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DECIPHERING TEMPERATURE-DEPENDENT ACOUSTIC SIGNALLING IN AMPHIBIANS: AN INTEGRATED ASSESSMENT OF ENVIRONMENTAL AND PHYSIOLOGICAL INFLUENCES

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Abstract*—It is acoustic communication that is being used as a critical reproductive tool in the attraction and recognition of mates, and in the coordination of reproduction in amphibians. While temperature effects on the advertisement call characteristics of ectotherms have been shown to affect their vocal behavior the relative importance of environmental and physiological factors is poorly known. This study assessed the effects of water temperature and physiological condition on average call duration and weighted average call rate while also examining interannual variation. A quantitative observational design was applied to 42 observations from 23 Sierran chorus frogs. Descriptive statistics, Spearman correlation, Mann–Whitney U tests, variance inflation factors, and multiple linear regression were performed, with HC3 robust standard errors applied to account for heteroscedasticity. Water temperature was strongly negatively correlated with average call duration ($\rho = -0.940$, $p < 0.001$) and positively correlated with weighted average call rate ($\rho = 0.859$, $p < 0.001$). Regression analysis showed that each 1°C increase in temperature reduced average call duration by 0.0155 s and increased weighted average call rate by 0.0519 units. Temperature was the only significant predictor in both models, explaining 88.2% and 74.6% of the variance, respectively, whereas frog condition and study year showed no significant effects. These findings demonstrate that amphibian acoustic signalling is strongly temperature dependent, with warmer conditions producing shorter and more frequent advertisement calls. Integrating thermal recordings within bioacoustic monitoring can enhance the understanding of reproductive behavior and bolster ecological monitoring in a dynamic environment.*

Keywords: *amphibian bioacoustics, advertisement calls, water temperature, reproductive behaviour, acoustic signalling*

I. INTRODUCTION

Amphibians require some of the most basic behaviours for successful reproduction, territorial behavior, and species recognition, and acoustic communication is one such behavior. Advertisement calls are mainly made by males to attract mates, promote reproductive isolation and allow individuals to broadcast their message in a complex natural environment. They are also shaped by the physiological state of the animal and external environmental conditions and are affected by the physiological state of the animal and external environmental conditions, and therefore they have great significance for understanding the physiological processes and population persistence that affect reproductive success and ecological processes (Wells, 2019). As a group of vertebrates, amphibians have a unique evolutionary position and make an important contribution to the functioning of ecosystems through their trophic interactions, nutrient cycling, and their bioindication of their surroundings. Many populations of these vital species are suffering from severe declines across many areas, and a lack of understanding of behavioural mechanisms that affect their reproductive success (Jetz & Pyron, 2018) is hindering knowledge of the factors leading to population declines.

Amphibian communication systems have evolved in such a way as to ensure that the signals are both maximally efficient and recognizable by their own species. Vocal behaviour is thus considered to be an important behavioural phenotype that correlates environmental condition with reproductive fitness. Physiological performance of ectothermic animals can be directly influenced by environmental variation (e.g., temperature) affecting muscle activity, metabolism and hence acoustic signal production. Because of the potential for reproductive communication to be influenced by changing climatic conditions and the impact this may have on population viability (Wake & Koo, 2018), understanding these relationships has become more important. In a conservation context, behaviour that reacts quickly to environmental change is one of the most sensitive parameters for the health of ecosystems and species resilience and acoustic behaviour is therefore an important parameter for amphibian conservation research (Pabijan et al., 2020).

The process of animal communication is well recognized as a dynamic process of exchange of biologically meaningful information that is relevant for survival and reproduction. An advantage of the acoustic signals is that they can be used bb to communicate over relatively long distances under conditions where visual or chemical signals might not be as useful. Ecological requirements for signal production and reception rely on coordinated physiological processes, and communication is particularly sensitive to environmental changes (Vickers, 2017). In recent years, the field of ecological acoustics has also shown that natural sound can offer important data regarding behavioral interactions, reproductive processes and environmental quality across a wide range of animal communities (Yadav et al., 2024). Bioacoustic monitoring is rapidly growing and can now be utilized in order to study wildlife populations without causing disturbance to nature. Passive acoustics techniques allow for long-term monitoring of vocal activity and have proven to be useful in ecological monitoring, biodiversity studies, and conservation planning. These techniques allow quantitative assessments of the behavioural responses, which can be directly associated with environmental variables and benefit the understanding of species ecology in response to environmental changes (Teixeira et al., 2019).

One of the most important environmental parameters influencing the acoustic communication of ectothermic vertebrates is temperature. The effect of rising temperature on neuromuscular performance and metabolic activity has been shown in a variety of aquatic species, affecting the frequency of signals, temporal structure of signals, and effort invested in the signals (Ladich, 2018). Terrestrial insects also show reproductive communication that depends on temperature, with the mating call characteristics changing significantly over the range of temperatures, suggesting that environmental temperature can influence reproductive communication and mate choice (Singh et al., 2020). The results indicate that thermal change in acoustic signaling is a widespread phenomenon in the biology of many taxonomic groups. Vocal communication in amphibians has received significant research attention and has greatly enriched the knowledge base of the evolution and physiology of vocal communication. Amphibian bioacoustics history shows the possible variability of advertisement calls, which have been closely linked to species recognition, adaptation to the environment and reproductive behaviour. The value of considering both ecological and physiological approaches in the study of amphibian acoustic communication is emphasized in these studies (Narins et al., 2023). Similar findings in avian systems also support the general function of thermal environments in communication across vertebrates: increased temperatures decrease vocal activity and alter the characteristics of the vocalizations (Coomes & Derryberry, 2021).

A relationship between environmental temperature and signalling behaviour is well correlated with physiological constraints of the ectotherms. The production of signals tends to rise with the activity of the organism up to thermal limits, and then eventually to fall, because of effects on behaviour. Thus, the trade-off between energy investment and reproductive output as a function of temperature seems to be a balancing act of adaptation (Ord & Stamps, 2017). The reproduction of many amphibians is mediated by acoustic communication, but other modes of communication are also used, such as chemical communication, which is used for recognition at several life stages. Acoustic signals are used in a multidimensional communication system, highlighting the complexity in the reproductive ecology of amphibians, and the need to consider such signals in a broader physiological context (Schulte et al., 2021). Further ecological studies have since confirmed the effect of environmental modification of breeding habitats on amphibian behaviour and habitat use, further highlighting the importance of understanding the behaviour of amphibians under different environmental conditions (McIntyre et al., 2025).

Previous studies have shown that the ambient temperature affects acoustic communication in ectothermic animals, and bioacoustic research of amphibians has revealed many behavioural modifications related to the signals of reproduction. The literature of acoustic behaviour, however, has been largely descriptive of individual aspects of the phenomenon and most studies have concentrated on aspects of the ecology. There have been comparatively fewer studies that combine multiple environmental and physiological variables in a single analytical model and assess the relative contribution of

each variable to each of the characteristics of the advertisement call. Furthermore, there are still limited statistical modelling approaches that account for assumptions, collinearity of predictors, and simultaneously model call duration and call rate. These limitations need to be addressed to enhance knowledge of the influence of environmental factors on amphibian reproduction communication.

The present study examines the effect of environmental and physiological factors on advertisement call characteristics of amphibians by looking at how water temperature and physiological condition affect the average duration of calls and the weighted average call rate. It also investigates if there are differences between study years in these acoustic properties and pinpoints the main determinants of temperature-driven acoustic signalling based on an integrated statistical approach.

2. METHODOLOGY

2.1 RESEARCH DESIGN

The research design for this study was quantitative with an observational approach and secondary data analysis to examine how environmental and physiological variables affect the characteristics of the advertisement calls of amphibians. The analytical framework was based on the comparison, correlation and regression analysis of water temperature, frog condition and acoustic signalling, as well as descriptive analysis. This design was chosen because it allows one to identify what environmental factors influence the acoustic reproductive behavior and retains the natural structure of the observations.

2.2 DATA SOURCE

This study utilized secondary data that was publicly available from Pekny et al. (2025). The data include repeated observations of individual Sierran chorus frogs from the 2022 and 2023 breeding seasons, and environmental, physiological and acoustic variables of relevance to Sierran chorus frog reproductive communication. For this study, 42 observations from 23 separate frogs were examined to determine how advertisement call characteristics changed with water temperature and frog condition (Pekny et al., 2025).

2.3 STUDY VARIABLES

The response variables that were considered were average call duration and Weighted average call rate. The water temperature was chosen as the main environmental predictor, and the frog condition was chosen as the physiological predictor. Possible interannual variation was accounted for by the inclusion of study year as a categorical variable. During data screening, frog mass and frog length were initially explored but were not included in the regression model because they were highly collinear with frog condition; this ensured that the parameters of the models were stable.

2.4 DATA PREPARATION

The data were loaded into Python and checked for completeness, duplication of observations, data structure and consistency. Descriptive statistics were used to summarise continuous variables, and the interquartile range method was used to check that there were no possible outliers. There were no missing values, and the missing values were assessed. The normality of the continuous variables was checked with the Shapiro-Wilk test and plotted graphically to be able to choose the proper statistical procedures for further analysis.

2.5 STATISTICAL ANALYSIS

The environmental, physiological and acoustic parameters of the objects of study were summarized by the calculation of descriptive statistics. Spearman's rank correlation was used to investigate relationships between continuous variables as some variables were not normal. Mann-Whitney U was used to assess differences between study years (2022 and 2023), and the rank-biserial correlation coefficient was used to quantify the effect size.

Several multiple linear regression models were then created to determine the factors predicting average call duration and weighted average call rate. To check for multicollinearity, the variance-inflation factor (VIF) was calculated before the model was fitted. Mass and length of frogs were not included in the final models as they were highly correlated with frog condition. The model assumptions were assessed using residual normality tests, Breusch–Pagan tests for heteroscedasticity, Cook's distance and residual diagnostic plots. Heteroscedasticity was identified, and HC3 (henceforth called heteroscedasticity-robust) standard errors were used to ensure reliable coefficient estimation and statistical inference. Because of this, standardised regression coefficients were also derived to be able to compare the effects of the predictors between the different models.

3. RESULTS

3.1 DESCRIPTIVE CHARACTERISTICS OF THE STUDY VARIABLES

There were 23 Sierran chorus frogs used in the experiment with 42 experimental observations. The mean values of water temperature, frog mass and length were 13.56°C, 3.59 g and 34.10 mm, respectively, with a body-condition index of 0.105. The average call duration was in the range 0.181-0.469 s, while the weighted average call rate was in the range 0.257-1.301. The distribution of the environmental, physiological and acoustic variables used in the analysis is given in Table 1.

Table 1. Descriptive statistics of environmental, physiological, and acoustic variables

Variable	N	Mean	SD	Median	Q1	Q3	Minimum	Maximum
Water temperature (°C)	42	13.557	4.805	13.850	10.550	17.875	4.700	21.200
Frog mass (g)	42	3.594	0.426	3.450	3.310	3.930	2.920	4.390
Frog length (mm)	42	34.104	1.658	33.860	33.460	34.920	30.540	38.440
Frog condition	42	0.105	0.011	0.104	0.098	0.116	0.087	0.126
Average call duration (s)	42	0.300	0.079	0.283	0.241	0.340	0.181	0.469
Weighted average call rate	42	0.771	0.289	0.754	0.553	0.952	0.257	1.301

3.2 Correlations Among Environmental, Physiological, and Acoustic Variables

The highest correlations were for water temperature with both acoustic outcomes. Average call duration ($\rho = -0.940$, $p < 0.001$) and weighted average call rate ($\rho = 0.859$, $p < 0.001$) were strongly and negatively and positively correlated, respectively. There was also a strong inverse correlation between average call duration and weighted average call rate ($\rho = -0.773$, $p < 0.001$), with shorter calls corresponding to higher call rates. In terms of the physiological variables, frog mass was positively correlated with frog length ($\rho = 0.488$, $p = 0.001$), and highly correlated with frog condition ($\rho = 0.903$, $p < 0.001$). There was no significant correlation between acoustic outcomes and frog mass, length or condition, however. Table 2 shows the complete Spearman correlation matrix.

Table 2. Spearman correlations among study variables

Variable	1	2	3	4	5	6
1. Water temperature	1.000					
2. Frog mass	0.104	1.000				
3. Frog length	0.094	0.488**	1.000			
4. Frog condition	0.026	0.903***	0.149	1.000		
5. Average call duration	−0.940***	−0.023	−0.094	0.050	1.000	
6. Weighted average call rate	0.859***	0.201	0.140	0.123	−0.773***	1.000

Note: **p < 0.01; **p < 0.001

3.3 Visual Pattern of the Correlation Structure

The pattern of correlations supported the hypothesis that water temperature is the major environmental factor correlated with advertisement-call characteristics. Most associations with body size and body condition were weak, while the strongest were around temperature, call duration and call rate. The magnitude, direction and statistical significance of the correlations between all the study variables are represented in Figure 1.

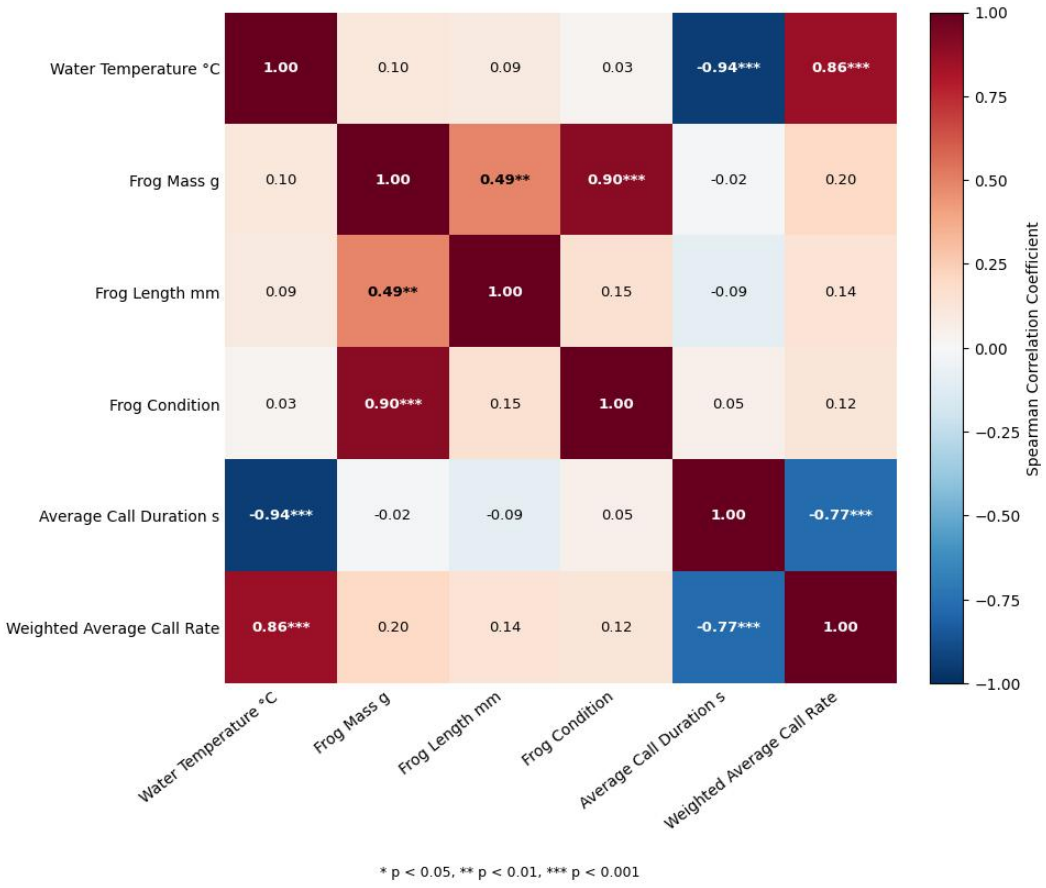

Figure 1. Associations among environmental, physiological, and acoustic variables

3.4 Interannual Differences Between 2022 and 2023

The Mann-Whitney U tests revealed that the majority of the environmental, physiological and acoustic measurements were not significantly different in the two study years. No significant differences were found between 2022 and 2023 in terms of frog temperature ($U = 225.0$, $p = 0.327$), frog condition, average call duration and weighted average call rate. The only variable that varied significantly over the years was frog length. The median frog length was 34.53 mm in 2022 and 33.49 mm in 2023 ($U = 287.0$, $p = 0.0076$), with a moderate-to-large rank-biserial effect size of −0.523. Year-wise comparisons with effect sizes are shown in Table 3.

Table 3. Comparison of study variables between 2022 and 2023

Variable	2022 Median	2023 Median	U Statistic	p-value	Rank-Biserial r	Interpretation
Water temperature (°C)	14.6000	11.4000	225.0	0.3273	−0.1936	Not significant
Frog mass (g)	3.4500	3.4200	205.0	0.6629	−0.0875	Not significant
Frog length (mm)	34.5300	33.4900	287.0	0.0076	−0.5225	Significant
Frog condition	0.1031	0.1120	144.0	0.2307	0.2361	Not significant
Average call duration (s)	0.2840	0.2825	165.0	0.5315	0.1247	Not significant
Weighted average call rate	0.7444	0.7644	194.0	0.8918	−0.0292	Not significant

3.5 Distribution of Environmental and Acoustic Variables by Study Year

Visual comparison confirmed the inferential analysis, and there were significant overlaps in annual distribution for water temperature and both of the acoustic metrics. Water temperature was observed over a similar range in 2022 and 2023, as shown in Figure 2A. The distributions and medians for average call duration were similar for both years, as seen in Figure 2B. The variability in weighted average call rate (WACR) between year-to-year observations can also be seen in Figure 2C.

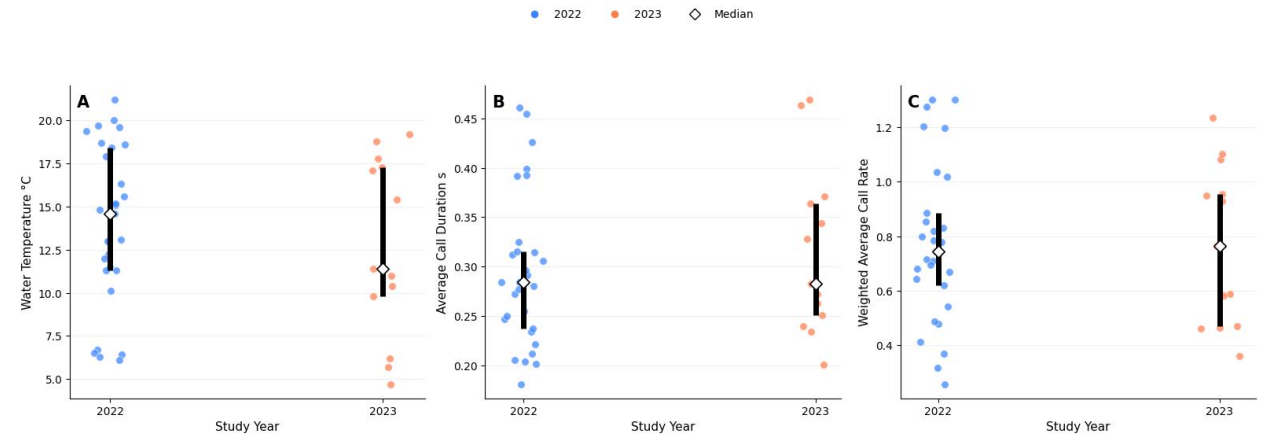

Figure 2. Interannual variation in environmental and acoustic characteristics: (A) water temperature by study year; (B) average call duration by study year; (C) weighted average call rate by study year

3.6 Temperature-Dependent Variation in Advertisement Calls

The regression-based relationships, adjusted for the influence of the other variables, showed differing temperature associations with the two acoustic outcomes. In Figure 3A, the average call duration decreased with increasing water temperature. This is reflected in the increase in weighted average call rate (WACR) seen in Figure 3B throughout the temperature range observed. The 95% confidence bands were fairly narrow throughout the range of temperatures, suggesting that the sign of both estimates was consistent after frog health and study year were factored in.

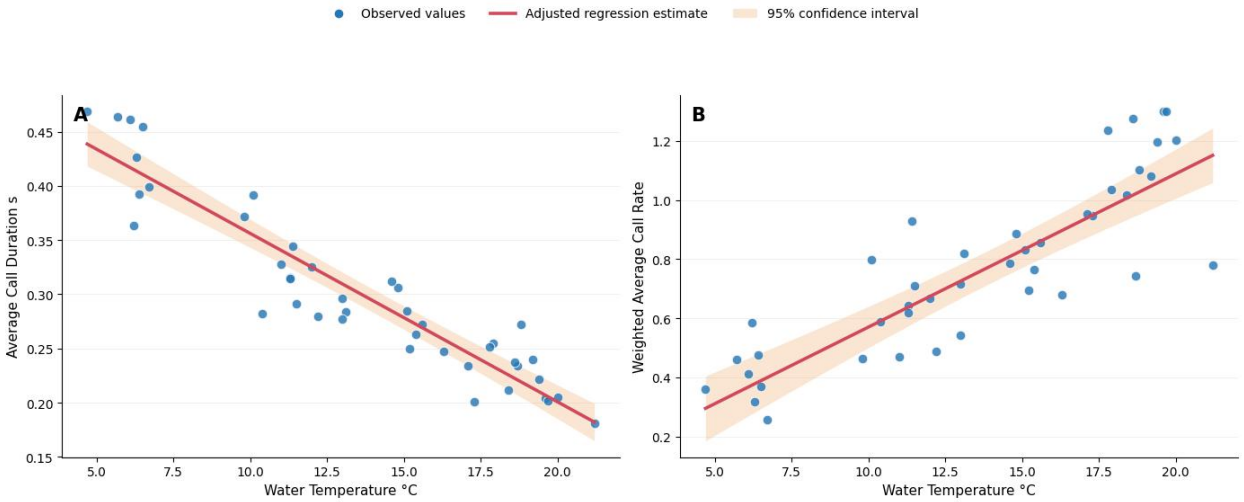

Figure 3. Temperature-dependent variation in advertisement-call characteristics: (A) adjusted relationship between water temperature and average call duration; (B) adjusted relationship between water temperature and weighted average call rate

3.7 Predictors of Average Call Duration

The multiple regression model for the average call duration was statistically significant, $F(3, 38) = 95.04$, $p < 0.001$ and accounted for 88.2% of the variance in the observed average call duration. The adjusted R2 value of 0.873 showed that the model remained highly predictive even for the number of predictors used. The only important predictor was water temperature. Each 1°C increase in water temperature was associated with a 0.0155-s reduction in average call duration after controlling for frog condition and study year (B = −0.0155, robust SE = 0.0011, $p < 0.001$, 95% CI [−0.0178, −0.0133]). The standardized coefficient was −0.942, which was very large and negative. Neither frog condition (B = 0.5016, p = 0.309) nor the 2023 study-year indicator (B = −0.0019, p = 0.874) independently predicted call duration. The average call duration estimates from HC3 are presented in Table 4.

Table 4. HC3-robust multiple regression predicting average call duration

Predictor	B	Standardized β	Robust SE	t	p-value	95% CI
Intercept	0.4588	—	0.0553	8.296	<0.001	[0.3469, 0.5708]
Study year: 2023	−0.0019	−0.024	0.0117	−0.160	0.874	[−0.0255, 0.0218]

Water temperature (°C)	−0.0155	−0.942	0.0011	−13.924	<0.001	[−0.0178, −0.0133]
Frog condition	0.5016	0.068	0.4868	1.030	0.309	[−0.4838, 1.4870]

Model statistics: $R^2 = 0.882$; adjusted $R^2 = 0.873$; $F(3, 38) = 95.04$; $p < 0.001$.

3.8 Predictors of Weighted Average Call Rate

A regression model was also statistically significant in predicting the weighted average call rate, $F(3, 38) = 37.18$, $p < 0.001$. The predictors overall explained 74.6% of the variance and the adjusted R^2 was 0.726. The call rate was found to be significantly influenced by water temperature. A 1°C increase in temperature was associated with an estimated increase of 0.0519 units in weighted average call rate (B = 0.0519, robust SE = 0.0056, $p < 0.001$, 95% CI [0.0406, 0.0632]). Its standardized coefficient value (0.863) indicated that temperature was the most significant predictor. The effects of frog condition (B = 1.7260, p = 0.550) and study year (B = 0.0511, p = 0.299) were not statistically significant. Table 5 shows the HC3-robust regression estimates for weighted average call rate.

Table 5. HC3-robust multiple regression predicting weighted average call rate

Predictor	B	Standardized β	Robust SE	t	p-value	95% CI
Intercept	−0.1306	—	0.3218	−0.406	0.687	[−0.7821, 0.5208]
Study year: 2023	0.0511	0.179	0.0485	1.054	0.299	[−0.0471, 0.1492]
Water temperature (°C)	0.0519	0.863	0.0056	9.289	<0.001	[0.0406, 0.0632]
Frog condition	1.7260	0.065	2.8638	0.603	0.550	[−4.0714, 7.5235]

Model statistics: $R^2 = 0.746$; adjusted $R^2 = 0.726$; $F(3, 38) = 37.18$; $p < 0.001$.

4. Discussion

Average call duration and weighted average call rate were found to be strongly negatively and positively related to water temperature, respectively, and were the most consistent factors across all amphibian groups to predict acoustic signalling. These associations were confirmed in the regression models, which accounted for frog condition and study year. The average call duration decreased about 0.0155 seconds per call, and the weighted average call rate increased about 0.0519 units per call with an increase of 1°C. The large standardized coefficients also suggest that the thermal conditions accounted for significant variance in both acoustic measures relative to each other and to the other predictors listed. From these results, it can be concluded that advertisement call timing is closely related to temperature-dependent physiological processes that are associated with muscle contractions, respiratory activity, and sound production. Opposing responses of call duration and call rate suggest a coordinated change in the structure of the signal rather than two separate behavioural changes. Short calls that occurred more often were related to warmer temperatures, while longer calls that occurred less frequently were related to cooler temperatures. This type of behavior might be a compromise between the length of time the signal can last and the number of calls that occur over a certain time. This interpretation is confirmed by the high inverse correlation found between call duration and call rate. Thus, the temporal organization of calling might be modified by temperature, while an adequate amount of signalling activity may be maintained during reproductive communication. After temperature and year were accounted for, frog condition was not a significant predictor of either of the acoustic outcomes. This does not mean that the physiological state is not important for reproductive ability. The contribution of this variation, however, was restricted because of the few frogs observed and their limited variation in condition. Moreover, the lack of significant differences in call duration and call rate between 2022 and 2023 indicates that the thermal relationship was fairly stable over the two sampling years. Frog length differed significantly between years; however, this morphological difference did not translate into significant differences in the principal acoustic traits.

The present results corroborate studies revealing that advertisement calls contain biologically relevant information that is utilized in mate assessment. Temporal signal properties can be linked directly to sexual selection and reproductive success, as females are influenced by call characteristics related to male reproductive quality (Pettitt et al., 2020). This strong temperature dependence found here is therefore ecologically relevant as environmental conditions could alter some of the signal characteristics that receivers use to choose mates. Inter- and intra-specific descriptions of frog advertisement calls also indicate that there has been considerable variation in call duration, call repetition, frequency, and call temporal organisation. This variability is essential for species recognition and reproductive isolation, including in a wide variety of frog communities (Amram & Zainudin, 2025). The findings herein contribute to this literature by demonstrating that part of the observed variation within a species could be attributed to the short-term thermal conditions rather than the fixed morphological differences. In the past, calling behaviour has been observed in the field and is part of a larger reproductive act that includes spatial positioning, courtship, and the interaction of males and females (Ringeis et al., 2017). A similar behavioural integration has been observed in terrestrial and semi-aquatic frogs, where vocal activity is part of the complex parental and reproductive behaviour (da Rocha et al., 2018). The present temperature effects should thus be assumed to be adjustments to a larger reproductive system.

There can be several sensory or physiological inputs to reproductive signalling. Experimental studies with túngara frogs demonstrate that visual, acoustic and social cues can affect behaviour and deep physiological reactions (Still et al., 2019). It is possible that the absence of any such frog-condition effect in the present analysis was due more to the role of unmeasured social, hormonal, or multimodal influences than to the lack of physiological regulation. Vocal repertoires have also been documented in the context of natural history (anurans) and their close association with breeding-site characteristics and species-specific reproductive modes (Malagoli et al., 2021). Signals can be shaped by environmental and morphological constraints over evolutionary time, such that immediate responses to temperature are within the evolutionary boundary of the species' anatomical traits (Muñoz et al., 2020). The thermal effect seen here is strong in this region, and has been identified as a possible interaction, though little independent explanatory power was observed in the fitted models. The environmental factors may also affect whether the signal is attractive or detectable to potential receivers. Despite the complexity of their reproductive calls, the effectiveness of such calls can be lowered by habitat structure, transmission conditions and background disturbance (Halfwerk et al., 2017). Variation in call length and call frequency with temperature may thus influence call production as well as the perception of calls within breeding habitat. Modelling of the timing and synchronization of frog calls also reveals that these can lead to synchronized communication at the group level (Aihara et al., 2019). Collective signalling dynamics and the structure of choruses may thus scale up to be affected by changes in individual call rates driven by changes in temperature.

The results show that water temperature is a major covariate that needs to be included in amphibian bioacoustic research. If thermal conditions are not measured at the same time as calls, differences in call duration or call rate among sites and/or sampling periods may be interpreted as errors. Acoustic monitoring, standardized by temperature, could enhance the ability to compare populations and better use vocal behaviour as an indicator of reproductive activity. The results are also of conservation significance. Changes in climate variability could have a significant impact on advertisement call frequency and timing, as well as the organisation of chorus and species recognition. The temperature-driven shifts in acoustics could be used as an early behavioural signature of environmental stress before changes in abundance.

Some limitations must be noted. These observations were made with a relatively small number of species, and only one species of amphibian, so there is not sufficient taxonomic generalization. A few frogs gave repeated measurements, but with a limited number of measurements per frog, it was impossible to estimate a mixed-effects model. The analysis was also made without including humidity, social density, hormone levels and ambient noise.

There is a need for additional studies with larger samples, multiple species, and expanded thermal ranges. To differentiate between individual stability and short-term plasticity, repeated observations over complete breeding seasons would be helpful. The incorporation of reproductive success, female preference, and chorus-level dynamics with acoustic measurements would yield a fuller picture of the effects of temperature-dependency of signalling on amphibian fitness and population persistence.

5. CONCLUSION

The main factor influencing variation in advertisement call was water temperature, resulting in shorter calls and higher calling rates with increasing water temperature. The contrasting responses are in favor of coordinated shifts in the temporal call structure, as opposed to shifts in acoustic features in isolation. Physiological condition and study year were not independently associated with either of the acoustic outcomes, and the strong regression effects persisted after these were controlled for. The thermal relationship was also relatively stable between years, as the interannual variations were small. The results reinforce the hypothesis that the calling behaviour of amphibians is a thermoregulatory response that signals reproductive activity. Thermal conditions are therefore important to measure in addition to the acoustic measurements for a site-to-site, season-to-season, and population-to-population comparison to be reliable. Temperature-standardised bioacoustic monitoring can enhance detection of changes in behaviour in response to changes in the environment and be used in conservation assessments prior to population-level impacts. More extensive studies with a larger number of species, longer breeding seasons, and more environmental parameters would help to assess whether the patterns observed are behavioural plasticity over the short-term or ecological adaptation over the long term.

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