose to measure CTmin because of its strong relationship with elevation and its importance for surviving nocturnal temperatures and high elevational climates (Muñoz, 2022; Muñoz et al., 2014). We also measured physiological indicators related to thermal tolerance and stress – specifically glucose, reactive oxygen metabolites (dROMs; an indicator of oxidative stress), and corticosterone – to determine whether the different thermal environments across elevation were causing a physiological response by the coqui. Finally, because sex can influence physiology (French et al., 2021; Haggerty, 2016; Moore and Jessop, 2003; Webb et al., 2019), we included sex and body size – which is correlated with sex in coqui frogs (O'Neill et al., 2018) – in our analyses to test their importance compared to elevation.

Our predictions were based on evidence of other phenotypic changes to the coqui population in Hawai'i across elevation (Gayle, 2020; O'Neill et al., 2018; O'Neill and Beard, 2011), and on evidence of narrow thermal tolerance breadths and plasticity in lowland tropical species (Ghalambor et al., 2006). We predicted that CTmin would be lower for frogs at higher elevations relative to low elevations, regardless of acclimation treatment, sex, or body size because of their regular exposure to cold environmental temperatures. Further, we predicted that glucose levels would be higher in high elevation frogs because it can serve as a cryoprotectant and as an energy source to cope with low, non-freezing temperatures. We also predicted glucose would not differ by sex or body size. We expected relatively low corticosterone and dROM levels in high elevation and cold-acclimated frogs due to prolonged exposure to cold temperatures, which would diminish the stress severity of acute cold temperatures of CTmin testing. Finally, we predicted dROMs would be higher in females relative to males due to the oxidative costs of reproduction (French et al., 2021; Webb et al., 2019).

2. Material and methods

2.1. Study site

We collected frogs from 12 sites across the eastern side of the island of Hawai'i, USA (Fig. 1). Our sites were visited twice; first between 25 June and July 8, 2021 to collect frogs for the short acclimation experiment, then between 13 and July 27, 2021 to collect frogs for the extended acclimation experiment. Although we collected different individuals for our two acclimation groups, we collected all frogs within five weeks of each other. Therefore, we assumed the thermal physiology of frogs collected for both acclimation groups would be similar because not enough time passed for seasonal changes in physiology to occur (Schroeder, 1993). We had four field sites located at low elevation (<150 m), four at medium elevation (350–550 m), and four at high elevation (>750 m). At each site, we employed three temperature loggers (Thermochron iButtons; Embedded Data Systems) by attaching
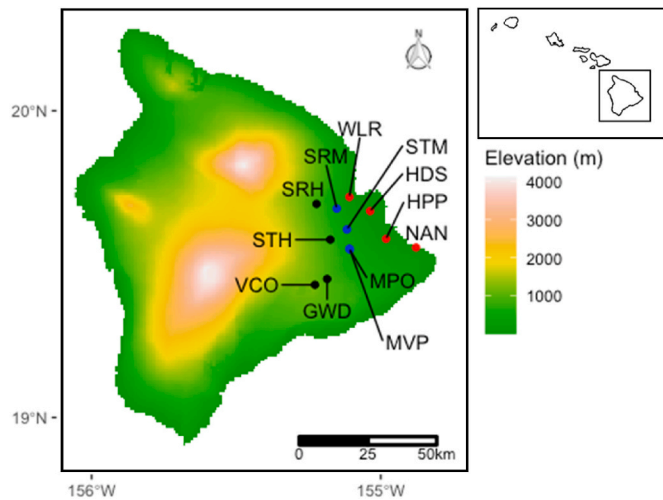
Fig. 1. Twelve coqui sampling sites in the present study on the island of Hawai'i. Site abbreviations are Glenwood (GWD); Hilo Drag Strip (HDS); Hawaiian Paradise Park (HPP); Mountain View Post Office (MPO); Mountain View Park (MVP); Nanawale Natural Area Reserve (NAN); Saddle Road High (SRH); Saddle Road Mid (SRM); Stainback Hwy High (STH); Stainback Hwy Mid (STM); Volcano (VCO); Wailuku River State Park (WLR). Low elevation sites are in red, mid-elevation are in blue, and high elevation sites are in black. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

them to trees at different heights off the forest floor (0, 1, 2 m) and shading them from direct sunlight via opaque plastic coverings. We left them in the field for eight weeks where they recorded ambient temperature every 15 min and showed mean temperature at low, medium, and high elevations to be 23 °C, 20 °C, and 18 °C, respectively, during the study. Annual mean temperatures at low, medium, and high elevation are 22.8 °C, 19.4 °C, and 15.6 °C, respectively (Giambelluca et al., 2014). Our temperature loggers recorded significantly different daily minimum temperatures among elevations (Appendix A), indicating nocturnal temperatures were consistently different among elevations. All of our sites were forested habitats, with dominant species varying by site. Dominant canopy species included *Bambusa vulgaris*, *Metrosideros polymorpha*, *Psidium cattleianum*, and dominant understory species included *Dicranopteris linearis*, *Hedychium* sp*.*, and *Melastoma malabathricum* (Choi and Beard, 2012). Across our sites, mean annual precipitation ranges from 1000–6000 mm/year (Price, 1983).

2.2. Animal collection

Both times we visited each site, we collected frogs between 1900 h and 0100 h, until 30 frogs were captured. We searched for frogs visually and captured them by hand within a 1 km radius of a central GPS point. We only captured frogs >25 mm to exclude juveniles from our study (Woolbright, 2005). Because we did not determine the sex until after euthanasia (see below), the sex ratio for both experiments was not balanced. Our sampling was male biased because calling males are easier to collect, and reflects the male-biased sex ratio of the species in Hawai'i (Woolbright et al., 2006). We received approval for all handling and procedures from Utah State University Institutional Animal Care and Use Committee, protocol #10250 and by the USDA NWRC Institutional Animal Care and Use Committee, protocol #QA-3350.

2.3. Short acclimation conditions

After collection, we placed frogs in plastic terrariums and transported them to the USDA-APHIS National Wildlife Research Center (NWRC) Field Station in Hilo, Hawai'i. We housed each frog we brought to the field station individually in 0.5 L plastic containers with a moist

sponge and we provided vitamin-dusted crickets and water *ad libitum*. We kept the frogs in a room with a 12L:12D light cycle and at a mean temperature of 23.6 °C ($\pm$0.9 SD). In Puerto Rico, high elevation coqui have been shown to prefer lower temperatures relative to low elevation frogs in thermal selection trials (Delgado-Suazo and Burrowes, 2022); however, due to logistical constraints we could only house frogs at ca. 23 °C, but all animals were housed and treated consistently. We kept all frogs in these conditions for 72 h prior to testing thermal tolerance. We used 3 days for the short acclimation period because anuran CTmin can change in response to temperatures experienced within the previous 12 h (McCann et al., 2014). Therefore, uniform conditions prior to CTmin testing were critical for assessing basal differences among populations. We fasted frogs 12 h prior to testing to reduce the impacts of food and digestion on metabolism, thermal tolerance, and other related physiological metrics (e.g., blood glucose). If fasting introduced stress to the frogs, the effect would have been similar for all animals.

2.4. Extended acclimation conditions

After we collected an additional 30 frogs from the same 12 field sites between 1900 h and 0100 h (as previously described), we shipped these coqui frogs overnight to a laboratory at Utah State University where they were randomly assigned to one of two acclimation temperature groups: 16 °C or 23 °C. These temperatures correspond to mean ambient temperatures at high and low elevation habitats in Hawai'i. We housed frogs in incubators (Percival Scientific Incubator Model DR-36VL, Perry, Iowa, USA) set to 16 °C or 23 °C ($\pm$0.1 SD) according to their assigned acclimation treatment for 3 weeks, after which their CTmin was tested. We assumed a 3-week acclimation was adequate because 1-week acclimation periods have been sufficient to induce changes in thermal tolerance in other anurans (Mittan and Zamudio, 2019). Our incubators were set to maintain 80% humidity and 12L:12D light cycle for the entire acclimation period. We housed each frog individually in 0.5 L plastic containers with a moist sponge and a small plastic cup as a retreat site. Our frogs were misted with water daily and given crickets *ad libitum,* and were fasted 12 h prior to CTmin testing. Frogs acclimated to lower temperatures may have had slower specific dynamic actions, and therefore 12 h may not have been long enough for these frogs to be fasted.

2.5. Critical thermal minimum testing

We used a custom-built Temperature Regulated Chamber System (TReCS) to test CTmin (Appendix A). The TReCS is a device in which five aluminum cradles sit on top of an aluminum heatsink. Each cradle is shaped to hold a clear plastic tube. The cradle temperature is controlled by two peltier devices (CUI Devices, Lake Oswego, Oregon, USA) beneath each cradle that can reach temperatures from −3 to 50 °C. Fans built into the heatsink are crucial to the TReCS's ability to reach low temperatures. The temperature of each cradle is monitored on a liquid-crystal display. We began CTmin testing at 2000h after the 3 day and 3 week acclimation periods for the short and extended acclimation frogs, respectfully. Each trial consisted of testing the CTmin of five frogs in the TReCS. Because we collected and tested 30 frogs per night, we ran 6 trials per night after collections. At the beginning of each trial, we placed frogs in the plastic tubes and the TReCS began cooling the cradles starting at 20–23 °C. Each plastic tube was sealed to prevent air from flowing through them and subsequent evaporative water loss by frogs. The TReCS cradles cooled at a mean rate of 0.97 $\pm$ 0.32 °C min^{-1} (SD), varying slightly by cradle number (range = 2.07 °C). Once a cradle temperature reached 15 °C, we periodically spun each tube on the cradles to flip the frog on its back and elicit a righting response. If the frog did not right itself after 30 s, we recorded the TReCS cradle temperature (righting loss temperature; hereafter RL-temp), then removed the frog from its tube and recorded its body temperature using a thermocouple sensor inserted into the cloaca (Model TP870, Extech Instruments,

Nashua, New Hampshire, USA). While cradle temperature differed slightly from the frog's internal body temperature, the latter being a true measure of CTmin, we chose this measurement because previous work has shown handling small frogs for even a few seconds can alter body temperature (Navas and Araujo, 2000). Therefore RL-temp served as a close and consistent approximation of CTmin, but not an exact measurement. We also recorded the elapsed time from the start of the trial to the time of righting loss (righting-loss time; hereafter RL-time). This was also important for approximating the coqui frog's CTmin, assuming frogs with lower CTmin will be able to tolerate colder temperatures for longer periods of time before losing their righting response. After losing righting response, we anesthetized frogs by submersion in a room-temperature benzocaine-water bath (>250 mg/L). Previous work did not find effects of benzocaine on blood metrics (Haggerty, 2016; von May et al., 2019). Moreover, all animals in this study are treated consistently for anesthesia, euthanasia and blood sampling so any effects would be consistent across all animals. Once unresponsive, snout-vent length (SVL; ±0.1 mm) was measured. We then euthanized coqui via rapid decapitation and a blood sample was collected using a heparinized capillary tube, to measure physiological responses to thermal testing (glucose, dROMs, corticosterone). Then, we determined sex by the presence of testes and/or eggs.

We spun collected blood samples for 10 min using a field centrifuge. We separated plasma from red blood cells and measured it using a Hamilton syringe (±1 μL), and then placed plasma in a separate tube. We stored blood and plasma samples collected in Hawai'i in a 0 °C freezer until we overnight shipped them to Utah State University. We stored all blood and plasma samples in a −80 °C freezer at Utah State University until we conducted assays.

2.6. Assays

We conducted several assays to assess the physiology of the coqui frogs in both acclimation groups after testing their thermal tolerance. Using an Accu-Chek Active blood-glucose meter (Roche Diagnostics, Indiana, USA), we measured circulating glucose concentration (mg/dL) with a drop of blood collected immediately after euthanasia. We measured reactive oxygen metabolites (dROMs; mg H_2O_2/dL).) using an assay kit (MC435, Diacron International, Italy), which detects levels of hydroperoxides that oxidize an alkyl-substituted aromatic amine (A-NH2) to assess chronic oxidative stress. Using the manufacturers instructions for 'endpoint' mode and an established protocol outlined by (French et al., 2017), we diluted each sample in the buffer solution provided (5 μl:100 μl) and we ran samples in duplicate on a 96-well microplate. This resulted in a color change that was measured with a spectrophotometer at 505 nm (xMark; Bio-Rad, Hercules, California, USA). We calculated values as absorbance change relative to the standard. The intra-assay coefficient of variation (CV) was 1.6%. Finally, we measured plasma corticosterone (ng/mL) as a proxy of the frog's acute stress response after exposure to extreme cold temperatures in the TReCS. We used an enzyme-linked immunosorbent assay (ELISA) kit (ADI-900-097, Enzo Life Sciences, Farmingdale, NY) following a validation protocol described by Hudson et al. (2020a,b) for blood plasma (8 μl). The corticosterone ELISA is based on competitive binding between the sample hormone and a corticosterone conjugate to sheep polyclonal antibodies and a microplate coated with donkey anti-sheep immunoglobulin. The intra-assay CV was 9.69% (short acclimation group) and 2.48% (extended acclimation group), and the interassay CV was 7.07%.

2.7. Statistical analyses

All analyses were run in R version 4.1 (R Core Team, 2019). To test our first hypothesis, generalized linear mixed models were built using the lme4 package (Bates et al., 2015) to test the effects of several predictor variables (see below) on CTmin, glucose, dROMs, and corticosterone in both our short and extended acclimation groups. Because both RL-temp and RL-time described a coqui frog's ability to withstand cold TReCS cradle temperatures and were therefore important components of thermal tolerance assessment, we conducted a principal component analysis (PCA) using these two variables and the first principal component (PC1) was used as a proxy for CTmin (hereafter CTmin index). In our models built for the short acclimation group, 'elevation' (categorical; low, med, or high), 'SVL' (continuous), and 'sex' (categorical; male, female) served as fixed effects, and the TReCS cradle number (categorical; 1–5) and field sites within elevation served as random effects. We ran six models for each response variable: a null model, three models each containing one fixed effect, and two models with an additive combination of elevation and SVL, or elevation and sex. We did not run SVL and sex in the same model as they were found to be highly correlated (short acclimation: Spearman's $\rho = -0.44$; extended acclimation: Spearman's $\rho = -0.5$). For the extended acclimation group, we used the same fixed effects as above, and added acclimation temperature (categorical; 16 or 23 °C) and an interaction between elevation and acclimation temperature. We used the same random effects as above. We ran nine models for each response variable: a null model, five models each containing one fixed effect, and three models with an additive effect between elevation and acclimation temperature, elevation, acclimation temperature and SVL, and elevation, acclimation temperature, and sex.

We used a model selection analysis using the *AICcmodavg* package (Mazerolle, 2020) for both acclimation groups, which ranks models based on Akaike information criterion (AIC_c), delta AIC_c (Δ AIC_c) and Akaike weight (W_i). We considered models with the lowest AIC_c and Δ $AIC_c < 2$ to be top models that best fit the data (Burnham and Anderson, 2004). We conducted a Shapiro-Wilk normality test on the residuals of our top model, as well as visual inspection of model residuals to check the validity of model assumptions using the *DHARMa* package (Hartig, 2021). We ran a Type II marginal Sum of Square test on top models to obtain F ratios and determine predictor variable significance using the *car* package (Fox and Weisberg, 2019). Finally, we ran pairwise comparisons to examine differences in response variable between categorical predictor variables on top model using the *emmeans* package (Lenth, 2021).

3. Results

3.1. Short acclimation group

After removing tests where the TReCS did not cool, we included a total of 306 individuals (n = 30 females, n = 276 males; Table A.1) in the analyses. The proportion of variance held by the first principal component (PC1) between RL-temp and RL-time was 0.505. PC1 eigen values were 0.707 (RL-temp) and −0.707 (RL-time), meaning a lower PC1 is indicative of a lower CTmin. Females were evenly distributed across elevations, and results were consistent with and without females included in the analyses.

The first of our top models for the CTmin index (PC1) contained elevation as the only fixed effect (Table 1; $F_{2,9} = 8.49$, P = 0.0086). Pairwise comparisons showed frogs from high elevations had significantly lower CTmin index than low elevation frogs (Fig. 2A; $t_{14} = -4.1$, P = 0.0029). Sum of square analyses showed that elevation was also significant in other top models (Table 1; second model: $F_{2,9} = 8.59$, P = 0.0082; third model: $F_{2,9} = 8.59$, P = 0.0084). Mean RL-temp (±SE) was 4.06 °C (±0.21), 3.74 °C (±0.19), and 3.08 °C (±0.11) for frogs collected from low, mid-, and high elevation, respectively (Table 2).

Our top model for glucose contained sex as a fixed effect (Table 1; $F_{1,295} = 26.8$, P < 0.0001), while pairwise comparisons showed glucose was significantly higher in females than males (Fig. 3A; $t_{298} = 5.17$, P < 0.0001). We confirmed this difference in glucose was driven by sex and not body size by removing females from our dataset and running a linear regression between glucose and SVL, which yielded insignificant results

Table 1

Model selection of linear mixed models analyzing the effects of multiple variables on CTmin index, glucose, dROMs and corticosterone in both acclimation groups. Only the top four models are shown here. Bold font indicates that the predictor variable is significant in subsequent sum of squares analyses (i.e., $p < 0.05$). Abbreviations: AIC_c, Akaike information criterion; ΔAIC_c, difference between model of interest AIC_c and lowest AIC_c in the model set; W_i, Akaike model weight; K, number of parameters.

Short Acclimation Group					
Response Variable	Model	AIC_c	ΔAIC_c	W_i	K
CTmin Index (PC1) ($n =$ 306)	**Elevation**	811.66	0.00	0.55	6
	Elevation + Sex	813.47	1.81	0.22	7
	Elevation + SVL	813.50	1.84	0.22	7
	Null	819.91	8.25	0.01	4
Glucose ($n = 300$)	**Sex**	2277.69	0.00	0.79	5
	Elevation + Sex	2280.39	2.70	0.21	7
	SVL	2298.42	20.73	0.00	5
	Elevation + SVL	2301.37	23.68	0.00	7
dROM ($n = 107$)	**Sex**	573.20	0.00	0.82	5
	Elevation + Sex	576.55	3.35	0.15	7
	SVL	580.50	7.30	0.02	5
	Elevation + SVL	584.89	11.69	0.00	7
Corticosterone ($n = 198$)	Null	3215.47	0.00	0.43	4
	Sex	3217.05	1.58	0.19	5
	SVL	3217.29	1.82	0.17	5
	Elevation	3218.16	2.70	0.11	6

Extended Acclimation Group					
Response Variable	Model	AIC_c	ΔAIC_c	W_i	K
CTmin Index (PC1) ($n = 317$)	Elevation + **Acclimation** + **SVL**	702.87	0.00	0.81	8
	Elevation*Acclimation	706.21	3.34	0.15	9
	Elevation + Acclimation + Sex	710.69	7.82	0.02	8
	Acclimation	711.36	8.49	0.01	5
Glucose ($n = 314$)	**Elevation** + Acclimation	2232.56	0.00	0.38	7
	Elevation	2233.82	1.26	0.20	6
	Elevation + Acclimation + Sex	2233.86	1.30	0.20	8
	Elevation + Acclimation + SVL	2234.32	1.76	0.16	8
dROM ($n = 27$)	Null	159.45	0.00	0.47	4
	Elevation	161.60	2.15	0.16	6
	Acclimation	162.10	2.65	0.12	5
	SVL	162.36	2.90	0.11	5
Corticosterone ($n = 99$)	Null	1576.62	0.00	0.32	4
	SVL	1577.42	0.81	0.21	5
	Acclimation	1577.51	0.89	0.21	5
	Sex	1578.24	1.62	0.14	5

($F_{1,268} < 0.001$, $P = 0.98$).

The top model for dROMs also had sex as a fixed effect (Table 1; $F_{1,104} = 20.4$, $P < 0.0001$), with females having higher dROMs than
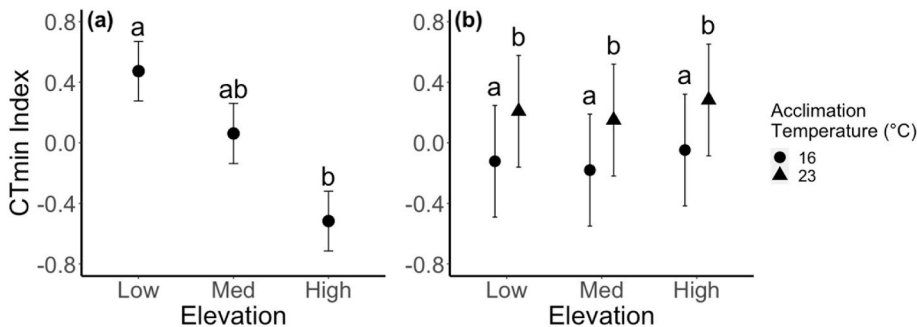
males (Fig. 3B; $t_{108} = 4.52$, $P < 0.0001$). Top models for corticosterone contained sex and SVL as fixed effects but sum of square analyses showed no significance (Table 1; sex: $F_{1,192} = 0.53$, $P = 0.47$; SVL: $F_{1,183} = 0.23$, $P = 0.63$).

3.2. Extended acclimation group

We tested CTmin on 317 frogs (n = 43 females, n = 274 males, Table A.1). The proportion of variance held by PC1 between RL-temp and RL-time was 0.506. PC1 eigen values were 0.707 (RL-temp) and −0.707 (RL-time), again indicating a lower PC1 represented a lower CTmin.

The top model for CTmin index contained acclimation temperature, SVL, and elevation as fixed effects, with both acclimation temperature and SVL as significant (Table 1; acclimation: $F_{1,301} = 17.8$, $P < 0.0001$; SVL: $F_{1,168} = 10.9$, $P = 0.0011$; elevation: $F_{2,9} = 0.94$, $P = 0.42$). The CTmin index was significantly lower for frogs acclimated to 16 °C compared to 23 °C (Fig. 2B; $t_{309} = -4.21$, $P < 0.0001$) and declined as SVL increased (Appendix A). Mean RL-temp ($\pm$SE) was 4.87 °C ($\pm$0.37) and 4.47 °C ($\pm$0.36) for frogs acclimated to 16 °C and 23 °C, respectively (Table 2).

The first of our top models for glucose contained elevation and acclimation temperature (Table 1; elevation: $F_{2,9} = 20.1$, $P = 0.00048$; acclimation: $F_{1,301} = 3.32$, $P = 0.07$). Glucose increased significantly with elevation (Fig. 4B; low-mid: $t_{16} = -3.48$, $P = 0.0081$; high-mid: $t_{16} = 2.81$, $P = 0.032$; high-low: $t_{16} = 6.35$, $P < 0.0001$). Glucose was also higher in frogs acclimated to 16 °C (25.3 mg/dL $\pm$ 0.81; mean $\pm$ SE) than 23 °C (23.6 mg/dL $\pm$ 0.8); this difference was marginally significant (Fig. 4B; $t_{302} = 1.83$, $P = 0.067$). Sum of square analyses showed elevation was significant in all top models (Table 1; second: $F_{2,9} = 20.2$,

Table 2

Mean ($\pm$SE) righting-loss temperature (RL-temp), time to righting loss (RL-time), and body temperature (cloacal temperature) of frogs from low, mid-, and high elevations in both acclimation experiments.

Elevation	RL-temp (°C)		RL-time (minutes)		Cloacal (°C)	
Short Acclimation						
Low	4.06 (0.21)		26.0 (0.40)		10.5 (0.15)	
Mid	3.74 (0.19)		28.2 (0.53)		10.3 (0.19)	
High	3.08 (0.11)		30.3 (0.45)		10.2 (0.21)	
Extended Acclimation						
	16 °C	23 °C	16 °C	23 °C	16 °C	23 °C
Low	4.85 (0.64)	4.56 (0.62)	18.8 (0.52)	16.0 (0.44)	12.3 (0.48)	13.0 (0.36)
Mid	5.47 (0.69)	4.77 (0.62)	17.9 (0.48)	18.4 (0.60)	12.5 (0.34)	12.5 (0.31)
High	4.38 (0.60)	4.09 (0.61)	17.7 (0.63)	15.5 (0.44)	11.1 (0.31)	11.6 (0.33)

Note: CTmin index values are taken from the first principal component (PC1) between RL-temp and RL-time of coqui frogs.

Fig. 2. Mean CTmin index ($\pm$SE) of coqui frogs collected at sites from low, medium, and high elevations in (a) the short acclimation group (low: $n = 108$; medium: $n = 96$; high: $n = 102$) and (b) the extended acclimation group (16 °C – low: $n = 53$; medium: $n = 47$; high: $n = 56$; 23 °C – low: $n = 54$; medium: $n = 53$; high: $n = 54$) divided by acclimation temperature. CTmin index values are taken from the first principal component (PC1) between RL-temp and RL-time of coqui frogs from the short and extended acclimation group. RL-temp refers to the TReCS cradle temperature at the time when a frog lost its righting response, and RL-time refers to the elapsed time from the beginning of the CTmin test to the moment of righting response loss. Different letters represent significance differences (i.e., p < 0.05) between groups.
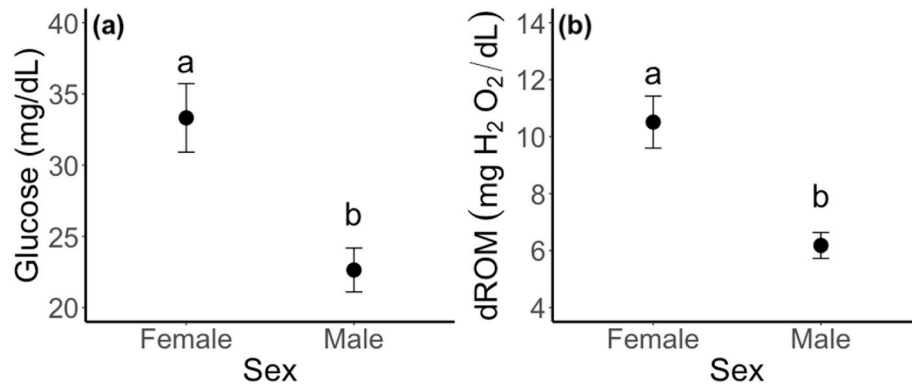
Fig. 3. Mean (±SE) (a) blood glucose (female: $n = 30$; male: $n = 270$) and (b) plasma reactive oxygen metabolite (dROM) levels (female: $n = 16$; male: $n = 91$) of female and male frogs from the short acclimation group collected and housed in Hawai'i. Different letters represent significance differences (i.e., p < 0.05) between groups.
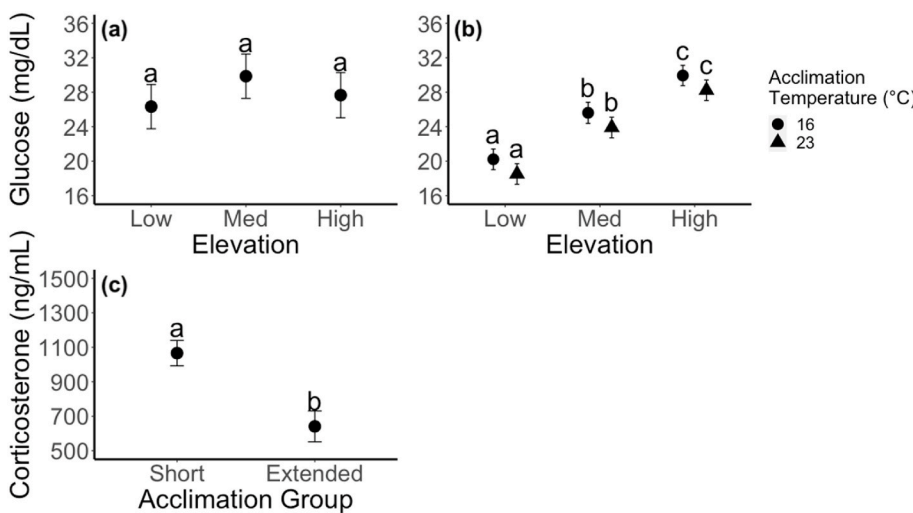

Fig. 4. Mean blood glucose levels (±SE) of coqui frogs collected at low, medium, and high elevations after (a) the short acclimation group (low: $n = 105$; medium: $n = 96$; high: $n = 99$) and (b) the extended acclimation group (16 °C – low: $n = 51$; medium: $n = 47$; high: $n = 56$; 23 °C – low: $n = 53$; medium: $n = 53$; high: $n = 54$). Circles indicate frogs acclimated to 16 °C and triangles indicate frogs acclimated to 23 °C during the extended acclimation period. (c) Mean corticosterone levels (±SE) of coqui frogs after the short acclimation period (three days) ($n = 198$) or extended acclimation period (three weeks) ($n = 99$). Different letters represent significance differences (i. e., p < 0.05) between groups.

P = 0.00048; third: $F_{2,9} = 21$, P = 0.00041; fourth: $F_{2,9} = 19$, P = 0.00062). Acclimation temperature in the other top models was marginally significant (P = 0.067 and P = 0.072) whereas sex and SVL were not (sex: $F_{1,305} = 0.66$, P = 0.42; SVL: $F_{1,254} = 0.44$, P = 0.51).

The top model for dROMs was the null model, but we did not perform subsequent analyses because other models had $\Delta AIC_c > 2$ (Table 1). Top models for corticosterone did not contain significant fixed effects according to sum of square analyses (Table 1; SVL: $F_{1,66} = 1.24$, P = 0.27; acclimation: $F_{1,94} = 1.16$, P = 0.29; sex: $F_{1,94} = 0.53$, P = 0.47).

4. Discussion

To better understand the ability of coqui frogs to acclimate to high elevation conditions, we tested basal thermal tolerance and physiology at different elevations, and their ability to acclimate to lower temperatures. We found thermal tolerance differed among elevations after the short acclimation in directions that suggest coqui are already acclimated to local temperatures. We also found that after only a few weeks thermal tolerance can increase to lower temperatures regardless of the elevation frogs were collected from. We also found evidence that glucose levels were higher in high elevation frogs and higher in cold-acclimated frogs suggesting that this is a physiological response that coqui have to lower temperatures. We did not find that coqui frogs had oxidative stress responses to with lower temperatures. In summary, our study shows that coqui frogs appear to be modifying physiology to deal with lower temperatures and these responses seem to occur relatively quickly which

may allow them to invade higher elevations than they currently occupy.

Similar to previous studies on other terrestrial anuran species, we found elevational differences in critical thermal minimum (Pintanel et al., 2019, 2022; von May et al., 2017). However, our results differ from other studies on coqui frogs that have not found CTmin differences with elevations (Christian et al., 1988; Delgado-Suazo and Burrowes, 2022; Haggerty, 2016). There are several potential reasons for this, including methodological differences that were used between studies. For example, because elevational range used here was larger than that used by Haggerty (2016) (26–1131 m vs. 19–688 m, respectively), the range in Haggerty (2016) may not have been a large enough to reveal CTmin differences. Moreover, lack of elevational differences in CTmin in Puerto Rico (Christian et al., 1988; Delgado-Suazo and Burrowes, 2022) may be the result of the different abiotic conditions between the ranges. Puerto Rico is on average warmer than Hawai'i at comparable elevations (O'Neill and Beard, 2011). In addition, coqui are experiencing colder temperatures in Hawai'i which may produce an acclimation response that is not needed in Puerto Rico.

Results from these two experiments taken together suggest that high elevation frogs are more resilient to cold temperatures than low elevation frogs due to adaptive plasticity. First, there was a basal elevational difference seen in the short acclimation group that was not present after the extended acclimation, which suggests that CTmin in the coqui is not locally adapted, but rather reflects a plastic response to temperatures where they are collected. Second, we found that coqui frogs can readily acclimate to cooler temperatures by lowering CTmin when exposed to

low temperature conditions for an extended period, regardless of the elevation at which they were collected. This acclimation of CTmin is likely important for remaining active in cold nocturnal conditions, especially in new habitats that may be colder than what frogs have previously experienced. Acclimation may also be an important factor in explaining how coqui have been such a successful invasive species in Hawai'i and has enormous implications for their ability to invade high elevation habitats.

We chose ecologically-relevant acclimation temperatures that were within the range encountered by coqui in Hawai'i which allowed us to elucidate how the temperature conditions of the coqui's current range influence its thermal tolerance. However, it is possible that more extreme thermal acclimation would yield different results and provide more insight into the invasion potential of the coqui in Hawai'i. A more extreme thermal acclimation might also be informative in assessing the limits of the coqui's acclimation ability, which could provide insight into the coldest possible environmental temperatures the coqui could tolerate. Relating these limits to the daily and annual minimum nocturnal temperatures across the island would be informative as to the potential spatial distribution of the coqui in Hawai'i (Bisrat et al., 2012).

While we predicted CTmin would not differ according to body size, we found that in the extended acclimation group, CTmin index decreased with increasing SVL. The significant inverse relationship in CTmin with SVL in the extended acclimation frogs supports past studies that have shown CTmin is lower in larger animals (Percino-Daniel et al., 2021; von May et al., 2019), and can result from thermal inertia in larger individuals. Haggerty (2016) found female coqui in Hawai'i had lower basal CTmin than males, possibly because females are larger than males (O'Neill et al., 2018), or because females have more lipid-rich organs (e. g., egg masses) which may help individuals retain heat.

As we predicted, we found differences in glucose according to elevation in our extended acclimation experiment, where glucose was higher in high elevation frogs. An increase in glucose with elevation has been observed in other anurans (Carvajalino-Fernández et al., 2021), and suggests coqui frogs may use glucose as a cryoprotectant (Storey, 1997) or to provide energy to cope with higher energy demands when experiencing colder temperatures at higher elevations (Farrar and Frye, 1977; Rocha and Branco, 1998). Our temperature loggers did not detect freezing conditions at our field sites, suggesting glucose more likely serves as an energy store for frogs at high elevation rather than a cryoprotectant. However, temperatures do drop below freezing on the island (Giambelluca et al., 2014), and so investigating the coqui's freeze tolerance should be considered for investigating their potential to survive such conditions. Cold-acclimated frogs also had marginally higher glucose levels than warm-acclimated frogs, indicating a trend towards increasing glucose when exposed to colder temperatures. This, and our CTmin data, reflects the coqui's ability to acclimate to cold temperatures by mounting a physiological response. While CTmin acclimation occurs within a few weeks, glucose acclimation appears to occur more slowly which suggests changes in glucose may not be a mechanism of acclimation to cold temperatures but rather a result of it.

Interestingly, we did not find an elevational difference in glucose in the short acclimation group. Because glucose levels change rapidly in response to stressors in other species (French et al., 2022; Gangloff et al., 2017; Kelley et al., 2021; Romero and Beattie, 2022), the stress of capture and novel housing in the short acclimation group could have overshadowed baseline differences in glucose by increasing glucocorticoid levels. In support of this idea, a study by Narayan et al. (2012) showed baseline urinary corticosterone levels in cane toads were highest after 4 days in captivity, while corticosterone levels at 14 days were similar to day 0. We found a similar effect on corticosterone, with lower corticosterone levels in the extended acclimation group (day 21) frogs relative to the short acclimation group (day 3) (Fig. 4C; $t_{138} = -4.4$, P < 0.0001). Had we kept frogs from this group for 3 weeks under uniform conditions (not possible because the experiment and blood samples were taken after 3 days), we likely would have observed the same relationship

between glucose and elevation found in the extended acclimation group. We suggest future studies measure blood glucose immediately and consider the length of time in captivity in determining when to measure blood glucose levels.

As expected, we also found sex differences in glucose, where females had higher levels of glucose than males in the short acclimation group but this conflicts with past work showing glucose levels in anurans do not differ with sex (Rocha and Branco, 1998) or body mass (Carvajalino-Fernández et al., 2021), which is correlated with sex in coqui and in other anuran species. However, sex-dependent differences in glucose are apparent in other species of ectotherms, especially during reproduction (Allender et al., 2006; Arguedas et al., 2018; Knafo et al., 2016; Oliveira et al., 2018). Furthermore, responses to stress are also sex-dependent (Bale and Epperson, 2015) which might explain why glucose differences between males and females remained after a stressful event such as thermal tolerance testing.

We also expected low reactive oxygen metabolite (dROMs) levels in cold-acclimated frogs. Elevation had no effect on dROMs in either acclimation group, suggesting elevational temperature differences do not have a significant influence on oxidative stress. dROMs were higher in females compared to males in the short-acclimated frogs, which supported our prediction that dROMs might be higher in females than males due to the high cost of reproductive investments, such as vitellogenesis (French et al., 2021; Webb et al., 2019). dROMs were not, however, significantly explained by sex (or any predictor variables) in the extended acclimation frogs. It is possible females may have stopped investing in reproduction during the extended acclimation period due to the prolonged exposure to unnatural conditions (Husak et al., 2016; Santana et al., 2015), resulting in no differences between sexes in oxidative stress.

While we expected relatively low corticosterone levels in high elevation and cold acclimated frogs due to prolonged exposure to cold temperatures, which would diminish the stressfulness of acute cold temperatures during CTmin testing, our prediction was not supported. Instead, our results align with previous research showing no differences in glucocorticoid expression in amphibians acclimated to different temperatures (Xia and Li, 2010). Past research has also suggested high or low levels of glucocorticoids may not be a simple or a direct indication of high or low stress levels because corticosterone is often highly variable for numerous reasons (Romero and Beattie, 2022). For example, Moore and Jessop (2003) found that male anurans had higher levels of corticosterone than females, likely due to the energetic demands of calling. While many of the males collected for this study were calling, no calling was observed in captivity. Similar to glucose, the laboratory conditions may have overshadowed any potential effects of our predictor variables on corticosterone levels.

5. Conclusions

In summary, our results suggest that coqui in Hawai'i differ in their thermal tolerance according to elevation in such a way as to help them cope with local temperatures, and that they can acclimate to new temperatures over an extended (i.e., 3 weeks) period of time. This could mean that lower temperatures are not as great a barrier against future invasion as previously thought (Beard and Pitt, 2005). We found that glucose increased significantly with elevation and with lower acclimation temperatures (albeit with p-values of 0.07), suggesting glucose might be important in the coqui's physiological response to cold temperatures by providing energy to meet higher energy demands under lower temperatures. Because stress metrics, such as oxidative status and corticosterone, did not appear to be influenced by elevation, this suggests the coqui's physiological status may not be negatively affected by further invasion into higher elevations. Altogether, further invasion by the coqui into high elevations seems probable, likely through known dispersal methods such as vehicular transport (Peacock et al., 2009) or intentional introductions (Kraus et al., 1999), which poses a threat to

endemic Hawaiian flora and fauna (Beard et al., 2009). Invasion of higher elevations by the coqui seems inevitable unless more effective control strategies are developed.

Data availability

The datasets generated during and/or analyzed during the current study are available in the Utah State University Digital Commons repository, https://doi.org/10.26078/p7tx-3402.

Author contributions

SSF and KHB conceived of the ideas and designed methodology. JRM, KHB, and EEV acquired the data. SCH helped with logistical support and permit acquisition. JRM, EEV, ELL, KCK, LOS and APF ran lab assays and assisted in animal husbandry at USU. JRM analyzed the data, and JRM, SSF, KHB, and SCH contributed to writing the manuscript. All authors contributed critically to the drafts and gave final approval to for publication.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103590.

References

Allender, M.C., Mitchell, M.A., Phillips, C.A., Gruszynski, K., Beasley, V.R., 2006. Hematology, plasma biochemistry, and antibodies to select viruses in wild-caught Eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) from Illinois. J. Wildl. Dis. 42 (1), 107–114. http://meridian.allenpress.com/jwd/article-pdf/42/1/107/2333899/0090-3558-42_1_107.pdf.

Andersson, M.N., Nilsson, J., Nilsson, J.Å., Isaksson, C., 2018. Diet and ambient temperature interact to shape plasma fatty acid composition, basal metabolic rate and oxidative stress in great tits. J. Exp. Biol. 221, 1–10. https://doi.org/10.1242/jeb.186759.

Angilletta, M.J., Wilson, R.S., Navas, C.A., James, R.S., 2003. Tradeoffs and the evolution of thermal reaction norms. Trends Ecol. Evol. 18 (5), 234–240. https://doi.org/10.1016/S0169-5347(03)00087-9.

Arguedas, R., Steinberg, D., Lewbart, G.A., Deresienski, D., Lohmann, K.J., Muñoz-Pérez, J.P., Valle, C.A., 2018. Haematology and biochemistry of the San Cristóbal Lava lizard (*Microlophus bivittatus*). Conserv. Physiol. 6, 1–9. https://doi.org/10.1093/conphys/coy046.

Bale, T.L., Epperson, C.N., 2015. Sex differences and stress across the lifespan. Nat. Neurosci. 18 (10), 1413–1420.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67 (1), 1–48.

Beard, K.H., Pitt, W.C., 2005. Potential consequences of the coqui frog invasion in Hawaii. Divers. Distrib. 11, 427–433.

Beard, K.H., Price, E.A., Pitt, W.C., 2009. Biology and impacts of Pacific island invasive species. 5. *Eleutherodactylus coqui,* the Coqui frog (Anura: Leptodactylidae) 1. Pac. Sci. 63 (3), 297–316.

Bisrat, S.A., White, M.A., Beard, K.H., Richard Cutler, D., 2012. Predicting the distribution potential of an invasive frog using remotely sensed data in Hawaii. Divers. Distrib. 18 (7), 648–660. https://doi.org/10.1111/j.1472-4642.2011.00867.x.

Briggs, D., Smithson, P., Addison, K., Atkinson, K., 1997. Fundamentals of the Physical Environment. Routledge.

Burnham, K.P., Anderson, D.R., 2004. Multimodel inference: understanding AIC and BIC in model selection. Socio. Methods Res. 33 (2), 261–304. https://doi.org/10.1177/0049124104268644.

Bury, S., Cichoń, M., Bauchinger, U., Sadowska, E.T., 2018. High oxidative stress despite low energy metabolism and vice versa: insights through temperature acclimation in an ectotherm. J. Therm. Biol. 78, 36–41. https://doi.org/10.1016/j.jtherbio.2018.08.003.

Carvajalino-Fernández, J.M., Argenis Bonilla Gomez, M., Giraldo-Gutierréz, L., Navas, C. A., 2021. Freeze tolerance in neotropical frogs: an intrageneric comparison using *Pristimantis* species of high elevation and medium elevation. J. Trop. Ecol. 37, 118–125. https://doi.org/10.1017/S026646742100016X.

Choi, R.T., Beard, K.H., 2012. Coqui frog invasions change invertebrate communities in Hawaii. Biol. Invasions 14 (5), 939–948. https://doi.org/10.1007/s10530-011-0127-3.

Christian, K.A., Nunez, F., Clos, L., Diaz, L., 1988. Thermal relations of some tropical frogs along an altitudinal gradient. Biotropica 20 (3), 236–239.

Cortes, P.A., Puschel, H., Acuña, P., Barthel, J.L., Bozinovic, F., 2016. Thermal ecological physiology of native and invasive frog species: do invaders perform better? Conserv. Physiol. 4 (1), 1–10. https://doi.org/10.1093/conphys/cow056.

Costanzo, J.P., Reynolds, A.M., Clara, M., Do Amaral, F., Rosendale, A.J., Lee, R.E., 2015. Cryoprotectants and extreme freeze tolerance in a subarctic population of the wood frog. PLoS One 10 (2). https://doi.org/10.1371/JOURNAL.PONE.0117234.

Delgado-Suazo, P., Burrowes, P.A., 2022. Response to thermal and hydric regimes point to differential inter- and intraspecific vulnerability of tropical amphibians to climate warming. J. Therm. Biol. 103, 103148 https://doi.org/10.1016/j.jtherbio.2021.103148.

Dupoué, A., Brischoux, F., Lourdais, O., Angelier, F., 2013. Influence of temperature on the corticosterone stress-response: an experiment in the children's python (*Antaresia childreni*). Gen. Comp. Endocrinol. 193, 178–184. https://doi.org/10.1016/j.ygcen.2013.08.004.

Dupoué, A., Rutschmann, A., Le Galliard, J.F., Clobert, J., Blaimont, P., Sinervo, B., Miles, D.B., Haussy, C., Meylan, S., 2018. Reduction in baseline corticosterone secretion correlates with climate warming and drying across wild lizard populations. J. Anim. Ecol. 87 (5), 1331–1341. https://doi.org/10.1111/1365-2656.12843.

Farrar, E.S., Frye, B.E., 1977. Seasonal variation in the effects of adrenalin and glucagon in *Rana pipiens*. Gen. Comp. Endocrinol. 33 (1), 76–81.

Fox, J., Weisberg, S., 2019. *An {R} Companion to Applied Regression* (Third). Sage.

French, S.S., Neuman-Lee, L.A., Terletzky, P.A., Kiriazis, N.M., Taylor, E.N., DeNardo, D. F., 2017. Too much of a good thing? Human disturbance linked to ecotourism has a "dose-dependent" impact on innate immunity and oxidative stress in marine iguanas, Amblyrhynchus cristatus. Biol. Conserv. 210 (December 2016), 37–47. https://doi.org/10.1016/j.biocon.2017.04.006.

French, S.S., Virgin, E.E., Ki, K.C., Maryon, D.F., Goode, A.B.C., Pasachnik, S.A., 2021. Reproductive stage and clutch size incur energetic and oxidative costs in an endangered Iguana, *Ctenosaura oedirhina*. J. Herpetol. 55 (2), 192–200. https://doi.org/10.1670/20-099.

French, S.S., Webb, A.C., Wilcoxen, T.E., Iverson, J.B., Denardo, D.F., Lewis, E.L., Knapp, C.R., 2022. Complex tourism and season interactions contribute to disparate physiologies in an endangered rock iguana. Conserv. Physiol. 10, 1–17. https://doi.org/10.1093/conphys/coac001.

Frisard, M., Ravussin, E., 2006. Energy metabolism and oxidative stress. Endocrine 29 (1), 27–32.

Gangloff, E.J., Sparkman, A.M., Holden, K.G., Corwin, C.J., Topf, M., Bronikowski, A.M., 2017. Geographic variation and within-individual correlations of physiological stress markers in a widespread reptile, the common garter snake (*Thamnophis sirtalis*). Comp. Biochem. Physiol., A : Mol. Integrat. Physiol. 205, 68–76. https://doi.org/10.1016/j.cbpa.2016.12.019.

Gayle, S.M., 2020. Thermal Dependence of Eleutherodactylus Coqui Vocalization on Hawaii Island. University of Hawaii at Hilo.

Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J., Wang, G., 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr. Comp. Biol. 46 (1), 5–17. https://doi.org/10.1093/icb/icj003.

Giambelluca, T.W., Shuai, X., Barnes, M.L., Alliss, R.J., Longman, R.J., Miura, T., Chen, Q., Frazier, A.G., Mudd, R.G., Cuo, L., Businger, A.D., 2014. Evapotranspiration of Hawai'i.

Haggerty, J., 2016. Thermal Tolerance of the Common Coqui Frog *(Eleutherodactylus Coqui)* in East Hawaii along an Elevation Gradient. University of Hawaii at Hilo.

Hartig, F., 2021. DHARMa: residual Diagnostics for hierarchical (Multi-Level/mixed) regression models (R package version 0.4.4). https://cran.r-project.org/package=DHARMa.

Hermansen, B., Jørgensen, C.B., 1969. Blood glucose in male toads (*Bufo bufo*): annual variation and hormonal regulation. Gen. Comp. Endocrinol. 12, 313–321.

Hill, S.A., Beard, K.H., Siers, S.R., Shiels, A.B., 2019. Invasive coqui frogs are associated with differences in mongoose and rat abundances and diets in Hawaii. Biol. Invasions 21, 2177–2190.

Hudson, S.B., Kluever, B.M., Webb, A.C., French, S.S., 2020a. Steroid hormones, energetic state, and immunocompetence vary across reproductive contexts in a parthenogenetic lizard. Gen. Comp. Endocrinol. 288, 113372 https://doi.org/10.1016/j.ygcen.2019.113372.

Hudson, S.B., Lidgard, A.D., French, S.S., 2020b. Glucocorticoids, energy metabolites, and immunity vary across allostatic states for plateau side-blotched lizards (*Uta stansburiana uniformis*) residing in a heterogeneous thermal environment. J. Exp. Zool. Part A: Ecological and Integrative Physiology 333 (10), 732–743. https://doi.org/10.1002/jez.2415.

Husak, J.F., Ferguson, H.A., Lovern, M.B., 2016. Trade-offs among locomotor performance, reproduction and immunity in lizards. Funct. Ecol. 30, 1665–1674. https://doi.org/10.1111/1365-2435.12653.

Janzen, D.H., 1967. Why mountain passes are higher in the tropics. Am. Nat. 101 (919), 233–249.

Jessop, T.S., Lane, M., Wilson, R.S., Narayan, E.J., 2018. Testing for short- and long-term thermal plasticity in corticosterone responses of an ectothermic vertebrate. Physiol. Biochem. Zool. 91 (4), 967–975. https://doi.org/10.1086/698664.

Jungreis, A.M., 1970. The effects of long-term starvation and acclimation temperature on glucose regulation and nitrogen anabolism in the frog, *Rana pipiens*—II. Summer animals. Compar. Biochem. Physiol. 32, 433–444.

Jungreis, A.M., Hooper, A.B., 1970. The effects of long-term starvation and acclimation temperature on glucose regulation and nitrogen anabolism in the frog, *Rana pipiens*—I. Winter animals. Compar. Biochem. Physiol. 32, 417–432.

Kelley, S., Farrell, T.M., Lind, C.M., 2021. Validating the use of a quick-read glucometer to assess the glycemic response to short-term capture stress in two species of snake, *Nerodia sipedon* and *Sistrurus miliarius*. Ichthyol. Herpetol. 109 (2), 436–442. https://doi.org/10.1643/h2020102.

Knafo, S.E., Norton, T.M., Mitchell, M., Stevenson, D.J., Hyslop, N., Poppenga, R., Oliva, M., Chen, T., Cray, C., Gibbs, S.E.J., Durden, L., Stedman, N., Divers, S., Dierenfeld, E., 2016. Health and nutritional assessment of free-ranging eastern indigo snakes (*Drymarchon couperi*) in Georgia, United States. J. Zoo Wildl. Med. 47 (4), 1000–1012. https://doi.org/10.1638/2015-0127.1.

Kraus, F., Campbell, E.W., Allison, A., Pratt, T., 1999. *Eleutherodactylus* frog introductions to Hawaii. Herpetol. Rev. 30 (1), 21–25.

Lenth, R.V., 2021. Emmeans: estimated marginal means, aka least-squares means (R package version 1.7.1-1.). https://cran.r-project.org/package=emmeans.

Lin, T.E., Chen, T.Y., Wei, H.L., Richard, R., Huang, S.P., 2019. Low cold tolerance of the invasive lizard *Eutropis multifasciata* constrains its potential elevation distribution in Taiwan. J. Therm. Biol. 82 (March), 115–122. https://doi.org/10.1016/j.jtherbio.2019.03.015.

Mazerolle, M.J., 2020. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c) (R package version 2.3-1.).

McCann, S., Greenlees, M.J., Newell, D., Shine, R., 2014. Rapid acclimation to cold allows the cane toad to invade montane areas within its Australian range. Funct. Ecol. 28, 1166–1174. https://doi.org/10.1111/1365-2435.12255.

Mittan, C.S., Zamudio, K.R., 2019. Rapid adaptation to cold in the invasive cane toad *Rhinella marina*. Conserv. Physiol. 7 (1), 1–12. https://doi.org/10.1093/conphys/coy075.

Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Homones Behav. 43, 39–47. https://doi.org/10.1016/S0018-506X(02)00038-7.

Muñoz, M.M., 2022. The Bogert effect, a factor in evolution. Evolution 76 (S1), 49–66. https://doi.org/10.1111/evo.14388.

Muñoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A., Bakken, G.S., Losos, J.B., 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proc. Biol. Sci. 281 (1778) https://doi.org/10.1098/rspb.2013.2433.

Narayan, E.J., Cockrem, J.F., Hero, J.-M., 2012. Effects of temperature on urinary corticosterone metabolite responses to short-term capture and handling stress in the cane toad (*Rhinella marina*). Gen. Comp. Endocrinol. 178, 301–305. https://doi.org/10.1016/j.ygcen.2012.06.014.

Navas, C.A., 2006. Patterns of distribution of anurans in high Andean tropical elevations: insights from integrating biogeography and evolutionary physiology. Integr. Comp. Biol. 46 (1), 82–91. https://doi.org/10.1093/icb/icj001.

Navas, C.A., Araujo, C., 2000. The use of agar models to study amphibian thermal ecology. J. Herpetol. 34 (2), 330–334.

Navas, C.A., Carvajalino-Fernández, J.M., Saboyá-Acosta, L.P., Rueda-Solano, L.A., Carvajalino-Fernández, M.A., 2013. The body temperature of active amphibians along a tropical elevation gradient: patterns of mean and variance and inference from environmental data. Funct. Ecol. 27, 1145–1154.

O'Neill, E.M., Beard, K.H., 2011. Clinal variation in calls of native and introduced populations of *Eleutherodactylus coqui*. Copeia 2011 (1), 18–28. https://doi.org/10.1643/CH-10-012.

O'Neill, E.M., Beard, K.H., Fox, C.W., 2018. Body size and life history traits in native and introduced populations of coqui frogs. Copeia 106 (1), 161–170. https://doi.org/10.1643/ce-17-642.

Oliveira, M.R., Braghirolli, F.M.H., Verrastro, L., Oliveira, G.T., 2018. Seasonal and sexual variation of intermediate metabolism and body condition indexes in the lizard *Tropidurus catalanensis* (Gudynas and Skuk, 1983) (Squamata: Tropiduridae). South Am. J. Herpetol. 13 (1), 85–95. https://doi.org/10.2994/SAJH-D-17-00073.1.

Peacock, M.M., Beard, K.H., O'Neill, E.M., Kirchoff, V.S., Peters, M.B., 2009. Strong founder effects and low genetic diversity in introduced populations of Coqui frogs. Mol. Ecol. 18, 3603–3615. https://doi.org/10.1111/j.1365-294X.2009.04308.x.

Percino-Daniel, R., Contreras López, J.M., Téllez-Valdés, Oswaldo, Méndez De La Cruz, F. R., Gonzalez-Voyer, A., Piñero, Daniel, 2021. Environmental heterogeneity shapes physiological traits in tropical direct-developing frogs. Ecol. Evol. 00 (1), 1–15. https://doi.org/10.1002/ece3.7521.

Pintanel, P., Tejedo, M., Merino-Viteri, A., Almeida-Reinoso, F., Salinas-Ivanenko, S., López-Rosero, A.C., Llorente, G.A., Gutiérrez-Pesquera, L.M., 2022. Elevational and local climate variability predicts thermal breadth of mountain tropical tadpoles. Ecography, e05906. https://doi.org/10.1111/ecog.05906.

Pintanel, P., Tejedo, M., Ron, S.R., Llorente, G.A., Merino-Viteri, A., 2019. Elevational and microclimatic drivers of thermal tolerance in Andean Pristimantis frogs. J. Biogeogr. 46, 1664–1675.

Price, S., 1983. Climate. In: Armstrong, R. (Ed.), Atlas of Hawaii. University of Hawaii Press, pp. 59–66.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.r-project.org/.

Reguera, S., Zamora-Camacho, F.J., Trenzado, C.E., Sanz, A., Moreno-Rueda, G., 2014. Oxidative stress decreases with elevation in the lizard *Psammodromus algirus*. Comp. Biochem. Physiol. Mol. Integrat. Physiol. 172, 52–56. https://doi.org/10.1016/j.cbpa.2014.02.018.

Rocha, P.L., Branco, L.G.S., 1998. Seasonal changes in the cardiovascular, respiratory and metabolic responses to temperature and hypoxia in the bullfrog *Rana catesbeiana*. J. Exp. Biol. 201, 761–768. https://doi.org/10.1242/jeb.201.5.761.

Romero, L.M., Beattie, U.K., 2022. Common myths of glucocorticoid function in ecology and conservation. J. Exp. Zool. Comp. Exp. Biol. 337, 7–14. https://doi.org/10.1002/jez.2459.

Santana, F.E., Swaisgood, R.R., Lemm, J.M., Fisher, R.N., Clark, R.W., 2015. Chilled frogs are hot: hibernation and reproduction of the Endangered mountain yellow-legged frog *Rana muscosa*. Endanger. Species Res. 27 (1), 43–51. https://doi.org/10.3354/esr00648.

Schroeder, T.A., 1993. Climate controls. In: Sanderson, M. (Ed.), Prevailing Trade Winds: Weather and Climate in Hawai'i. University of Hawaii Press, pp. 12–36.

Sin, H., Beard, K.H., Pitt, W.C., 2008. An invasive frog, *Eleutherodactylus coqui*, increases new leaf production and leaf litter decomposition rates through nutrient cycling in Hawaii. Biol. Invasions 10 (3), 335–345. https://doi.org/10.1007/s10530-007-9133-x.

Smith, R.L., Beard, K.H., Koons, D.N., 2018. Invasive coqui frogs are associated with greater abundances of nonnative birds in Hawaii, USA. Condor 120 (1), 16–29. https://doi.org/10.1650/CONDOR-17-109.1.

Souri, A.M., Rastegar-Pouyani, N., 2018. Morphological characters, antioxidant defenses and oxidative stress in the lizard *Ophisops elegans* at different altitudes. Biharean Biol. 12 (2), 97–101.

Storey, K.B., 1987. Glycolysis and the regulation of cryoprotectant synthesis in liver of the freeze tolerant wood frog. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 157 (3), 373–380. https://doi.org/10.1007/BF00693364.

Storey, K.B., 1997. Organic solutes in freezing tolerance. Comp. Biochem. Physiol. A Physiol. 117 (3), 319–326. https://doi.org/10.1016/S0300-9629(96)00270-8.

Velo-Antón, G., Burrowes, P.A., Joglar, R.L., Martínez-Solano, I., Beard, K.H., Parra-Olea, G., 2007. Phylogenetic study of *Eleutherodactylus coqui* (Anura: Leptodactylidae) reveals deep genetic fragmentation in Puerto Rico and pinpoints origins of Hawaiian populations. Mol. Phylogenet. Evol. 45 (2), 716–728. https://doi.org/10.1016/j.ympev.2007.06.025.

von Humboldt, A., 1849. Aspects of nature in different lands and different climates; with scientific elucidations. trans. Elizabeth J.L. Sabine, London: Longman, Brown, Green and John Murray. https://doi.org/10.5962/bhl.title.151955.

von May, R., Catenazzi, A., Corl, A., Santa-Cruz, R., Carnaval, A.C., Moritz, C., 2017. Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. Ecol. Evol. 7 (9), 3257–3267.

von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A.S., Moritz, C., Rabosky, D.L., 2019. Thermal physiological traits in tropical lowland amphibians: vulnerability to climate warming and cooling. PLoS One 14 (8), 1–18. https://doi.org/10.1371/journal.pone.0219759.

Wang, X., Zhong, M., Yang, S., Jiang, J., Hu, J., 2022. Multiple β - diversity patterns and the underlying mechanisms across amphibian communities along a subtropical elevational gradient. Divers. Distrib. 00, 1–14. https://doi.org/10.1111/ddi.13618.

Webb, A.C., Iverson, J.B., Knapp, C.R., DeNardo, D.F., French, S.S., 2019. Energetic investment associated with vitellogenesis induces an oxidative cost of reproduction. J. Anim. Ecol. 88, 461–472. https://doi.org/10.1111/1365-2656.12936.

Winwood-Smith, H.S., Alton, L.A., Franklin, C.E., White, C.R., 2015. Does greater thermal plasticity facilitate range expansion of an invasive terrestrial anuran into higher latitudes? Conserv. Physiol. 3 (1), 1–11. https://doi.org/10.1093/conphys/cov010.

Woolbright, L.L., 2005. A plot-based system of collecting population information on terrestrial breeding frogs. Herpetol. Rev. 36 (2), 139–142.

Woolbright, L.L., Hara, A.H., Jacobsen, C.M., Mautz, W.J., Benevides, F.L., 2006. Population densities of the Coquí, *Eleutherodactylus coqui* (Anura: Leptodactylidae) in newly invaded Hawaii and in native Puerto Rico. J. Herpetol. 40 (1), 122–126. https://doi.org/10.1670/79-05W.1.

Xia, J., Li, X., 2010. Effect of temperature on blood parameters of the salamander *Batrachupems tibetanus* (Schmidt, 1925) (Amphibia: Hynobiidae). Russ. J. Ecol. 41 (1), 102–106. https://doi.org/10.1134/S1067413610010194.

Zhang, C., Hamilton, K., Wang, Y., 2017. Monitoring and projecting snow on Hawaii Island. Earth's Future 5, 436–448.