The Effects of Male Advertisement Call Characteristics and the Microclimate on Calling Behavior and Potential Reproductive Success in *Oophaga pumilio* at La Selva Biological Station, Heredia, Costa Rica.

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Abstract

Amphibian behavior is influenced by environmental conditions, particularly temperature and humidity, which may affect acoustic communication and reproductive success. I investigated the relationships between microclimate, male advertisement call characteristics, and qualitative behavioral indicators of mating opportunities in *Oophaga pumilio* at La Selva Biological Station, Heredia, Costa Rica. From June 23 through June 29, 2026, I observed fifteen male strawberry dart frogs, recorded their advertisement calls, and monitored air temperature and relative humidity. During each sampling period, I measured distance from each focal male and recorded the presence or absence of male territorial deterrence and female visitation. I quantified calling effort, lower and upper frequency bounds, and distance-corrected relative amplitude through acoustic analysis. I found that calling effort displayed little linear relationship with either air temperature or relative humidity, but air temperature showed a stronger negative relationship with the lower frequency bound of male *O. pumilio* advertisement calls. I also found that calling effort showed a negative relationship with distance-corrected relative amplitude and was higher in focal males that experienced female visitation. These findings suggest that environmental conditions may affect characteristics of amphibian acoustic communication, with potential implications for mating behavior as climates change.

Methods

Study Site

Field studies and data collection were conducted at La Selva Biological Station, Puerto Viejo de Sarapiquí, Heredia Province, Costa Rica, from June 23 through June 29, 2026. La Selva is in the Caribbean lowlands of northeastern Costa Rica, in which it contains 1,600 hectares or 3,954 acres of lowland tropical rainforest, with an elevation approximately 35 m above sea level (Organization for Tropical Studies, 2026). La Selva is managed by the Organization for Tropical Studies (OTS) and is renowned for its vast biodiversity and historical ecological research (Organization for Tropical Studies, 2026). The region receives high precipitation and maintains a warm temperature annually (Organization for Tropical Studies, 2026). That particular climate provides an ideal habitat for many amphibian species, particularly *Oophaga pumilio*. Male *O. pumilio* were sampled on three different trails, Arboleda, SHO, and STR.

Study Species

Oophaga pumilio, or strawberry poison dart frog, is a small diurnal frog from the family Dendrobatidae distributed across sections of Central and South America (Penner, 2011). Male *O. pumilio* are territorial and produce advertisement calls associated with the defense of territories from other males and the attraction of females (Penner, 2011). Therefore, *O. pumilio* advertisement calls are a significant factor in male reproductive communication (Penner, 2011). Advertisement calls have measurable temporal, spectral, and amplitude characteristics (Ringler et al., 2017). In this study, calling effort, upper and lower frequency bounds, and relative amplitude were measured as variables of acoustic communication.

Field Sampling

Fifteen territorial male *O. pumilio* were observed from June 23 through June 29, 2026. Each individual male was located along the SHO, STR, and Arboleda forest trails within La Selva Biological Station. Audio recordings of male advertisement calls were taken using a Zoom H4n Pro Handy Recorder equipped with a directional microphone. Individual focal males were identified after displaying persistent calling activity for at least one minute alongside trails. Once a calling male was identified, the microphone was adjusted toward the direction of the focal male and remained stationary until the end of the recording period. Following the predetermined one minute period of persistent calling to qualify a focal male, the recording period began after the focal male resumed calling activity. An appropriate recording distance was observed based on maintaining an undisturbed calling process, with recordings primarily remaining on the concrete pathing at an approximate distance of 1 meter from the focal male. Following each recording period, the distance between the focal male and the microphone was measured in meters to produce a correction of recorded amplitude to a relative distance of 1 m. Recording periods ranged from approximately 8 to 12 minutes, with the majority lasting exactly 10 minutes. Air temperature and relative humidity were recorded simultaneously during the recording period using a Kestrel DROP D3 Environmental Data Logger. Air temperature and relative humidity values were taken at one minute intervals with a setting applied on the Kestrel logger. These values were then averaged based on the associated focal male's recording period and then paired with their corresponding observation.

Additionally, qualitative observations were made whilst recording to document female visitation and male territorial deterrence. Female visitation was defined by a female's approach to a focal male during the observational period. Male deterrence was defined by an observed calling

interaction between the focal male and another individual male, the focal male physically removing another male from its territory, or the apparent deterrence of another male from the territory by the focal male's calling. Female visitation and male deterrence were recorded as yes/no variables for each focal male.

Bioacoustics Analysis

Audio recordings were uploaded to Raven Lite Pro in the form of .WAV files to produce spectrograms for each recording period. The entirety of each recording was examined and every instance of the focal male calling was annotated. A focal male's advertisement call was identified within each recording based on the timing of calling once the recording period began. Additionally, the calling signal intensity displayed on the spectrogram was used as a secondary characteristic to differentiate between the focal male and other calling males nearby.

Spectrograms were analyzed to determine the upper and lower frequency bounds, calling effort, and amplitudes for each focal male. After all calls produced by a focal male were annotated, the annotations were selected together to record upper and lower frequency bounds (Hz). The lower frequency measurement (Hz), which represents the lower frequency bound of all calls from a focal male during the recording period, was used from each focal male during analysis. Calling effort of each male was calculated as a percentage based upon total duration of call throughout the recording period divided by the total recording period, which is represented by the equation:

$$\text{calling effort (\%)} = \frac{Total\ calling\ duration\ (s)}{Total\ recording\ period\ (s)} \text{ x } 100,$$

where *total calling duration (s)* represents the summed duration of all annotated calls produced by a focal male, and *total recording period (s)* represents the duration of each focal male's recording period. Amplitudes were recorded in decibels relative to full scale (dBFS) after each

call annotation from a focal male was selected in order to get a range in amplitude throughout the calling period. The lower amplitude measurement from the range recorded for each focal male was used in the relative distance correction equation. Since the distance between the microphone and frog varied across focal males, the amplitude measurements were corrected to a relative distance of one meter using the equation:

$$A_{1m} = A_{measured} + 20log_{10}(d),$$

where A_{1m} represents the distance-corrected relative amplitude at 1 m, $A_{measured}$ represents the lower recorded amplitude in dBFS, and d represents the distance between the microphone and focal male in m. The resulting values were recorded as distance-corrected relative amplitudes (dBFS at 1 m).

Statistical Analysis

Statistical analyses and data visualizations were performed using Microsoft Excel for Mac. The relationships between acoustic and microclimate variables were examined using scatterplots and linear regression analysis. Scatterplots and linear regressions were generated to examine the relationships between calling effort and air temperature, calling effort and relative humidity, lower frequency bound and air temperature, and calling effort and distance-corrected relative amplitude. For each linear regression, the regression equation and coefficient of determination (R^2) were calculated to characterize the direction of the corresponding relationship and the proportion of total variation displayed by the model.

Additionally, calling effort was compared between focal males with and without observed female visitation and between focal males with and without observed male territorial deterrence. Calling effort among focal males with and without observed female visitation was modeled using box plots that represent the distribution of the corresponding calling effort (%) between a

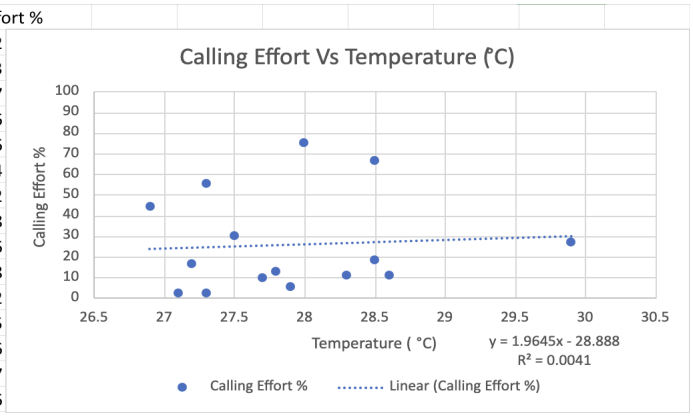
female-visitation presence and absence groups. Calling effort among focal males with and without observed male territorial deterrence was also modeled using box plots that represent the distribution of the corresponding calling effort (%) between male-deterrence presence and absence groups.

Results

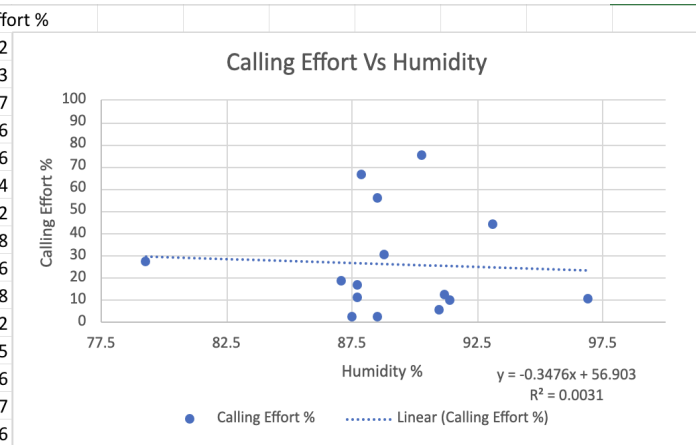
Fifteen male *O. pumilio* were sampled between June 23 and June 29, 2026. Calling effort, lower frequency bound, distance-corrected relative amplitude, air temperature, and relative humidity varied between focal males during the sampling period.

Calling Effort and Microclimate

Temperatu	Calling Effort %
27.5	30.2
28.5	18.3
28.6	10.7
28.5	66.6
27.9	5.6
28	75.4
26.9	44.2
27.7	9.8
27.8	12.6
28.3	10.8
29.9	27.2
27.1	2.5
27.3	2.6
27.2	16.7
27.3	55.6



Humidity !	Calling Effort %
88.8	30.2
87.1	18.3
96.9	10.7
87.9	66.6
91	5.6
90.3	75.4
93.1	44.2
91.4	9.8
91.2	12.6
87.7	10.8
79.3	27.2
88.5	2.5
87.5	2.6
87.7	16.7
88.5	55.6



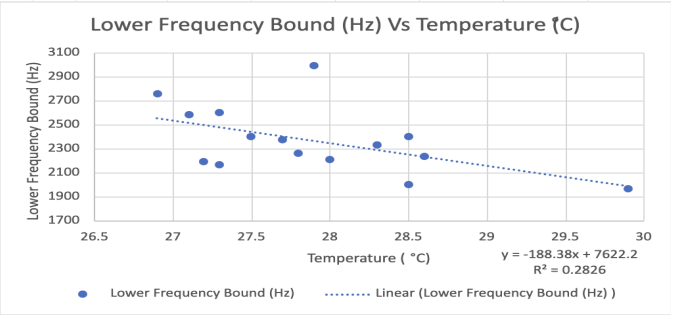
(Figure 1 seen above & Figure 2 seen below)

Air temperature during focal male sampling ranged from 26.9 to 29.9 °C (Figure 1), while relative humidity ranged from 79.3 to 96.9% (Figure 2). Calling effort varied considerably among the 15 focal males, with a range from 2.5% to 75.4% (Figures 1 and 2).

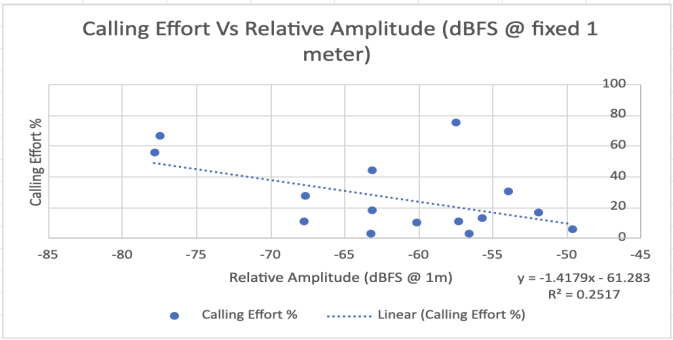
Calling effort displayed a very weak positive relationship with air temperature (Figure 1). The linear regression equation was y = 1.9645x - 28.888, with a coefficient of determination of R^2 = 0.0041, which indicates that air temperature accounted for 0.41% of the variation in calling effort in the context of the linear model. Calling effort displayed a very weak negative relationship with relative humidity (Figure 2). The linear regression equation was y = -0.3476x + 56.903, with a coefficient of determination of R^2 = 0.0031, which indicates that relative humidity accounted for 0.31% of the variation in calling effort in the context of the linear model (Figure 2)

Advertisement Call Characteristics

Temperatu	Dominant Frequency (Hz)
27.5	2400
28.5	2400.1
28.6	2235.3
28.5	2004.6
27.9	2992.6
28	2211.5
26.9	2756.2
27.7	2377.1
27.8	2260.5
28.3	2336.1
29.9	1967.2
27.1	2582
27.3	2171
27.2	2198
27.3	2604.9



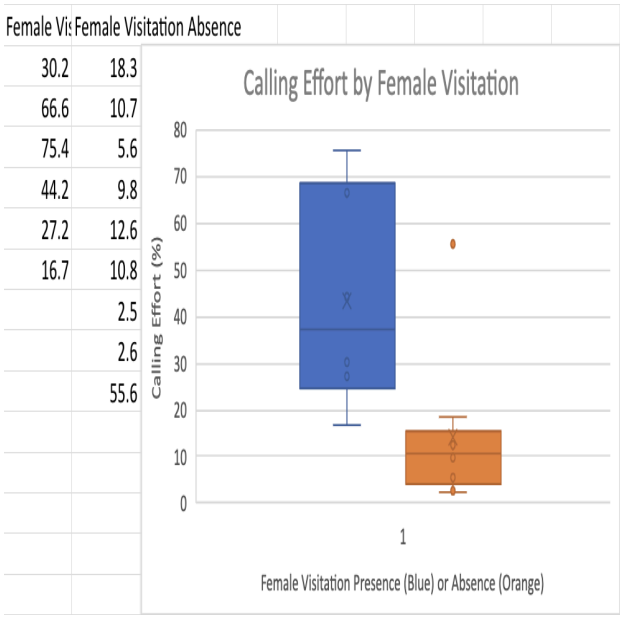
Relative Ai	Calling Effort %
-53.9	30.2
-63.1	18.3
-67.7	10.7
-77.4	66.6
-49.6	5.6
-57.5	75.4
-63.1	44.2
-60.1	9.8
-55.7	12.6
-57.3	10.8
-67.6	27.2
-56.6	2.5
-63.2	2.6
-51.9	16.7
-77.8	55.6



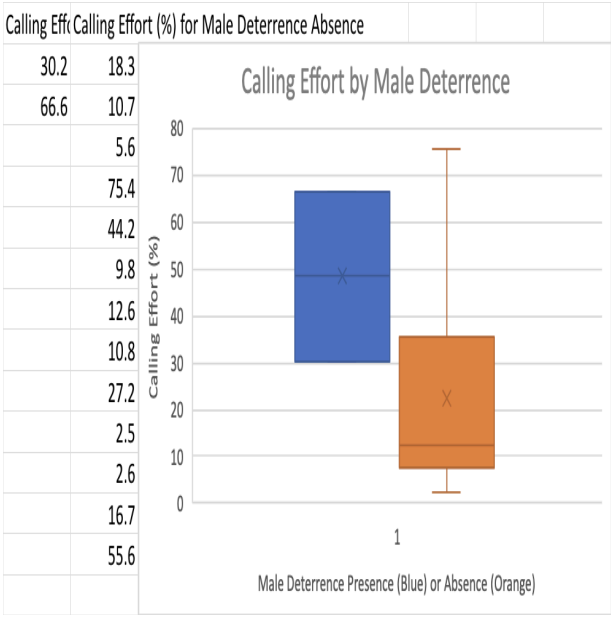
(Figure 3 seen above & Figure 4 seen below)

The lower frequency bound varied among the 15 focal males that were sampled, with a range from 1967.2 to 2992.6 Hz. Distance-corrected relative amplitude varied as well, with a range from -77.8 to -49.6 dBFS at 1 m. The lower frequency bound displayed a moderate negative relationship with air temperature (Figure 3). The linear regression equation was y = -188.38x + 7622.2, with a coefficient of determination of R^2 = 0.2826, which indicates that air temperature explained 28.26% of the variation in lower frequency bound in the context of the linear model (Figure 3). Calling effort displayed a negative relationship with distance-corrected relative amplitude (Figure 4). The linear regression equation was y = -1.4179x - 61.283, with a coefficient of determination of R^2 = 0.2517, which indicates that distance-corrected relative amplitude explained 25.17% of the variation in calling effort in the context of the linear model (Figure 4).

Behavioral Observations



(Figure 5)



(Figure 6)

Female visitation was counted present for 6 out of 15 focal males, while female visitation was counted absent for the remaining 9 focal males. Calling effort differed between focal males with a female visitation presence and absence (Figure 5). Median calling effort was 37.2% among focal males with female visitation present compared to a median calling effort of 10.7% among focal males with female visitation absence. Calling effort ranged from 16.7-5.4% when female visitation was counted present and from 2.5-55.6% when female visitation was counted absent (Figure 5). Male territorial deterrence was counted present for 2 out of 15 focal males, while male deterrence was counted absent for the remaining 13 males (Figure 6). The two focal males with male deterrence presence had calling efforts of 30.2 and 66.6%. Calling effort among the 13 focal males with male deterrence counted absent had a median of 12.6% and ranged from 2.5-75.4% (Figure 6).

Discussion

Microclimate and Calling Effort

The relationship between calling effort and microclimate conditions such as air temperature and relative humidity was insignificant, with each measurement representing less than 1% of the variation. This implies that under the conditions sampled, neither microclimate variable strongly corresponded with calling effort within this study's sampling conditions. Even so, the small sample size, small study parameters, and short sampling period may have denied the ability to detect these relationships, while at the same time, additional biological factors such as heterospecific acoustic interference could have influenced calling activity (Wong et. al., 2009).

Advertisement Call Characteristics

The relationship displayed between temperature and lower frequency bound was stronger, taking account for approximately 28% of variation, in which lower frequency bounds generally decreased as temperature increased. Furthermore, calling effort showed a negative relationship with distance-correct relative amplitude, where it represented approximately 25% of variation. The models implied that individual characteristics of advertisement calls could be differently associated with environmental conditions and calling behavior. However, the amplitude measurements represent relative dBFS values instead of absolute sound pressure, and the frequency measurement is taken in lower frequency bound instead of dominant frequency, so the full relationship is not determined.

Behavioral Observations

Focal males with a validated female visitation had a considerably greater median calling effort in comparison to focal males without a validated visitation, which implies a potential relationship between calling activity and female presence. However, the model cannot directly verify that greater calling effort caused female visitation, and at the same time, mating success is not indicated by female visitation. Additionally, territorial deterrence was only observed in two males, which is unreliable in analysis and prevents any definite conclusions about its relationship to calling effort.

Limitations, Future Research, and Ecological Implication

The main limitations of this study were the seven-day sampling period, 15-male sample size, naturalistic sampling, and a limited amount of behavioral observations. In the future, studies should examine larger sample sizes across an extended duration with the measurement of more direct reproductive behavior and broader environmental factors, while still integrating acoustic measurements. Despite the limitations, the observed relationships imply that analyzing multiple

variables of acoustic communication could provide more comprehension as to how environmental factors relate to *O. pumilio* calling behavior.

Conclusion

This field study examined the relationships between microclimate conditions, advertisement call conditions, and behavioral observations in male *Oophaga pumilio* at La Selva Biological Station. Calling effort displayed little relationship with air temperature and relative humidity, whilst air temperature showed a stronger relationship with the lower frequency bound of male advertisement calls. Additionally, calling effort varied with distance-corrected relative amplitude and was considerably higher among focal males with female visitation present. Even so, due to the limited sample size and confined sampling period, the applicability of these trends are restricted. Overall, the results suggest that environmental conditions might associate differently to distinct factors of acoustic signaling. Despite the restrictions, continued research with a larger sample size and broader environmental conditions might present a clearer understanding of how climate variation influences acoustic communication and the reproductive behaviors of *O. pumilio*.

References

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