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Response to thermal and hydric regimes point to differential inter- and intraspecific vulnerability of tropical amphibians to climate warming

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A B S T R A C T

In Puerto Rico, an island threatened by climate warming, only one of two species of frogs that share part of their distribution has undergone a recent range contraction to higher elevations. We questioned if differences in their physiological response to temperature and dehydration might explain this distributional change. We studied a lowland and a highland population of *Eleutherodactylus coqui*, a widespread generalist, and *E. portoricensis*, an endangered species that is currently found only above 600 m. We compared various physiological aspects: operative temperature; temperature selection; critical temperatures; and their response to jumping performance tests at various thermal and hydric regimes. Results revealed that *E. portoricensis* had the highest CT_{min} and lowest CT_{max} and selected a cooler range of temperatures from the experimental gradient. Jumping performance increased with temperature for the three populations until attaining maximum performance. Afterwards, performance dropped drastically until reaching CT_{max}. Dehydration had a negative effect on performance for both species, particularly on maximum performance. This effect was greatest for *E. portoricensis,* followed by high-elevation *E. coqui*. The significantly greater thermo-hydric physiological limitations of *E. portoricensis* may explain its recent range contraction, potentially, as a response to climate warming. Low-elevation *E. coqui* had the lowest operative warming tolerance and was the only population to select temperatures like those encountered in their environment, indicating it may be narrowly adapted to local thermal conditions and thus, also vulnerable to climate change. Our results point towards plasticity in the response of *E. coqui* to varying climatic conditions, and present evidence of different physiological responses between closely related species at the same locality. This work highlights the importance of studying the combined effects of temperature and hydration to understand the response of ectotherms to warming environments and presents further evidence that desiccation may be a limiting factor determining which species may survive.

1. Introduction

Empirical studies and theoretical modeling have provided substantial evidence indicating that modern climate change will affect various taxa worldwide (Parmesan, 2006; Parmesan and Yohe, 2003). Effects of climate change include alterations in species phenology (Richardson et al., 2013; Thackeray et al., 2016), breeding behavior (Hitch and Leberg, 2007), disease occurrence (Rohr et al., 2011), and distributional ranges (Sinervo et al., 2010). Temperature, a key environmental factor intrinsic to climate change, has relevance at various levels of organization, ranging from driving ecosystem processes (Running and Coughlan, 1988) to determining proper physiological functions at an organismal level (Angilletta et al., 2002). Among vertebrate animals, we are especially concerned with ectotherms because many lineages are dependent on environmental temperature to regulate body heat and maintain proper internal environment for physiological functions (Huey and Stevenson, 1979). Moreover, tropical ectotherms are expected to have limited acclimation abilities because they have evolved in relatively constant environments (Diele-Viegas et al., 2018; Huey et al., 2009; Janzen, 1967). Recent research has demonstrated that tropical ectotherms tend to have higher thermal physiological specialization making them susceptible even to relatively small temperature changes (Deutsch et al., 2008). Thus, although warming trends are predicted to be lower in the tropics than at higher latitudes (Allen et al., 2018), tropical ectotherms may be facing greater risks (Deutsch et al., 2008).

Among ectotherms, amphibians might be particularly vulnerable to climate warming because their permeable skin tends to lose water via evaporation as temperature increases (Hutchison and Dupré, 1992;

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Lillywhite, 2006). In addition, amphibians depend on water and humidity to maintain moist skin for complementary gas exchange and to ensure the survival of their unprotected anamniotic eggs (Duellman and Trueb, 1994). As a response to climate warming, several amphibian species in the tropics have altered their distribution either by seeking cooler and more mesic microhabitat in their current range (Burrowes et al., 2020), or by displacing to higher elevations (Raxworthy et al., 2008; Seimon et al., 2007). Nonetheless, species encountering environmental temperatures outside ranges for their best performance may face suboptimal conditions for physiological functioning, having adverse consequences on fitness-related traits such as foraging, reproductive behavior, or predator escape (Feder and Londos, 1984; Greenberg and Palen, 2021). Thus, amphibians at greater extinction risk are those that live closer to their critical thermal limits, or those that will face temperatures higher than optimal for their performance (Deutsch et al., 2008; Gerick et al., 2014). In this manner, we expect species to respond to suboptimal climate conditions by modifying their behavior or physiology, leading to thermoregulation (Köhler et al., 2011; Rohr et al., 2018), or by changing their distribution to areas that offer more favorable climates (Seimon et al., 2007; Raxworthy et al., 2008; Burrowes et al., 2020).

In the past 20 years, herpetologists in Puerto Rico have noticed a marked range contraction to higher elevations of the endemic, terrestrial direct-developing frog *Eleutherodactylus portoricensis* (Barker and Ríos--Franceschi, 2015; Burrowes et al., 2004; Joglar, 1998; Woolbright, 1997). In the past, this species was reported from elevations >300 m in forested areas within the western, central and eastern slopes of the island (Drewry and Rand, 1983; Woolbright, 1997), but at present it can only be found in the eastern mountains of El Yunque National Forest at elevations above 600 m (Barker and Ríos-Franceschi, 2015; Campos-Cerqueira and Aide, 2017), and it is classified as Endangered by the International Union for the Conservation of Nature (IUCN, 2021; https://www.iucnredlist.org/species/56875/110924108). However, it is possible that some altitudinal records for *E. portoricensis* prior to 1966 may have been mistaken with those of the broadly distributed *E. coqui* because until then, they were considered the same species (Thomas, 1965). Studies conducted in the 1980′s revealed physiological limitations in *E. portoricensis* (Beuchat et al., 1984; Christian et al., 1988). Particularly, *E. portoricensis* showed relatively low tolerance to high temperatures and died at 30 °C, while congeners that occur at low elevations, *E. coqui* and *E. antillensis,* survived, and their jumping performance was respectively less affected by temperature even when dehydrated (Beuchat et al., 1984). Thus, despite the similarities in morphology, call, and ecology (Joglar, 1998; Rivero, 1978) and their close phylogenetic relatedness (Pyron and Wiens, 2011), *E. portoricensis* seems to have a greater physiological sensitivity to higher temperatures and dehydration than *E. coqui*.

In contrast to *E. portoricensis*, *E. coqui* remains widely distributed across the entire elevational gradient of Puerto Rico. Thus, we questioned if the observed range contraction of *E. portoricensis* could be attributed to physiological differences with *E. coqui* in the way individuals respond to changes in body temperature and hydration state. To address this, we compared the physiological responses of these two species at a high elevation site where they occur sympatrically. We also compared populations of *E. coqui* from low and high elevation sites to evaluate potential intraspecific plasticity in the thermo-hydric response of this generalist species. Our study is different from those done by colleagues in the 1980′s in that we examined the effect of a broader range of temperatures in the physiological response of *E. coqui* and *E. portoricensis* that currently co-occur at what seems to be the elevational refugia of the latter species. Furthermore, our questions are timely because we have observed a recent range contraction to high elevations in only one of these two species, and because we address the consequences of modern climate change. In the Caribbean, studies have shown that minimum temperatures, which typically occur at night when amphibians are active, have increased significantly since the late 1900′s (Peterson et al., 2002; Stephenson et al., 2014), and specifically in the eastern mountains of Puerto Rico where this work was conducted (Burrowes et al., 2004; Narins and Meenderink 2014; Van Beusekom et al., 2015). Also, while changes in precipitation patterns are not as clear (Burrowes et al., 2004), some studies have shown a significant drying trend in the Caribbean (Karmalkar et al., 2013; Neelin et al., 2006) with an increase in the number of consecutive dry days (Stephenson et al., 2014) that can potentially affect the ability of amphibians to retain moisture (Köhler et al., 2011; Van Berkum et al., 1982). The hydrothermal state of amphibians can affect their performance (Beuchat et al., 1984; Greenberg and Palen, 2021; Rozen-Rechels et al., 2019), and fitness trade-offs between temperature and moisture may dictate habitat, behavioral and/or distributional changes (Burrowes et al., 2020; Greenberg and Palen, 2021). Thus, if *E. portoricensis* is indeed more sensitive to warmer temperature and desiccation than *E. coqui*, we can hypothesize that modern climate change in the Caribbean may have driven the distributional change observed in this species.

The persisting broad distribution of *E. coqui* in contrast to *E. portoricensis*, and the fact that these two species overlap at high elevations, allow us to address questions regarding plasticity in the response to different environmental factors, as well as ecological adaptation (Riddell et al., 2018). This study is significant from an evolutionary perspective because it comprises intraspecific and interspecific comparisons that can enhance our understanding of the speciation processes that take place in islands subjected to radiations, as is the case of the *Eleutherodactylus* in the Caribbean (Hedges 1989; Hedges et al., 2008). Studies on replicate radiations on Caribbean species of both *Anolis* lizards (Losos et al., 1998) and *Eleutherodactylus* (Dugo-Cota et al., 2019) have shown that those living under similar selective pressures in similar microhabitats, have analogous morphologies, and that these are convergent across islands, highlighting the effect of the environment on evolutionary processes. Our work questions if the mechanisms that lead to similar morphologies among distant populations of the same species (low- and highland *E. coqui*), or among sympatric congeners (*E. coqui* and *E. portoricensis* at high elevation), also result in similar physiological responses. We expect to distinguish intraspecific and interspecific physiological responses that arise as a result of adaptation to local environments, versus those that reflect distinct phylogenetic signals (Huey and Bennett, 1990). From a conservation standpoint, being able to link distributional responses to hydrothermal physiological limitations that render species more vulnerable to environmental change is important because it allows us to identify risk factors and use this knowledge to design effective management plans.

2. Materials and methods

2.1. Study site

We conducted the field work at El Yunque National Forest, Río Grande, Puerto Rico, at two different sites that vary in elevation, forest composition, and annual temperature and rainfall. The low elevation site (180 m) corresponds to the Subtropical Wet Forest type according to Holdridge Life Zones and receives between 2000 and 4000 mm of rain each year (Ewel and Whitmore, 1973). This site is located within Angelito Trail near road PR-988 (18.323024°, −65.747160°). The high elevation site (875 m) corresponds to the Lower Montane Wet Forest Holdridge classification and sustains between 1000 and 4000 mm of rain annually (Ewel and Whitmore, 1973). This site is located within the elfin forest formation near road PR-191 (18.301942°, −65794685°). At the low elevation study site, the average air temperature below the canopy is 22.82 ± 0.70 °C (mean ± SD) during the cool-dry season (December–April) and 25.76 ± 1.37 °C during the warm-wet season (May–November). In contrast, the high elevation site has an average air temperature below the canopy of 17.83 ± 0.47 °C during the cool-dry season and 20.99 ± 0.81 °C during the warm-wet season. The temperature data reported above were collected in the field during 2015–2016 using HOBO® (U23-002) data loggers.

2.2. Operative temperature

We measured operative environmental temperatures in the field as a proxy for frog body temperatures (T_b; Bakken and Gates 1975) in their diurnal and nocturnal habitats. To do this, we used thermocouples (Onset HOBO® U23-003) fitted into brown-colored agar models built to scale in the size and shape of an adult *Eleutherodactylus* (Rowley and Alford, 2010). We placed four thermocouples each with an agar model at frog retreat sites, specifically, inside bromeliad axils at 1 m above the ground where canopy cover ensured that they would not receive direct sunlight. The models were in bromeliads on trees separated approximately 2 m from each other in the forest to obtain four replicate readings at both the low- and high elevation sites. We programed the thermocouples to record operative temperatures every 15 minutes for seven days at both study sites during the warm-wet season of 2016. We were interested in determining differences in frog's operative temperature at night (6:00 p.m.–6:50 a.m.) when they are active, and during two periods of the day: morning (7:00 a.m.–10:50 a.m.) when activity stops, and afternoon (11:00am–5:50pm) when temperatures are typically the highest in Puerto Rico. Because of the high relative humidity at El Yunque (89–100%), frog agar models placed in retreat sites remain moist, with negligible change in weight over a 24-h period (Burrowes, unpublished). Thus, there was no need to replace them during the time in which operative temperatures were monitored at our study sites.

2.3. Animal collection and husbandry

We performed hydrothermal physiology experiments on individuals of two populations of *Eleutherodactylus coqui* and a single population of *E. portoricensis* collected at El Yunque during the warm-wet season of 2017. We collected 20 adult frogs from each of the three populations studied: 20 *E. coqui* frogs from the low-elevation site (low-elevation *E. coqui*), 20 *E. coqui* frogs from the high-elevation site (high-elevation *E. coqui*) and, 20 *E. portoricensis* frogs at this high-elevation site where they occur sympatrically (Table 1).

All animals were kept in a dedicated animal-maintenance room (2 x 3 x 2.5 m [W x L x H]) in the laboratory. We used a rodent breeding rack (Ancare) retrofitted for terrestrial, direct development frogs such as *Eleutherodactylus* spp. to house the frogs in individual terrariums during experiments (Colón-Piñeiro et al., 2017). We maintained room temperature on average at 22.13 °C (±0.96 SD). To maintain humid conditions in the terraria, we sprayed them daily every morning using an automatic irrigation system and kept a layer of sphagnum moss as bedding. All frog husbandry methods followed the protocol described by Colón-Piñeiro et al. (2017).

We allowed frogs to adjust to laboratory conditions and their terrariums for at least four days before running experiments because this is the time recommended for studies of critical temperatures and metabolic rates in anurans (Brattstrom and Lawrence, 1962; Dunlap, 1969; Rohr et al., 2018). Prior to any test, we assumed that frog T_b was the same as room temperature (22.13 °C ± 0.96 SD). This assumption is valid for small vertebrate ectotherms, especially when kept in relatively small terrariums at constant room temperature and high levels of humidity because potential changes due to behavioral thermoregulation or evaporation are minimal (Kirk and Hogben, 1946). Animals were housed in the laboratory for a maximum of six weeks after collection date until experiments ended and then returned to their respective collection sites.

Table 1
Number of individuals (N) of each population studied, elevation at collection site, and average body size given by snout-to-vent length in mm (SVL).

Population	N	Elevation (m)	SVL (±SD)
Low *E. coqui*	20	180	36.19 (4.85)
High *E. coqui*	20	875	41.85 (4.90)
E. portoricensis	20	875	36.30 (3.61)

2.4. Critical thermal minima and maxima

For every individual of *Eleutherodactylus* studied, we evaluated the critical thermal minimum (CT_{min}) and maximum (CT_{max}), defined as the temperature at which frogs could no longer right themselves after being turned ventral side up for a period of at least 5 s (Brattstrom and Lawrence, 1962; Navas et al., 2007). These measurements were taken when frogs were fully hydrated only. We placed each frog in a dry plastic container (7 x 7 x 5 cm; Rubbermaid®) that was set over a water bath with initial water temperature of 22 °C. The water bath consisted of another plastic container (11 x 11 x 7.5 cm; Rubbermaid®) holding an initial water volume of 300 ml that would be replaced by cooler or warmer water. For CT_{min}, we progressively added 20 ml of water at 0 °C per minute, to obtain a 0.50 °C/min decrease in water temperature. For CT_{max}, we progressively added 10 ml of water at 100 °C every minute, to obtain a 0.80 °C/min increase in water temperature. We used an infrared thermometer (General Tools & Instruments LLC, model IRT850K) to measure the frogs' T_b every minute until they reached their critical temperature. We opted for an infrared thermometer, opposed to a cloacal probe, because it is less invasive and does not require frog manipulation, which may alter T_b (Navas and Araujo, 2000).

We also calculated each population's average thermal tolerance range (difference between CT_{max} and CT_{min}) and operative warming tolerance (OWT), defined as the difference between CT_{max} and the average maximum operative temperature at the site where they occur for each population studied (Catenazzi et al., 2014). We used the average mid-afternoon operative temperature as a proxy of the average maximum operative temperature. At this time of the day, *Eleutherodactylus coqui* and *E. portoricensis* are in their retreat sites, and high temperatures may determine if they resume activity at night or not.

2.5. Thermal selection

It has been shown that the thermal preference of a species is dependent upon thermotaxis (Navas et al., 2021). Because we did not specifically test if our study species showed positive or negative thermotaxis, we will refer to the results of the preference experiments as selected, rather than preferred, temperatures (Navas et al., 2021). The setup for thermal selection (T_{sel}) trials consisted of four rectangular galvanized steel chambers 22 x 40 x 5 cm. These chambers were tightly covered with clear thick transparent plastic (Fig. 1). We used paper clamps to hold covers in place and prevent frogs from escaping, while controlling relative humidity. Underneath one end of each chamber, we taped a 7.62 cm wide heating tape (THGHeat; 4 W/30.5 cm) controlled by a rheostat (ReptiTemp®, Zoo Med Laboratories, Inc.) that provided a temperature range of 17.3–33.3 °C lengthwise which included slightly cooler and warmer extremes that frogs may encounter in their environment. For insulation purposes we placed a 2.5 cm-wide extruded polystyrene foam layer underneath the chamber and along its walls (Fig. 1). Some studies have found that when exposed to a temperature gradient, the risk of dehydration may deter amphibians from choosing high temperatures (Tracy and Christian 2005; Köhler et al., 2011). Thus, to prevent this tendency, we made sure that humidity was high (95–100%) all over the gradient by placing a double layer of paper towels moistened with 20 ml of tap water on the floor of the chambers. This level of ambient relative humidity is comparable to conditions in the forest at night when the frogs are active (range 93.8–100%, during the warm-wet season, Burrowes, unpublished). A trial consisted in placing a frog in the middle of the chamber and allowing it to get used to the setting for 15 min before taking any readings (as in Freidenburg and Skelly 2004). Afterwards, we used an infrared thermometer to measure the frogs' T_b at the position where it was placed within the gradient

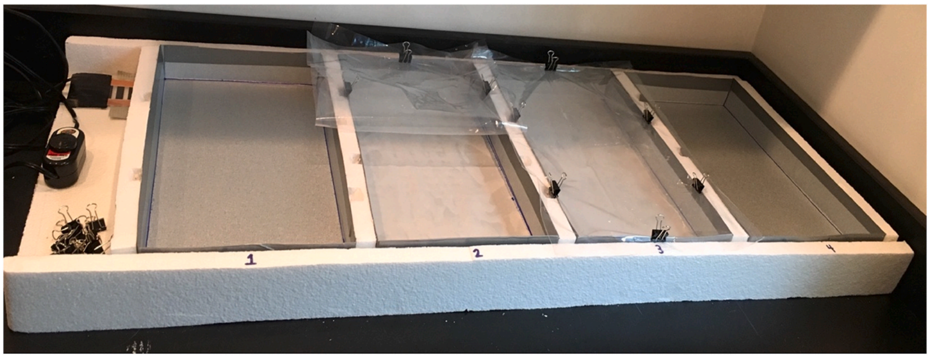

Fig. 1. Thermal selection setup showing the rheostat and heating tape on the left, and the plastic liners used as covers to maintain moisture and prevent frogs from escaping during trials.

every 5 min for 90 min. Finally, we defined each population's T_{sel} as the median T_b taken for all individuals during the 90 min trials.

2.6. *Jumping performance*

To study the effect of temperature and hydration on frog performance, we examined two response variables, average jumping distance, and average jumping speed, under a set of five experimental temperatures (18, 21, 24, 27, and 30 °C) and two hydric regimes (100% hydrated and 95% of initial hydrated frog mass). We measured the average performance during each trial, opposed to the maximum distance or speed of a single jump, because average values are better estimators of an individual's potential along the length of the trial and thus, may be more realistic indicators of performance under environmental conditions (Moore and Gatten, 1989). Performance trials were run for each individual (N = 60), such that every frog experienced the five experimental temperatures at both hydric regimes. We chose this temperature array because it includes the temperature range that *E. coqui* and *E. portoricensis* experience at both elevations where they were collected (18–24 °C), and it also accounts for higher readings that may come as a result of climate warming in the Caribbean (Alexander and Eischeid, 2001; Burrowes et al., 2004; Taylor et al., 2018). We measured the effect of dehydration and temperature in this test, because when frogs are active, they are exposed (versus protected in their retreat sites), and thus more likely to lose water through evaporation, particularly on dry nights (Pough et al., 1983), and also, because the combined effect of temperature and moisture represents a more realistic way to evaluate the effect of modern climate change in amphibians (Greenberg and Palen, 2021).

To dehydrate frogs to 95% of their initial hydrated mass, we built a desiccation setup as illustrated in Fig. 2. This setup consisted of an air pump (Topfin™) that pushed air through an initial chamber containing anhydrous calcium sulfate to remove moisture, and three additional chambers that housed frogs individually. Frogs were first weighed and then placed in the corresponding chamber (Fig. 2C). During dehydrations trials, individuals were weighed every 10–15 minutes using a digital scale (PESOLA, model PPS200) until they weighed 95% of their initial hydrated mass. We chose this level of dehydration because on preliminary experiments we noticed that further dehydration (90%) caused frogs to exhibit lethargic behavior in which they barely jumped, and another study showed that further dehydration (~75%) of initial mass could result in frog death (Beuchat et al., 1984).

Once frogs were dehydrated, we exposed them to the desired experimental temperature in a calibrated thermal incubator (Exo Terra®, model PT 2499) for 15 minutes prior to jumping trials. This time is considered appropriate for frogs to reach a target temperature that may cause thermal stress, but without breaching to lethal exposure times (Köhler et al., 2011; Navas et al., 2007; Whitehead et al., 1989).

To evaluate frog locomotor performance at specific temperature and hydration levels, we built a 2.5 m^2 cardboard arena with 10 cm high walls and covered the bottom with plasticized grid paper (Fig. 3). Inside the arena, we induced frogs to jump by gently tapping their urostyle with the blunt edge of a tweezer. We used a camera (iPhone 6S, Apple Inc.) set directly above the arena to record videos of each trial. Using Tracker software (version 4.95, copyright Douglas Brown 2018), we

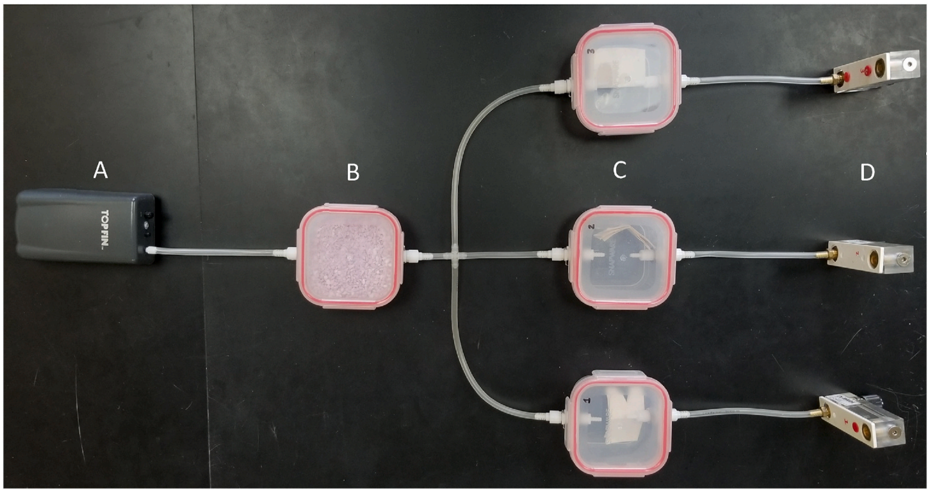

Fig. 2. Setup for frog desiccation trials consisting of an air pump (A), air drying chamber containing anhydrous calcium sulfate (B), and three additional chambers to house frogs receiving the treatment (C). Air valves (D) were used to control air pressure in each lane.

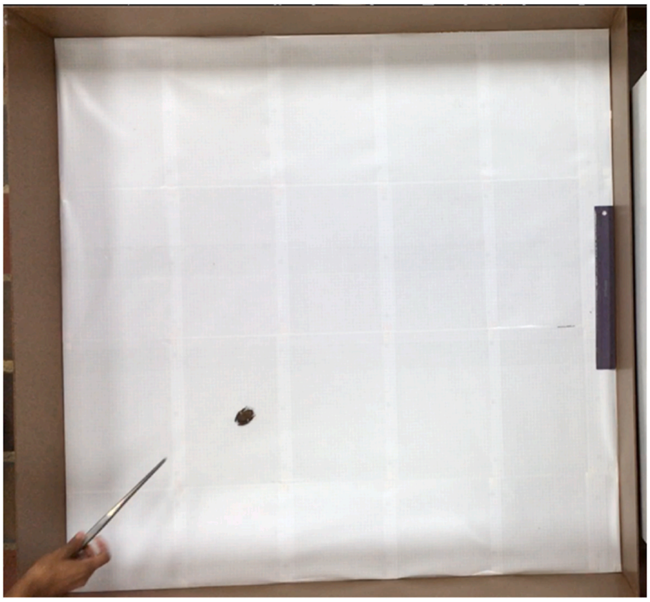

Fig. 3. Jumping performance arena (1.6 x 1.6 m), showing an *Eleutherodactylus coqui* during a performance trial and a 30.5 cm ruler for scale.

analyzed each video and measured the Euclidean distance and speed of frog jumps during trials. Each trial lasted a maximum of 1 min or until the frogs reached exhaustion and were no longer able to jump when tapped up to five times. To prevent biased results from cumulative stress we did not dehydrate individuals for more than two consecutive days, nor subjected individual frogs to more than one jumping performance trial per day.

2.7. Data analysis

We used an ANCOVA to correct for body size when comparing critical temperatures because it varied between populations (Table 1), and differences in frog mass can influence body heat transfer (Navas and Araujo, 2000). We used Tukey's HSD post-hoc tests to assess statistical differences between critical temperatures. To determine if the temperature chosen by each population was not different from random, we first compared each of their T_{sel} medians with the median temperature provided by the experimental chambers using a Mann-Whitney Wilcoxon test. We also used a Kruskal-Wallis test to compare the realm of temperatures explored by frogs in each population and the time spent at particular temperatures (expressed as T_{sel} distributions), followed by pairwise comparisons using Conover's post-hoc test. This comparison allowed us to test if frogs from a certain population chose particularly lower or higher temperatures.

We used non-linear mixed effect models to study the effect of temperature and hydration state on all three frog populations. We fit thermal performance curves based on a growth function as described by Yin et al. (2003). Specifically, we compared maximum performance and thermal optimum (i.e. temperature where maximum performance was attained) between populations and hydration levels (Appendix I). Because each individual was tested 10 times (five temperatures under two hydration states), we considered individual-level variation as a random effect in our models. Additionally, we used CT_{min} and CT_{max} data to determine the temperature where frog jumping performance would be 0 at the colder and warmer ends of the curve, respectively. Since critical temperatures were only measured on hydrated individuals, we used the same CT_{min} and CT_{max} data for both hydration levels. All data were analyzed using R statistical computing environment (version 3.6.0; R Core Team, 2016).

3. Results

3.1. Critical thermal minima and critical thermal maxima

Eleutherodactylus portoricensis had the highest average critical thermal minima, $CT_{min} = 6.52$ °C, and the lowest average critical thermal maxima, $CT_{max} = 34.69$ °C, compared to the two populations of *E. coqui* studied (Table 2). Correspondingly, *E. portoricensis* had the narrowest thermal tolerance range (28.17 °C) and had a lower operative warming tolerance (13.72 °C) than sympatric high-elevation *E. coqui* (Table 2). Operative temperatures exhibited by frogs at their field sites increase as the day progresses from morning to afternoon and drop at night when frogs are active (Fig. 4). As expected, frogs at the low-elevation site (180 m) experience higher temperatures (+6 °C on average) than those that occur at the high elevation locality (Fig. 4). Maximum daily frog operative temperatures are reached by midafternoon (12:00–16:00 h), when the mean is 25.78 °C and 20.98 °C at low and high elevation sites, respectively (Fig. 4).

Results from a one-way ANCOVA to assess potential differences in CT_{min} and CT_{max} revealed a significant population effect on CT_{min} after controlling for frog mass ($F_{3,56} = 4.31$, $p < 0.05$, $R^2 = 0.14$). A Tukey HSD post-hoc test was significant for the difference between the CT_{min} of high-elevation *E. coqui* and *E. portoricensis* ($p < 0.05$; Fig. 5). Additionally, these results showed a significant population effect on CT_{max} ($F_{3,56} = 29.91$, $p < 0.05$, $R^2 = 0.55$). There are significant differences in the CT_{max} among all populations (Tukey HSD post-hoc test $p < 0.05$ for all comparisons), the most notable being between low-elevation *E. coqui* and *E. portoricensis* (Table 2, Fig. 5).

3.2. Thermal selection

The range of temperatures available in the chamber used for the thermal selection experiments was 17.3–33.3 °C and is represented with kernel density estimates along with the range of temperatures chosen by frogs in Fig. 6. The median temperatures selected by individuals from each one of the three frog populations differed significantly from that offered by the chamber (Mann-Whitney Wilcoxon $U = 19019$, $p < 0.05$ for Low *E. coqui*; $U = 20484$, $p < 0.05$ for High *E. coqui;* $U = 24742$, $p < 0.05$ for *E. portoricensis*; Fig. 6), indicating that the temperatures at which frogs positioned themselves when given a gradient were not chosen at random. Selected temperature also differed significantly among populations (Kruskal-Wallis $\chi^2 = 48.41$, $df = 2$, $p < 0.05$, see Fig. 7), and post-hoc pairwise comparisons using Conover's test showed that *E. portoricensis* selected the lowest temperatures, followed by high-elevation *E. coqui*, and lastly by low-elevation *E. coqui* ($p < 0.05$ for all comparisons).

3.3. Jumping performance

Thermal performance curves had similar shapes for the three populations studied (Figs. 8 and 9). Average jumping distance and speed increased with temperature until attaining maximum performance, after which it dropped drastically until reaching CT_{max}, as is typical for thermal performance curves of other ectotherms (Angilletta, 2006; Burrowes et al., 2020; Khelifa et al., 2019; Sinclair et al., 2016). Our model estimates for the effect of temperature and hydration state on

Table 2
Average critical thermal values, thermal tolerance range, and operative warming tolerance (OWT) for each population of *Eleutherodactylus* studied.

Population	CT_{min} °C (±SD)	CT_{max} °C (±SD)	Tolerance range (°C)	OWT (°C)
Low *E. coqui*	6.06 (1.02)	36.72 (0.77)	30.66	10.94
High *E. coqui*	5.54 (0.63)	35.39 (1.07)	29.85	14.42
E. portoricensis	6.52 (1.25)	34.69 (0.75)	28.17	13.72

performance revealed that when hydrated *E. portoricensis* individuals jumped significantly longer distances and at a faster speed than both high and low-elevation *E. coqui* (Table 3, Appendix 1).

Dehydration had a negative effect on thermal performance, mainly on maximum performance (Y_{max}), but not on the thermal optimum for both average jumping distance and speed on all three populations (Table 3, Figs. 8 and 9). Although the effect of dehydration was

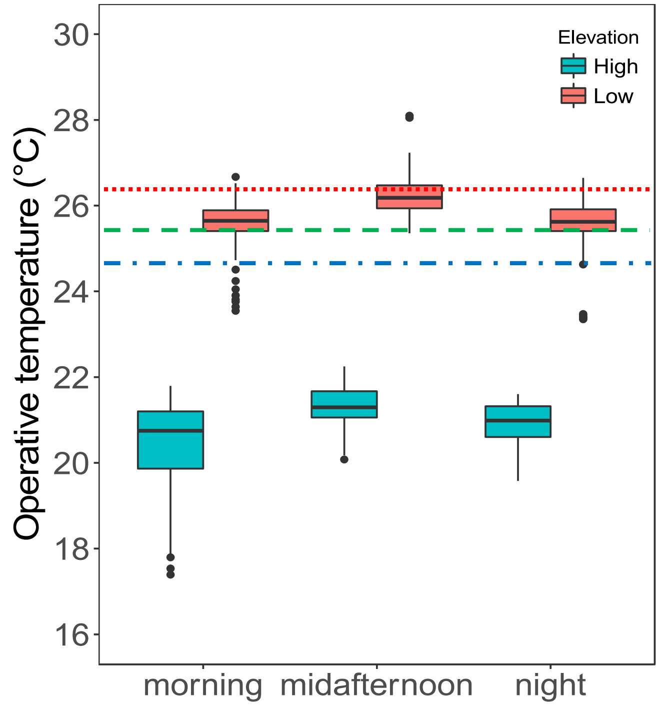

Fig. 4. Boxplots showing daily operative temperatures for the two elevation sites studied during three timeframes: morning (7:00–10:50am); mid-afternoon (11:00am–5:50pm); and night (6:00pm–6:50am). The dashed lines across show the median T_{sel} for low-elevation *E. coqui* (red dots), high-elevation *E. coqui* (green dashes) and *E. portoricensis* (blue dashes with dots). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

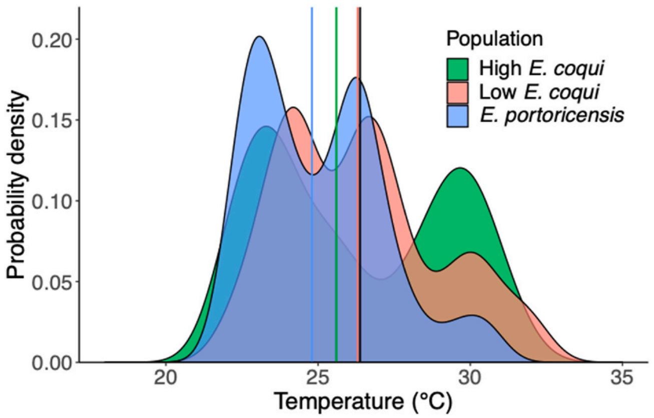

Fig. 7. Plot contrasting the probability densities of the selected temperatures by frogs in the three populations studied: Low *E. coqui* (red), High *E. coqui* (green), and *E. portoricensis* (blue). Multiple pairwise comparisons using Conover's test show that *E. portoricensis* selected the lowest temperature, followed by High-elevation *E. coqui* and finally Low-elevation *E. coqui* ($p < 0.05$ for all comparisons). The black vertical line represents the median chamber temperature while the colored vertical lines show the median (T_{sel}) for the corresponding populations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

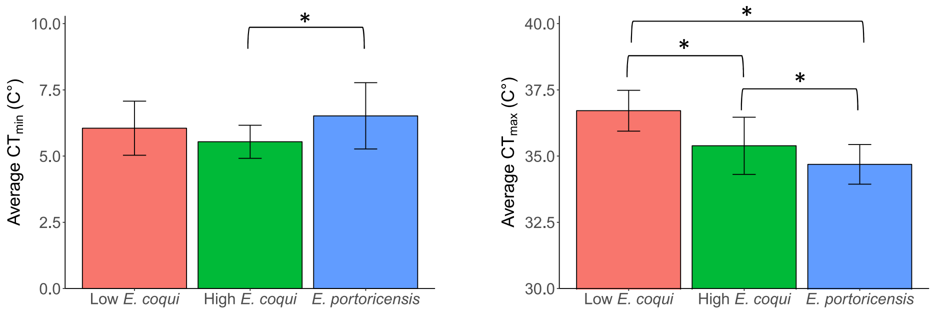

Fig. 5. Average and critical thermal minima (CT_{min}, left) and critical thermal maxima (CT_{max}, right). Asterisks (*) represent statistical differences between groups.

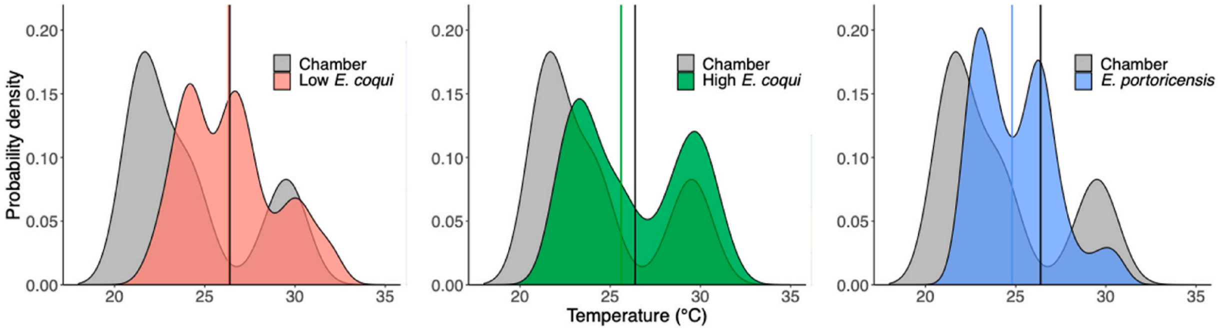

Fig. 6. Plots showing the probability densities for temperatures provided by the thermal gradient chamber (gray) in comparison with those where frogs selected to position themselves (color). The three frog populations studied are color coded from left to right: Low-elevation *E. coqui* (red), High-elevation *E. coqui* (green), and *E. portoricensis* (blue). The black vertical lines represent the median temperature of the chamber (26.38 °C), while the colored vertical lines show the median selected temperature (T_{sel}) for the corresponding populations (Low *E. coqui* 26.3 °C; High *E. coqui* 25.6 °C; *E. portoricensis* 24.8 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

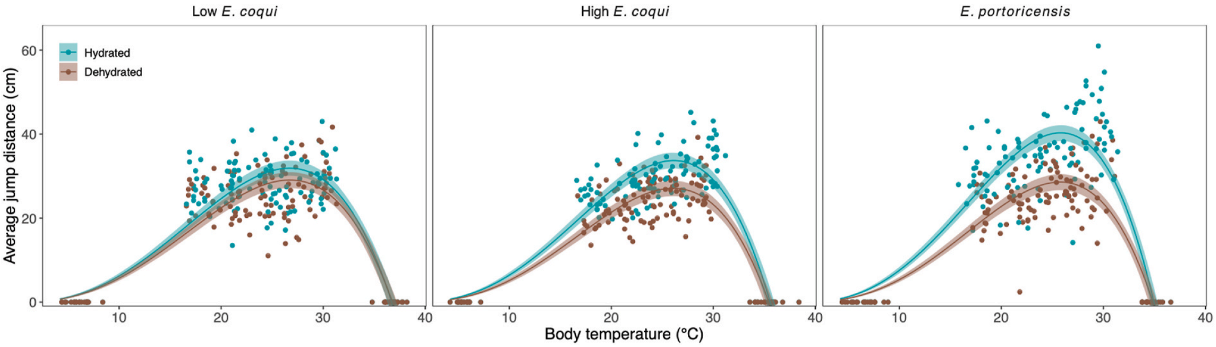

Fig. 8. Thermal performance curves on average jumping distance based on a nonlinear mixed-effects model for the three *Eleutherodactylus* populations studied. Performance trials were done under a set of five experimental temperatures (18, 21, 24, 27, and 30 °C) and two hydric regimes: 100% hydrated (blue) and 95% of initial hydrated frog mass (brown). CT_{min} and CT_{max} values correspond to hydrated individuals. The shaded areas around the curves represent the 95% confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

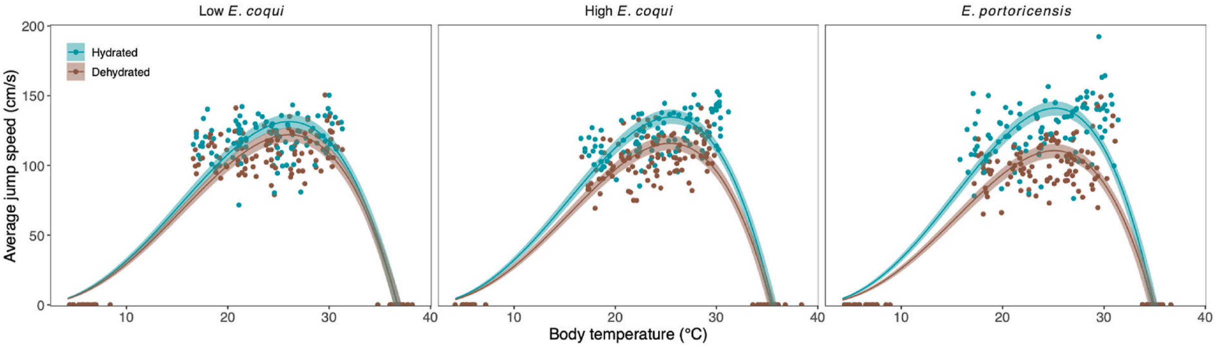

Fig. 9. Thermal performance curves on average jumping speed based on a nonlinear mixed-effects model for the three *Eleutherodactylus* populations studied. Performance trials were done under a set of five experimental temperatures (18, 21, 24, 27, and 30 °C) and two hydric regimes: 100% hydrated (blue) and 95% of initial hydrated frog mass (brown). CT_{min} and CT_{max} values correspond to hydrated individuals. The shaded areas around the curves represent the 95% confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3

Nonlinear mixed-effects model estimates for maximum performance (Y_{max}) and thermal optimum (T_{opt}) on trials of average jumping distance and jumping speed under a set of five experimental temperatures (18, 21, 24, 27, and 30 °C) and two hydric regimes (100% hydrated and 95% of initial hydrated frog mass). For each parameter we calculated the percent difference (Δ%) between hydration treatments per population and report the level of significance where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (See Appendix1 for the model output).

Jumping distance		Ymax (cm)			T_{opt} (°C)	
Population	Hydrated	Dehydrated	Δ%	Hydrated	Dehydrated	Δ%
Low *E. coqui*	31.87	29.06	−8.82***	26.7	26.73	0.11
High *E. coqui*	33.7	27.1	−19.58***	26.13	26.06	−0.27
E. portoricensis	40.31	28.58	−29.09***	25.81	25.76	−0.19
Jumping Speed		**Ymax (cm/s)**			**T_{opt} (°C)**	
Population	**Hydrated**	**Dehydrated**	**Δ%**	**Hydrated**	**Dehydrated**	**Δ%**
Low *E. coqui*	131.46	122.03	−7.17**	26.07	26.09	0.08
High *E. coqui*	134.74	115.92	−13.98***	25.5	25.43	−0.27
E. portoricensis	141.11	110.63	−21.6***	25.17	25.12	−0.2

significant in all populations, the decrease in performance was always greatest on *E. portoricensis* (Table 3, Appendix 1, Figs. 8 and 9). Dehydration reduced the maximum jumping distance of *E. portoricensis* by 29.09% compared to hydrated animals, while the reduction was only of 19.58% and 8.82% for high and low-elevation *E. coqui* respectively (Table 3, Fig. 8). The same was true for the maximum jumping speed where dehydrated *E. portoricensis* were slower than hydrated animals by 21.6%, compared to a reduction of only 13.98% for high-elevation *E. coqui*, and 7.17% for low-elevation *E. coqui* (Table 3, Fig. 9). We did not find differences on the thermal optimum performance among populations (Table 3). Independent of the level of hydration, the three populations attained their maximum jumping distance between 25.76 and 26.73 °C (Fig. 8) and their maximum jumping speed between 25.12 and 26.09 °C (Fig. 9). It is noteworthy that the effect of dehydration on performance was less drastic on low-elevation *E. coqui*, contrasting with highland populations, and pointing towards interspecific and intraspecific differences in the response to desiccation (Table 3).

4. Discussion

We found that the three populations of *Eleutherodactylus* differed in the three aspects of thermal physiology studied: critical temperatures, thermal selection, and jumping performance under different hydration levels. These differences were significant even though the two species are closely related (Pyron and Wiens, 2011), and two of these populations belong to the same species (low-elevation and high-elevation *E. coqui*). Thus, our results point towards local adaptation or plasticity in the response of *E. coqui* to varying environmental conditions with elevation, and present evidence of different physiological responses between closely related species at the same locality. These findings suggest that despite convergent morphologies between ecomorphs, physiological responses may have a deeper phylogenetic signature than implied by studies based on microhabitat utilization such as Dugo-Cota et al. (2019).

4.1. Temperature tolerance and selection

Experiments on thermal tolerance revealed that *E. portoricensis* had a narrower thermal tolerance range (28.17 °C) and a lower operative warming tolerance (13.72 °C) than *E. coqui* at the high elevation site where they co-occur (Table 2). This finding was not surprising considering that *E. portoricensis* recently exhibited a range contraction to cooler, higher elevations (Fig. 4), and because among congeners, species with narrower geographic distributions tend to have more restricted physiological responses (Brattstrom, 1970; Rohr et al., 2018). Intraspecific comparisons of critical temperatures in *E. coqui* yielded contrasting results. We found that while CT_{min} was similar for low and highland *E. coqui*, CT_{max} differed significantly between these populations (Table 2, Fig. 5). However, more than 33 years ago, Christian et al. (1988) did not find differences in the CT_{max} of *E. coqui* from different elevations and interpreted this as a lack of evolutionary plasticity in this generalist species. Thus, it is possible that the effect of recent climate warming has influenced the response of *E. coqui* to increasing temperatures at both ends of their elevational gradient. While similar behavior and microhabitat choice may lessen selective pressure for intraspecific variation (Beuchat et al., 1984; Dugo-Cota et al., 2019; Pough et al., 1983), reduced gene flow between populations may result in adaptation to local conditions (Van Valen, 1965). We show differences in physiological response of high- and low-elevation populations of *E. coqui* that may occur as a result of adaptation to local thermal and hydric regimes within an elevational gradient. The advertisement call of this species also differs with elevation, and certain parameters of the call have changed at the same elevation belts in recent years, suggesting a shift of populations to higher elevations as a response to an increase in temperature in Puerto Rico (Narins and Meenderink, 2014). Thus, it is plausible that *E. coqui* has the underlying genetic variation to respond differently to environmental temperatures, and that this plasticity is observable in elevational gradients, such as that available at El Yunque National Forest. One caveat of our study is that we did not include a mid-elevation *E. coqui* population, which if distinct and intermediate in terms of their physiological responses to temperature and dehydration, would further support this hypothesis.

Interpreting patterns in temperature selection (or preference) among populations of ectotherms of the same species that are exposed to different thermal environments is difficult because there is much variation in their response (Trochet et al., 2018). Studies on temperate frogs with aquatic larvae (Catenazzi and Kupferberg, 2017; Freidenburg and Skelly, 2004; Skelly, 2004), rainforest lizards (Llewelyn et al., 2017), and fish (Fangue et al., 2009), show intraspecific variation in selected (or preferred) temperature reflecting a counter-gradient pattern, where populations that occur in cooler environments prefer higher experimental temperatures than conspecifics from warmer environments. In contrast, other species of lizards (Gvoždík and Castilla, 2001), crickets (Kuyucu and Çağlar, 2016), and fish (Wagner and Wahl, 2006), show no differences in thermal selection among populations following an elevational or latitudinal gradient. We found neither of these responses; while high-elevation *E. coqui* showed a bimodal response (almost reflecting that offered by the chamber), low-elevation frogs exhibited a bell shape curve, with a high probability of selecting mid-range temperatures even though these were offered only in a small area of the chamber (Fig. 6). The difference in the realm of temperatures explored by high- and low-elevation *E. coqui*, suggests intraspecific variation, points towards plasticity in the response of this generalist species, and provide further evidence of local adaptation to temperature regimes (Fig. 6).

The temperature range explored by individuals of both populations of *E. coqui* was broader than that of *E. portoricensis* (Fig. 7). This may be explained by the narrower thermal tolerance (Table 2) and the higher sensitivity to temperature of this highland specialist, compared to *E. coqui* as evidenced in by the differences in thermal performance curves in this study as well as in Beuchat et al. (1984). Also, the temperatures selected by both populations of *E. coqui* were warmer than those chosen by *E. portoricensis* (Fig. 7), further highlighting a phylogenetic signal and the importance of species-specific studies when predicting vulnerability to changing climatic conditions. Our data suggest that *E. portoricensis* is clearly of greatest concern, having physiological limitations that may have served as drivers for the range contraction observed, potentially as a response to the trends of modern climate change (warming and increased drought) described for the Caribbean.

4.2. Performance under different thermal and hydric regimes

As expected for tropical ectotherms, locomotor performance increased gradually with experimental temperature in the three populations of *Eleutherodactylus* until reaching a peak in performance (Figs. 8 and 9). However, *E. portoricensis*, appear to be more responsive to temperature increase than congeners because hydrated individuals attained a higher peak in jumping performance at about the same temperature (T_{opt}) than *E. coqui* populations, in spite of having the narrowest thermal tolerance range (Tables 2 and 3, Figs. 8 and 9). Our results differ from those of Beuchat et al. (1984), who found no difference in the performance of *E. portoricensis* exposed to 20 and 25 °C. By expanding the range of experimental temperatures to 30 °C we were able to explore a more realistic temperature scenario that included environmental temperatures that may occur in Puerto Rico during very warm days (Van Beusekom et al., 2015), and that span within this species' selected temperatures (Fig. 7), while still below its CT_{max}.

Our research supports previous findings on the vulnerability of tropical, terrestrial, direct-developing frogs to desiccation (Burrowes et al., 2020) and confirm the importance of studying the combined effects of temperature and hydration to understand the response of amphibians to climate warming (Greenberg and Palen, 2021). Dehydration hindered jumping performance in the three populations of *Eleutherodactylus* studied (Figs. 8 and 9). However, this response was greatest in *E. portoricensis*, for which peak jumping distance and speed was reduced the most when dehydrated, followed by High *E. coqui*. Considering that evapotranspiration rates of amphibians typically increase with warming temperature (Tracy, 1975), this may explain the range contraction toward higher, cooler and more humid areas observed for this Endangered species (Barker and Ríos-Franceschi, 2015), especially at El Yunque National Forest, where rising temperatures have become evident (Burrowes et al., 2004; Narins and Meenderink 2014; Van Beusekom et al., 2015).

Another notable finding was that the operative warming tolerance was lowest for low-elevation *E. coqui* compared to high elevation conspecifics, and to *E. portoricensis.* These lowland frogs selected temperatures similar to those encountered in their environment (Fig. 4) suggesting they may be thermoconformers, narrowly adapted to local thermal conditions, hence more vulnerable to climate change (Huey et al., 2009). Studies along an elevational gradient in the Andes showed similar results and suggested that low-elevation tropical amphibians may also be susceptible to climate warming (Catenazzi et al., 2014). However, the fact that dehydration had a smaller effect on jumping performance of low-elevation *E. coqui* populations when compared to highland conspecifics (Table 3), suggest that lowland *E. coqui* frogs are more tolerant to dehydration and consequently may withstand effects of climate warming on evaporation rates, even if their operative warming tolerance range is low (Table 2).

4.3. Concluding remarks

While *E. portoricensis* performed best at almost 26 °C, prolonged exposure to temperature close to 30 °C results in mortality (Beuchat et al., 1984). Thus, risk of mortality and high sensitivity to desiccation may explain why *E. portoricensis* no longer occurs at lower elevations, even though its temperature choices in a thermal gradient (Fig. 6) and CT_{max} (Fig. 5) may indicate tolerance to higher temperatures. Our results corroborate other studies (Burrowes et al., 2020; Catenazzi et al., 2014; Navas et al., 1999; Tracy and Christian, 2005; von May et al., 2017) that show that tropical terrestrial amphibians have high tolerance for temperature (high CT_{max}) and experience a large breadth in temperature selection trials. Our findings support the idea that the interaction between body temperature and evaporation rate leads to physiological trade-offs in amphibians. These trade-offs, subjected to phylogenetic constraints, may explain the observed behaviors, performance curves, and the distributions of species. In some cases, limitations on the physiological response to changing thermal and hydric regimes can lead to range expansions or contractions of species particularly vulnerable to climate warming, as shown herein for *E. portoricensis*. Finally, this research underscores the value of combining field and experimental studies because, together, they can elucidate how local environmental conditions may shape species physiological traits in response to new stressors like recent climate warming. Further studies should consider the thermo-hydric physiological response of these and other amphibian species in a finer elevational gradient. Combined, these data may help define patterns that will inform conservation prioritization for species and habitats under greater risk of the effects of global climate change.

Declaration of competing interest

None.

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Author contributions

Both authors P.D-S. and P.A.B. conceived the study and wrote the manuscript. P.D-S. conducted the field work and the laboratory experimental work with input and collaboration from P.A.B. P.D-S. was responsilble for data analysis and P.A.B. contributed to data interpretation and significance of the results.

Data availability

Data will be made available on request.

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Appendix I

Table A.1

Nonlinear mixed-effects model[a] coefficients for two jumping performance parameters, maximum performance (Y_{max}) and thermal optimum (T_{opt}) in response to population and hydration level. Jumping performance parameters correspond to jumping distance and speed under five experimental temperatures (18, 21, 24, 27, and 30 °C). Statistics compare the dehydrated state of populations arbitrarily to “Dehydrated *E. coqui*”, and within a population it compares the effect of hydration level. The added values of Y_{max} and T_{opt} coefficient estimates are reported in Table 3. Asterisks indicate the level of significance where * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

	Jumping Distance			Jumping Speed		
	Estimate	*Std. Error*	*t-value*	*Estimate*	*Std. Error*	*t-value*
Y_{max}						
Dehydrated High *E. coqui*	27.1	0.96	28.13*	115.92	2.63	44.03*
Hydrated High *E. coqui*	6.6	0.84	7.81*	18.84	2.65	7.1*
Dehydrated Low *E. coqui*	1.96	1.36	1.44	6.11	3.72	1.64
Hydrated Low *E. coqui*	−3.79	1.2	−3.17*	−9.41	3.75	−2.51*
Dehydrated *E. portoricensis*	1.48	1.36	1.08	−5.29	3.72	−1.43
Hydrated *E. portoricensis*	5.13	1.2	4.27*	11.65	3.77	3.01*
T_{opt}						
Dehydrated High *E. coqui*	26.06	0.17	157.38*	25.43	0.15	171.77*
Hydrated High *E. coqui*	0.06	0.11	0.6	0.07	0.09	0.74
Dehydrated Low *E. coqui*	0.67	0.16	4.09*	0.66	0.15	4.37*
Hydrated Low *E. coqui*	−0.1	0.16	−0.63	−0.09	0.13	−0.67
Dehydrated *E. portoricensis*	−0.29	0.16	−1.85	−0.31	0.15	−2.03*
Hydrated *E. portoricensis*	−0.02	0.15	−0.13	−0.02	0.13	−0.13

[a] Model: $Y_{max} + T_{opt} \sim$ Hydration level + Population + Hydration level*Population, where individual-level variation was considered a random effect, and the curve inflection point did not vary.

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