

Comparative Microhabitat Use of Two California Native Ranids, California red-legged frog (*Rana draytonii*) and foothill yellow-legged frog (*Rana boylei*), in a Riparian Woodland

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ABSTRACT

Amphibians are the most threatened group of vertebrates in the world, due to human-caused impacts of climate change and habitat alteration on amphibian accessibility to moisture and appropriate temperatures for thermoregulation. Two imperiled species, the California red-legged frog (*Rana draytonii*) and the foothill yellow-legged frog (*Rana boylei*), overlap in some ranges and may differ in how they use shared microhabitats. Understanding how these species partition their use of microhabitat features is critical for species management. I conducted a nocturnal microclimate-based study in Sonoma County, CA along Copeland Creek, taking frog body temperatures and microclimate variables from June to October 2021. Using mixed models, I evaluated the relationship between body size, body temperature, and microhabitat features. I found that body size was a prominent driver in body temperature and microhabitat use. As body size increased in California red-legged frogs, distance to water and elevation from water line increased, and relative humidity and body temperature decreased. Thermal images showed that although individuals were basking in locations warmer than the frog itself, there was no evidence that basking location was determined by the fine-scale thermal landscape. However, water balance characteristics of larger frogs appeared to allow them to spend time further from water with a reduced risk of desiccation. Studies of microhabitat use are critical for understanding how frogs living in sympatry use their habitat, as changing microhabitat conditions effecting temperature and moisture levels will likely play a critical role in species management in the face of changing climates.

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INTRODUCTION

Amphibians are the most threatened group of vertebrates in the world, with declines from severe habitat loss and alteration, disease, climate change, and introduced species (Jennings & Hayes 1994, Bishop et al. 2014). With their high dependence on moisture and reliance on specific temperatures for functioning, they are particularly susceptible to threats from alterations of their environment. As ectothermic organisms, amphibians rely directly on their surrounding environment for regulation of internal body temperature and water balance. As human-altered environmental change, particularly with regards to changing climates, impacts these organisms, they may not be able to adapt rapidly enough to changing conditions to avoid extirpation of some populations (Taylor et al. 2020, Nowakowski 2016). However, phenotypic plasticity, particularly behavioral responses to spatial and temporal heterogeneity at the microclimate level, may buffer these impacts and allow opportunity for adaptive responses at the genetic level to accrue (Pincebourd & Suppo 2016, Sears et al. 2011, Sih 2013).

Much of the work done to evaluate the likely impacts of climate change on ectothermic amphibians stems from analyses of species distribution models, which employ a scale of variation more than 10,000 times that experienced by the individual organism (Potter et al. 2013). It is well established that microhabitats can reduce an animal's exposure to climate extremes (Scheffers et al. 2014). Thus, efforts to bridge understanding of the spatial gap about how ectotherms, in general, and amphibians, in particular, interact with the spatial and temporal heterogeneity in their environment are warranted (Isaak et al. 2017, Nowakowski et al 2018). Moreover, differences in size can influence how individuals experience the immediate environment, allowing two individuals to have different responses to the same proximal environmental conditions. As a practical matter, this may lead to species-specific behavioral

responses by different species within the same collection of landscape features (Potter et al. 2013, Sears et al. 2011). As a management concern, understanding the fine-scale spatial and temporal needs of the species in the landscape is a fundamental component of scaling up to broader management practices (Nowakowski et al. 2018).

Anurans are one of the most imperiled taxa throughout most of their geographic range in the state of California, particularly because of their dependence on access to aquatic habitats (Jennings and Hayes 1994). As ectotherms, they are affected by temperature and moisture level changes more than many other taxa and provide good models for examining the effects of climate change (Taylor et al. 2020). With ongoing loss of wildlands and changes in landscape use, these species may be forced into refugia where they encounter overlapping ranges with other similar species more frequently. Thus, the need for studies that not only improve understanding of how anuran species, particularly species of special concern, use the landscape, but also how they partition their use of microclimates where they overlap, is of growing urgency (Hoffman et al. 2021).

Two native anuran species that serve as ideal candidates for the study of microclimate-based habitat use are the California red-legged frog (*Rana draytonii*) and foothill yellow-legged frog (*Rana boylei*), both of which have been designated with special status species listings by federal and state agencies through significant portions of their known ranges (USFWS 2002, Hayes et. al, 2016). Although generally infrequent in their co-habitation, these two species do overlap in few known locations throughout California (Surber 2019; Alvarez and Wilcox 2021b) and may increasingly have to respond to sharing refugial habitat (Hayes and Jennings 1988). Further understanding of how these species utilize the same riparian habitat where they overlap

is critical to the protection of both species, as actions to protect one may hinder the success of the other (Catenazzi and Kupferberg 2017, Hoffman et al. 2021).

The California red-legged frog is a native anuran found at elevations ranging from 0 to 3400 feet and is located throughout much of the state, although thought to be extirpated throughout former ranges in southern California and the Sierras (Jennings and Hayes 1994). Early exploitation for human consumption and range decreases of up to 70% from human-altered landscapes led to the listing of this species as “threatened” in 1996 by the United States Fish and Wildlife Service (USFWS 2002, Alvarez et al. 2013, Jennings and Hayes 1994). This protected status is in effort to protect the species from its many threats, including introduced species, habitat destruction and fragmentation, water diversions, and disease (Jennings and Hayes 1994, Fellers and Kleeman 2006).

The foothill yellow-legged frog is primarily a stream obligate species found in California from the San Gabriel River up to the Oregon border (Storer 1925, Jennings and Hayes 1994). As a poorly studied species, most of what is known about this frog is in relation to its breeding habitat and associated movements (Wheeler, 2008). The decline of this species is primarily driven by water diversions and dams, which alter the hydrological structure of their habitat which drives much of their life history. Other factors putting this species at risk include mining, dredging, habitat loss, invasive species, and climate change (Hayes et al. 2016)

Insufficient documentation on habitat use for these two species contributes to a lack of understanding as to why these two species show an overall pattern of population decline (Hayes and Jennings, 1988). However, habitat protection practices for these imperiled species are not created equal: there is a need to study and protect non-breeding sites as well as breeding sites (Bishop et al. 2014). Although foothill yellow-legged frog is unique in its restricted stream

habitat use (rivalled only by *Rana muscosa* in the state) (Storer 1925, but see Wilcox and Alvarez 2019, Alvarez and Wilcox 2021b). Current trends show California red-legged frogs breeding in ponds and using riparian habitat for other functions (Storer 1925). And although foothill yellow-legged frog males have shown site fidelity to breeding locations outside of the breeding season, females spend the majority of their life elsewhere, separating breeding and non-breeding habitat within stream (Bourque 2008, Wheeler and Welsh 2008).

Previous research has raised the question of the role of non-breeding creek habitat in the life history of native Ranid species and how aspects of microhabitat use facilitate their coexistence in riparian habitat. Surber (2019) compared adult movements of California red-legged frogs and *Lithobates catesbeianus* (American bullfrog) in Sonoma County, CA, USA and found differences in how these two species utilized their shared habitat, with California red-legged frog found further and higher from the stream bed. Surber highlighted the potential importance of the upland component of this habitat surrounding the streams in allowing cohabitation of similar Ranid species. Recent observations (Alvarez and Wilcox 2021; Alvarez et. al 2021; Pierce et al. 2021) in other drainages validate Surber's assertions.

One study to date has explored larger scale habitat requirements of California red-legged frog and foothill yellow-legged frog in their overlapping habitat. Hayes and Jennings (1988) assessed habitat variables in habitat shared by these two species in the central valley of California and found that California red-legged frogs were primarily found in intermittent streams with emergent vegetation, deep pools, and a lack of non-native predators. Additionally, the presence of foothill yellow-legged frogs was best determined by shallow, partly shaded perennial streams and native species assemblages (Hayes and Jennings, 1988). Although these are important variables to explore novel areas and determine potential presence or absence of

these species, more detail is needed on use of microhabitat components along shared waterways, particularly where these two frogs are known to occur (Pierce et al. 2021).

Studies on other anuran species assemblages have found microhabitat elements such as substrate type (Kopp and Eterovick, 2006), distance from water and elevation from water line (Kopp and Eterovick, 2006; Gillespie et al. 2004) to have significant effects on interspecies partitioning of shared habitat. Additionally, although several studies on anuran habitat partitioning focused on the presence of calling males during the breeding season (Nakanishi et al., 2020, Kopp & Eterovick, 2006, Gillespie et al., 2003), there appears to be a lack of investigation on what drives species microhabitat partitioning during the non-breeding season. Furthermore, these studies consider presence of aquatic resources, but do not account for the potential of moisture (i.e. relative humidity) and temperature as factors in species microhabitat distribution.

In the case of California red-legged frog and foothill yellow-legged frog, body size may also provide an important morphological difference between the two species, with adult California red-legged frogs growing up to six times the mass of an adult foothill yellow-legged frog (pers. Obs). As a result, simple differences in body size may also influence how these species differentially use upland microhabitat which may be associated with multiple potential explanations related to heat balance and/or water balance, two of the most important physiological components known to affect anuran life history patterns (Ollala-Terraga et.al. 2009, Mokhatla et al. 2019).

Heat balance incorporates both heat gain and heat loss for ectotherms as they interact with their environment (Cushman et al. 1993). On the larger geographic scale, adaptation of Bergmann's rule, that endotherm body sizes tend to increase as temperature decreases with

latitude, to ectothermic amphibians shows that species which have the ability to thermoregulate often show a relationship between size and temperature in the environment (Ashton 2002).

Anurans are known to be able to thermoregulate through behavioral control of their environmental interactions (Ollala-Terraga et.al. 2009). At the latitudinal scale, anurans that can thermoregulate are predicted to show a relationship between body size and energy availability (heat) in the environment (Ollala-Terraga and Rodriguez 2007, Yu 2021). However, it remains to be determined whether these large-scale relationships are maintained among individuals at the microhabitat scale. Recent work by Surber (2019) suggested thermoregulation might play a critical role in upland riparian microhabitat use in California red-legged frog.

In amphibians, water balance is another critical regulatory concern and a major driver of amphibian adaptation and behavior (Ashton 2002, Mokhatla et al. 2019). Resistance to evaporative water loss has been shown to be a driver of interspecific habitat partitioning at larger scales (Amado et al. 2018). In addition, moisture in the environment has also been shown to be a driver of microhabitat selection in other anuran species (Smith et al. 2003). Here again, species' size may play a role in microhabitat selection with regards to water balance in that the smaller surface area to volume ratio in larger species of frogs reduces the likelihood of water loss and can provide for better resistance to desiccation allowing for access to dryer areas of the microhabitat (Ollala-Terraga et.al. 2009).

In this study, we examine how body size and microclimate characteristics influence patterns of upland microhabitat use in two native California Ranid frogs in a sympatric population during the non-breeding season. We determined whether species distinctions or body size impact body temperature, elevation above water line (vertical distance from water surface to frog position), and distance from water (horizontal distance of water's edge to frog position) in

California red-legged frogs and foothill yellow-legged frogs engaged in nocturnal basking. In addition, we also examined whether localized moisture (relative humidity) levels played a role in patterns of microhabitat use. Finally, we employed the use of thermal imaging as a noninvasive approach to evaluate the thermal characteristics of the individuals relative to their surrounding environment to search for potential patterns in their use of the thermal landscape during nocturnal basking activities.

METHODS

Study Area

All field work was conducted within the Mitsui Ranch, a 632-acre property owned by Sonoma Mountain Ranch Preservation Foundation (SMRPF) (Figure 1). This property sits eight kilometers east of Petaluma on Sonoma Mountain in Sonoma County, California, USA (elevation 611-732 meters). The ranch includes two California red-legged frog breeding ponds, Leaky Lake and Bonnie's Pond, and the headwaters of Copeland Creek. Copeland Creek is a tributary of Laguna de Santa Rosa in the Russian River watershed, and is home to both California red-legged frog and foothill yellow-legged frog (Figure 1).

The length of wetted creek fluctuates throughout the year with different weather events, but maintains a fairly consistent one-kilometer span where both frog species are found. Upstream riffles dry up by June, with deeper pools maintained through the summer (Surber 2019). Copeland Creek consists of a variety of pools and runs of varying depths. Plant communities and canopy cover vary along the corridor, and is predominantly characterized as oak savannah with the community assemblage made up of Oregon white oak (*Quercus garryana*), black oak (*Quercus kelloggii*), live oak (*Quercus agrifolia*), horsetail (*Equisetum sp.*), California bay-laurel

(*Umbellularia californica*), Himalayan blackberry (*Rubus armeniacus*), and a variety of naïve and invasive grasses.

California red-legged frogs and foothill yellow-legged frogs are native to the area, but their numbers were previously low to the point that repeated searches by biologists in the 1990s produced negative results on the occurrence of these species. Both species were present when surveys resumed in 2010, and their populations have steadily increased since (J. Wilcox pers. Comm).

Research Methods

Visual Search Surveys and Frog Demographics

For the past several years, biologists on the ranch have conducted research for both native frog species. Integral to this research, a variety of demographic data was collected, including frog weight (in grams) using a Pesola (spring) scale, snout-urostyle length (SUL) and gape width in millimeters, and sexing adult individuals. Over 4 years, >400 adults of both native frog species were marked with individual tags (see below); and >1000 newly transformed California red-legged frogs were also individually tagged with a passive integrated transponder (Biomark MiniHTP8, Boise, ID, USA) (PIT) and released. Visual surveys were used to locate individual frogs. Surveyors used headlamps and flashlights to find frogs along Copeland Creek through retinal (tapetum lucidum) eye shine. After capture by hand or net, a remote reader (HPR Lite, Biomark, Boise, ID) was employed to determine whether the individual frog was previously PIT tagged so that its individual number and movements could be recorded. We captured thermal images of the frog in its natural surrounding using a FLIR camera for later processing (see below). When frogs were encountered that had not been previously tagged, they were captured by hand or net to place a PIT tag, and measurements of weight and SUL were taken. Frogs were captured, handled, and marked under guidelines described in a United States Fish and Wildlife

Service permit (TE-068745-5) and a California Department of Fish and Game Scientific Collectors Permit (SC-005654) issued to J. Wilcox.

Frog body temperature

Frog body temperature was taken through the cloaca with a small, beaded thermocouple and reader (FLUKE 51 II Thermometer). Because of the relatively unknown reliability of using thermal imaging to extract exact internal body temperature measurements, this traditional method was used for most accurate comparison and analysis against body size and microhabitat variables.

Marking Techniques: Passive Integrated Transponder (PIT) Tagging

Identification of individual frogs includes using a hand held reader to scan for a previously implanted PIT tag. PIT tags give the frog an individual code so the frog can be identified throughout its lifespan. The tags are 8mm in length and 1.2mm in diameter. The tags are placed in the frog by making small chevron shaped cut in the dermis in the frog's upper dorsal side with sterilized scissors. The tag is then inserted with sterilized forceps, and pushed gently with a finger to the back of the frog over the sacral hump to prevent the tag slipping back through the incision.

Microhabitat Classification

An animal's habitat and body size are the most important factors when measuring body temperature (Taylor et al. 2020). Many temperature-based studies tend to use macroclimatic data, which does little to reflect the finer microhabitats that amphibians use to control their body temperature (Brattstrom 1963). Microhabitats along Copeland Creek were classified and assigned to each individual according to where they were located. A general pool or run location

was associated with each frog upon discovery. Microhabitat variables collected at the site of frog capture were air temperature, water surface temperature, elevation above nearest water line, distance from water edge, maximum water depth of pool closest to animal, relative humidity, and basking substrate. Air temperature (in Celsius) and relative humidity were taken with a hygrometer (Reed R6001). Water temperature (in Celsius) was taken with a thermometer (Traceable).

To measure a frog's distance from the water, a pin flag was placed at the point of capture to ensure an accurate measure on frog capture location. A five-meter tape measure (to the nearest centimeter) was used to measure the linear distance from the flag to the nearest water edge to the basking frog. To measure elevation from the water line, a laser level (Bosch GL55) was set directly over the top of the pin flag. The distance between the laser and the base of the pin flag was then subtracted from the total measurement. Frogs found in the water were marked with a distance and elevation from water as zero.

Thermal Imaging

The Forward-Looking Infrared (FLIR) cameras provides a non-invasive technique to obtain a thermal reading of a biological organism as well as its surrounding habitat. I used the FLIR E76 camera with FLIR Thermal Research Studio post-field processing. The primary function of the camera is to provide a thermal regime and detect minute temperature differences within the photo frame. Photos were taken 3.5 to 4 meters from the subject to capture sufficient substrate temperature gradients surrounding the frog.

Previous studies using FLIR technology have noted the need to assign a value of emissivity, an important value to ensure accurate temperature readings. Emissivity was set at 0.95, a standard value to sufficiently capture the low reflection from a frog's moist skin (Tattersall 2016, Barroso et al. 2020, Taylor et al 2020). The camera was calibrated and tested in the field before

data collection, as calibrating thermometers and sensors is noted as a best practice for any temperature-based studies (Taylor et al. 2020).

To assess frog body temperatures relative to the surrounding thermal landscape, a grid was overlaid onto the image. One square on each of the four sides of the frog was averaged to sample a “near frog temperature”. Using a random number generator, eight squares on the remainder of the grid were selected, and FLIR Research Studio was used to average the temperature inside the square. If the frog or another obstacle (i.e. overhanging vegetation) was within the randomly selected square, the number generator was done again to avoid misreading temperature of the substrate suitable for a frog to be found on. Frog body temperature was extracted by drawing a polygon around the frog and again averaging the resulting temperature. Averages were used to minimize the influence of any outliers found in the temperature from one pixel, as this is not as representative of the thermal landscape as a whole.

Statistical Analysis

All statistics were analyzed using JMP Pro (Version 16, SAS Institute) and statistical significance was accepted at $\alpha=0.05$ level. Body size was recorded both as mass and snout-urostyle length (SUL) for body size comparisons between individuals and between species. I used a regression to test for correlation between SUL and mass to avoid duplication of a body size variable. I ran two regressions, one for each species, with SUL (in mm) as the fixed effect and PIT tag as a random variable, with mass (in grams) as the response variable.

To determine if body temperature varies with body size, I ran a linear mixed model with a cross of SUL and species to assess the effect on body temperature. Air temperature and water temperature were added as covariates, as body temperature fluctuates with these environmental temperatures because frogs are ectothermic and cannot fully separate from these temperatures. PIT tag was added as a random variable to avoid pseudoreplication. Given a significant result, I

ran additional linear mixed models, one for each species, with body temperature as my response variable and body size (in SUL) as my fixed effects. Air temperature and water temperature were added as covariates. Model residuals were assessed for normality.

To evaluate whether microhabitat variables are correlated with body temperature, I ran a linear mixed model with body temperature as my response variable and all microhabitat variables (air temperature, water temperature, relative humidity, elevation from water line, distance to water, substrate type, and water depth) added to the model individually and crossed with species. PIT tag was added as a random effect. Non-significant effects were eliminated for the final model. Model residuals were assessed for normality. This analysis was also run with three different species by size classes: small-bodied California red-legged frogs, large-bodied red-legged frogs, and foothill yellow-legged frogs. Within this study, body size for red-legged frogs was divided at the size of the largest foothill yellow-legged frog within our study with a snout-urostyle length that is generally accepted as indicative of sexual maturity within red-legged frog species.

When evaluating the effects of body size on microhabitat variable selection, I ran a series of linear mixed models for each species with body size as the fixed effect, PIT tag as the random variable, and elevation above waterline and distance from water as response variables.

To examine whether nocturnally basking frogs had different temperatures from their surrounding substrate, I ran a mixed model with body temperature of the frog extracted from the FLIR Research Studio as the y-value, and temperature of immediate surrounding substrate and temperature average of more distant grids in the thermal regime as fixed effects. Date was also added as an ordinal variable to account for temperature fluctuations that may impact results

during seasonal changes, particularly as multiple photos were analyzed that were taken on the same night.

RESULTS

We collected a total of 275 nocturnal observations over the course of the non-breeding, non-rainy summer season (mid-June to mid-October 2021). Of these, 242 observations were of California red-legged frogs among 36 individual subjects, and 33 foothill yellow-legged frog observations among 16 individual subjects. We captured 195 digital thermal images, and analyzed 22 images in FLIR Research Studio.

Body size and body temperature

Mass and Snout-Urostyle length (SUL) were strongly correlated across California red-legged frogs ($r^2 = 0.99$, $F_{1,214.1}=222.46$, $p<0.0001$) and foothill yellow-legged frogs ($r^2 = 0.89$, $F_{1,16.91}=164.88$, $p<0.0001$). SUL was chosen over mass for analysis because SUL is less likely to fluctuate over time. For all frogs measured, body temperature in California red-legged frogs ranges from 8.8 to 23.5°C, and in foothill yellow-legged frogs from 12.4 to 23°C. Assessed within a single model, the interaction of SUL and species affected body temperature ($r^2 = 0.70$, $F_{1,50.46}=5.51$, $p=0.0229$). In a post-hoc analysis with models separated for each species, body temperature decreases with increasing body size in California red-legged frogs ($r^2 = 0.68$, $F_{1,37.14}=21.41$, $p<0.0001$) (Figure 2), but does not change with changing body size in foothill yellow-legged frogs ($r^2 = 0.71$, $F_{1,10.15}=0.16$, $p=0.70$). In our species by size assessment, SUL for red-legged frogs ranged from 39mm to 108mm, whereas yellow-legged frog SUL ranged from 40mm to 67mm. Based on these distinctions in size and the fact that red-legged frogs tend to reach maturity for breeding at sizes greater than 67mm, we divided the red-legged frogs into

two size classes separated at 67mm. Using these size classes, we found that the average temperatures of similar sized California red-legged frog and foothill yellow-legged frog have no difference in average body temperature, whereas both of these groups are different than the larger California red-legged frog size class ($F_{2, 87.75}=9.04, p=.0003$) (Figure 3).

Body temperature and microhabitat

Species ($F_{1,111.5}=4.81, p=.03$), air temperature ($F_{1,258.8}=25.34, p<.0001$), water temperature ($F_{1,257.6}=107.98, p<.0001$), substrate type ($F_{4,255.4}=14.67, p<.0001$), and the crosses of species with air temperature ($F_{1,258.2}=4.39, p=.04$) showed a significant correlation with body temperature. The cross of species and relative humidity had a marginal effect on body temperature ($F_{1,257.7}=2.95, p=.09$). When run in two separate models, one for each species, in California red-legged frogs, body temperature increases with increasing water temperature ($F_{1,229.6}=96.60, p<.0001$), and increasing air temperature ($F_{1,222.8}=57.82, p<.0001$). Body temperature decreases with increasing relative humidity ($F_{1,228.8}=4.21, p=.04$). Body temperature varies between different substrate types ($F_{4,227.6}=13.58, p<.0001$), with post-hoc analysis showing that temperatures within water are lower than temperatures on all other substrates (dry vegetation, moss, mud, and soil). In foothill yellow-legged frogs, body temperature increases with increasing water temperature ($F_{1,26.56}=15.21, p=.0006$) and marginally increases with increasing air temperatures ($F_{1,27.14}=3.84, p=.06$).

Body size and microhabitat

In California red-legged frogs, individuals with larger snout-urostyle length were found at deeper pools ($F_{1,36.47}=7.73, p=.0085$). Distance from water ($F_{1,36.39}=4.95, p=.03$) and elevation from water line ($F_{1,27.39}=8.96, p=.006$) also increased with increasing body size. Air temperature

at point of capture relative to nearest water temperature (whether in or near water) was not associated with body size. Relative humidity decreased significantly with elevation from water line ($F_{1,190}=10.2290$, $p=0.0016$), and body size ($F_{1,53.9}=3.77$, $p=0.0573$; Figure 4 and 5) and decreased marginally with an increase in distance from water ($F_{1,190}=3.2408$, $p=.0734$). In foothill yellow-legged frogs, we did not detect any association between body size and microhabitat variables ($p>.05$). Species of frog did not impact distance from water ($F_{1,66.76}=1.56$, $p=.22$), but had a marginal effect on elevation from water line ($F_{1,61.58}=3.78$, $p=.06$). There was no difference in microhabitat variables used between smaller California red-legged frogs and foothill yellow-legged frogs ($p>.05$), but both were different than larger California red-legged frogs ($p>.05$) (Figure 6 and 7).

FLIR imaging

We captured 195 thermographs and analyzed 22 thermographs between both California red-legged frog ($n=20$) and foothill yellow-legged frog ($n=2$) from August to October 2021. Images of frogs taken in water or with significant obstruction to assessing body and substrate temperatures (e.g. overhanging vegetation) were omitted from analysis. Analysis focused on images of frogs basking out of the water to determine whether basking site choice was the warmest spot in their immediate surrounding habitat (Figure 8). Frogs were found to be much colder than the surrounding substrate. When accounting for temperature fluctuations due to time of year, neither the nearest ($F_{1,11}=0.78$, $p=0.40$) nor surrounding substrate temperatures ($F_{1,11}=0.13$, $p=0.73$) had a significant effect on frog body temperature extracted from the FLIR image.

DISCUSSION

The results indicate that sympatric populations of California red-legged frog and foothill yellow-legged frog have size driven differences in use of their shared upland microhabitat during the dry, non-breeding season. As a general pattern, larger frogs appear to use nocturnal basking sites that are further from and higher out of water. Although frogs may be leaving the water for thermoregulatory purposes, as they were found to be significantly cooler than the upland substrate, there is no indication the pattern of sites they choose for basking is associated with differential temperature. Rather, their body size, as it relates to water balance, may impact how far individuals can move away from water for nocturnal basking.

Body size and body temperature

Body temperature decreased with increasing body size in all California red-legged frogs studied. The majority of theories regarding body size in relation to environmental variables are associated with endothermic organisms, including the role surface area-to-volume ratio (Mayr 1956, Walters and Hassal, 2006). Several studies examining the extent to which these theories may hold with ectothermic organisms have shown mixed results. Theories regarding the role of surface area-to-volume ratio in ectotherms suggest that a smaller surface area to volume ratio, typically found in animals with a larger mass, leads to reduced heat loss and these organisms would fare better in colder climates (Walters and Hassal, 2006). However, much of the association of larger body size and cooler temperatures in ectotherms is related to rearing temperatures and/or is evaluated on larger geographic scales, across species (Atkinson 1994, Walters and Hassal 2006, Kingslover and Huey 2008). In this study, where microhabitat use is occurring in adults who have completed development, body temperature decreased with

increasing size, contrary to the larger-scale patterns of body size and climate noted in the literature.

The data indicate that body size rather than species is highly associated with body temperature for frogs engaged in nocturnal basking. I found that body temperature decreases with increasing body size in California red-legged frogs, but there was no difference in body temperature based on size in foothill yellow-legged frogs. Moreover, when California red-legged frogs were split into large and small size classes, body temperature in smaller red-legged frogs was not different that of foothill yellow-legged frogs but was significantly different from their larger conspecifics (Figure 3). These results suggest patterns of body temperature are dependent on size and not on other traits that distinguish these species.

Body size and microclimate

Previous studies have hypothesized that frogs take advantage of spatiotemporal distribution of environmental temperatures to maximize energy use (Hutchinson and Maness, 1979), and may be leaving aquatic resources to take advantage of higher temperatures. A visual of this can be seen in the thermal images (e.g. Figure 8): frogs were consistently defined in cooler tones (blue/purple), whereas the surrounding substrate, both ground and vegetation, were comprised of warmer tones (red/orange/yellow), indicating the surrounding substrate was higher in temperature than the frog in all images studied. Results from these thermal images and from cloacal body temperature data suggest that although the frogs studied here may be using basking sites out of water that are of a higher temperature than the frog is itself, the exact basking site choice in their microhabitat may be of less importance from a thermal stand-point.

Many amphibians are poor thermoregulators and the need for water conservation outweighs their ability to thermoregulate, although some species are more active

thermoregulators that will seek out hotter and colder parts of their environment to maintain their body temperature (Brattstrom 1979). As both foothill yellow-legged frog and California red-legged frog were primarily seen unmoving and not in the warmest parts of their environment, based on thermal imaging, this is strongly indicative that both Ranids are poor thermoregulators. These species have adapted to either stay close to water, in the case of foothill yellow-legged frog, or maintain a larger body size for overland travel, as in California red-legged frog.

Previous research conducted within this same study area hypothesized that California red-legged frogs were travelling further from the water than American bullfrogs possibly in order to meet thermoregulatory requirements (Surber 2019). In this study, larger California red-legged frogs were also found further from and higher out of the water than either the smaller conspecifics or the yellow-legged frogs. However, we did not find any evidence that suggests that being further from water or higher on the bank provided access to any differential temperature interaction. Air temperatures increased marginally as distance and elevation from water increased, but with no significant effect. Although body temperature of nocturnally basking California red-legged frogs decreased with increasing size, large-bodied frogs did not have body temperatures that were higher at distances farther from the water. Moreover, analyses of thermal images showed that temperatures adjacent to the frog basking sites were not significantly different than temperatures randomly selected from the surrounding substrate. These results indicate that frogs in this size class may be travelling farther from the water to meet other ecological needs, such as foraging for terrestrial prey, which has been found to make up a majority of their diet (Bishop et al. 2014)..

Despite the lack of evidence that the larger frogs are traveling further from the water strictly for thermoregulation, it may be that their larger size explains the negative relationship

between size and body temperature. Larger bodied individuals have greater thermal inertia, which helps minimize heat loss because of their larger mass relative to the surface area of skin exposed to the environment (Atkinson 1994). However, this provides that the opposite is also true: less surface area relative to mass in larger-bodied frogs means there is less exchange of thermal radiation from the ground, vegetation, and atmosphere (Tracy et al. 2010). Additionally, California red-legged frog are considered a nocturnal species, active predominantly at night when temperatures are cooler and heat exchange occurs more slowly, furthering the difficulty of larger sized frogs achieving higher temperatures as quickly as smaller-bodied frogs do (Tracy 1975).

Body size, distance from water, and water balance

An alternative explanation for the pattern of larger frogs basking further from the water may lie in consideration of water balance, which is well known to influence amphibian adaptation and behavior. Water balance is critical for the survival and performance of organisms, influencing aquatic breeding success, dispersal, and foraging time (Peterman et al. 2013).

Water availability may be a more important factor on body size clines than temperature (Olalla-Tarraga and Rodriguez, 2007 & Gouveia and Correia, 2016). Omitting data taken for California red-legged frogs in water, relative humidity decreased as body size, distance to water, and elevation from water line increased. This is critical to note, as body temperature decreases with increasing body size in this species. Large-bodied individuals have relatively low rates of water loss considering their greater mass (Mokhatla, 2019). In a study using plaster models, smaller sized amphibian models lost water at a significantly greater rate than larger models (Peterman et al. 2013). Overall, these large body individuals can handle more arid conditions,

which is consistent with our findings that larger frogs are found at slightly higher air temperatures and at lower relative humidity than smaller bodied frogs.

Larger frogs that maintain lower body temperatures have a lower rate of desiccation which allows them to be further from water and for longer periods of time. As the California red-legged frogs are highly terrestrial frogs that can travel over a mile to reach breeding ponds, it is important for individuals to maintain moisture levels while in the uplands, where this species is hypothesized to spend a majority of their time (Bulger et al. 2003; Fellers and Kleeman 2007; Tatarian 2008; Halstead and Kleeman 2017; Surber 2019; Alvarez and Wilcox 2021a). Previous research has also found that frogs that more effectively avoid rapid desiccation are more terrestrial (Tracy et al. 2010). Conversely, foothill yellow-legged frogs are known to be a highly aquatic species, rarely travelling from streams. These data may indicate that a driving factor in their smaller body size is they are able to maintain their water balance through a near constant proximity to water. Putting resources into a larger body size is not necessary for yellow-legged frogs which do not require large-scale overland travel as occurs in California red-legged frogs.

The threat of desiccation has implications both for dispersal and foraging. Threat of desiccation can be mitigated by having skin resistant to water loss, having a larger body size, selecting moist microhabitats, and through nocturnality (Tracy et al, 2010). The “water time limit” is a concept in which amphibians are limited in time spent foraging by their internal water balance (Feder and Landos 1984). Active and mobile amphibians are at greater risk for water loss than those that are inactive (Feder and Landos 1984), particularly if less active frogs are utilizing moist shelters (Seebacher and Alford 2002). Additionally, amphibians have been found to abandon foraging activities sooner in environments with lower humidity (Feder and Lands 1984). Larger-bodied California red-legged frog were observed in unsheltered areas and were

largely immobile during the course of the study and are therefore able to maintain water balance while foraging, as they are sit and wait predators (Roth 1986).

Time to desiccation can be greatly reduced by nocturnality of individuals with rates of water loss during the night thought to be 10 to more than 50 times slower than during the daytime (Tracy et al, 2010). Observation of California red-legged frogs indicate they are nocturnal in their upland movements (Surber 2019; Pierce et al. 2021; Alvarez and Wilcox 2021a), which allows for a frog to move farther from moist refugia at night without increased risk of desiccation (Tracy et al, 2010). The thermal landscape is more homogenous during the night (Peterman et al. 2013) because the lack of direct solar radiation increases the time a frog can spend out of the water meeting ecological needs, such as foraging.

Lastly, rates of water loss can limit the ability of dispersal to different parts of the species' habitat. Species more susceptible to desiccation are more limited in their dispersal between habitat patches, a potentially detrimental trait in the face of increasing habitat fragmentation globally (Watling and Brava 2015). Species that are able to travel farther and avoid desiccation may fare better travelling between suitable habitat patches, whereas species more prone to desiccation will be greatly limited in dispersal. This again provides evidence to California red-legged frogs being a much larger and more terrestrially-adapted frog than foothill yellow-legged frogs, although small metamorphic California red-legged frogs undergo a large dispersal event, typically following the first big rain in late summer or early fall (Allaback et al. 2010). This may indicate a species behavior to minimize water loss, although little is known about microhabitat use and use of moist habitats for metamorphs as they leave the breeding ponds.

Potential for Predation and Competition

A lack of aquatic predators may also be important for the maintenance of sympatry of these two similar species (Hayes and Jennings 1988). There are no known aquatic predators along Copeland Creek within the Mitsui Ranch, and although bullfrogs have been previously documented along this stretch of the creek (Surber, 2020), none were observed during this study. This indicates that smaller frogs can stay closer to the water to meet thermoregulatory and water retention needs with reduced chance of predation by invasive aquatic predators.

There has been little research done on the degree of competition and predation potential between these two species in their shared habitat. Previous work in overlapping habitat suggests competition is reduced due to microhabitat and resource partitioning (Gonsolin, 2010). However, our findings indicate there is a high degree of overlap in the use of shared habitat of smaller red-legged frogs and yellow-legged frogs. The difference in habitat use between smaller and larger individuals is more likely the driver of habitat partitioning, rather species differences, with the larger California red-legged frogs able to travel further from water to acquire additional resources without risk of desiccation. Further understanding of how these two species are partitioning their habitat is needed to determine what is driving resource use in shared habitat. In particular, exploring microhabitat choice and microclimate in foothill yellow-legged frogs is particularly critical in this species, as there are still many unknowns in regards to species ecology.

CONCLUSIONS

In this study, body size appears to be the primary driver in microhabitat partitioning where California red-legged frogs and yellow-legged frogs share riparian habitat, rather than being driven solely by other interspecific behavioral differences. Air and water temperature may

be a significant a factor for nocturnal movement out of water during non-breeding, summer periods, however, substrate temperature does not appear to drive specific microhabitat-level basking site choice in these two species; rather, body size in conjunction with relative humidity and capabilities associated with water balance allow larger frogs to travel further from the water to acquire additional resources. However, as congeners, risk of predation and potential for competition between size classes of these co-occurring species may be another driver in microhabitat partitioning for resources. To further explore this hypothesis, studies should be conducted in other overlapping habitats as well as stream habitats in which these species individually occur to explore the degree to which one species is influencing the other, and whether the findings of our study are consistent throughout their range. Previous habitat restoration of the uplands within a riparian corridor led to an increase in California red-legged frogs seen using these uplands, further exemplifying the need to maintain both breeding and non-breeding habitat through protection and restoration (Alvarez and Wilcox 2021). Understanding microhabitat use is critical for the management of these species and determining potential for species presence, best protection of breeding and non-breeding aquatic habitat, and how habitat alteration and fragmentation may drive changes of ecological interactions between congeners.

LITERATURE CITED

- Allaback, M. L., D. M., Laabs, D. S. Keegan, and J. D Harwayne. 2010. RANA DRAYTONII (California red-legged frog). Dispersal. *Herpetological Review* 41(2):204-206. Alvarez, J. A., Cook, D. G., Yee, J. L., Van Hattem, M. G., Fong, D. R., & Fisher, R. N. (2013). Comparative microhabitat characteristics at oviposition sites of the California Red-legged frog (*Rana draytonii*). *Herpetological Conservation and Biology*.
- Alvarez, J. A., & Wilcox, J. T. (2021). Observations of Nocturnal Upland Habitat Use by the *Rana Draytonii* (California Red-Legged Frog), and Implications for Restoration and Other Activities. *Ecological Restoration*, 39(3), 155-157.
- Alvarez, J. A., M. A. Shea, and J. T. Wilcox. 2021a. Upwardly mobile: vertical movement of California red-legged frogs (*Rana draytonii*) and its management implications. *Herpetology Notes* 14:839–841.
- Alvarez, J. A., and J. T. Wilcox. 2021b. Observations of atypical habitat use by foothill yellow-legged frogs (*Rana boylei*) in the Coast Range of California. *Western North American Naturalist* 81:293–299.
- Amado, T. F., Bidau, C. J., & Olalla-Tárraga, M. Á. (2019). Geographic variation of body size in New World anurans: energy and water in a balance. *Ecography*. <https://doi.org/10.1111/ecog.03889>
- Ashton KG. 2002. Do amphibians follow Bergmann’s rule? *Can J Zool.* 80:708–716
- Atkinson, D. 1994. Temperature and organism size: a biological law for ectotherms? *Advances in Ecological Research* 25:1–58.
- Barroso, F. M., Riaño, G., Sannolo, M., Carretero, M. A., & Rato, C. (2020). Evidence from *Tarentola mauritanica* (Gekkota: Phyllodactylidae) helps validate thermography as a tool to infer internal body temperatures of lizards. *Journal of Thermal Biology*. <https://doi.org/10.1016/j.jtherbio.2020.102700>
- Bishop, M. R., Drewes, R. C., & Vredenburg, V. T. (2014). Food web linkages demonstrate importance of terrestrial prey for the threatened California red-legged frog. *Journal of Herpetology*. <https://doi.org/10.1670/12-288>
- Bolochio, B. E., Lescano, J. N., Cordier, J. M., Loyola, R., & Nori, J. (2020). A functional perspective for global amphibian conservation. *Biological Conservation*, 245, 108572.
- Brattstrom, B. H. (1963). A Preliminary Review of the Thermal Requirements of Amphibians. *Ecology*. <https://doi.org/10.2307/1932171>
- Brattstrom, B. H. (1979). Amphibian temperature regulation studies in the field and laboratory. *Integrative and Comparative Biology*. <https://doi.org/10.1093/icb/19.1.345>
- Bulger, J. B., N. J. Scott, Jr., and R. B. Seymour. 2003. Terrestrial activity and conservation of adult California red-legged frogs *Rana aurora draytonii* in coastal forests and grasslands. *Biological Conservation* 110:85–95.

- Catenazzi, A., & Kupferberg, S. J. (2017). Variation in thermal niche of a declining river-breeding frog: From counter-gradient responses to population distribution patterns. *Freshwater Biology*, 62(7), 1255–1265. <https://doi.org/10.1111/fwb.12942>
- Fellers, G. M., & Kleeman, P. M. (2006). Diurnal Versus Nocturnal Surveys for California Red-Legged Frogs. *Journal of Wildlife Management*. [https://doi.org/10.2193/0022-541x\(2006\)70\[1805:dvnsfc\]2.0.co;2](https://doi.org/10.2193/0022-541x(2006)70[1805:dvnsfc]2.0.co;2)
- Halstead, B. J. and Kleeman, P. M., 2017. Frogs on the beach: Ecology of California Red-Legged Frogs (*Rana draytonii*) in coastal dune drainages. *Herpetological Conservation and Biology*, 12(1), pp.127-140.
- Hayes, M. P., & Jennings, M. R. (1988). Habitat correlates of distribution of the California red-legged frog (*Rana aurora draytonii*) and the foothill yellow-legged frog (*Rana boylei*): implications for management. *General Technical Report - US Department of Agriculture, Forest Service*.
- Hayes, M. P., Wheeler, C. A., Lind, A. J., Green, G. A., & Macfarlane, D. C. (2016). Foothill yellow-legged frog conservation assessment in California. *United States Department of Agriculture, Forest Service, Pacific Southwest Research Station*.
- Hoffmann, E. P., Williams, K., Hipsey, M. R., & Mitchell, N. J. (2020). Drying microclimates threaten persistence of natural and translocated populations of threatened frogs. *Biodiversity and Conservation*. <https://doi.org/10.1007/s10531-020-02064-9>
- Hutchison, V. H., & Maness, J. D. (1979). The role of behavior in temperature acclimation and tolerance in ectotherms. *American zoologist*, 19(1), 367-384.
- Gillespie, G. R., Lockie, D., Scroggie, M. P., & Iskandar, D. T. (2004). Habitat use by stream-breeding frogs in south-east Sulawesi, with some preliminary observations on community organization. *Journal of Tropical Ecology*. <https://doi.org/10.1017/S0266467404001361>
- Gonsolin, T. E., Jr, 2010. Ecology aspects of foothill yellow-legged frogs in the Diablo mountain range on upper Coyote Creek, Santa Clara County, California. Masters Thesis, San Jose State University, San Jose, California. 128 pp.
- Isaak, D. J., Wenger, S. J., & Young, M. K. (2017). Big biology meets microclimatology: Defining thermal niches of ectotherms at landscape scales for conservation planning. *Ecological Applications*. <https://doi.org/10.1002/eap.1501>
- Jennings, M. R., & Hayes, M. P. (1994). Amphibian and Reptile Species of Special Concern in California. In *California Department of Fish and Game*.
- Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research*.
- Kopp, K., & Eterovick, P. C. (2006). Factors influencing spatial and temporal structure of frog assemblages at ponds in southeastern Brazil. *Journal of Natural History*. <https://doi.org/10.1080/00222930601017403>

- Lillywhite, H. B. (1970). Behavioral Temperature Regulation in the Bullfrog, *Rana catesbeiana*. *Copeia*. <https://doi.org/10.2307/1441983>
- Luna, S., & Font, E. (2013). Use of an infrared thermographic camera to measure field body temperatures of small lacertid lizards. *Herpetological Review*.
- Mayr, E. 1956. Geographical character gradients and climatic adaptation. *Evolution* 10:105–108.
- Mokhatla M, Measey J, Smit B. 2019. The role of ambient temperature and body mass on body temperature, standard metabolic rate and evaporative water loss in southern African anurans of different habitat specialisation. *Peer* 7:e7885 <https://doi.org/10.7717/peerj.7885>
- Nowakowski, A. J., Watling, J. I., Whitfield, S. M., Todd, B. D., Kurz, D. J., & Donnelly, M. A. (2017). Tropical amphibians in shifting thermal landscapes under land-use and climate change. *Conservation Biology*, 31(1), 96-105.
- Pierce, M. T., J. T. Wilcox, A. L. Erway, V. L. Brunal, and J. A. Alvarez. 2021. RANA DRAYTONII (California red-legged frog) HABITAT USE. *Herpetological Review* 52(3):619–620.
- Olalla-Tárraga, M. Á., & Rodríguez, M. Á. (2007). Energy and interspecific body size patterns of amphibian faunas in Europe and North America: Anurans follow Bergmann's rule, urodeles its converse. *Global Ecology and Biogeography*. <https://doi.org/10.1111/j.1466-8238.2007.00309.x>
- Pincebourde, S., & Suppo, C. (2016). The vulnerability of tropical ectotherms to warming is modulated by the microclimatic heterogeneity. *Integrative and Comparative Biology*. <https://doi.org/10.1093/icb/icw014>
- Potter, K. A., Arthur Woods, H., & Pincebourde, S. (2013). Microclimatic challenges in global change biology. *Global Change Biology*. <https://doi.org/10.1111/gcb.12257>
- Roth, G. 1986. Neural mechanisms of prey recognition: an example in amphibians, pp. 42-68. In M. E. Feder and G. V. Lauder [eds.], *Predator-prey relationships: perspectives and approaches from the study of lower vertebrates*. The Univ. of Chicago Press, Chicago, IL.
- Scheffers, B.R., Edwards, D.P., Diesmos, A., Williams, S.E., & Ebvans, T.A. 2014. Microhabitats reduce animal's exposure to climate extremes. *Global Change Biology* (2014) 20, 495–503, doi: 10.1111/gcb.12439
- Sears, M. W., Raskin, E., & Angilletta, M. J. (2011). The world is not flat: Defining relevant thermal landscapes in the context of climate change. *Integrative and Comparative Biology*. <https://doi.org/10.1093/icb/icr111>
- Seebacher, F., & Alford, R. A. (2002). Shelter microhabitats determine body temperature and dehydration rates of a terrestrial amphibian (*Bufo marinus*). *Journal of Herpetology*. [https://doi.org/10.1670/0022-1511\(2002\)036\[0069:SMDBTA\]2.0.CO;2](https://doi.org/10.1670/0022-1511(2002)036[0069:SMDBTA]2.0.CO;2)
- Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*. <https://doi.org/10.1111/j.1752-4571.2010.00166.x>

- Smith, G. R., Todd, A., Rettig, J. E., & Nelson, F. (2003). Microhabitat selection by Northern Cricket Frogs (*Acris crepitans*) along a west-central missouri creek: Field and experimental observations. *Journal of Herpetology*. [https://doi.org/10.1670/0022-1511\(2003\)037\[0383:MSBNCF\]2.0.CO;2](https://doi.org/10.1670/0022-1511(2003)037[0383:MSBNCF]2.0.CO;2)
- Storer, T. I. 1925. A synopsis of the Amphibia of California. University of California Publications in Zoology 27: 1–342, 18 pl..
- Surber, L. L. 2019. Comparison of habitat use and movement patterns of native and invasive frogs in a grassland and oak savanna habitat. Masres Thesis, Sonoma State University, Rohnert Park, California. 47pp.
- Tatarian, P. J. 2008. Movement patterns of California red-legged frogs (*Rana draytonii*) in an inland California environment. *Herpetological Conservation and Biology* 3(2): 155–169.
- Tattersall, G. J. (2016). Infrared thermography: A non-invasive window into thermal physiology. In *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*. <https://doi.org/10.1016/j.cbpa.2016.02.022>
- Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., Rödder, D., Rollinson, N., Spears, S., Sun, B. jun, & Telemeco, R. S. (2021). The thermal ecology and physiology of reptiles and amphibians: A user’s guide. In *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*. <https://doi.org/10.1002/jez.2396>
- U.S. Fish and Wildlife Service. (2002). Recovery plan for the California red-legged frog (*Rana aurora draytonii*). *US Fish and Wildlife Service, Portland, Oregon*. <https://doi.org/10.1007/s10725-013-9828-1>
- Vences, M., Galán, P., Vieites, D. R., Puente, M., Oetter, K., & Wanke, S. (2002). Field body temperatures and heating rates in a montane frog population: The importance of black dorsal pattern for thermoregulation. *Annales Zoologici Fennici*.
- Walters, R. J., & Hassall, M. (2006). The temperature-size rule in ectotherms: May a general explanation exist after all? *American Naturalist*. <https://doi.org/10.1086/501029>
- Wheeler, C. a, & Welsh Jr., H. H. (2008). Mating Strategy and Breeding Patterns of the Foothill Yellow-Legged Frog (*Rana Boylii*). *Herpetological Conservation and Biology*.
- Wilcox, J. T. and J. A. Alvarez. 2019. Wrestling for real estate: male-male interactions in breeding foothill yellow-legged frogs (*Rana boylii*). *Western Wildlife* 6:14–17.
- Yu, T. L., Jia, G., Sun, H. Q., Shi, W. H., Li, X. L., Wang, H. B., Huang, M. R., Ding, S. Y., Chen, J. P., & Zhang, M. (2021). Altitudinal body size variation in *Rana kukunoris*: the effects of age and growth rate on the plateau brown frog from the eastern Tibetan Plateau. *Ethology Ecology and Evolution*. <https://doi.org/10.1080/03949370.2021.1913448>

FIGURES



Figure 1. Overview of Mitsui Ranch, in Sonoma County, CA, USA with relevant water features. Probably need inset showing where this in California, or coordinates for the ranch.

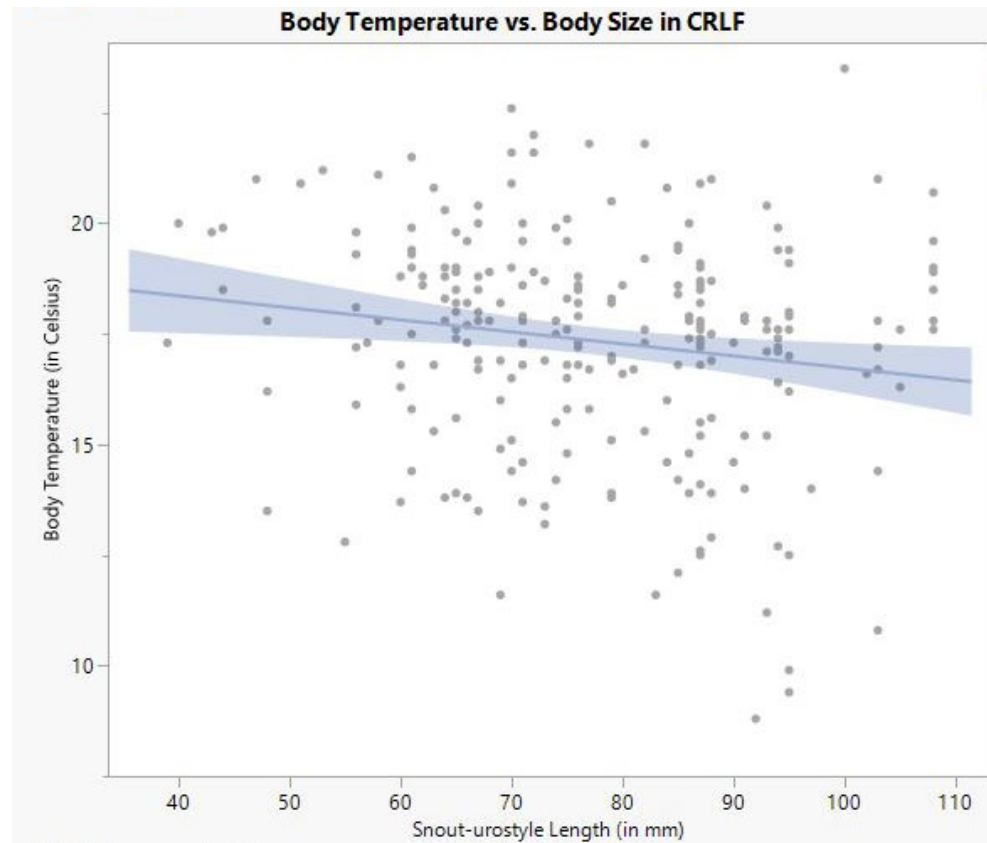


Figure 2. Body temperature decreased with increasing body size (snout-urostyle length) in California red-legged frogs ($r^2 = 0.68$, $F_{1,37.14}=21.41$, $p<0.0001$).

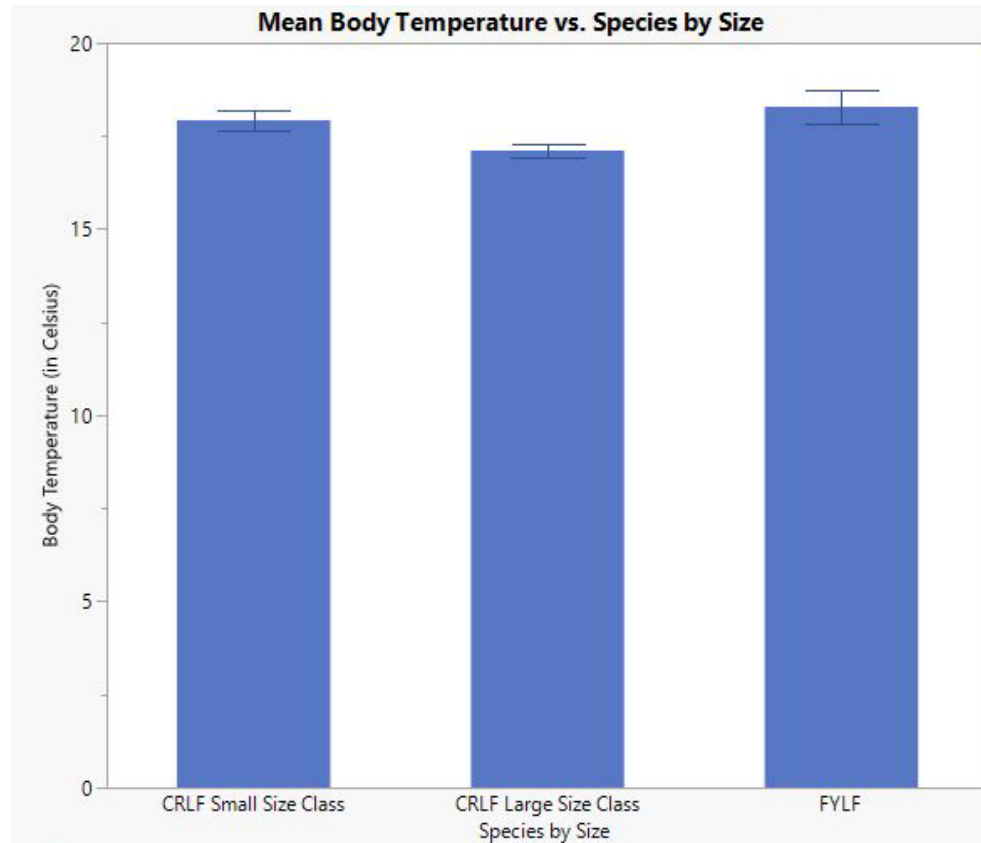


Figure 3. The body temperature of small-bodied California red-legged frogs and foothill yellow-legged frogs are more similar to each other than to large-bodied California red-legged frogs ($F_{2, 87.75}=9.04$, $p=.0003$).

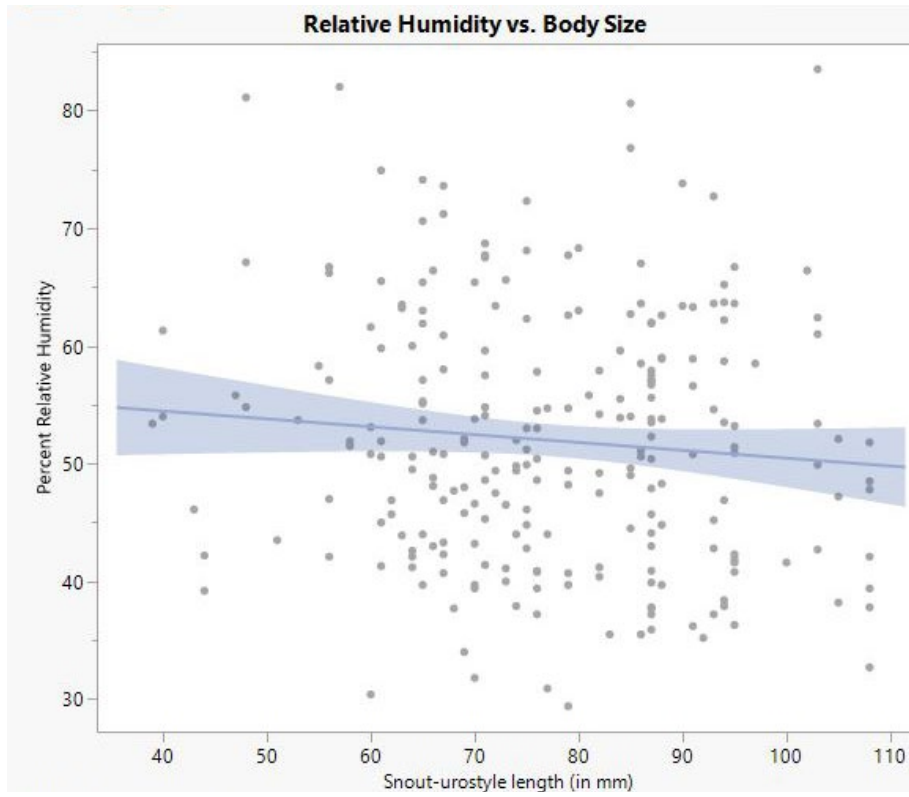


Figure 4. Body temperature decreases with increasing relative humidity ($F_{1,228.8}=4.21$, $p=.04$).

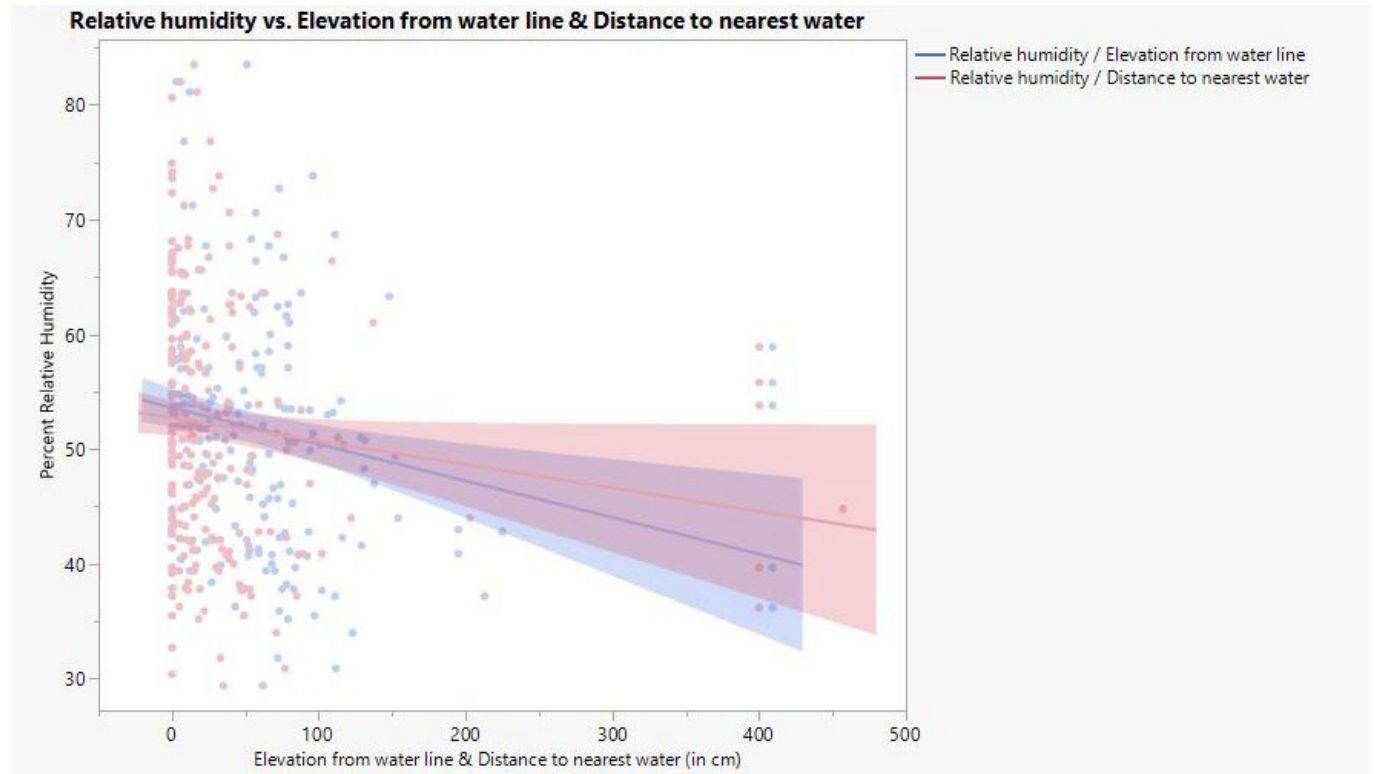


Figure 5. Relative humidity decreased significantly with elevation from water line ($F_{1,190}=10.2290$, $p=0.0016$), and decreased marginally with an increase in distance from water ($F_{1,190}=3.2408$, $p=.0734$).

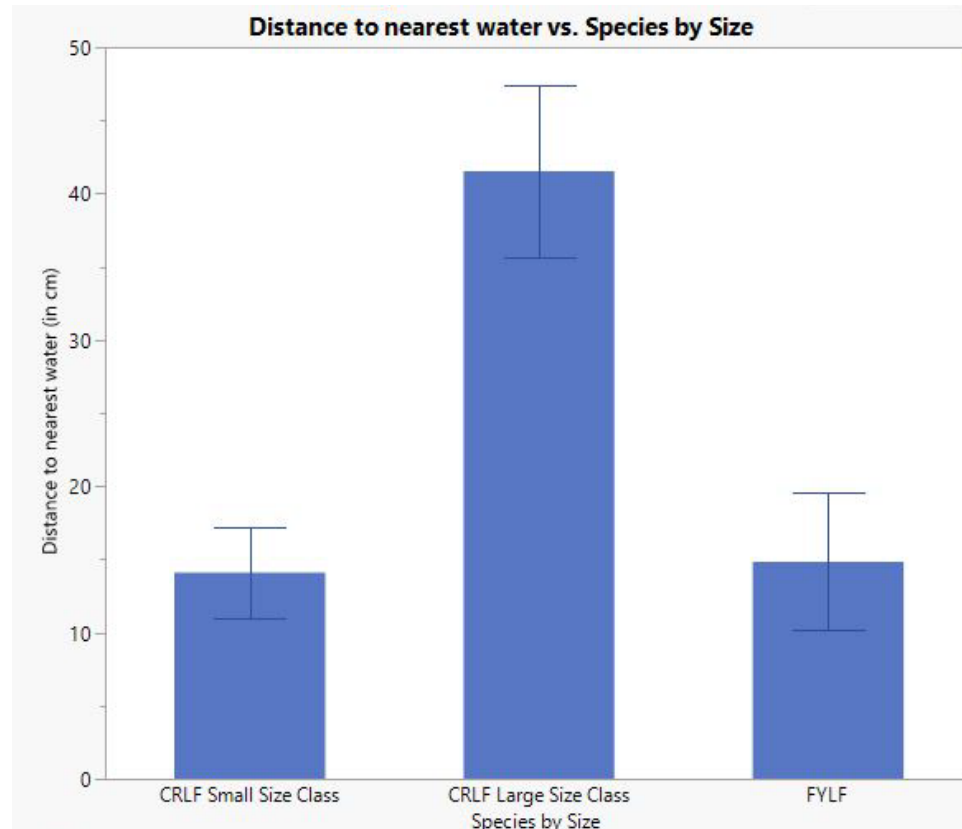


Figure 6. Distance to nearest water is more similar between small-bodied California red-legged frogs and foothill yellow-legged frogs than to large-bodied California red-legged frogs ($F_{2,86.58}=2.94$, $p=.05$).

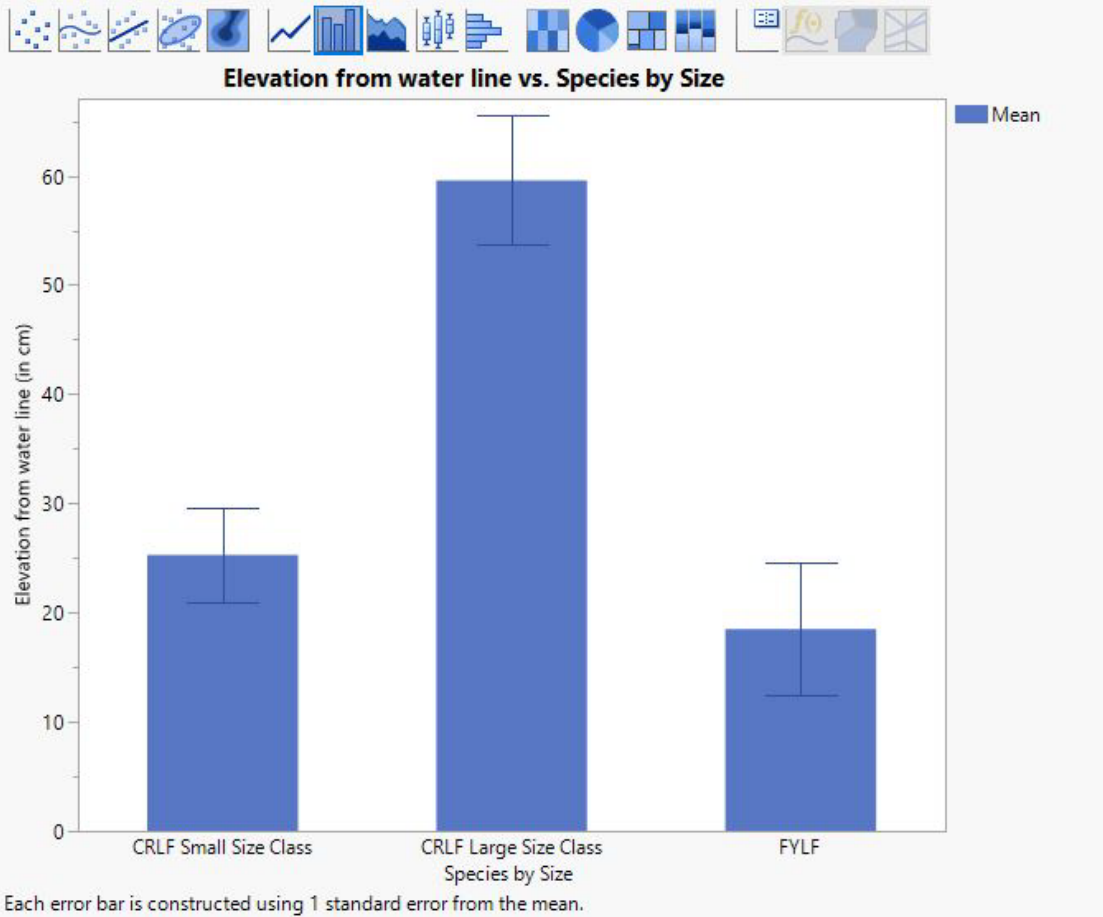


Figure 7. Elevation from water line is more similar between small-bodied California red-legged frogs and foothill yellow-legged frogs than to large-bodied California red-legged frogs ($F_{2,72.62}=5.6, p=.005$).

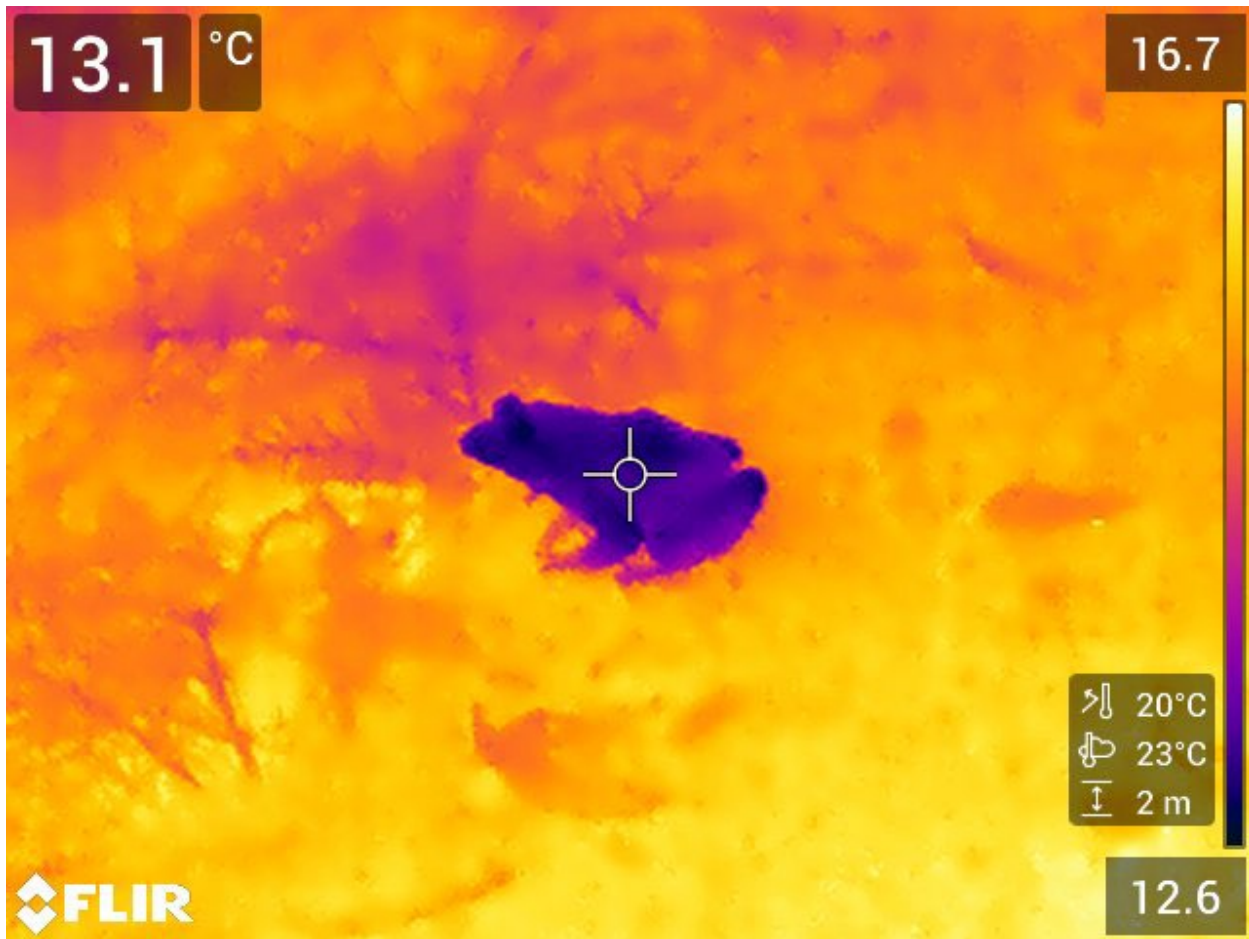


Figure 8. Sample thermal image analyzed with FLIR Research Studio showing that frog body temperature (average of pixels within polygon) is considerably lower than temperature of surrounding substrate ($F_{1, 11} = 0.78$, $p = 0.40$, $F_{1, 11} = 0.13$, $p = 0.73$). Temperature of substrate was averaged separately for sample sites adjacent to the frog and sample sites randomly selected from the substrate.