Fortune may favor the flexible: Environment-dependent behavioral shifts in invasive coqui frogs

Katharina M Soto[1,2], Devin Edmonds[3] , Andrea L Colton[3], Michael R Britton[4], Faith O Hardin[1], Eva K Fischer[1,5,6]

[1] Department of Evolution, Ecology, and Behavior; School of Integrative Biology; University of Illinois Urbana-Champaign

[2] Program of Evolution, Ecology, and Conservation; School of Integrative Biology; University of Illinois Urbana-Champaign

[3] Illinois Natural History Survey; Prairie Research Institute; University of Illinois Urbana-Champaign

[4] School of Life Sciences; Arizona State University

[5] Carl R. Woese Institute for Genomic Biology; University of Illinois Urbana-Champaign

[6] Department of Neurobiology, Physiology, and Behavior; University of California Davis

*Corresponding author:

Eva K Fischer

Department of Evolution, Ecology, and Behavior

School of Integrative Biology

University of Illinois Urbana-Champaign

505 S Goodwin Ave

Urbana, IL 61801

Abstract

Biological invasions are a major driver of global biodiversity loss, impacting endemic species, ecosystems, and economies. As invasive species introductions continue to rise, addressing their impact has become a priority for conservation management. While the influence of life history traits on invasive success is well-established, the role of behavior remains an evolving area of research. The coquí frog, *Eleutherodactylus coqui*, an invasive species in Hawai'i, serves as an ideal model for studying the role of behavior, complementing previous research on its physiology, morphology, and the environmental factors that drive its invasive success. We examined behavior in the field across ecological gradients of population density and elevation. We observed that variations in exploratory behavior and activity were influenced by an interaction between density and elevation. Additionally, we evaluated behavior following prolonged acclimation to a novel lab environment. We found widespread plasticity in boldness, exploration, and activity, with all behaviors decreasing in the lab. In contrast, we observed consistent correlations between exploratory behavior and activity, both in the field and lab. The persistence of these correlations across environmental contexts highlights the potential for predicting behavioral responses of invasive species in novel environments. Lastly, we investigated the relationship between resting metabolic rate and behavior in the lab. We found positive correlations between resting metabolic rate and behavior, expanding our understanding of the interactions between physiology, behavior, and ecological influences. Taken together, our results suggest that behavior may be predicted based on a combination of environmental and physiological responses.

Keywords: biological invasions, behavioral plasticity, invasive species, physiology, behavior

Introduction

Biological invasions are one of the major causes of global biodiversity loss, impacting endemic species, ecosystems, and economies (Kumschick et al. 2012; Lennox et al. 2015). Invasive species are often equipped with a competitive advantage due to their abilities to exploit limited resources (Zwerschke et al. 2018; Dueñas et al. 2021), which affects the fitness and long-term survival of less competitive native species, leading to their decline (Polo-Cavia et al. 2009; Bellard et al. 2016; Zwerschke et al. 2018). Moreover, invasive species possess the capacity to reshape ecosystems by modifying nutrient cycles (Abernethy et al. 2014; Foxcroft et al. 2017), disrupting trophic interactions (David et al. 2017), and even altering the physical structure of habitats (Howard et al. 2019; Rowe et al. 2019). These ecosystem transformations, instigated by invasive species, carry substantial implications for the conservation of biodiversity and the stability of ecosystems. Taken together, these challenges give rise to substantial economic costs, with estimates exceeding $423 billion annually (Roy et al. 2023). While the impacts of invasive species have been thoroughly examined, we are still in the process of understanding the attributes that contribute to invasive species' success.

When examining the invasive success of a species, research has demonstrated the importance of life history traits, such as high reproductive rates (Alcaraz et al. 2005), rapid population growth, dispersal capabilities, and generalist diets (Perkins et al. 2013; Sakai et al. 2001). However, these traits alone do not provide a comprehensive explanation of invasion success. Beyond life history traits, behavior can also play a pivotal role in the spread of invasive species (Sol and Lefebvre 2003; Weis 2010; Hudina and Hock 2012; Damas-Moreira et al. 2019). For instance, Candler & Bernal (2015) revealed that introduced cane toads (*Rhinella marina*) displayed reduced neophobia and increased exploration of novel prey and objects compared to toads from native populations. Damas-Moreira et al. (2019) found similar results when comparing behavior among invasive (*Podarcis sicula*) and non-invasive (*Podarcis virescens*) wall lizards. These studies highlight how behavior drives invasive success, enabling

the exploitation of new resources and range expansion. Furthermore, behavioral plasticity may be particularly crucial during the initial stages of invasion when adaptation to novel environments, prey, predators, and competitors is paramount (Chapple et al. 2012). Moreover, behavioral plasticity can facilitate survival and range expansion in the absence of adaptation, which may be slowed in invasive species due to low genetic diversity resulting from founder's effect and bottleneck events during colonization (Tsutsui et al. 2000; Peacock et al. 2009; Wright et al. 2010).

A comprehensive understanding of the role of behavior in invasive success also requires consideration of physiology. Behavioral traits are closely tied to physiological processes, particularly in ectotherms that regulate body temperature through their behavior (Myles-Gonzalez et al. 2015; Mathot et al. 2019). A meta-analysis by Mathot et al. (2019) reinforces this assessment, showing a stronger relationship between behavior and metabolic rate in ectotherms compared to endotherms. High metabolic rates, often associated with a 'fast pace of life'—marked by rapid growth, early reproduction, and a generalist diet—are traits already recognized as key contributors to invasive potential (Lagos et al. 2017). Additionally, high metabolic rates have been correlated with behaviors such as boldness, exploration, and activity (Biro and Stamps 2010; Myles-Gonzalez et al. 2015; Baškiera and Gvoždík 2022), which are increased in invasive as compared to native organisms (Candler and Bernal 2015; Damas-Moreira et al. 2019). Nevertheless, the multifaceted roles that behavioral and physiological traits play in biological invasions has only been examined in a few systems (Mathot et al. 2019; Mowery et al. 2021; Myles-Gonzalez et al. 2015).

The common coquí (*Eleutherodactylus coquí*), a nocturnal, arboreal tree frog, serves as an ideal model for a multicomponent investigation of mechanisms that contribute to invasion success. Coquí are endemic to Puerto Rico, where they are an integral part of the island's ecosystem and culture (Beard et al. 2002; Westrick et al. 2022). However, their introduction to other regions has led to them becoming one of the 100 worst invasive species in the world

(Lowe et al. 2000). These frogs have successfully colonized Hawai'i, Guam, and various other Pacific islands (Kraus and Campbell 2002; Beard and Pitt 2005), in addition to establishing invasive populations in Central America (Barrantes-madrigal et al. 2019). Their successful establishment extends across the Hawaiian archipelago, including the main islands of Hawai'i, Maui, Oahu, and Kauai (Beard et al. 2009). Their loud and incessant calls have made them an unmistakable presence in the Hawaiian nightscape, impacting property values and tourism (Beard and Pitt 2005).

Since their introduction to Hawai'i in the 1980s, coquí frogs have undergone remarkable ecological changes associated with their invasive success (Beard and Pitt 2005). Invasive populations have reached staggering numbers of up to 91,000 frogs per hectare, exceeding densities two to three times higher than in their native habitat, (Beard et al. 2009). The coquís reproductive efficiency, marked by direct development without a tadpole stage, allows year-round reproduction and independence from freshwater (Beard et al. 2009), fostering their rapid population growth. These elevated densities likely intensify competition for resources, territories, and mating opportunities.

The population density of coquí frogs typically decreases at higher elevations, though this pattern is not consistently observed (Beard et al. 2008). Still, coquí frogs are expanding to higher elevations in Hawai’i (Marchetti et al. 2023), with the island’s unique topography offering suitable habitats up to 1219 meters above sea level (Strohecker 2011). These higher elevations pose distinct challenges compared to Puerto Rico, including increased predation from invasive birds (Smith et al. 2018) and colder temperatures (Marchetti et al. 2023). O’Neill et al. (2018) noted that coquí frogs exhibit larger body sizes at higher elevations, likely due to temperature-induced plasticity. Moreover, Marchetti et al. (2023) found that coquí frogs are adjusting their thermal tolerance to align with Hawai'i's elevational gradients. This range expansion to higher elevations and increased population density may lead to a situation where frogs face a tradeoff

between escaping high densities at lower elevations and coping with extreme environmental conditions higher uphill.

Like many other invasive species, Hawaiian coquí exhibit reduced genetic diversity when compared to their native counterparts, presumably due to founder's effects (Tsutsui et al. 2000; Peacock et al. 2009; Wright et al. 2010). Nonetheless, their populations have thrived, establishing a presence in diverse habitats and ecosystems across the island. This versatility in the face of low genetic diversity suggest a role for behavior plasticity in their invasive potential. While previous studies have characterized the invasive presence of coquí in Hawai'i (Beard et al. 2008; Beard & Pitt, 2005; Kraus & Campbell, 2002; Smith et al. 2018) and explored some physiological aspects (Peacock et al. 2009; Rollins-Smith et al. 2015; O'Neill et al. 2018; Marchetti et al. 2023), there remains a noticeable gap in our understanding of their behavior.

Our study investigated how behavior contributes to coquí frogs' invasive success. To achieve this goal, we adopted a stepwise approach, involving field and lab experiments. First, we characterized behavioral variation across elevational and density gradients in Hawai'i to understand whether (and how) behavior varies across environmental conditions at the invasive center verses edge. Second, we examined if (and how) behavioral traits changed when we introduced coquí to a novel, lab environment. This allowed us to investigate behavioral plasticity and differentiate short-term behavioral changes from long-term behavioral differences that could indicate developmental influences or local adaptation. In both the field and the lab, we evaluated correlations between behavioral traits (i.e., behavioral syndromes) as these are known to influence immediate and evolutionary responses to changing environments (Dochtermann and Dingemanse 2013; Michelangeli et al. 2019). Lastly, we examined if resting metabolic rate predicted behavioral variation in the lab. Taken together, our findings contribute a behavioral perspective to complementary life history studies, providing a more holistic understanding of the coquís invasive success.

Methods

All frog husbandry and experimental methods were approved by our university Animal Care and Use Committee (Protocol # 20147). Frog collection and export were approved by the State of Hawai'i Department of Land and Natural Resources (Permit # H0422-04; EX-22-11). Given the invasive nature of coquí frogs in the Hawaiian Islands, our collection posed no conservation concerns.

Field Collection: We collected 60 male coquí on the Big Island of Hawai'i. We collected from four 'site types,' each representing a combination of density and elevation levels (e.g., high-density/low-elevation (HL), low-density/low-elevation (LL), high-density/high-elevation (HH), and low-density/high-elevation (LH)). We collected from populations at low (~300 m) and high (~700 m) elevations. We classified population density in the field based on call intensity, with low-density sites having an average frequency of 500 Hz at 40.7 dB, and high-density sites having an average frequency of 2639 Hz at 55.6 dB. The post-field analysis of intensity was conducted using Audacity v. 3.0.2 (Audacity Team 2021). We collected 16 frogs at HL site types, 15 frogs at HH and LH site types, and 14 frogs at the LL site type due to limited availability. We collected frogs from Lava Tree State Monument Park (LH, [19.48278, -154.90257]), 'Ōla'A Forest Reserve (HL, [19.4528, -155.18733]), Watershed Forest Reserve (LH, [19.4174, -155.16275]), Upper and Lower Stainback Highway (HH, [19.57462, -155.190996]; HL, [19.62948, -155.09364]), Homestead Road (LL, [19.842645, -155.113838]), and Hilo Watershed Reserve (HH, [19.843408, -155.185547]).

Frogs were hand-captured between 19:00 and 00:00 hours, then individually placed in 16 oz clear plastic cups with ventilation holes and damp paper towels for moisture and humidity. Frogs were transported from each collection site to our field site in Leilani Estates, Hawai'i County, HI, and held for 24 hours before behavioral testing the following night. Additionally, we

conducted acoustic recordings at each site type as well as ambient sound recordings at our field site using a shotgun microphone (Sennheiser ME 66/K6) and a handheld recorder (Zoom H1 Handy Recorder).

Field Behavioral Assays: We conducted field trials (N=60) between May and June 2022, from 19:00 to 03:00 hours at our field site. We used two behavioral arenas constructed from ½" PVC pipes and white window screen mesh fabric, with dimensions measuring 82.55 x 57.79 x 48.26 cm. The arenas were illuminated by red light lamps and infrared illuminators (Univivi IR Illuminator) (Figure 1a), and we recorded behavior using infrared camcorders (Sony DCR-SR85). Each arena was placed on opposite sides of the field site to prevent trial interference. We constructed emergence chambers (Figure 1b) using a ½" PVC pipe held vertical by a deli cup base and a retractable cardboard cover.

To examine behavior, we used two tests: (1) an emergence test, which quantified latency to emerge (a commonly used metric of boldness see Magnhagen et al. 2014; Myles-Gonzalez et al. 2015; Yuen et al. 2017)), and (2) an open field test, which measured activity and exploration (Figure 2). For the emergence test each frog was placed in the emergence chamber and given a 3-minute acclimation period. After the acclimation period, the cover of the chamber was removed from outside the arena. Each frog was allowed a 10-minute period to emerge voluntarily from the chamber. Individuals that did not emerge after 10 minutes were gently removed from the PVC pipe. Latency to emerge (i.e., boldness) was recorded during trials as the moment when the majority of the frog's body had exited the PVC pipe. Immediately following the emergence test (or removal of frogs that did not emerge on their own), we performed the open field test for 15 minutes. During the open field test, we quantified exploration as the proportion of areas visited, and activity as the number of times a frog crossed between defined areas (Figure 2 and below).

Lab Transfer and Husbandry: Within four days of capture, we transported all frogs to our university's frog facility. Each frog was placed in a 16 oz deli cup with a moist paper towel and dead leaves. Cups were packed into styrofoam containers with insulation and padding material and shipped via UPS overnight. All frogs arrived alive and well. Upon arrival in the lab, frogs were individually housed in tanks measuring 30.48 x 20.32 x 15.24 cm, with ~ 2-3 cm of soil and sphagnum moss as a substrate. Additionally, each frog was provided with an 8” length of ½” diameter PVC plastic pipe for shelter. We maintained humidity >70% and temperature 69 - 74 °F on a 12L:12D reversed light cycle and fed frogs brown crickets (A*cheta domesticus*) gut loaded with vitamins three times weekly. Four individuals died over the course of the study due to escape and subsequent desiccation.

Lab Behavioral Assays: We conducted lab trials (N=56) after an 18-week acclimation period between September and November 2022 within our lab, from 13:00 to 17:00 hours. To replicate the conditions of field capture and acclimation, we isolated individuals from their enclosures within 16 oz. deli cups lined with moist paper towel 24 hours prior to the lab trials. These trials were conducted in an unoccupied animal room at our university, with temperature controls maintained at between 70 - 75-degrees °F. In the lab trials, we used the same arena as in the field assays. However, we implemented curtains to dampen sound waves and maintain humidity levels (Figure 1c). Behavioral assays in the lab closely followed the field protocol, consisting of a 13-minute emergence test (3 minutes acclimation and 10 minutes to emerge) and a 15-minute open field test. Ambient sound recordings were used to replicate the background noise experienced during field trials.

Physiological Assays: We conducted physiological assays (N=55) in the lab during February and March of 2023, 12 weeks after the lab-based behavioral assays. Given that resting

metabolic rate (RMR) can be influenced by numerous factors, including diet, temperature, age, sex, and environmental conditions (particularly in ectotherms), prior studies have highlighted that a common garden approach is a preferred method for controlling these variables (Biro and Stamps 2010; Burton et al. 2011).

We used an intermittent flow-through respirometry system to measure RMR. We placed frogs in 127 mL chambers made from ½" diameter PVC plastic pipe and PVC end caps. We configured a flow-through system with an open circuit and incurrent flow measurement using a PP2 Dual Pump, FB8 Flow Measurement System, RH-300 Water Vapor Analyzer, Fox Box, and UL2 Universal Interface (all from Sable Systems International). The incurrent airstream was scrubbed of CO_2 and water vapor. After allowing frogs to acclimate in the chamber for 20 minutes, we sampled CO_2 production and O_2 consumption for each individual every 5 seconds over a 40-minute window. We switched from a chamber containing a frog to a control chamber between individuals to establish a baseline measurement of ambient CO_2 and O_2 and account for drift. Measurements were recorded following a 2-day fast to eliminate the energy expenditure associated with digestion (Naya et al. 2009). Before calculating RMR, we first matched each frog to its associated control taken directly after the trial. For three individuals, we substituted controls from the following trial on the same day because their controls were too short or appeared to have errors. We then deleted 5 minutes from the start of each trial to remove any error associated with switching tubing between the control and frog. We also deleted 7.3 minutes from the end of each trial to remove the lag between sequential CO_2, O_2, and H_2O sensors in our system. To determine the length of the time lag, we visually inspected plots of the three variables, noting the maximum lag was 7.3 minutes. After trimming data to remove inaccuracies and adjusting for the time lag, we then calculated the fractional concentration of CO_2 and O_2, and H_2O adjusted for barometric pressure.

To calculate RMR, we first calculated the rate of O_2 consumption (VO_2) and CO_2 emission (VCO_2) using Lighton (2008) equations 10.1:

$$VO_2 = FR * {}^{O_{2\,diff}}\!/_{1 - O_2}$$

and 10.8:

$$VCO_2 = \frac{FR * (CO_{2\,diff} - (CO_2 * (1 - H_2O)) * \frac{(FR * O_{2\,diff})}{(1 - O_2)})}{1 - (CO_2 * (1 - H_2O))}$$

where FR = flow rate, $O_{2\,diff}$ is the average O_2 during the control minus the O_2 produced by a frog, $CO_{2\,diff}$ is the CO_2 produced by a frog minus the average CO_2 during the control, and CO_2, O_2, and H_2O are the average values during control. We then calculated RMR as the lowest 3-minute average VO_2 and VCO_2 during each trial. Next, we calculated the respiratory quotient (RQ) by dividing the lowest 3- minute average VCO_2 by the lowest 3- minute average VO_2. Finally, we calculated mass specific RMR by dividing each frog's RMR by its mass (g), hereafter referred to as RMR. Data manipulation was performed using packages ‘zoo’ and ‘tidyverse’ (Wickham et al. 2019; Zeileis et al. 2023).

Statistical Analysis: Due to technical issues (a hard drive failure), we lost field recordings for five frogs from low-density/low-elevation (LL) and eight frogs from high-density/low-elevation sites (HL). The analysis of field behavior (i.e., exploration and activity) is based on the remaining dataset (N=49). Field emergence data was recorded prior to the data loss, and all available data (N=60) were utilized. We used Behavioral Observation Research Interactive Software (BORIS) version 8.13 (Friard and Gamba 2016) to code exploration and activity from videos. We analyzed data in R version 4.2.4 (R Core Team 2022)in Rstudio v.4.2.2 (RStudio Team 2021). We analyzed the response variables (i.e., behavior and RMR) using the raw data for those with a normal distribution. However, since activity did not adhere to a normal distribution, we applied

square root transformations. For all analyses, significance was determined at $p \leq 0.05$, and a 95% confidence interval was used.

We used the 'lm' function from the 'stats' package (R Core Team 2022) for linear models, with behavior and RMR as the response variables, and density, elevation, and their interaction as predictor variables. We used the ‘lmer’ function in the ‘lmerTest’ package (Kuznetsova et al. 2017) for linear models comparing field and lab trials, with frog ID as a random effect and trial, density, elevation, and their interactions as fixed effects. We conducted Type III ANOVAs on our linear models to generate test statistics and p-values for main effects and interactions. Additionally, we used the ‘emmeans’ package (Lenth 2021) for pairwise post-hoc comparisons as needed. We calculated Pearson correlation coefficients between behaviors and RMR using the 'rcorr' function from the ‘Hmisc’ package (Herrell Jr and Dupont 2023). We interpreted correlation coefficients following the criteria where ≈0.1 indicated a small effect, ≈0.3 was a moderate effect, and values >0.5 represented a strong effect (Garamszegi et al. 2013).

Results

Field Behavioral Assays: We found an effect of the interaction between density and elevation (i.e., site type) on exploration ($F_{1,37}$= 6.257, p= 0.017) (Table 1). Post hoc tests revealed marginal differences between both the high-density/high-elevation (HH) frogs and the high-density/low-elevation (HL) frogs (t_{37}= -2.319, p= 0.112), and the low-density/high-elevation (LH) frogs (t_{37}= -2.217, p= 0.138) (Figure 3a). There was no main effect of density ($F_{1,37}$= 0.817, p= 0.372) or elevation ($F_{1,37}$= 0.708, p= 0.401) on exploration (Table 1). Similarly, there was no main effect of density (F1,37= 0.696, p= 0.409) or elevation (F1,37= 0.224, p= 0.639) on activity. However, we found a marginal effect of the interaction of density and elevation (F1,37= 3.945, p= 0.054) on activity (Figure 3b). We did not observe any effects of density ($F_{1,56}$= 1.913, p= 0.172), elevation ($F_{1,56}$= 0.718, p= 0.400), or their interaction ($F_{1,56}$= 0.560, p= 0.457) on

boldness (Figure 3c) (Table 1). We found a strong correlation between exploration and activity (t_{38}=17.366, r=0.941, p< 0.001) (Figure 4). Boldness was not correlated with either activity (t_{39}=0.152, r=0.024, p= 0.880) or exploration (t_{39}= -0.285, r=-0.046, p= 0.777) in the field (Table 2).

Lab Behavioral Assays: We did not observe any effects of density, elevation, or their interactions on boldness, exploration, or activity in the lab (Table 1). We found strong correlations between exploration and activity (t_{54}= 12.633, r= 0.880, p< 0.001) (Figure 4), and no correlations between boldness and activity (t_{52}= 0.145, r= 0.020, p=0.885) or exploration (t_{52}= -0.179, r= 0.025, p= 0.858) (Table 2).

Comparison of Field and Lab Behaviors: When comparing field and lab assays, we observed significant effects on exploration ($F_{1,43}$= 5.414, p= 0.025) (Figure 5a), activity ($F_{1,47}$= 7.393, p= 0.009) (Figure 5b), and boldness ($F_{1,52}$= 25.983, p< 0.001) (Figure 5c). In general, frogs took longer to emerge, moved less frequently, and explored less in the lab as compared to the field. For exploration, we found an effect of the interaction of site type and trial ($F_{3,42}$= 3.266, p= 0.031) (Table 1). Post-hoc tests revealed that high-density/low-elevation (HL) frogs significantly decreased exploration in the lab (t_{51}= 2.968, p= 0.005). In the case of activity, there was a marginal effect of the interaction between site type and trial ($F_{3,46}$= 2.233, p= 0.097) (Table 1). We observed significant decreases in movement in the lab for high-density/low-elevation (HL) (t_{49}= 2.484, p= 0.016) and low-density/high-elevation (LH) frogs (t_{59}= 1.994, p= 0.051). Lastly, for boldness, a significant interaction effect between site type and trial was observed ($F_{1,52}$= 25.983, p< 0.001). Again, we found significant decreases in boldness for the high-density/ low-elevation (HL) and high-density/high-elevation (HH) frogs (t_{51}= -5.057, p< 0.001 and t_{52}= -2.431, p= 0.019, respectively). However, only a marginal effect was observed for the low-density/low-elevation (LL) (t_{54}= -1.649, p= 0.105) and low-elevation/high-density (t_{56}= -1.324, p= 0.191) frogs.

Physiological Assays: We found no effect of density ($F_{1,51}$= 0.035, p= 0.852), elevation ($F_{1,51}$= 0.707, p= 0.404), or their interaction ($F_{1,51}$= 0.800, p= 0.376) on RMR within the lab. However, we observed significant correlations between RMR and exploration (t_{54}= 3.497, r= 0.430, p< 0.001) (Figure 6a) and activity (t_{54}= 2.749, r= 0.353, p= 0.008) (Figure 6b). There was no correlation between boldness and RMR (t_{52}= 0.445, r= 0.062, p= 0.658) (Table 2).

Discussion

In this study, we investigated behavioral traits, ecological gradients, and physiological traits in invasive coquí frogs across Hawai'i. During their expansion, coquí have experienced extreme changes in their ecological conditions, in particular, increased population densities and more variable and on average lower temperatures at higher elevations. Previous research has predominantly focused on understanding the consequences of these shifting conditions on ecosystem dynamics (Beard and Pitt 2005; Beard 2007; Beard et al. 2009; Smith et al. 2018), morphology (O'Neill et al. 2018), and physiology (Peacock et al. 2009; Haggerty 2016; Marchetti et al. 2023), but coquí behavior has not been studied in this context. To address this research gap, we conducted a series of experiments, examining variations in behavior across environmental and experimental contexts. We found that behavior varied based on interactions between density and elevation (i.e., site types) across the invasive range. Interestingly, these field-based variations disappeared after acclimation to lab conditions, suggesting behavioral variation in the field was primarily influenced by the environment, rather than local adaptation. However, we observed consistent correlations between specific behaviors (i.e., activity and exploration) in both field and lab settings, indicating their reliable relationship across different contexts. Additionally, we observed correlations between physiological (i.e., resting metabolic reate) and behavioral traits within the lab. These findings contribute to our understanding of the

behavioral factors influencing the expansion and persistence of coquí frogs in the unique ecological landscape of Hawai'i.

In the field, we observed significant variations in exploratory behavior, with frogs living at high densities displaying increased exploratory tendencies at low elevations (HL) compared to high elevations (HH) (Figure 3a). We observed similar differences in activity across site types, with high density variations also differing by elevation (Figure 3b). Coquí frogs were originally introduced at low elevations and have since thrived in high-density populations (Beard et al. 2009; Bisrat et al. 2012), making these high-density/low-elevation (HL) sites their invasive epicenter. The increased exploration and activity observed in coquí frogs from high-density/low-elevation (HL) sites could contribute to their dispersal from the invasive center throughout Hawai'i, as observed in other invasive species (reviewed in Chuang and Peterson 2016). Alternatively, elevation-related temperature variations may contribute to the behavioral differences between the HL and HH frogs, as higher elevations impose physiological constraints, potentially limiting coquí frogs' capacity for extensive movement and their competitive abilities, particularly in high-density situations (Beard et al. 2008; Haggerty, 2016). These ideas are not mutually exclusive, and variation in behavior is likely the result of dynamic interactions involving multiple factors, including site-specific conditions, climatic variables associated with elevation, and inherent physiological constraints.

Additionally, our study provided evidence of behavioral plasticity in coquí frogs when introduced to a novel, lab environment. Behavioral plasticity may enhance invasion success by enabling rapid responses to new environmental conditions (Komers 1997; Wright et al. 2010). In the lab, all frogs exhibited a reduction in exploration and activity, as well as a decrease in bold behavior (Figure 5). We propose two potential explanations for these behavioral shifts. First, decreased activity and exploration may signal a shift towards cryptic behavior, which is a key factor in the success of invasive species, enabling them to evade predation, reduce competition, and thrive in new environments (Jarić et al. 2019). Alternatively, this behavioral shift may result

from reduced need or motivation due to extended acclimation in the controlled lab environment, where the absence of resource competition and mating opportunities could play a role (Hughes and Duncan 1988). Although we cannot distinguish these alternatives, the uniform behavioral response to a novel environment suggests a generalized strategy across individuals from different site types, pointing toward plasticity, rather than local adaptation, in facilitating these behavioral shifts.

Despite overall changes in behavior, we found consistent correlations between exploration and activity in both field and lab settings (Figure 4). The persistence of correlated behaviors, known as 'behavioral syndromes', demonstrates their stability and predictability in an organism's behavior, offering the potential to anticipate responses to changing environments (Garamszegi et al. 2013; Dochtermann & Dingemanse, 2013; Michelangeli et al. 2019). The persistence of behavioral syndromes across contexts demonstrates the potential for predicting behavioral responses to changing environments. For instance, when coquí frogs are introduced into a new habitat with varying resource availability and environmental challenges, these adaptable amphibians can consistently modify their activity and exploratory behavior. This behavioral flexibility allows them to survive, find ecological niches, and contributes to their ability to establish themselves in novel environments. Chapple et al. (2012) suggested that collections of correlated behaviors that enhance invasive success could be classified as an 'invasion syndrome'. For example, correlations between aggression and activity in invasive crayfish (*Pacifastasus leniusculus*) at higher densities facilitates their dominance over native species in the communities they invade (Pintor et al. 2009). However, research on the concept of invasive syndromes remains limited (but see Chapple et al. 2012; Perkins & Nowak, 2013), presenting an opportunity for future investigations to understand the connections between behavior, plasticity, and invasiveness.

Correlations between behavior and physiology have also been observed to play a role in enhancing invasive success (Candler and Bernal 2015; Damas-Moreira et al. 2019). We found

correlations between resting metabolic rate (RMR) and exploration (Figure 6a) and activity (Figure 6b). Higher RMR was associated with more active and exploratory behavior, while lower RMR was associated with reduced activity and exploration. These results highlight the role of physiological factors, such as metabolic rate (Biro and Stamps 2010; Myles-Gonzalez et al. 2015; Baškiera and Gvoždík 2022), in shaping behavior, and suggest a mechanistic basis for behavioral syndromes/invasive syndromes. In conjunction with previous studies demonstrating associations between elevation gradients and coquí metabolism (Chaparro 2023; Marchetti et al. 2023), these findings suggests a complex interplay of factors shaping coquí plasticity, contributing to their invasive success.

Conclusions

Our findings highlight the relationships between behavioral traits, physiological characteristics, and environmental factors in one of the world's most impressive invaders. Collectively, our findings emphasize the ability of coquí frogs to thrive in diverse ecological conditions and suggest that their behavioral plasticity plays a pivotal role in their success as invaders. This study adds to the growing body of research on the importance of behavior as invasive species establish and expand into novel environments (Weis 2010; Hudina and Hock 2012; Candler and Bernal 2015; Damas-Moreira et al. 2019).

This research holds broader implications for invasive species management and conservation. Understanding the mechanisms that drive invasion success, including the relationships between behavior and physiological traits, as well as the influences of environmental conditions, can inform more effective strategies for monitoring and controlling invasive species. For example, prior studies have demonstrated that both native and invasive coquí frogs exhibit lower metabolic rates at higher elevations and in colder temperatures (Chaparro 2023; Marchetti et al. 2023). Our research suggests that this reduction in metabolic rates at higher elevations may lead to decreased activity and exploratory behavior. This

knowledge enables us to foresee potential implications for conservation and management, showing a need for protective measures in areas where invasive coquí may be less competitive. Additionally, tailored monitoring and control strategies could be developed for the reduced activity and exploratory behavior in colder, higher-elevation habitats. Further research in this direction could offer valuable insights into the underlying genetic, physiological, and ecological factors that govern these behavioral traits and syndromes.

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Data Availability

Data will be made available upon publication.

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Tables

Table 1 Linear Model Results for Behaviors and Resting Metabolic Rate Across Ecological Gradients

Density x Elevation (i.e., site type)	**Field**	**Lab**	**RMR**
Exploration	**$F_{1,37}$= 6.257, p= 0.017***	$F_{1,52}$= 0.022, p= 0.882	$F_{3,31}$= 1.556, p= 0.220
Activity	**$F_{1,37}$= 3.945, p= 0.054.**	$F_{1,52}$= 0.070, p= 0.794	$F_{3,31}$= 2.162, p= 0.112
Boldness	$F_{1,56}$= 0.560, p= 0.457	$F_{1,50}$= 0.030 p= 0.866	$F_{3,31}$= 1.603, p= 0.218
Density	**Field**	**Lab**	**RMR**
Exploration	$F_{1,37}$= 0.817, p= 0.372	$F_{1,52}$= 0.052, p= 0.820	$F_{1,39}$= 0.786, p= 0.381
Activity	$F_{1,37}$= 0.696, p= 0.409	$F_{1,52}$= 0.022, p= 0.882	$F_{1,39}$= 0115, p= 0.736
Boldness	$F_{1,56}$= 1.913, p= 0.172	$F_{1,50}$= 1.277. p= 0.264	**$F_{1,39}$= 3.504, p= 0.069.**
Elevation	**Field**	**Lab**	**RMR**
Exploration	$F_{1,37}$= 0.708, p= 0.401	$F_{1,52}$= 2.457, p= 0.123	**$F_{1,39}$= 3.514, p= 0.068.**
Activity	$F_{1,37}$= 0.224, p= 0.639	$F_{1,52}$= 0.619, p= 0.435	**$F_{1,39}$= 6.298, p= 0.016***
Boldness	$F_{1,56}$= 0.718, p= 0.400	$F_{1,50}$= 1.260, p= 0.267	$F_{1,39}$= 0.687, p= 0.412

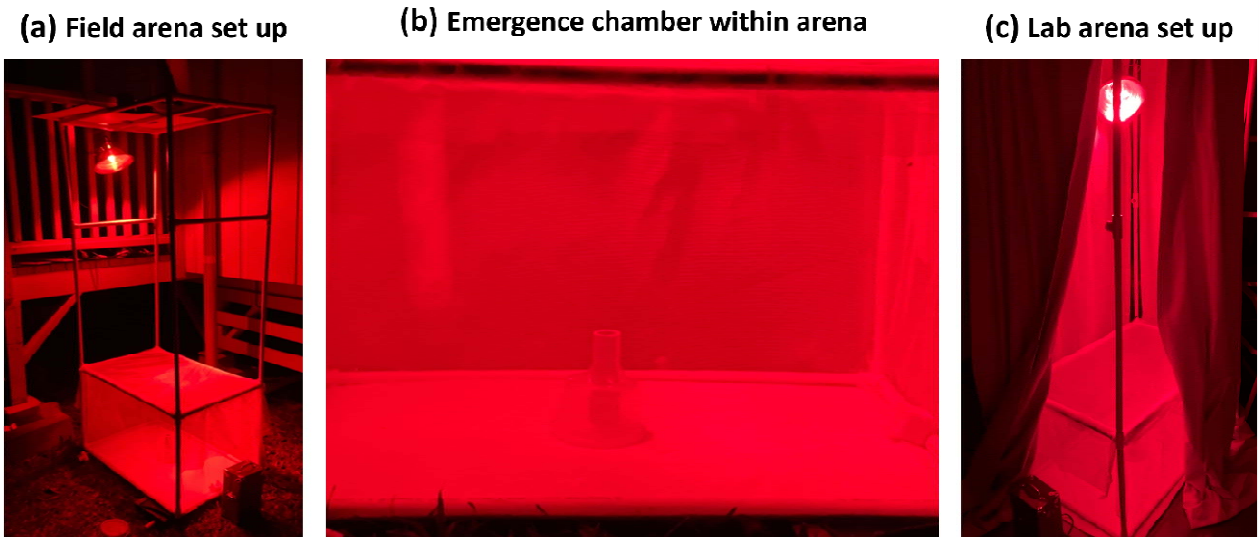
White boxes depict significant (p≤ 0.05) (*) and/or marginal significant (p≤ 0.10) (.) results. Grey boxes depict non-significant results (p≥ 0.05).

Table 2 Correlation Analysis of Behaviors and Resting Metabolic Rate

Field	Activity	Exploration	Boldness
Exploration	**r= 0.941, p< 0.001***	r= 1	r= 0.024, p= 0.880
Boldness	r= 0.024, p= 0.880	r= -0.046, p= 0.777	r= 1
Lab	**Activity**	**Exploration**	**Boldness**
Exploration	**r= 0.880, p< 0.001***	r= 1	r= 0.025, p= 0.858
Boldness	r= 0.020, p= 0.885	r= 0.025, p= 0.858	r= 1
RMR	**r= 0.353, p= 0.008***	**r= 0.430, p< 0.001***	r= 0.063, p= 0.658

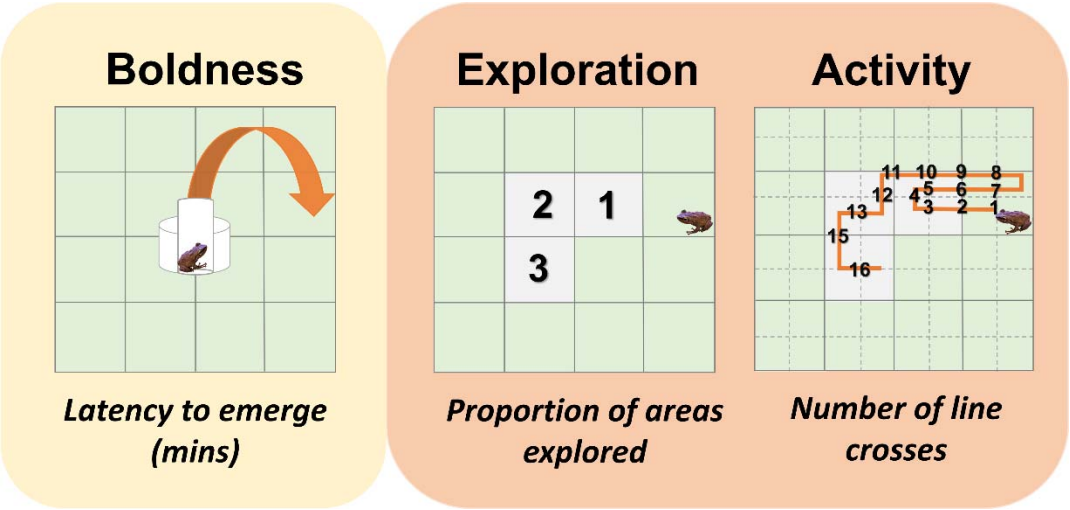
White boxes depict significant (p≤ 0.05) (*) results. Grey boxes depict non-significant results(p≥ 0.05).

Figure 1



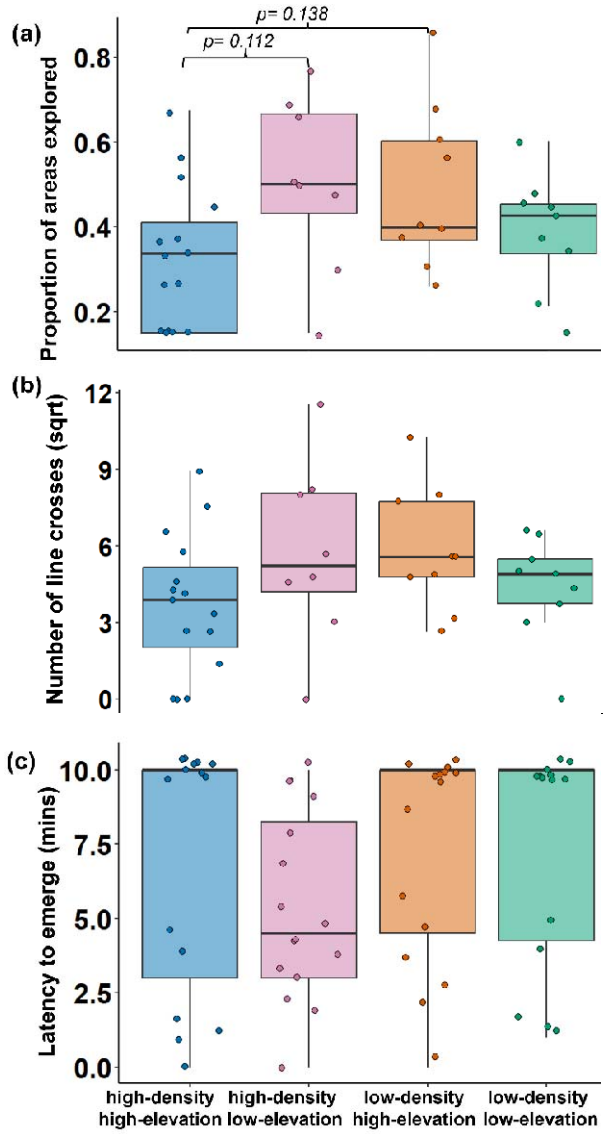
(a) Field arenas were placed below the field site porch and illuminated with red light. (b) Wide shot of the arena with emergence chamber in the center. (c) Lab arena setup featuring curtains to regulate temperature and humidity.

Figure 2



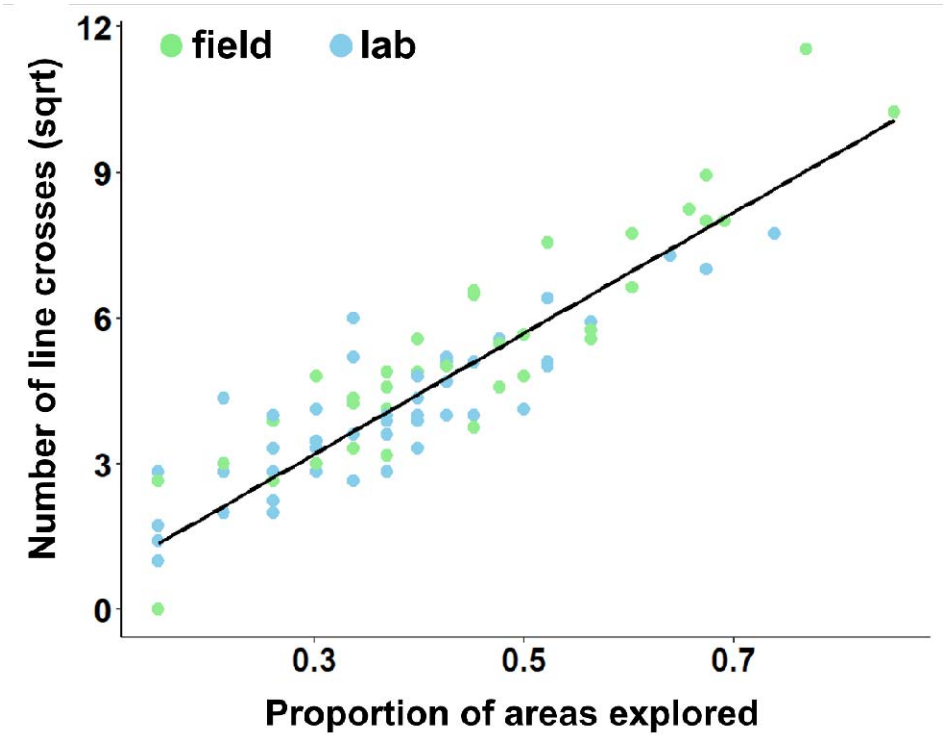
Each frog underwent two behavioral assays: an emergence test (in yellow) and an open field test (in orange). After a 3-minute acclimation period, frogs were given 10 minutes to emerge on their own or were gently removed from the emergence chamber. The 15-minute open field test started immediately after emergence. Behaviors recorded were boldness (latency to emerge), exploration (proportion of areas explored), and activity (number of line crosses). Exploration and activity are not inherently correlated, as frogs can be highly active without engaging in exploratory behavior.

Figure 3



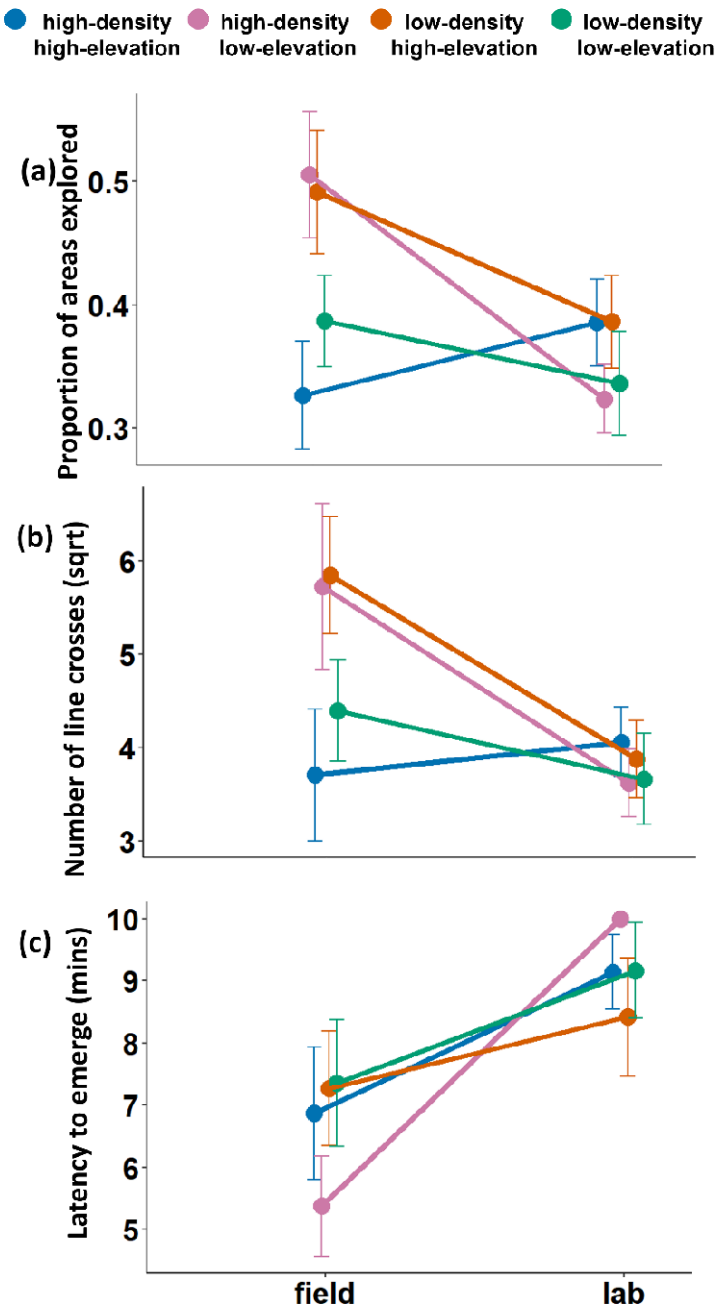
Behaviors across ecological gradients, involving elevational and density gradients (i.e., HH is high density high elevation). Key findings include a significant difference for (a) exploration ($F_{1,37}$= 6.257, p= 0.017) across site types with marginal differences between HL and HH, marginal significance for (b) activity ($F_{1,37}$= 3.945, p= 0.054), and no significant differences in (c) boldness (p≥ 0.05). Complete statistics in Table 1.

Figure 4



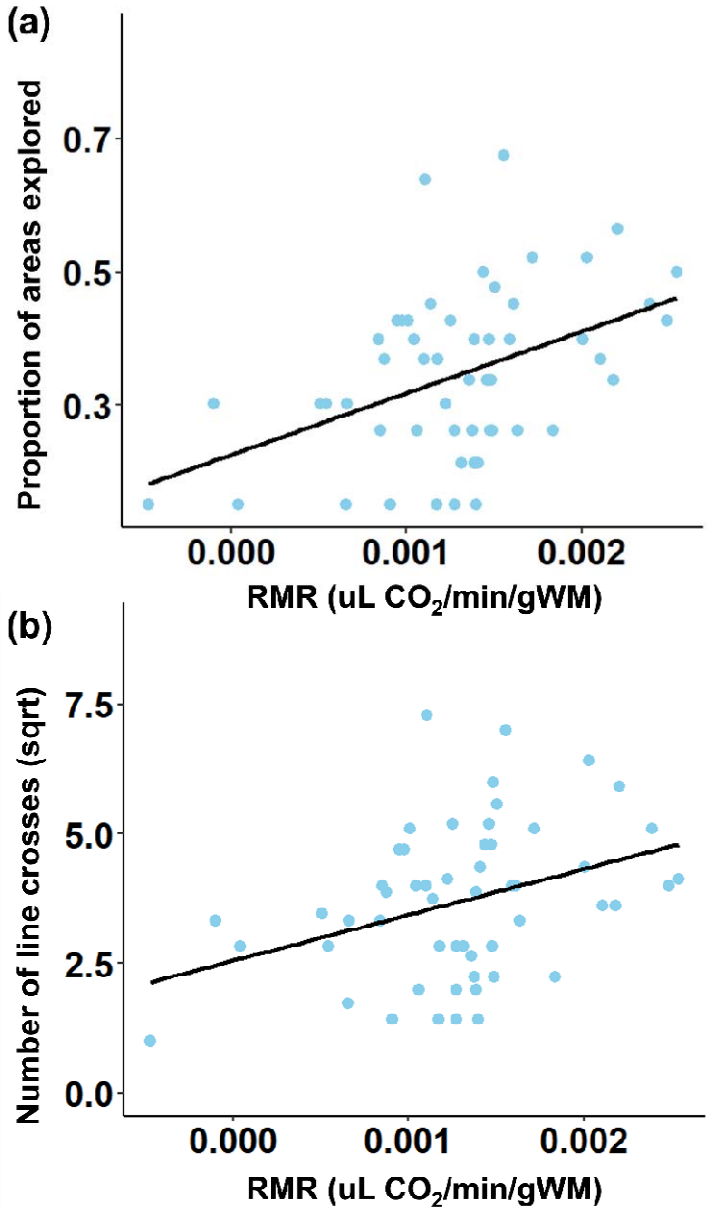
Significant correlations between exploration and activity in both lab and field trials ($p \leq 0.001$). A single line is drawn because the relationship between exploration and activity was the same in the lab and the field. Complete statistics in Table 2.

Figure 5



Behaviors across ecological gradients (i.e., density and elevation), involving the transition between field and lab environments. In the lab, significant ($p \leq 0.05$) decreases in (a) exploration, (b) activity, and (c) boldness compared to the field for most site types, except for HH (i.e., high density high elevation), which trends towards increased activity and exploration in the lab without reaching statistical significance ($p \leq 0.10$).

Figure 6



Significant correlations between (a) mass-specific Resting Metabolic Rate (RMR) and exploration ($p \leq 0.001$), and (b) mass-specific RMR and activity ($p \leq 0.05$) exclusively within lab trials. RMR is represented as uL CO2/min/gWM, indicating the volume of CO2 produced per minute per gram of wet mass. Complete statistics in Table 2.