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The use of bioacoustics to assess the impact of environmental parameters on male pool frog (*Phelophylax lessonae*) call characteristics

ABDELLATIF BOUKHEMAL

SUPERVISOR
Lars Korslund

University of Agder, 2022
Faculty of Engineering and Science
Department of Natural Sciences

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This masters' thesis is carried out as a part of the education at University in Agder and is therefore approved as a part of this education. However, this does not imply that the University answers for the methods that are used or the conclusions that are drawn.

University in Agder

Faculty for Engineering and Sciences

Department of Natural Sciences

Gimlemoen 25

4604 Kristiansand

http://www.uia.no

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Whilst not used in the final investigation, it should be acknowledged that a GoPro Hero 10 was used to record video of frog activity on the following dates: 27/05/2021, 06/06/2021, 07/06/2021, 09/06/2021, 15/06/2021, 17/05/2021. This was due to the initial concept of the thesis being centered around linking audio recordings of frog calls to the video recordings of their activities. I would thereby be able to establish whether frog calls played an important role in coordinating frog activity. However, once frog calls had been isolated and video footage obtained, it proved impossible to actively align video footage with audio recordings of calls. Therefore this objective, and the use of video footage, was discarded, as it would likely have resulted in inaccurate results and an unacceptable amount of guesswork.

I would also like to thank my family for their continued support during my master's thesis.

Summary

The purpose of this investigation was to use current methods of bioacoustic analysis to determine whether the environmental parameters of water temperature and background noise significantly affect the characteristics of male *Pelophylax lessonae* (Pool frog) mating calls. The secondary purpose was to ascertain whether call characteristics (call length, pulse length, pause length, number of pulses, and peak frequency), are useful indicators of frog activity. This study was conducted during the predicted peak mating season, over a period of several months. Environmental readings of the study site were obtained using a variety of primary and secondary research, whilst frog calls were recorded using a microphone. Linear regression modelling was used to determine to what extent water temperature and background noise explained the variation in each call characteristic. Analysis of call characteristics produced results which suggested that call length, pulse length, pause length and number of pulses are significantly influenced by environmental parameters. As no relationship was found between peak frequency and the environmental parameters, alternative methods of study and predictor variables for peak frequency were put forward.

It was also concluded that environmental parameters vary in their influence upon frog calls and that variance of each environmental parameter must be considered when studying their effect on frog calls. Emphasis is put on the great extent to which background noise influences call length and pause length, as this is a new development in the field of bioacoustics. Call characteristic analysis were found to be a very useful study method for several interactions, not limited to environment. The call characteristics which showed a relationship with the environment also proved to be useful indicators of frog activity, as frog activity is intrinsically linked to changes in in water temperature.

Content

1. Introduction

1.1 Phelophylax lessonae/ Pool frog/ Damfrosk

Pelophylax lessonae, commonly known as the pool frog, is located throughout northern and western Europe. Post glacial recolonization in the northern clade has affected *P. lessonae* migration throughout these areas (Zeisset & Hoogesteger, 2018). Norway is a prime area for analysis of *P. lessonae* as northern Europe is the only area where *P. lessonae* can be found isolated from other water frog species and hybrid species such as *P. esculenta* (Zeisset & Hoogesteger, 2018). Whilst *P. lessonae* and the two other predominant frog species in Norway, *Rana temporaria* and *Rana arvalis*., belong to different genera, all three are placed with the family Ranidae (Dolmen, 2019).

P. lessonae inhabit freshwater, for 3 -5 months of the year, with their spawning season usually lasting from May until June (Dolmen, 2019). The spawn produced remain tadpoles for a period of several months before beginning their metamorphosis into adults roughly in August (Dolmen, 2019). *P. lessonae* populations face several extrinsic pressures, largely based on significant changes in the environment such as disruption in pond refilling cycles due to climate change (Engemyr & Reinkind, 2019). However, the focus of my study is not on assessing larger environmental disruptions such as this, but rather gradual changes in environment that would naturally occur regardless of human impact. The study will assess if and how environmental parameters influence the call characteristics of male *P. lessonae* calls.

1.2 Bioacoustics

Bioacoustics is a term that encompasses many aspects of sound analysis in biology. It can refer to vocalizations produced by animals, and is a cornerstone of species propagation and survival, as concise and clear vocalizations between individuals are essential for interactions such as intimidation between males, attracting the opposite sex, or warning other individuals of the presence of danger (Rossing, 2007). Some organisms invest massively in their vocalizations, given how it is their primary method of information transfer (Rossing, 2007). There are several reasons for why animals produce certain vocalizations. Vocalizations can, on a base level, signal existence and locations of other individuals within the species (Rossing, 2007).

Bioacoustics also refers to the analysis of soundwaves in a habitat, not only through vocalizations between individuals of a species but also through other sound sources such as wind, vibrational communication, hunting via echolocation and abiotic sources which impact the habitat in question (Rossing, 2007). These analyses have a myriad of applications, from species identification to establishing soundscapes of environments over long periods of time, to test for synchronicity with environmental factors (Lindseth & Lobel, 2018).

Various features within the calls of a certain species can result in different signals, whilst a combination of features within a call can produce several effects simultaneously. This can be seen in species such as the vervet monkey *Chlorocebus pygerythrus*, who produce calls that not only function as alarm calls to warn of predators, but also serve to deter the predators themselves (Isbell & Bidner, 2016). These features are referred to as call characteristics and focus on the structure of each individual call.

1.3 Impact of environmental factors on animal vocalization

The subject of animal vocalization in relation to activity under various conditions has been explored in past research. It has been observed that in rats, vocalization is more frequent in cold environments compared to warm environments (Kraebel et al, 2002). A similar result has been observed in the large treefrog (*Rhacophorus dennysi*), with relatively high water temperature and lower humidity inhibiting vocalization (Jichao et al., 2012). However, this directly contrasts with data on pool frog call activity, with vocalization being measured as more frequent at high water temperatures (Wahl, 1969). As *Rana esuclenta* is a hybrid of *P. lessonae*, formerly known as *Rana lessonae*, and *Pelophylax ridibundus* is also found in similar European habitats to the one where our study was carried out, we would expect more frequent call activity at higher water temperatures, as the two species undoubtedly have similar adaptations. This is corroborated by the fact that *P. lessonae* exhibit a preference for warmth, reportedly requiring a water temperature of 16°C to spawn (Sjögren et al, 1988). Increased reproductive activity at higher water temperatures was exhibited for both sexes (Sjögren et al, 1988). Another variable shown to have an effect is background noise, with green tree frogs *(Hyla cinerea)* in noisy environments producing higher frequency calls to make themselves discernible from background noise, as females will otherwise often fail to detect the calls (Gerhardt & Klump, 1988).

Background noise can also be reflective of wind speed, as a large portion of background noise is caused by wind. In this case windspeed will be encapsulated under the umbrella of background noise, as measuring background noise potentially also provides information on other sources of background noise such as water disturbances, rather than purely wind speed. Variation in winds speeds impacts background noise in a number of ways. A good example is wind induced vibration of up to 100hz in the plant *P. trifoliata*, and gusts of wind inhibiting insect calls, due to these species attempting to make themselves discernible during periods with less winds (McNett et al., 2010). These signals would certainly contribute to background noise (McNett et al., 2010).

1.4 Bioacoustics visualization and applications of secondary research

Oscillograms are used to visualize audio recordings of calls as they display changes in amplitude over time, allowing a pattern to be formed based off the structure of the sound (Köhler et al., 2017) (Figure 1). One of the significant call characteristics is known as pulse groups, which are distinct groups of sound energy bursts (Köhler et al., 2017). These pulse groups are separated by inactive sections of the call, or significant differences in frequency (Köhler et al., 2017).

When assessing how call characteristics deviate, we must establish a rough idea of which call characteristics should be examined. Radvan and Schneiders 1988 paper identifies several significant call characteristics (Schneider & Radwan, 1988). More relevant are the call characteristics they choose to measure; namely call duration, pulse repetition rate and number of pulses within a pulse group.

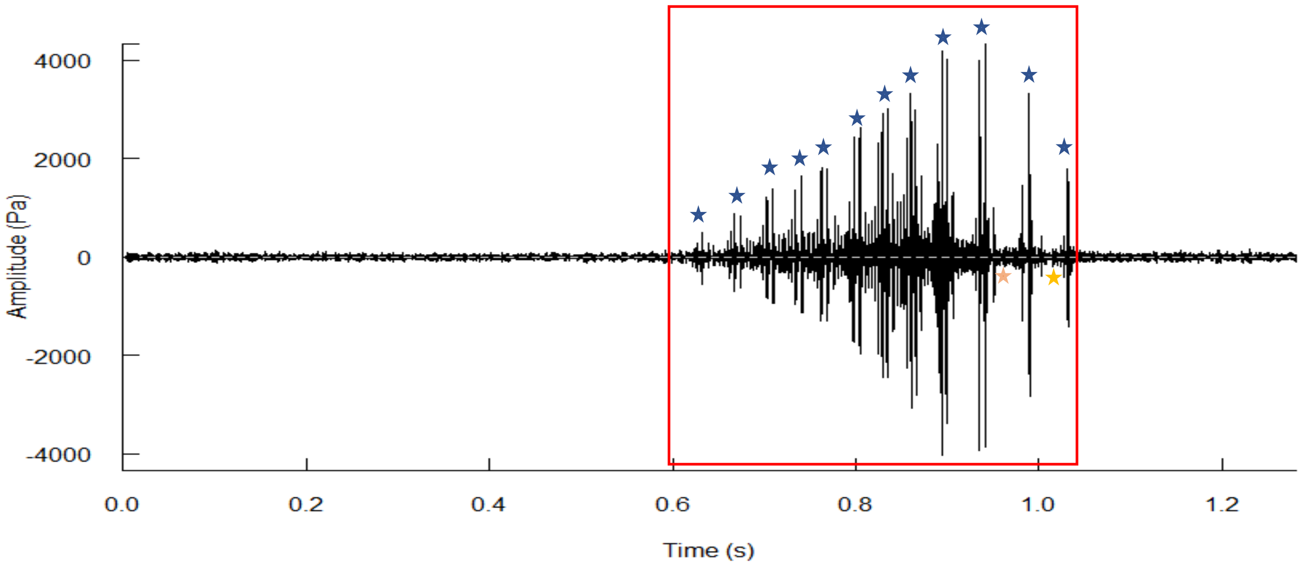

Figure 1-Oscillogram of an isolated P. lessonae call, visualizing the features of a call spectrum. The spectrum within the red rectangle would be considered a call. This is also the call length. The start and the end of the call are distinct and are used to calculate call length and pause length. In this spectrogram it is evident that the call contains 10 different pulse groups, with each pulse group consisting of approximately 3-4 pulses. Each pulse group is indicated by a blue star. Examples of pauses are indicated by orange stars.

Radwan and Schneider (1988) assess the effect of water temperature on these characteristics, stating that, independent of frog size, call duration decreases as water temperature rises, whilst the frequency and repetition of the pulse groups increases (Schneider & Radwan, 1988).

Ergo, this study provides a guideline on which call characteristics to focus on and what results we may be able to expect. It will be interesting to determine whether similar results are found in our studies, although it should be noted that the study by Radwan and Schneider (1988) was carried out in Bonn, Germany, whereas the population in my study was within the northern clade of *P. lessonae*. Therefore, there may be differences in the temperatures of the two different sites, and the populations may have adapted differently to the environments. However, the call characteristic data should be sufficient in determining impacts of environmental parameters. Let us consider how the call characteristics will likely be influenced by each predictor variable. First, when water temperature increases, I would expect shorter call length, pulse length and pause length. This is because frogs are cold blooded so they will be more active at higher water temperatures. Frogs would produce more frequent, shorter bursts of noise, because they are more aggressive and more active in general. Radwan and Schneider (1988) support the idea that calls will be shorter, and more frequent. I expect number of pulses to increase, as calls will likely be denser, with more signals per call as the frogs will communicate more, due to higher activity. I also expect peak frequencies to be higher at higher water temperatures, as more active individuals usually produce higher frequency calls.

In terms of increase in background noise, it is doubtful whether there would be a significant correlation between call length, pause length, and number of pulses. However, if there is a correlation, I would expect it to be negative. This is because higher background noise could be an indicator of stronger winds, which increase evaporation from the skin, hence frog body temperature is likely to decrease, so it would reduce their activity. However, peak frequency is likely to have a positive correlation with background noise. This is because higher frequency background noise will mean that the frogs will produce higher frequency calls in order to distinguish their calls more from the background noise. Whilst the focus on Schneider and Radwans (1988) study was on types of *P. lessonae* calls, I will instead be focusing purely on call characteristics on a population level, rather than individuals, and assessing the potential effect of not only water temperature but also other environmental variables.

Thus far two environmental variables of significance have been established: water temperature and background noise. Rainfall, or in other terms, precipitation, is another environmental variable that will be considered. Heavy rainfall can directly reduce water temperature, along with being another source of the background noise, which can affect frequency of frog calls (Iveland & De Boer, 2021).

1.5 Aims

This study is focused on whether changes in environmental parameters significantly impact call length, pulse length, pause length, number of pulses and peak frequency in frog calls during spawning season, rather than the call as a whole. An emphasis is placed on the variation and patterns of each call characteristic, establishing the relationships of each characteristic with environmental parameters. Expectations prior to the study are that water temperature has a significant relationship with call duration, pulse length, pause length and number of pulses, whilst background noise is significantly related to peak frequency (Schneider & Radwan, 1988). The other aims of this study will be to determine how significant variance of the individual environmental parameters is in influencing frog vocalizations, whether background noise can be used as an 'umbrella parameter' for other environmental parameters such as wind speed, and whether call characteristics can be used as an indicator of frog activity. My findings will provide a basis for future studies as I will have established which call characteristics are most useful as metrics for significant changes in frog vocalization, and how they relate to the environment.

2. Materials and methods

2.1 Location

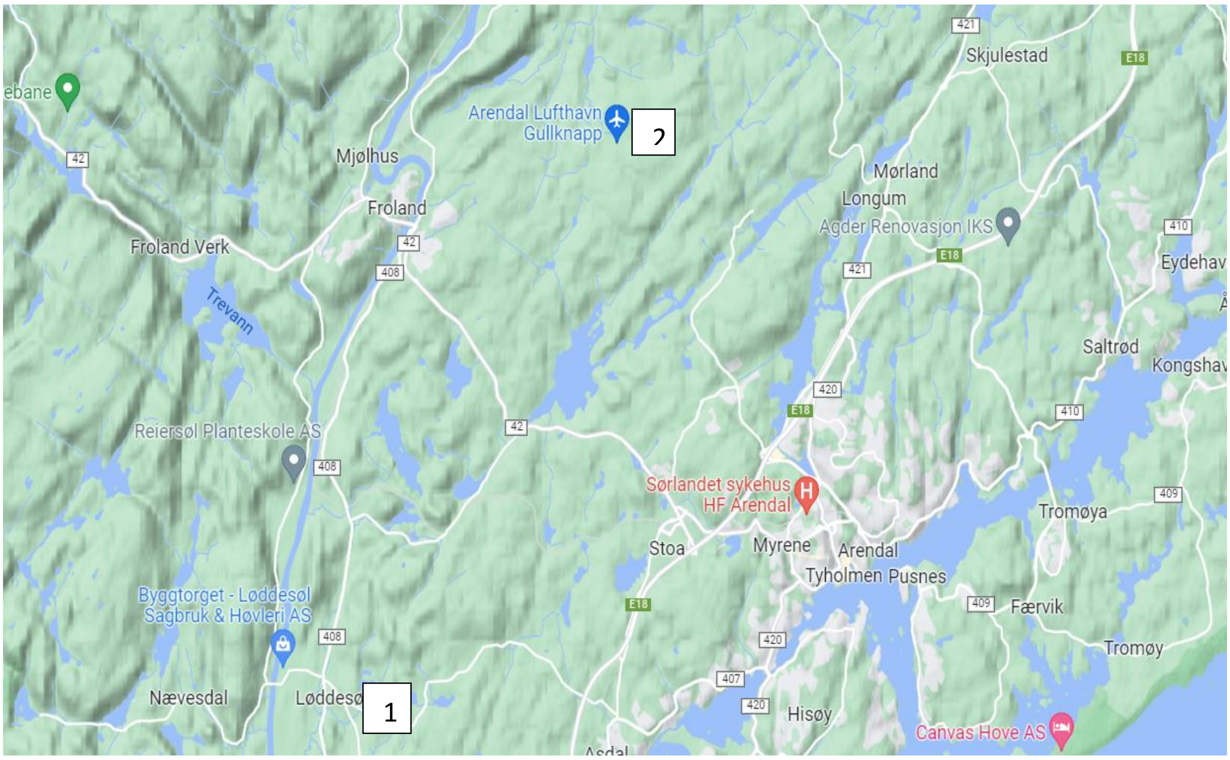

Figure 2- Topographical view of the Froland/ Løddesol areas. The 2 points of interest in this study are marked, with (1) being the approximate location of the study site and (2) the location of the weather station at Arendal Lufthavn.

Three potential locations in the Arendal area were considered suitable for investigating. However, it was decided upon that location 3 was ideal as it was more isolated from the main road, meaning there would be less background noise in the recordings, from outside sources such as passing cars (Figure 2). Furthermore, previous studies show that this was the location the with the highest population density, and therefore with the most data gathering potential (Engemyr & Reinkind, 2019). Since *P. lessonae* is critically endangered in Norway the exact position of this location will not be provided, according to the wish of the Norwegian Environment Agency (L. Korslund, personally communicated). The body of the pond encompasses around 40m and is surrounded by approximately 15 meters of peat bog on all sides, with the predominant flora being reeds (*Phragmites australis*) (Figure 3). The bog is surrounded by forest on all sides. The elevation of the area is approximately 100 meters above sea level.

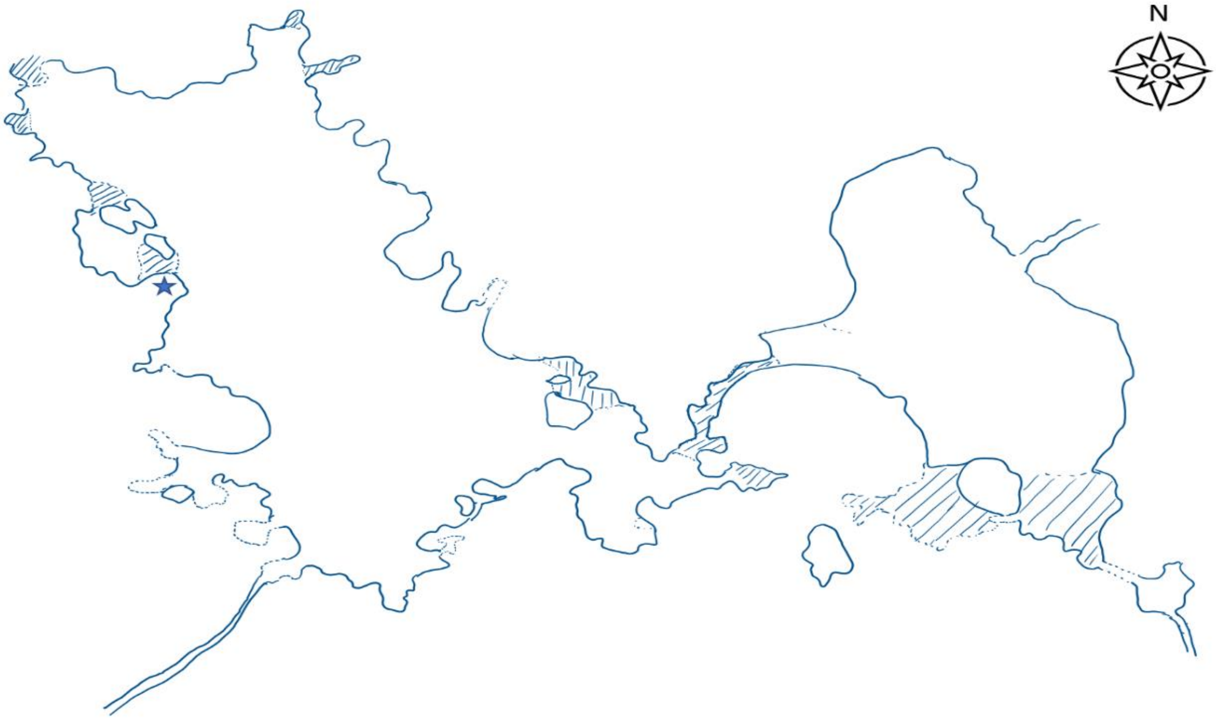

Figure 3- A sketch of the pool body at the study site. The location of the microphone is marked by the star symbol, whilst areas that are very shallow (0-10 cm), are hatched. Note that peat bog is not visualized in this diagram, and that there are two distinct segments of the pond.

The data on windspeed and precipitation was obtained from the nearest meteorological station, Arendal Lufthavn (2) , that could be found on Seklima.No, which was 14.4km from the study site (Figure 2).

2.2 Timeframe of study period

Water temperature readings were made hourly via a data logger (HOBO MX2203, Onset Computer Corporation, U.S.A.) at the determined location, from the 14/04/2021-17/09/2021.

A data logger and a microphone were set up at the study site well ahead of the expected beginning of the spawning season (14th April). Data collection was continuous from this point and stretched into the month of September, with the last day of the study period being 17/09/2021. However, this is not reflected in my data (see section 2.3).

There were long stretches of time during the recording period which lacked useful audio. This was due to a lack of discernable frog calls on these days, or, if there were frog calls recorded, they were of very low amplitude and thus unsuited for further analysis. This was because they proved indiscernible from the background noise when run through the R analysis (Figure 6). A sufficiently large dataset with audible, singular calls was obtained for the following days ;27/05/2021, 06/06/2021, 07/06/2021, 09/06/2021, 10/06/2021, 17/06/2021 (Figure 7).

For all days the audio recordings used were those from 13.00-15.00 (the peak period of day of frog call activity) and 01.00-03.00 (taken to assess how patterns in call characteristic data change between day and night, and whether there was a significant difference).

2.3 Data collection

Water temperature readings were made hourly via a data logger (HOBO MX2203, Onset Computer Corporation, U.S.A.) at the determined location. The data logger was placed in shallow water, inside a perforated white plastic tube to reduce the heating effect of solar irradiation, close to the microphone at the edge of the pond (Figure 4).

Figure 4-The placement of the data logger at the study site. It was placed in an area of the pond with an expected high volume of calls, as indicated by the number of frogs observed.

A passive acoustic recorder (SM4, Wildlife Acoustics Inc. U.S.A) with an external SMM-A2 Acoustic Microphones (Wildlife Acoustics Inc.) was set up close to the pond, attempting to cover as much of the sound coming from the pond as possible (Figure 5). These recorded continuously, but split into 1-minute files, over the entire period. The sample rate of the audio was 24000Hz, and recordings were done in mono (only one audio channel). The batteries of the recorder were replaced, and the data downloaded, on a bi-weekly basis.

Figure 5- Example of the passive acoustic recorder used for recording audio at the study site.

An important aspect of this study was establishing which days of the season the analysis should be based on, as it was untenable to isolate calls and conduct analysis for the entire spawning period. The days were chosen based on when the greatest fluctuation in water temperatures was, during the known spawning season, were recorded. In other words, the days with the greatest lowest and highest water temperatures were selected to ensure that there would be sufficient variation in the primary environmental variable of interest. With water temperature being established at the benchmark variable, readings for precipitation and average wind speed per day, on the chosen days, were obtained from the weather station at Arendal Lufthavn , via The Norwegian Centre for Climate Services (www.seklima.net.no). This weather station was located 14.4 kilometers from the study site (Figure 2).

2.4 Isolating calls

The moment long audio files recordings obtain from the microphone were visually inspected using the Kaleidoscope software, and all distinguishable calls were isolated from the recording and saved as their own wav file. The following conditions had to be met for the call to be of sufficient quality; the call file must have a start and an end visible in the spectrum, there must be no overlap with other frog calls, it must clearly be only produced by one individual, and it had to have a high enough amplitude to be distinguishable from the background noise (Figure 7). Furthermore, there must be no discernible medium frequency bird song (i.e., audible bird song that was within the 400-3000 Hz range) in the call file.

2.5 Sound analysis and statistics

R was used to run analysis of the isolated calls and determine their call characteristics (R Core Team. 2019). Sueur (2018) provided a very useful guide to understanding the basics of bioacoustics analysis using R.

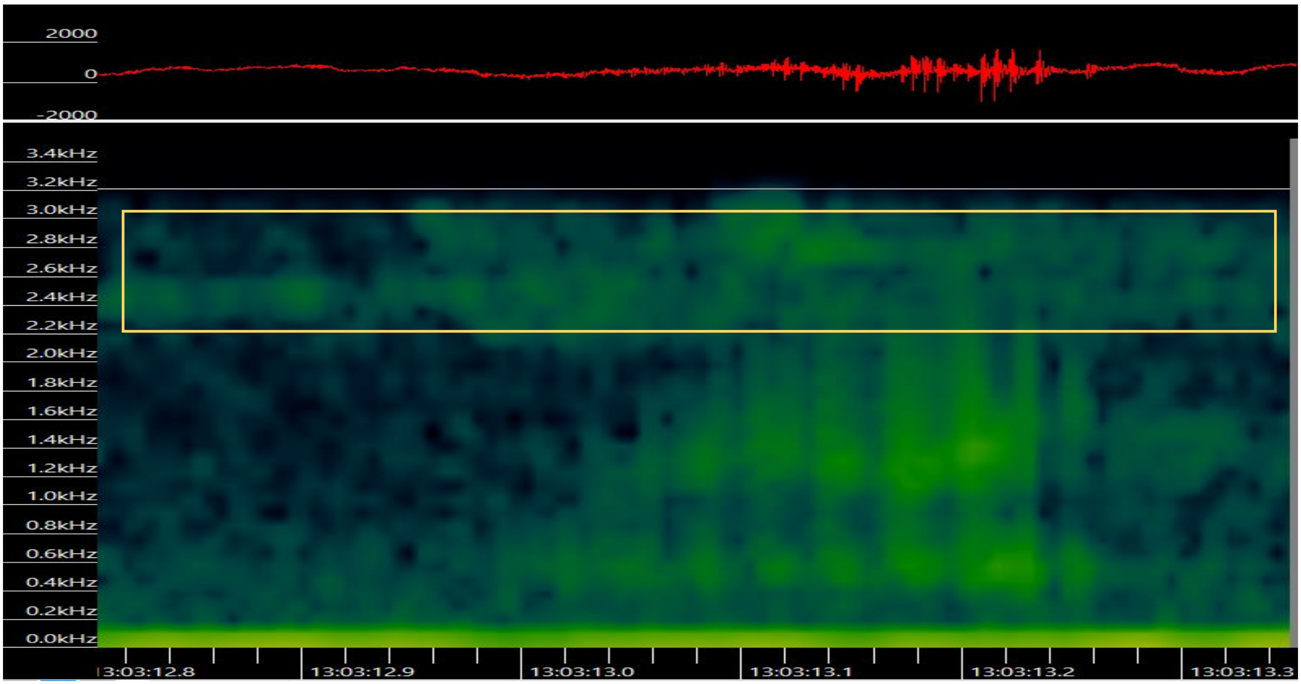

Figure 6- Example of a poor-quality call file. It is very difficult to ascertain the start and the end of the call, and the amplitude of the few pulses that are present is very low. High frequency bird calls are clearly visible in the spectrum which will affect peak frequency results, as represented by the yellow rectangle (spectrum obtained from Kaleidoscope).

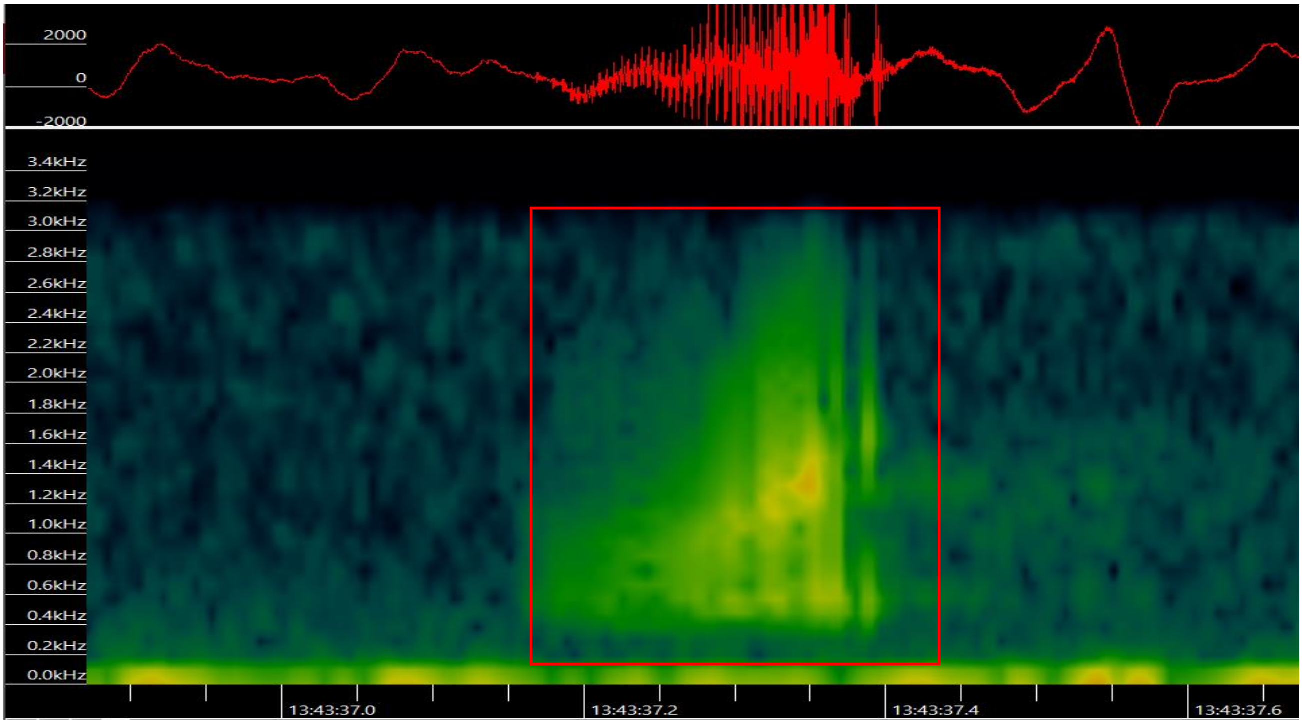

Figure 7- Example of a high-quality call file. There is a distinct start and end to the call (represented by the red rectangle), with visible pulse groups, in relation to an otherwise very low frequency spectrum. Bandpass filter is represented by the frequency range encompassed by the red rectangle. There is no visible bird song in the spectrum. There also sufficient space between the end of the call and the end of the sound file, allowing R to clearly identify the entire call (spectrum obtained from Kaleidoscope).

Using the Seewave and tuneR packages, various levels of smooth functions, bandpass filters and window length visualisation settings were tested on the single call audio files and the subsequent spectrograms examined (Sueur et al, 2008), (Ligges et al, 2018). This was done to determine which parameters were best for producing reliable outputs in the batch processing of all single call files. The methodology which consistently produced outputs of highest quality, and therefore was adopted for the batch audio analysis of all calls, was as follows; a bandpass filter of 400-3000hz was applied to all single call files, to remove low frequency background noise and potential high frequency sounds originating from sources such as bird calls. Peak frequency value was detected by first creating a spectrogram with a window length (wl) of 350, and the fpeaks function was used to extract the highest frequency peak (Sueur,2018). To allow for detection of call length, pulse length, pause length, number of pulses, number of pulse groups, and average number of pulses per group, a timer2 function was used at points when call duration and pulse detection were exhibited. A moving average sliding window (msmooth) of 50 was applied to the timer2 function to create a smoother amplitude envelope and allow for improved pattern visualization and pulse detection, whilst attempting to minimize the impact this had on the original spectra (Sueur,2018). Any calls files which did still not produce useful spectra were discarded, as they were deemed of poor quality. Following this, call length, pulse length, pause length, number of pulses, number of groups, and average number of pulses per group were calculated by creating functions based on the position and number of the detected pulses (see Figure 1 for an example of an oscillogram of a call where pulse groups with pulses can be identified).

This R analysis produced output for the following characteristics: call length, pulse length, pause length, number of pulses, number of pulse groups, average number of pulses per group and peak frequency. The call characteristics pause length consisted of the mean value of all the pauses detected within the call (Figure 1). However, the results for number of pulse groups and number of pulses were deemed unreliable and were thus discarded (See section 4.5).

To obtain a measure of the overall background noise during a call the minute long audio recordings that the isolated call swas taken from were analysed, The relative mean amplitude of the envelope of each minute long audio file was produced, once the band pass filter of (<400Hz), had been applied to each file (Sueur, 2018).

Extreme outliers of any of the call characteristics in the dataset were identified by plotting and visual inspection. Call files were once again visually inspected in the Kaleidoscope software to establish if the value represented a true characteristic of the call or if persistent background noise or other issues with the call file was the cause of the issue. If the latter was the case, the call file was removed from the dataset.

To account for the predictor variables having a mean and variance of different magnitudes, all predictor variables were scaled, and due to a clear positive skew, all response variables they were log transformed for regression analysis. Coefficient of variance was used to compare dispersion around the mean for the environmental variables, regardless of their scale. CoV was calculated by dividing the standard deviation of the call characteristic (for all data) by the mean of the call characteristic. Higher CoV indicates greater dispersion around the mean, with provides a useful aid for determining which call characteristics have the greatest variation and would therefore be useful in bioacoustics analysis, whilst also accounting for differing scales (Hashim et al., 2021).

Pearsons correlation was calculated between the three environmental variables; water temperature, background noise and averaged windspeed per day, in order to test for co variance(Appendix 1), (Appendix 2). Average windspeed per day was not used as a predictor variable in the regression models, as I wished to determine whether background noise can be used as an indicator of average wind speed when testing environmental parameters.

Regression models were used to assess potential relationships between each call characteristic and the environmental parameters. All possible combination of models were tested for each call characteristic, with the goal being to determine which environmental parameters best explained the variation of the response variable. Four different regression models were made for each predictor variable: the multiple regression using both predictors, a model with water temperature, a model with background noise, and a null model (no predictor variables).

The competing models were then compared using the AIC() function (R Core Team. 2019). This produced an output of an Akaike information criterion (AIC) value for each of the competing models which allowed for comparison between them. The model with the lowest AIC was deemed of the highest quality and the best model for explaining the spread of the data for the response variable. Visualising the regression models and their respective p values allowed me to determine whether the predictor variables had a statistically significant relationship on the response variable.

Finally, a two tailed t-test was done between the calls recorded during the day and those recorded at night, to test for differences in call characteristics between night and day

3. Results.

3.1 Overview of environmental data

Dates are presented as follows in the following section. 27/05/2021= day 1, 06/06/2021= day 2, 07/06/2021= day 3, 09/06/2021= day 4, 10/06/2021= day 5, 17/06/2021= day 6.

The highest peak water temperature was recorded on day 5 at 20.4 °C. The greatest water temperature difference between days was day 5 and day 1, which had a maximum water temperature of 14.5°C (Figure 8). However, water temperature remained relatively stable throughout the month of June, with the highest average temperatures being on days 3 and 5 (Figure 8). Water temperature remained relatively stable within the days themselves with the largest recorded water temperature difference within a day being 4.6 °C. As expected, water temperature and background noise were higher during the day. Median water temperature during day and night were quite similar, however there was a greater difference between max recorded temperature. A higher range of temperatures was observed during the day.

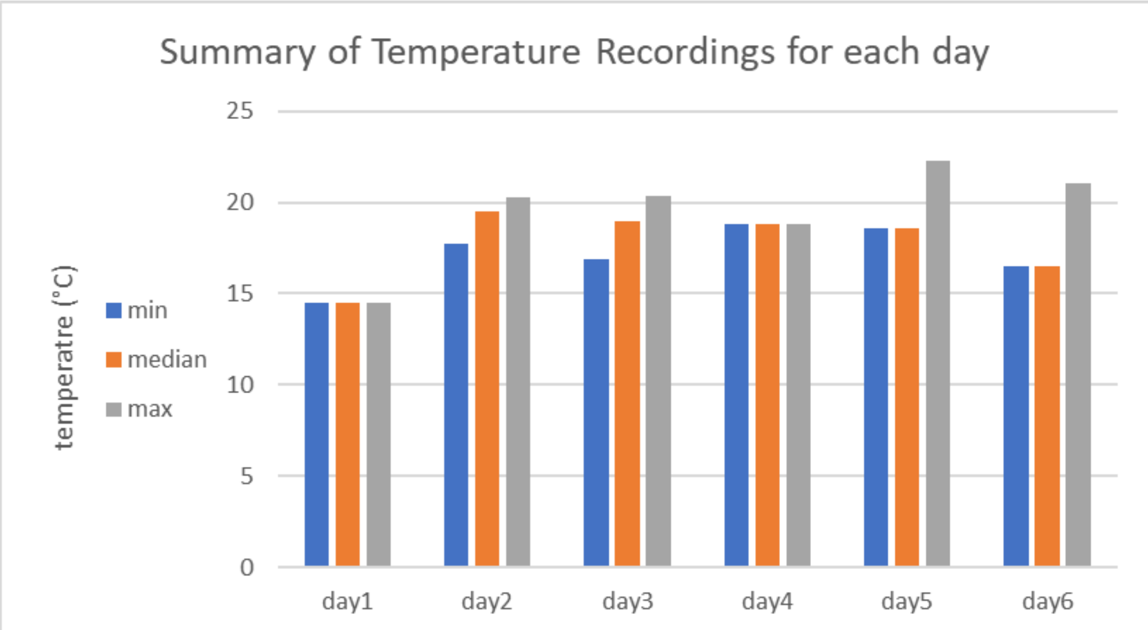

Figure 8-Mean, max and median water temperature recordings for each day. Day 1= 27/05/2021, day 2= 6/6/2021, etc.

Maximum background noise recorded for each day remained relatively stable, with the exception of day 1, where it was noticeably lower at 4.4 (Figure 9). The highest maximum background noise was on day 4, at 5.1 (Figure 9). Average background noise was highest on day 1 (4.4) and lowest on day 4 (3.5) (Figure 9). There is a pattern of background noise varying to a greater extent within each day rather than between days. The range of background noise values during the day and night are very similar, however the median background noise is higher during the day, as is the max background noise recorded. Compared to the background noise of randomly selected days of the season, it was clear that the background noise levels of the 6 days that the study focuses on was in general representative of the pattern for the whole season.

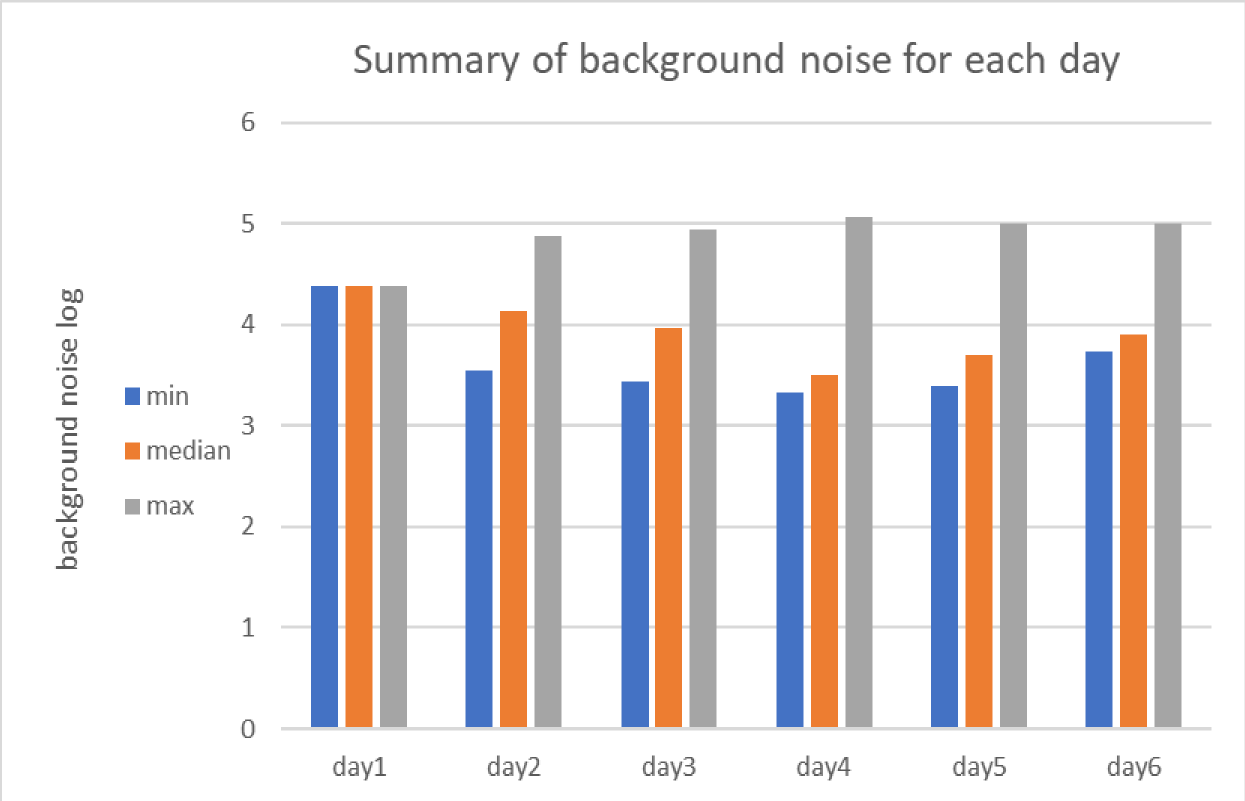

Figure 9- Mean, max and median background noise measurements for each day. Day 1= 27/05/2021, day 2= 6/6/2021, etc.

The assertion that water temperature varied less than background noise across the entire recorded period is not only visually represented in the Figures 8 and 9, but is also supported by the coefficient of variance values (Table 1). Water temperature was the lowest coefficient of variance value at (0.052, Table 1) lower than the background noise coefficient of variance value (0.097, Table 1). Coefficient of variance is of limited use and thus will not be used for interpretation but rather merely to provide a generalized overview of variance within the environmental variables.

Table 1- Coefficient of variance for water temperature and background noise. Calculated by dividing the standard deviation by the

Environmental variable	Normalized sd/coefficient of variance
Temperature	0.052
Background noise	0.097

3.2 Correlation between environmental variables

Pearson's correlation revealed that there was a statistically significant positive relationship between water temperature and background noise (Figure 10, r=.40, p < .001), (Appendix 2). There was also a significant negative relationship between water temperature and average wind speed per day (Figure 11, r= -.29, p < .001), (Appendix 2) and between background noise and average windspeed per day (Figure 12, r= -.41, p < .001). Whilst there is no evidence of very close correlation (i.e., r=1), background noise and average windspeed per day co vary the most (Figure 12), (Appendix 1).

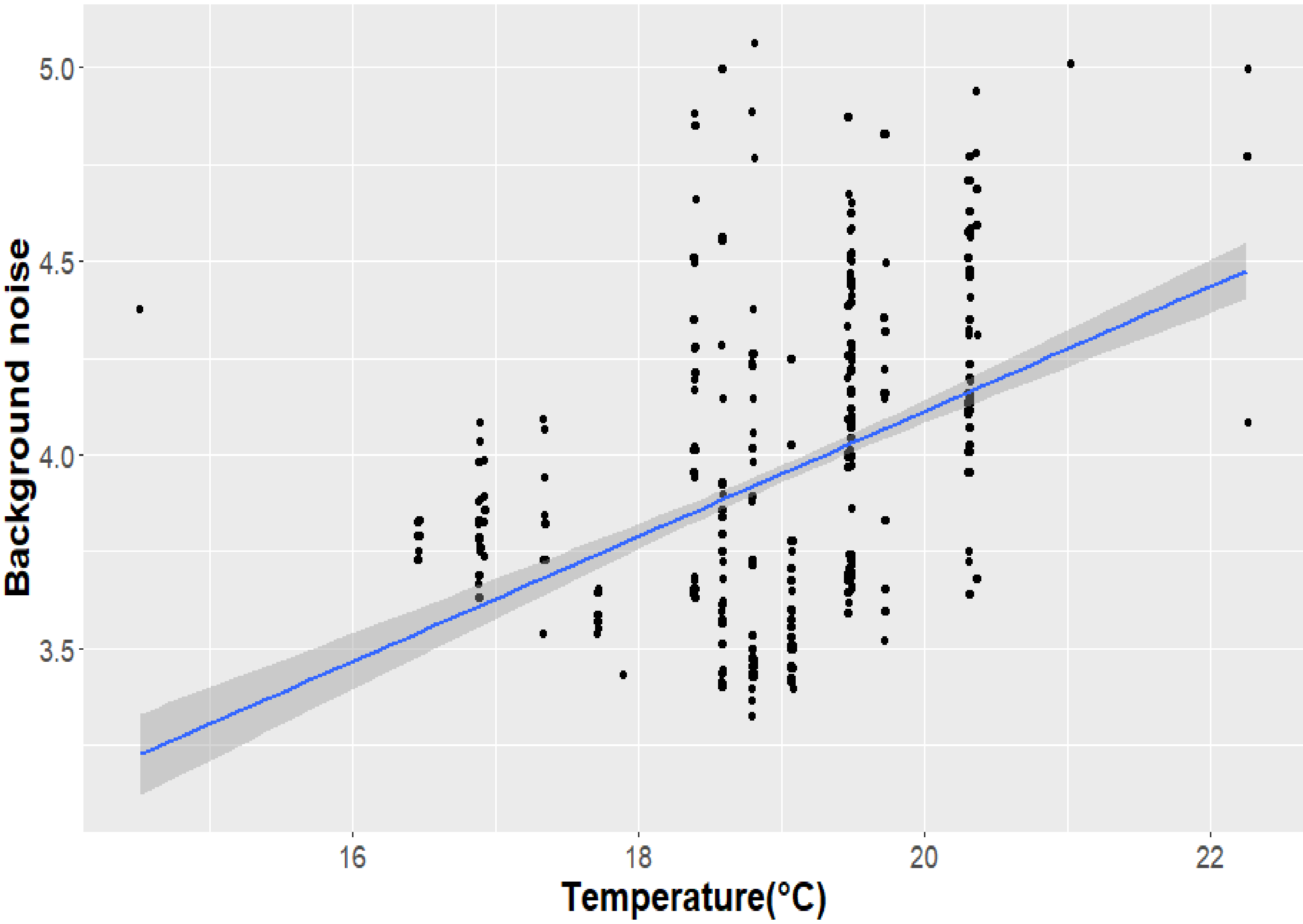

Figure 10- Scatterplot for water temperature vs background noise data (data from all days), with residuals and regression line plotted.

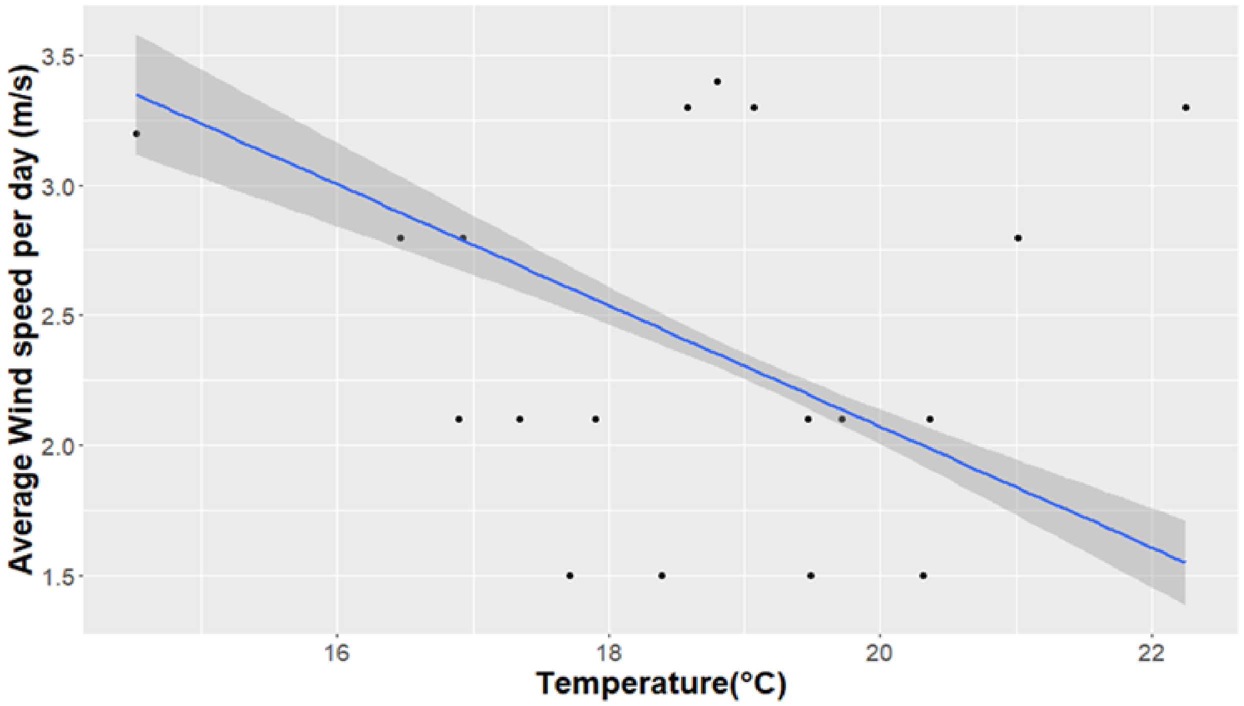

Figure 11- Scatterplot of water temperature vs average wind speed per day (data for all days), with the residuals and regression line plotted. Data points for average wind speed were recorded on a daily basis, whilst data for temperature was hourly, hence the low number of data points for wind speed (see section 4.5). .

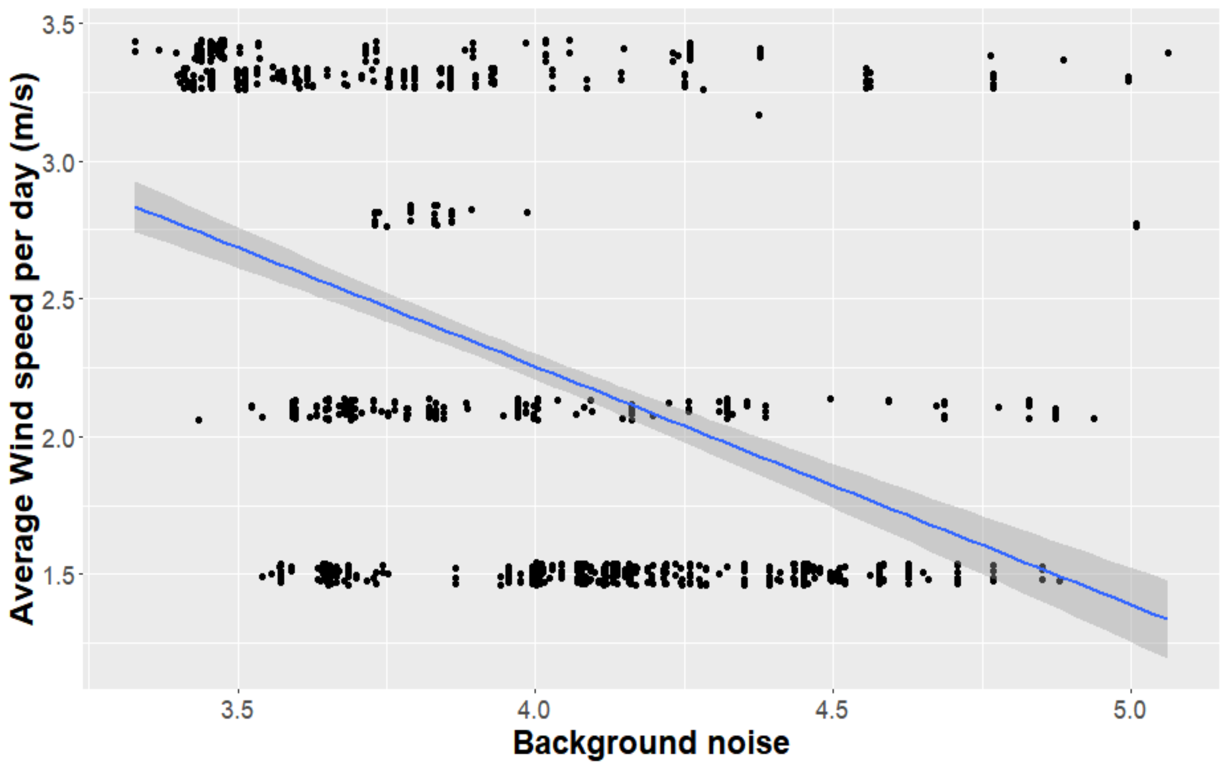

Figure 12- Scatterplot for background noise vs average wind speed per day (data for all days). Data points for average wind speed were recorded daily, whilst data for background noise was based on the minute long audio files that contained the isolated calls, hence the low number of data points for wind speed (see section 4.5).

Median water temperature during day and night were quite similar, however there was a greater difference between max recorded temperature (Figure 13). A higher range of temperatures was observed during the day (Figure 13). The range of background noise values during the day and night are very similar, however the median background noise is higher during the day, as is the max background noise recorded (Figure 13). Two tailed t test confirms that there is a very significant difference in both water temperature values (Table 2, $p < .001$), and background noise values (Table 2, $p < .001$), during the day vs during the night.

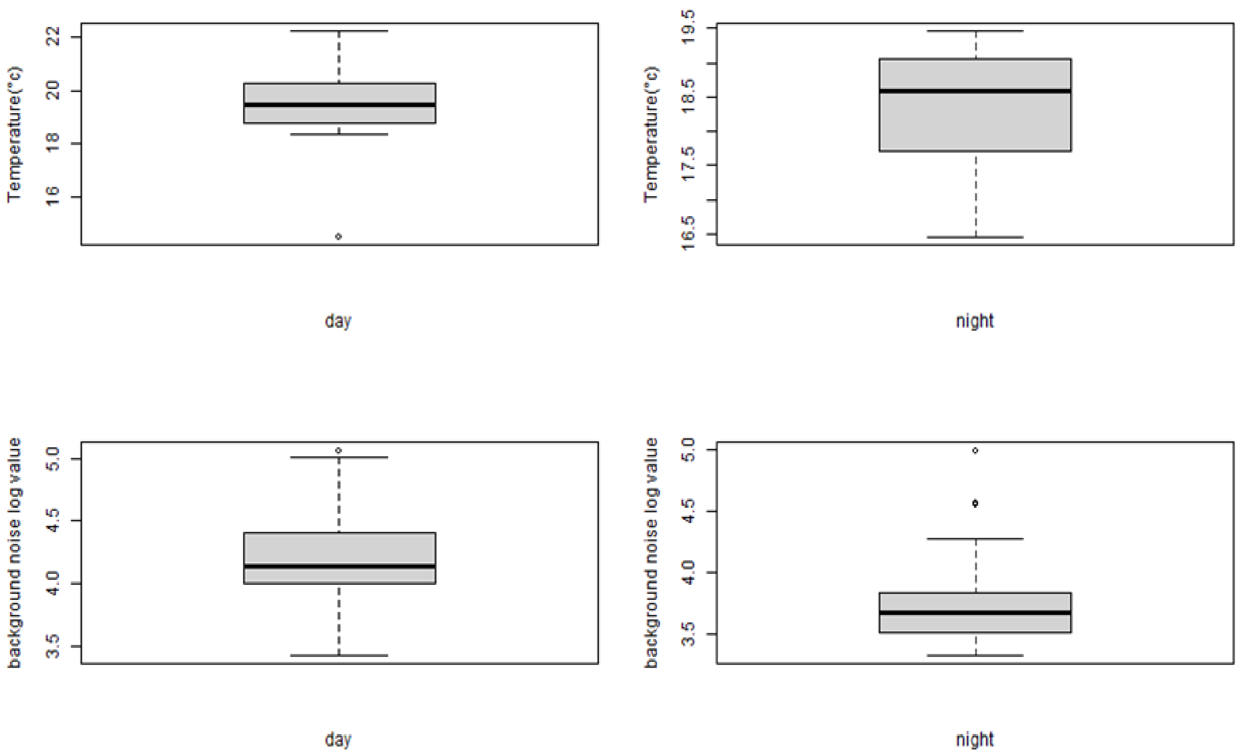

Figure 13- Boxplots of data for temperature and background noise, separated into day and night.

Table 2- P value and t value for all variables, when unpaired t test was done for values during the day vs values at night. Significant p values are in bold.

	Temperature	Background noise	Call length	Pulse length	Pause length	Number of pulses	Peak frequency
p value	**p < .001**	**p < .001**	**p < .001**	0.928	**p < .001**	0.1	0.574
t value	20.24	21.99	-9.62	-0.09	-15.53	1.65	-0.56

3.3 Regression modelling for response variables

When comparing AIC values, the model with that lowest AIC value is considered the best fit for the variance in data. However, parsimony must also be accounted for i.e., establishing a compromise between the explanatory power of the model and its simplicity. Therefore, if the AIC output differs by less than two units, the one with the fewest degrees of freedom is considered the best model. The use of the term temperature in all figures and tables below refers to water temperature.

3.3.1 Call length

AIC output suggests that the linear regression model incorporating background noise is the most parsimonious (Table 3). Call length had a significant negative relationship with water temperature (Figure 15, β= -0.078 ±0.02, $p < .001$ and an even greater significant negative relationship with background noise (Figure 16, β=-0.127±0.02, p= $p < .001$). Effect size is represented in Figure 14, which supports the assertion that call length has a stronger relationship with background noise. Multiple R- squared (0.06) and adjusted R- squared (0.06) are very similar indicating that the model is not overfit. However, these very low r squared values suggests that this model has low ability in explaining the variation in call length, despite it being the best model.

Table 3- AIC output and degrees of freedom for each regression model. Model in bold is the most parsimonious.

Model	df	AIC
Temperature + Noise	4	1462.1
Temperature	3	1497.1
Noise	3	**1462.7**
Null model	2	1515.3

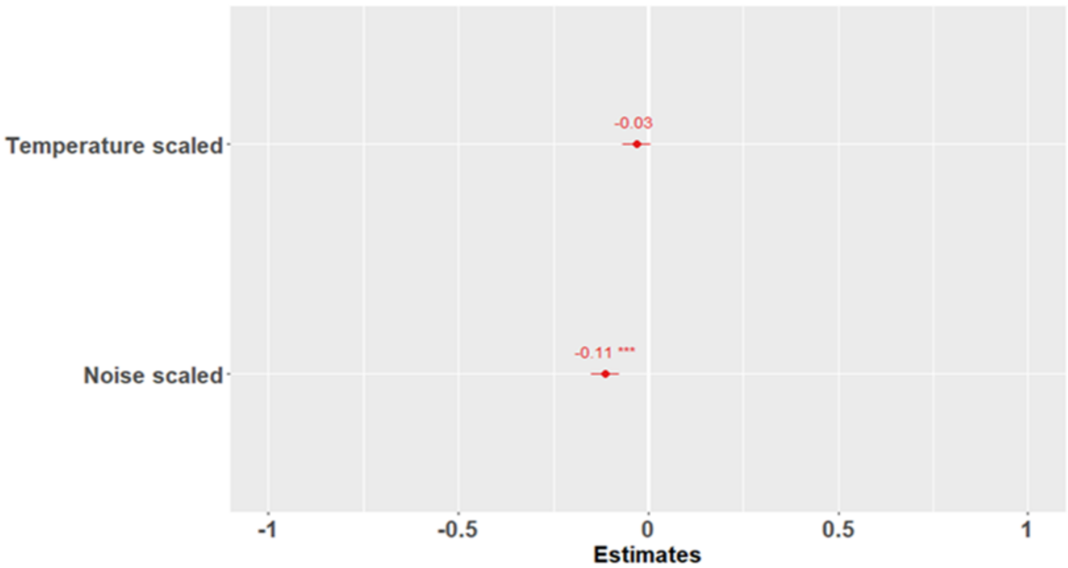

Figure 14- Effect model for the multiple regression model, visualizing the effects of the environmental predictors on call length. Water temperature and noise are scaled, and call length converted to its log form, to allow for easier comparison of effect size.

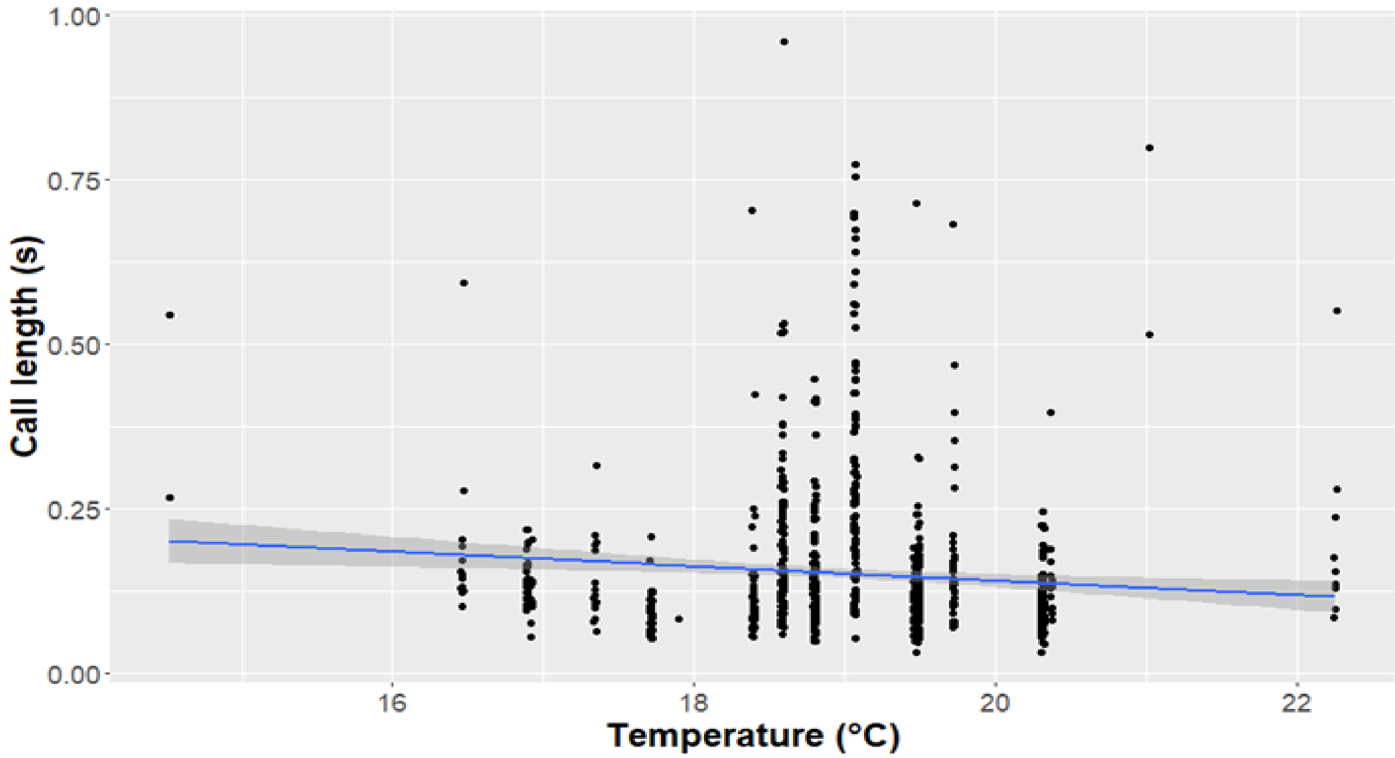

Figure 15- Scatterplot for call length against water temperature, with the regression line plotted. There is a high level of variation along the regression line, as reflected in the R- squared value.

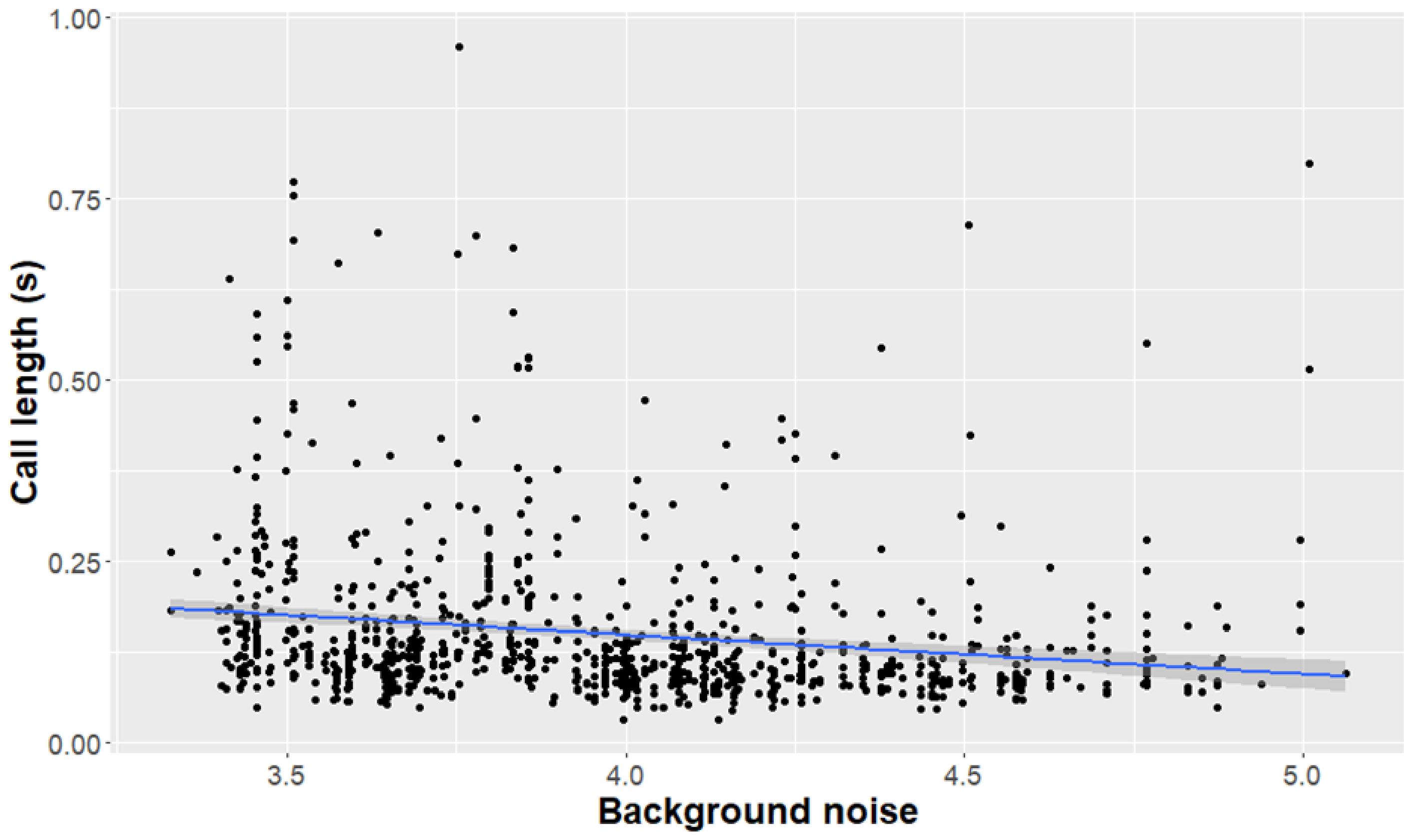

Figure 16- Scatterplot for call length against background noise, with the regression line plotted. There is a high level of variation along the regression line, as reflected in the R- squared value.

3.3.2 Pulse length

AIC output suggests that the most parsimonious model for explaining pulse length variation is the one incorporating water temperature (Table 4). Pulse length had a significant negative relationship with water temperature (Figure 18, β= -0.016±0.01, p= 0.013). However, pulse length did not have a significant relationship with background noise (Figure 19, β= 0.003±0.01, p= 0.693). Figure 17 visualizes the very small effect size of background noise. Similar values for multiple r squared (0.007) and adjusted R squared (0.005) indicate that the model is not overfit. However, these very low R squared values indicate that the best model still has a low ability to explain the variation of pulse length data. The null model is more parsimonious than the model incorporating noise, supporting the assertion that noise has no effect on pulse length (Table 4).

Table 4- AIC output and degrees of freedom for each regression model. Model in bold is the most parsimonious.

Model	df	AIC
Temperature + Noise	4	-345.0
Temperature	**3**	**-344.5**
Noise	3	-338.5
Null model	2	-340.4

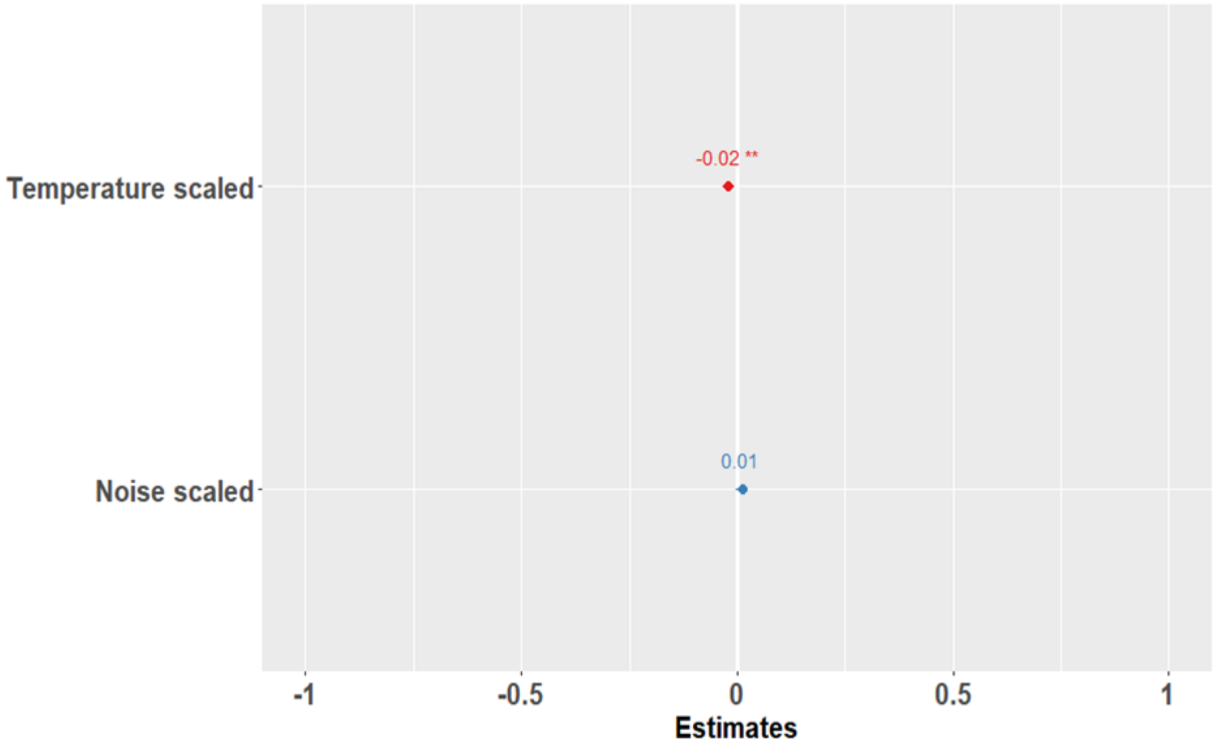

Figure 17- Effect model for the multiple regression model, visualizing the effects of the environmental predictors on pulse length. Water temperature and noise are scaled, and pulse length converted to its log form, to allow for easier comparison of effect size.

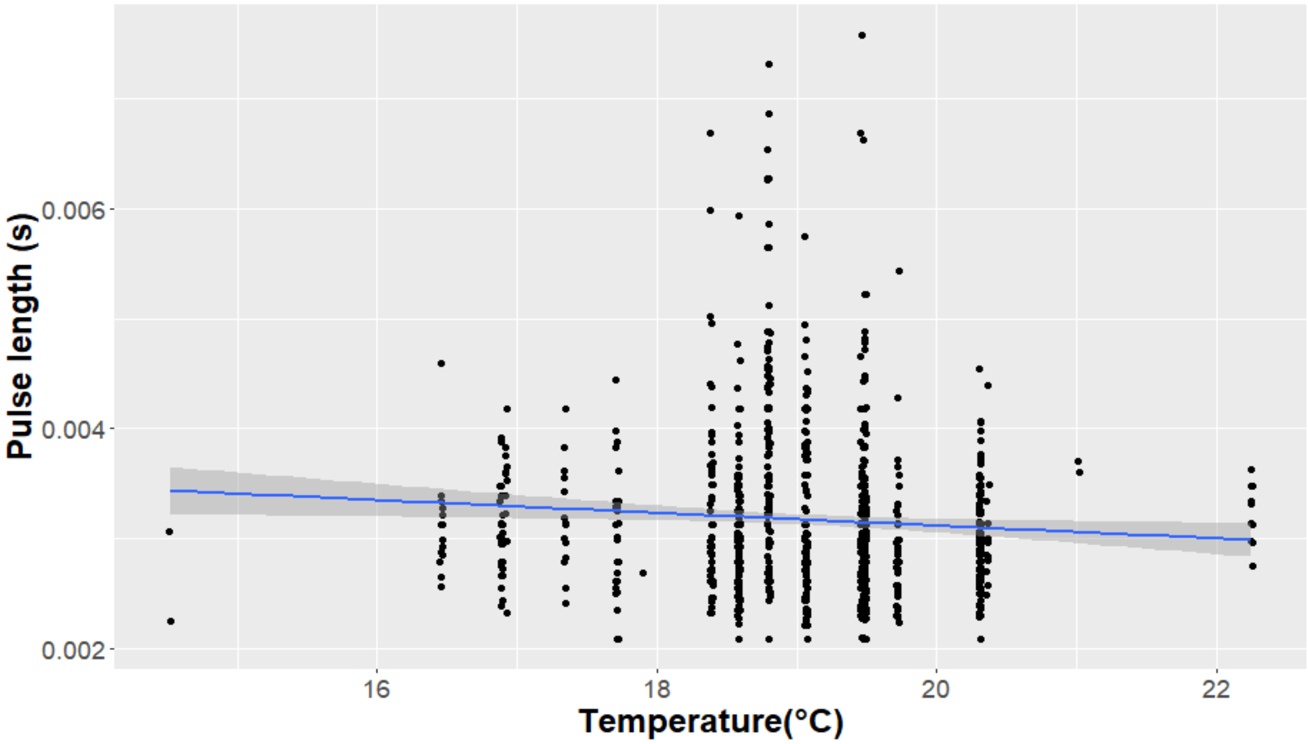

Figure 18- Scatterplot for pulse length against water temperature, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

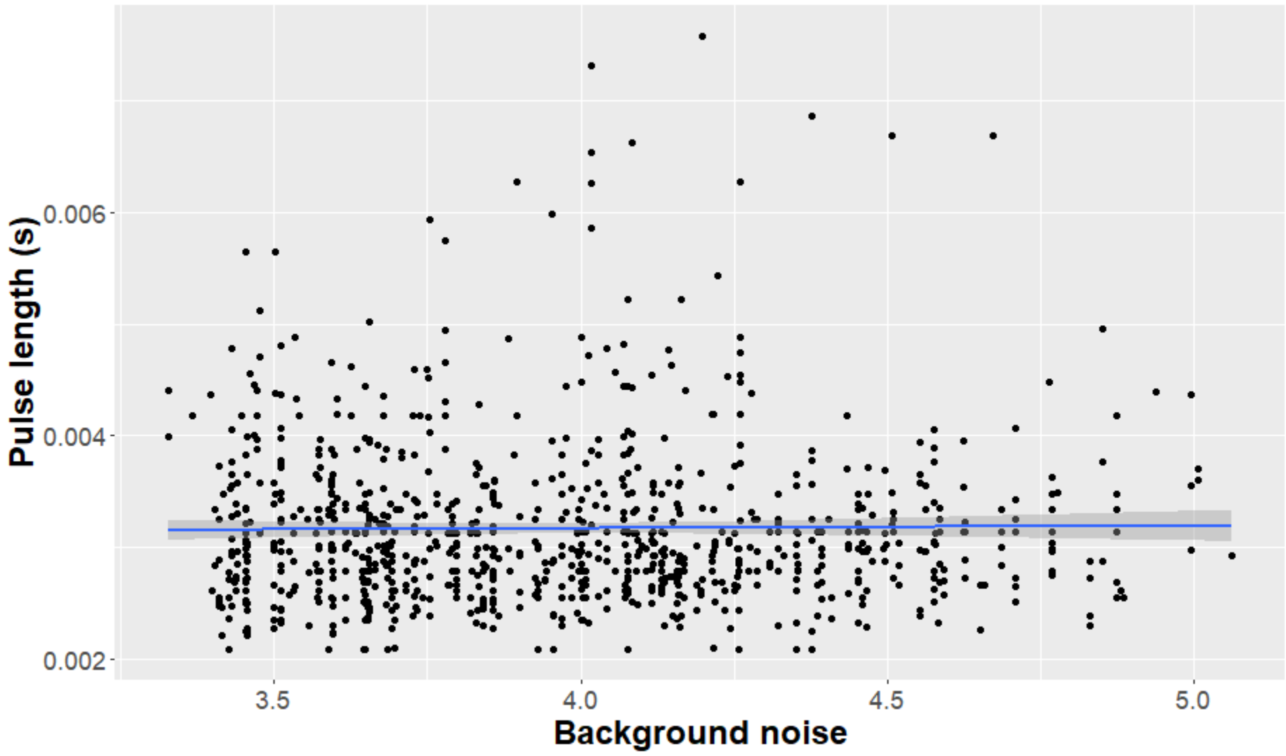

Figure 19- Scatterplot for pulse length against background noise, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

3.3.3 Pause length

AIC output confidently suggests that the best model for explaining pause length variation is the multiple regression model using both water temperature and noise (Table 5). Pause length had a significant negative relationship with water temperature (Figure 21, β= -0.145$\pm$ 0.02, $p < .001$), and an even greater significant negative relationship with background noise (Figure 22, β=-0.175 $\pm$0.02, $p < .001$). Effect sizes of both predictor variables on pause length were large (Figure 20). Multiple R-squared (0.116), and adjusted R-squared are very similar (0.114), meaning the model is likely not overfit. Whilst these R- squared values are still low, they are relatively high when compared to my other multiple regression models . Therefore, while the model pause length ~temperature +noise has a low ability to explain pause length variation, it has the highest ability to explain data variation for its respective response variable, of all the multiple regression models produced in my study.

Table 5- AIC output and degrees of freedom for each regression model. Model in bold is the most parsimonious.

Model	df	AIC
Temperature + Noise	**4**	**1500.7**
Temperature	3	1550.7
Noise	3	1519.7
Null model	2	1613.8

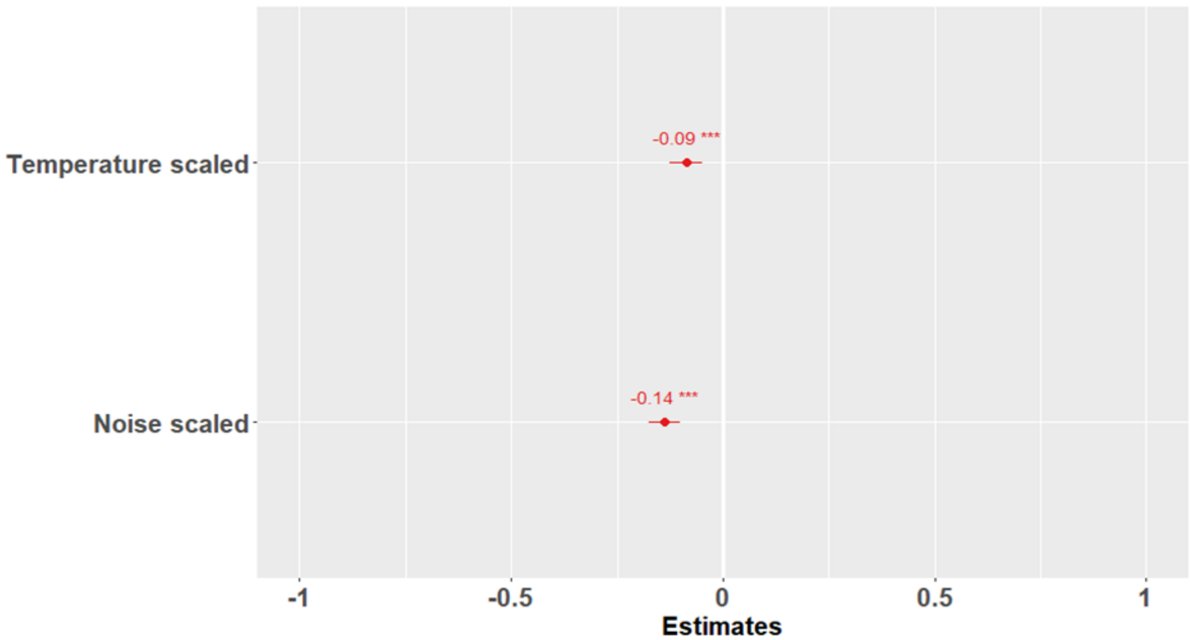

Figure 20- Effect model for the multiple regression model, visualizing the effects of the environmental predictors on pause length. Water temperature and noise are scaled, and pause length converted to its log form, to allow for easier comparison of effect size.

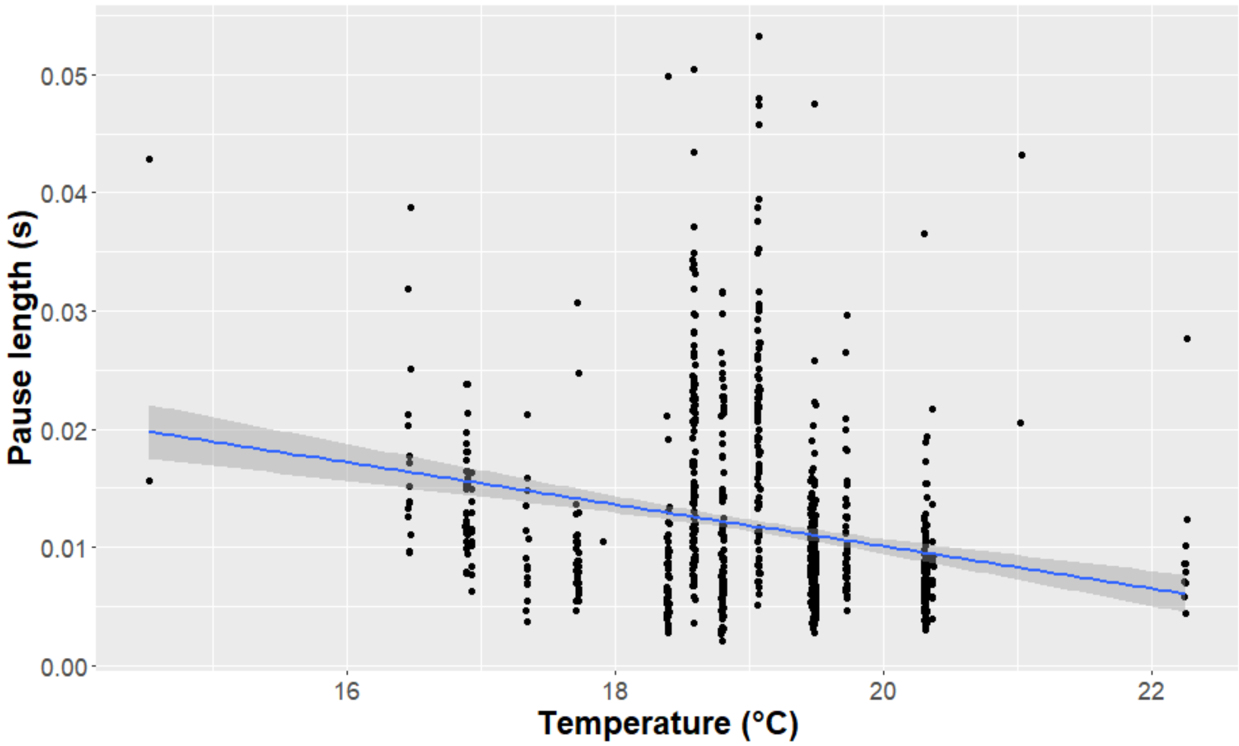

Figure 21- Scatterplot for pause length against water temperature, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

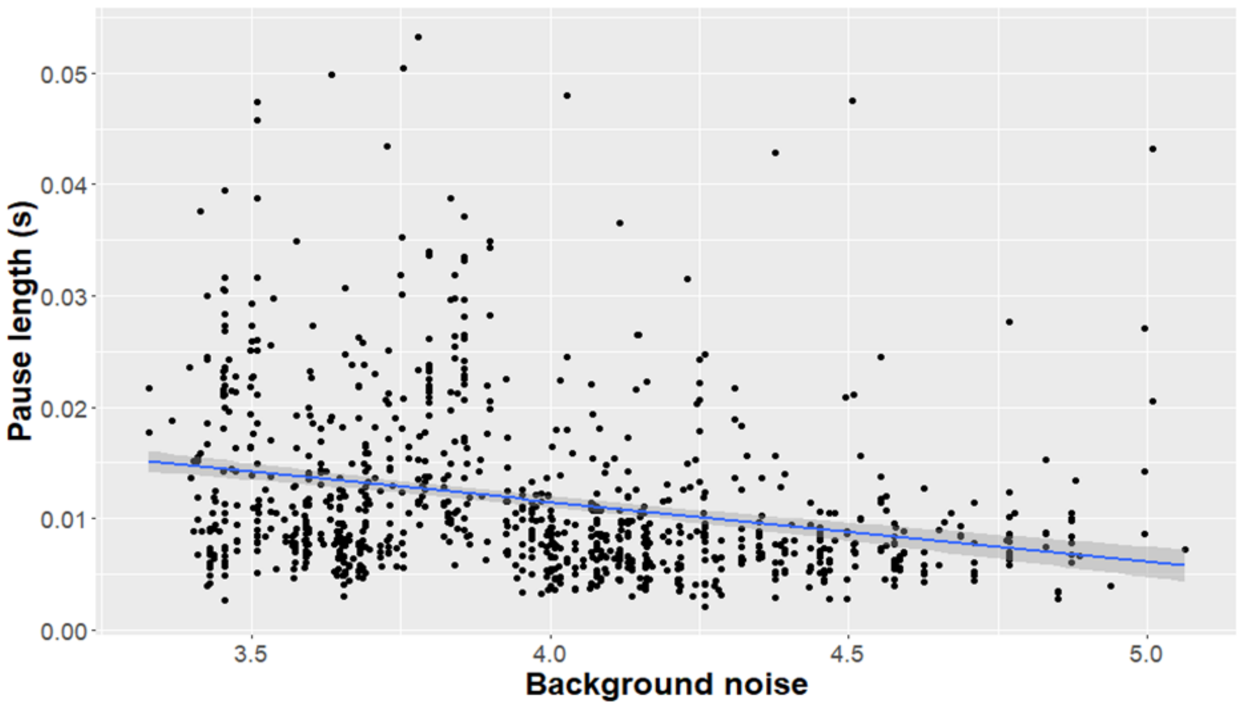

Figure 22- Scatterplot for pause length against background noise, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

3.3.4 Number of pulses

AIC output allows me to conclude that the most parsimonious model for explaining the variation in number of pulses is the one incorporating water temperature (Table 6). Number of pulses had a significant positive relationship with temperature (Figure 24, β= 0.034± 0.001 , p= 0.001). However, there is no significant relationship between number of pulses and background noise (Figure 25, β= 0.001±0.001, p= 0.900). The differing effect sizes of temperature and background noise on number of pulses are visualized in Figure 23. The multiple R- squared value (0.013) and adjusted R- squared value (0.012) are very similar for the regression model using temperature as an explanatory variable, suggesting it is not overfit. These low R- squared values suggest that the best model still has a low ability to explain the variation of the number of pulses. The fact that the null model is better than the model with background noise supports the notion that noise had no effect on number of pulses (Table 6).

Table 6- AIC output and degrees of freedom for each regression model. Model in bold is the most parsimonious.

Model	df	AIC
Temperature + Noise	4	428.2
Temperature	**3**	**428.4**
Noise	3	440.5
Null model	2	438.5

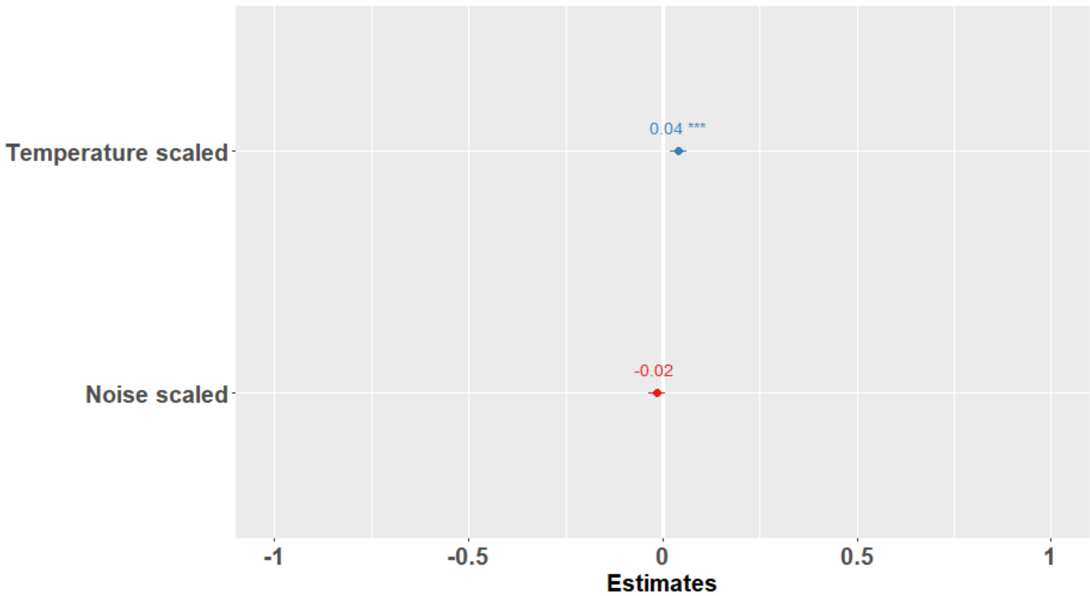

Figure 23- Effect model for the multiple regression model, visualizing the effects of the environmental predictors on the number of pulses. Water temperature and background noise are scaled, and number of pulses converted to its log form, to allow for easier comparison of effect size.

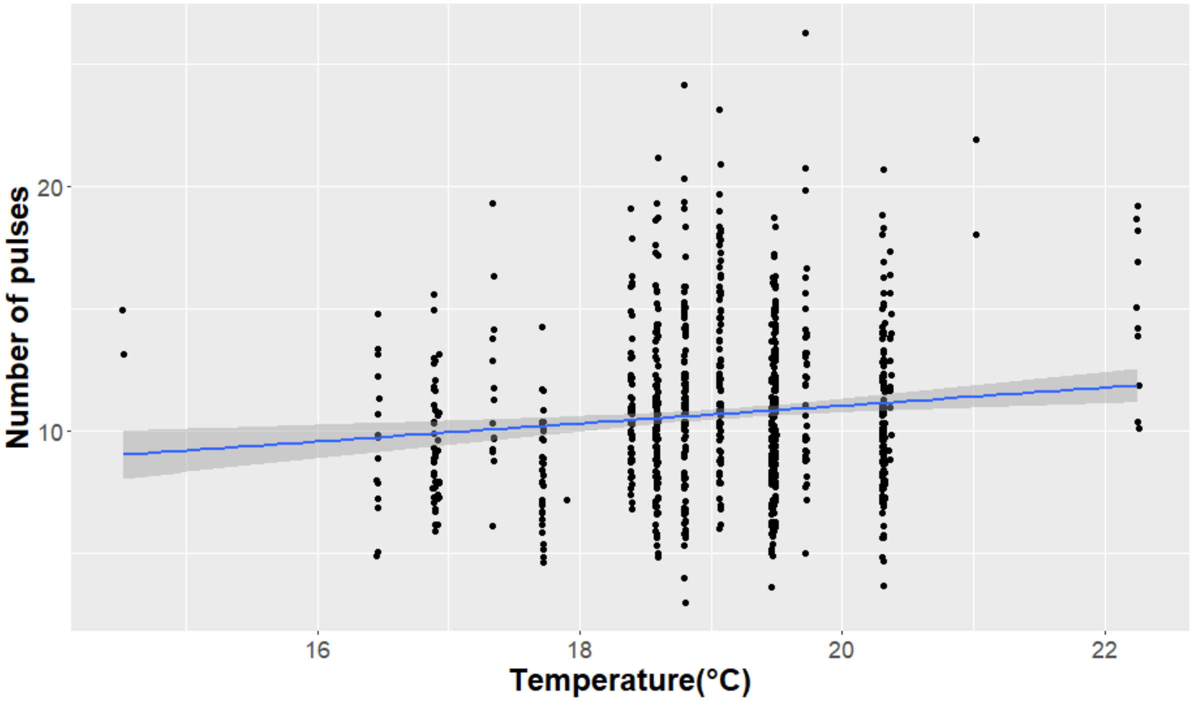

Figure 24- Scatterplot for number of pulses against water temperature, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

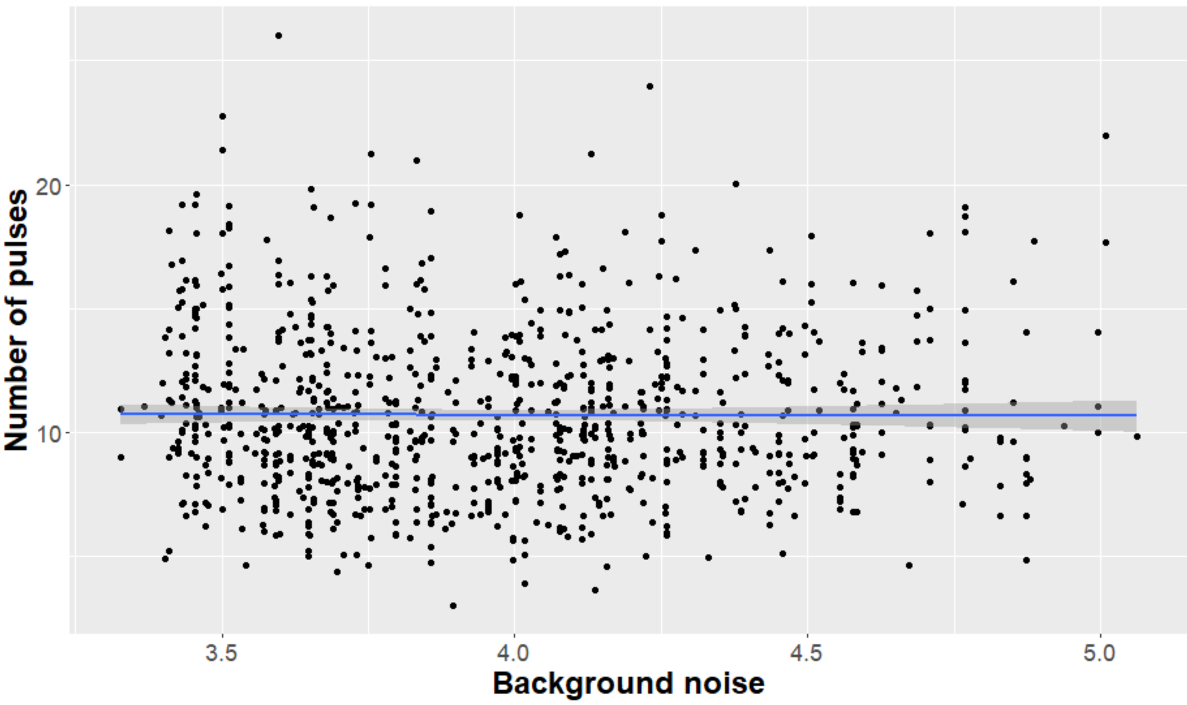

Figure 25- Scatterplot for number of pulses against background noise, with a plotted regression line. There is a high level of variation along the regression line, as reflected in the R- squared value.

3.3.5 Peak frequency

AIC output suggests the most parsimonious model for variation in peak frequency is the null model (Table 7). The p values support this, as no significant relationship was found between peak frequency and temperature (Figure 27, β= 0.019± 0.01, p= 0.128). Furthermore, no significant relationship was found between peak frequency and background noise (Figure 28, β= 0.016± 0.01, p= 0.184). The small and similar effect sizes of the predictor variables on peak frequency are visualized in Figure 26. As the null model is favoured here, no R-squared values were produced.

Table 7- AIC output and degrees of freedom for each regression model. Model in bold is the most parsimonious.

Model	df	AIC
Temperature + Noise	4	889.1
Temperature	3	888.2
Noise	3	887.7
Null model	**2**	**888.0**

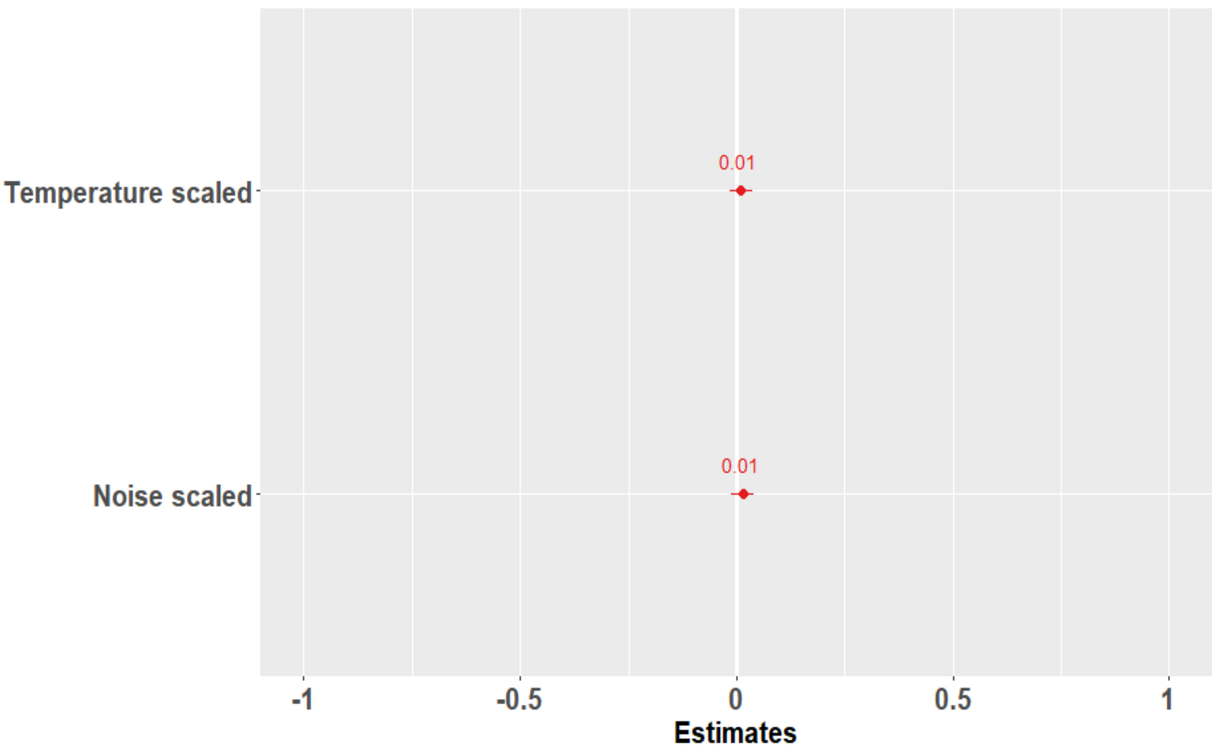

Figure 26- Effect model for the multiple regression model, visualizing the effects of the environmental predictors on the peak frequency. Water Temperature and background noise are scaled, and peak frequency converted to its log form, to allow for easier comparison of effect size.

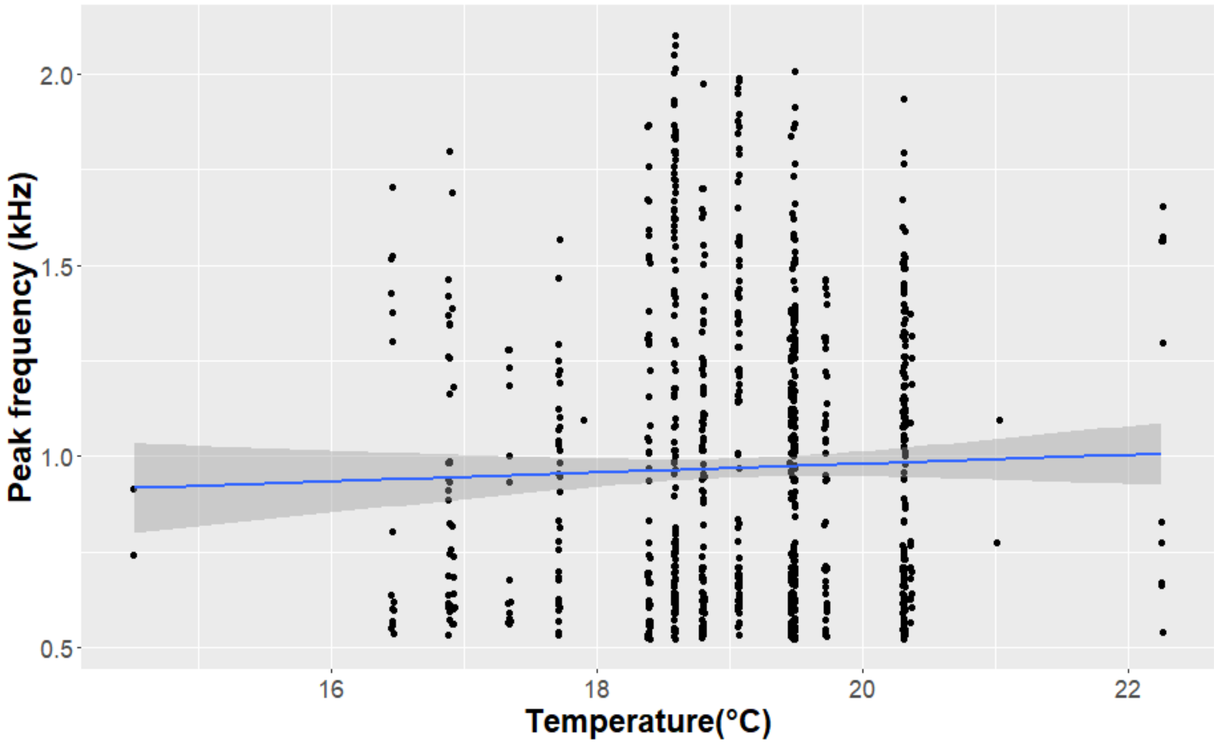

Figure 27- Scatterplot for peak frequency against water temperature, with a plotted regression line. There is a high level of variation along the regression line.

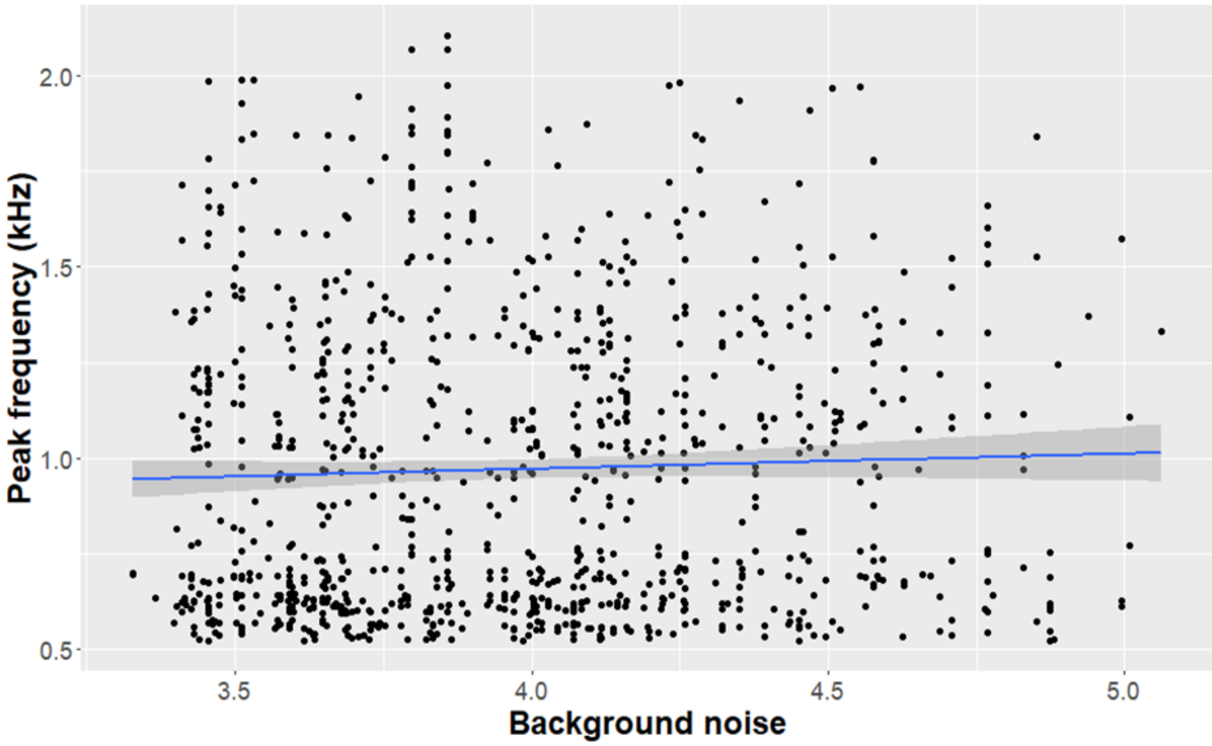

Figure 28- Scatterplot for peak frequency against background noise with a plotted regression line. There is a high level of variation along the regression line.

3.4 Night vs day

Two tail t test f for variables when comparing night and day reveal that there is a significant difference in the results for background, call length and pause length. The p value is most significant for pause length (Table 2 , p < .001), indicating that this is the characteristic with the largest change between night and day, with higher pause length at night.

Call length pattern during the night and during the day was very similar. However, data is more skewed towards longer calls at night, with calls at night being significantly longer than calls during the day (Figure 29), (Table2, p < .001). There was a greater range of pulse lengths during the day, along with overall higher pulse length (Figure 29). However, the difference in pulse length between night and day is not significant (Table 2, p= 0.929).

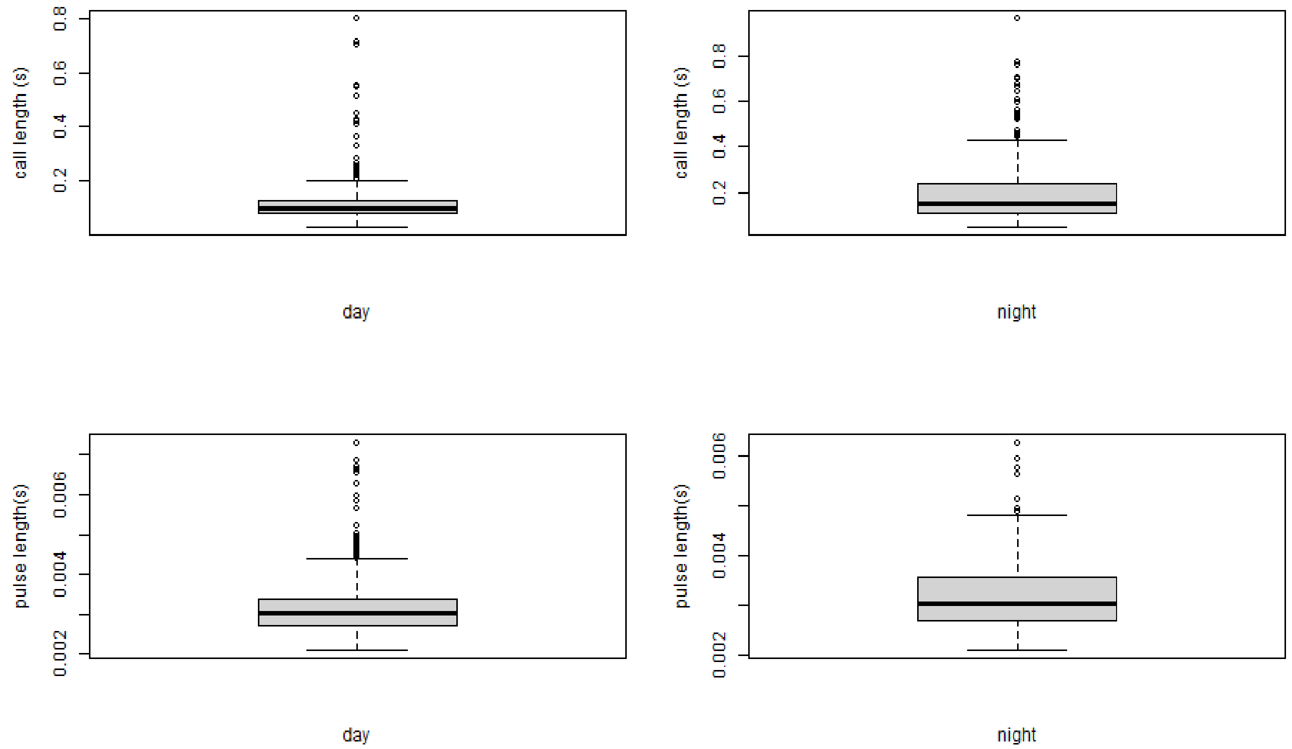

Figure 29- Boxplots of data for call length and pulse length, separated into day and night.

The upper quartile for pause length was far higher at night, and data seems far more skewed towards lower values during the day (Figure 30). There is a significant difference between pause length data during the day than during the night (Table 2, p=0.100). The data for the number of pulses remains similar from day to night, as reflected in the t test (Table 2, p=0.100).

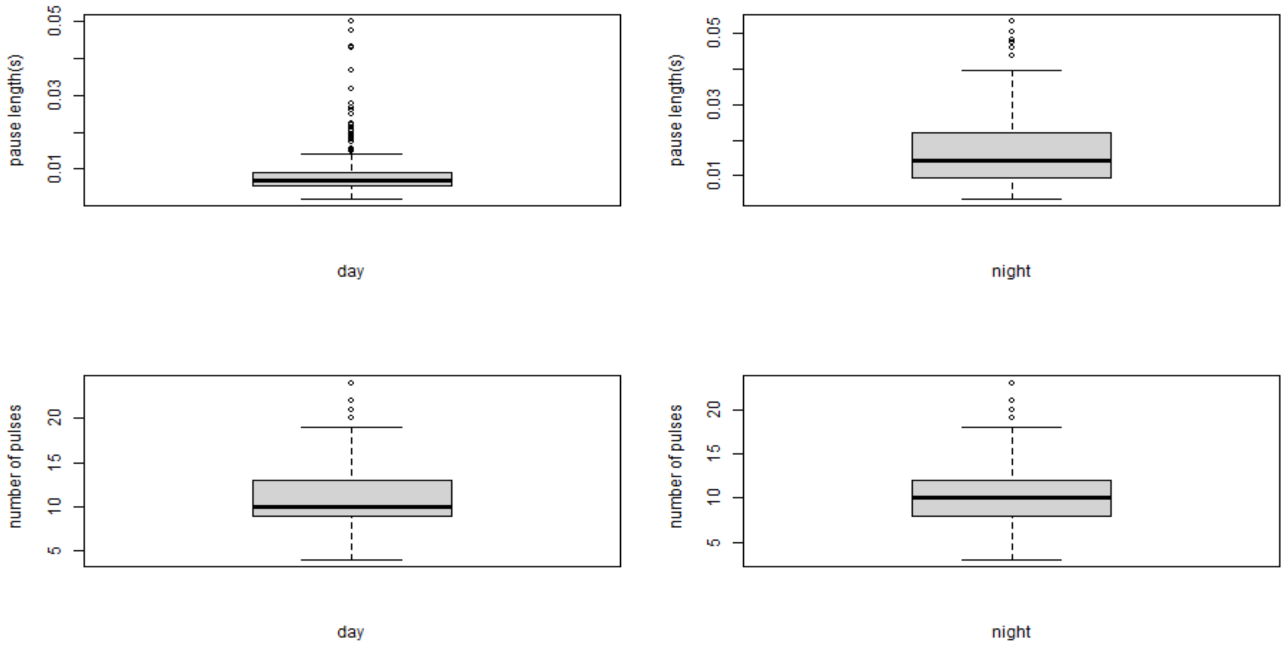

Figure 30- Boxplots of data for pause length and number of pulses, separated into day and night.

Average peak frequency was higher during the day (Figure 31).however, the box plots show us that the highest peak frequency values were present at night Figure 31). Overall range of peak frequency values is higher at night. However, the difference is overall not significant as reflected in the t test (Table 2, p=0.574)

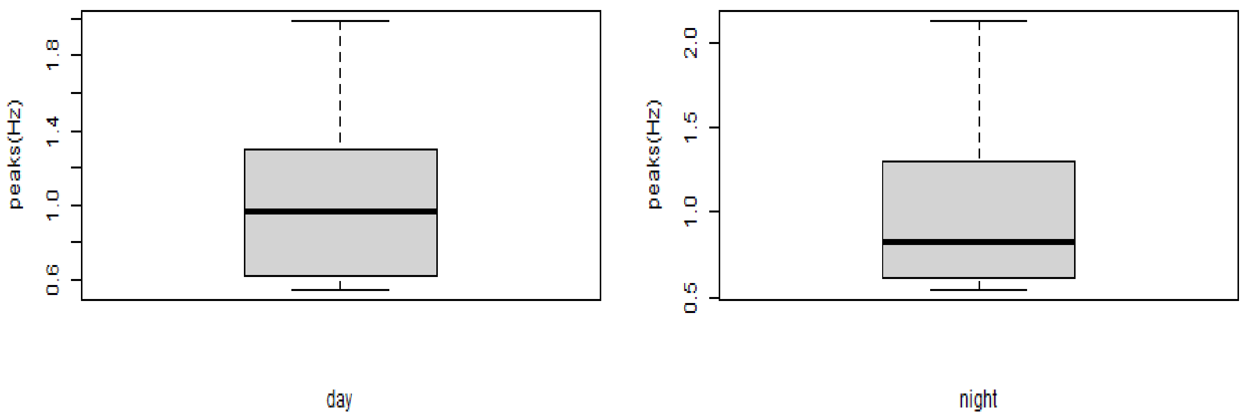

Figure 31- - Boxplots of data for peak frequency, separated into day and night.

4. Discussion

4.1 Environmental parameters and call characteristics

The purpose of this study was to determine whether temporal call characteristics; call length, pulse length, pause length, and auditory characteristics; number of pulses and maximum pulse frequency are affected by environmental parameters, and which of these characteristics are useful for future studies regarding the impact of the environment on frog call vocalization. To summarize, I expect water temperature to have a greater influence on variation of the temporal call characteristics, and background noise to have a greater influence on variation of the auditory characteristics

Results from call characteristic regression modelling do not match my expectations. I would expect the linear model for temperature to best explain variance in call length data, as outlined in section 1.3. Instead, the regression model incorporating only background noise the most parsimonious. The relationship between call length and background noise is unexpected, but it does open an interesting avenue in call analysis. It can be argued that increased background noise promotes a reduction in call *complexity* of *P. Lessonae*. This would make sense considering how increased ambient noise means there are a larger array of signals and frequencies. Hench there would be a need for *P.lessonae* to produce shorter, more discernable calls. A reduction in call length would result in a 'simpler' call, in the sense that the time period that other frogs must process is shorter. Such phenomenon has been observed in other vocal species, such as dolphins. Elevated background noise results in a reduced contour whistle complexity, a feature used by dolphins to identify individuals (Fouda et al., 2018). Of course, dolphin calls are far more complex than frog calls, due to their high level of intelligence (Fouda et al., 2018). However, given my results, I believe that this concept is well worth looking into when studying frog calls.

Linear regression for pulse length meets my expectations, as the regression model incorporating only water temperature as an explanatory variable was the most parsimonious. The negative relationship between pulse length and water temperature is due to increased temperature resulting in increased activity and hence shorter, more frequent pulses. Whilst pulse length exhibits a relationship with the environment, it can be argued that the relevance of pulse length to this study can be brought into question. There is little research regarding the effect of the environment on pulse length, and it is possible that the water effect of temperature on pulse length is merely a secondary effect, due to call length and pause length having negative relationships with the environments. These three call characteristics are likely closely interlinked as they form the temporal structure of the frog call (Appendix 1).

The dynamic between pause length and call length is reflected in the multiple regression model for pause length being the best model, as is supported by all of the output data. The decrease in call length will mean that frogs can call more frequently and hence will do so in order to communicate more effectively over increased background noise. The negative effect of temperature on pause length is explained by increase temperature leading to increased activity in amphibians, meaning they produced calls in shorter, more frequent bursts as is outlined by Radwan and Schneider (1988). Pause length is closely related to call length, hench, the impact of background noise on pause length will be the same as on call length, as reflected in linear regression results, where pause length had a significant negative relationship with background noise. These results show that elevated background noise not only induces reduction in frog call length, but also that frogs pause less within the call. I would suggest that this supports the idea of frogs making their calls more succinct in the presence of elevated background noise. Producing these shorter calls with less pauses in them means that it will be easier for other individuals to understand the vocalizations. Previous studies suggest that auditory systems in the gray treefrog *(Hyla chrysoscelis)* have evolved to be sensitive to statistical irregularities in the presence of high background noise environments (Lee et al., 2017). These irregularities include physical properties of the natural sounds (Lee et al., 2017). This supports my assertion that decreases in pause length and call length in response to background noise are due to *P. lessonae* differentiating their calls from the temporal structure of the background noise.

AIC values for number of pulses indicate that the best model for explaining the variance in number of pulses is the one incorporating temperature as predictor variable. A positive relationship between number of pulses and temperature would be explained by increased activity at higher temperatures, hence number of pulses increases as *P. lessonae* convey information at faster rates. However, previous studies contradict these findings somewhat. Lüddecke and Sanchez (2002) propose that number of pulses is not greatly affected by temperature, due to the highland species *Hyla labialis* being cold adapted (Lüddecke & Sánchez, 2002). As Norway and Scotland are similar in terms of terms of environment, we can hypothesis that *P. lessonae* are also cold adapted, and hence have some call characteristics that are in general quite resistant to the slight changes in temperature observed. This may explain a lack of variance in certain call characteristics. Navas and Bevier (2001) also highlight a lack of significant variation in number of pulses in the frog species *Colostethus subpunctatus* ,at different temperatures (in this study number of pulses is encompassed within the call characteristic of pulse repetition rate), with a p value of 0.37 (A. Navas & R. Bevier, 2001). Methodology for obtaining number of pulses data was satisfactory, and there were no visible discrepancies in the data set. However, I would suggest that pulse repetition rate would perhaps be a less limiting characteristic for analysis of pulse data, as this provides information on the number of pulses over a timespan. This also provides information on the temporal characteristics of the call, rather than just the raw number of pulses.

Another unexpected result is that variance in peak frequency data was best explained by the null model, and there is no significant relationship with either environmental parameter. The idea that background noise will lead to higher frequency calls is therefore not supported in this study. It should be noted that it was difficult to determine the results for peak frequency estimation. The methodology had to be adjusted several times to account for uncharacteristically high values that did not match the spectrum. The resulting data was quite noisy, and therefore difficult for R to interpret. Nevertheless, past studies support my conclusion that peak frequency is not primarily influenced by the environment. Ziegler et al instead highlight very significant scaling between body size and call frequency (Ziegler et al., 2015). Regression models testing note frequency against body mass, temperature, and body condition, showed that there was a significant negative relationship between body mass and note frequency, and that the model for this fitted the frequency note data far better than those for the other two variables (Ziegler et al., 2015). In other words, larger frogs produced lower frequency calls. Frog size was not considered in my study given difficulties present in assessing the size of individuals due to the study not being carried out in a controlled environment. It was therefore very difficult to identify individual frogs and measure their size. It is possible that the frog sizes vary between recordings, as different size frogs may be active at different times. As frog size is not an environmental parameter, I conclude that peak frequencies are generally not useful for assessing the impacts of temperature and background noise, but rather closely linked to frog size.

It was ascertained that the environmental parameters did indeed significantly impact most of the call characteristics. The results suggest that temporal characteristics are, surprisingly, influenced by background noise to a large extent, whilst number of pulses is affected by temperature. The degree of variation of the environmental parameter, rather than merely the parameter itself, seems to be of great importance in impacting call characteristics.

4.2 Correlation of predictor variables

The results from Pearson's correlation analysis shows a significant positive correlation between temperature and background noise (Appendix 2). This is unexpected, however when considering the addition of wind speed , it is possible to explain this correlation. Both water temperature and background noise have a negative correlation with windspeed (Appendix 2). Whilst the correlation between wind speed and temperature is to be expected, as wind speed is shown to decrease the temperature of the environment, one would expect wind speed and background noise to have a positive correlation. However, there is evidence of wind in fact having a negative correlation with background noise (Appendix 2). This can however be explained by considering the potential effect of wind on other species, which were perhaps within the frequency of frog call, but not discernable from them, or of too low an amplitude to be audible, yet still contributing to background noise. Insects such as the grasshopper species *Enchenopa binotata* signal through chirps more in between wind gusts than when the wind is blowing, in an attempt to make themselves better discernible (McNett et al., 2010). I would argue that wind speeds were not high enough to produce a large amount of noise directly, but were present enough to influence the calling periods of insects in the aforementioned manner, meaning there was more background noise in periods of lower wind speed. Other species in the habitat have adapted their signaling to take place between gusts of wind. Here we see how inter species bioacoustics relationships are intrinsically linked to my study.

Water temperature is shown to have a strong positive seasonal relationship with air temperature. Web and Nobilis conclude via a linear regression model, that over a 90-year span, fluctuations in air temperature monthly mean explained the fluctuations in water temperature monthly to a statistically significant level (WEBB & NOBILIS, 1997). Importantly, the amplitude of annual variation was much greater for air temperature than for water temperature, dropping to -10 degrees Celsius in 1928, whilst water temperature did not drop below 0 degrees during the study period (WEBB & NOBILIS, 1997). The air temperature and water temperature relationship is only prevalent over long periods of time, hench it was not assessed in my study (WEBB & NOBILIS, 1997). The addition of air temperature to future, longer spanning studies, would be useful. It is unlikely that *P. lessonae* calls were directly affected by air temperature in my study, as they were only observed as calling in the water. However, it has been established that increased air temperature induces increased vocal activity in species that were relevant to my study, namely birds (Pérez-Granados & Schuchmann, 2021). Therefore air temperature, is an important consideration when attempting to determine the sources of background noise, and when investigating relationships between environmental parameters.

A possible explanation for the relationship between temperature and background noise is that increased temperature leads to increased frog activity, which in turn increases background noise. Background noise comes from a large variety of sources, and I would suggest that increased frog activity, in other words, more aggression between frogs, physical contact, mating and movement within water are all potential sources of background noise. Such activities were observed at the site in question. As with the example of *P. trifoliata*, kinetics motion clearly results in noise production. The assertion that temperature increases activity is supported by the regression analysis as temperature increase results in an increased number of pulse and a decrease in pulse length. Increases in background noise are partially due to increased frog activity resulting in more frog motility. Sjøgrens 1988 paper displays an increase in male frog chorusing and oviposition in warmer pond areas (Sjögren et al, 1988). It can be argued that increased motion during warmer periods, causes disturbances in the pond water, which is in itself a source of background noise. This was observable for several of inspected minute long audio files with high background noise. However, I feel that the amount of audio files this was observed in is not sufficient to confidently make this conclusion. I believe a study focusing on the contribution of kinetics from fauna to background noise is needed in order to make an informed decision on whether it is worth considering.

It is important to note that topography of the environment can lead to spectral degradation, meaning call characteristics such as pulse repetition rate sre not propagated from one individual to the next at the optimal level, due to physical barriers that impede the soundwave (Penna and Solis, 1998). This can influence the spectrum of the signal as it travels across the environment, meaning the signal is not received it its entirety (Penna and Solis, 1998). Therefore, topography would be important if one were to compare call characteristics in different habitats.

4.3 Utility of day vs night analysis

The results from the two tailed test allow us to conclude that day vs night is a useful metric of which to separate measurements by, as we see that there is a significant difference in environmental variables between day and night (Table 2, water temperature $p < .001$, background noise $p < 0.001$). Water temperature is expected to be significantly higher during the day, as sunlight warms water. Results from the unpaired two tailed t test match these expectations.

Whilst there is a significant difference in temperature during the day vs during the night, the mean difference for water temperature ,1.3 °C, which perhaps not a big enough difference to significantly affect pulse length (Table2 , $p=0.928$), number of pulses (Table 2, $p= 0.1$) and peak frequency (Table 2,$p = 0.,574$) between night and day, although number of pulses and pulse length showed a significant relationship with temperature across the entire study period.

However, although mean background noise during the day and mean background noise during the night only varies by 0.5 units, the response variables that were found to have a relationship with background noise overall, call length and pause length, were also significantly different during the day vs during the night (Table 2, call length $p < 0.001$, pause length, $p < 0.001$) . Further research is needed, but it appears that less variance in background noise is needed in order to have an effect on its relevant response variables.

4.4 Call characteristics as indicators of activity

The extent of which call characteristics are influenced by environmental parameters is outlined in this investigation. Call characteristics play a significant role in frog activity, due to frog calls of various frequencies and pitch being their main method of communication. *P. lessonae* females deduce the source of male calls using call characteristics, as a form of identifier (Farris & Ryan, 2011). Therefore, frog call characteristics play a major role in facilitating frog activity and can be used as an indicator for frog activity. The call characteristics found to have a significant relationship with water temperature (pause length, pulse length, number of pulses), were the most useful as indicators of frog activity, as frog activity is shown to be influenced by temperature. Highest frequency peak has very low utility as an indicator for frog activity, due to the distinct lack of relationships with the environmental parameters.

4.5 Potential drawbacks in methodology/ investigation

There are a number of potential constraints on the investigation that should be noted, although the utmost has been done to alleviate them. Isolating frog calls from audio recordings proved very time consuming, hence the need to only do so for days with the highest peaks and troughs in temperature. It would of course have been preferable to isolate frog calls for the audio recordings in the entire period. However, the dataset that was produced was reasonably large, consisting of 952 isolated calls. Furthermore, when isolating individual frog calls, there were several aspects that had to be considered. It proved at times difficult to discern individual calls as many frogs were often calling simultaneously. Any call that was overlapped by another call was therefore regarded as useless to our study. There was a higher probability of short calls being successfully isolated, as longer calls often had some overlap with other calls. This will inevitably lead to the dataset being biased towards shorter calls, as is reflected in the results. However, this should not be regarded as a significant drawback, as the study focuses on call characteristics in relation to environment rather than attempting to assess the raw call characteristic data of the population. Precipitation was non-existent on most days, with day 2 have the only noticeable amount of precipitation, at 8.2 mm. This will of course severely limit our ability to compare precipitation between days. This is reflected in my final results, where precipitation was discarded in favour of using windspeed as an additional comparative parameter. Furthermore, it would have been preferable to obtain average wind speed measurements on a hourly basis rather than daily, as a greater spread of data would have allowed for more precise analysis. Unfortunately, due to access to recording equipment being limited, I could not make these measurements myself, and the wind speed measurements obtained from Arendal Lufthavn weather station were not frequent enough to facilitate presenting average wind speed per hour.

Another potential drawback was that a compromise had to be made regarding the parameters used for producing results in R. The aim was to determine which parameters produced a useful output for the majority of the isolated frog calls. Therefore some calls had to be discarded as they did work under these parameters, due to inconsistencies in their characteristics, for example the amplitude being too low. In some cases, a high amount of background noise caused by wind meant some poor-quality recordings were produced, as the background noise would be indistinguishable from the lower amplitude frog calls. This did however provide an additional method of improving the quality of the sample, as calls with poor quality output were re-visualized in kaleidoscope, with some of them being deemed as poor quality regardless of analysis parameters. The use of the smoothing function in order to allow for data visualization resulted in a distortion of pulse groups identified in R, meaning the two call characteristics related to these pulse groups, number of groups and pulses per groups, had to be discarded in this study.

The potential impact of the size of the individual frog must also be considered, as bigger frogs have been shown to produce calls of lower frequency (Ziegler et al., 2015). It is therefore possible that the frog sizes vary between recordings, as different size frogs may be active at different times. Therefore, some of the differences in call characteristics are likely due to frog size, rather than environmental factors. As I was unable to account for frog size in my study, due to it being carried out in the field. It is likely that the frogs of various sizes were calling at the same time, and that there is therefore a much larger variation in call characteristic data in my study, than if frog size had been accounted for. This large amount of variation would explain the very low R-squared values for all the models, as high variation in the dataset results in lower R-squared values, as R2 is calculated by determining what proportion of variance in the response variable is explained by the predictor variable (Everitt & Howell, 2005). If the response variable has high variation, the predictor variable is less able to predict this variance. This a major caveat when doing fieldwork, and highlights the need for controlled experiments in this area of research.

4.6 Other considerations and future applications

There are several other environmental parameters that may be better reflected in the call characteristics. Whilst precipitation had very low utility in our study, this may be due the location of the study. A controlled environment would provide opportunity for analysis of many other environmental parameters, as the parameters can be adjusted. For example, there is substantial evidence for environmental pollution affecting the activity of animal calls. Pollution of the environment leads to changes in precipitation and humidity, which high precipitation leading to longer call durations in bats (Penar et al., 2020).

Population density has also been shown to play a role in environmental stress upon species. Băncilă et als 2015 study on density dependence in frog populations concludes that density dependence can buffer amphibian populations against environmental stress (Băncilă et al, 2015). This is primarily due to competition from other male frogs (Băncilă et al, 2015). I would suggest that an approach centered around the effect density dependence has on call characteristics should be undertaken, as frog call frequencies are potentially affected by the regularity of other frog calls, rather than purely background noise level. The results support this assertion, due to the null model best reflecting peal frequency data.

Through this study I have determined the utility of the measured call characteristics. Temporal call characteristics, i.e., call length, pulse length and pause length are useful indicators of temperature and log noise, whilst peak frequency can potentially be used to assess intraspecies dynamics rather than environmental pressures. I suggest the following approach going forward. A larger scale investigation of *P.Lessonae* , done over the course of the several decades. Two populations of the same species, of different population sizes, should be observed for call characteristics. The advent of automated sensors on a large scale would allow for this (Lapp et al., 2021). The demand for species level identification is large, and my study provides a potential alternative method of species monitoring, to popular eDNA methods in use. The current lack of species level pool frog data has resulted in DNA hybridization and the mass spread of invasive species in *P.lessonae* habitats, meaning that there is a critical need for *P. lessonae* conservation in Europe (Dufresnes et al., 2020).

Given the conclusion that call frequency is more closely linked to frog size than background noise, establishing a range of frequencies for different frog species of different sizes would provide the basis for an automated recognition tool, that could be used to distinguish the species of frogs in the large sample area. Calls could also be divided into sub-categories based off their characteristics, much in the same manner that Schneider and Radwan (1988) categorized *P. lessonae* frog calls into mating calls, territorial calls and release calls. Categorizing calls allows future researchers to identify frog behaviours using only audio recordings, rather than needing to also visually observe frog movements/ behaviour manually. Through this method one could produce a comprehensive database of frog call characteristics. A time series of call length, pulse length and pause length would indicate changes in environment over a span of decades, whilst peak frequency would serve to discern the frog species. A long-term investigation would alleviate the issue regarding frog call characteristics not deviating greatly, due to little variation in the environment. Further research into the impact of precipitation on frog calls is necessary in order to provide a baseline for future investigations. This would preferably take place in an environment with great variance of rainfall. Knowledge of this is still lacking, and, as exemplified in my study, somewhat difficult to ascertain.

5. Conclusion

Through this study I defined call characteristics into temporal call characteristics and auditory (i.e., relating to frequency). Call length, pause length and pulse length were classed as temporal characteristics, whilst number of pulses and peak frequency were classed as auditory. Variance in temporal characteristics and number of pulses were well explained by the environmental parameters tested. Peak frequency can potentially be used as an indicator of frog size and body condition and showed little relationship with the environmental parameters. The results placed emphasis on *P. lessonae* calls adapting to background noise in order to make themselves discernible via shorter, more frequent calls. Consideration must be given to how the extent of variation in the environment affects call characteristics rather than purely whether significant variation is present. My results provide a foundation for future studies, highlighting which call characteristics should be analyzed when testing for environmental effect e.g., the impacts of climate change on frog vocalizations. I was also able to identify several key flaws in current bioacoustics methods, predominantly that it is very difficult to produce consistent and accurate results for individual frogs in a natural environment, due to a significant number of simultaneous calls. It was also determined that background noise cannot be used as a parameter that encompasses wind speed, due to the myriad of different background noise sources which can have varying effects on background noise level, and their complex relationships with each other. Therefore sources of background noise should be assessed individually or categorized further. Call characteristics are useful in bioacoustics as they can be used for inter-species comparisons and are universal features, not specific to amphibians. Further study should focus on how significant environmental change must be to affect call characteristics, and potentially on whether *P. lessonae* adapt their communication through methods other than simply altering their call characteristics.

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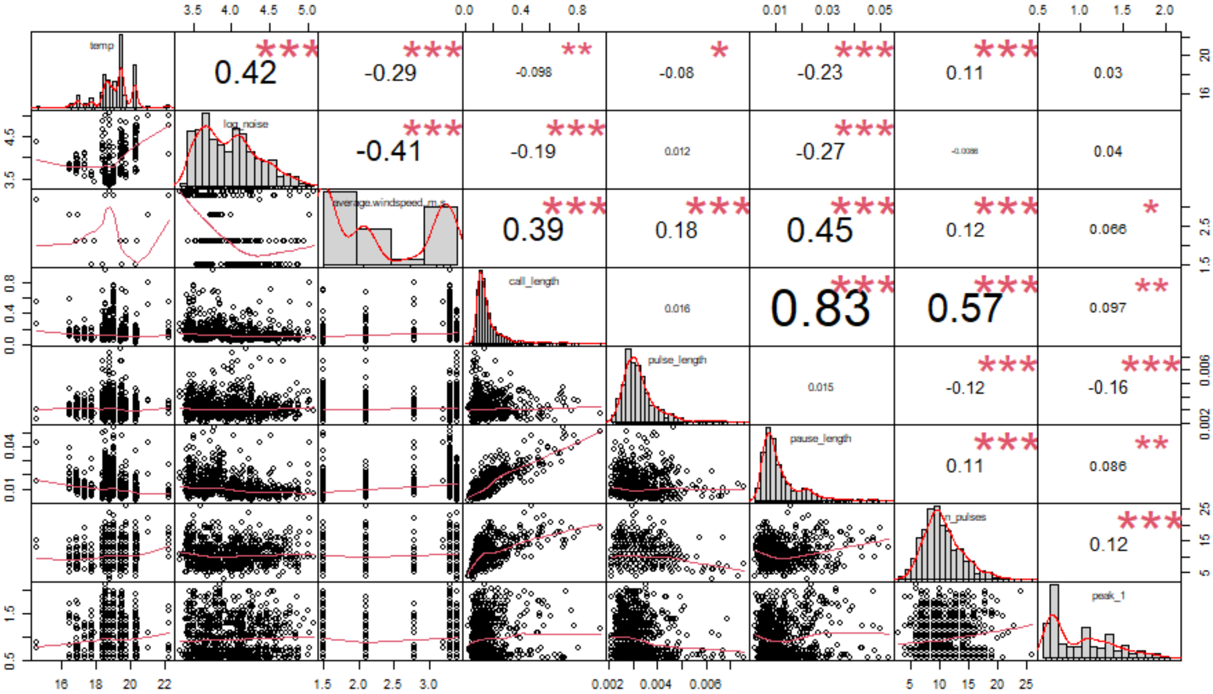
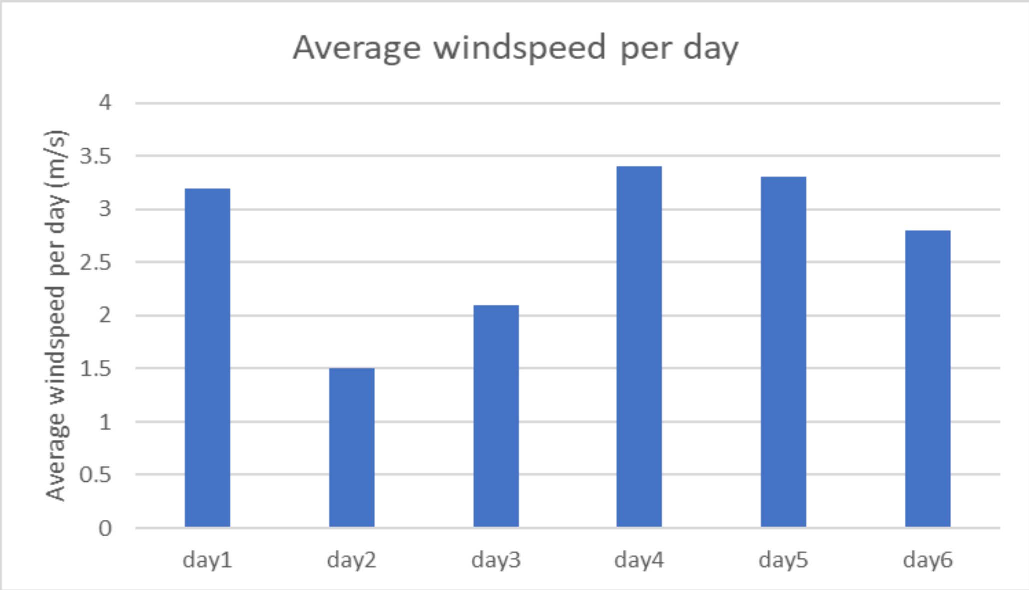
7. Appendix



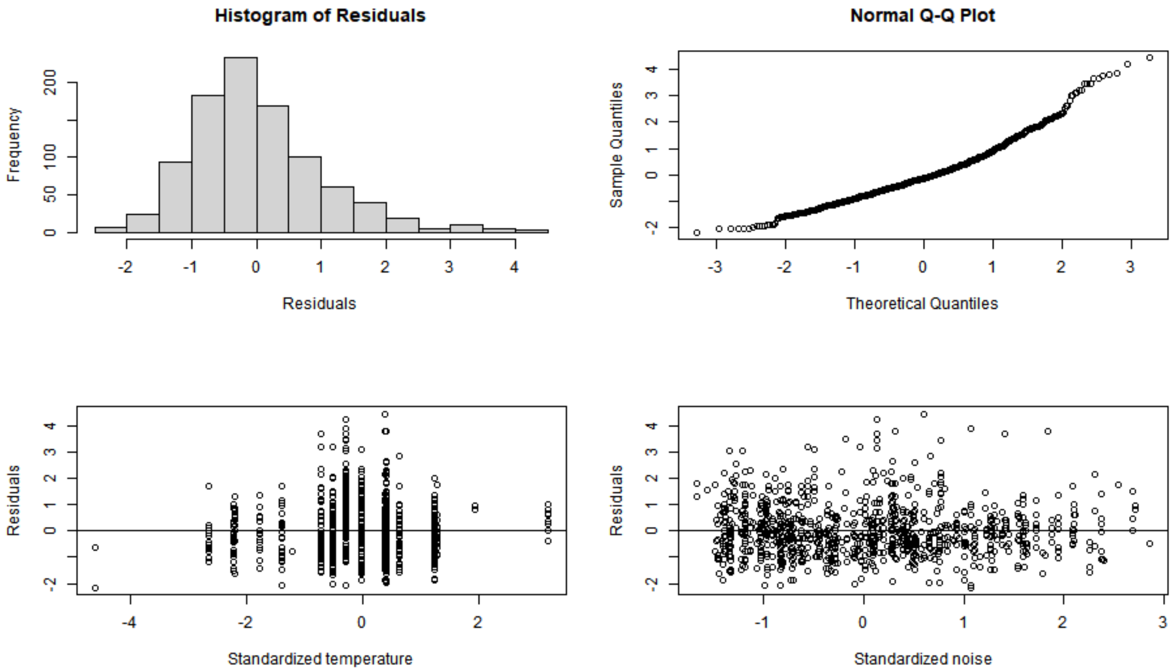
Appendix 1- Correlation matrix for all the variables used in the study. The strongest correlation found was between pause length and pulse length. Call length also exhibited a strong correlation with number of pulses (n_pulses). Peak frequency (peak_1) exhibited little correlation with any variables. The environmental variables all exhibited similar, moderately strong correlation with each other.

Test	Correlation coefficient (r)	P value
Temperature and Background noise	0.40	p < .001)
Temperature and average windspeed per day	-0.29	p < .001)
Background noise and average windspeed per day	-0.41	p < .001)

Appendix 2- Correlation coefficients and p value outputs of all combinations of Pearson's correlation test between the environmental variables.



Appendix 3- Average wind speed per day for each day that measurements were taken on. Day 1= 27/05/2021, day 2= 6/6/2021, etc.



Appendix 4- Model diagnostics produced for the multiple regression model for call length. Diagnostics suggest high model quality, as residual values were not very skewed, and were in general evenly spread around average standardized temperature and standardized background noise. Model diagnostics produced very similar graphs for the majority of the regression models. Ergo, model diagnostics were not considered in this study, due to AIC being a better metric for determining model quality.