

# Altitudinal Effects on Bioacoustics and Body Size Allometry on the Invasive Coquí Frogs in Hawai‘i

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## Abstract

The island of Hawai‘i is a tropical and isolated island with unique endemic species. Over time, Hawai‘i has become a breeding ground for invasive species, the common Coquí (*Eleutherodactylus coqui* Thomas, 1966) being one of them. These small ectotherms have intense vocalizations used for establishing territory and locating potential mates. On the island of Hawai‘i, the Coquí has become persistent across many islands. There have been acoustic studies done on the frogs in their native habitat, Puerto Rico, yet little is known about the possible differences in calls between these two geographically distant groups. According to past studies, climate change in Puerto Rico could cause the Coquí frogs to expand their ranges into higher, cooler altitudes with larger body sizes and lower pitch ranges. This phenomenon has yet to be studied in Hawai‘i. Through this study, I directionally recorded *Eleutherodactylus coqui* to obtain information on the peak frequency, body size, and altitude differences. All frogs were handled with proper permits from Cornell University.

## Introduction

The common Coquí (*Eleutherodactylus coqui* Thomas, 1966) gets its name from the sound of vocalizations and is a native amphibian to Puerto Rico. The Coquí was introduced to Hawai‘i in the late 1980’s through imported nursery plants. Despite the frog’s small body size, their unique and piercing calls can reach up to 70-80 decibels. Male Coquí’s produce variations of their vocalizations, “coqui”: The “co” is used to establish territory and therefore deter the other males, whereas the high-pitched “qui” is used to attract females (Narins & Capranica, 1978). However, with their common call being “co-qui,” they will also produce variations such as “co-co-co-qui” depending on the stage of calling (Narins & Capranica, 1978). These frogs can call at levels that make them the perfect candidate for acoustic monitoring. Understanding how, when, and where these frogs primarily vocalize and how they differ from the native calls could provide useful information for future behavior and preventative methods in Hawai‘i.

Acoustic monitoring is a non-invasive method that can collect presence/absence information

as well as gather principal characteristics of individual vocalizations. Coquí frogs are ectotherms that rely on the environment as a heat source for metabolic processes and sound production (Percino-Daniel et al., 2021). In Puerto Rico, populations of coquí frogs tend to show larger body size as altitude increases, indicating a positive linear relationship between altitude and body size (Narins & Meenderink, 2014). However, due to the inverse rule of bioacoustics, those with larger body size will create calls with lower frequencies (Narins & Meenderink, 2014). As global temperatures continue to increase due to climate change, it has been hypothesized that populations will shift their spatial distribution upwards, to higher altitudes, which would lead to larger bodied individuals occupying higher elevations (O’Neill & Beard, 2011). As a consequence, the allometric relationship between body size and frequency should also shift (Narins & Meenderink, 2014).

Overall, this study aims to explore the relationship between body size and bioacoustics of Coquí frogs along an altitudinal gradient in the island of Hawai‘i. This study also confirms

the possibility that previously established patterns of how altitude effects body size and vocalizations in native frogs may be similar in invasive frogs.

## Goals/Questions/Hypothesis

The focus of this study is to explore the possibility of differences in vocalizations at different altitudes and how body size could play a role in this. I expect that higher altitudes would lead to increases in body sizes and a decrease in peak frequency. This is due to body sizes' direct inverse relationship with wavelengths. I would therefore expect a negative relationship between higher altitudes and frequency; at higher altitudes, there should be lower frequency calls and larger body sizes (Narins & Meenderink, 2014). Coquí frogs are quite cold tolerant, so it is possible that they will expand their regions to higher elevations even in areas that are not registered on iNaturalist (Narins & Meenderink, 2014). Temperature change in Hawai'i could result in a change in calls or amplitude.

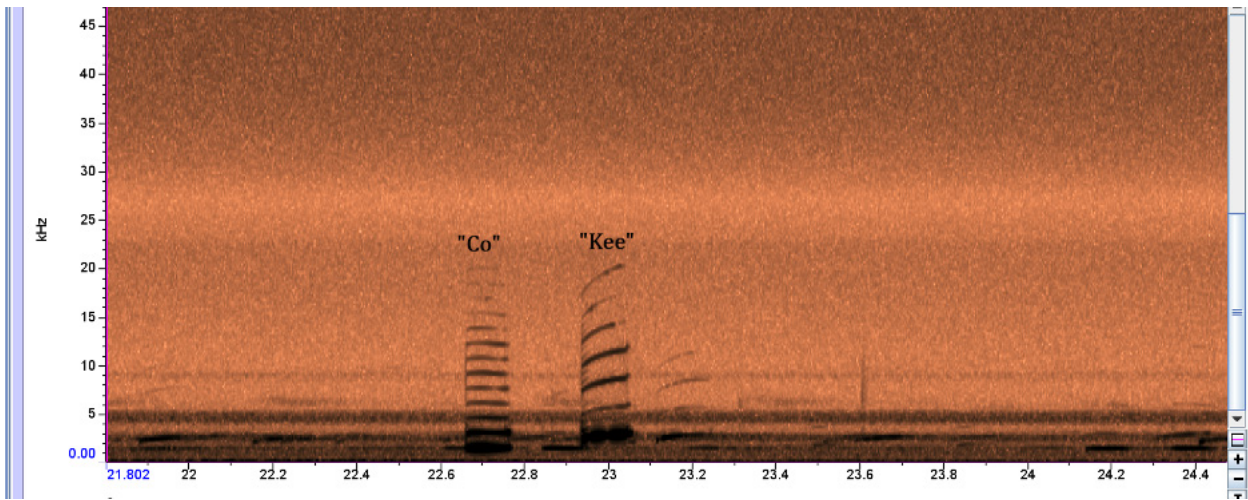
The second foundation of this project focused on whether there are any differences between the native and invasive frogs. For this, I expect that there would be no difference between the native Coquí and the invasive Coquí, because species usually have fixed traits that do not vary much in location, especially if climates across their biomes are similar elevations (O'Neill & Beard, 2011).

## Detailed Methodology

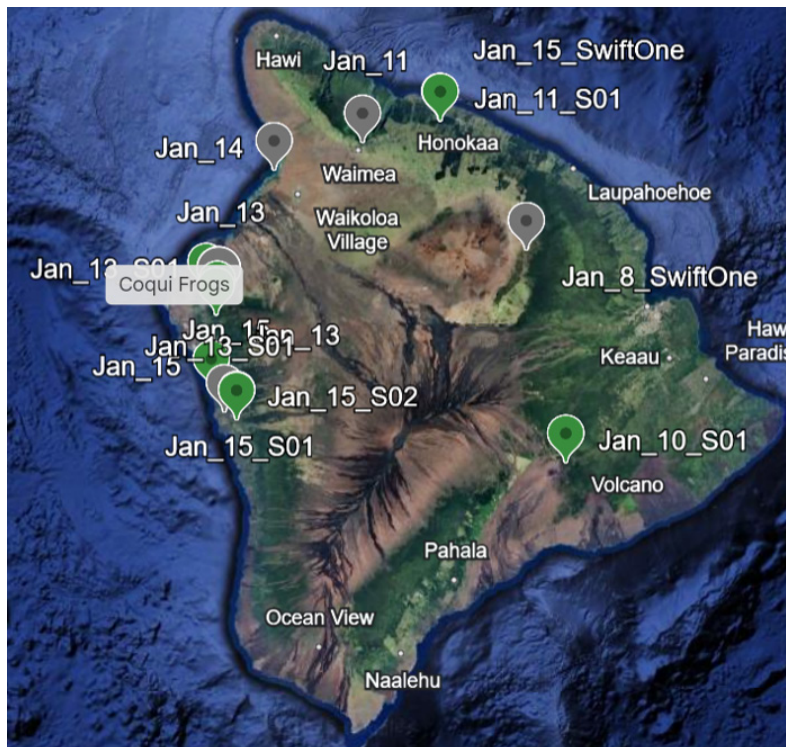
To locate the sampling areas, we used GBIF and iNaturalist to determine locations where people had heard or seen the Coquí frogs. I registered these onto a Google Map so that it was easily accessible. Given that Coquí frogs call from dawn until dusk we started sampling at 5:30 pm Hawai'i Standard Time (HST). We would drive to the area and detect if frogs were singing; if we could not hear the frogs, or if they were not easily accessible for us to collect data

on (i.e., in private property), another location was chosen. Once we could find a location, we would record a GPS coordinate on GPSTracks and the altitude. We would begin by locating where the frogs were calling, and once we found the individual that we heard, we would turn the headlamp off due to the frog's sensitivity to light and then start recording. We would record for 2 minutes at a time, save the voice memo, and then attempt to collect the frog. Once the frog was captured, he was placed in a plastic bag, and given an individual ID based on site, collector, and date. We also collected very few females to get size estimates on them as well. We then would use a datalogger to take the temperature of the outside environment, and a digital caliper to obtain the frog's length from tip of nose to cloaca. This was then recorded on an Excel spreadsheet. We would spend 30 minutes in an active search, and then either drive to a new location or begin processing the individuals. Once the night excursion was completed, data was backed up, properly ejected, converted to correct formats, and renamed with a unique code. As an example, this is what the code looked like: "11\_S1\_Kay\_1", where 11 was the date in January, S1 is site one of that night, Kay is the collector, and one is the first individual of the night caught. The data was subsequently uploaded to RavenPro to obtain information on peak frequency and change in (delta) time. RavenPro was also used to make annual annotations in an effort to obtain an average dominant frequency (Yang, 2024). The specific measurements that were captured were begin time (s), end time (s), low frequency (kHz), high frequency (kHz), peak frequency, delta time (s), and two annotation columns to describe the individual ID, and what call type it was using.

Additionally, two SwiftOne units (autonomous recording units developed at the K. Lisa Yang Center for Conservation Bioacoustics, Cornell Lab of Ornithology) were deployed to obtain data on diel variations throughout the night. The SwiftOne unit was configured with the default settings and then tied to a tree where Coquí frogs had been heard previously.



**Figure 1:** A spectrogram showing the different variations in the “co” and “qui” calls. The intensity of the sound is seen on the axis labeled “kHz”, and the higher the frequency the higher the pitch of the call. The background in the red is the background noise and the black lines are the harmonics of the frog.



**Figure 2:** A map of the Island of Hawai‘i, where data was collected between 01/10/2025 through 01/16/2025. The green markers indicate active coquí frog captures and recordings, whereas the grey markers are spots where there were either no or unattainable frogs.

The locations that were visited between the 10th-16th of January are located above. The entries labeled with month\_date\_site with a green marker are ones where I conducted an active search. Those that are just month\_date and are green are ones where Coquí frogs were heard, but an active search was not conducted due to

research limitations. Grey location markers are areas that I heard no Coquí frogs present. Additionally, seventeen of the twenty-two individuals caught have body measurements and directional recordings. The figure below shows the data collection sheet that explains how individuals were documented.

Selection	View	Channel	Begin Time	End Time	Low Freq	High Freq	Peak Freq	Delta Time	ID	Call Type	Altitude	SVL
4	Spectrogr	1	5.890183	5.999859	901	19658.7	0.1097	1500	27	co	507	1.2
6	Spectrogr	1	8.286822	8.400153	1064.8	14907.8	0.1133	1500	27	co	507	1.2
8	Spectrogr	1	10.72543	10.85704	819.1	16955.6	0.1316	1500	27	co	507	1.2
10	Spectrogr	1	13.02478	13.16371	901	17037.5	0.1389	1500	27	co	507	1.2
12	Spectrogr	1	16.35572	16.48002	737.2	16873.7	0.1243	1500	27	co	507	1.2
14	Spectrogr	1	20.11249	20.23679	982.9	16709.9	0.1243	1500	27	co	507	1.2
16	Spectrogr	1	23.80583	23.93744	901	15727	0.1316	1500	27	co	507	1.2
18	Spectrogr	1	26.5822	26.70284	1146.8	16955.6	0.1206	1500	27	co	507	1.2
15	Spectrogr	1	20.37936	20.5256	1638.2	23344.7	0.1462	2437.5	27	qui	507	1.2
1	Spectrogr	1	0.208384	0.361929	1146.8	21051.2	0.1535	2531.25	27	qui	507	1.2
3	Spectrogr	1	3.2475	3.379111	901	20477.8	0.1316	2531.25	27	qui	507	1.2
5	Spectrogr	1	6.129642	6.290499	1146.8	20723.5	0.1609	2531.25	27	qui	507	1.2
7	Spectrogr	1	8.53542	8.674342	1310.6	20477.8	0.1389	2531.25	27	qui	507	1.2
13	Spectrogr	1	16.61529	16.7469	1556.3	21624.6	0.1316	2531.25	27	qui	507	1.2
17	Spectrogr	1	24.06539	24.20066	1965.9	18511.9	0.1353	2531.25	27	qui	507	1.2
19	Spectrogr	1	26.83445	26.98069	1965.9	19413	0.1462	2531.25	27	qui	507	1.2
20	Spectrogr	1	30.09545	30.24534	1884	21215	0.1499	2531.25	27	qui	507	1.2
9	Spectrogr	1	10.97037	11.12757	1228.7	22443.7	0.1572	2625	27	qui	507	1.2
11	Spectrogr	1	13.26707	13.43249	1228.7	22116	0.1645	2625	27	qui	507	1.2

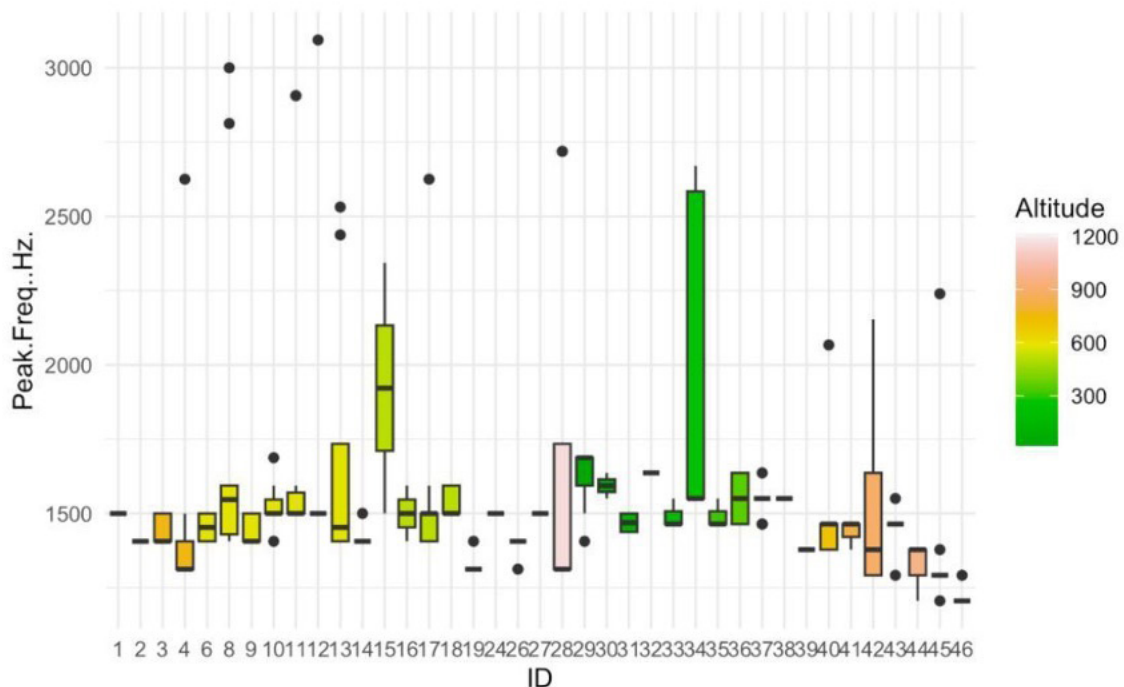
**Figure 3:** Selection table containing annotations made in RavenPro from the frogs captured in Hawai'i. The selection table is a summary of the manual annotations including begin time (s), end time (s), low frequency (kHz), high frequency (kHz), peak frequency, delta time (s), and two annotation columns to describe the individual ID, and what call type it was using.

## Preliminary Results

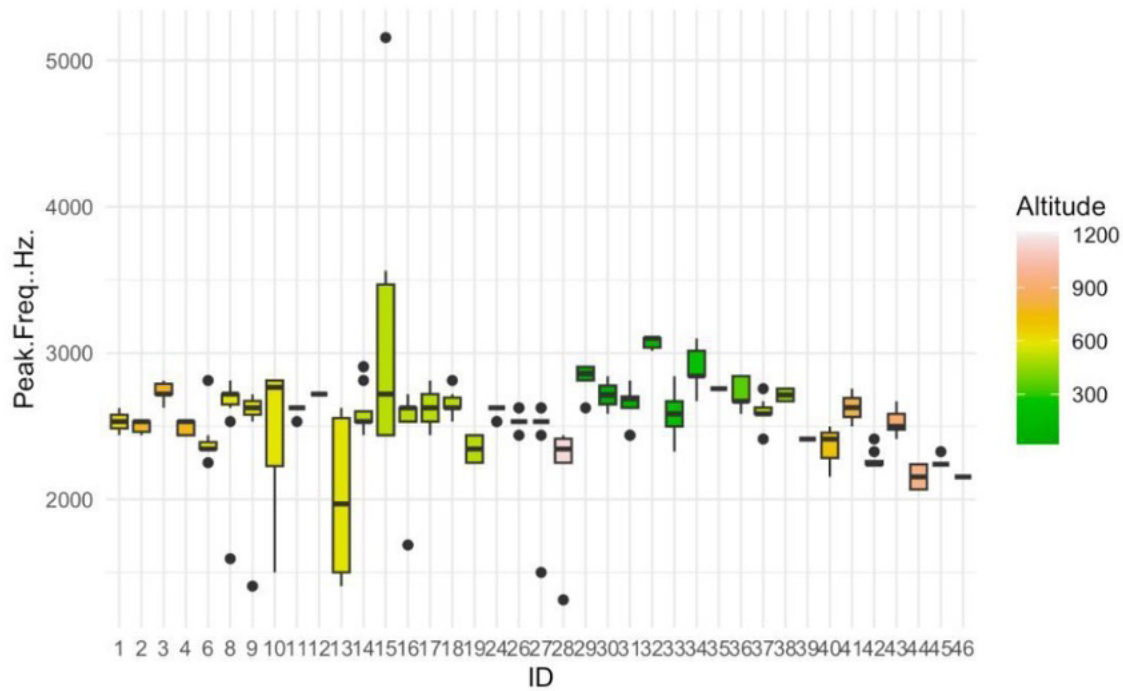
Due to time constraints, the results of this study may be slightly skewed as there are gaps in the data collection locations. Regardless, this study shows results of a negative relationship between peak frequency of the Coquí frog vocalizations and altitude. The negative relationship results correlating with the invasive Coquí are what

were anticipated given the previous knowledge on the native Coquí frogs.

In order to understand intra-individual variability in peak frequency, I visually explored the distribution “co” and “qui” peak frequencies (Figure 4a-b). Overall, individuals showing small and high variability were not related to lower or high altitudes.



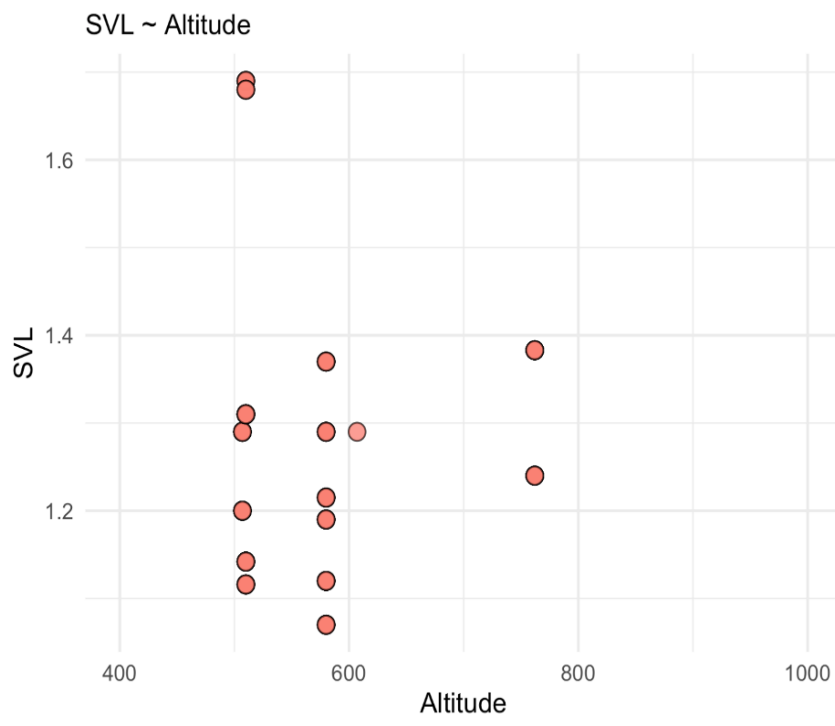
**Figure 4-a:** Box plot with peak frequency of “co” notes of Coquí frog individuals sampled in the island of Hawai'i. The color represents the altitude these individuals were sampled at.



**Figure 4-b:** Box plots with peak frequency “qui” notes of Coquí frog individuals sampled in the island of Hawai‘i. The colors represent the altitude these individuals were sampled.

Using the average peak frequency, I fitted linear models to visually explore the allometric relationship with body size. Following initial expectations, I found a negative relationship

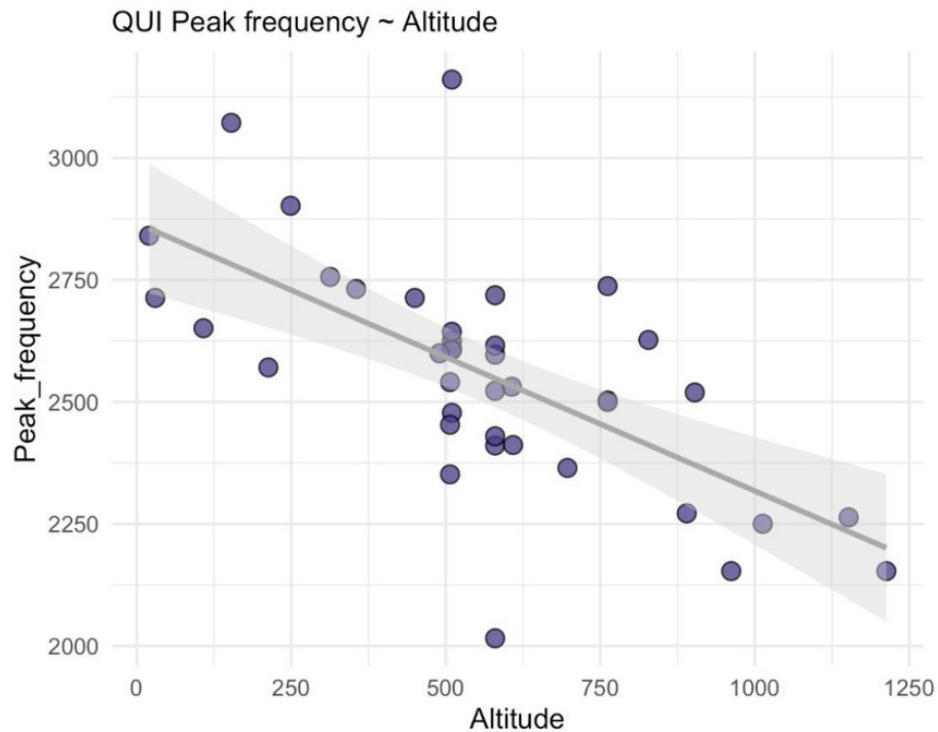
between the two variables (Figures 6a-b). The relationship seems to be especially stronger for the “qui” note (larger slope).



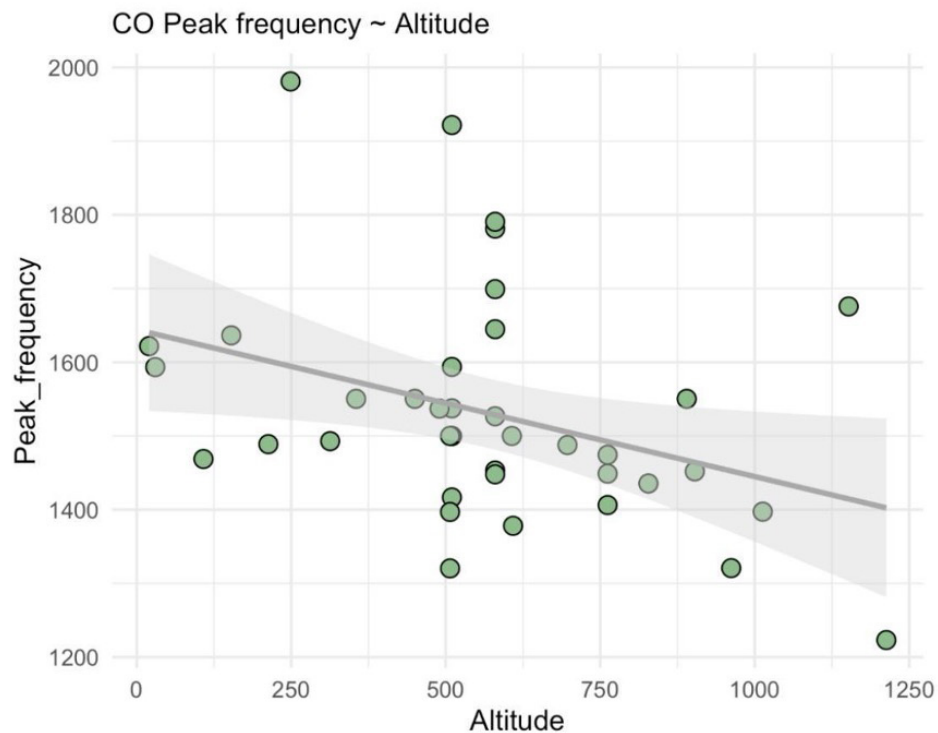
**Figure 5:** A dot plot showing the relationship between Altitude and Body Size.

For body size, it is apparent that there is a relationship that as altitude increases so does body size (Figure 5). However, these results are

ambiguous because there is lack of samples from the higher altitudes of the study.

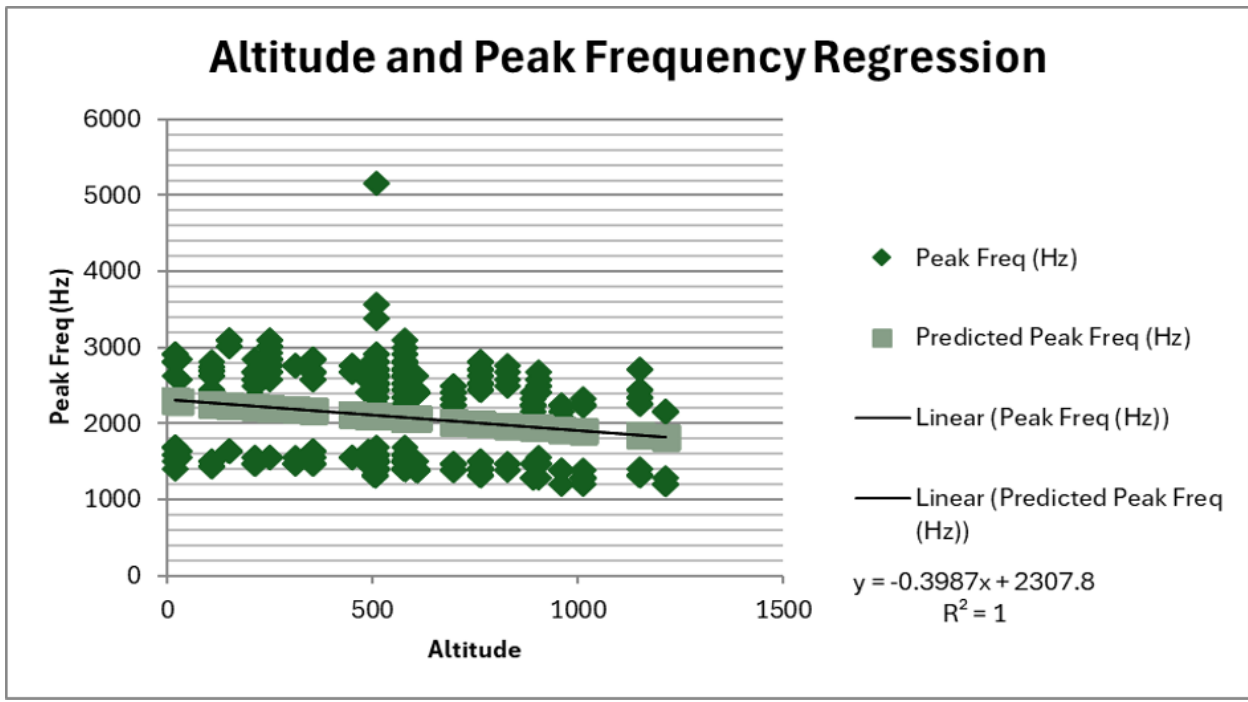


**Figure 6-a:** Dot plots with peak frequency of the “co” notes of Coquí frog individuals sampled in the island of Hawai’i. The other axis considers the altitudes of the captured frogs.



**Figure 6-b:** Dot plots with peak frequency the “qui” notes of Coquí frog individuals sampled in the island of Hawai’i. The other axis considers the altitudes of the captured frogs.

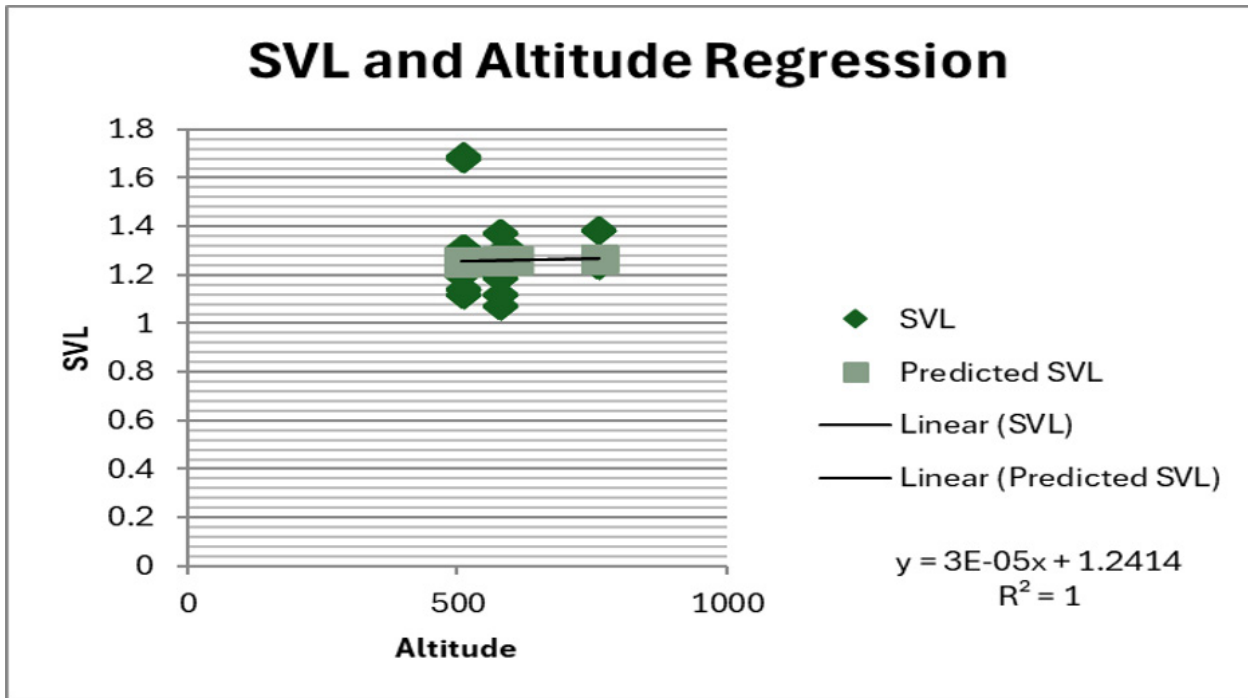
Using a 95% confidence interval and a .05 alpha, I tested the null hypothesis that there is no relationship between altitude and peak frequency vocalizations. These results indicate a clear negative relationship between the two variables. With a negative slope of  $-.3987$ , a P-value of  $3.3E-185$ , and an adjusted  $R^2$  of  $0.027484$ , we reject the null hypothesis and conclude that there is a relationship between these two variables.



**Figure 7:** A linear regression plot between Peak Frequency (Hz) and Altitude (m).

Using a 95% confidence interval and a .05 alpha, I tested the null hypothesis that there is no relationship between SVL and altitude. These results indicate a slightly positive relationship between the two. With a slope of 3E-05, a

P-value of 3.86E-62 and an adjusted R<sup>2</sup> value of -0.00298 we reject the null hypothesis and conclude that there is a relationship between SVL and altitude.



**Figure 8:** A linear regression model fit between SVL and Altitude.

## Discussion

In their native habitats, Coquí frogs show lower call characteristics with increasing altitude because of larger individuals occupying higher altitudes. I found a similar relationship with invasive Coquí frogs on the island of Hawai'i. This finding establishes a new baseline from which the phenomena of upward shifting due to climate change can be compared and monitored over time. In Hawai'i, temperatures typically decrease by 3.5 degrees Fahrenheit per 1,000 ft of altitude increase and overall lower as altitude increases (Diaz et al., 2011). This temperature gradient is similar to that of Puerto Rico, where every 1,000 feet is associated with a decrease of 5.4 degrees Fahrenheit (Van-Beusekom et al., 2015). While the Hawai'i gradient may not be as drastic, it provides a similar baseline to compare locations.

Peak frequency altitude graphs matched initial expectations based on native populations: as altitude increased, the peak frequency, of both the “co” and the “qui” vocalizations decreased, a result consistent with past studies in Puerto Rico. When tested with a regression model (Figure 7), the slope indicated a negative relationship of  $-.3987$ , as well as the P-value of  $3.3E-185$  establishes that this is likely not due to chance. However, the adjusted  $R^2$  of  $0.027484$  indicates that only a very small portion, about 2.75%, of the variation in the dependent variable around the mean. This indicates that while there is a relationship, it is a very weak relationship. This phenomenon is explained by the fact that for larger frogs, the amount of area that they have for a vocal sac also increases, allowing for a larger vocal sac. These larger vocal sacs increase the amount of air that can be inhaled and vibrated to create larger sound waves. Frequency is described as the number of wave cycles that pass through a point in a second measured in Hertz. Larger waves are extended over time, resulting in lower frequencies. This relationship is clearly reproduced in the figures of this study. Due to being ectotherms as the weather becomes less favorable for rain conditions and overall

becomes warmer which would lead to more of these low-frequency calls.

Additionally, the relationship between SVL and altitude was tested with a regression model. The slope indicated a positive relationship of  $3E-05$  as well as the P-value of  $3.86E-62$  shows that there is a relationship between these variables. However, with such a small adjusted  $R^2$  value of  $-0.00298$  this relationship is very weak. This is because of the time constraints leading to not having enough SVL data. The positive relationship is due to the cooler temperatures up in higher elevations. In ectotherms, colder temperatures allow for more body growth, enabling larger body sizes. Higher temperatures can inhibit the efficiency of cellular processes, leading to smaller cells, and consequently smaller body sizes (Angilletta et al., 2004).

Further analysis using this same dataset will give more insights into the spatial distributions of this invasive species. For instance, the large variations in individual calls shown in figure 4 could be due to analyzer bias, which is common in the field of bioacoustics when doing manual annotations. Although utilizing software-based models could be used in future studies to annotate recordings, many programs are designed for bird calls, not frog vocalizations. Bias is also created around the natural subjectivity of what constitutes a vocalization. This could also be due to some annotations coming from iNaturalist, a citizen science website for recordings. iNaturalist recordings also do not account for the distance between the frog and the physical recorder, leading to possible inaccuracies in the data.

Other environmental factors may be at play when it comes to the frequencies of vocalizations. For example, rain was an important bioindicator for when the frogs were vocalizing. On every night that we sampled it had either rained during the day or was raining while we were searching. This is not an uncommon occurrence for amphibians as “calling activity of frogs is influenced by

local environmental factors such as relative humidity” (Hatano et al., 2002). This is because amphibians breathe through their skin which makes moist environments ideal to move around and find mates.

Overarchingly, some results were ambiguous, however the clear negative relationship between frequency and altitude (Figures 6a and 6b) are consistent with the expected results from the native frog research. This indicates that if this pattern holds true, with more studies we could determine that altitude does have a large effect on these vocalizations, and that overtime we may see these frogs shift into higher altitudes where they have a bigger body size, but a smaller peak frequency as expected in Puerto Rico.

## Further Research

This study establishes a baseline which can be reassessed further as the global climate continues to warm. It also remains to be explored if climate will also impact the inverse relationship of the calls and change the calls of the frogs even at higher altitudes. It is possible that producing short and quick calls at higher frequencies may be less energy-intensive, so a future study should investigate if even at the higher elevations we are seeing distinct changes in the calls. Furthermore, a more in-depth process should redo manual review of the different variations in the “co” and “qui” to make sure each annotation is correctly identified. There could also be a study looking at the entire duration of one frog call to see if duration is also impacted by altitude gradients and anthropometrics.

## Acknowledgements

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