Calling ecology of micro frogs (*Microbatrachella capensis*): A case study using Acoustic Spatial Capture Recapture

Photo credit: Jerry Khalo

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To the CB class of 2019, thanks for a fun and enjoyable year.

Abstract

Amphibians worldwide are declining, increasing the demand for monitoring populations of many threatened amphibians, including the South African Critically Endangered micro frog (*Microbatrachella capensis*). I attempted to improve ongoing monitoring efforts by determining the calling ecology of the micro frog population on the Cape Flats. I used acoustic spatial capture recapture to calculate call density and identify the main factors that determine periods of maximum frog calls. Increased calling behaviour was found early in the season and in response to rainfall. In addition, micro frogs were found to call more at night than during the day. This suggests that future monitoring of this species should occur at night, after rain, and early in the winter breeding season. From the call density estimates, I calculated the size of the micro frog population on the Cape Flats to be about 200 adult frogs when an equal sex ratio of adult males and females is assumed. Future monitoring and conservation efforts should take into consideration this baseline population estimate and keep track of any demographic trends in the population.

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Introduction

Animal populations of conservation concern are often monitored to inform conservation interventions. When combined with a baseline population estimate, periodic monitoring at regular intervals can be used to assess population trends (Butcher et al. 1990; Collins et al. 2020; Ortiz et al. 2020). These trends can be used by conservationists to evaluate the effectiveness of protected areas and management (Kiffner et al. 2020; Ortiz et al. 2020). In addition, monitoring of indicator species can be used to assess habitat quality, the impact of invasive species, or the effects of management actions (Underwood & Fisher 2006; Koehler et al. 2015). Monitoring is essential following translocation efforts in order to evaluate whether the effort was successful or if the population needs to be supplemented (Germano & Bishop 2009). Furthermore, population size and demographic trends are both factors used by the IUCN to decide a species' red list status (IUCN 2001). For monitoring to serve these purposes and provide useful data, it needs to be consistently performed over the long term in order to show quantifiable trends in population numbers.

Frog population numbers have been declining worldwide (Wake 1991; Houlahan et al. 2000; Green et al. 2020). Since the 1980s, amphibians have been found to be decreasing all over the world, with an estimated 41% threatened with extinction (Semlitsch 2003; IUCN 2020). Measey (2011) states that 35% of endemic South African frogs are considered threatened. Within South Africa, the main threat towards frog populations is habitat loss as urbanization destroys wetlands in favour of human development and agriculture (Measey 2011). As a consequence of this on-going destruction of habitat, the ranges of many frog species have become fragmented, limiting dispersal between sites. These sites are then effectively isolated, potentially increasing their risk of extinction (Gibbs 1998). Monitoring of these sites is essential to effectively manage and preserve these populations.

Many species of frogs are difficult to spot and catch, making populations difficult to count using capture-recapture or visual surveys. Therefore, monitoring of frog populations often relies on acoustic methods (de Solla et al. 2006; Bedoya et al. 2014; Stevenson et al. 2015). Males of many species of frogs emit mating calls during the breeding season. Individual frog species can be identified by these species-specific calls and counted, allowing for a population estimate (Bedoya et al. 2014). One method of acoustically monitoring frogs is via manual calling surveys. These surveys require trained human observers to listen for calls of each species. The resulting population estimate is often in the form of semi-quantitative categorical variables (such as "50 to 100 frogs calling"). This method is subject to observer bias and has low detection probability (de Solla et al. 2006). An additional limitation of manual surveys is that there is no calculation of the study area, making it impossible to know what area the detected frogs are distributed over and therefore the resulting population estimates cannot be converted into population density estimates (Stevenson et al. 2015).

Due to the limitations of traditional acoustic monitoring, the method of acoustic spatial capture recapture (aSCR) was developed. This method estimates population abundance from acoustic signals through combining the methods of distance sampling and capture-recapture studies (Stevenson et al. 2015). An array of microphones is set up in a rough circle allowing for some microphones to pick up a frog call while others do not (Dawson & Efford 2009). Information on which microphones picked up each call is recorded in the form of a capture history (Dawson & Efford 2009; Stevenson et al. 2015). Location of each call is extrapolated from the capture history, time when the call arrived at each microphone, and the amplitude of the call at each microphone (Stevenson et al. 2015). Detection functions similar to those used in distance sampling are calculated by modelling the distance of each detected animal from the microphone "traps" (Efford et al. 2009). These data can also be used to estimate an effective sampling area, allowing for the acoustic survey data to be converted into a call density (Borchers & Efford 2008; Stevenson et al. 2015).

Calls are recorded electronically, allowing for playback and reanalysis, unlike traditional manual estimates. This effectively eliminates the risk of observer bias as multiple researchers can listen to the calls and compare results. The recording equipment can be set up by someone who does not know how to identify frog calls and the resulting recordings can be sent to an expert for analysis or archived for future use. This limits the need for experts in the field, potentially increasing the number of monitoring studies that can be carried out. This method has been tested on the Cape Peninsula Moss frog (*Arthroleptella lightfooti*) where it was found to have negligible bias and more precise estimates than found in traditional monitoring (Stevenson et al. 2015) and has been used on other taxa such as birds, primates, and whales (Dawson & Efford 2009; Marques et al. 2012; Kidney et al. 2016). ASCR is a useful method for species that are difficult to see but easy to identify by their calls (Dawson & Efford 2009).

To accurately assess population trends, one must consistently monitor populations when the probability of detecting all individuals is high. Therefore, acoustic monitoring should occur when the maximum number of individuals are calling. Understanding the calling ecology of the species in question is crucial to determining the optimum time for monitoring. Frogs are known to vary their calls in response to rainfall, temperature, moon phase, or hours of day length (Blankenhorn 1972; Brooke et al. 2000; Both et al. 2008; Grant et al. 2009; Yoo & Jang 2012). In addition, the community composition of calling frogs in a pond can affect when different species call, as different species within the pond may call at different times from each other in order for all the calls to be heard (Wong et al. 2009). Therefore, understanding the effect of these environmental variables on a species is essential to effectively monitor the species.

Micro frogs (*Microbatrachella capensis*) are listed as Critically Endangered by the IUCN Red list due to the species inhabiting a total area of less than ten square kilometres (de Villiers 2004; IUCN & SA-FROG 2017). The remaining fragmented populations of micro frogs are threatened by urban development and agriculture as a result of habitat degradation in the form of flooding or elimination of wetlands, pollution, changes in water quality and acidity, and alien vegetation (Baard & de Villiers 2000; de Villiers 2004). Due to its small size (maximum length of 18 mm) and physical similarity to, but distinctive calls from, the sympatric genus *Cacosternum* (de Villiers 2004), the micro frog population is monitored acoustically.

Due to the continued threats to the remaining micro frog population in Kenilworth Racecourse Conservation Area (KRCA), the City of Cape Town and Cape Nature are considering translocating some micro frogs to the Cape Flats (A. de Villiers 2020, personal communication). In order to prepare for this translocation, the planning team would first like to know how many micro frogs are currently in the KRCA. Although the micro frog population in Kenilworth Racecourse Conservation Area has been acoustically monitored by Cape Nature since 1995 (Measey et al. 2019), these manual survey estimates are categorical estimates, not precise population estimates. Furthermore, these surveys occur once a year during the daytime when a trained individual is available. Little is known about the optimum time for monitoring micro frogs. Consequently, these estimates vary from year to year without providing clear trends in population size. Therefore, the goal of this study was to measure the calling ecology of the micro frog in order to better inform these ongoing monitoring efforts. Furthermore, this study aims to provide a baseline population estimate to compare against future population trends. This study also attempts to study the community composition of calling frogs that co-inhabit ponds with micro frogs in an attempt to understand whether these other frogs have an effect on the calling behaviour of micro frogs. In addition, this study represents the first time this method of acoustic spatial capture recapture was used on micro frogs, providing more insight into the effectiveness and best use of this method.

The objectives of this study were to identify the optimal time to monitor the micro frog by:

1. Determining whether rainfall, ambient temperature, percentage of moonlight visible, time of day, or day length influences the calling behaviour of micro frogs.

2. Determining a population estimate for the micro frogs in Kenilworth Racecourse Conservation Area using acoustic spatial capture recapture (aSCR).

3. Describing the community composition of calling frogs in ponds occupied by micro frogs and the effect this has on micro frog calling behaviour.

Methods

Study Site

Kenilworth Racecourse Conservation Area (KRCA) (18° 29' E; 34° 00' S) consists of 52 hectares of Cape Flats Sand Fynbos in the centre of the oval of the racecourse track (Kenilworth Racecourse Conservation Area 2019). The area within the racetrack is the largest, good quality tract of Cape Flats Sand Fynbos remaining with high water quality (Brown 1991) and the only known remaining population of micro frogs within the Cape Flats (de Villiers 2004). The Cape Flats Sand Fynbos vegetation found within the KRCA is part of the Cape Floristic Kingdom, characterized by small evergreen shrubs and high levels of endemism (Brown 1991). During the winter rainfall breeding season, Hopkins (2006) found micro frogs, flat cacos (*Cacosternum platys*), clicking stream frogs (*Strongylopus grayii*), Cape sand frogs (*Tomopterna delalandii*), and platannas (*Xenopus laevis*) breeding within the KRCA.

*Study Species*The micro frog (*Microbatrachella capensis,* Boulenger 1910), a monotypic species, is endemic to the Western Cape Province of South Africa (de Villiers 2004) where it is found in acidic black water temporary ponds of the fynbos biome (Baard & de Villiers 2000). The Cape Flats region that is known for these ponds has been heavily developed, leading to significant habitat loss for this species (Baard & de Villiers 2000). Male micro frogs call while perched on vegetation near the water's surface (de Villiers 2004). The call is short and raspy (Channing 2001). Micro frogs call during the winter rainy season, between the months of May and October (de Villiers 2004). This species is ideal for a study using aSCR because it calls in high densities, calls both day and night, and is thought to have high population densities within its limited habitat (de Villiers 2004). The call of the micro frog is described as 0.1 seconds long with a dominant frequency of 4.9 kHz (Channing 2001).

The flat caco (*Cacosternum platys,* Rose 1950) coexists with the micro frog in the Western Cape Province of South Africa (Hopkins 2006; Channing et al. 2013). Male flat caco frogs call from beneath vegetation at the water level (Channing 2001). These calls are described by Channing et al. as lasting 0.28 seconds long with eight clicks in which the clicks start slowly and then speed up (2013). Channing (2001) found a dominant frequency of 7.5 to 8 kHz.

The clicking stream frog (*Strongylopus grayii*, Smith 1849) occurs in the southern part of South Africa (Tolley et al. 2010). In the winter rainfall region of the Western Cape, male clicking stream frogs call during winter from the base of vegetation close to water (Channing 2001). Channing describes the clicking stream frog call as a single click lasting six milliseconds long with a dominant energy at 2.3 kHz (2001).

Permit

A permit (CN44-31-10000) was obtained from Cape Nature to conduct acoustic recordings in Kenilworth Racecourse Conservation Area.

Study Methodology

Within the KRCA, three ponds were chosen as study sites. The ponds selected were estimated to have the greatest number of micro frogs heard calling regularly. This was essential as Louw (2018) found that this method of aSCR produces unreliable density estimates when fewer than 111 calls per minute (about five Cape Peninsula Moss Frogs *Arthroleptella lightfooti*) were detected by the microphone array. For ponds adjacent to one another, only one pond was selected in order to eliminate the risk of overlap in recording area. In this way, I avoided double-counting the same frogs when calculating the total population of micro frogs in Kenilworth Racecourse Conservation Area.

Figure 1. Map showing approximate location of the microphone arrays (white circles) and effective sampling area (black circles) within the Kenilworth Racecourse Conservation Area. Ponds with calling micro frogs are represented by blue shapes. Ponds one, two, and three were recorded using aSCR while ponds four and five were manually surveyed. Map figure provided by Google Maps.

Within the Racecourse Conservation Area, Pond one was relatively centrally located while Pond two was located near the racetrack and Pond three was close to the fence line and the horse stalls (Figure 1). The microphone array for pond two was placed towards the middle of the pond while the arrays for the other two ponds were located in the corners of the ponds. Due to the small number of calling frogs in Ponds four and five, these ponds were not recorded using aSCR but were instead manually surveyed on 8 August 2019.

Sites were visited six to nine times each from 26 July through 16 October. Few visits occurred during the months of July and August due to difficulties with the equipment, scheduling conflicts and frequent rainfall. In September and October visits occurred weekly. Sites were visited between 8 and 17:30 hours. Rainy days were avoided as the recording equipment was not waterproof. Days in which a race occurred at Kenilworth Racecourse Conservation Area were also avoided due to the difficulties of access to the infield on a race day and the potential of the higher volume of noise to mask or prevent frog calls. When the area of a pond that was used for the recording array dried up and multiple visits yielded no recorded micro frogs, I stopped visiting that site. Site One retained its water for the duration of the study. Therefore, I stopped visiting Site One when fewer than five micro frogs were heard calling by human observers as Louw (2018) suggested this was too few frogs to be accurately determined by aSCR.

When possible, all three sites were visited on the same day. When this was not possible, the remaining sites would be visited the next available day. Time of each visit and order of sites visited each day were determined by availability of researchers, park management, and other studies and management activities occurring in the same area. A random number generator was used late in the season (September to October) to determine site visitation order but was largely overruled by conflict of use of wetland study areas.

Array Setup

For each recording, an array of six omni-directional microphones (Audio-Technica AT8004) connected to a six-track recorder (Tascam DR 680, TEAC, Wiesbaden, Germany) via 15.5m cables was set up in an imperfect circle along the edge of a pond. Where possible, microphones were placed on land at the edge of the water or on an island in the pond to minimize the risk of electrical equipment falling in the water. Some microphones were placed directly over water. The ponds studied were too big to completely surround with the array, so the array was placed in a corner of the pond with vegetation at water level thought to provide calling habitat for the micro frogs. Microphones were placed three to nine metres apart from each other on one-metre tall wooden dowels. The dowels were placed inside PVC pipe tubes planted in the ground to guarantee that they were placed in the exact same locations each time. Numbered microphones were placed in counter-clockwise order and plugged into the corresponding number on the recorder. The recorder was always placed on land next to microphone one. All microphone channels were set to the same sensitivity for all recordings.

Recordings occurred in 40-minute segments. Once the recording started, researchers walked 100 m away from the pond to avoid disturbing the frogs.

Data Collected

Following the completion of each recording, I measured the distance between each microphone pair within the array to the nearest centimetre using measuring tape. Measurements were recorded from the centre of the head of the microphone to the centre of the other microphone head. For each recording sample I also drew a sketch of the microphone array, labelling all the microphones. The date and time of each recording were also noted.

Environmental Variables

Rain and ambient temperature data were obtained from Weather Underground, Wynberg station (Trovato1 I Cape-T7, elevation 113 m above sea level, 34.00°S, 18.46°E) (Weather Underground 2020). The Wynberg station is about 1.5 km west of the Kenilworth racecourse (elevation 28 m above sea level) (Helme & Trinder-Smith 2006; Norwegian Meteorological Institute and Norwegian Broadcasting Corporation 2020). Since weather varies across Cape Town due to variations of elevation and distance from the coast, it was thought that as the closest available weather station to the study site, the Wynberg station would have the most similar weather. The weather at this station was reported every five minutes for nearly every day during the study period from July to October.

The rain variable used in this study represents the cumulative amount of rain falling in Wynberg from 8 am two days before the date of the recording to 7:59 am the day before the day of recording. (For example, for a recording on 6 August, the rainfall was measured as the cumulative amount of rain between the hours of 8 am on 4 August and 7:59 am on 5 August.)

Ambient temperature data from the Wynberg station were taken at the beginning of each recording. It was reasoned that frogs are responding to current temperature when they call and therefore the temperature at the beginning of the recording would affect whether frogs would call during the recording.

Percentage of moonlight visible was collected from Moon Phase calendar app (ProbadoSoft 2020) for the dates of study. This represents the percentage of the moon visible to earth (zero = new moon, 50 = half-moon, 100 = full moon). This was seen as the most accurate measure of moonlight available to the frogs.

Day length was gathered from Timeanddate.com (2020) for the duration of the study and measured in hours of sunlight per day. This variable was used to represent the effect of date or season on calling behaviour.

Density Estimates Pre-Processing

Call Identification

The program Raven Pro 1.5 (Cornell Lab of Ornithology) was used to convert the acoustic recordings into data that stated the times of each call, which microphone(s) picked up each call, and the amplitude of each call.

To distinguish unique multi-syllable calls, I programmed RavenPro to classify calls as distinct individual calls if the time between syllables was greater than 0.02133 seconds. In order to distinguish the desired frog calls from sounds made by other frogs, birds, traffic, aircraft, and general background noise, I programmed a Band Limited Energy Detector in Raven. I found a lot of variation between calls of the same species both within and between recordings making it difficult to estimate the exact settings for the Detector. As Detectors are unable to detect 100% of true positives (Charif et al. 2010; Stowell et al. 2018) and reducing the number of false positive detections increases the number of false negative detections (Crump & Houlahan 2017; Knight et al. 2017; Shonfield et al. 2018), one must decide which to eliminate (Crump & Houlahan 2017; Shonfield et al. 2018). Capture-recapture models typically assume imperfect detection but are incapable of dealing with false positives (Blanc et al. 2014), thus for this study, I attempted to eliminate false positive detections while still picking up most true positive detections. Therefore, I used settings that seemed to work relatively consistently across all recordings in picking up roughly 80 to 90% of the calls with a few false positives in the form of birds or wind. Table 1 lists species specific settings used for the Detectors.

Table 1. Detector settings for analysing array data in Raven Pro.

	Flat caco	Clicking stream frog	Micro frog
Minimum Frequency (Hz)	4600	2200	2300
Maximum Frequency (Hz)	7000	2700	4900
Minimum Duration (s)	0.20267	0.00533	0.03733
Maximum Duration (s)	0.90133	0.02667	0.144
Minimum Separation (s)	0.04267	0.02133	0.02667
Minimum Occupancy (%)	20	100	70
Signal to Noise Threshold (SNR)	10	9.5	9.1
Block Size (s)	1.99467	1.99467	0.08533
Hop Size (s)	0.50133	0.50133	0.06933
Percentile (%)	20	20	20
Exclusion Band Minimum Frequency (Hz)	N/A	4000	5500
Exclusion Band Maximum Frequency (Hz)	N/A	8000	7000
Exclusion Band SNR	N/A	18	20

Since the short raspy calls of micro frogs and flat cacos are similar, I distinguished between the calls of the two species by classifying all calls below 5 kHz as micro frog calls, and those above as flat caco. In addition, the flat caco has multiple calls, one of which is similar to that of the micro frog (A de Villiers, A

Turner 2019 personal communication). Since all flat cacos make each type of call (A Turner 2019 personal communication), I programmed the flat caco detector to pick up only the long calls.

Subsample Selection

Each Detector was run for each species on each 40-minute recording, using all six channels. The output file contained the following measurements for each detection: Start time, End time, Peak Power, Peak Frequency, and Channel (microphone). This file was converted to a .CSV (MS-DOS) file to be used in R (version 3.6.1, R Core Team 2019).

For each recording I chose ten one-minute subsamples to process in R, as analysing the entire 40-minute recording would require too much computing power. The default choice for these minutes was minutes 10,13,16,19,22,25,28,31,34,37. This allowed ten minutes for the frogs to resume calling following the disturbance of us setting up the array and avoided the last minute of our footsteps returning to the array in order to shut off the recorder. When the desired minute subsamples detected false positives in the form of bird calls or sounds from aircraft, alternative subsamples were used. Late in the season, frogs were not found to call consistently throughout the recording so only minutes with frog calls were used. When necessary, minutes prior to minute ten were considered for subsamples if there were frogs calling and no disturbance. In several recordings fewer than ten subsamples were found and used for the final analysis. Some recordings only had one subsample analysed. Figure 2 shows the breakdown of how many recordings yielded ten or fewer subsamples.

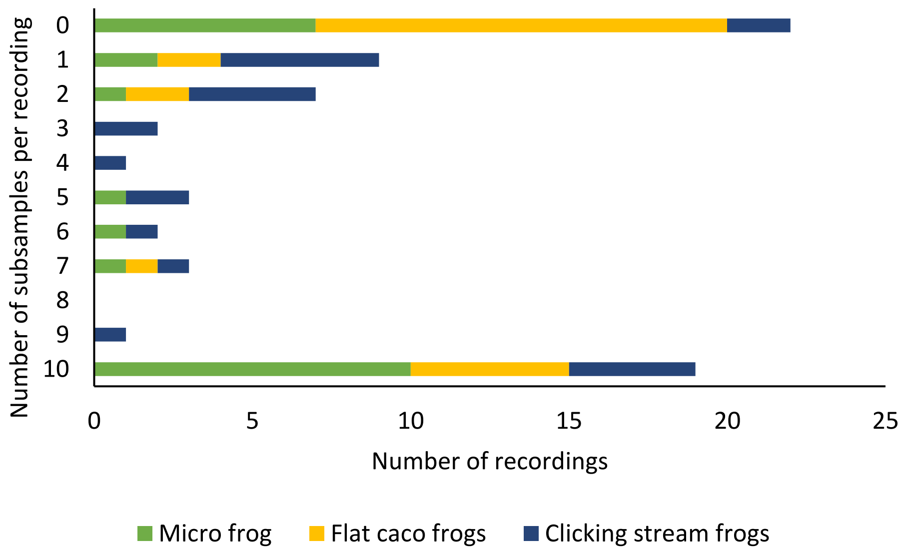

Figure 2. Number of subsamples analysed for each recording. The x-axis represents the number of recordings and the y-axis shows the number of subsamples analysed. For example, there were ten recordings with ten subsamples analysed for the micro frog. A recording with zero detected calls would yield zero subsamples for analysis. A total of 23 recordings were collected. The same 23 recordings were processed for each study species. Each recording was originally 40 minutes long. Each subsample is one-minute long.

Microphone Location

To estimate the relative location of each microphone within the array, I used the array field sketch and the measured distances between microphone pairs to estimate the coordinate locations of each microphone on a planar coordinate system measured in metres. For these estimated locations, microphone one was used as the origin and the other microphones' locations were estimated relative to microphone one. I then used the R code locest.R, and locestfuns.r, to calculate the actual relative locations of the microphones. This code estimates microphone locations by comparing the estimated locations with the measured distances. The resulting output file lists the coordinate locations of each microphone.

Density Estimates

To calculate the estimated population density of each species of frog for each subsample I used the R (v. 3.6.1, R Core Team 2019) package aSCR (Stevenson & Borchers 2017) which requires the packages secr (Efford 2019), and data.table (Dowle & Srinivasan 2019). This required the input files of the capture history of each call on each microphone as well as the microphone location file.

For each subsample, time of the arrival of each call to each microphone was calculated and used to estimate the locations of each calling frog as described by Stevenson et al. (2015). Signal strength of each call was also calculated and used to generate a detection function representing the probability of a call being picked up by the microphone array. The number of calls per hectare, number of frogs per hectare, and effective sampling area recorded by the microphone array were also calculated.

For this study, I used the call rate of Cape Peninsula moss frogs (*Arthroleptella lightfooti)* of 16.25 calls per minute (Measey et al. 2017). This call rate was used as a substitute for the unknown call rates of the study species. Call rate can vary widely both within and between species. Call rate within anuran species has been found to be influenced by many factors including individual differences such as size (Kamath & Sreekar 2016), and regional differences such as environment (Kamath & Sreekar 2016), temperature (Wong et al. 2004), number of frogs calling (Wong et al. 2004), presence of other species (Bleach et al. 2015) or anthropogenic noise (Sun & Narins 2005; Kaiser & Hammers 2009; Kruger & Du Preez 2016; Caorsi et al. 2017). In addition, regional "accents" have been identified within some species and are thought to be a result of genetic evolution (Wycherley et al. 2002). Call rate between species has been found to vary along genetic differences such as species (Philippe et al. 2017) and common ancestry (Yang et al. 2019). As the closest-related, similarly-sized species (American Museum of Natural History 2020; FrogMAP 2020) for which the call rate is currently available, the Cape Peninsula moss frog was deemed the best proxy until the micro frog call rate is known. These results therefore have an unknown bias and future studies should attempt to estimate a species-specific individual call rate for each of these species in order to verify or correct this assumption.

Bootstrapping was used to estimate standard errors and confidence intervals for the population estimates. A Monte Carlo Error estimate was also calculated. When subsamples showed a Monte Carlo Error above 0.5, I first found alternate subsamples, and then increased the bootstrapping from 300 iterations to 500. Due to time constraints, I was unable to run the analyses at higher iterations than 500. Although many of the error estimates remain high, this did not affect the actual estimates used for the analysis, but rather affected the confidence interval of each estimate.

The median density estimate of the subsamples was used to represent the density for that sample. Since the densities used in the analysis are all estimates, some of the outlier density estimates could be erroneous. Both the mean density estimate and the maximum density estimate would place too much weight on an outlier estimate. However, the median is less susceptible to influence by erroneous outliers. Thus, the median was chosen for use in further analysis as a robust measure of the average calling frequency per recording. For the recordings in which no calls were heard, a zero was used to represent frogs per hectare. If only one call was heard in the entire 40-minute recording, a one was listed as the number of frogs per hectare and the recording was not analysed in aSCR. While running a few recordings through the aSCR package in R, the error message "Error allocating init bounded number min b = 4.78749 max b = 4.77238 Error in file(con, "r") : cannot open the connection” indicated that the number of frogs calling was not sufficient to run the analysis. For these subsamples, a two was used as a placeholder in the frogs per hectare category. The exact number of frogs heard within these subsamples is unknown.

Seasonal Variation

Using R, I ran linear models with estimated frog density (frogs per hectare) as the response variable and day length measured in hours, rainfall (mm), ambient temperature, start time of the recording, and percentage of moon visible as the explanatory variables. Each recording represented one sample. Frog density for the recording sample was determined by taking the median subsample population density estimate of all the subsample estimates for each recording. I was unable to find rainfall or ambient temperature data for 26 July 2019 on Weather Underground, so I only included data from 6 August to 16 October in the analysis. Site was included in all models as each site had numerous unique characteristics and different numbers of frogs calling. Different combinations of covariates were also considered in the models.

Diel Calling Patterns

To determine the diel calling patterns of micro frogs over a 24-hour period, I placed two Song Meters (SM3, Wildlife Acoustics Inc.) by study sites one and three. They were each placed along the bank of the pond, facing the pond, with one microphone pointing towards each half of the pond (Figure 3). To avoid double counting the same calls, the microphone facing away from the aSCR array was turned on, while the other microphone was not used. The Song Meters were positioned approximately one metre directly above the bank of the pond and were fastened to a nearby tree or post with wire. The Song Meters were deployed from 3 September to 24 October 2019. They were checked weekly when I replaced memory cards and batteries.

Figure 3. Map showing approximate location of Song Meters (white stars) within the Kenilworth Racecourse Conservation Area. Ponds with calling micro frogs are represented by blue shapes. Ponds one, two, and three were recorded using aSCR while ponds four and five were manually surveyed. White circles represent microphone arrays while black circles represent the effective sampling area of the microphone arrays. Map figure provided by Google Maps.

The Song Meters were set to record continuously with each hour-long segment representing one file. Initially, I set the recording frequency to 8kHz as recommended for frogs by the Song Meter manual (Wildlife Acoustics, Inc. 2018), but after two weeks I realised this was too low to record the flat caco frogs and reset both Song Meters to a recording frequency of 20kHz on 19 September. The higher setting resulted in memory and battery drain and a few days of data (n= seven days at Site One [21-27 September], n = three days at Site Three [25-27 September]) were consequently lost.

Analysis of Song Meter Data

I used the same Detectors in Raven Pro that I had programmed for the array data. However, due to the difference in the recording quality, Raven Pro automatically changed some of the settings. These new settings might reduce the proportion of correct detections. The new settings are listed in Table 2.

Table 2. Adjusted Detector Settings for analysing the Song Meter Data in Raven Pro.

	Flat caco	Clicking stream frog	Micro frog
Minimum Frequency (Hz)	4600	2200	2300
Maximum Frequency (Hz)	7000	2700	4900
Minimum Duration (s)	0.20267	0	0.032
Maximum Duration (s)	0.896	0.02133	0.14933
Minimum Separation (s)	0.04267	0.02133	0.02133
Minimum Occupancy (%)	20	100	70
Signal to Noise Threshold (SNR)	10	9.5	9.1
Block Size (s)	1.99467	1.99467	0.08533
Hop Size (s)	0.50133	0.50133	0.064
Percentile (%)	20	20	20
Exclusion Band Minimum Frequency (Hz)	N/A	4000	5500
Exclusion Band Maximum Frequency (Hz)	N/A	8000	7000
Exclusion Band SNR	N/A	18	20

Note: From 3 September to 19 September the Song Meters recorded at eight kHz. This resulted in aliasing when I attempted to use my Detectors as the micro frogs' dominant frequency is about 4.9 kHz (Channing 2001). Aliasing is found in recordings in which the desired frequency is more than half the recorded frequency (Charif et al. 2010). Upon noticing this error, the Song Meter settings were subsequently changed. However, the recordings with aliasing still picked up and identified the micro and clicking stream frog calls. Table 3 shows the revised Detector settings used for these recordings. Since the dominant frequency for flat caco calls is well above four kHz, I was unable to use the flat caco Detector to identify flat caco calls in these recordings.

Table 3. Detector Settings for recordings with an aliasing error. The maximum usable frequency in these recordings was 4000Hz.

	Flat caco	Clicking stream frog	Micro frog
Minimum Frequency (Hz)	3500	2200	2300
Maximum Frequency (Hz)	4000	2700	4000
Minimum Duration (s)	0.192	0	0.032
Maximum Duration (s)	0.896	0.032	0.16
Minimum Separation (s)	0.032	0.032	0.032
Minimum Occupancy (%)	70	100	70
Signal to Noise Threshold (SNR)	10	9.5	9.1
Block Size (s)	1.984	1.984	0.096
Hop Size (s)	0.512	0.512	0.064
Percentile (%)	20	20	20
Exclusion Band Minimum Frequency (Hz	N/A	4000	N/A
Exclusion Band Maximum Frequency (Hz	N/A	4000	N/A
Exclusion Band SNR	N/A	18	N/A

The program Raven Pro was used to save the start times of the detection. I eliminated incomplete days of recording (n = four), leaving only dates in which data were generated from 12 am to 11:59 pm. These data were then compiled and sorted by time of day to create boxplots.

Population Estimate

To calculate the adult population estimate of micro frogs, I multiplied the population density estimate calculated by the aSCR program by the area of the pond. This number was then multiplied by two to include females in the estimate as only male frogs call. Estimates from manual observations were used for Sites Four and Five as these ponds were not recorded using aSCR.

Results

1.Call Behaviour

Twenty-three array recordings (nine from Site One, six from Site Two, and eight from Site Three) were collected between the dates of 26 July and 16 October 2019. Of these, 21 were analysed with a linear model. The two recordings conducted on 26 July were not included in the analysis due to the lack of corresponding rain and temperature data.

The Song Meter at Site One recorded for a total number of 43 days while the Song Meter at Site Three recorded for 47 days.

Seasonal Variation

A collinearity test found that none of the explanatory variables used had a collinearity value of greater than 0.6 (Appendix G and H).

Model selection resulted in the combined effect of the variables of day length and rainfall being chosen as the best model to explain calling behaviour in micro frogs, where the variable of rainfall represents a rainfall event two days preceding the recording sample (Table 4). Increased calling behaviour was found two days after rain (Figure 4). However, very few rainfall events were included in this analysis. Calling decreased as day length increased (Figure 4).

Table 4. Model Selection table for the micro frog. Site was included in all models. Moon Phase represents percentage of moonlight visible.

Variables	Number of Parameters	Log Likelihood	Delta AIC_c	Weight
Day length + Rain	6	-88.68	0.00	0.6292
Start time + Rain	6	-90.35	3.34	0.1182
Day length + Start time	6	-90.60	3.84	0.0921
Start time + Temperature	6	-90.63	3.90	0.0895
Start time	5	-93.07	4.78	0.0576
Start time + Moon Phase	6	-93.05	8.73	0.0080
Day length	5	-96.05	10.75	0.0029
Rain	5	-96.96	12.55	0.0012
Day length + Temperature	6	-95.76	14.16	0.0005
Day length + Moon Phase	6	-96.04	14.72	0.0004
Rain + Moon Phase	6	-96.73	16.10	0.0002
Rain + Temperature	6	-96.80	16.24	0.0002
None	4	-101.95	19.05	0.0000
Temperature	5	-101.11	20.87	0.0000
Moon Phase	5	-101.92	22.49	0.0000
Temperature + Moon Phase	6	-101.09	24.83	0.0000

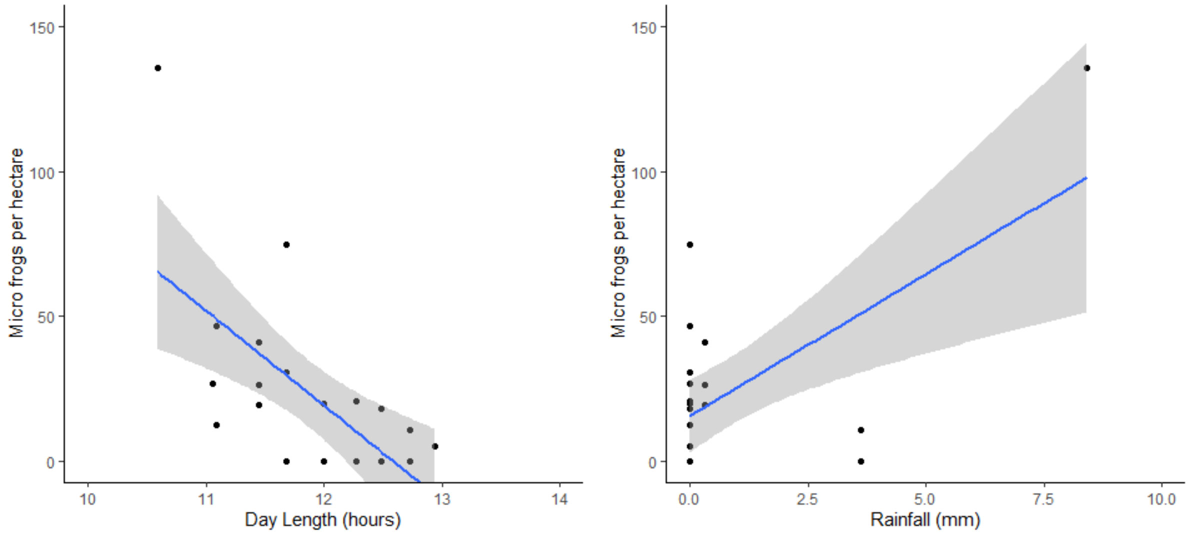

Figure 4. Linear model reflecting the influence of day length and rainfall on micro frog calling behaviour. Shaded area represents confidence interval. The line equation is Y = 403.067 + -31.876 * Day length + -18.402 * Site 2 + -9.401 * Site3 + 8.598 * Rainfall. The Adjusted R^2 value is 0.655. The p value is 0.0002286. The Durbin-Watson test statistic is 2.194906.

Effective Sampling Area

The area covered by the array of microphones varied from recording to recording (Figure 5). The effective sampling area of Site One varied by a factor of almost five (0.09 hectares to 0.41 hectares). Sites Two and Three had fewer data points in which the effective sampling area was calculated (n = three and four, respectively) but the sampling area for Site Three never exceeded 0.12 hectares while the sampling area for site two ranged from 0.06 to 0.16 hectares. Site One consistently had the largest effective sampling area of the three ponds surveyed.

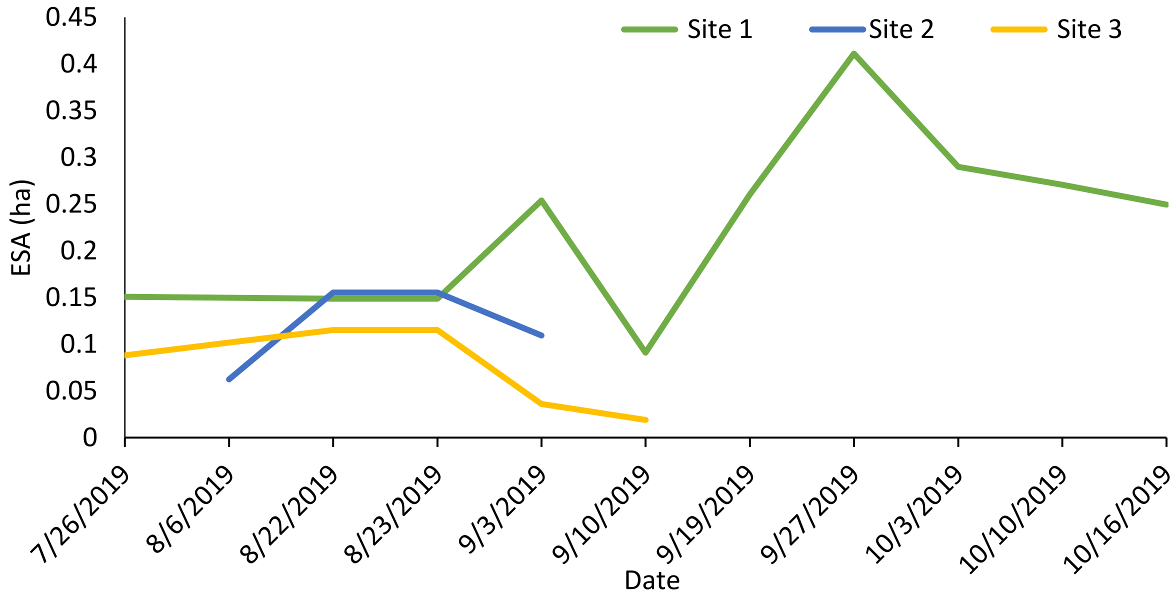

Figure 5. Effective sampling area (ESA) of micro frog recordings. Graph depicts median ESA values for each date.

Diel Calling Patterns

In both Sites One and Three, peak half-hour recording periods representing at least 14 percent of daily recorded calls fell between the hours of 19:00 and 0:30 (Figure 6). At Site One, increased levels of calling continued until 6:00. This corresponds with the period of darkness as sunrise times during the study period occurred from 6:00 to 7:00 while sunset times ranged from 18:30 to 19:00 (Timeanddate.com 2020). At site three, calling behaviour was at its lowest from 1:00 to 6:00, and then slightly increased during daylight hours. Both sites demonstrated high variability of frog calls in the evening.

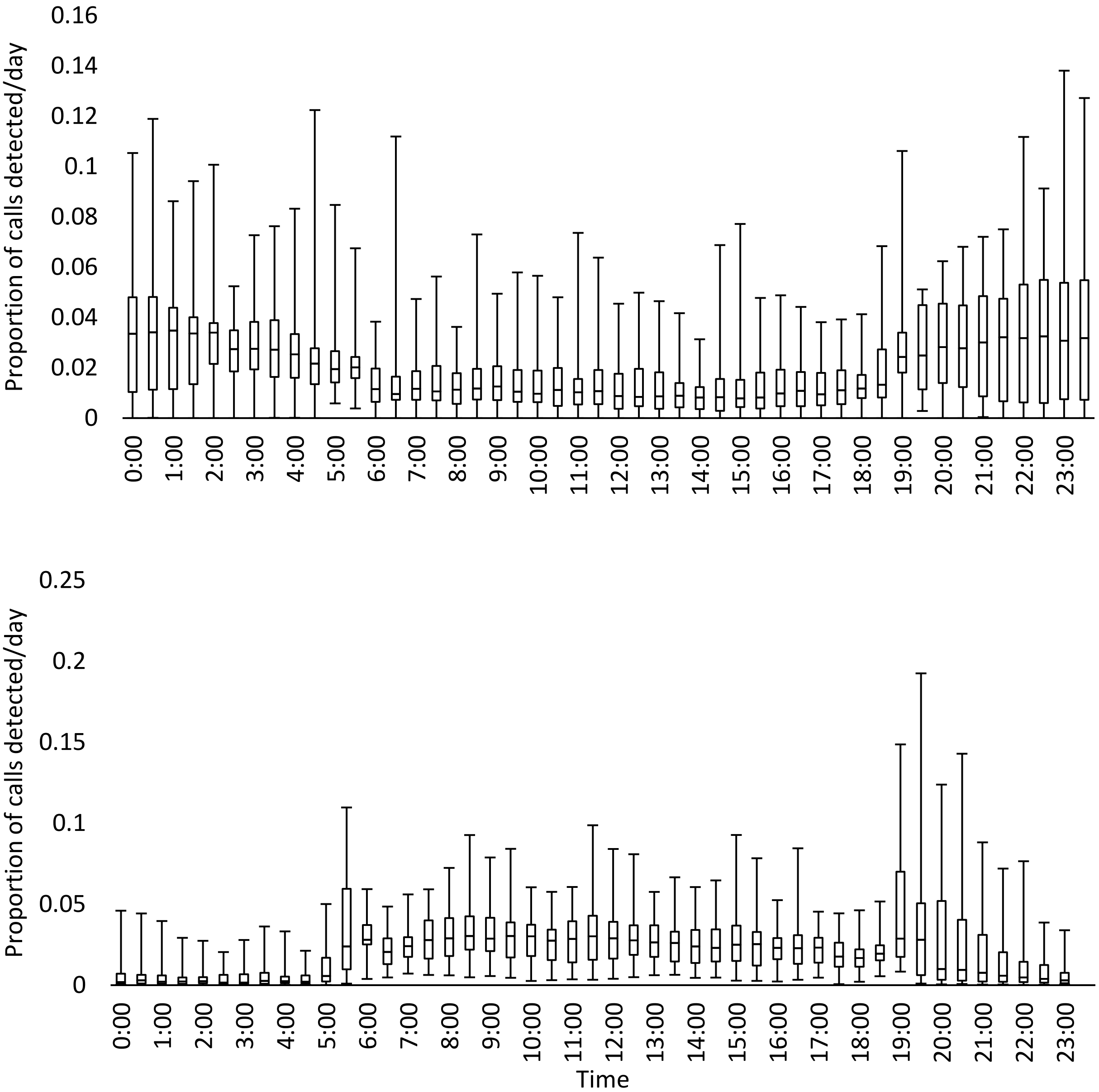

Figure 6. Relative daily phenology of micro frog calls at Sites One and Three. Each box plot represents the calls recorded during one half-hour period as a proportion of the daily number of calls. Top graph depicts Site One while bottom graph represents Site Three.

2. Population Estimate

Pond two was the largest pond of the three surveyed at 0.57 hectares. Pond one was the next largest at 0.21 ha while Pond three had an area of 0.15 hectares. The mean distance between ponds was 284.00 m [standard deviation 138.93]. Pond distances are displayed in Table 5.

Table 5. Distance from microphone array or human observer to nearby ponds (m). Ponds are represented as 1 through 5. Distance measurements were calculated from centre of array (Ponds one, two, and three) or human observer location (Ponds four and five) to nearest point of remaining ponds representing micro frog habitat. Distances were measured using Google Map data. All measurements are in meters.

	1	**2**	**3**	**4**	**5**
1	-	220	184	341	27
2	156	-	419	485	216
3	279	482	-	162	255
4	385	550	167	-	365
5	59	267	254	407	-

Effective Sampling Area

Effective sampling area did not overlap with any other ponds or micro frog calling habitat, suggesting that frogs were not double counted.

Density Estimates

Site Two initially had a higher density of micro frogs but was the first site in which the frogs stopped calling during the recording period (Figure 7). Micro frogs stopped calling in Site Two by 10 September. The recording area was dry by 19 September although the rest of the pond retained water. Site Three was the second site in which the frogs stopped calling with a recording on 19 September yielding no micro frog calls. The recorded sampling area was dry by 10 October. In contrast, Site One retained its water habitat for the duration of the study and micro frogs continued calling into mid-October. Frog calls late in the season were interspersed with lulls of silence.

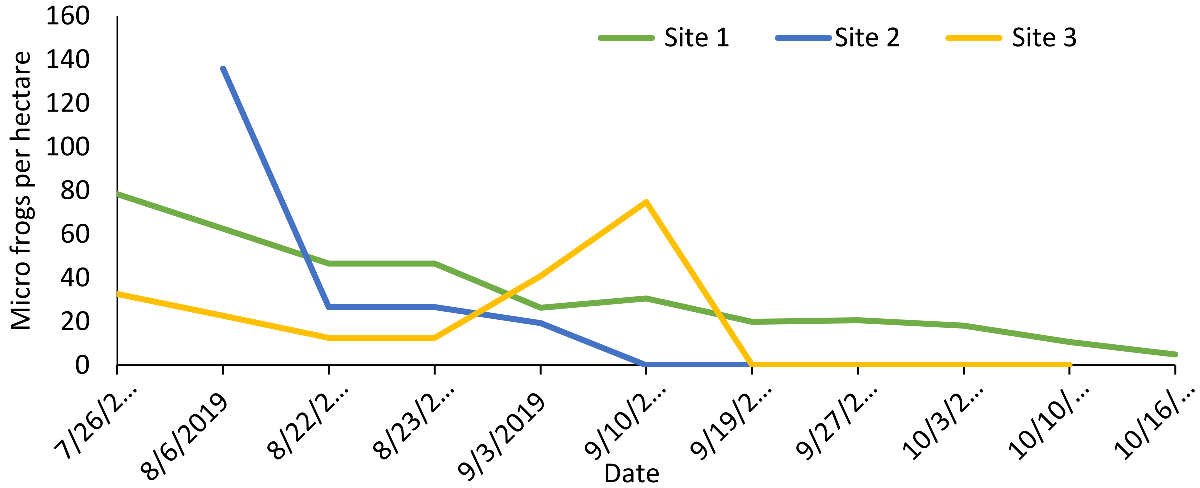

Figure 7. Population density estimates of micro frogs per hectare by site. Graph depicts median density values for each date.

Population Estimate

I calculated a total adult breeding population of 217 micro frogs in the Kenilworth Racecourse Conservation Area (Table 6). Site Two had the most frogs with a calculated total of 154 adults while Site Three had the least frogs of all the recorded sites at ten adult frogs. This population estimate was calculated by multiplying the density estimate of calling adult male frogs for each pond by the area of the pond and then multiplying that total by two to include females. These density estimates were calculated by aSCR from the call rate of the Cape Peninsula moss frog, as the call rate of the micro frog is unknown, resulting in an unknown bias. When recording, I selected for areas thought to be better quality calling habitat. As a result, these population estimates could be overestimating the number of frogs in the study area. Estimates from manual observations were used for sites four and five. Like the call density calculations, these observations of calling male frogs were also multiplied by two to include females.

Table 6. Conversion of population density estimates to population estimate of micro frogs. Density estimates are represented by the median estimate for that date. Effective Sampling area (ESA) and Standard Error are calculated only for the density estimates generated by aSCR. Estimates from manual observations were used for sites 4 and 5.

Site	Date	Density (frogs per ha)	Standard Error	ESA (ha)	Standard Error	Pond Area (ha)	Number of calling frogs	Total frogs including females
1	26/07/2019	78.40	26.36	0.16	0.03	0.21	16.40	33
2	6/8/2019	135.95	58.74	0.06	0.02	0.57	76.93	154
3	26/07/2019	32.66	189.78	0.09	0.33	0.15	5.01	10
4	8/8/2019	5	-	-	-	0.07	5	10
5	8/8/2019	5	-	-	-	0.03	5	10
TOTAL						1.02	108.34	217

3.Community Composition

In addition to the micro frog, I found the following frogs calling in the Kenilworth Racecourse Conservation Area during my study period of 26 July through 23 October: flat caco (*Cacosternum platys*), clicking stream frog (*Strongylopus grayii*), Cape rain frog (*Breviceps gibbosus*), and the Cape sand frog (*Tomopterna delalandii*).

For this study, I focused on flat cacos and clicking stream frogs as these were the most vocal species detected in the areas where the recording units were placed.

Call Frequency

The calls of micro frogs (2300 to 4900 Hz) and flat cacos (4600 to 7000 Hz) overlap in frequency, while the clicking stream frogs (2200 to 2700 Hz) call at a lower frequency than the other two species.

Density

In all five ponds that had micro frogs calling, all three studied species were present and calling during the day. Micro frogs tended to call in higher numbers than the other two although flat caco frogs called in similar numbers to micro frogs.

Effective Sampling Area

Effective sampling area varied between species. Clicking stream frogs often had the highest effective sampling area, although several recordings lacked enough calling frogs to calculate an effective sampling area. Micro frogs generally had the smallest sampling area estimates.

Flat Caco (*Cacosternum platys*)

Seasonal Variation

Table 7. Flat caco frog model selection table. Site was included in all models. Moon Phase represents percentage of moonlight visible.

Variables	Parameters	Log Likelihood	Delta AIC_c	Weight
Day length + Rain	6	-86.25	0.00	0.884899
Start time + Rain	6	-88.66	4.82	0.079522
Rain	5	-91.99	7.47	0.021089
Rain + Moon Phase	6	-90.58	8.67	0.011591
Rain + Temperature	6	-91.98	11.47	0.002862
Start time	5	-98.98	21.46	0.000019
Start time + Temperature	6	-97.75	22.99	0.000009
Day length + Start time	6	-98.56	24.62	0.000004
None	4	-102.66	25.33	0.000003
Start time + Moon Phase	6	-98.95	25.39	0.000003
Day length	5	-101.70	26.91	0.000001
Day length + Temperature	6	-101.45	30.41	0.000000
Temperature	5	-103.62	30.75	0.000000
Day length + Moon Phase	6	-101.66	30.82	0.000000
Moon Phase	5	-104.12	31.74	0.000000
Temperature + Moon Phase	6	-103.50	34.51	0.000000

Similar to the micro frog, the flat caco linear model selected the variables of day length and rainfall where the variable of rainfall represents a rainfall event two days preceding the recording sample (Table 7). This suggests flat caco frogs also exhibit increased calling behaviour early in the season and two days after a large rainfall event (Figure 8). It is worth noting, however, that very few recordings were conducted following rain.

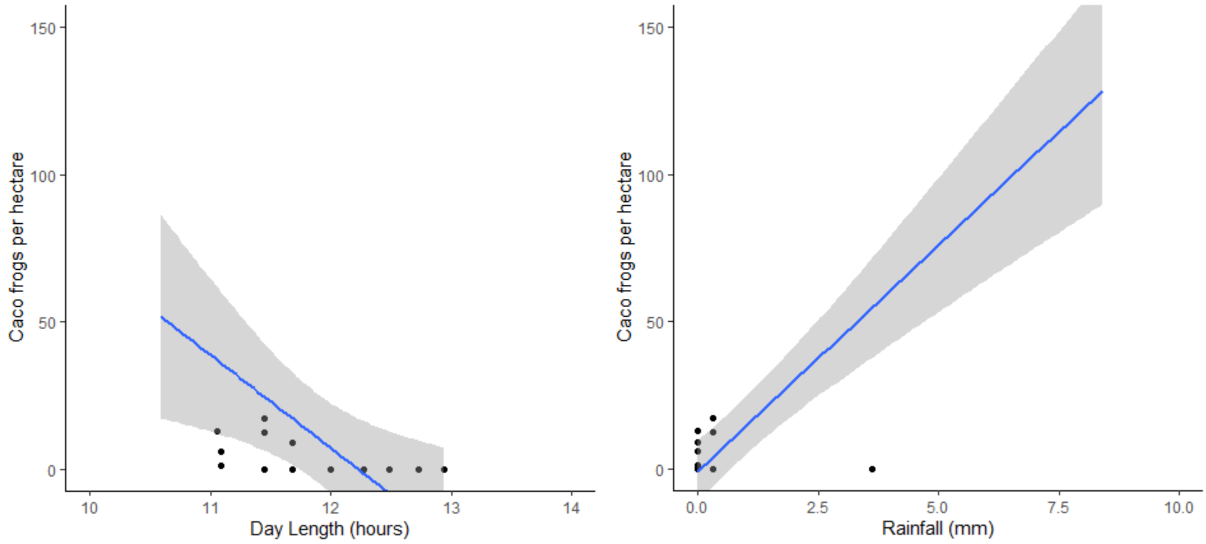

Figure 8. Linear model reflecting the influence of day length and rainfall on flat caco frog calling behaviour. Shaded area represents confidence interval. The line equation is Y=263.574+ -22.101 *Day length + 1.797*Site 2 + -4.472*Site3 + 13.903* Rainfall. The Adjusted R^2 value is 0.8017. The p value is 3.098e-06. The Durbin-Watson test statistic is 1.456761.

Diel Calling Patterns

Flat cacos recorded at Site Three showed a trend of increased calling behaviour during the day and decreased calling behaviour at night (Figure 9). However, at Site One, higher median call rates were found from 20:00 to 1:00 although variability in calling behaviour was highest during daytime hours.

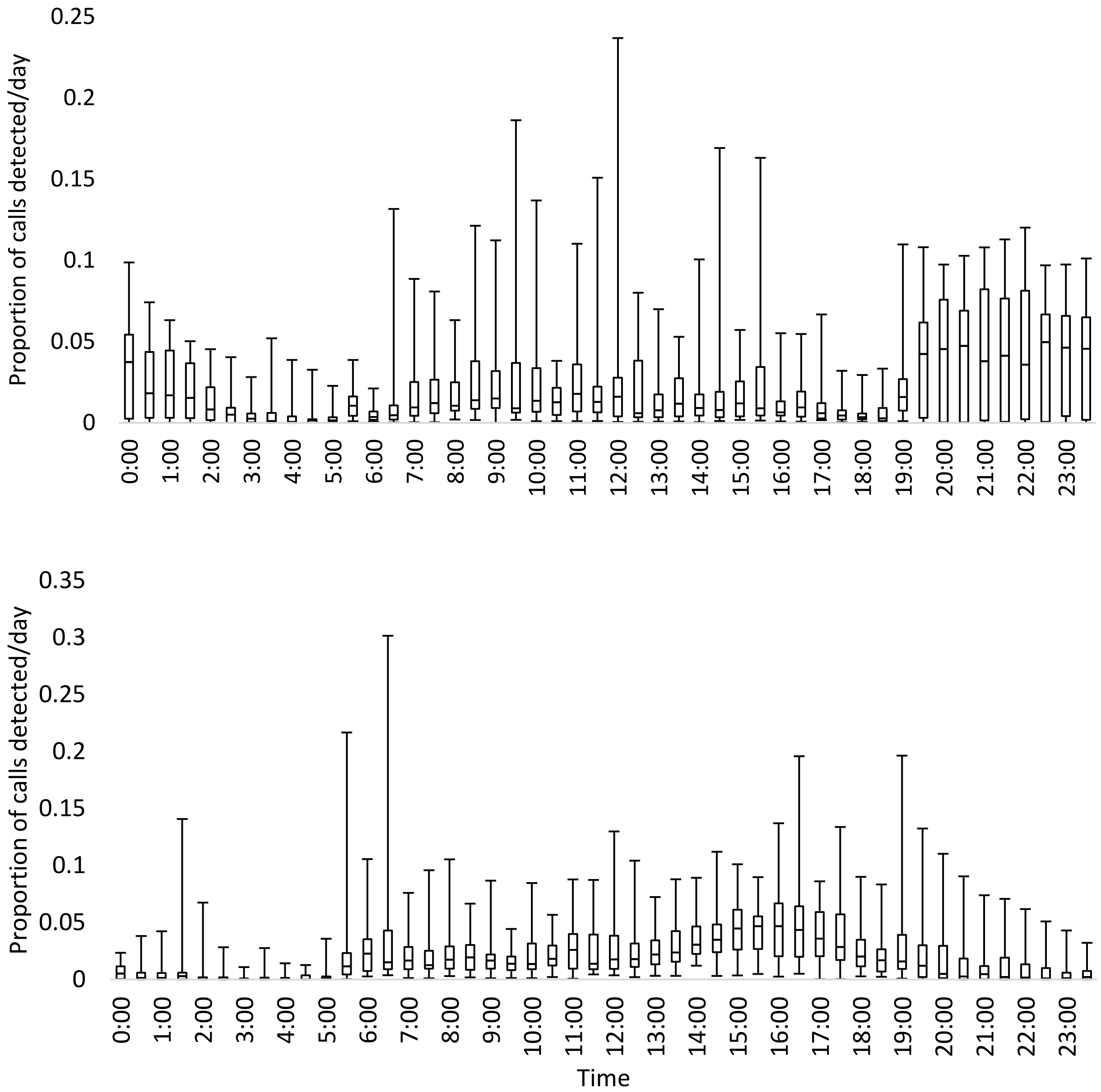

Figure 9. Relative daily phenology of flat caco frog calls at Sites One and Three. Each box plot represents the calls recorded during one half-hour period as a proportion of the daily number of calls. Top graph depicts Site One while bottom graph represents Site Three.

Clicking Stream Frog (*Strongylopus grayii*)

Seasonal Variation

Table 8. Clicking stream frog Model Selection Table. Site was included in all models. Moon Phase represents percentage of moonlight visible.

Variables	Parameters	Log Likelihood	Delta AIC_c	Weight
None	4	-102.66	0.00	0.388
Moon Phase	5	-102.19	2.55	0.109
Start time	5	-102.23	2.63	0.104
Temperature	5	-102.35	2.87	0.093
Rain	5	-102.61	3.39	0.071
Day length	5	-102.62	3.41	0.071
Start time + Moon Phase	6	-101.62	5.40	0.026
Temperature + Moon Phase	6	-101.89	5.95	0.020
Start time + Temperature	6	-101.92	6.02	0.019
Day length + Moon Phase	6	-102.09	6.36	0.016
Rain + Moon Phase	6	-102.16	6.49	0.015
Rain + Temperature	6	-102.16	6.49	0.015
Day length + Start time	6	-102.21	6.58	0.014
Day length + Temperature	6	-102.22	6.60	0.014
Start time + Rain	6	-102.23	6.63	0.014
Day length + Rain	6	-102.57	7.32	0.010

The selected model for the clicking stream frog was the null model (Table 8).

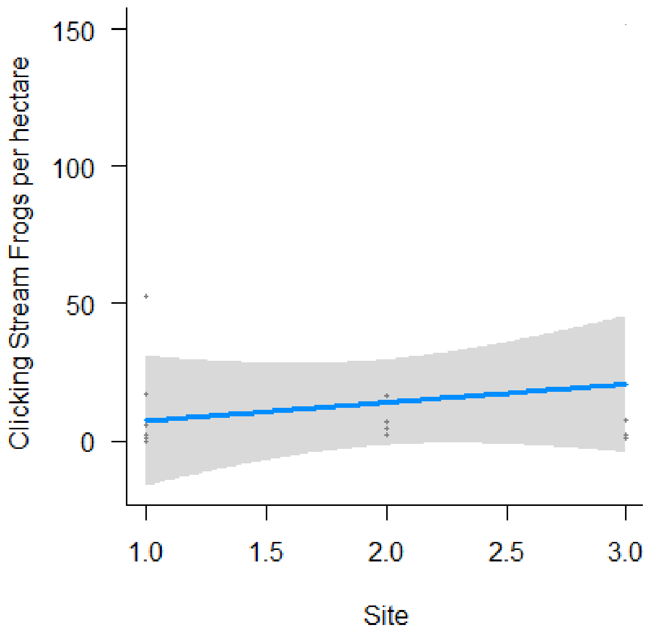

Figure 10. Linear model depicting the null model of different sites. Shaded area represents confidence interval. The line equation is Y=10.310+ -4.795 *Site 2 + 13.906*Site3. The Adjusted R^2 value is -0.04974. The p value is 0.5997.

Diel Calling Patterns

Clicking stream frogs appear to exhibit increased calling behaviour in the evening (Figure 11). Calls were at their lowest between the hours of midnight and 5:00 in the morning.

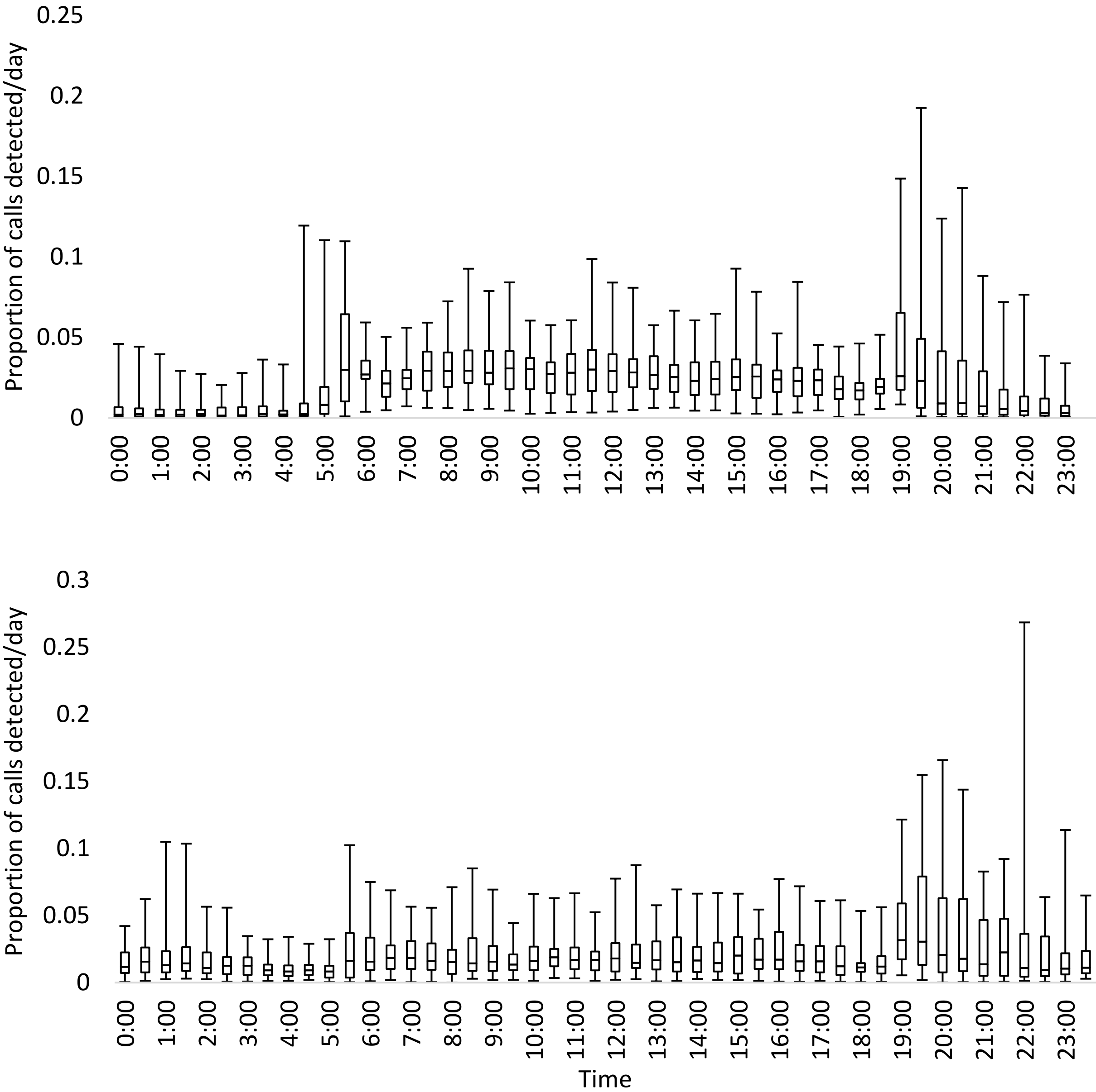

Figure 11. Relative daily phenology of clicking stream frog calls at Sites One and Three. Each box plot represents the calls recorded during one half-hour period as a proportion of the daily number of calls. Top graph depicts Site One while bottom graph represents Site Three.

Discussion

I found that micro frogs call more two days after rainfall, early in the recorded winter breeding season (July and August), and in Site One, after dark, indicating that these variables dictate the optimum time of monitoring micro frogs. I calculated a baseline total population estimate of 217 adult micro frogs which can be used to assess future population trends. I also found that micro frogs and flat cacos call in response to the same variables but often at different times of day from one another, suggesting that monitoring of micro frogs should take into consideration the calling habits of other species.

My finding that micro frogs and flat cacos are more likely to call after a rainfall event is consistent with other studies (Blankenhorn 1972; Yoo & Jang 2012), but unlike others on the Cape Peninsula (Measey et al 2017). This suggests that these frogs may be adapted to the short breeding seasons within these small rain-fed seasonal ponds. However, the limited number of rainfall events included in my analysis suggests that more studies may be needed to confirm this relationship. The clicking stream frog did not follow this pattern which could be a result of the fact that the eggs can pause their development and wait until sufficient rainfall occurs before hatching into tadpoles, decreasing the importance of rainfall to timed breeding events (Channing 2001).

I will address each of these objectives below.

1. Call Behaviour

Seasonal Variation

Seasonality is linked to the presence of water in the ponds at the Kenilworth Racecourse Conservation Area. The ponds are fed from the winter rains and typically fill with water in June and dry up by December (Hopkins 2006). My results indicate a seasonal effect on the calling behaviour of both the micro frog and the flat caco as represented by hours of day length. This is likely a result of the limited time available for breeding before the ponds dry up. Many other frogs have also been found to call in response to season or photoperiod, including a relative of the flat caco and micro frog, the Cape Peninsula moss frog (*Arthroleptella lightfooti*) (Brooke et al. 2000; Both et al. 2008; Canavero et al. 2008; Measey et al. 2017).

Effective Sampling Area

The effective sampling area varied widely from recording to recording. This is consistent with other surveys using this same method as Measey et al. (2017) also found large variation in the effective sampling area both within and between sites but found that this variation did not affect the calculation of call density estimates.

Diel Calling Patterns

Frogs have been found to vary the timing and frequency of their calls in response to heterospecific frog calls or other noise (Wong et al. 2009). As the purpose of a mating call is to attract a mate, any competing sound in the same frequency could mask one's calls and limit one's success (Wong et al. 2009). Calling is energetically demanding to frogs (Taigen et al. 1996), and therefore, to avoid wasting energy, frogs should only call when their calls can be heard. As the calls of micro frogs and flat caco frogs overlap in frequency, it is possible that they may be competitors in calling, and this may require them to call at different times from one another. At one site, I found that micro frogs call more at night while flat caco frogs tend to call more during the day. This could be a response of one species to the presence of another. At the other site studied, micro frogs called more in the evening while flat cacos called more in the afternoon. These site-specific temporal shifts in calling behaviour could be in response to different environmental variables such as anthropogenic noise or other species' presence. Future studies could investigate the calling behaviours of the species assemblages found in the other micro frog populations near Betty's Bay and Cape Agulhas, where the flat caco is replaced by other members of the genus *Cacosternum*, to estimate whether this is a species-specific adaptation.

The change in number of micro frog calls heard by the Song Meters at Site One (Appendix A) could be a result of the changed recording frequency settings on 19 September. However, the same settings were also applied to the Song Meter at Site Three (Appendix B) which does not display this same pattern. In addition, several of the Song Meter recordings contained some false positive detections picked up by the Song Meters in the form of birds, planes, and wind. Due to the sheer amount of data, I was unable to pick out all of these false detections. However, these sounds seemed to occur only during the day, suggesting that the night-time detections were accurate. Removing the false positives from the day-time detections would thus highlight the difference in day and night-time calling in micro frogs.

2. Micro Frog Population Estimate

Frog densities and calculated frog numbers varied from pond to pond with the largest pond (Site Two) containing the both the highest density and the highest number of frogs.

Effective Sampling Area

Effective sampling area did not overlap with any other ponds suggesting that frogs were not double counted. As dispersal generally occurs during the juvenile stage of development in anurans and frogs typically return to the same pond every year to breed (Semlitsch 2003), it is unlikely that any calling micro frog would move from one pond to another during the sampling period, further reducing the risk of double counting.

Population Estimate

I estimated a total of 217 adult frogs in Kenilworth. Due to the lack of information on sex ratios and survival rates on the micro frog, this calculation assumes a ratio of 1:1 between calling male frogs and non-calling females. This calculation is biased, but the direction of the bias is unknown. Many frog studies report a male-biased sex ratio at breeding sites (Swannack & Forstner 2007; Madsen & Loman 2010). While increased female mortality is a potential factor in some species (Madsen & Loman 2010), male bias at breeding sites is often a factor of delayed maturity in female frogs compared to male frogs (Swannack & Forstner 2007; Madsen & Loman 2010). In addition, this calculation does not take into consideration non-calling males but instead assumes all adult males call. If this assumption proves to be incorrect, these estimates would be biased low. Madsen and Loman (2010) found that up to 41% of female Common toads (*Bufo bufo*) opted to not breed each year while only five% of males sat out a breeding season on any particular year.

One of the assumptions of using aSCR to calculate adult frog density is that all adult male frogs call during the recording or that a known percentage of male frogs call during the recording (Dawson & Efford 2009). Therefore, for calculating the population estimate, I used the earliest recordings in the season, which had the highest number of male callers. Consequently, this estimate represents the population of calling males in Kenilworth on 26 July and 6 August 2019. If all the male frogs in Kenilworth were not calling during this recording, this estimate would be an underestimate of the actual adult male population size. However, if a known percentage of males were calling, the population size could be extrapolated from this data. These density estimates also rely on the call rate of the Cape Peninsula moss frog as a surrogate to the unknown call rate of the micro frog. As call rates can vary from individual to individual even within a species (Sullivan & Walsberg 1985; Kamath & Sreeker 2016), there is an unknown bias involved in using this call rate. This population estimate should be recalculated when the micro frog call rate becomes known. This population estimate assumes that calling frogs are equally spaced across the study area in a constant density. This does not seem likely, as calling habitat defined as vegetation at water level (De Villiers 2004) is found in shallower areas and on the perimeter of the pond more than in the deeper centre regions. Since I sampled in areas of better calling habitat, this estimate is biased high. However, this method can also be used to triangulate the exact locations of the frogs, allowing one to bypass this assumption. Another assumption of this calculation is that there are no calling adult frogs outside of the pond area. As micro frogs are reported to call while clinging to vegetation at water level (De Villiers 2004), it is unlikely that any frogs would be calling away from the pond, allowing for calculations to take into consideration the pond area alone. Despite all these assumptions, this number is more precise, with estimated error, than estimates gained from traditional monitoring and provides a baseline population estimate from which to assess future population trends.

Capture-mark-recapture surveys have generally been found to be reliable in estimating population density of anurans, although the method is not ideal for every species and requires ethical considerations on the handling and marking of individual animals (Nelson & Graves 2004; Grafe et al. 2011). Studies have determined monitoring via call counts shows the same population trends as those found by capturing animals (Fogarty & Vilella 2001; Nelson & Graves 2004). This method of aSCR, therefore, can be used as a reliable method for monitoring frogs to assess population trends. Additional studies could be performed on the micro frog population in Kenilworth using physical traps and capture-recapture techniques to estimate the proportion of calling males (Fogarty & Vilella 2001). This would allow a more precise recalculation of the Kenilworth population.

3. Community Composition

During the winter breeding seasons in 2003 and 2004, Hopkins (2006) reported finding micro frogs, flat cacos, clicking stream frogs, the Cape sand frog (*Tomopterna delalandii*), and the platanna (*Xenopus laevis*) breeding in a study pond within the infield of the Kenilworth Racecourse. I did not record the platanna, as it calls underwater, but I did find the other species she listed. I also found calls from the Cape rain frog (*Breviceps gibbosus*), which Hopkins did not consider in her study as this species develops directly from an egg into a frog without an intermediary tadpole stage (Hopkins 2006). I found fewer calls from the Cape sand frog than any of the three species that I studied. This is consistent with Hopkins' findings of fewer calls (2006).

Density

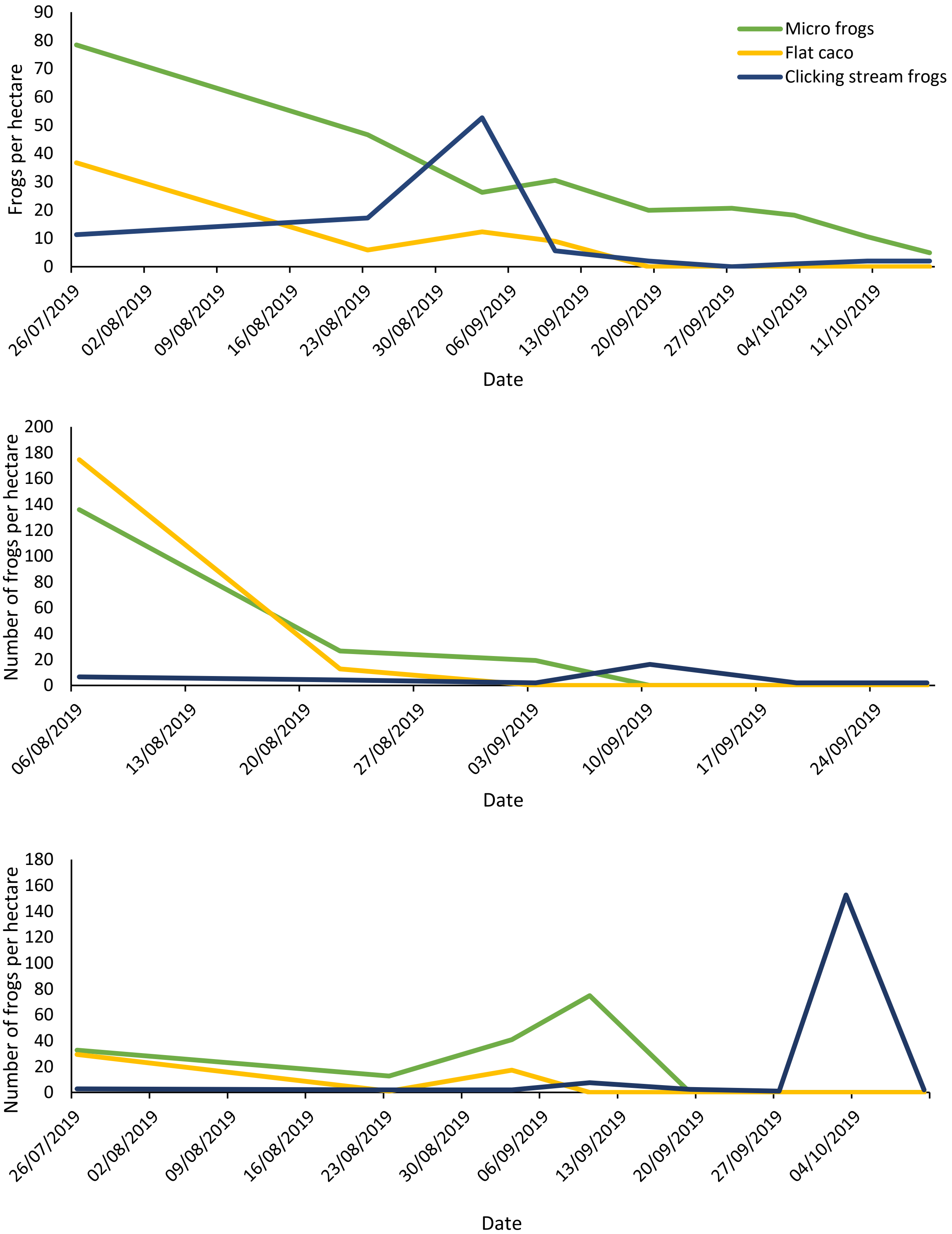

Figure 12. Median density of frogs measured in number of frogs per hectare for each recording date. The top graph represents Site One, the middle graph represents Site Two, and the bottom graph represents Site Three. Micro frogs are represented by the green line, flat caco frogs are represented by the yellow line and clicking stream frogs are represented by the blue line.

I found higher densities of micro frogs than flat cacos or clicking stream frogs within the studied ponds (Figure 12). This finding is also dependent on the assumption that the call rate of each of these species is similar to the estimated call rate of the Cape Peninsula moss frog. The relatively low numbers of clicking stream frogs calling could be a result of the lateness of the season as they are often reported as calling and breeding early in the season (Channing 2001; Hopkins 2006). Future studies should compare these findings with the other populations of micro frogs near Betty's Bay and Agulhas.

Clicking Stream Frogs

The linear model for the clicking stream frogs (Figure 10) selected for the null model. This suggests that the variance found in the calling behaviour of the clicking stream frog can be explained by the difference in the three sites. Channing (2001) states that clicking stream frogs along the southern coast generally begin calling in response to changes in temperature rather than rainfall. This is consistent with Hopkins' report that the clicking stream frogs in Kenilworth began calling in May or June before the pond even had water (2006). As I started recording in July, I would have missed the initial cue for the clicking stream frogs to start calling. This explains why my model results did not find temperature to explain much variation in clicking stream frog call densities.

Conservation Implications

Monitoring of Micro Frogs

Annual monitoring of micro frogs should use this population estimate as a baseline from which to assess population trends. This will allow for studies on whether the population is increasing or decreasing in Kenilworth as well as for demographic calculations such as carrying capacity or the minimum viable population. These data can also be used to measure the population of micro frogs' response to changes in habitat quality or management regimes such as burning of the neighbouring fynbos vegetation.

Ideally monitoring should occur early in the winter breeding season when the maximum number of frogs are calling. If possible, monitoring should be done 2 days following rainfall between 19:00 and 22:00 when calling behaviour increases. The usage of aSCR in future monitoring would allow for a more precise and comparable population estimate to the estimate I calculated. However, the analysis of the field data is time-consuming and may require a dedicated researcher, just like the traditional method of manual monitoring required a dedicated researcher.

Future research should be done to determine if micro frogs call more at the beginning of the season (before the beginning of this study) or if peak calling occurs around late July when this study began. It would also be of interest to determine if the greater number of calls detected at night is a function of more frogs calling or merely a result of the same frogs calling more frequently. This could potentially be determined by setting up an aSCR microphone array and triangulating the exact locations of the calling frogs. Alternatively, one could calculate a species-specific call rate for the micro frog for both daytime and night-time calling.

Translocation

Dodd and Seigel (1991) suggest that all translocation attempts should be preceded by a baseline population estimate and followed by regular monitoring at the introduced site. The population estimate of adult micro frogs in this paper can be used as this baseline population estimate for future translocation attempts. In addition, the method of aSCR can be used to monitor both the translocated population and the remaining Kenilworth population following translocation. Even if traditional methods are used, however, monitoring should follow the above recommendations of occurring at times of maximum calling behaviour, namely after dark, following a rainfall event, and towards the beginning of the calling season.

While Seigel and Dodd point out that most translocations of amphibians fail (2002), Germano and Bishop (2009) find that releasing over 1000 individuals leads to greater chances of success. Trenham and Marsh (2002) go so far as to caution that translocation of frogs should only be attempted if failure is considered an acceptable outcome of the translocation attempt. Keeping this in mind, conservationists of micro frogs face the difficult dilemma of deciding how many individuals can be potentially lost in a failed translocation attempt while realizing that translocating a greater number of individuals reduces that same risk. Despite this, it is important that consideration be taken to preserve the existing Kenilworth population. The Kenilworth population of micro frogs is significant in being the only remaining population left of the historical Cape Flats distribution as well as being genetically distinct from the Agulhas population from which it was estimated to have split one to 1.5 million years ago (Will 2005). In addition, Will (2005) recommended that any translocation attempts of micro frogs to Rondevlei Nature Reserve should rely on the Kenilworth population as a source population due to it containing a high amount of genetic variability while also being the closest historical population.

A previous translocation effort in 1999 of 140 tadpoles and three adults was unsuccessful in establishing a new population of micro frogs at Rondevlei (de Villiers 2004). A future attempt to translocate micro frogs to somewhere on the Cape Flats is being investigated (A. de Villiers 2020, personal communication) as Will (2005) recommends translocation efforts as necessary for the survival of micro frogs.

As translocation attempts of frogs often rely on tadpoles or eggs (Germano & Bishop 2009), knowing the population size of these for the micro frog would be useful. To calculate this from my population estimate, one would need to know how many females breed each year, how many eggs a female lays each year, and how many eggs hatch into tadpoles. A current literature search yields fragmentary information: eggs are laid in clusters of twenty (de Villiers 2004) but no mention is found as to how many clusters of eggs one female lays each season. Hopkins (2006) found only four tadpoles in Kenilworth, but a 1999 translocation effort involved 140 tadpoles from Kenilworth (de Villiers 2004). Further studies should attempt to fill in these gaps in our knowledge.

While Bubac et al. (2019) found that most post-translocation monitoring only lasts four years, Germano and Bishop (2009) recommend that monitoring should follow translocation for ten to 15 years to evaluate if the translocation was successful or if the population needs additional assistance. My findings can help create monitoring protocol for the translocated micro frogs.

Conclusion

The goal of this study was to improve monitoring efforts of micro frogs on the Cape Flats by determining the calling ecology, community composition, and a population estimate of the micro frogs in Kenilworth Racecourse Conservation Area. I have found that micro frogs tend to call more two days after rainfall, early in the winter breeding season, and after dark. Flat caco frogs also exhibit increased calling behaviour early in the winter breeding season and two days after rainfall but tend to call more during the daytime than the night-time. Assuming an even sex ratio and a constant call rate, I have found an estimated 217 adult micro frogs in Kenilworth Racecourse Conservation Area.

Monitoring of these species should occur at times of maximum calling behaviour. For the micro frog monitoring should occur between 19:00 and 22:00, early in the calling season, and after a rainfall event. The use of the method of aSCR for monitoring efforts would allow for consistent precise estimates that would clearly indicate population trends. Translocation efforts from Kenilworth Racecourse Conservation Area should take into consideration the current number of frogs in Kenilworth to avoid removing too many frogs from this population. Future studies should gather more demographic and survival data for this species to better improve translocation and management efforts.

Data Storage

Recordings are in the South African Environmental Observation Network (SAEON) database.

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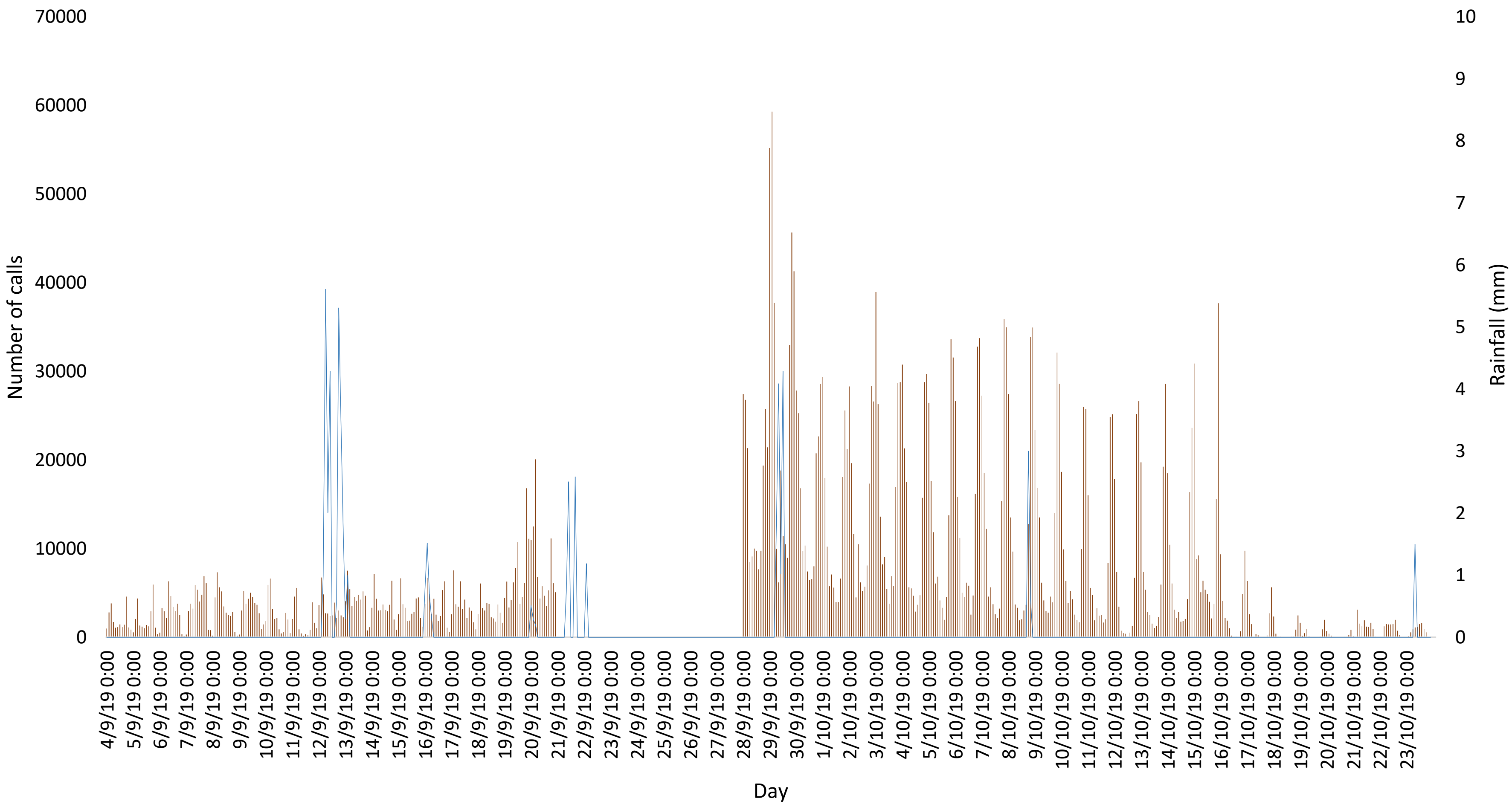
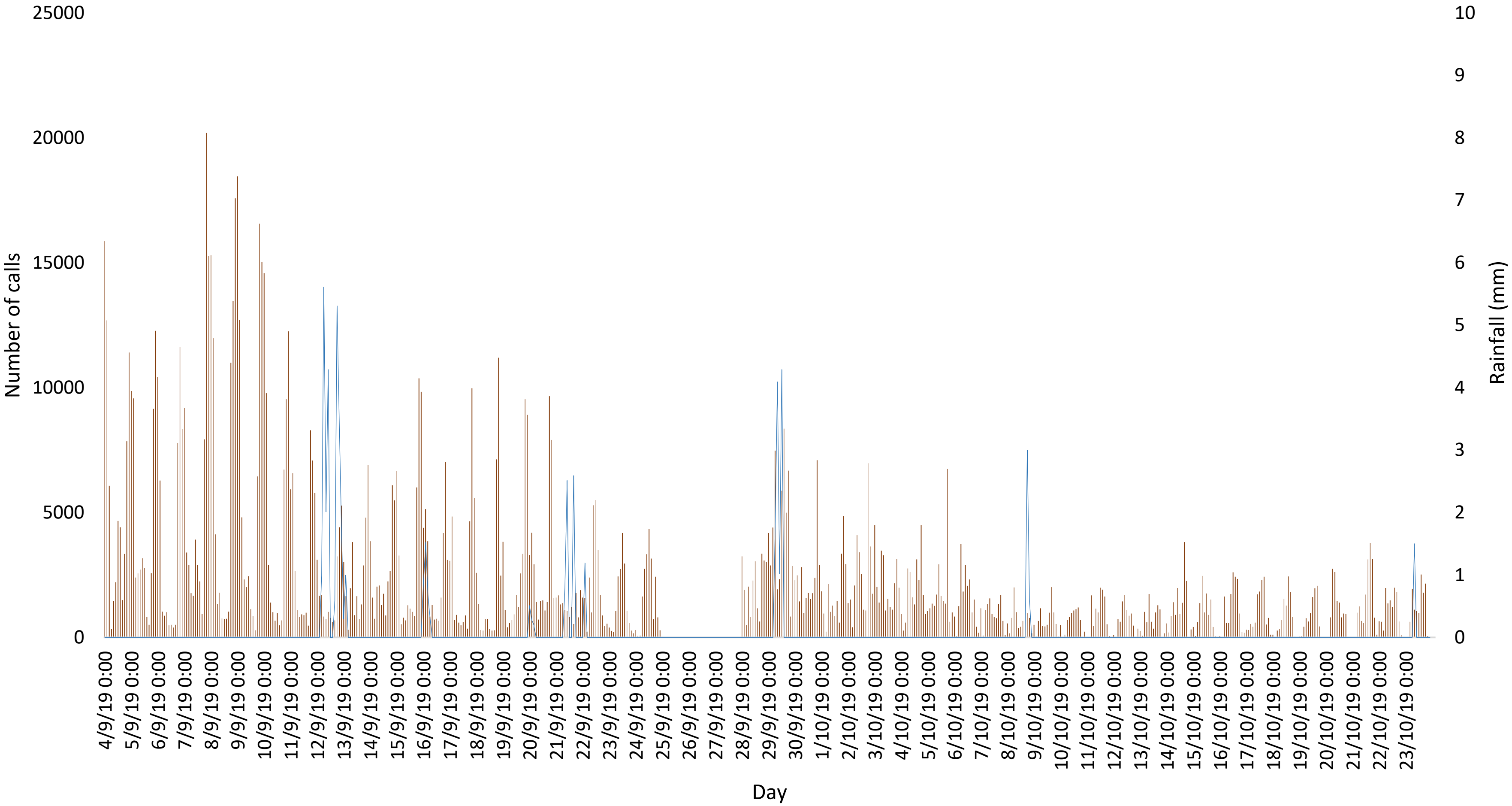
Appendix A



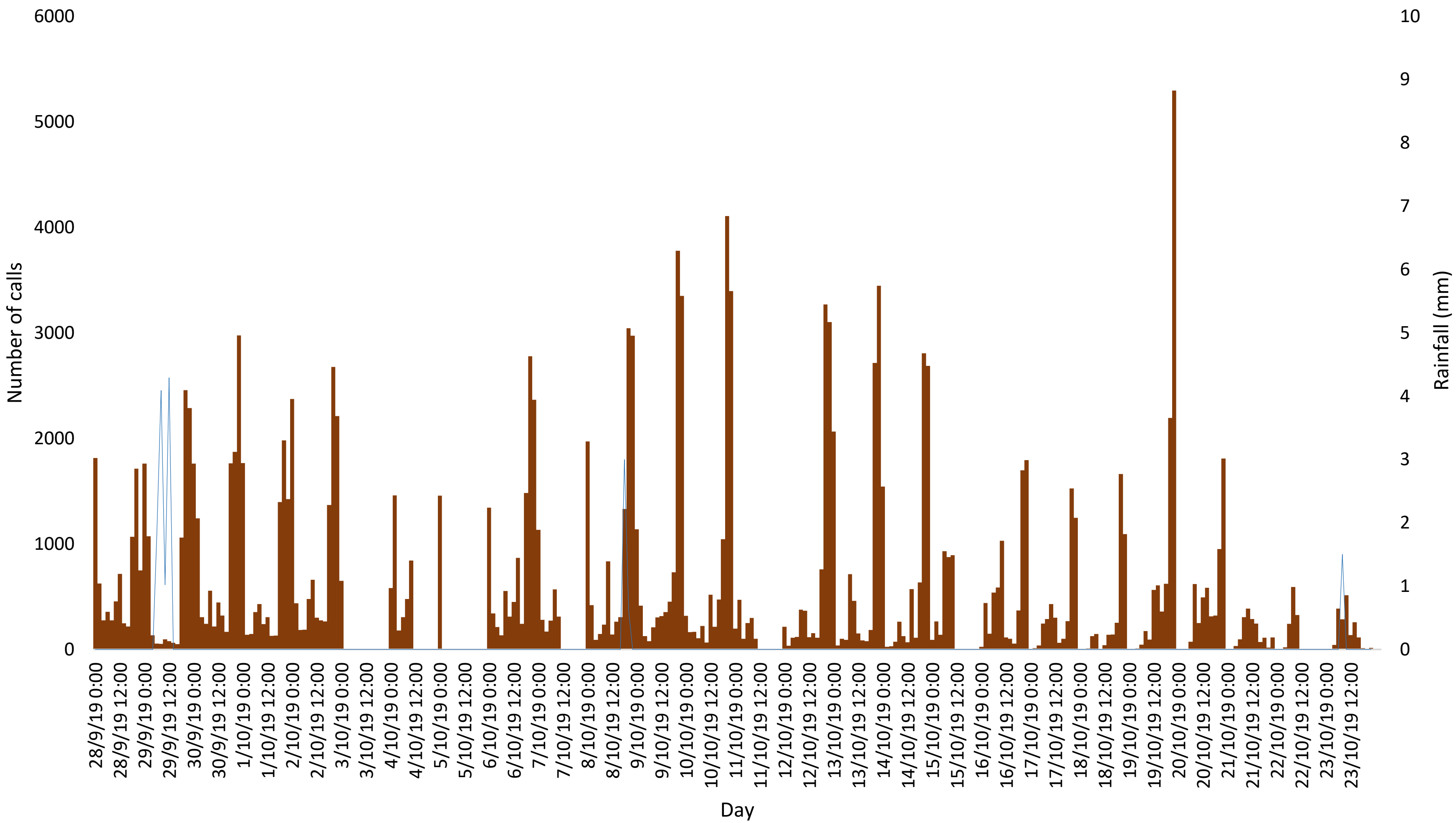
Histogram of micro frog calls across season as compared to rainfall in Site one. Each bar represents the number of frog calls in a two-hour period. Rainfall (blue) is represented as millimetres of rainfall in Wynberg over the same two-hour period. No data available for the dates of 21 through 27 September. Note: Recording frequency settings were changed from eight kHz to 20 kHz on 19 September.

Appendix B



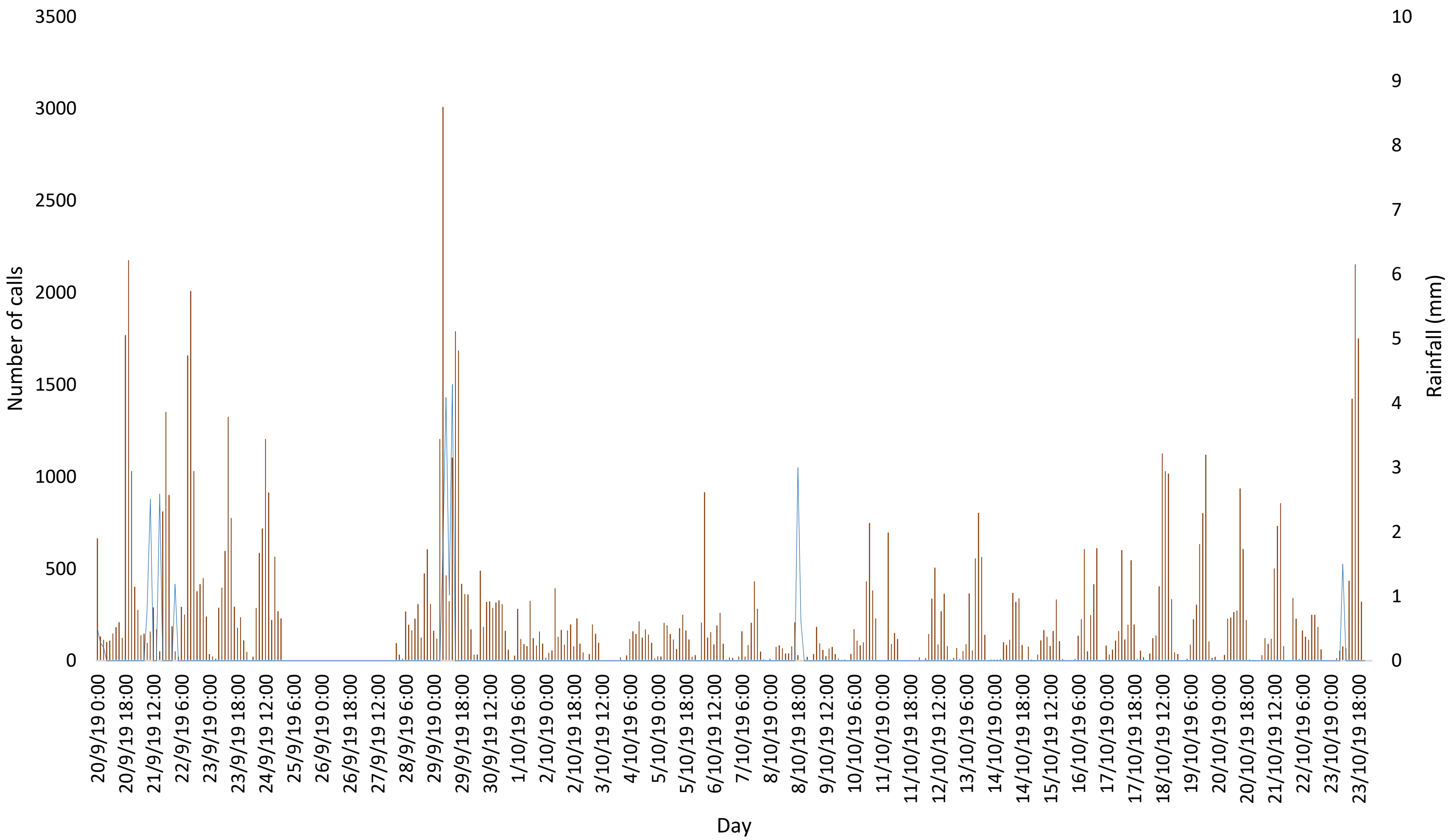
Histogram of micro frog calls across season as compared to rainfall in Site three. Each bar represents the number of frog calls in a two-hour period. Rainfall (blue) is represented as millimetres of rainfall in Wynberg over the same two-hour period. No data available for the dates of 25 through 27 September. Note: Recording frequency settings were changed from eight kHz to 20 kHz on 19 September.

Appendix C



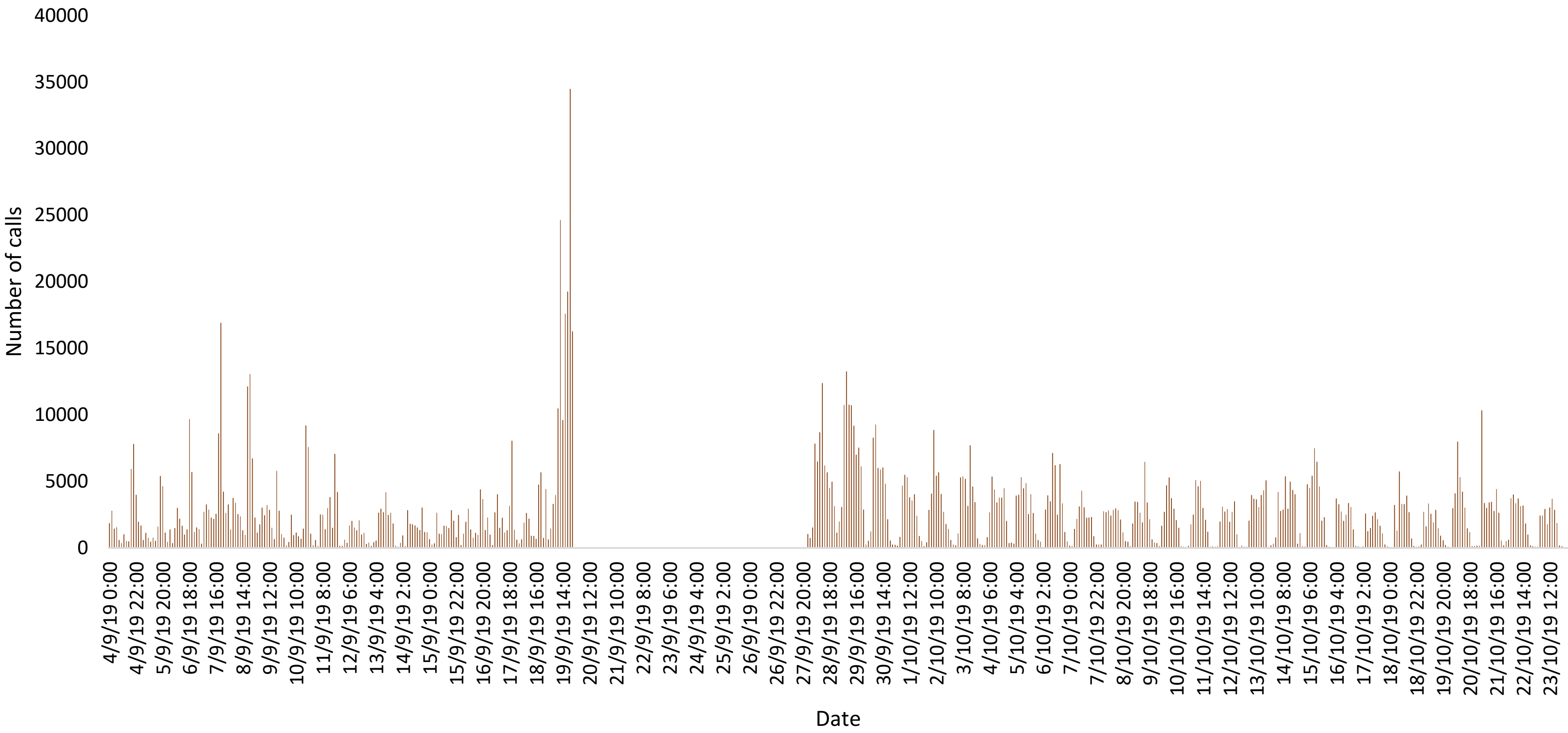
Histogram of flat caco frog calls across season as compared to rainfall in Site one. Each bar represents the number of frog calls in a two-hour period. Rainfall (blue) is represented as millimetres of rainfall in Wynberg over the same two-hour period.

Appendix D



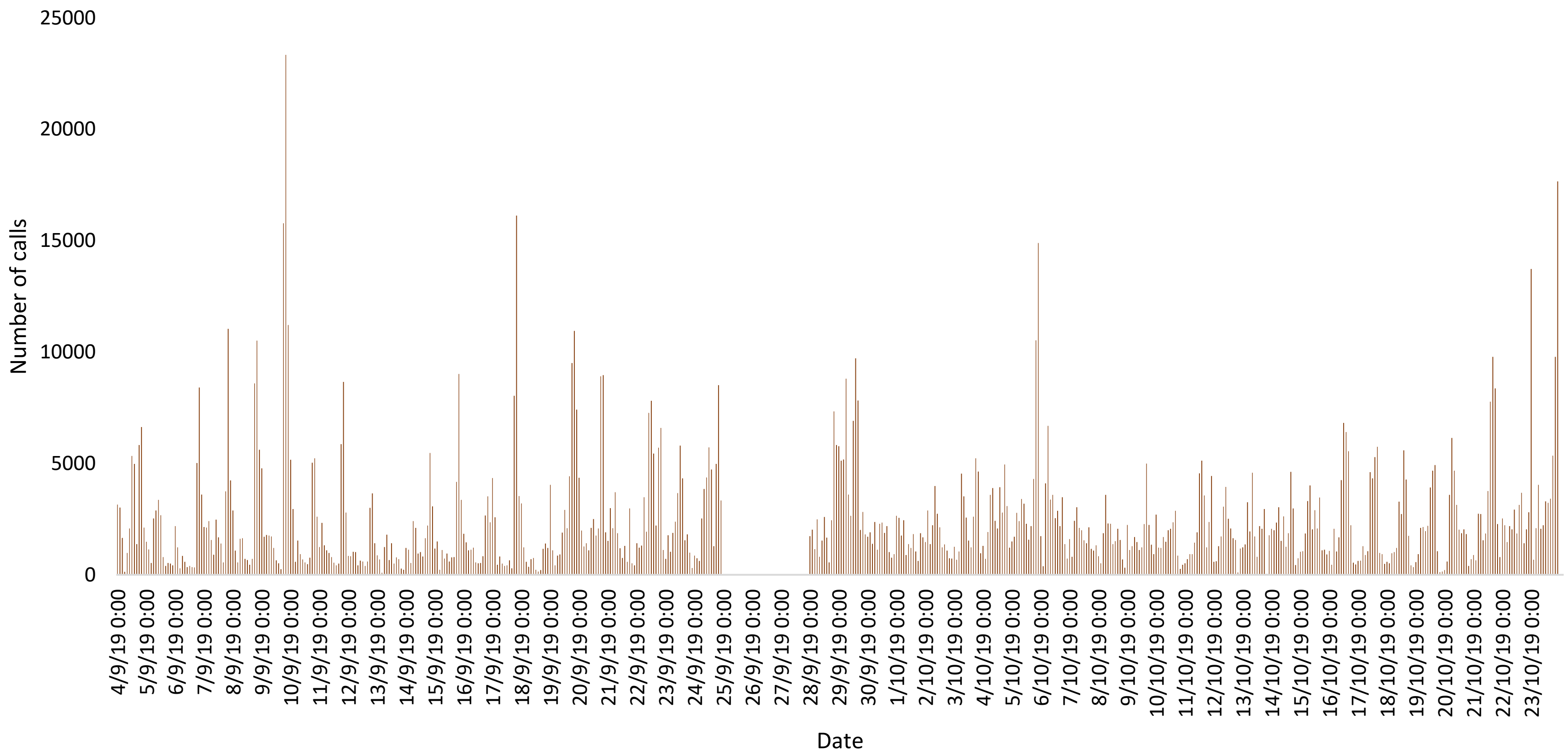
Histogram of flat caco frog calls across season as compared to rainfall in Site three. Each bar represents the number of frog calls in a two-hour period. Rainfall (blue) is represented as millimetres of rainfall in Wynberg over the same two-hour period. No data available for the dates of 25 through 27 September.

Appendix E



Histogram of clicking stream frog calls across season in Site one. Each bar represents the number of frog calls in a two-hour period. No data available for the dates of 21 through 27 September. Note: Recording frequency settings were changed from eight kHz to 20 kHz on 19 September.

Appendix F



Histogram of clicking stream frog calls across season in Site three. Each bar represents the number of frog calls in a two-hour period. No data available for the dates of 25 through 27 September. Note: Recording frequency settings were changed from eight kHz to 20 kHz on 19 September.

Appendix G

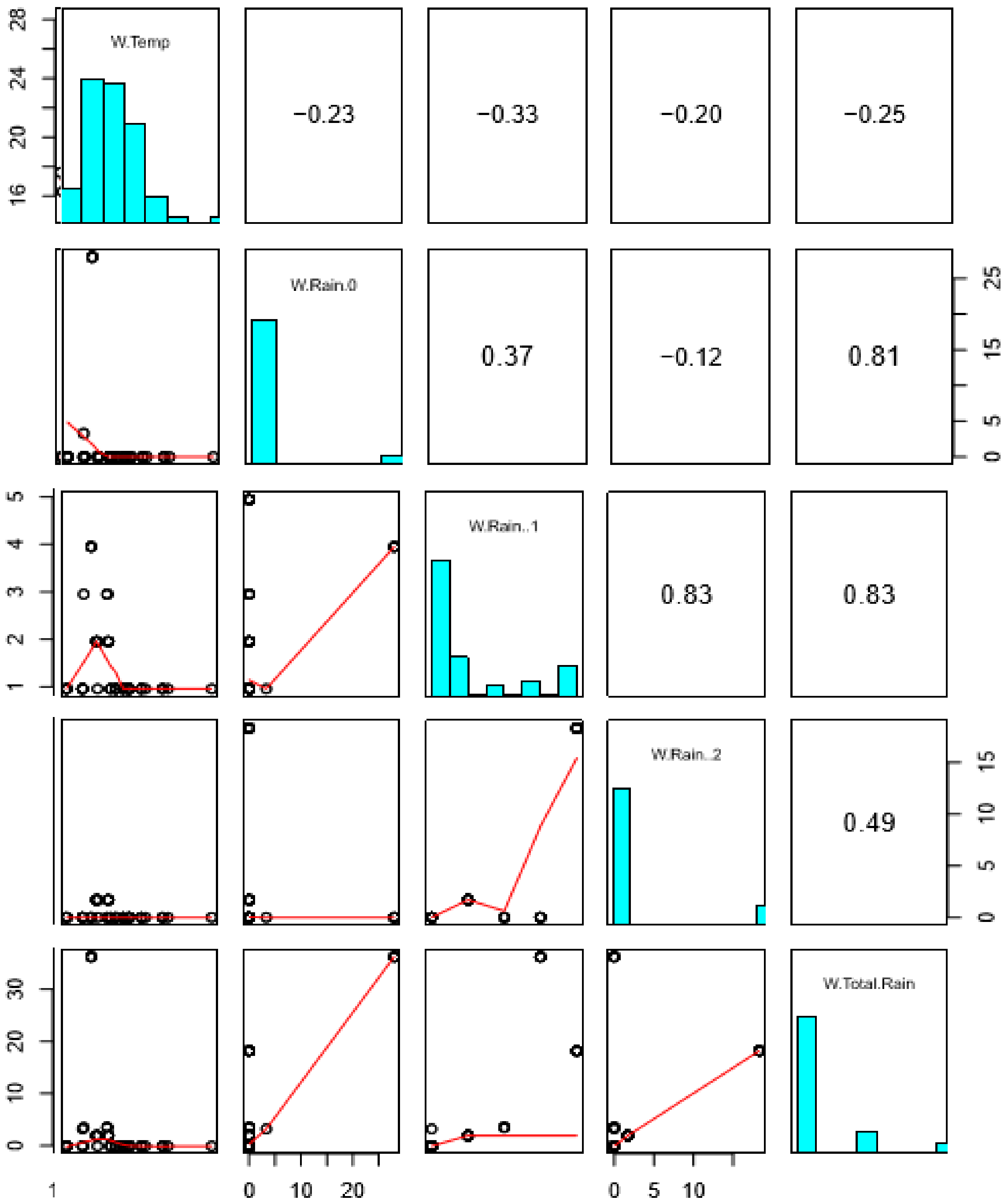


Table of collinearity comparing variables of temperature and rainfall. Variables chosen for this analysis were Temperature (W. Temp, top-most line) and Rainfall after 2 days (W.Rain.2, second from bottom). The cut-off value used for determining collinearity was 0.6.

Appendix H

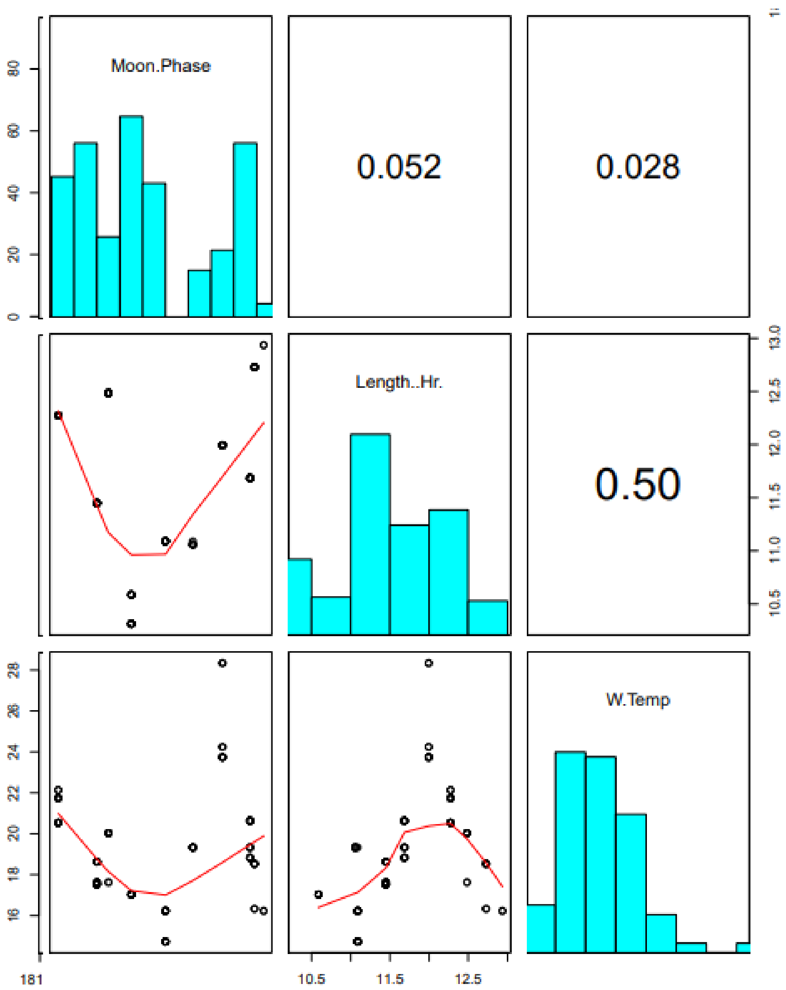


Table of collinearity comparing variables of moon phase (Moon.Phase), day length (Length.Hr), and temperature (W.Temp). The cut-off value used for determining collinearity was 0.6.