

Aspects of the breeding behaviour of Queckett's river frog (*Amietia queckettii*)

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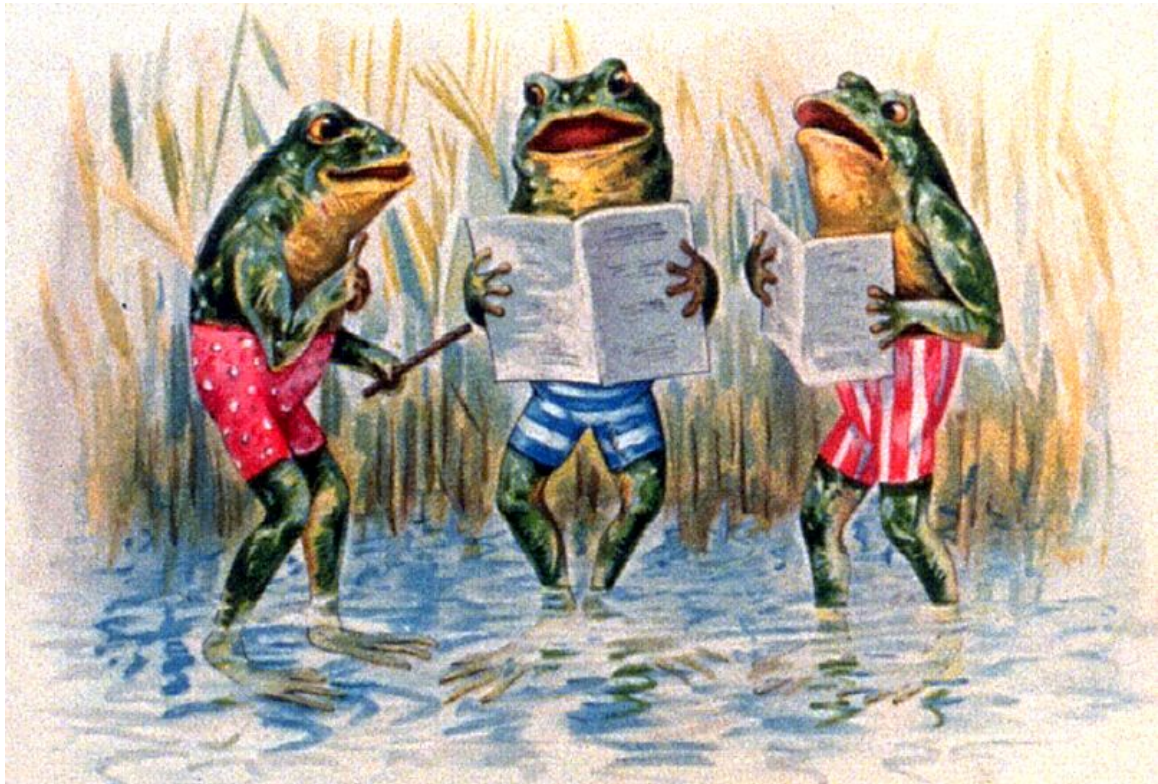
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This is dedicated to my long-suffering husband, my amazing parents and my awesome brother. Without all your love, support and encouragement I wouldn't have been able to get this far.



The chorus frogs in the big lagoon
Would sing their songs to the silvery moon.
Tenor singers were out of place,
For every frog was a double bass.
But never a human chorus yet
Could beat the accurate time they set.
The solo singer began the joke;
He sang, 'As long as I live I'll croak,
Croak, I'll croak,'
And the chorus followed him: 'Croak, croak, croak!
- A.B. Paterson

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Acoustic communication in anurans plays a critical role in species recognition, defending territories and resources, and finding a mate. Anurans use a number of different call note types to communicate, from which the most notable are advertisement- and aggression calls. Optimum weather conditions are a precursor to the commencement of the breeding season for all frog species and their calling behaviour is therefore readily influenced by atmospheric conditions. Male frogs within a chorus also tend to call from territories within a specific range of distances from one another. The objectives of this study were to 1) determine a possible context in which call note types are produced, 2) to determine the effect of atmospheric conditions on calling behaviour and 3) to determine the spacing distances between males and females in a chorus of Queckett's River Frog (*Amietia queckettii*). Pre-recorded note types were used in a playback experiment to determine a context for elicited responses. A context was derived for six of the responses. Advertisement (clicks and whines), aggression (creaks), encounter (tonal notes), territorial (whine-tonal notes), and release calls (squeaks) were described. Calls and atmospheric conditions were recorded and correlated for an entire breeding season. Water temperature, wind speed, humidity and barometric pressure had a significant effect on calling intensity. As water temperature decreased calling intensity increased, while increased wind temperature led to increased calling intensity. *Amietia queckettii* calls from the water, explaining the effect while increased wind speed decreases water temperature and can carry sound further. Both humidity and barometric pressure showed increased calling intensity only at specific levels. Humidity and barometric pressure have a direct effect on one another, which most likely causes the correlation between calling intensity and both these variables. In this study *A. queckettii* was shown to have breeding ponds for males and resting ponds and positions for non-gravid females. This prevents unwanted or unnecessary amplexus. Males showed much smaller and less variable territory sizes than females. This is most likely because males have a small range of optimal spacing distance while females move towards and away from males. The presence of vegetation resulted in smaller territories. This is possibly because smaller males act as satellite males and cannot be seen by larger males in vegetation. The size of males did not affect territory size. Males have a specific inter-male spacing distance regardless of size.

Keywords: *Amietia queckettii*, acoustic monitoring, atmospheric conditions, bioacoustics, breeding behaviour, call description, calling intensity, call repertoire, inter-male spacing

Akoestiese kommunikasie speel 'n kritiese rol by paddas veral vir spesieherkenning, verdediging van gebiede en bronne en om 'n wyfie te vind. Paddas gebruik 'n aantal verskillende roeptipes, meestal advertensie- en aggressiewe roepe om te kommunikeer. Optimale weerstoestand is 'n voorloper tot die aanvang van die broeiseisoen vir alle paddaspesies en dus word hulle roepsgedrag beïnvloed deur atmosferiese toestande. Die mannetjies in 'n koor is ook geneig om bepaalde afstande van mekaar af te roep. Die doelwitte van hierdie studie was om 1) 'n moontlike verduideliking vir 'n aantal van die roeptipes te bepaal, 2) die effek van atmosferiese toestande op die roepgedrag te bepaal en 3) die spasiëringafstande tussen mannetjies en wyfies in 'n koor van Queckett se Rivier Padda (*Amietia queckettii*) te bepaal. Opnames van roeptipes is gebruik in 'n eksperiment om 'n konteks te ontlok vir die verskillende roepe. 'n konteks is afgelei vir ses van die roepe, naamlik advertensieroepe (“klikke”), aggressieroepe (“krake”), ontmoetingsroepe (“toonagtige note”), territoriale roepe (“ween-tonale note”), en die vrylatingsroepe (“skwieks”) is beskryf. Roepe en atmosferiese toestande is aangeteken en gekorreleer vir 'n hele broeiseisoen. Watertemperatuur, windspoed, humiditeit en barometriese druk het 'n betekenisvolle uitwerking op die roepintensiteit gehad. As water temperatuur gedaal het, het die hoeveelheid roepe toegeneem, terwyl verhoogde windspoed tot 'n verhoogde roepintensiteit. *Amietia queckettii* roep vanuit die water, wat verduidelik hoekom water temperatuur so 'n groot effek het terwyl hoër wind spoed water temperatuur kan verlaag en klank verder kan laat versprei. Beide humiditeit en barometriese druk het 'n verhoogde roepintensiteit gewys, maar slegs by spesifieke lesings. Humiditeit en barometriese druk het 'n direkte invloed op mekaar, wat waarskynlik die korrelasie verduidelik, alhoewel die rede agter dit nie duidelik is nie. In hierdie studie was daar bewyse dat *A. queckettii* 'n broeidam het vir beide mannetjies en wyfies asook 'n rusdam vir nie-dragtige wyfies. Dit verhoed ongewenste of onnodige paring. Mannetjies het veel kleiner en minder veranderlike grondgebiede as wyfies. Dit is waarskynlik omdat mannetjies 'n optimale spasiëringsafstand het terwyl wyfies rondbeweeg tussen mannetjies. Die teenwoordigheid van plantegroei het gelei tot kleiner territoriums vir mannetjies. Dit is moontlik dat kleiner mannetjies optree as satellietmannejjies en plantegroei keer dat groter mannetjies hulle sien en verskrik. Die grootte van die mannetjies het geen invloed gehad op die grootte van 'n grondgebied nie. Mannetjies het 'n spesifieke afstand tussen mekaar gehandhaaf, ongeag hul grootte.

Sleutelwoorde: *Amietia queckettii*, akoestiese monitering, atmosferiese toestande, bioakoestiek, inter-mannetje spasiëring, paringsgedrag, roepbeskrywing, roepintensiteit, roep repertoire

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A chorus of frogs sang and thumped and snapped the elastics God had for some reason stretched in their throats. – Stephen King

1.1 Anuran vocal communication

Animals communicate in a variety of ways using visual signals and cues, pheromones and acoustic signals. Acoustic communication is used by a large number of taxa, including insects, fish, amphibians, birds and mammals (Ehret, 1980; Zelick *et al.*, 1999; Pollack, 2000; Kumar, 2003). These signals are used for three main reasons, namely for species recognition, to attract a mate, and to mark and defend resources such as territory (Ryan, 1988; McGregor, 1993; Jones, 1997). Acoustic signals are especially useful as they are detectable from a distance and can relay information such as location, identity and condition without requiring close contact (Endler, 1993; Tibbets and Dale, 2007; Wilkens *et al.*, 2012). Acoustic communication is used to intimidate any potential rivals and ward them off, but it is also crucial for reproduction as this is how males compete for and attract females (Garcia-Rutledge and Narins, 2001; Arch *et al.*, 2011).

1.2 Vocal repertoires of frogs and toads

Among vocalising vertebrates, frogs are one of the most well-known groups with a vast variety of calls. Acoustics are of vital importance to frogs as it is their primary means of communication in every aspect, specifically to attract mates, but also to intimidate rivals, defend territories and even ward off predators (Wells, 1977; Parris, 2002). Frogs produce vocalisations in order to convey a variety of messages such as body size, physical fitness and even quality of genes to conspecific males and females (Gerhardt, 1992). Calls are produced mainly by males and are considered either advertisement calls or aggression calls, though a variety of call note types can be used for either call (Klump and Gerhardt, 1992; Kime *et al.*, 2010). A number of call characteristics influence the effectiveness as well as aid in the distinction and preference of frog calls. These include pulse rate, dominant frequency, call duration and amplitude (Drewry and Rand, 1983; Lopez *et al.*, 1988).

Certain species such as *Dendrosophus ebraccatus* have complex calls in that they produce a single call which acts as an advertisement call and aggression call simultaneously (Wells and Greer, 1981). Other species such as *Engystomops pustulosus* produce more complex calls when a large number of males are calling, even though they are metabolically more expensive, as females prefer the complexity (Rand and Ryan, 1981). These calls are made more complex by adding a number of call note types. Note types can be classified onomatopoeically as “clicks” or “chucks”, or according to traditional rhythm definitions as “tonal” or “iambic”, where appropriate (Feng *et al.*, 2002).

Advertisement and aggression calls are sometimes easily differentiated as certain species produce a unique call for each, while it is more difficult in other species where the only difference is slight variations in call aspects such as call rate (Grafe, 1995; Marler *et al.*, 1995). Aside from advertisement and aggression calls, which all species produce, certain species also produce territorial calls, distress calls and release calls (Van Gelder *et al.*, 1978; Given, 1987; Wells, 1977; Bourne *et al.*, 2001; Emerson and Boyd, 1999).

1.2.1 Advertisement calls

Apart from the key function of attracting females to a breeding site, frog advertisement calls also serve a range of functions, including species identity, sexual receptivity, position, size and, in some cases, the individual identity of males in a chorus (Gerhardt and Huber, 2002; Wells and Schwartz, 2006; Backwell and Passmore, 1991; Garcia-Rutledge and Narins, 2001). Each of these functions plays an important role in calling behaviour and therefore in breeding.

Recognition of conspecifics is important to allow successful breeding and in frogs this ability is so finely honed that different species can call and breed within centimetres of each other without easily mistaking one species for the other (Backwell and Jennions, 1993). Frogs often alternate their calls to avoid interference and distortion. Playback experiments with *Engystomops pustulosus* have shown that females will prefer conspecific calls to any other, though closely related species may illicit a response as well (Ryan and Rand, 1993). Studies using the sibling species *Hyla versicolor* and *H. chrysoscelis* have shown that females from

both species show broadly similar preferences in mating calls (Bush *et al.*, 2002). Hybridization also occurs between anuran species, usually in multi-species choruses where opportunistic males will try and mate with any females of the correct size while females of related species seem to show no discrimination (Gerhardt *et al.*, 1994). Hybridized male offspring seem to produce a call similar to both parental species (as they are usually closely related, their calls tend to be similar to begin with) though being non-specific it may not attract any females, while female offspring would be attracted to the calls of either species (Haddad *et al.*, 1994). Cases of hybridized tree frogs have shown that males produce a unique call, distinct from either parent, which female hybrids prefer to either parent's calls (Doherty and Gerhardt, 1983). Though frogs are usually attracted and react to only conspecific calls, eavesdropping on the advertisement calls of other species can yield certain advantages. The calls of species that breed at the same time and place or species that are closely related like *Engystomops pustulosus* and *Leptodactylus mystacinus* can act as breeding cues for each other in multi-species choruses (Phelps *et al.*, 2007).

Female preference greatly affects the selection pressure for advertisement calls, as females would rather choose the males with more desirable calls than simply the males closest to them (Wollerman, 1998; Gerhardt *et al.*, 1996). Most female frogs prefer lower frequency calls as they can indicate a larger male which is usually considered more attractive (Ryan *et al.*, 1992; Ramer *et al.*, 1983). The females of certain species such as *Hyla versicolor* also prefer longer call durations as this is seen as a sign of genetic superiority (Gerhardt *et al.*, 2000; Welch *et al.*, 1998). Females of a number of species including *Dendrosophus microcephalus* and *E. pustulosus* also prefer more complex calls (Schwartz, 1987a; Ryan and Rand, 2003). Advertisement calls can also indicate sexual receptivity as hormones present during breeding times influence how calls are produced (by the males) as well as received (by the females), allowing females to choose the most receptive partner (Arch and Narins, 2009). Hormones can also alter which calls females find attractive, with female mate choices changing depending on the female's reproductive stage (Lynch *et al.*, 2006).

1.2.2 Aggression calls

Unlike advertisement calls, which are fairly easy to determine, aggression calls are usually rather implied as such based on context and reactions (Wells and Schwartz, 1984; Roy *et al.*, 1995). Aggression calls can trigger a number of responses, including response aggression calls, increased call rates, attack and, in certain cases, retreat (Bee and Perrill, 1996; Given, 1987; Wells, 1977). Though all species produce advertisement calls, aggression calls are more likely to be produced by prolonged breeders where males defend specific calling sites from rivals (Wells, 1977). Explosive breeders may call to attract females to a breeding site, but have no need to defend territories from rivals and as such have no need to produce aggression calls (Ryan, 1983).

Aggression calls are usually graded in the sense that males become more aggressive the closer an intruder comes and will react more aggressively to larger intruders as opposed to smaller ones (Grafe, 1995; Wagner Jr, 1989). Higher levels of aggression are indicated by an increased number of calls as well as an increased duration of calls (Wells, 1989). Certain species, e.g. *Hypsiboas faber*, actually produce varying aggression calls depending on the phase of the encounter, i.e. how close the intruder is (Martins *et al.*, 1998). These calls can be seen as defensive aggression calls to alert intruders (Van Gelder *et al.*, 1978). Other species like *Pseudophilautus leucorhinus* produce calls with increasing frequency and notes with both males matching the other (Arak, 1983). *Dendrosophus ebraccatus* produce more complex aggression calls in larger choruses, possibly to attract females at the same time (Wells and Greer, 1981). Many species, such as *Pseudophryne bibronii*, *Pelophylax lessonae* and *Pelohylax ridibundus*, and their hybrid *Rana temporaria*, also produce territorial calls which are considered a type of aggression call, specifically in the defense of territory (Wolkowiak and Brzoska, 1982; Brzoska *et al.*, 1977; Wells, 1977; Brzoska, 1982; Byrne, 2008).

Some frogs, like *Lithobates clamitans*, cheat when it comes to aggression calls and produce lower frequency calls than expected, which sends a false signal about size to potential rivals, i.e. they appear bigger than they really are (Bee *et al.*, 2000). *Acris crepitans blanchardi* have also been known to lower their call frequencies during aggressive encounters, but it was

found that this is less a deception and more a sign of size-independent fighting ability, which shows that aggression calls can signal more than what is expected (Wagner, Jr., 1992). When it comes to intruders in their territories, resident males tend to win during aggressive calling encounters, regardless of size. Size becomes a deciding factor during fights (Given, 1988).

1.2.3. Female vocal behaviour

As mentioned, choruses are mainly males calling to attract females, while females voicelessly choose a mate. This, however, seems to be slightly inaccurate as a number of species have quite vocal females, a trait which seems to have evolved independently numerous times (Emerson and Boyd, 1999). These vocalizations by females do not include the release or distress calls which all species produce (Boyd, 1992; Gans, 1973).

Female vocalization is used primary to attract the attention of males, which *Xenopus* females do by “rapping” to indicate impending oviposition (Tobias *et al.*, 1998). Females that vocalise do not necessarily produce identical calls to the males of their species, which can be heard in female *Odorrana tormota* whose calls have higher frequencies and harmonics, and shorter call duration than their male counterparts, as well as in the calls of the female *Lithobates virgatipes*, with higher frequencies and less distinct harmonics (Shen *et al.*, 2008; Given, 1993). Females that produce calls in response to male advertisement calls (or even to elicit them) seem to do so to elicit male phonotaxis and indicate fertile/gravid females (Shen *et al.*, 2008; Roy *et al.*, 1995). In very few species, such as *Eleutherodactylus coqui*, the females may also produce an aggression call while defending a retreat from both male and female conspecifics (Stewart and Rand, 1991).

1.2.4. Release and distress calls

As mentioned, excited males tend to grasp any females (and sometimes even males) they encounter that are the right size. In the case of unwanted clasping or amplexus, many species give a release call, designed to signal erroneous coupling and cause release (Boyd, 1992). This is advantageous to the clasping males as it prevents energy wasted in clasping the wrong sex or releasing sperm that will not be used (Marco and Lizana, 2002). Release calls tend to

be higher pitched and have a negative correlation to body size (Sullivan and Wagner, Jr., 1988). Gravid females produce arginine vasotocin, a hormone, which appears to inhibit release calls and allows mating to occur (Diakow and Raimondi, 1981; Tito *et al.*, 1999).

Many species of frogs appear to produce a distress call when annoyed, in danger or having been caught by a predator (Ridpath, 1977; Green, 1988; Van Gelder *et al.*, 1978; Gans, 1973). What differentiates distress calls from all other calls is that all frogs produce them, regardless of sex or sexual maturity (Kanamadi *et al.*, 1993; De Toledo *et al.*, 2009). Distress calls, also known as alarm calls, are usually only produced once a frog has been captured. A possible explanation for this is the predator-attraction hypothesis which states that the call (and any pheromones released at the same time) will attract more predators which will hopefully give the prey a chance to escape (Mathis *et al.*, 1995). Distress calls in general are made with the mouth open, with a wide frequency range and at a high frequency, potentially to have the signal spread as far as possible (Penna and Veloso, 1987; Gridi-Papp, 2008; Kanamadi *et al.*, 1993).

It should be noted that there is a distinct difference between a release call and a distress call. Release calls are only produced when erroneous or unwanted coupling occurs from another frog. This will not produce a distress call. Distress calls are only produced when a frog has been caught by a predator. This will not produce a release call.

1.2.5 Static and dynamic properties of frog calls

All frog calls have dynamic and static properties where the former are highly variable and may differ from call to call for a single individual, while the latter remain relatively constant throughout a breeding season for the entire species (Gerhardt, 1991; Opazo *et al.*, 2009). Different populations of the same species can show significant differences in dynamic properties, while static properties still remain the same, allowing for species recognition between populations (Ryan *et al.*, 1996). Dynamic properties are influenced over short (moment-by-moment) periods of time by the calling frogs as they compete to attract mates while static properties are influenced by female preference over much longer (evolutionary) periods of time (Martínez-Rivera and Gerhardt, 2008).

Static properties usually have certain constraints e.g. frequency is dependent on the size of the males; while dynamic properties are much more variable and flexible e.g. call duration that varies as a function of temperature (Castellano and Giacoma, 1998). Though the attractiveness of males is determined acoustically by both static and dynamic properties, it is suggested that static properties are more important for conspecific recognition while dynamic properties are more important for mate selection (Gerhardt, 1991). Though more focused on conspecific recognition, static properties are still influenced by female preference as it exerts directional or stabilizing selection on properties such as dominant frequency (females prefer lower frequencies as they indicate greater size) (Wollerman, 1998).

Dynamic properties used for mate selection can vary enough to effectively differentiate between individuals within a chorus, which also helps males identify neighbouring calls versus actual intruder calls (Gerhardt, 1991; Bee *et al.*, 2001). Because dynamic properties have such a large amount of variation, the recognition system (used for conspecifics) is very tolerant of variation in general and static properties make up a very small amount of the actual call produced (Wilczynski *et al.*, 1995). Dynamic properties are more important for mate selection because they are so variable which allows for female preference regarding certain properties such as inter-call interval (Bosch *et al.*, 2000). Dynamic properties can vary wildly from the mean values within a single night of calls, most likely for the benefit of female preferences, which also tend to vary (Gerhardt *et al.*, 1996). Because of their importance in mate selection and female preference, dynamic properties have a much larger influence on mating success than static properties (Pröhl, 2003).

1.2.6 Complex and extensive repertoires

Many species, such as *Physalaemus spiniger*, have extremely complex and varied repertoires, with *Boophis madagascariensis* currently holding the record at 28 distinct call note types (Costa and Toledo, 2013; Narins *et al.*, 2000). Variation in call types can be a result of different combinations of call note types, i.e. one call note type is not only used for one call type (Christensen-Dalsgaard *et al.*, 2002). Call types can include advertisement, aggression, territorial, release and distress, with variation possible in each one with regard to harmonics,

call duration, frequency and pulses per call, which leads to extensive repertoires (Costa and Toledo, 2013). As mentioned, females of the *Physalaemus* genus prefer more complex calls, a trait that seems to have been present in an ancestor as well, as the larynx is designed for complex calls, indicating a directional pressure for complexity (Ryan and Drewes, 1990). Complex calls seem to be reserved for advertisement in general and courtship in particular, again showing directional pressure from female preference (Owen *et al.*, 2006). More complex calls also make it easier for females to discriminate between calls and choose their optimal mate (Richardson and Lengagne, 2010).

1.3 Calling activity and species response to weather variables

Literature has shown that frog calling activities are correlated with atmospheric variables such as ambient and water temperature, humidity, wind, air pressure and ambient light (Blankenhorn, 1972; Obert, 1975; Woolbright, 1985; Banks and Beebee, 1986; Henzi *et al.*, 1995; Brooke *et al.*, 2000; Friedl and Klump, 2002). Atmospheric conditions act as cues for the commencement of the breeding season, which is advertised by calling males, and therefore affect calling behaviour (Moreira *et al.*, 2007; Schad, 2007). Aside from simply acting as cues, atmospheric conditions can also affect the acoustic physical properties of call propagation as they can cause attenuation and atmospheric absorption of sound, resulting in calling behaviour being dependent on the most favourable conditions for optimal signalling (Wiley and Richards, 1978).

1.3.1 Temperature, humidity and barometric pressure

Temperature plays a significant role in frog calling behaviour. Walker (1975) proposed a hypothesis, which states that there is a linear relationship between temperature and nervous system rates in poikilotherms. This was confirmed for frogs when it was discovered that calling rate is a linear function of temperature (Gayou, 1984; Wells *et al.*, 1996). The Walker hypothesis was found to hold true for both ambient and water temperature and thus the effect remains the same whether frogs call from water or not (Radwan and Schneider, 1988). However, the role that temperature plays becomes less important when temperatures are

above the required threshold, at which point other atmospheric conditions become more significant (Oseen and Wassersug, 2002).

Due to their permeable skin and tendency to dehydrate easily, the availability of moisture, especially in the form of humidity, can be an important limiting factor for any activities (Cree, 1989). Frogs prone to dehydration are less likely to call during dry periods, as calling is metabolically expensive and dehydration lowers their aerobic metabolism (Pough *et al.*, 1983). Humidity also acts as a breeding cue, because high levels of humidity mean lower levels of evaporation and thus a better chance of survival for frog eggs and larvae (Hauselberger and Alford, 2005). Furthermore, humidity levels also affect the acoustics of calls as resonant transmission, like that produced when frogs call, are more efficient in humid air (Harris, 1966).

It is well known that animals react to a drop in barometric pressure and frogs are no different (Wuethrich, 2000; Cryan and Brown, 2007; Heupel *et al.*, 2003; Schofield *et al.*, 2010; Brooke *et al.*, 2000). Calling behaviour increases with a drop in barometric pressure, usually because this signals approaching rain (which is especially important for species that breed in areas with distinctive wet and dry seasons), though it may also just signal a general change in weather and therefore other significant atmospheric conditions e.g. a cold front approaching (Obert, 1976; Hauselberger and Alford, 2005; Oseen and Wassersug, 2002).

1.3.2 Other meteorological variables

The majority of frog species are significantly influenced by rainfall when it comes to breeding, and thus calling behaviour (Blankenhorn, 1972; Henzi *et al.*, 1995; Zina and Haddad, 2005). Rainfall plays such a significant role because it can greatly affect the survival of both eggs and larvae (Telford and Dyson, 1990). Frogs who spawn in temporary pools are obviously more dependent on rainfall, because breeding would not be possible if not for the rain (Byrne and Roberts, 2004). However, rainfall is just as important for frogs breeding at permanent water bodies indicating that it might act as a cue for favourable conditions and to initiate calling and breeding behaviour for certain species (Oseen and Wassersug, 2002; Silva *et al.*, 2005).

High wind speeds are not favourable for frog calling behaviour or acoustics (Larom *et al.*, 1997; Waxler, 2004). For frogs prone to dehydration, high wind speeds result in less calling due to higher evaporation rates (Henzi *et al.*, 1995). High wind speeds have also been found to have a negative correlation to calling activity due to the fact that calling is energetically costly and high wind speeds cause high levels of signal distortion (Steelman and Dorcas, 2010). Looking at the effect on acoustics, irregularities in wind structure such as sudden gusts can be a more important factor in sound attenuation over short distances and short periods of time than any other weather variable (Ingård, 1953). High wind velocity can also cause wind shear, which greatly affects call directionality (Larom *et al.*, 1997).

Ambient light and light intensity have also been known to affect anuran calling activities (Blankenhorn, 1972; Oseen and Wassersug, 2002). Light intensity is an important factor in signalling breeding times and thus initiating calling activity for certain species (Pengilley, 2010). Certain species have been found to call more on moonlit nights as it is easier to spot predators such as bats, which locate the frogs by their calls (Tuttle and Ryan, 1982). Other species prefer the dark to avoid detection by predators and dim ambient light will even influence female choice by making closer males a safer choice regardless of how attractive they are (Baugh and Ryan, 2010).

1.3.3 Winter-breeding species

As frogs are poikilothermic, few species prefer to breed during the winter. Some species only breed during winter as these months are the only ones that provide adequate moisture/precipitation for breeding, while others make use of year-long water availability to reduce competition for resources such as space (Saenz *et al.*, 2006).

Winter-breeding species may be more strongly influenced by weather variables such as temperature, humidity and rainfall to determine optimal migratory and breeding times as these tend to fluctuate more during the winter (Pechmann and Semlitsch, 1986; Kirilin *et al.*, 2006). This also results in unpredictable breeding habitats, which favour dispersion and migration of juveniles (Caldwell, 1987). Many species do, however, have certain adaptations

such as faster embryonic development and lower embryonic temperature tolerance (Licht, 1971). *Rana sylvatica*, one of the most well-known winter-breeding frogs, is actually freeze tolerant and produces hepatic glycogen which is converted to glucose which then acts as a cryoprotectant when the frog freezes (Costanzo *et al.*, 1998). Many winter-breeding species such as *Rana aurora aurora* and *R. pretiosa pretiosa* prefer to call from the water rather than from perches, as water temperatures tend to fluctuate less than ambient temperatures (Licht, 1969).

1.4 Effects of calling behaviour on male density

1.4.1 Inter-male spacing

Calling males prefer to have their own personal space from which to call as this allows them to defend prime calling locations and to prevent other males from stealing a potentially interested female. This makes male density and spacing extremely important for calling behaviour (Klump and Gerhardt, 1992). Inter-male spacing is generally not observed among explosive breeders, but rather among prolonged breeders where their very calling, oviposition or courtship sites are a part of what makes the males attractive and so is defended constantly (Wells, 1977).

Inter-male spacing is not done randomly; males distribute themselves to ensure each individual receives the most benefit possible (Brenowitz, 1989; Tárano, 2009). Males have also been shown to effectively space themselves so that the call of a neighbour is above the resident's own auditory threshold, thus preventing any incoming females from being distracted by a rival (Gerhardt *et al.*, 1989; Murphy and Floyd, 2005). Ideal inter-male spacing distances are most likely determined by the amplitude of a neighbour's call, as calling is the most effective recogniser of neighbours and signal to others (Wilczynski and Brenowitz, 1988; Brenowitz *et al.*, 1984). Males also tend to space themselves to prevent an overlap of their calls as much as possible, with greater inter-male spacing resulting in less call overlapping (Schwartz *et al.*, 2002). Prevention of call overlapping can be very important for certain species such as *Hyla microcephala*, where females prefer non-overlapping calls (Schwartz, 1993). Call overlapping can also result in females of certain species, like *Hyperolius marmoratus*, not wanting to approach the caller or not even being able to locate

him (Grafe, 1996). Most choruses alternate calls. This allows individuals to gauge neighbours' call intensity, which in turn can facilitate inter-male spacing (Schwartz, 1987b). There is also some selection pressure towards adequate male spacing as females of certain species prefer wider spaced calls (Telford, 1985).

1.4.2 Chorus attendance

The importance of adequate spacing distances can be seen in species where the removal or addition of just a few males has been shown to affect entire choruses in both call rate and duration, both of which can enhance the attractiveness of calls (Whitney and Krebs, 1975; Schwartz *et al.*, 2002). Changes in calling rate and duration can also reduce the chance of call overlapping (Klump and Gerhardt, 1992) in increased aggregates, while in decreased aggregates it can allow for a leading call in the chorus (Greenfield and Rand, 2000). A number of species also show site fidelity among males during their entire breeding season, returning to and defending the same sites every night, while females wait at indiscriminate resting sites and only move towards males when gravid (Ringler *et al.*, 2009).

Larger choruses of species such as *Crinia georgiana* with less inter-male spacing distance available, can cause smaller, less attractive males to stop calling and just become satellite males with the hope of intercepting females (Byrne and Roberts, 2004). Satellite calling is a form of sexual parasitism by which smaller males stop calling, but position themselves close to larger calling males in the hope of intercepting any interested females (Forester and Lykens, 1986). It is usually seen in species such as *Epidalea calamita* (formerly known as *Bufo calamita*), where mating success is directionally proportionate to body size and calling intensity (Arak, 1988). This is a very useful tactic for species such as *Uperoleia rugosa*, which can form extremely dense choruses, where satellite males do not elicit any aggression from calling males, which prevents costly aggressive interactions from either side (Robertson, 1986). However, satellite males are also opportunistic as in the case of *Hyla cinerea*, where they will gladly start calling if the larger calling male moves away (Perril *et al.*, 1982). It should be noted that, though denser choruses provide a large benefit to both males and females by providing a larger opportunity for males to mate and more options for females to choose from, it also comes with a cost in that higher chorus densities mean higher

risk of predation for both males and females, which has been observed for *Physalaemus pustulosus* and their vast number of predators (Bernal *et al.*, 2007; Ryan *et al.*, 1981).

1.4.3 Aggressive encounters

Aside from competition, adequate distance between neighbours also means less chance of aggressive encounters as aggressive tendencies have been shown to decrease as distances between males increase (Brenowitz and Rose, 1994). Certain species such as *Hyla regilla* produce encounter calls when an intruder's call reaches a threshold amplitude, meaning it is getting too close (Rose and Brenowitz, 1997). Encounter calls, which are usually modified advertisement calls, are useful in deciding whether to start producing aggression calls and attack an advancing intruder and so increases the time spent attracting females (Whitney, 1980). As mentioned, males show a graded response with regard to aggression, with distance and size of nearest neighbours playing the most important roles. However, for certain species such as *Rana clamitans* size is not always a decisive factor and the graded aggression response may be used to reduce the costs of an aggressive encounter (Owen and Gordon, 2005). Again, call amplitude plays a large role as species like *Pseudacris crucifer* show a positive correlation between the amplitude of the nearest neighbour's advertisement call and the amplitude which triggers an aggressive response (Marshall *et al.*, 2003). Identifying individuals plays an important role in male-male communication in these cases as it allows residents to distinguish the calls of their neighbours from the calls of intruders and possible rivals (Bee *et al.*, 2001). Even without considering amplitude, nearest neighbours of species such as *Rana nicobariensis* still influence calling behaviour, where the type of call produced and its complexity depend on the call produced by the nearest neighbour (Jehle and Arak, 1998). It should be noted that in certain species such as *Rana catesbeiana*, further neighbours also influence calling behaviour. Where nearest neighbours may inhibit calling, further neighbours may promote it (Boatright-Horowitz *et al.*, 2000). However, when limited space is available, inter-spacing distances will become less important (Whitney and Krebs, 1975; Dyson and Passmore, 1992). Though inter-male spacing distances will become smaller in high density choruses, the spacing will become more regular (Ovaska and Hunte, 1992). Males of *Acris crepitans* will even tolerate and ignore intruders early in the breeding season, though they will become aggressive towards the end (Burmeister *et al.*, 1999). Inter-male

spacing and aggressive encounters because of it seem to be restricted to the breeding season in many species such as *Rana clamitans* (Shepard and Lannoo, 2004).

1.4.4 Vegetation “bunkers”

For many species such as *Rana virgatipes*, ideal calling sites include vegetation such as submerged shrubs, which provide hiding spots from predators (Given, 1988). Vegetation also provides shelter and nesting areas for insects providing food for a number of frog species such as *Pseudis bolbodactyla* (Brandao *et al.*, 2003). Along with providing safety and food, certain species such as *Rana sylvatica* lay eggs under or attached to vegetation (Egan and Paton, 2004). These reasons make calling sites with vegetation highly sought after and heavily defended. Calling from beneath or surrounded by vegetation also causes sound attenuation (Wells and Schwartz, 1982). Because of this, males in dense vegetative calling sites may require smaller territories as their calls degrade faster (Roithmair, 1992).

1.5 Focal species

This study will focus specifically on *Amietia queckettii*. This frog was first described as *Amietia angolensis* in 1866, but after a molecular-, advertisement call- and morphological study by Channing and Baptista (2013) it was declared a separate species. *Amietia queckettii* is found all over southern Africa where it lives on the banks of permanent water bodies. They can be heard calling throughout the year, but their breeding season is from late May until middle September during the dry winter, when calling increases (Du Preez and Carruthers, 2009). Though this frog was described more than a hundred years ago, very little is known about its breeding and calling behaviour.

1.6 Research aims and objectives

The principle aim of this study was to form a better understanding of the breeding behaviour of *A. queckettii* by looking at its calling behaviour and the different factors that influence it. The reason calling behaviour was investigated for this study is because calling and breeding

behaviour are fundamentally linked; neither will exist without the other (Mendonça, 1985; Marler and Ryan, 1996).

This study had the following main objectives:

- To elucidate the context in which the different calls are produced by the males of *A. quecketti* as described by Kruger (2014), by means of playback stimuli (Chapter 3).
- To investigate the influence of six meteorological variables on the calling activity of *A. quecketti* (Chapter 4).
- To investigate whether spacing is influenced by sex in *A. quecketti* (Chapter 5).
- To investigate whether the absence or presence of vegetation in a male's territory affects inter-male spacing distance in *A. quecketti* (Chapter 5).
- To investigate whether the size of an individual affects inter-male spacing distance in *A. quecketti* (Chapter 5).
- To examine the effect of male density (distance to nearest male) on the calling intensity of *A. quecketti* (Chapter 5).



2.1 Study Area

Fieldwork for this study was performed in the North-West University Botanical Garden in Potchefstroom. The garden contains a number of ponds, varying in size from 1m^2 to about 200m^2 , supporting a large population of *Amietia queckettii*. Frogs were studied in the two largest ponds, Ponds 1 and 6, but individual females in Ponds 2, 3 and 5 were used for tissue sampling (Figure 2.1). Three different aspects of breeding behaviour were studied and divided into the three result chapters, namely 1) describing call note types and investigating call responses to playback stimuli, 2) determining the effects of atmospheric conditions on calling behaviour during a breeding season, and 3) determining the effect that male density (distance to the nearest male) has on calling behaviour and intensity.

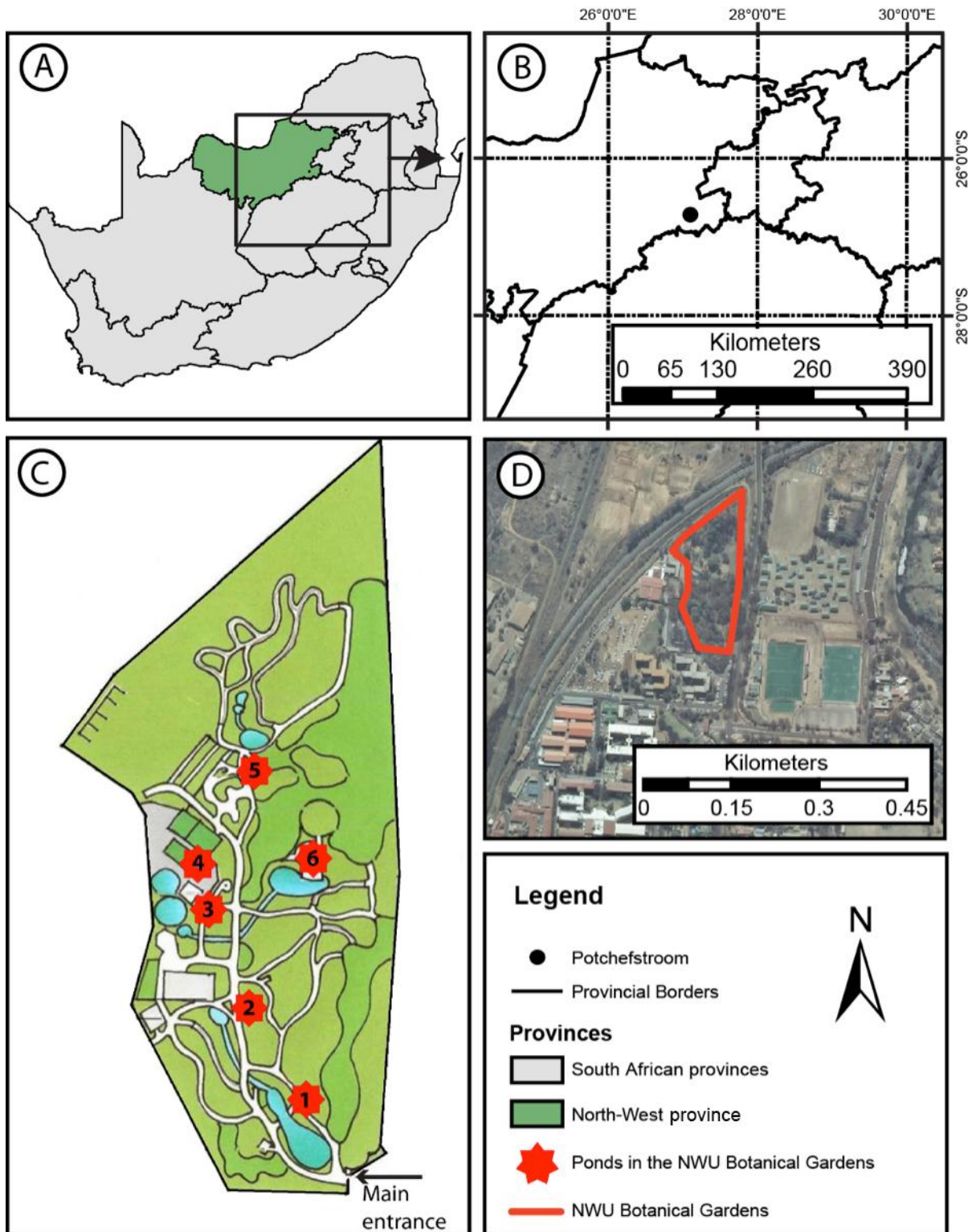


Figure 2.1 A map of South Africa (A) showing the location of Potchefstroom in the North-West Province (B) and of the North-West University Botanical Garden, showing the locations of the six ponds (C), supplemented with an aerial image of the garden (D).

2.2 Molecular analysis

In order to confirm the identity of the *Amietia* population in the North-West University Botanical Garden, fragments of the 16S mt gene were amplified from the tissue samples of two adult female specimens. Toe clippings for tissue samples were taken from two individuals in the NWU Botanical Garden. The clippings were preserved in 70% molecular ethanol and used for DNA extraction. Extraction was performed using the KAPA Biosystem Express Extract Kit and the manufacturer's instructions were followed. Mitochondrial (mt) 16S gene fragments were then amplified using the primer pair 16 SaR-F and 16 SbR-R. PCR reaction mixtures contained the PCR mastermix, primer, supernatant solution and PCR-grade water to provide a total of 25 μ l. Cycling conditions were set at 95°C for 90 seconds followed by 34 cycles of 45 seconds of denaturation at 95°C, 45 seconds of annealing at 51°C, 90 seconds of extensions at 72°C and a 5 minute final extension step at 72°C. PCR amplifications were confirmed using an agar gel. A fragment of approximately 600 base pares of the mt 16S gene was amplified for each of the samples. Resulting sequences were edited by generating chromatogram-based contigs using the Geneious (Ver. 7.1) bioinformatics software package (Biomatters, available from <http://www.geneious.com>). Sequences were matched to existing Genbank sequences and were entered into the Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/>) to confirm their identities. Additionally, 10 comparative *Amietia* species sequences were obtained from GenBank. A Maximum Likelihood phylogenetic tree was constructed in Geneious, with 1000 bootstrap replicates and based on the General Time Reversible + Gamma model (GTR+G) identified in jModelTest 2.1.5 (Posada, 2008), based on having the lowest Bayesian information criteria relative to other models.

2.3 Playback experiments

2.3.1 Playback experiment: Population responses

To investigate the context in which the different call note types are produced by the males of *A. quecketti*, six playback stimuli were used and the note type responses of individuals were documented. Pre-recorded calls were used for playback experiments at Pond 1 over a period of four nights (12/08/2014 to 15/08/2014) during the breeding season between 23h00 and 02h00 when the frogs are most vocally active (Chapter 4). Four different pre-recorded call note types were used from one male, namely, a click note, a whine note, an iambic note and a creak note (recorded on 15 September 2014 at an ambient temperature of 14°C and water temperature of 18°C). Train noise (30 seconds) and a recording of a running river (10 minutes) were used as additional stimuli to determine whether they had any effect on the ratios in which *A. quecketti* produce different call-types. Oscillograms and spectrograms of each stimulus can be seen in Figures 2.2 – 2.7. A portable DB Opera 110 Mobile speaker (Model K162, 65 Watts) was used to propagate stimuli at a constant sound pressure level (SPL) of 100 dB.

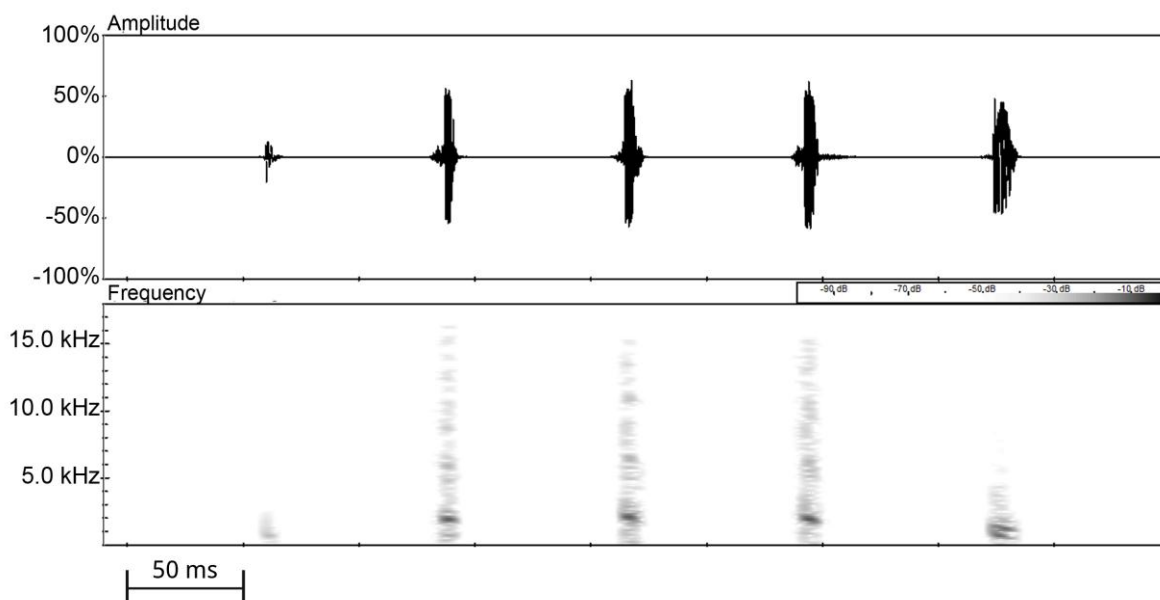


Figure 2.2 The spectrogram (bottom) and associated oscillogram (top) of the click note stimulus.

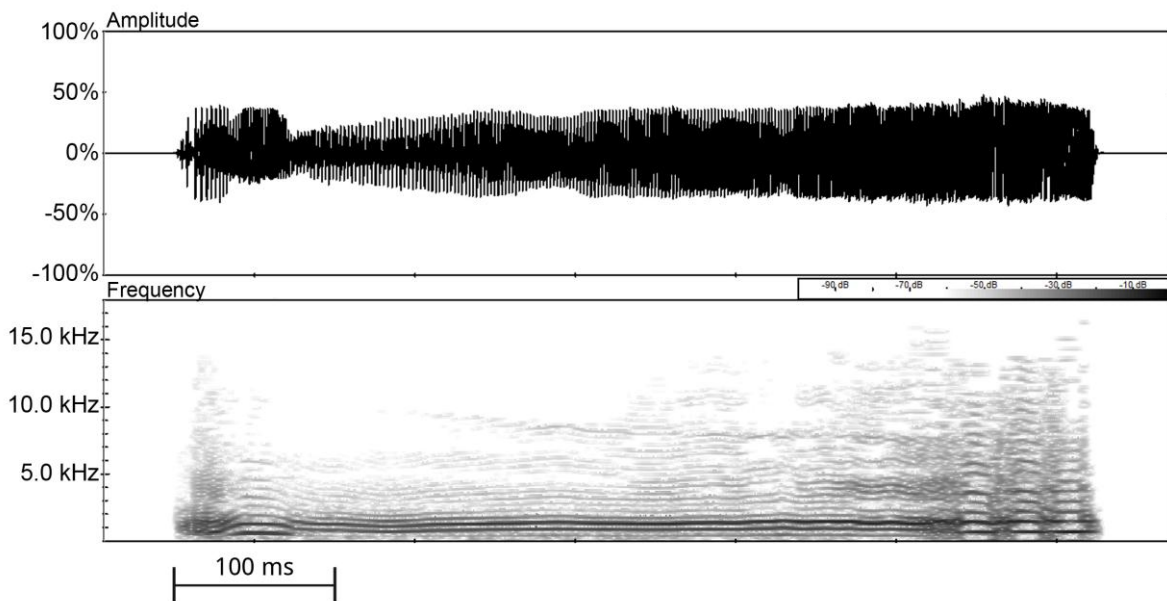


Figure 2.3 The spectrogram (bottom) and associated oscillogram (top) of the whine note stimulus.

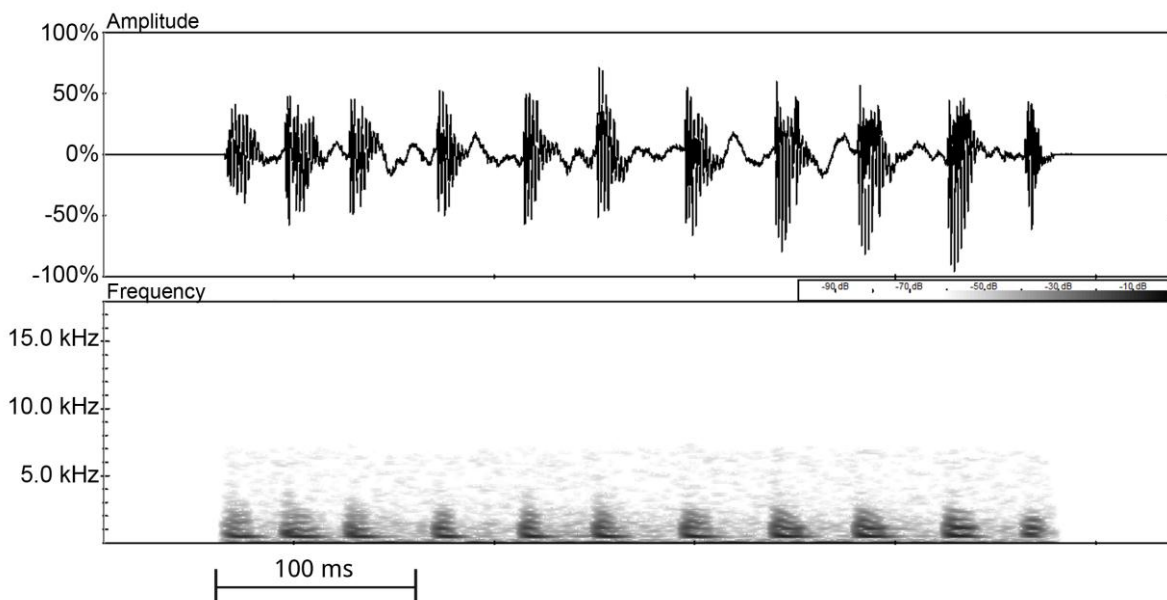


Figure 2.4 The spectrogram (bottom) and associated oscillogram (top) of the iambic note stimulus.

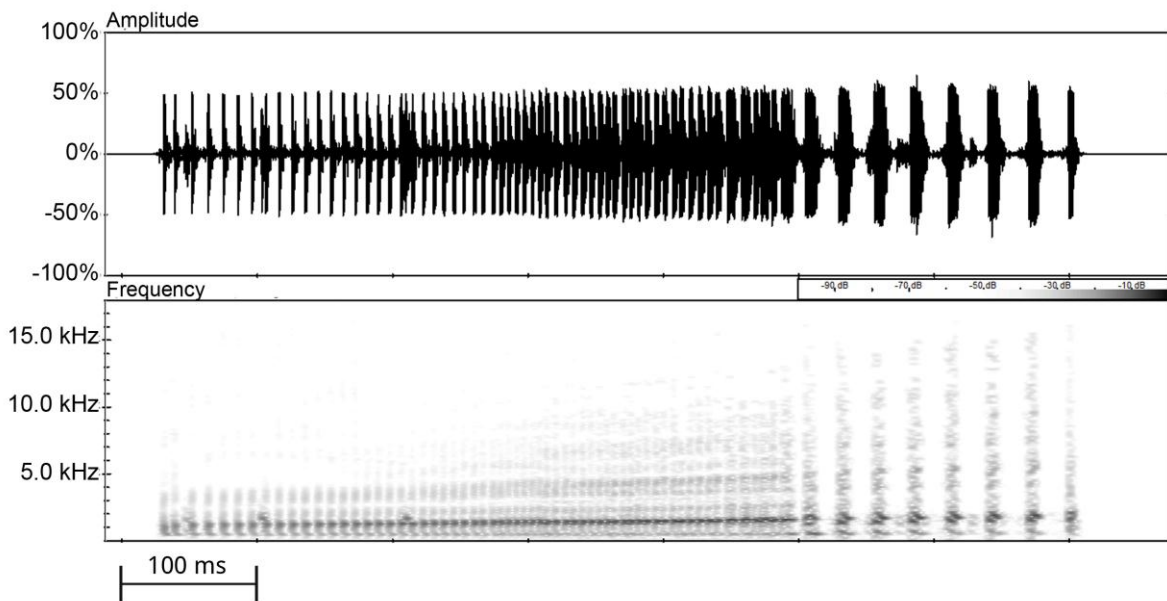


Figure 2.5 The spectrogram (bottom) and associated oscillogram (top) of the creak note stimulus.

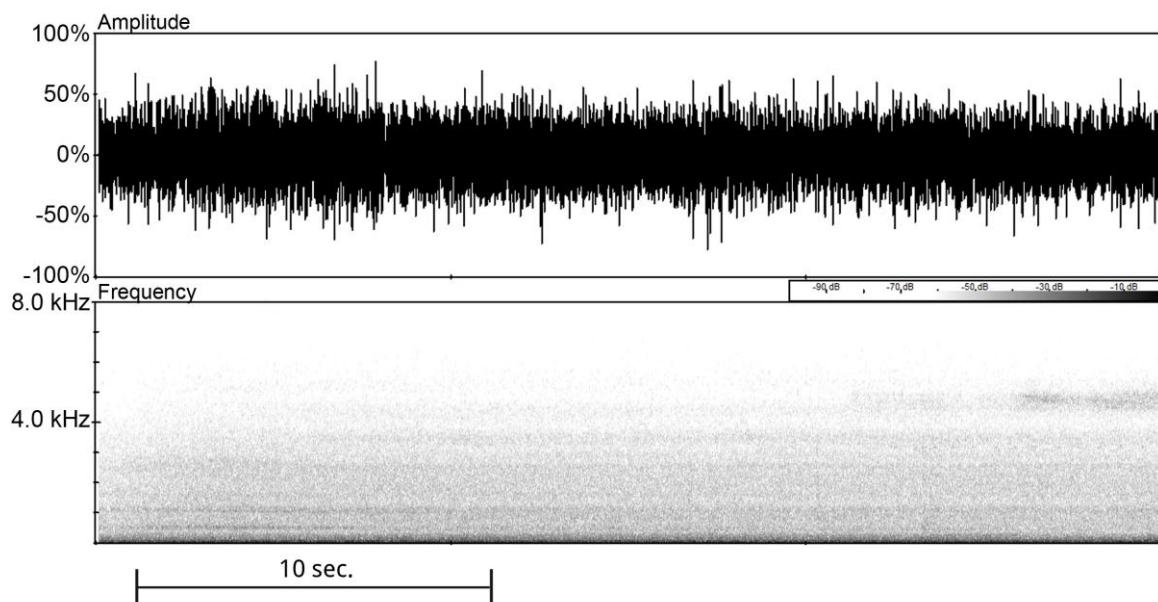


Figure 2.6 The spectrogram (bottom) and associated oscillogram (top) of the train stimulus.

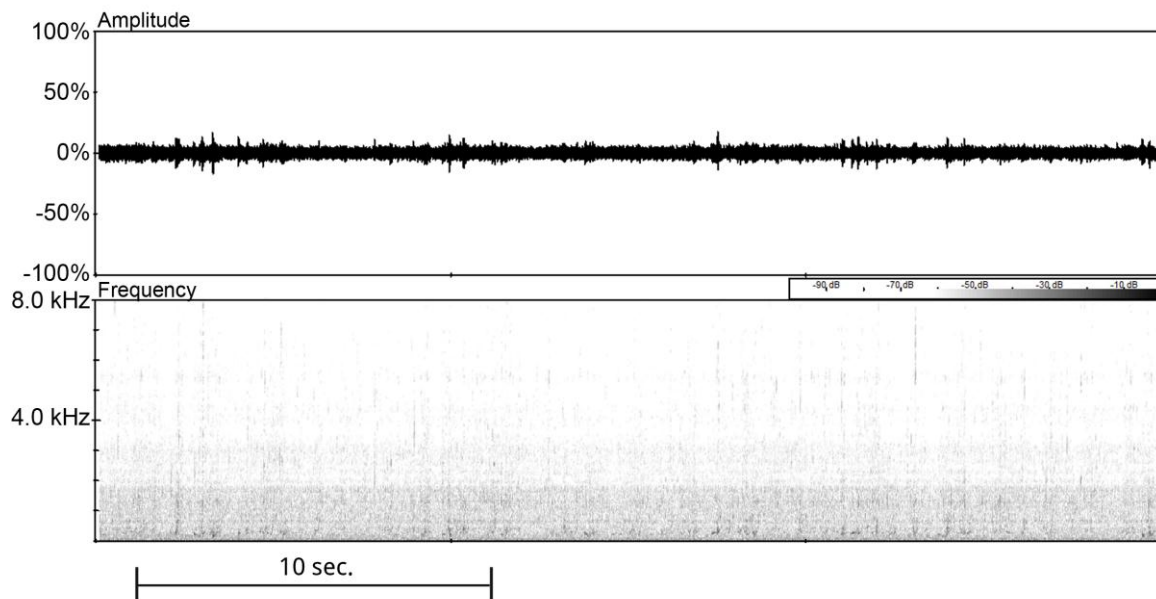


Figure 2.7 The spectrogram (bottom) and associated oscillogram (top) of the river stimulus.

The sequence in which stimuli was played varied each night to reduce possible bias. Each call stimulus played for five minutes, with a five second silence between each call, while the train and river stimuli played continuously for thirty seconds and ten minutes, respectively. After each stimulus, ten minutes of silence followed to allow the chorus to recover from interference. An example of how the stimuli were played can be seen in Figure 2.8. All calls were recorded on a Song Meter SM2 (Wildlife Acoustics) fitted with two SMX microphones. Recordings were analysed on SongScope software and call note types from the pond population were counted to determine the response to the different stimuli. The number of responses were documented for the duration that a stimulus was played.

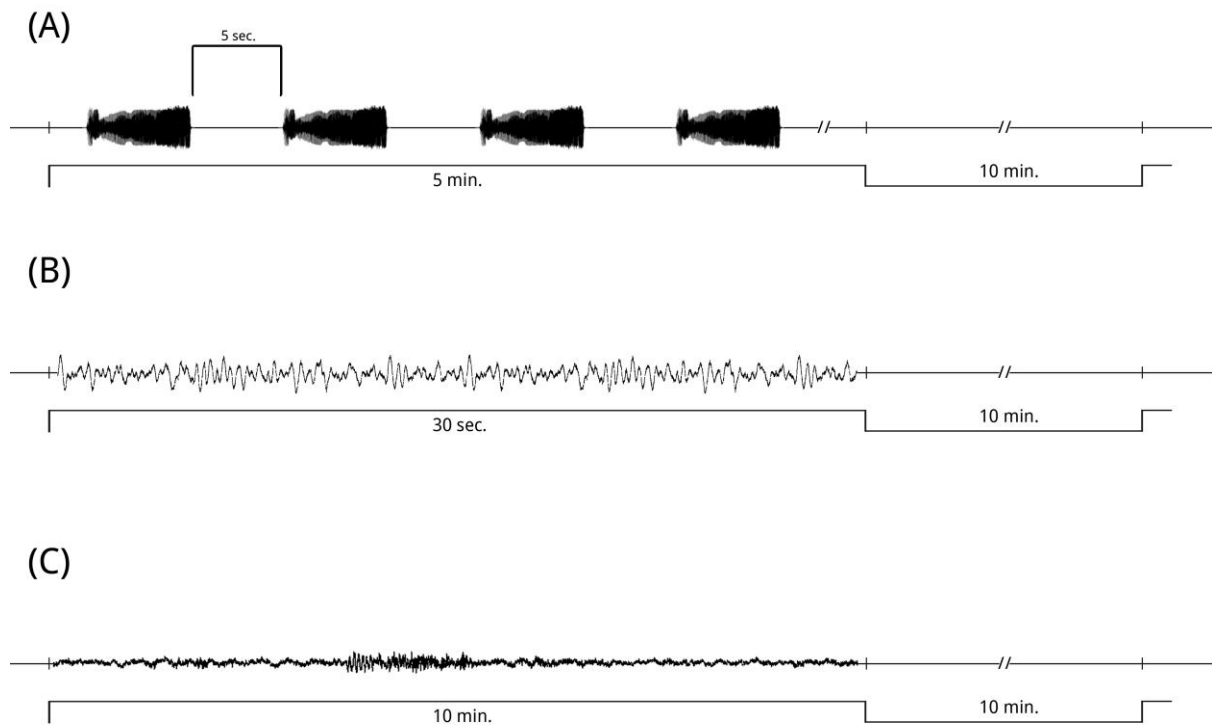


Figure 2.8 The call stimuli were broadcasted by playing a loop of a specific call for 5 minutes with 5 second intervals between each call, followed by 10 minutes of silence before the next stimulus was played (A). The train stimulus was broadcasted continuously for 30 seconds, followed by 10 minutes of silence before the next stimulus was played (B). The river stimulus was broadcasted continuously for 10 minutes, followed by 10 minutes of silence before the next stimulus was played (C).

Responses were categorised into seven note types, according to Kruger (2014), and an additional two note types were added after consulting responses on a spectrogram. Responses were classified according to sound, specific descriptions of visual call aspects (e.g. pulses or harmonics) and spectrogram images as seen in Figure 2.9.

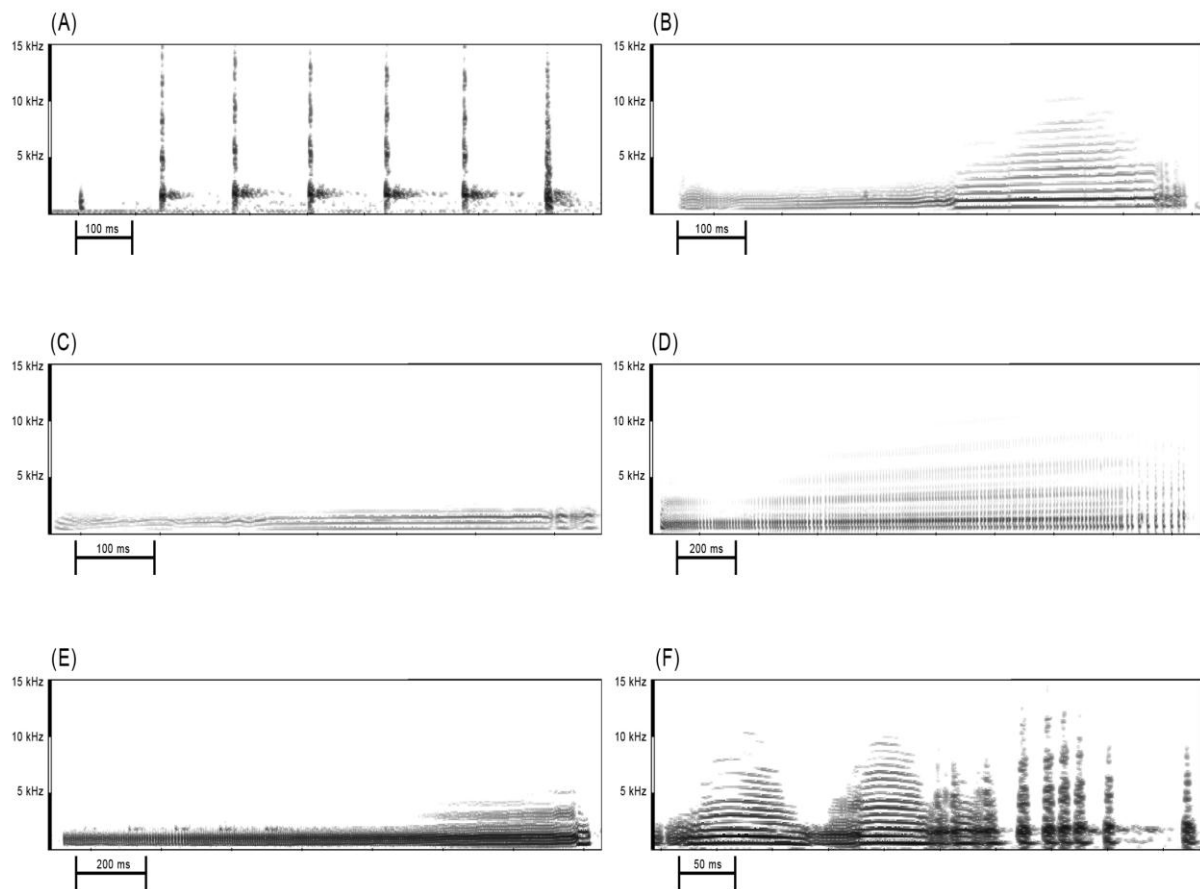


Figure 2.9. Note types used to distinguish different calls. Click notes consist of evenly spaced pulses (A). Whine notes are multi-phase calls that consist of tonals and harmonics (B). Tonal notes consist of constant tonals (C). Creak notes consist of rapid pulses with increased inter-pulse intervals (D). Whine-tonal notes consist of rapid pulses and a harmonic rich phase as seen in whine notes (E). Two-tonal whine notes are also multi-phase calls that consist of two tonal phases followed by strong pulses with chaotic white noise between each phase (F).

2.3.2 Playback experiment: Individual responses

An additional playback experiment was conducted at Pond 1 during the 2014 breeding season from the 13th of August to the 15th of August. A ShoX Maxi speaker (Model ESX301, 2.5 Watts) was used to propagate call note types directly to specific individuals. These males were injected subcutaneously with passive integrated transponders (PIT tags) after the sex, weight and snout-vent length (SVL) were determined. Males had prominent swollen nuptial thumbs, which were absent in females. Weight was measured with a Pesola hanging scale (100g max) and snout-vent length was measured with a Vernier calliper. The four call note types from Section 2.3.1 were used as stimuli for this playback experiment, but the train and

river stimuli were excluded. Responses were recorded using a Nagra ARES-M digital hand-held recorder equipped with a Sennheiser ME66 unidirectional microphone. Recordings were analysed on SongScope software and note types from each individual were categorised and counted to determine the response to the different stimuli. Note-types recorded were classified as described in section 2.3.1.

2.3.3 Additional call note type discovery

A female preference playback experiment was attempted in a laboratory setting. Eight females were caught from a number of ponds in the NWU Botanical Garden and each placed in a separate container filled with two centimetres (cm) of water from their respective ponds. They were taken to the laboratory and left overnight. The following night at 22:30, one female was placed on a damp, dark-blue towel on the laboratory counter, facing a ShoX Maxi speaker (Model ESX301, 2.5 Watts) at a distance of 60 cm. The lights were switched off and the click and whine stimuli were played at a SPL of 80 dB. Unfortunately, the female showed little interest in the call stimuli and repeatedly jumped off the counter. The experiment was repeated with two more females. The second female reacted like the first, but when the third female was introduced to the playbacks, she produced a tonal “squeak” sound whilst jumping away from the speaker. We abandoned the experiment thereafter.

While recording individual responses of males to stimuli (described in section 2.3.2), the speaker was placed between two males sitting close together (distance of about 100 mm between them). The creak stimulus resulted in one male jumping towards the speaker, but landing on top of the second male. This elicited a similar “squeak” sound to that produced by the female in the laboratory.

2.4 Calling behaviour and atmospheric conditions

To determine the effect of atmospheric conditions on calling behaviour, both calls and atmospheric conditions were recorded for the breeding season of 2013 from the 29th of April to the 19th of September at Pond 6. In order to investigate the effects of atmospheric conditions at two different time scales, we used two Song Meter SM2 recorders. The

recorders were equipped with two directly mounted SMX microphones as well as an ambient temperature sensor and an external water temperature sensor. Recorder one was set up against a tree next to the pond, one meter above the ground. It was set to record 20 minutes per hour from 17h00 – 08h00 each day from 29/04/2013 to 30/09/2013. The second recorder was set up on a tripod next to the first recorder, recording continuously for 15 hours from 17h00, but only every second week. Recordings were captured in 16-bit WAV files at a sampling rate of 16 kHz.

The recorded calls were counted using an automated detection setting in the SongScope software. Recognisers were built using a number of the recorded calls at different frequencies and decibel levels (i.e. training data) to ensure the largest number of click note pulses would be recognised by the software. The recogniser documented the number of click note pulses and assigned a date- and time stamp to each pulse. Each click was counted as a separate call because counting a series of clicks as one call resulted in too many false positives. The recordings were also manually screened using the spectrogram to visually inspect the results of the recognisers to determine true positives (pulses correctly identified), false positives (pulses incorrectly identified) and false negatives (pulses missed) in order to calculate a more accurate estimation of the calling intensity. A manual count and comparison was done for 20 calls for every 30 minutes of the recording to provide an error margin for false positives and false negatives. For the 20 minutes/hour recordings, one day was visually inspected each week to determine detection accuracy. The click note was used as the marker to count the number of calls produced. Though other call note types can be heard, click notes are the most easily discernable for training data and are also the note type produced most within a bout of calling (Chapter 3). Though a number of recognisers were built, a single one, producing the least false positives and false negatives, was used throughout the study. Settings that were standardised for the recogniser included: background filter (1 second), FFT size (256), FFT overlap (3/4) and frequency range (500–8000 Hz).

Atmospheric conditions were recorded using a constructed weather station placed in the Botanical Garden. The weather station consisted of a \pm 9 meter high tower with sensors installed for temperature (Model 41003), relative humidity (RH), precipitation (Model 61002), wind speed and wind direction (Model 05103). The sensors were of the brand R.M.

Young. All data were logged with a Campbell Sci Logger (CR10X). Data for percentage moon illumination were obtained from Time and Date AS (<http://timeanddate.com/s/2kvd>). As mentioned, the Song Meter SM2 recorder was also fitted with an ambient temperature sensor and an external water temperature sensor. Information from the weather station was downloaded and used for correlation with calling intensity.

2.5 Inter-male spacing

This study was done during the breeding season of 2014 over a period of four weeks from the 21st of July to the 14th of August at Pond 1. During this time, each individual frog found was captured and injected subcutaneously with a passive integrated transponder (PIT) tag. This allowed for identification of individuals if recaptured. Length (mm) was measured with a set of calipers, weight (g) was measured with a spring scale and sex was determined by looking at the presence/absence of swollen nuptial pads on the insides of the front feet. A Song Meter SM2 recorder was set up to record male calling activity from 22h00 to 02h00 for the duration of the study (Chapter 4).

Each PIT tag can be scanned using a hand-held scanner allowing for the identification of individuals. During each night of data collection, all visible individuals were scanned and their unique barcodes noted along with their positions in the pond, the presence or absence of vegetation at the calling site and distances between individuals. Distances between individuals were measured in metres using an electronic range finder. The distance of the two nearest neighbours of each individual was measured. In certain cases only one nearest neighbour was visible, so only one distance was recorded. Whether individuals were in/under vegetation was also noted as it could potentially affect inter-male spacing distances. All calls were recorded for the four week period of data collection together with atmospheric data. This was also correlated to eliminate the effect of atmospheric conditions on the calling behaviour of the population.

2.6 Statistical analysis

All statistical analyses were performed by using IBM SPSS Statistics ver. 22 (SPSS, 2013).

2.6.1 Playback experiments

The data obtained for the playback experiment was considered non-parametric as it did not show a normal distribution and there was an unequal sample size. The Kruskal-Wallis test was used as it is suitable for non-parametric data due to the fact that it does not require normal distribution or sample size. The null-hypothesis stated that none of the stimuli would show an effect on any of the note types produced. The different stimuli were the independent variables for this study while the note types produced in response were the dependent variables. A Monte Carlo permutation test was used to test for the significance ($p < 0.05$) (Nordstokke and Zumbo, 2007; Nordstokke and Zumbo, 2010). Box-and-whisker plots were drawn for each stimulus to show the variation in the number of different call note types encountered.

2.6.2 Calling behaviour and meteorological conditions

For both time scales the different variables that were tested for were wind speed, humidity, barometric pressure, change in barometric pressure, ambient temperature, water temperature and moon phases. The phases of the moon were measured as the percentage illumination of the moon. Both wind direction and rain were included in the initial analyses, but rain was removed due to the fact that there was no data available and wind direction was removed as the numerical value given and the subsequent results were considered to not reflect the true effect of the variable. To determine the effects of atmospheric conditions on a smaller time scale, the ambient temperature during the day as well as the solar radiation was measured and included in the analysis.

Data was tested for normality, Kurtosis and Skewness and it was found that a non-parametric statistical analysis was appropriate. Because a large number of dependent variables were used, a biplot diagram was drawn using results from a Principle Components Analysis (PCA)

to determine correlation between the number of calls and the different meteorological conditions for the larger time scale. This technique was considered appropriate as it projects the relationships between dependent and independent variables (Abdi and Williams, 2010). Principle component analysis was performed in the software program CANOCO v. 4.5.

A 2-tailed Pearson-Correlation and a model coefficient were used to determine whether there were any statistically significant correlations ($p < 0.05$) for the larger time scale data (15 hour recordings). For the smaller time scale data (20 min recordings), a Spearman's Rho correlation matrix was performed to determine statistical significance ($p < 0.05$). The variables that were found to be significant were used to run a General Linear Model (GLM) to explain variance in the dependent.

2.6.3 Inter-male spacing

To test the significance of the affects that vegetation has on the distances at which males and females position themselves, as well as whether distances at which females position themselves among males differ significantly from inter-male spacing, a Mann-Whitney U test was performed. To investigate whether the distances between larger males are significantly different than the distances between smaller males and their nearest neighbours, and whether calling intensity is affected by the average inter-male distances, a Kruskal Wallis ranks test was used. To determine whether size had an effect, males were categorised into three groups; small (<20g), medium (20–23g) and large (24–39g).

3.1 Molecular analysis

Based on phylogenetic analysis, all individuals tested formed a monophyletic clade with other *A. queckettii* previously published in GenBank (Channing and Baptista, 2013), confirming their taxonomic identity (Figure 3.1). The Potch 1 sample shows a similarity of 99.3% to sample KC756354, 98.7% to sample KC756357 and 98.9% to sample KC756358 from Gen Bank. The Potch 2 sample showed 99.6%, 99% and 99.1% similarity for the same Gen Bank samples. This falls within the 97% species boundary generally accepted as variation within a species (Channing and Batista, 2013).

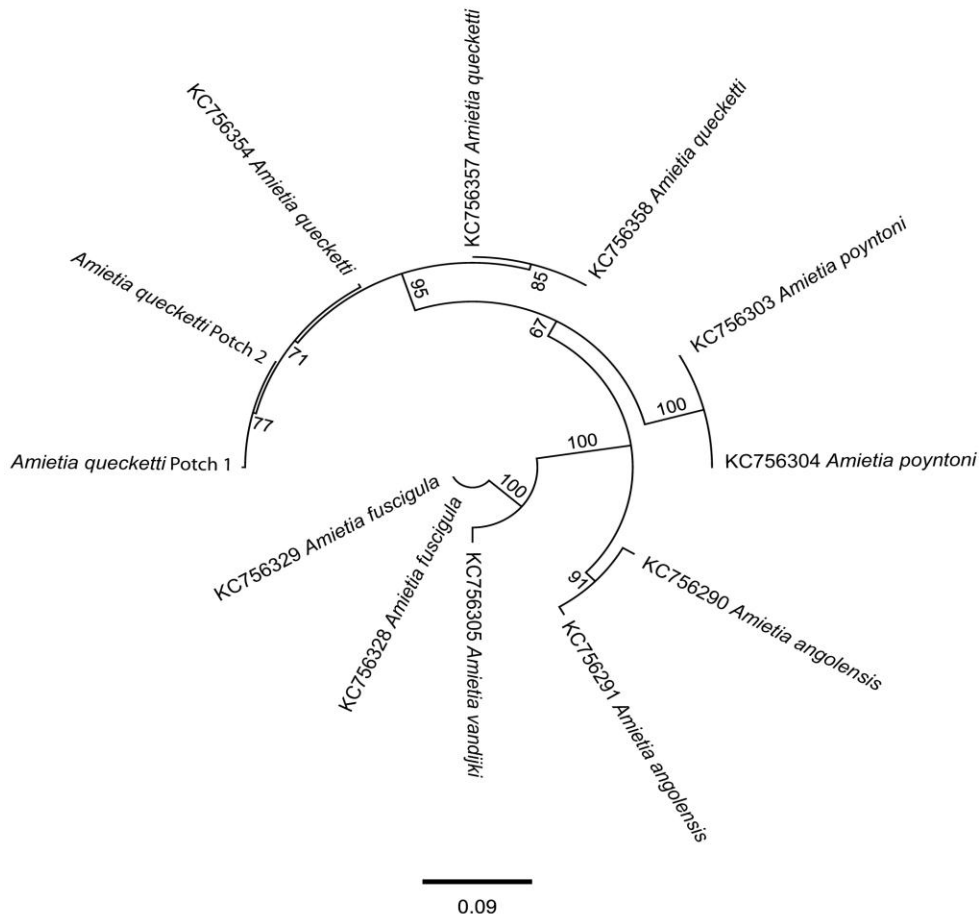


Figure 3.1 Molecular tree showing the relatedness of *Amietia* species in South Africa including samples from the study site in Potchefstroom.

3.2 Call note responses

This study confirms that *A. queckettii* has an extremely complex call and the number of note types indicates its large and diverse call repertoire. When no stimulus was played, the most abundant call note type observed was click notes (57%), followed by creak notes (25%), whines (9%) and then tonal notes, whine-tonal notes and two-tonal whine notes (each 3%) (Figure 3.2). Call note responses recorded from the playback experiment included click notes (Figure 3.3), whine notes (Figure 3.4), tonal notes (Figure 3.5), creak notes (Figure 3.6), whine-tonal notes (Figure 3.7) and two-tonal whine notes (Figure 3.8). See Appendix A (CD) for audio examples of all call types described.

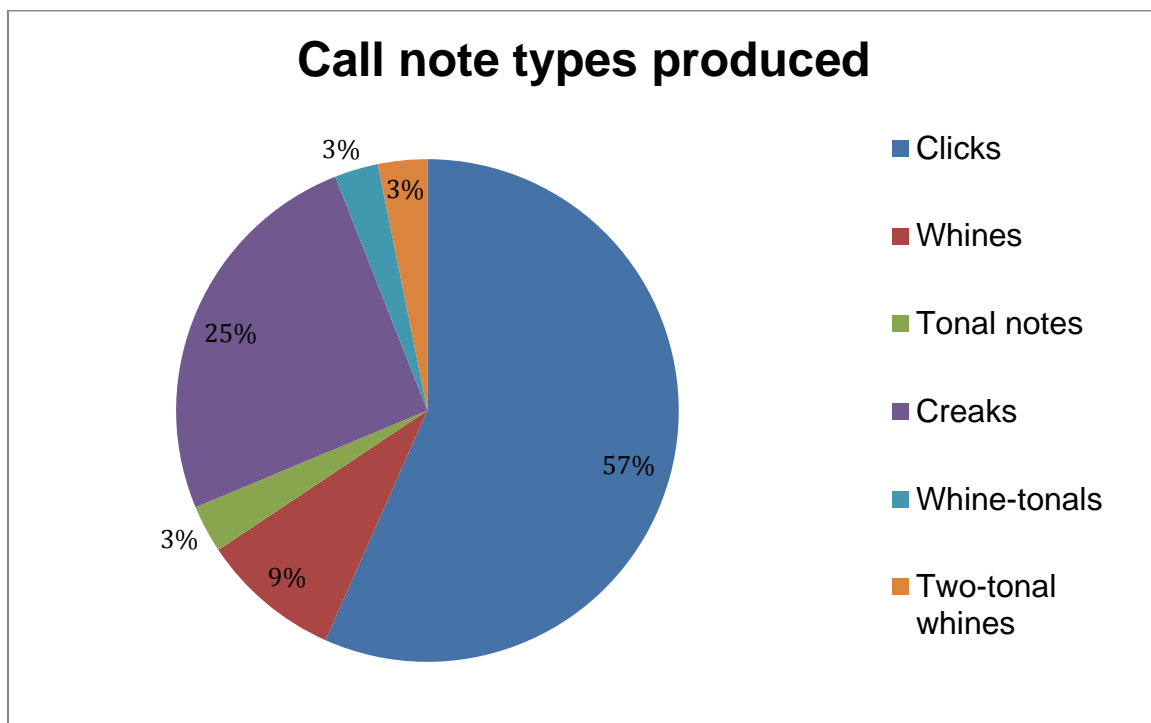


Figure 3.2 Pie chart denoting the percentage of each call note type produced over the course of the experiment when no audio stimulus was presented.

The click note produced consisted of a number of evenly spaced click-like pulses (Figure 3.3). Call duration lasted an average of 684 ms with an average of six pulses per call (Table 3.1). The inter-pulse interval lasted an average of 99 ms, while the pulse rate was nine pulses per second on average (Table 3.1). The average dominant frequency observed was 1728 Hz

(Table 3.1). Little variation was found in pulse rate ($SD = 1$), pulse number ($SD = 1$) or inter-pulse interval ($SD = 9$, Table 3.1) between individuals. A large variation in call duration ($SD = 164$) and dominant frequency ($SD = 188$, Table 3.1) was observed between individuals. Frogs had slower pulse rates in small choruses and at low temperatures. Pulse rate and pulse number increased with increased chorus size and slightly warmer temperatures (Table 3.1).

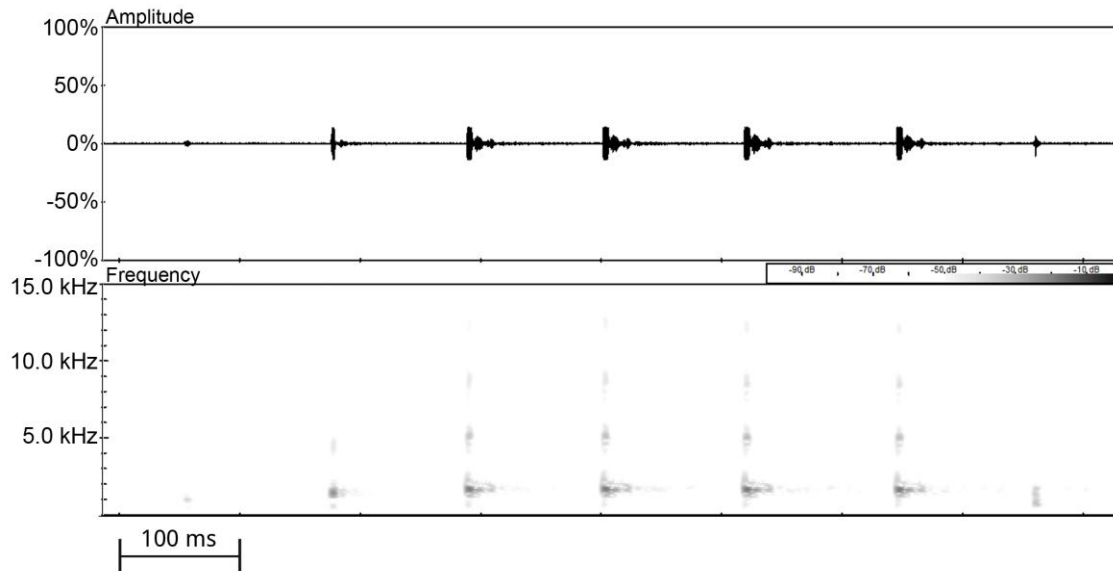


Figure 3.3 The spectrogram (bottom) and associated oscillogram (top) of the click note response produced.

Table 3.1 The analysis of the click note response ($n = 10$).

	Call Duration (ms)	Pulse Number	Inter-pulse Interval (ms)	Dominant Frequency (Hz)	Pulse rate (p/s)
Mean	684	6	99	1728	9
Min	372	4	87	1310	8
Max	986	8	115	2060	11
SD	164	1	9	188	1

The whine note produced consisted of a number of short pulses stacked close together, which develop into tonal harmonics that increase in frequency and number of harmonics (Figure 3.4). Once again, there was a large variation in call duration ($SD = 89$), but also in the fundamental frequency ($SD = 220$) as opposed to the dominant frequency as seen in the click note (Table 3.1, Table 3.2). On average, the whine note is only 476 ms long and has a

dominant and fundamental frequency of 1357 Hz and 745 Hz, respectively (Table 3.2). For this note type, the dominant frequency was one harmonic higher than the fundamental frequency (Figure 3.4, Table 3.2).

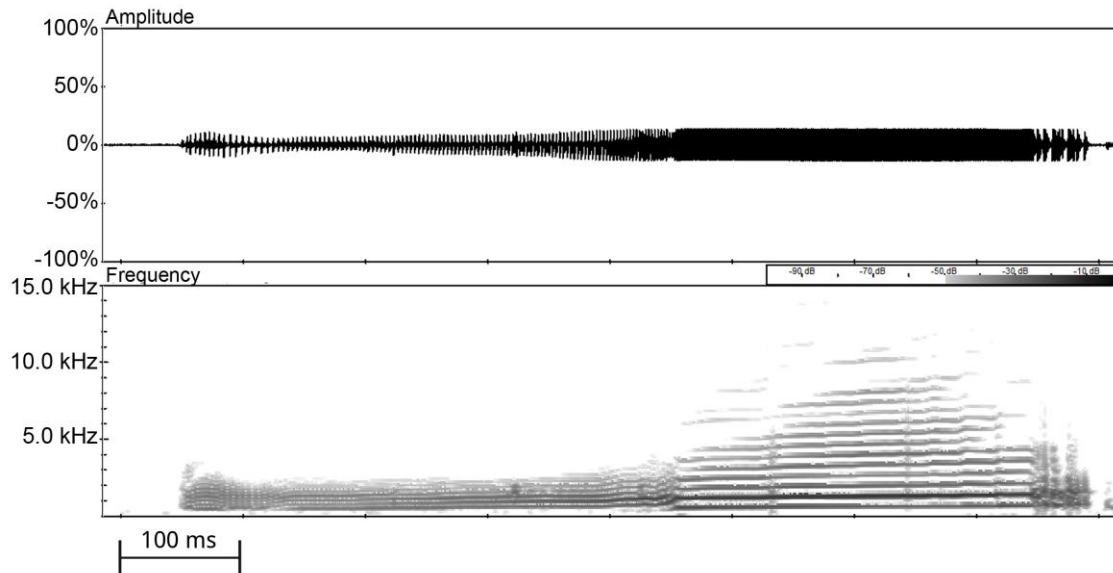


Figure 3.4 The spectrogram (bottom) and associated oscillogram (top) of the whine note response produced.

Table 3.2 Call analysis of the whine note response (n = 10).

	Call duration (ms)	Dominant Frequency (Hz)	Fundamental Frequency (Hz)
Mean	476	1354	745
Min	382	1185	498
Max	628	1464	1197
SD	89	93	220

The tonal note response is a simple harmonic call that remains at the same frequency and with the same number of harmonics throughout the call (Figure 3.5). Call duration showed a large amount of variation (SD = 89, Table 3.2). Though both the whine note and the tonal note have tonal elements, in the tonal note it is the fundamental frequency that shows the largest amount of variation (SD = 220, Table 3.3). In the case of the tonal note the dominant frequency is three harmonics above that of the fundamental frequency (Figure 3.5), while in the whine note the dominant frequency is only one harmonic above the fundamental

frequency (Figure 3.4). On average, the tonal note is slightly longer than the whine note with a call duration of 582 ms (Table 3.3). It also shows a higher dominant frequency (1318 Hz), which is not surprising due to the fact that there are more harmonics between the dominant and fundamental frequency in the tonal note, but the fundamental frequency in the tonal note was also higher on average at 622 Hz (Table 3.3, Table 3.2). In the field it was observed that whine notes are produced much louder than tonal notes, despite the higher frequencies in tonal notes.

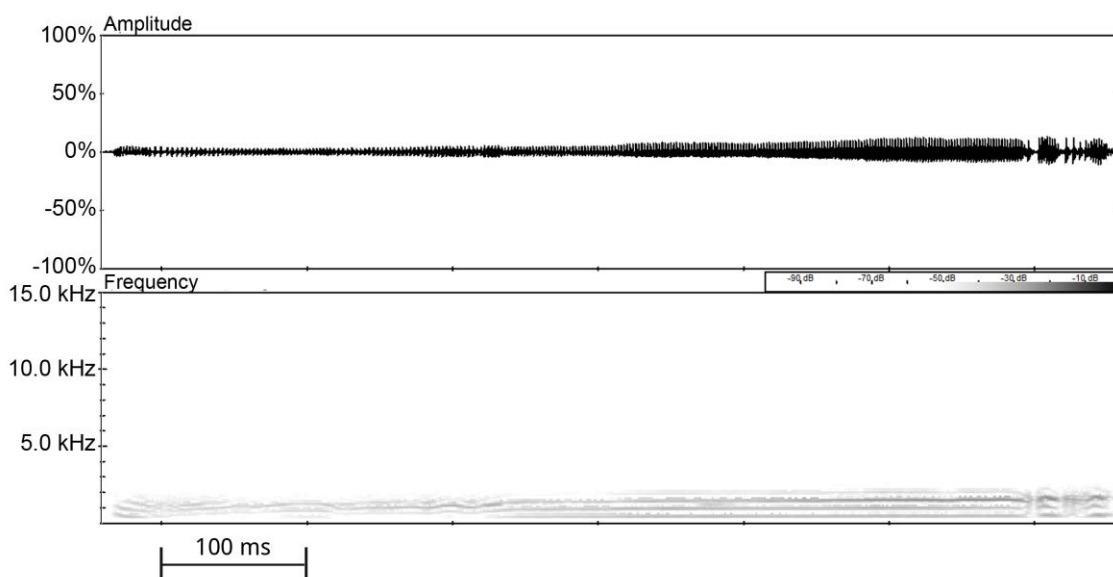


Figure 3.5 The spectrogram (bottom) and associated oscillogram (top) of the tonal note response produced.

Table 3.3 Call analysis of the tonal note response (n = 10).

	Call Duration (ms)	Dominant Frequency (Hz)	Fundamental Frequency (Hz)
Mean	582	1318	622
Min	386	870	560
Max	752	1750	690
SD	116	268	50

The creak response consisted of a number of short pulses in rapid succession with increasing inter-pulse intervals and increasing frequency (Figure 3.6). The creak note showed variation in each aspect; call duration (SD = 327), pulse number (SD = 12) and dominant frequency (SD = 3853, Table 3.4). Compared to the other note types, the creak note had the longest

average call duration at 1480 ms and the highest dominant frequency at 2538 Hz, as well as a high 81 pulses per second (Table 3.4). This note type was observed to be very loud in the field and often led to a male attacking the speaker during the individual playback experiment.

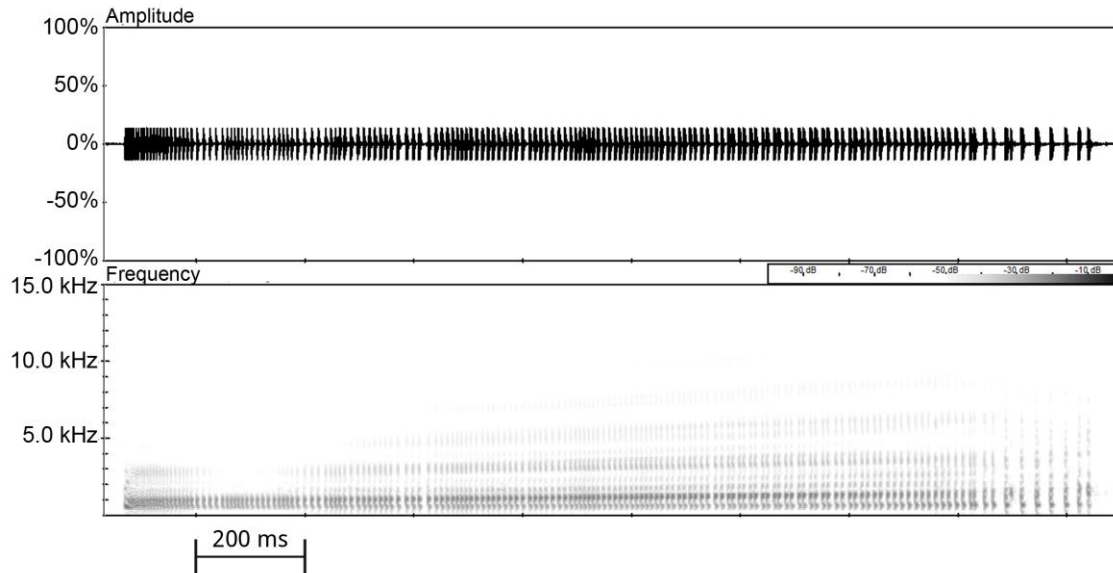


Figure 3.6 The spectrogram (bottom) and associated oscillogram (top) of the creak-note response produced.

Table 3.4 Call analysis of the creak note response (n = 10).

	Call Duration (ms)	Pulse Number	Dominant Frequency (Hz)
Mean	1480	81	2538
Min	948	64	1110
Max	1884	104	13500
SD	327	12	3853

The whine-tonal response consisted of a whine and a tonal section. The whine section, however, more closely resembles a creak on a spectrogram with a number of closely spaced pulses, though the sound is similar to a whine (Figure 3.7). Similar to the creak note, there was a large variation in each of the call aspects; call duration (SD = 668), the dominant and fundamental frequency of the whine aspect (SD = 195, SD = 218) and the dominant and fundamental frequency of the tonal aspect (SD = 170, SD = 258, Table 3.5). The average call duration was quite long at 1187 ms, though not as long as a creak (Table 3.5, Table 3.4). The average dominant frequency was higher in the tonal section (1294 Hz) than in the whine

section (1124 Hz), but the fundamental frequencies for the whine (741 Hz) and the tonal section (729 Hz) were very similar (Table 3.5).

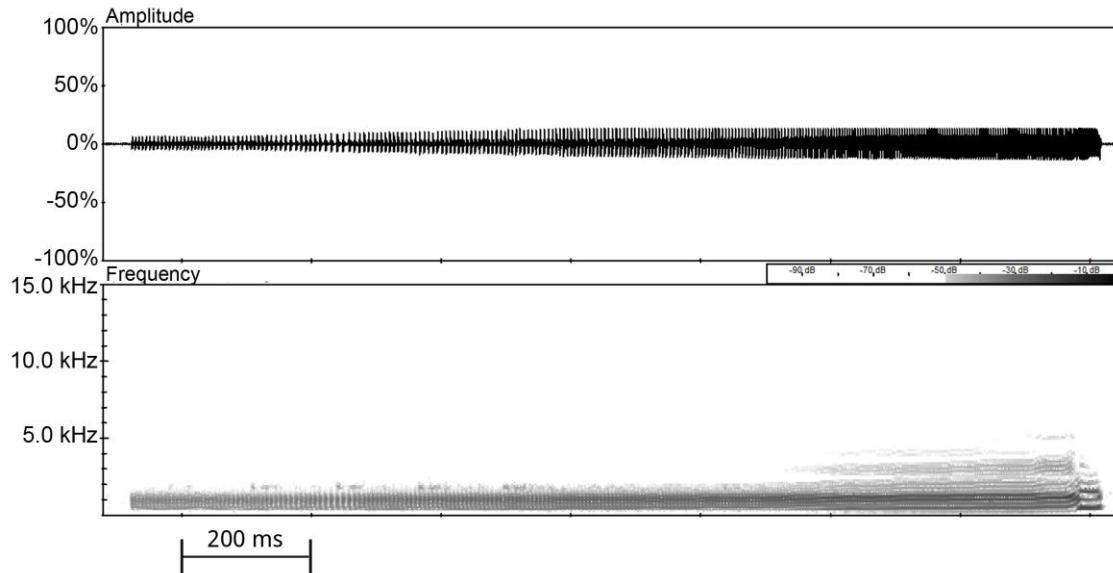


Figure 3.7 The spectrogram (bottom) and associated oscillogram (top) of the whine-tonal note response produced.

Table 3.5 Call analysis of the whine-tonal note response (n = 10).

	Call Duration (ms)	Whine Dominant Frequency (Hz)	Whine Fundamental Frequency (Hz)	Tonal Dominant Frequency (Hz)	Tonal Fundamental Frequency (Hz)
Mean	1187	1124	741	1294	729
Min	386	660	550	1090	550
Max	2266	1350	1130	1570	1330
SD	668	195	218	170	258

The two-tonal whine note responses consisted of two dome-shaped tonal aspects followed by a number of large pulses with varying inter-pulse intervals, with white noise between each aspect (Figure 3.8). Once again, the whine aspect does not visually resemble the whine note, but only sounds similar. There was very little variation in the first tonal part, both in the dominant frequency (SD = 120) and the fundamental frequency (SD = 54), as well as in the call duration (SD = 56, Table 3.6). In the second tonal part there was a large amount of

variation in the dominant frequency ($SD = 314$), but not much in the fundamental frequency ($SD = 51$, Table 3.6). In the whine aspect the dominant and fundamental frequencies were the same and they showed a large amount of variation ($SD = 387$, Table 3.6). This is a short call with an average call duration of just 490 ms (Table 3.6). The highest dominant frequency was found in the first tonal part (1146 Hz) and the lowest was found in the second tonal part (1079 Hz), though it did not differ much from the dominant frequency in the whine part (1084 Hz, Table 3.6). The highest fundamental frequency was found in the whine part (1084 Hz) while the lowest was found in the first tonal part (571 Hz, Table 3.6).

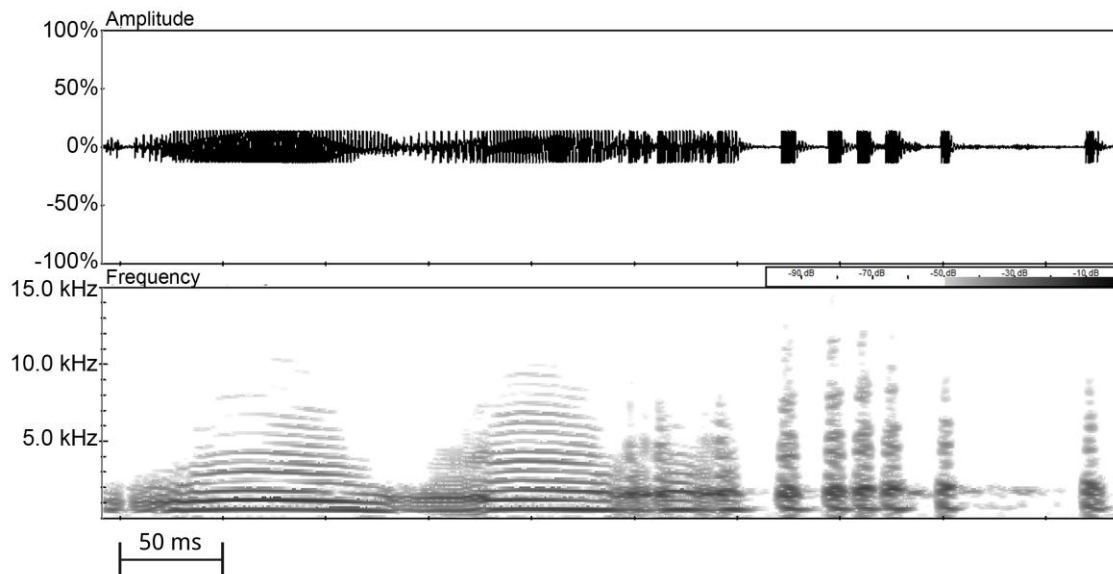


Figure 3.8 The spectrogram (bottom) and associated oscillogram (top) of the two-tonal whine note response produced.

Table 3.6 Call analysis of the two-tonal whine note response ($n = 10$).

	Call Duration (ms)	Whine Dom. Freq. (Hz)	Whine Fund. Freq. (Hz)	Tonal 1 Dom. Freq. (Hz)	Tonal 1 Fund. Freq. (Hz)	Tonal 2 Dom. Freq. (Hz)	Tonal 2 Fund. Freq. (Hz)
Mean	490	1084	1084	1146	571	1079	638
Min	419	640	640	984	504	620	571
Max	592	1591	1591	1342	662	1421	717
SD	56	387	387	120	54	314	51

3.3 Relationship between stimuli and call note types produced

A significant difference was found between the effects of different stimuli on the production of clicks, whines, tonal notes, creak notes and whine-tonal notes ($p < 0.01$), as well as for the two-tonal whine notes ($p < 0.05$, Table 3.7). The different stimuli elicited a different number of responses for each call note type. A large number of click notes were produced in response to each stimulus, though the creak stimulus elicited the most while the river stimulus elicited the least (Figure 3.9). Very few whine notes were produced, regardless of the stimulus, but the most were produced in response to the whine stimulus, while the least were produced in response to the river stimulus (Figure 3.9). Tonal notes were produced the most in response to the creak and iambic stimuli as well as the silence (no stimulus), while the train stimulus resulted in almost no responses (Figure 3.9). For the whine-tonal note, the highest number of calls were observed in response to the whine stimulus, followed by the iambic stimulus, while the train and river stimuli produced very little response (Figure 3.9). The stimuli that resulted in the most creak notes were clicks and creaks, though a number of calls were also produced in response to the whine and iambic stimuli. The least amount of creak notes produced was in response to the river stimulus (Figure 3.9). The two-tonal whine note was produced in response to each of the call stimuli with the whine stimulus eliciting the most responses. The train and river stimuli, however, once again produced very few calls (Figure 3.9).

Table 3.7 Statistical values for testing the null-hypothesis using the Kruskal-Wallis test ($n = 6$) in determining note context for *A. queckettii*.

	Clicks	Whines	Tonal notes	Creak	Whine-tonal	Two-tonal Whines
Chi-Square	29.00	26.77	16.20	26.03	20.10	13.59
df	6	6	6	6	6	6
Monte Carlo Sig.	.000 ^c	.000 ^c	.007 ^c	.000 ^c	.000 ^c	.024 ^c

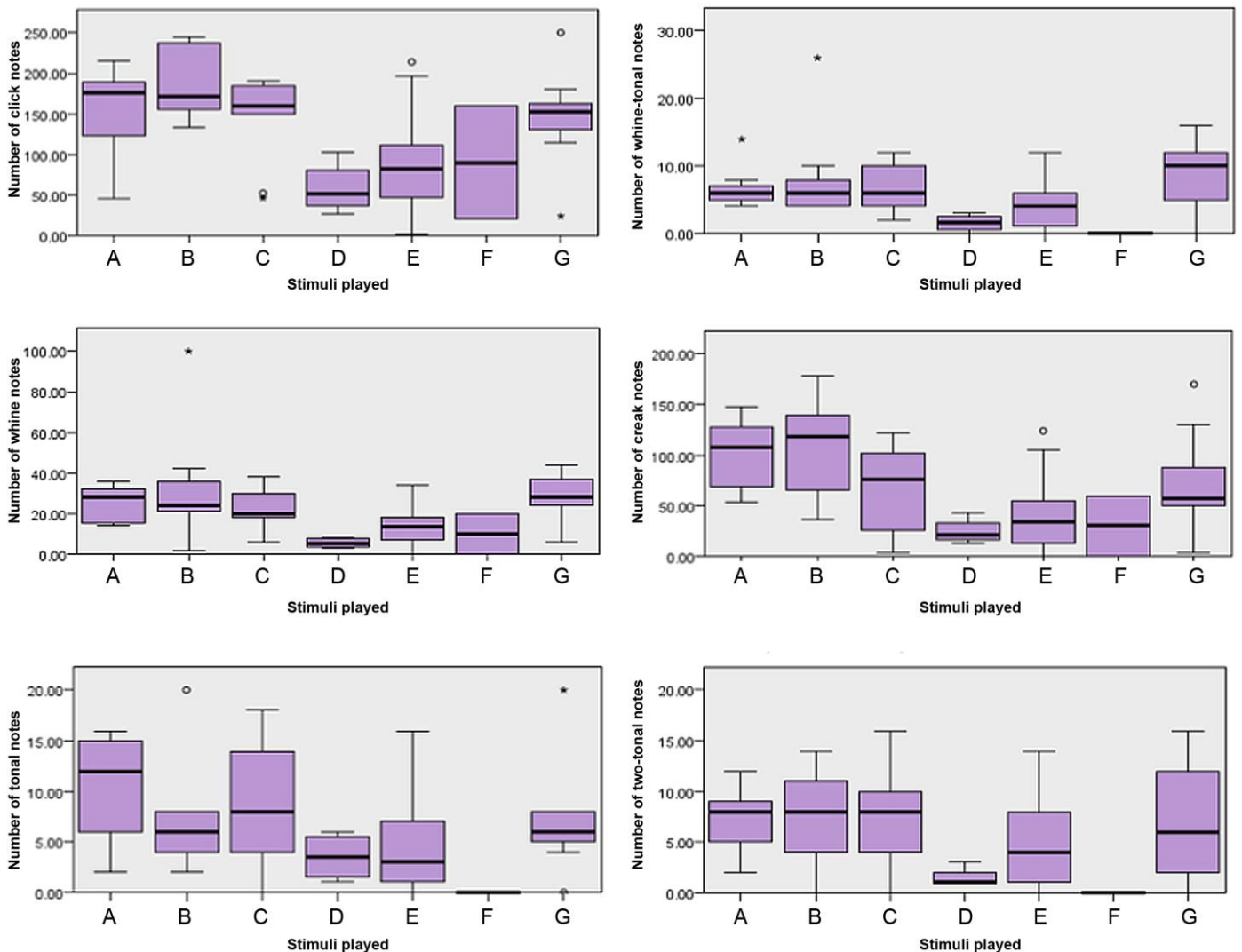


Figure 3.9 Independent-sample Kruskal-Wallis box-and-whisker plots denoting the effect of stimuli on the note type produced. The different stimuli used were click notes (A), creak notes (B), iambic notes (C), a flowing river (D), no stimulus/silence (E), a passing train (F) and whine notes (G). Circles on the diagrams represent outliers with stars representing extreme outliers. Dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

3.4 Additional tonal “squeak”

The tonal squeak note is a short tonal harmonic note with an average call duration of just 105 ms (Figure 3.10, Table 3.8). There is very little variation in the call duration ($SD = 16$), dominant frequency ($SD = 77$) or fundamental frequency ($SD = 77$, Table 3.8). The dominant frequency is the same as the fundamental frequency in this call. This call was observed in

both a male and a female, though the male produced it in response to a physical stimulus and the female produced it in response to a playback stimulus (Chapter 2). It was observed that both the female and the male moved away from the stimuli.

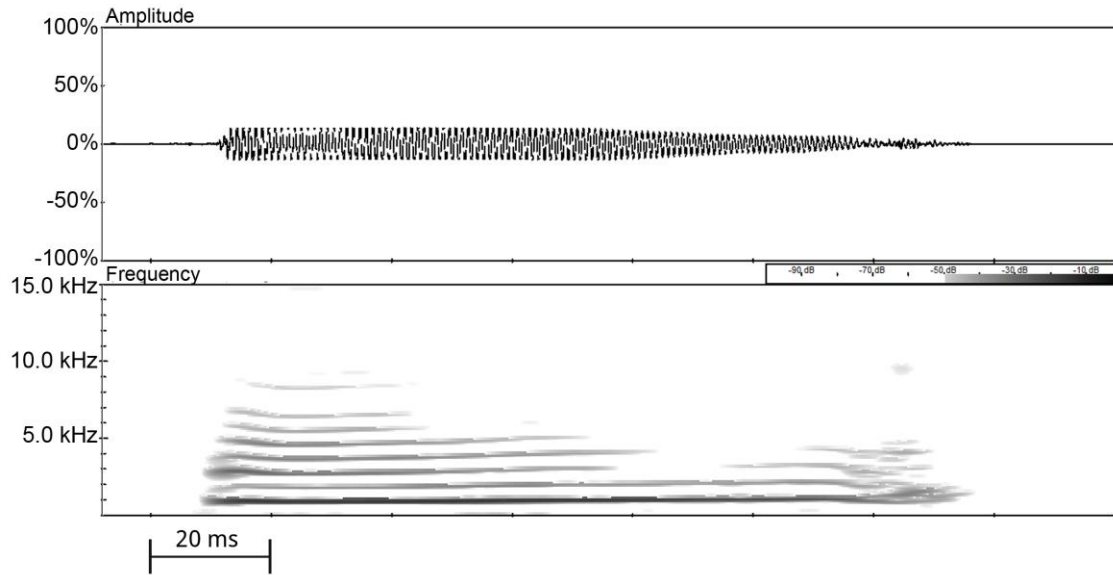


Figure 3.10 The spectrogram (bottom) and associated oscillogram (top) of the tonal squeak note produced by both males and females.

Table 3.8 The analysis of the tonal squeak note (n=5).

	Call Duration (ms)	Dominant Frequency (Hz)	Fundamental Frequency (Hz)
Mean	105	956	956
Min	88	870	870
Max	130	1060	1060
SD	16	77	77

4.1 Peak calling times

Amietia queckettii may be heard calling throughout the year, but they have a specific breeding season when calling intensity greatly increases. The breeding season of *A. queckettii* starts at the beginning of May and ends around the middle of September. Though the frogs breed during this entire period, there are certain peak times in the breeding season when calling intensity greatly increases. Based on passive acoustic monitoring (PAM) using a song meter, it was determined that call intensity peaked between 2nd of May and 15th of May with around 2500 calls per day, followed by a second longer peak of around 3500 calls per day lasting from the 4th of June until the end of the breeding season. During this second peak, a technical problem was experienced with the Song Meter, hence the gap in the data (Figure 4.1). The second, longer peak showed a number of fluctuations. Calling intensity during this peak remained high with between 1000 and 3500 calls per day (Figure 4.1).

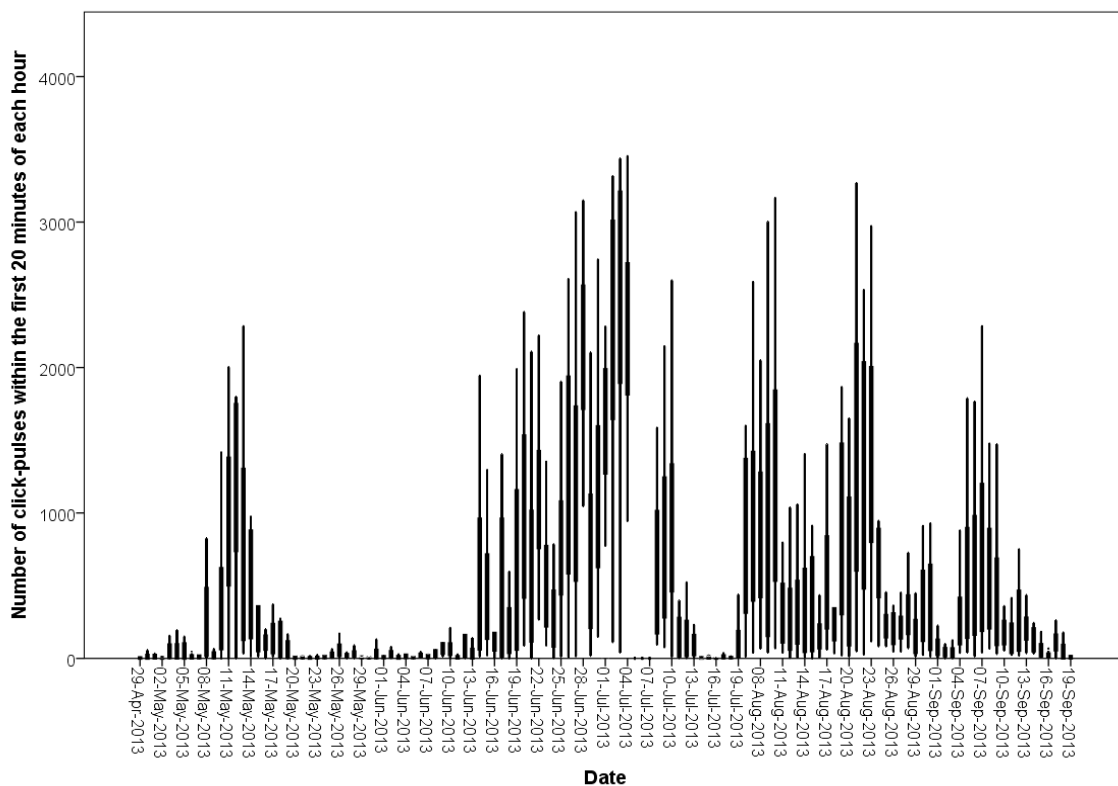


Figure 4.1 Mean daily call activity (18:00-7:00, 20min/h) during the 2013 breeding season.

Though *A. quecketti* can be heard calling during the day, their actual active calling time is at night. In general, calling activity during the breeding season starts around 19h00 and lasts until about 06h00 the following morning (Figure 4.2). As the days got shorter in winter and the nights longer *A. quecketti* was heard calling earlier in the evening and calling until later in the early morning hours. Call intensity peaked between 22h00 and 02h00, with the largest number of calls produced around 01h00 (Figure 4.2). Following a decrease in call intensity after 02h00, an increase was noted again around 05h00. Call intensity decreased drastically at sunrise.

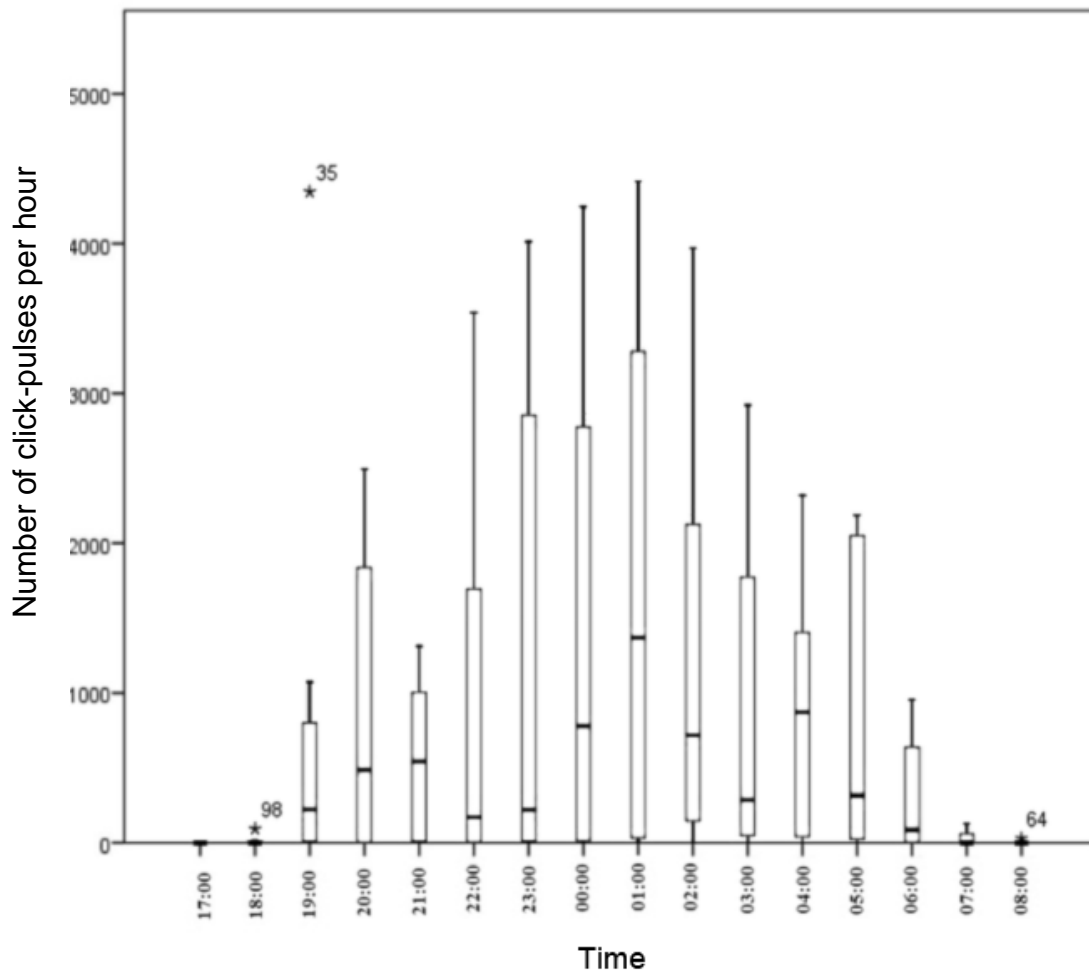


Figure 4.2 The average calling intensity based on the 15 hour uninterrupted recordings (17:00 to 08:00) for the entire breeding season. Stars represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

4.2 Larger time scale effects (15-hour recordings)

When observing the effect of different atmospheric conditions on calling intensity, it is important to bear in mind that not only can they affect calling intensity, but each atmospheric condition can also affect/be affected by the other atmospheric conditions. For this study only the effect of individual atmospheric conditions on calling intensity were observed. However, the relationships between different atmospheric conditions were also taken into account. Humidity showed a very strong positive correlation with calling intensity, while both air (or ambient) temperature and water temperature showed a strong negative correlation (Figure

4.3). Wind speed, barometric pressure, change in pressure and percentage moon illumination did not show a strong positive or negative correlation with calling intensity (Figure 4.3).

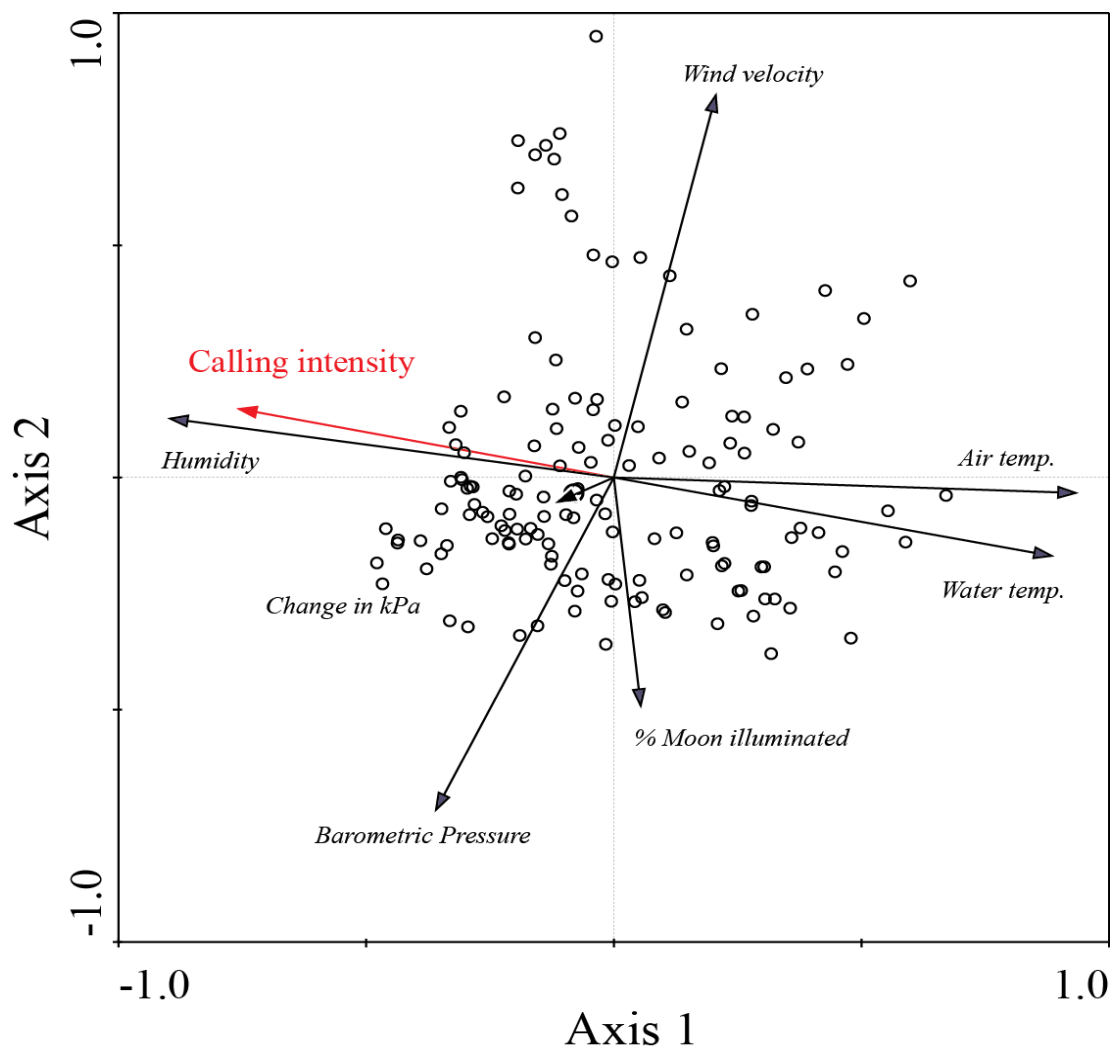


Figure 4.3 PCA Graph illustrating the correlation between different variables, with 90° or less indicating a positive correlation and more than 90° indicating a negative correlation.

Though there was a strong negative correlation between ambient temperature and calling intensity (Figure 4.3), it was not found to be statistically significant ($p = 0.621$). Calling intensity increased as temperature decreased with almost no calls produced at temperatures higher than 17°C (Figure 4.4). There was also a very definite range in which calls are produced as there were no calls at all at temperatures below 3°C (Figure 4.4). Most calls were produced between 5°C and 13°C (Figure 4.4). With regard to ambient temperature, A.

quecketti showed much more tolerance for higher temperatures than for lower temperatures, with a very sudden decrease in calling intensity below 5°C, while there were still a few calls in temperatures as high as 27 °C (Figure 4.4).

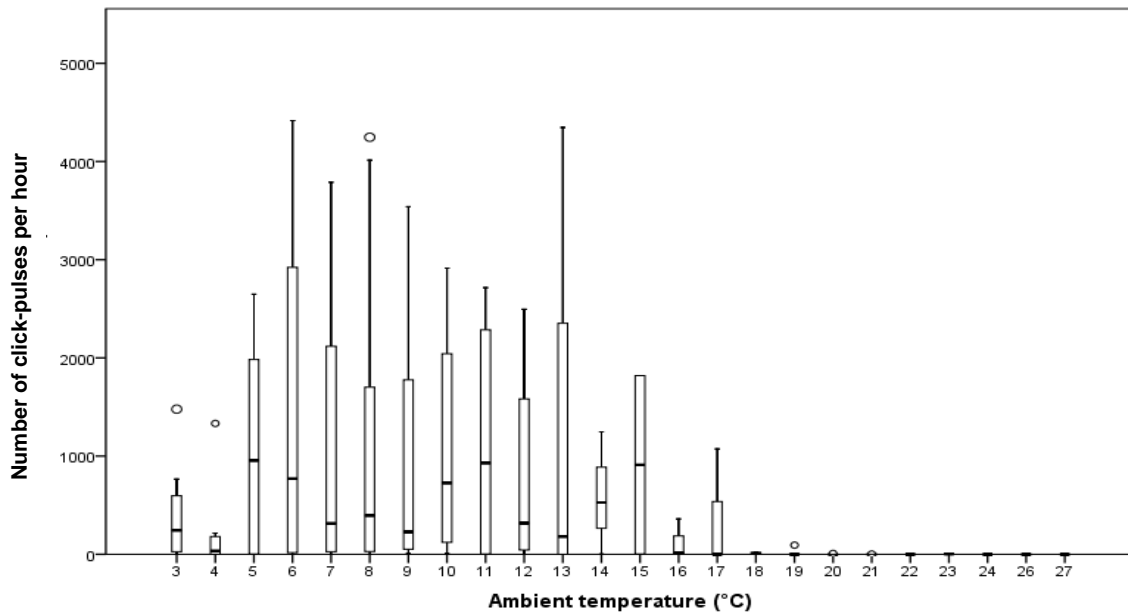


Figure 4.4 The effect of ambient temperature on calling intensity per hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

Unlike with ambient temperature, the strong negative correlation between water temperature and calling intensity (Figure 4.3) was found to be statistically significant ($p = 0.05$). The correlation between water temperature and calling intensity was very clear, with calling intensity increasing as the water temperature decreased (Figure 4.5). As with ambient temperature, there was a very clear range of temperatures in which the most calls were produced from 14°C to 9°C (Figure 4.5). Though water temperature is positively correlated with ambient temperature (Figure 4.3), water can retain more heat, which is why the frogs may call at air temperatures of 3°C (Figure 4.4), but do not call when water temperature is below 9°C.

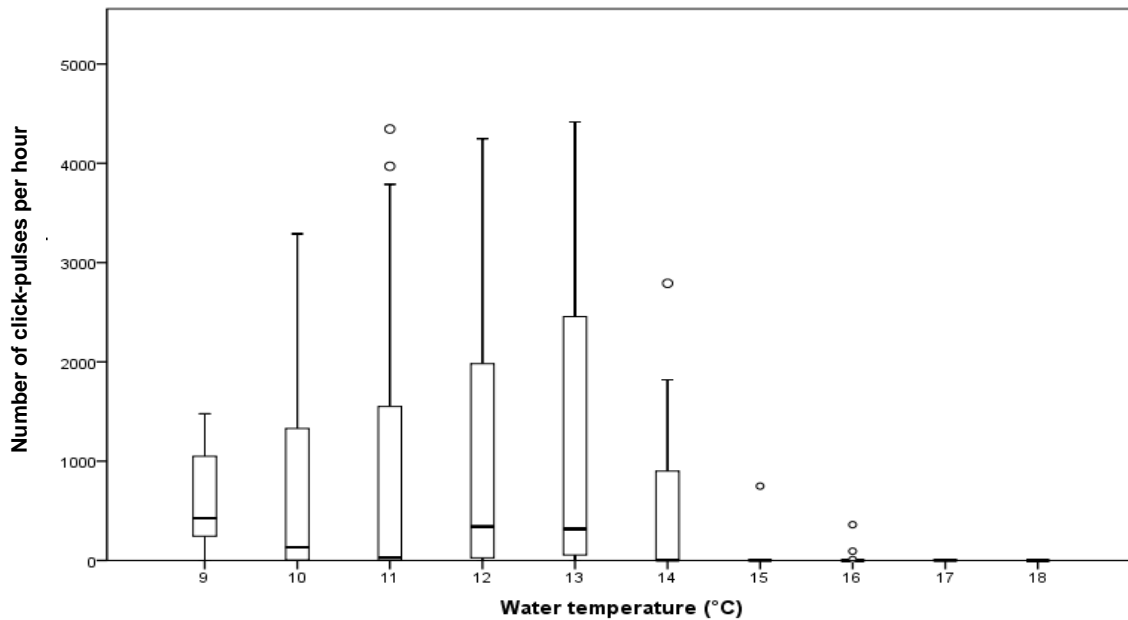


Figure 4.5 The effect of water temperature on calling intensity per hour. No water temperatures below 9°C were recorded during the study. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

Wind speed did not show a very strong positive or negative correlation with calling intensity (Figure 4.3), but it did show a very strong statistical significance with calling intensity ($p < 0.01$). Analysing the data using a Pearson correlation showed that there is indeed a positive correlation between the two, with call intensity increasing as wind speed increases (Figure 4.6). The highest number of calls was produced with the highest wind speeds recorded (12-19km/h), while the lowest number of calls was produced with the lowest wind speeds recorded (<1km/h) (Figure 4.6).

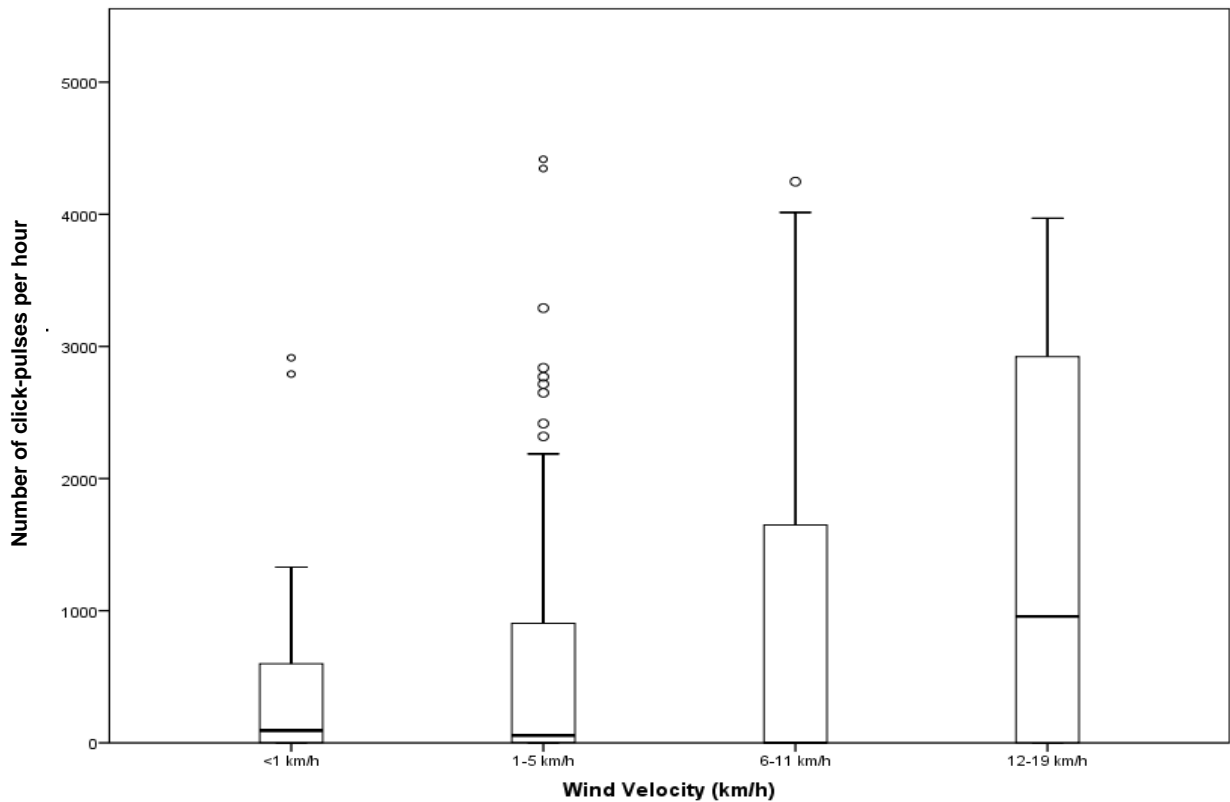


Figure 4.6 The effect of wind speed on calling intensity per hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

Because wind speed did not initially show a strong correlation with calling intensity, it was tested as a function of water temperature since this variable plays a definite role in calling intensity and might be influenced by wind speed. Water temperature, as a function of wind speed, still showed a negative correlation with calling intensity, showing the possible effect of wind speed on water temperature (Figure 4.7).

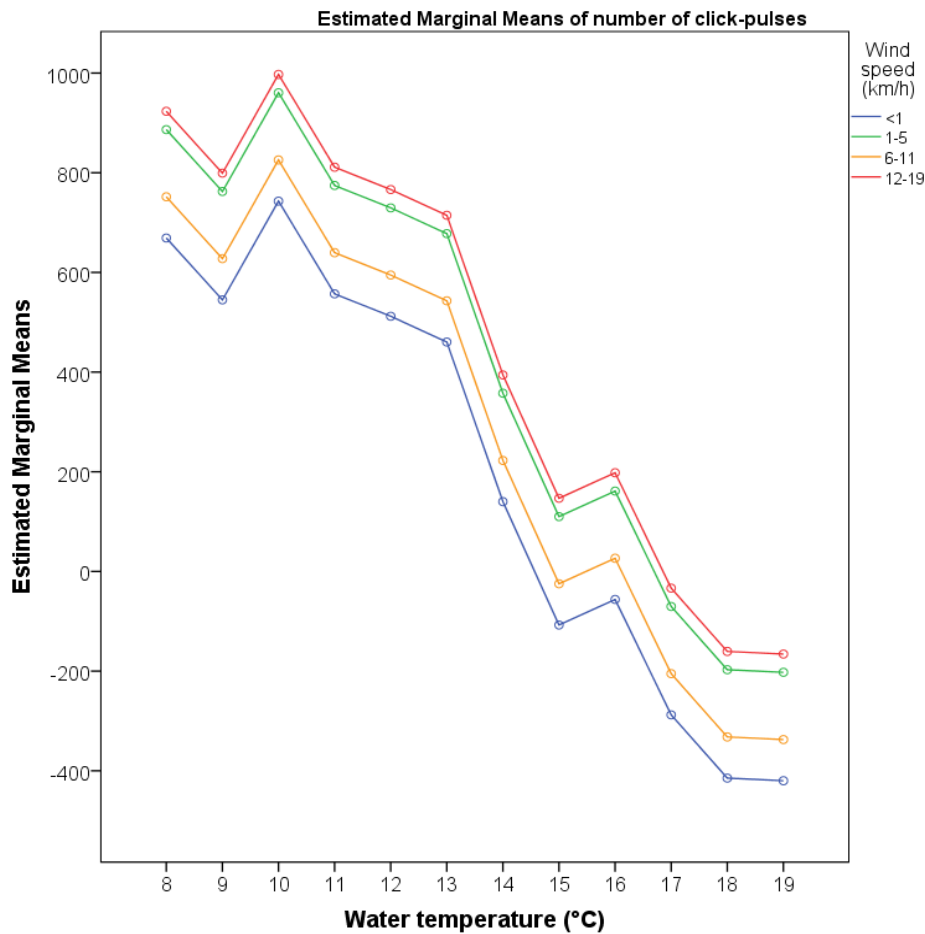


Figure 4.7 Estimated marginal means of calling intensity from the general linear model (GLM) showing the effect of water temperature as a function of the four wind velocity categories.

There was a strong positive correlation between calling intensity and percentage humidity initially (Figure 4.3), and a statistical significance was found ($p = 0.05$). The effect of the percentage humidity on calling intensity was different from the effects of previous variables, in that there was no linear relationship. What the results showed though, was that there were certain percentages where calling intensity peaked (36%, 69% and 81%), while there were almost no calls at 27-33%, 42-45%, 60%, 66%, 75% and 93% (Figure 4.8). None of the peaks represented drastically more calls than the others and percentages humidity with no calls were scattered between the peaks, so there was no clear, single percentage range that resulted in very high or very low calling intensities (Figure 4.8).

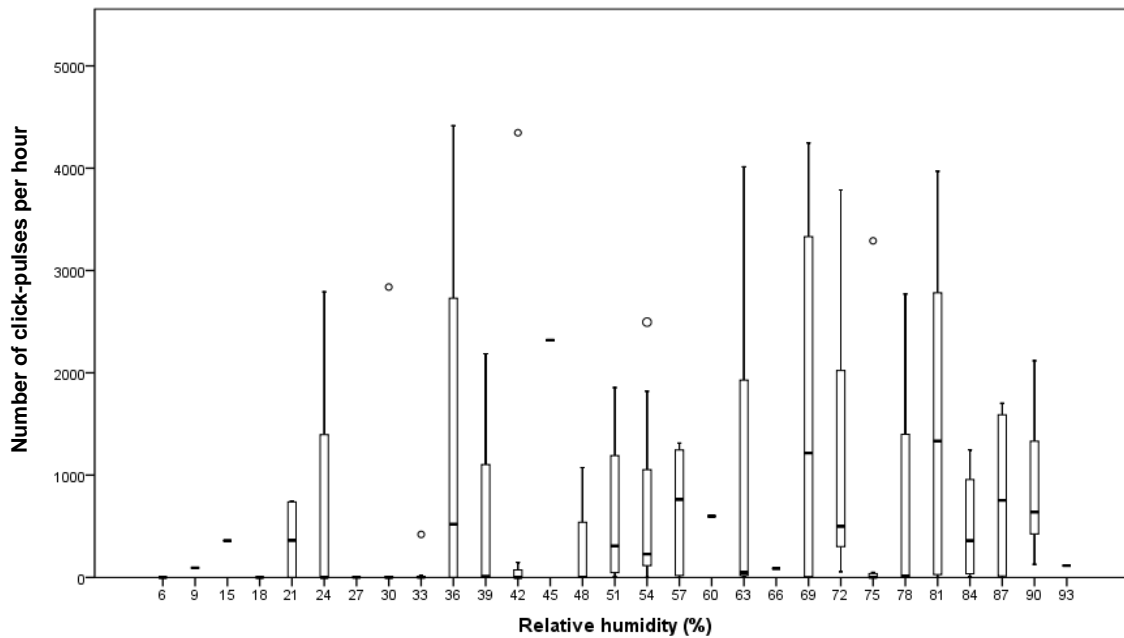


Figure 4.8 The effect of relative humidity on calling intensity per hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

The effect of barometric pressure on calling intensity was similar to that of humidity. There was no clear correlation initially (Figure 4.3), but it was found to be statistically significant ($p = 0.01$). Unlike humidity, however, there were two distinct groupings of barometric pressure readings where calling intensity had increased, 867.5-870.0 Pa and 874.0-875.5 Pa, with almost no calls in between (Figure 4.9).

I also tested to see whether the change in barometric pressure might show a clearer relationship. There was no significant difference between the change in barometric pressure and calling intensity ($p > 0.05$). There was also no clear linear relationship or even groupings, because although there was a sporadic increase in calling intensity as the barometric pressure dropped, the same happened at other times when the barometric pressure increased (Figure 4.10).

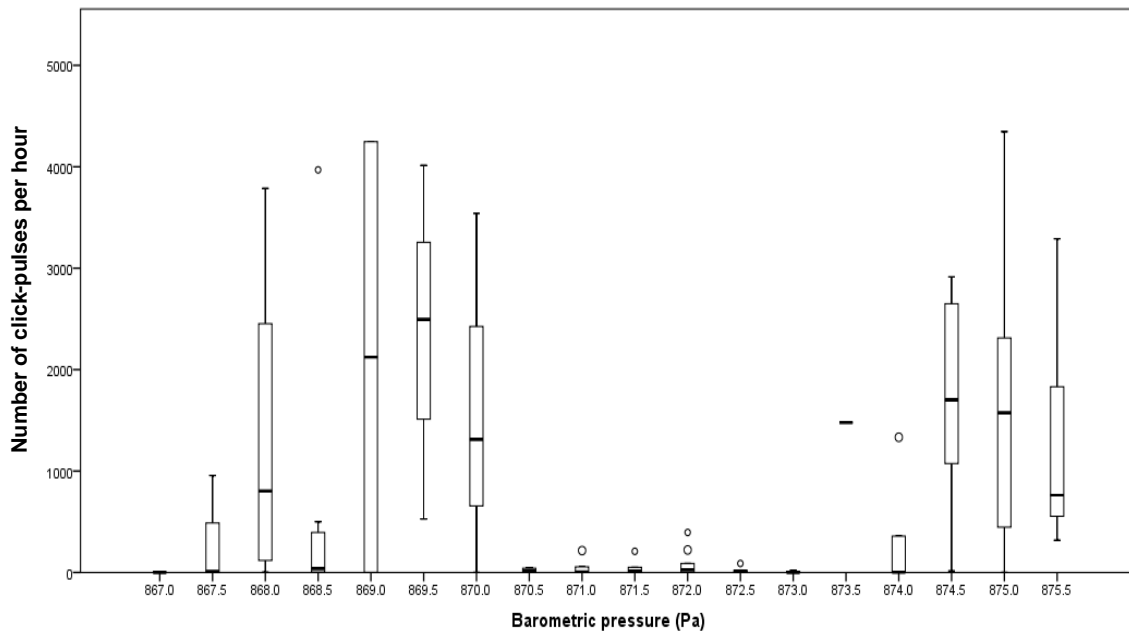


Figure 4.9 The effect of barometric pressure on calling intensity per hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

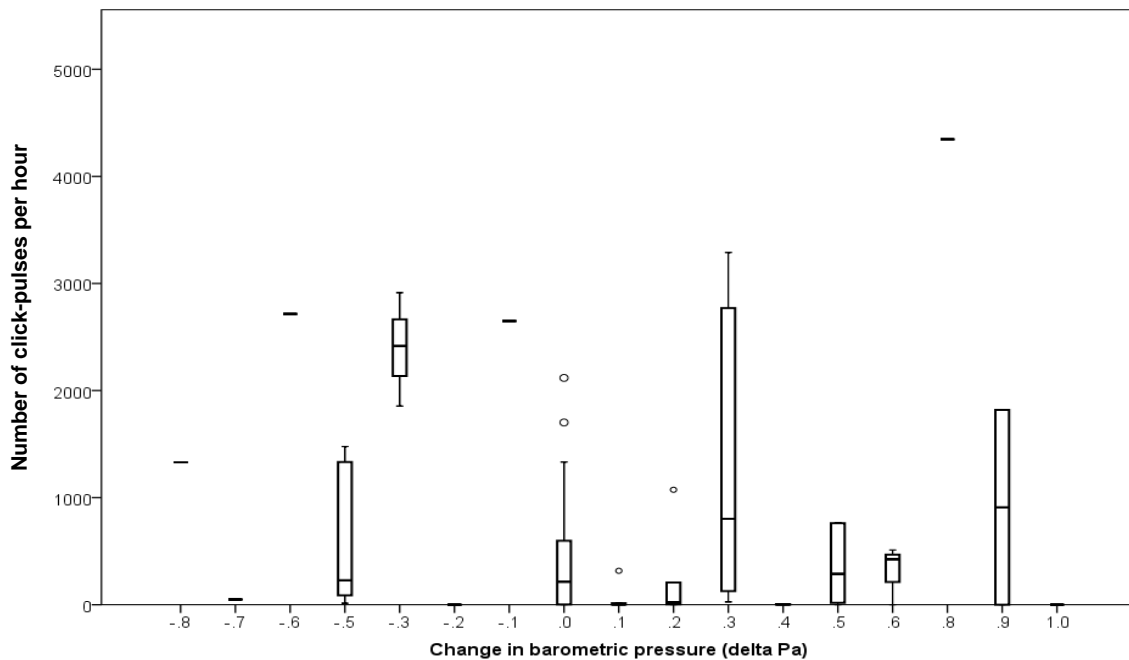


Figure 4.10 The effect of a change in barometric pressure on calling intensity per hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

The effect of the percentage moon illumination was also tested and it was found to be statistically significant ($p = 0.02$). However, because no clear correlation was initially found

(Figure 4.3), it was decided to rather use the data from the smaller time scale (20 min) recordings, as these were more likely to show a clear correlation.

4.3 Smaller time scale effects (20-minute recordings)

The results from the smaller time scale did not vary much from those obtained from the larger time scale. For the variables found significant, water temperature, wind speed, humidity and pressure ($p < 0.05$), the General Linear Model (GLM) drawn showed an r^2 value of 0.246.

Similar to the larger time scale, the ambient temperature was not found to be statistically significant ($p > 0.05$). Though there was still a negative correlation, with the call intensity increasing as the ambient temperature decreased, it was not seen as clearly as on the larger time scale. On the smaller time scale, a number of calls could already be observed at 21°C and calls were recorded for temperatures as low as -4°C (Figure 4.11), a large variation on the range seen in the larger time scale (3°C to 17°C (Figure 4.4)). The temperature at which the most calls were produced also differed between the two time scales, with the largest number produced at 13°C on the larger time scale (Figure 4.4), while the largest number of calls were produced at 5°C on the smaller time scale (Figure 4.11). It would appear that the effect of ambient temperature on calling intensity decreases on a smaller time scale, giving a clearer indication of its effect.

The effect of the maximum daytime ambient temperature was also measured against the calling intensity of the following night, but it was not statistically significant ($p > 0.05$). There was also no clear linear relationship between daytime ambient temperature and calling intensity, though there was a surprisingly high calling intensity at daytime temperatures between 22°C and 26°C, temperatures not normally seen during the winter i.e. the breeding season of *A. quecketti* (Figure 4.12). However, these are maximum temperatures, and temperatures are known to fluctuate during winter months.

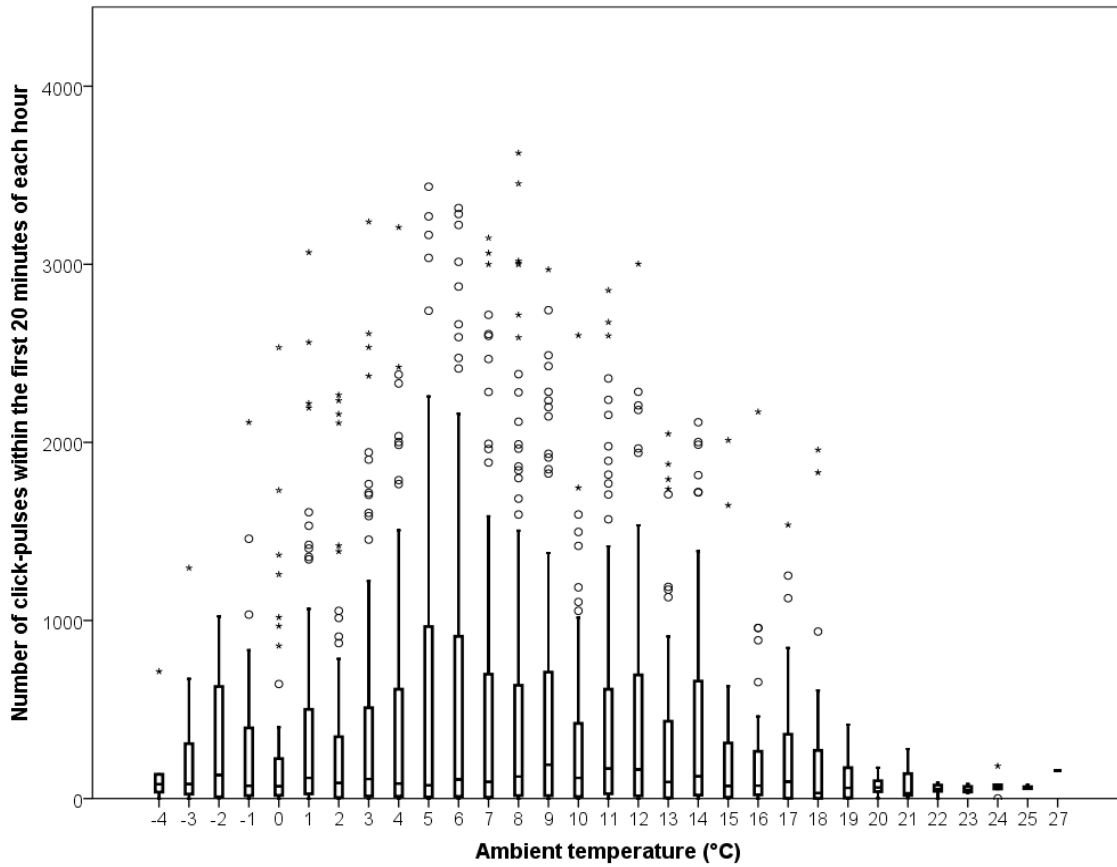


Figure 4.11 The effect of ambient temperature at night on calling intensity, for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

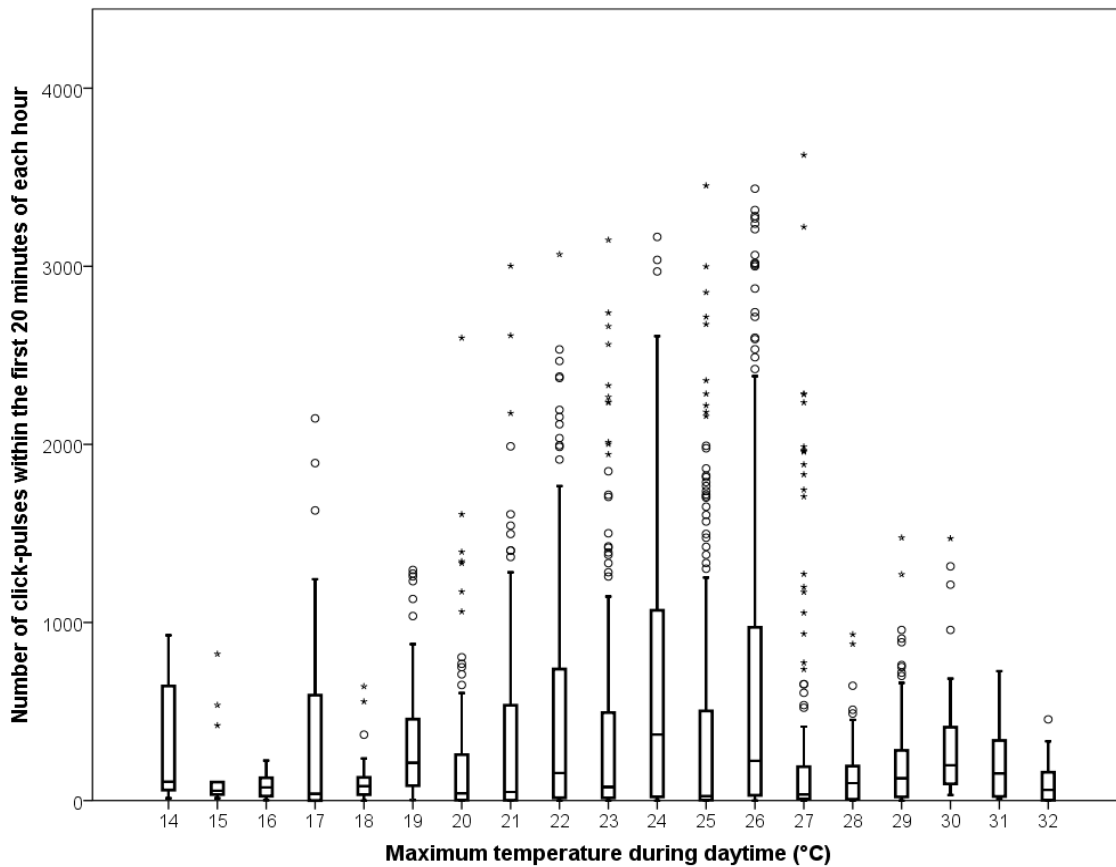


Figure 4.12 The effect of maximum daytime temperature on calling intensity during the night for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

As on the larger time scale, the effect of water temperature on calling intensity was statistically significant, but the significance was much stronger for the smaller time scale recordings ($p < 0.01$). The temperature for high calling intensity varied very little on the larger time scale, ranging from 9°C to 14°C (Figure 4.5), while on the smaller time scale it ranged from 8°C to 17°C (Figure 4.13). Again, *A. quecketti* showed a larger tolerance for higher temperatures, with the number of calls tapering off as temperatures increased, than for lower temperatures, where the calls immediately stopped below 8°C (Figure 4.13). The highest number of calls were produced at 10°C (Figure 4.13), though the second highest was produced at 13°C, the temperature where the highest number was produced on the larger time scale (Figure 4.5).

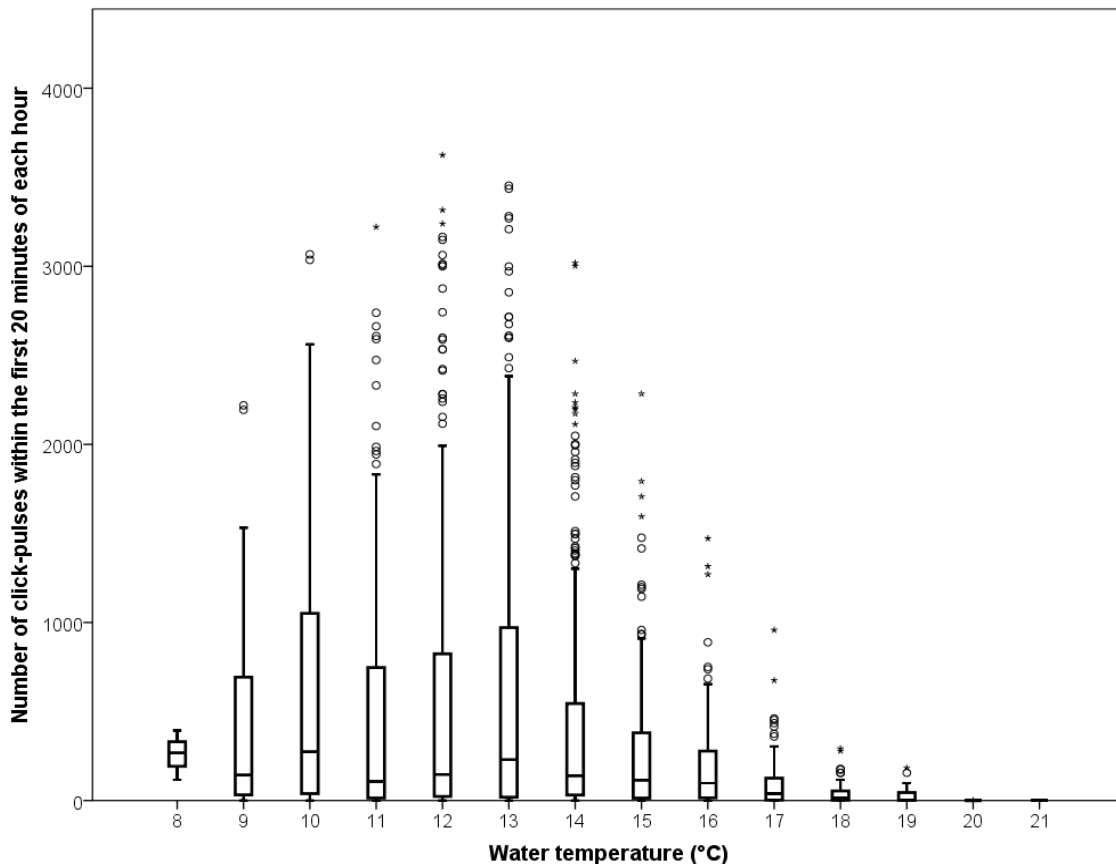


Figure 4.13 The effect of water temperature at night on calling intensity for the first 20 minutes of every hour. No water temperatures below 8°C were recorded during the study. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

Wind speed on the smaller scale was again statistically significant ($p = 0.046$). The exact same linear relationship was present as on the larger time scale, with the number of calls increasing as the wind speed increased. As on the larger time scale (Figure 4.6), the highest call intensity was found with the highest wind speeds (12-19km/h), while the lowest call intensity was found with the lowest wind speeds (<1km/h) (Figure 4.14). On the smaller time scale, however, the number of calls produced was almost the same for the lowest wind speed and the second lowest wind speed (1-5km/h) (Figure 4.14), while on the larger time scale there was a definite difference in the number of calls produced at each speed (Figure 4.6). As a correlation was found between wind speed and water temperature, and water temperature had a significant effect on the smaller time scale as well, it was expected that wind speed would also have a significant effect on the smaller time scale.

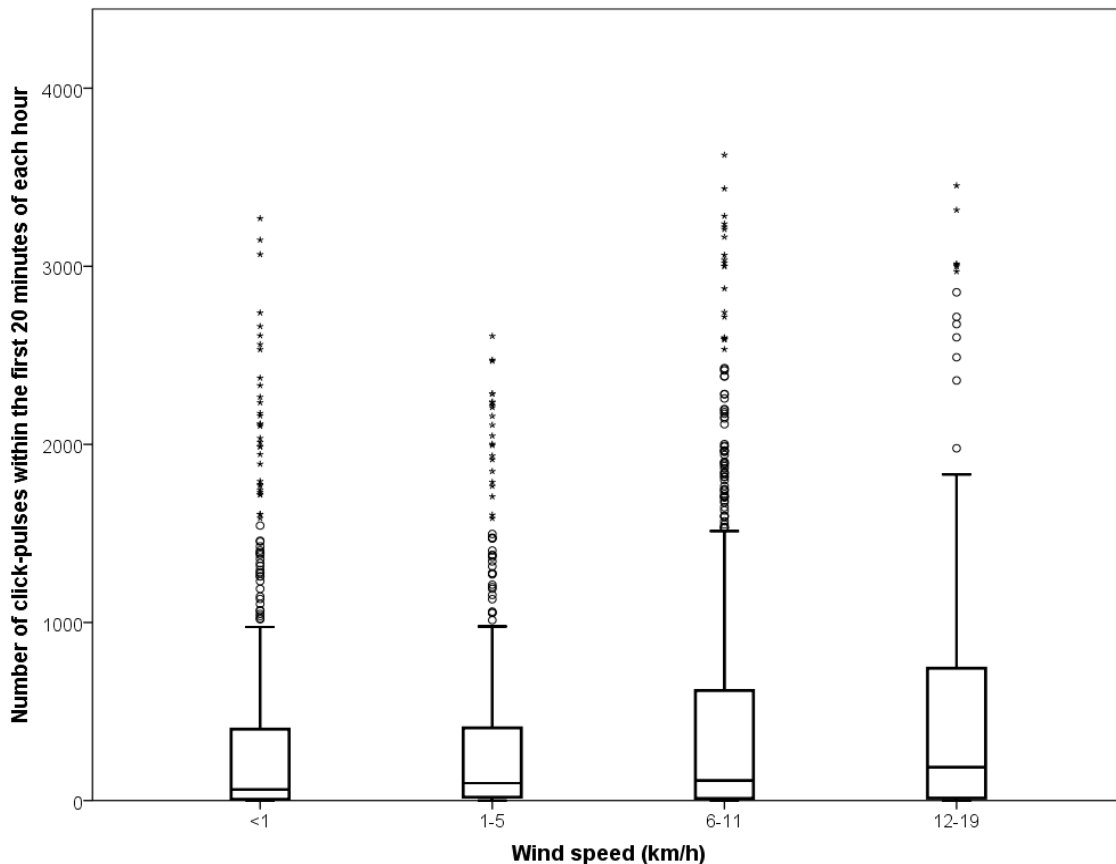


Figure 4.14 Graph showing the effect of wind speed at night on calling intensity for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

The effect of relative humidity on calling intensity was found to be significant on the smaller time scale as well ($p < 0.01$). As seen on the larger time scale (Figure 4.8), the effect of humidity on calling intensity on the smaller time scale was not linear either (Figure 4.15). Once again, there were certain percentages with high calling intensity, (39%, 45% (showed the highest number of calls) and 48%), while others showed very low calling intensity, (12%, 15%, 87% and 93% (Figure 4.15)). The biggest difference seen between the time scales was that on the smaller time scale there was less variation in the number of calls produced overall, and fewer percentages where there were almost no calls at all (Figure 4.15). On the larger time scale the most calls produced were almost a 1000 calls more than on the smaller time scale (Figure 4.8). This, however, might be because of how calls were sampled.

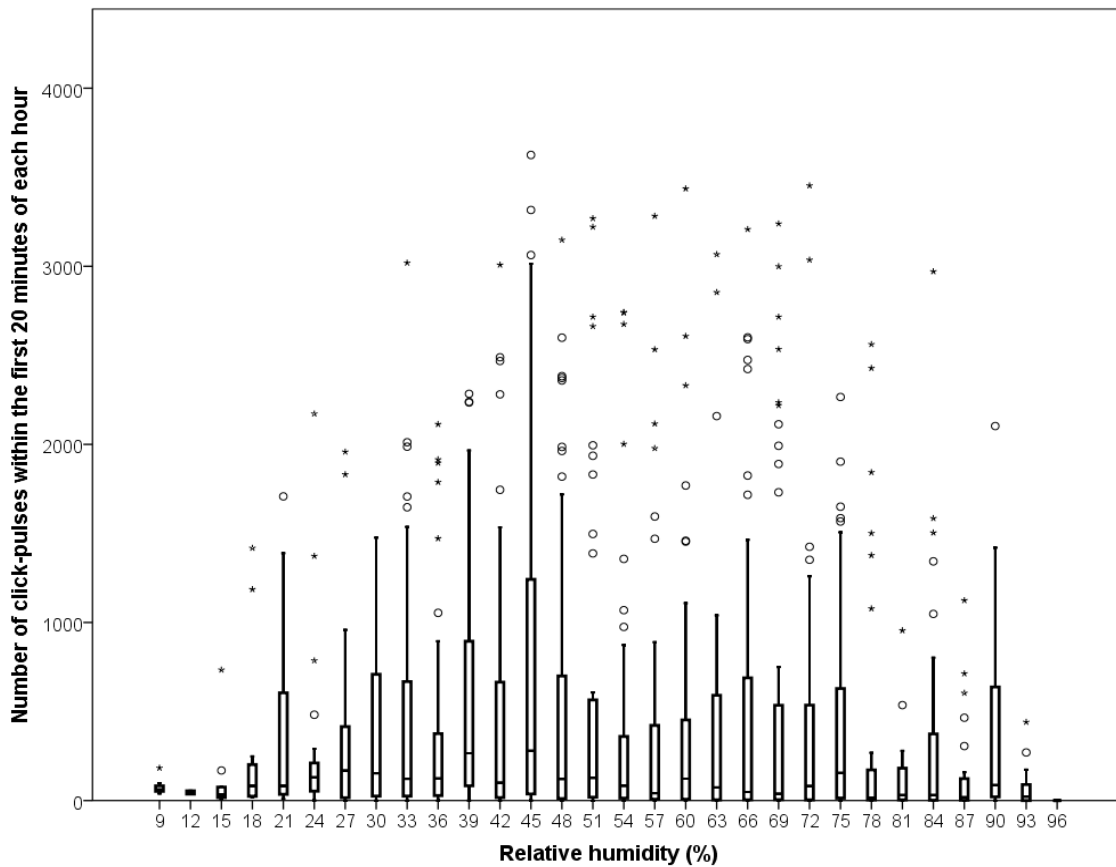


Figure 4.15 The effect of relative humidity at night on calling intensity for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

Barometric pressure also showed a statistically significant relationship with calling intensity ($p < 0.01$), as on the larger time scale. As on the larger time scale (Figure 4.9), there was again no linear relationship for the effect that barometric pressure had on calling intensity (Figure 4.16). Unlike on the larger time scale though, there were not two distinct groups where calling intensity increased (Figure 4.9). Rather, the calling intensity fluctuated with peaks at 866 Pa, 868 Pa and 872 Pa and decreased calling rates in between, though the only real low calling intensity was found between 860-865 Pa (Figure 4.16).

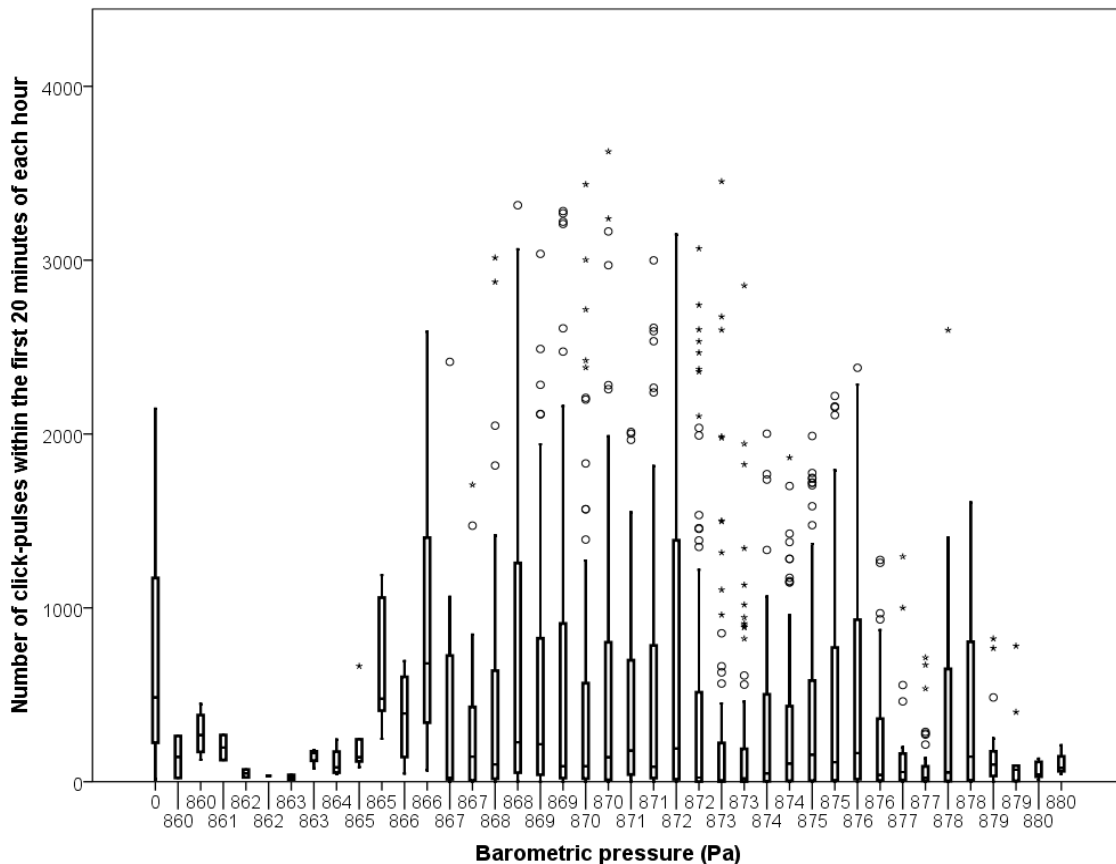


Figure 4.16 The effect of barometric pressure at night on calling intensity for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

As previously mentioned, the effect of the percentage moon illumination was found to be significant on the larger time scale ($p = 0.02$), but I decided to rather look at the data on the smaller time scale, which would explain the data better. On the smaller time scale the effect of the moon was found to not be significant ($p > 0.05$). The highest call intensities were found to be when there was a full moon, but also when there was a new moon and when there was 10% illumination (Figure 4.17). The lowest calling intensities (at 70% and at 80% illumination) were found when the moon was waxing and waning, i.e. in the middle of a lunar cycle as opposed to during a full moon or a new moon (Figure 4.17).

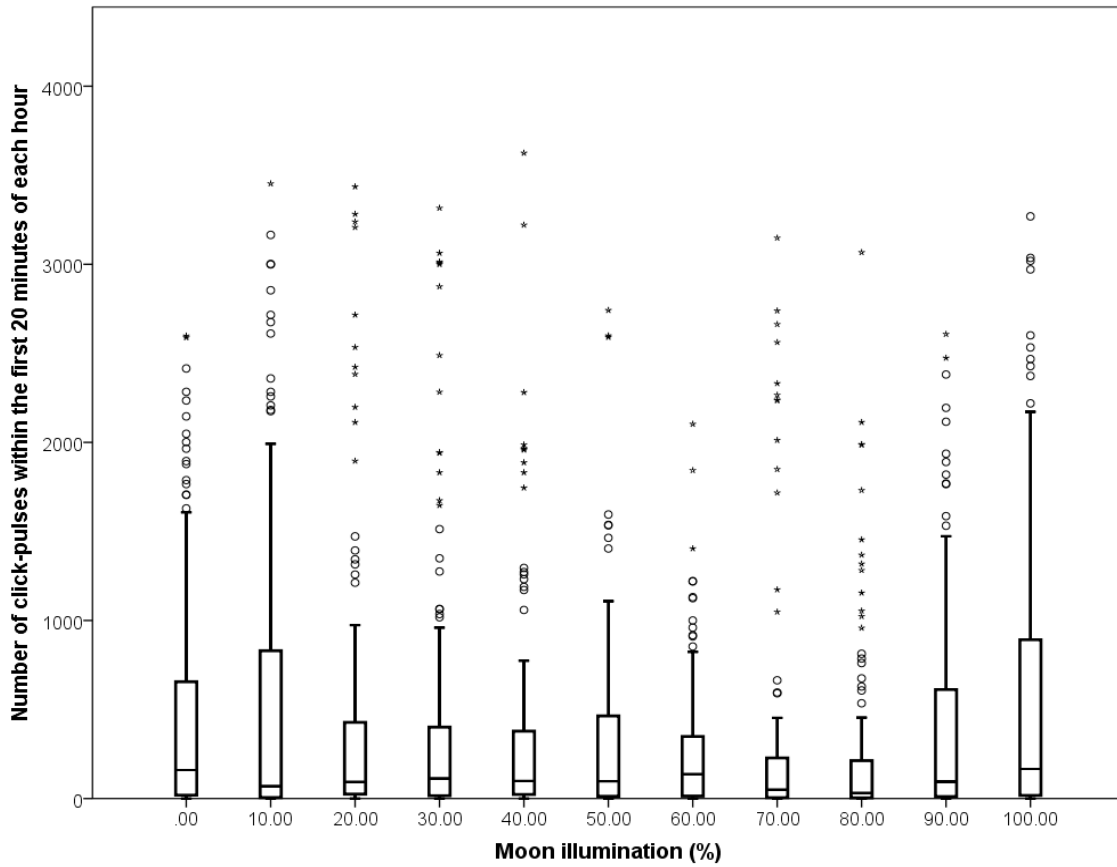


Figure 4.17. The effect of the percentage moon illumination on calling intensity for the first 20 minutes of every hour. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

5.1 Male and female attendance

In order to determine the population of *Amietia queckettii* in the study area and to determine the attendance profile, a survey was undertaken over a 19-day period. From 33 individually pit-tagged frogs, 23 were males and 10 females. Only 26 individuals (21 males and 5 females) were found on at least one night following the tagging event, which means that 7 frogs were not found again during the 19-day scanning survey. Females were the most elusive, with females 1 and 12 found on only three nights, females 13 and 19 on two nights and female 23 on one night. Male 4 was found most frequently, with 18 nights of attendance. During the 19-day survey, females were found to attend only 63.2% (12) of the nights (Figure 5.1). Three to nine males were found to attend between the nights of 21 and 29 July 2014, where after male attendance increased to between 13 and 18 between 1 and 14 August 2014 (Figure 5.1). Amplecting pairs were found on the 6th and 8th of August (Figure 5.1). The most females found on one night were four on the 25th of July (Figure 5.1).

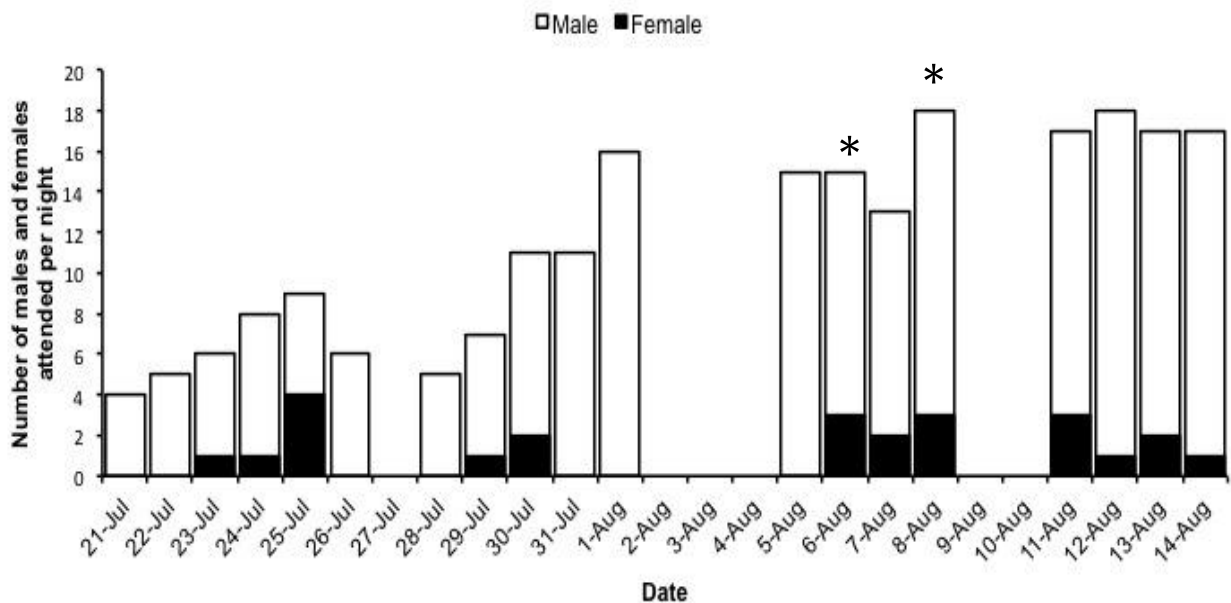


Figure 5.1 The number of males and females found at the pond during the nights surveyed. Dates with no data were not surveyed. Asterisks show dates on which amplecting pairs were found.

5.2 Effect of an individual's sex on territory size

A significant difference was found between individual males and their nearest male and female neighbours (mean = 1.52 m) and individual females and their nearest male and female neighbours (mean = 4.03 m, $Z = -2.851$ and $p = 0.003$, Figure 5.2). The average size of a male's territory, as defined by the distance to its nearest male neighbor, showed much less variation ($SD = 1.04$) than the average size of a female's territory ($SD = 2.52$). The average inter-male spacing distance, i.e. the distance between two males, was 1.76 m. The range varied from 0.10 – 7.76 m, while 50% of the individuals' nearest neighbours were 1.75 – 2.45 m away (Figure 5.2). Females were elusive at the study site (Pond 1, Figure 5.1). However, ponds 2, 4 and 5 hosted only female individuals. Measurements were not taken to determine the spacing distance at these ponds.

Site fidelity was also observed for both males and females, though it was more common amongst males (Figure 5.3). Male 4 showed the highest site fidelity by positioning himself at the same spot on 18 of the 19 nights that the study lasted and could be located under an overhanging sedge near the edge of the pond (Figure 5.3). Male 33 observed the second most nights at one position (16 out of 19 nights) and was also hidden in vegetation, but males 22 (13 out of 19 nights), 20 (10 out of 19 nights) and 5 (10 out of 19) nights were out in the open (Figure 5.3). The female observed was only in the pond for four nights, but stayed in one spot amongst vegetation (Figure 5.3).

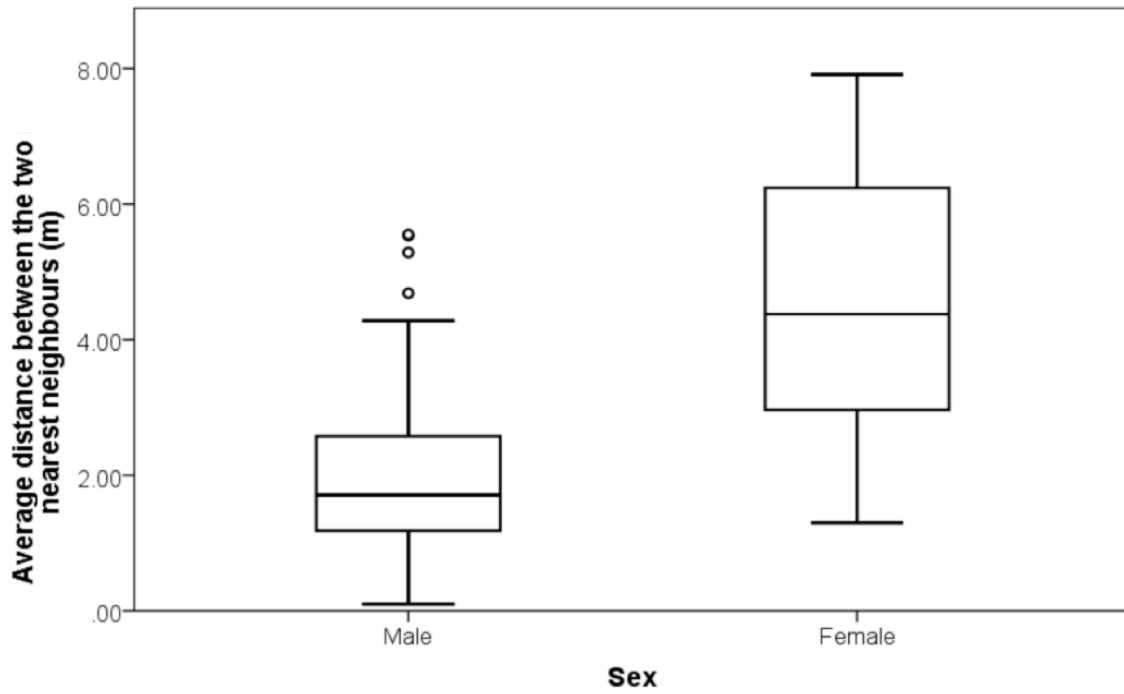


Figure 5.2 Box-and-whisker-plot showing the effect of an individual's sex on spacing distances. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

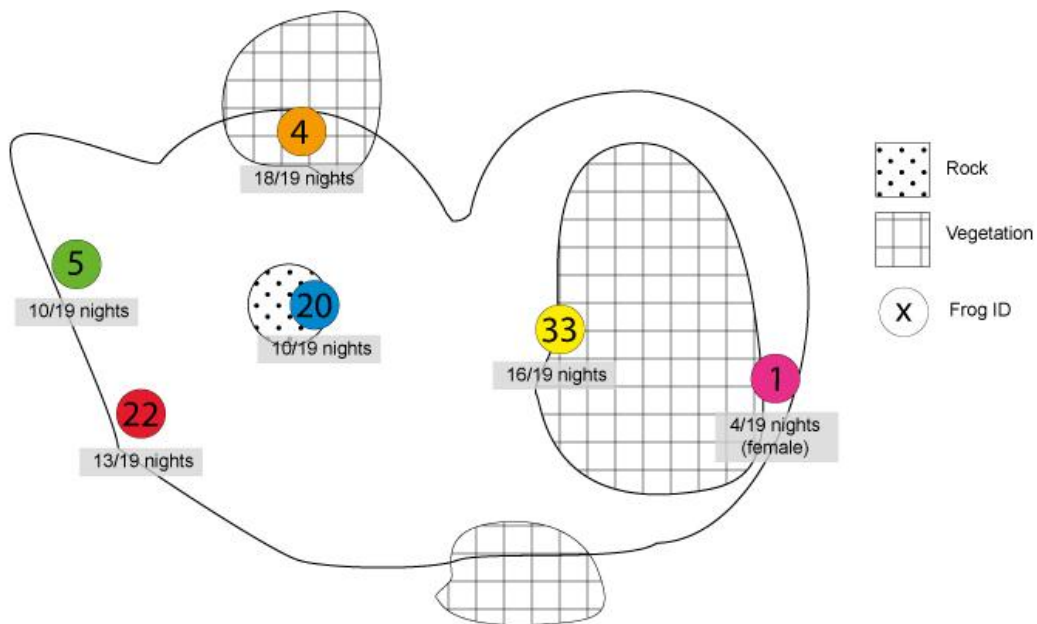


Figure 5.3 A graphical representation of Pond 1 showing site fidelity of five males and one female.

5.3 Effect of vegetation on inter-male spacing distances

A significant difference in the inter-male spacing distance was found based on the absence or presence of vegetation ($Z = -3.91$, $p > 0.001$). Males sitting in or under vegetation were found to have smaller inter-male spacing distances on average (1.26 m) than those who sat in the open (1.78 m) (Figure 5.4). A number of the males were found to show site fidelity, though this did not appear to be related to the absence or presence of vegetation. This part of the study focused specifically on males, but we also saw from the raw data that females were found in/under vegetation as well as in the open with no clear preference for either one.

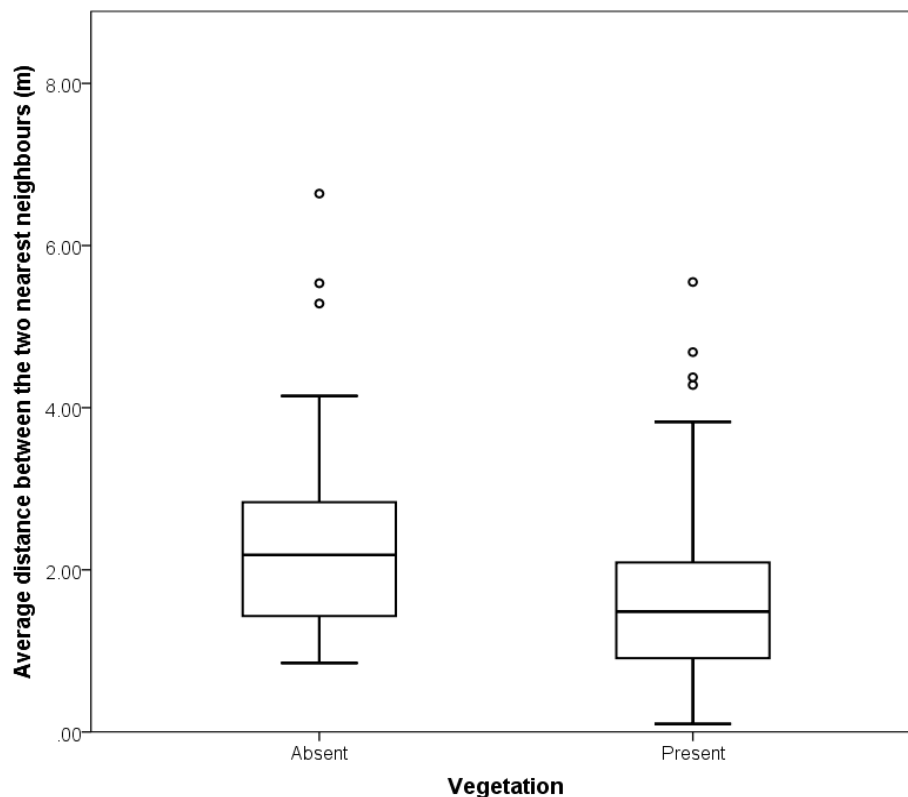


Figure 5.4 Box-and-whisker plot showing the effect of the absence/presence of vegetation on inter-male spacing distance. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

5.4 Effect of weight on inter-male spacing distances

No significant difference was found between the size of a male and its territory i.e. the inter-male spacing distance ($p > 0.05$). The small frogs (< 20 g) had a range of 0.05–4.5 m (Figure 5.5). The small frogs had the most outliers and showed a range of up to 8 m when including the outliers, giving it the largest range (Figure 5.5). Medium sized frogs (20–23 g) showed the largest range in territory size excluding outliers (0.1–4.9 m), while large frogs (23–39 g) showed the smallest range in territory size (0.05–3.8 m) (Figure 5.5). The medium sized frogs also showed the largest maximum distance while the largest frogs showed the smallest maximum distance (Figure 5.5).

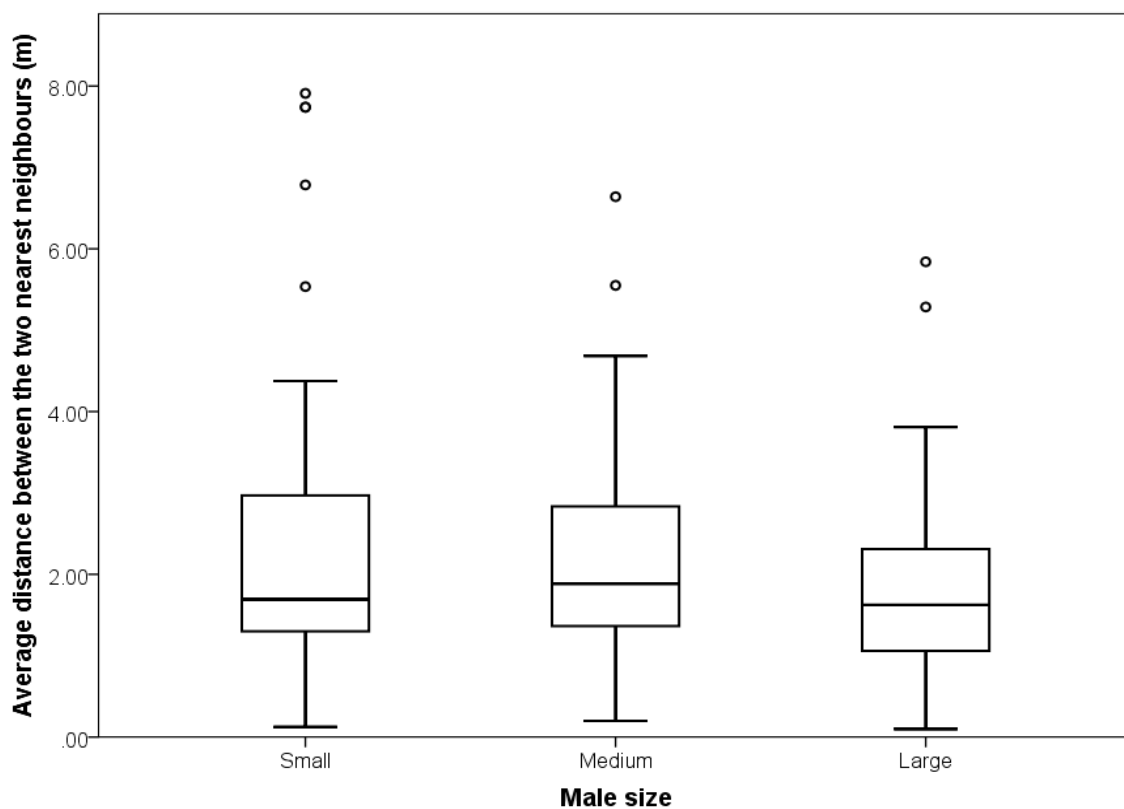


Figure 5.5 Box-and-whisker plot showing the effect of male size on inter-male spacing distance. Circles represent outliers, dark lines in the middle of each box indicate the median and box poles indicate interquartile values.

5.5 Calling intensity and inter-male spacing distances

A significant negative correlation was found between calling intensity and inter-male spacing distances ($p < 0.01$). As the distance between males increased, the calling intensity decreased and as the distance between males decreased the calling intensity increased (Figure 5.6). The number of males found at the pond increased over the course of the study, coinciding with an increase in chorus size and calling intensity and consequently a decrease in male spacing distance (Figure 5.1).

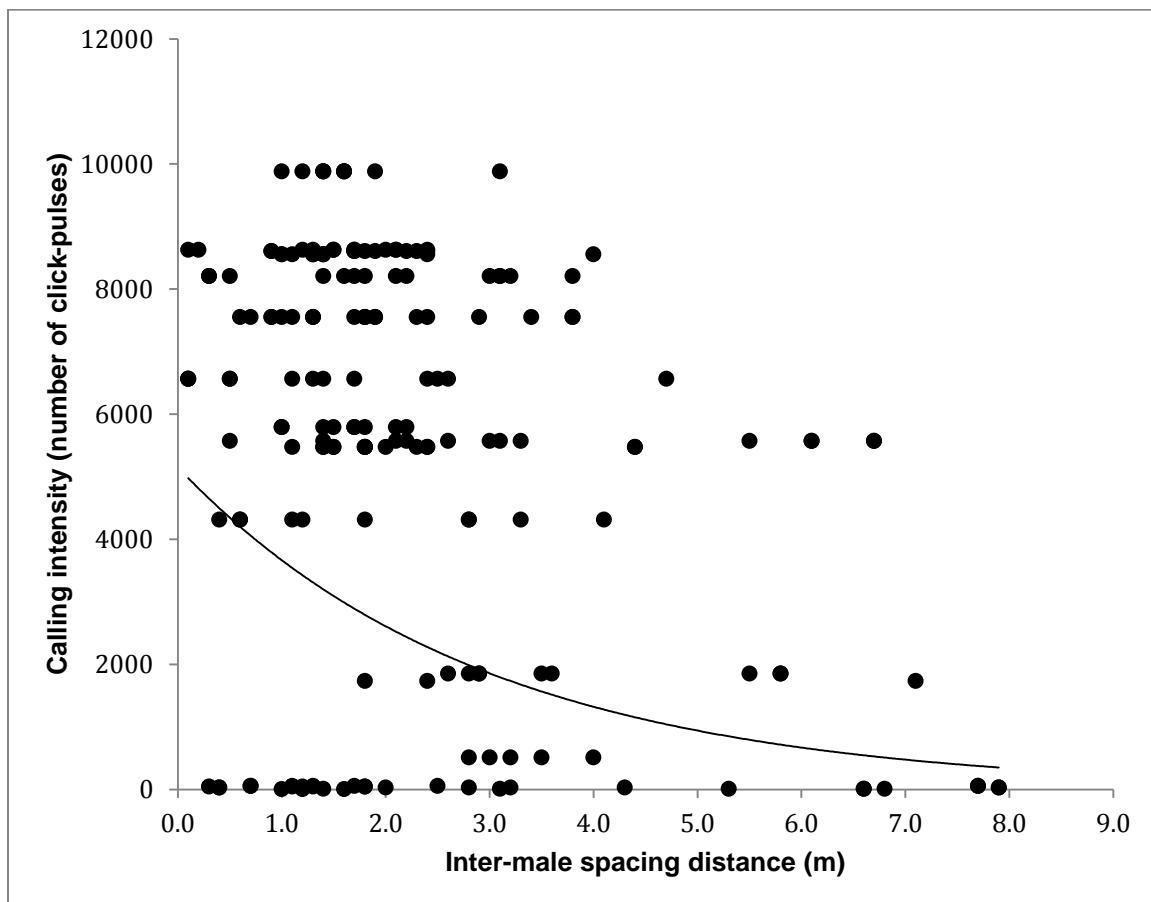


Figure 5.6 Scatter-plot showing the correlation between calling intensity and inter-male spacing distances.



If I can discover the meaning in the trilling of a frog, perhaps I may understand why it is for us not merely noise but a song of poetry and emotion. – Adrian Forsyth

6.1 Playback experiment

6.1.1. Calling repertoire

Frogs were the first vertebrate group in which vocalization evolved and today frogs are one of the most vocally active groups of animals on the planet. As loud and incessant as they may be, calls are species specific with only one or two different call types, showing very little variation (Du Preez and Carruthers, 2009). These call types are usually restricted to an advertisement call, an aggression call and in some cases, a release or a distress call (Feng *et al.*, 2002). However, even within a limited number of note types, calls can show quite a large amount of variation with regard to call duration or dominant frequency (Wagner Jr., 1989). Some species, however, do have large and extensive repertoires with a vast number of note types (Narins *et al.*, 2000; Feng *et al.*, 2002; Costa and Toledo, 2013). *Amietia queckettii* shows an unusually large and varied calling repertoire with seven identified call note types recorded in this study. *Amietia queckettii* shares certain similarities in note type with the Concave-eared torrent frog (*Amolops tormotus*), a frog known for its large and complex vocal repertoire (Feng *et al.*, 2002), specifically the onset of selective harmonics in a call note which can be heard in the whine note (Figure 3.4), whine-tonal note (Figure 3.7) and two-tonal whine note (Figure 3.8) of *A. queckettii*. The creak note and the tonal note (Figure 3.5) produced by *A. queckettii* are also similar to the iambic and tone-like notes produced by *Boophis madagascariensis* (Narins *et al.*, 2000). Comparatively speaking, the repertoire of *A. queckettii* is more similar to that of *Amolops tormotus* (Feng *et al.*, 2002), which contains a variety of click-like, iambic and tonal notes, than that of *Boophis madagascariensis* (Narins *et al.*, 2000), where the most variety is in the iambic notes it produces. The repertoire of *A. queckettii* can also be compared to that of *Odorrana tormota*, as both show a large amount of non-linear phenomena where note types show frequency jumps, frequency modulation and chaos (white noise) as part of the call (Feng *et al.*, 2009).

Variations between note types and within note types can be attributed to many factors. Variation within note types is usually related to male size e.g. larger males producing lower frequency calls (Ramer *et al.*, 1983; Tárano, 2001). The amount of variation between note types can be due to selection pressure, with female preference for more complex calls playing a defining role (Ryan, 1988). Speciation, female preference and call variation are also closely linked, with diverse female preferences resulting in speciation (Boul *et al.*, 2007). The environment and habitat of a frog can have a definite effect on the note types produced as well, with selection favouring calls that produce the clearest signals in a specific area (Ryan and Wilczynski, 1991). *Amietia queckettii* call from a number of permanent water bodies, including ponds and streams, in a number of habitats, all of which could influence the evolution of its diverse repertoire (Bro-Jørgensen, 2010). It also shares a distribution range with a large number of species (Du Preez and Carruthers, 2009) and could possibly have developed a diverse repertoire as a way to compete with sympatric species, a phenomenon often seen in Hylidae species (Duellman and Pyles, 1983).

6.1.2. Call note types

The function of a note type is usually inferred from the context in which it is given or the reaction of a conspecific male or female on hearing it (Roy *et al.*, 1995). However, more than one call note type can be produced for a specific reason (Odendaal *et al.*, 1983). The note types produced by *A. queckettii* appear to have different meanings, as each stimulus used for the playback experiment elicited a different response with regard to the number of each note type produced (Figure 3.9). Though a number of call properties were measured, e.g. call duration, dominant frequency and fundamental frequency, calls are considered effective, i.e. attractive or aggressive, based on variation in more than one aspect (Gerhardt, 1994).

The click note (Figure 3.3) was the most distinctive and most frequently produced note type in the absence of a stimulus (Figure 3.2). Call duration and dominant frequency were the two call properties that showed a large amount of variation (Table 3.1). Dominant frequency is considered a static property, while call duration is considered a dynamic property. Large variation in the dominant frequency of advertisement calls may allow for individual identification in a chorus, which can help a male to recognise whether the frog next to him is

merely a neighbour or rather an intruder who needs to be dealt with (Bee *et al.*, 2001). Dominant frequency is dependent on body size, with larger males producing lower frequencies, which imply that the variation in call frequency is determined by body size (Gerhardt, 1994). Call duration, though highly variable, can also be used as a means of identifying individuals (Gerhardt, 1991). In certain species, such as *Incilius valliceps*, call duration is positively related to SVL size, making it a good way to identify individuals (Sullivan and Wagner Jr., 1988). A variation in call duration may also allow frogs to call for longer periods when there is less interference and vice versa, which prevents call overlap (Ayre *et al.*, 1984). Certain species, like *Hyla versicolor*, use call duration as a measure of genetic quality where longer call rates equal better genes. This may also explain the variation in call duration (Welch *et al.*, 1998). The fact that almost all the stimuli in the case of *A. quecketti* elicited a large number of click notes (Figure 3.9), coupled with the fact that the click notes are the most frequently produced call in the absence of a stimulus, suggests that it is most likely an advertisement call.

The whine note (Figure 3.4) is not heard very often without a stimulus (Figure 3.2). Once again, only call duration and frequency showed any variation, though for the whine note the fundamental frequency plays a bigger role than the dominant frequency (Table 3.2). Fundamental frequency is also considered a static property. Fundamental frequency can vary greatly between species that share similar call structures, making it a useful tool for recognising conspecifics (Cocroft and Ryan, 1995). Fundamental frequency can also be used in the same way as dominant frequency to allow for discrimination between neighbours and intruders, though fundamental frequency can be influenced by more than just body size (Bee *et al.*, 2001). Fundamental frequencies have been shown to elicit responses from other males, with variation accounted for by the size of the calling individual (Ryan and Rand, 1990). These frequencies, considering that the most whine responses were elicited by the whine stimulus (Figure 3.9), are used to convey messages between males in a chorus. It is possibly a form of roll call to determine who is participating in the chorus or whether an individual close by is a neighbour or an intruder. Another possible explanation is that whine notes are used as matching calls. Matching calls are used to direct a threat at a specific individual with the idea that it could escalate into an aggressive response, even an attack, as the exchange continues (Arak, 1983).

The tonal note (Figure 3.5) is a very soft call, produced quite rarely in the absence of a stimulus (Figure 3.2). Though it has similar call properties to that of the whine note, in the tonal note it is the call duration and dominant frequency that show variation (Table 3.3). It is possible that the variation is more prominent in the dominant frequency in the tonal note, because the tonal note sends a different message than the whine note. The stimuli that produced the most responses were the clicks, the iambic notes and the silence (Figure 3.9). The iambic notes, though not counted as one of the responses, are considered an aggressive call. The tonal notes are possibly encounter calls, or warning calls, which are produced when an intruder male insists on producing advertisement calls in a resident's territory (Wells, 1978). The intruder would usually retreat, but if he continued calling, it could lead to an aggressive encounter (Robertson, 1986). Aggressive encounters are costly, both metabolically and for the time spent not advertising for a mate, and encounter calls serve to warn intruders so that they will move along without engaging (Bee *et al.*, 1999). Encounter calls are only produced by resident males to warn calling intruders and are not used for non-calling satellite males and females (Robertson, 1986; Rose and Brenowitz, 1997).

The creak note (Figure 3.6) is the second most heard call in the absence of a stimulus (Figure 3.2). It also tends to be a very loud call. Every aspect of this call is highly variable (Table 3.4). The variation in both call duration and number of pulses can be explained by an increase in the presence of other calling males (Bee and Perrill, 1996). Dominant frequency is once again a possible measure of size. The stimuli that resulted in the most responses were clicks and creaks, though the whine and iambic stimuli also produced a fair amount (Figure 3.9). Creak notes are possibly aggression calls. The fact that males will sometimes attack the speaker when presented with a creak stimulus supports this. In the case of prolonged breeders such as *A. quecketti*, males have territories from which they call and which they will defend (Give, 1988; Wagner Jr., 1989). If other males start producing advertisement calls, in this case clicks, too close to their territories or above the amplitude threshold used to determine distance, they will produce an encounter call. If the encounter call is too close to home, the resident will then respond with an aggression call. Though tonal notes are considered the true encounter calls, only whines were used as a stimulus and most likely fulfilled a similar function to tonal notes during the playback experiment. The fact that creaks were heard more

often than tonal notes or whines when no stimuli were played (Figure 3.2) is possibly due to the fact that a single encounter call may be given to warn intruders while a number of aggressive calls may be given during a single encounter.

The whine-tonal note (Figure 3.7) is a soft call that is not heard very often without stimuli present (Figure 3.2). Just like the creak note, this call also shows large variation across all the call properties; call duration, the dominant and fundamental frequencies of the whine part and the dominant and fundamental frequencies of the tonal part (Table 3.5). This means that a large amount of information is sent through this call. The two stimuli that elicited the most responses from this call were the whine and the iambic stimulus (Figure 3.9). Considering the most likely explanations for the functions of the creak note (the whine portion resembles a creak note, Chapter 3) and the tonal note, it is possible that this call is a combination of the two and acts as a graded aggression signal. Graded aggression calls imply increasingly agonistic calls as the intruder comes closer (Wagner Jr., 1989). This call seems to combine an aggressive call (creak note) with an encounter call (tonal note). Because aggressive encounters are metabolically expensive, this interaction can be avoided by increasingly agonistic calls instead of proceeding directly to a full aggression call or even attack (Wells, 1977). This calling behaviour is often seen in aggressive species such as *Hypsiboas rosenbergi* (previously *Hyla faber*) (Martins *et al.*, 1998). Unlike certain other species such as *Hyperolius marmoratus*, which also produce graded aggression calls, this specific call of *A. quecketti* is not one part of a continuum of graded calls, and is a call on its own (Grafe, 1995). Certain species such as *Pseudophryne bibronii* also produce territorial calls with the purpose of defending a territory (Wells, 1977; Byrne, 2008). Territorial calls can also show a similar graded response (Martins and Haddad, 1988). Because an interaction between frogs was not observed, it is difficult to determine whether this call is ultimately an aggressive encounter call or a territorial call.

The two-tonal whine note (Figure 3.8) has a distinct sound and is also not heard often in the absence of any stimuli (Figure 3.2). Variation was once again found in almost each call aspect for two parts of the three phases of the call, the second tonal and the whine aspect, but very little variation was seen in the first tonal phase (Table 3.6). All the variations are related to either body size (dominant and fundamental frequency) or could be responses to other calls

(call duration). All call stimuli produced a response, with the whine stimulus producing the biggest response (Figure 3.9). It is difficult to infer the role of the two-tonal whine note, as there are no clear relationships between any of the stimuli. Because the whine stimulus elicited the most responses, it is possible that the two-tonal whine note serves a similar function, i.e. to communicate with other males about size or position. Male-male communication is important to determine inter-male spacing, to determine the size of an opponent in aggressive situations, and to determine whether the frog next to it is a known neighbour or a strange intruder (Brenowitz *et al.*, 1988; Bee *et al.*, 1999; Bee *et al.*, 2001).

The tonal squeak note (Figure 3.10) is a short, high-pitched call produced by both males and females and was elicited by acoustic and physical stimuli. There is very little variation within the call (Table 3.8). The fact that both a male and a female produced this call, together with the fact that it is high pitched and the fact that both sexes move away from the stimulus, shows that this is most likely a release call. In general, release call frequency is negatively correlated to body size (Sullivan and Wagner Jr., 1988). Most likely, little variation is seen because there were only two individuals sampled, though there were five calls recorded. A release call is intended to convey that a male has clasped the wrong individual, whether another male or an uninterested/non-gravid female, and that he should release them (Boyd, 1992). This would be consistent with the conditions under which the calls were produced in my presence.

6.2 Atmospheric conditions

6.2.1 Common atmospheric conditions that affect calling behaviour

The main reason that frogs call is to attract a mate during breeding season. It stands to reason that environmental variables favouring reproductive success would act as cues for the beginning of the breeding season and for the frogs to start calling (Woolbright, 1985; Henzi *et al.*, 1995). Each species responds to different environmental variables even if they call at the same time (Oseen and Wassersug, 2002). Though there are threshold levels for certain variables, e.g. temperature, it is a combination of factors that influence activity and calling behaviour (Oseen and Wassersug, 2002). Calling is also metabolically expensive, which

means that frogs only tend to call when conditions are ideal and they obtain maximum benefit from it (Taigen and Wells, 1985). The influence of atmospheric variables becomes even more obvious with winter-breeding species, such as *A. quecketti*, as atmospheric conditions can fluctuate more during the winter (Kirlin *et al.*, 2006). The importance of each atmospheric variable may also change as the breeding season progresses (Oseen and Wassersug, 2002).

It should be noted that the two most common factors found to influence breeding behaviour and calling activity in anurans in general are rainfall and ambient temperature (Roy *et al.*, 2004). However, this study found that ambient temperature did not play a significant role. The breeding season of *A. quecketti* is also not during the rainy season and so rainfall also plays no significant role. Barreto and Moreira (1996), however, suggest that to truly understand the effect of rainfall on breeding and calling behaviour, rainfall should be measured throughout the year and not just during the breeding season. *Amietia quecketti* is known to live in permanent water bodies and its tadpoles will delay metamorphosis for up to 18 months waiting for ideal conditions, which suggest that a single dry year might not affect these frogs too much (Du Preez and Carruthers, 2009).

6.2.2 Effect of water and ambient temperature on calling behaviour

The temperature that affects the calling behaviour of frogs is related to the microhabitat from which the frogs call (Gayou, 1984). *Amietia quecketti* often calls while sitting/floating in water and therefore it makes sense that water temperature would have a greater effect than ambient temperature. Water temperature had a significant negative correlation ($p = 0.05$, $p = < 0.01$), with the number of calls increasing as the water temperature decreased (Figure 4.5 and Figure 4.13). This was in line with the assumptions about the linear effect that temperature has on the calling behaviour of ectotherms (Walker, 1975). However, there was a distinct threshold as the frogs stopped calling abruptly below 8°C and 9°C, depending on the time scale measured (Figure 4.5 and Figure 4.13). The ambient temperature, by comparison, showed no significant correlation (Figure 4.4 and Figure 4.11). Other species that call from the water are similarly affected by both ambient and water temperatures (Schneider *et al.*, 1984; Zweifel, 1959; Howard and Young, 1998). Water temperature also varies much less

than air temperature (Mohseni and Stefan, 1999). This more stable environment, especially when atmospheric conditions fluctuate more during the winter, shows why calling from the water is more beneficial for the frog. Microhabitat temperature is also important for metabolic processes, which might be one of the explanations for the very specific range of temperatures in which this frog calls (Fouquette, 1980). Water temperature has also been shown to affect the fundamental frequency of a frog call (Bee *et al.*, 2001). It is difficult to speculate on whether the currently preferred fundamental frequency developed as a result of a colder breeding season, or whether the colder breeding season led to a preference for the current fundamental frequency. Maximum ambient temperatures during the day also showed no significant effect on calling intensity at night (Figure 4.12). Day length and temperature are usually correlated, suggesting that though there is no direct or significant relationship, it may still play a role (Saenz *et al.*, 2001).

6.2.3 Effect of wind speed on calling behaviour

The literature shows wind and increased wind speed to have a negative influence on calling activity (Steelman and Dorcas, 2010; Johnson and Batie, 2001). It would seem logical for wind to have a negative influence, e.g. because of increased desiccation risk, though desiccation is usually influenced by a combination of temperature and wind speed (Shoemaker *et al.*, 1987). However, wind speed was found to have not just a significant correlation ($p < 0.01$, $p = 0.05$), but also a positive relationship, with the number of calls increasing as wind speed increased (Figure 4.6 and Figure 4.14). A possible explanation for this is that wind speed actually affects another variable that plays a larger role in influencing calling activity. Increased wind speeds are known to decrease water temperature, which might be an explanation for the increase in call intensity (Klemetson and Rogers, 1985). When water temperature is tested as a function of wind speed, it was found that water temperature does indeed decrease with an increase in wind speed (Figure 4.7). Another possible explanation is that an increased wind speed will allow females in other ponds to hear the calls. This signal, however, would only reach females in ponds downwind from the calling males. Increased wind speed combined with a temperature inversion would mean that a signal would reach much further (Larom *et al.*, 1997). Wind speed is at its slowest on the ground, increasing as height increases, resulting in sound at the ground level spreading

further in a horizontal direction (Wiley, 2009). A number of ponds with only females were observed and their numbers in the ponds varied. A more detailed explanation is given in 6.3.1, but here it is sufficient to say that females migrate to breeding ponds and stronger winds might mean that the calls from breeding ponds will be heard from further away.

6.2.4 Effect of humidity on calling behaviour

It stands to reason that the frogs would be more likely to call at times of higher humidity. As mentioned, humidity may also be an important cue for breeding as a way of determining whether eggs and tadpoles will survive (Hauselberg and Alford, 2005). Humidity was shown to have a significant effect ($p = 0.05$, $p < 0.01$) and a positive correlation with the number of calls (Figure 4.3). This was expected as many amphibians rely on a moist environment to prevent desiccation (Cree, 1989). Humidity increases the efficiency of resonant transmission and so calling at more humid times will send a clearer message (Harris, 1966). Though humidity is indicated to have a positive correlation with calling intensity, plotting the relationship on a graph shows that it is not as straight forward. *Amietia queckettii* shows very specific percentage ranges where there is high calling intensity while there is almost no calling activity between these ranges, even if humidity levels are higher (Figure 4.8 and Figure 4.15). Temperature, barometric pressure and humidity are all inter-linked. For this reason it is possible that though humidity is important due to desiccation, the specific percentages at which *A. queckettii* calls is linked to preferred temperatures or barometric pressure.

6.2.5 Effect of barometric pressure on calling behaviour

It was expected that there would be a negative correlation between barometric pressure and calling intensity as a drop in barometric pressure can act as a cue for the onset of rain, an important factor for most frogs (Oseen and Wassersug, 2002). For this reason, it was also expected that the change in pressure would actually be more indicative as that is what the frogs would react to. As mentioned, however, the breeding season for *A. queckettii* is during the dry winter and so a drop in pressure does not predict rain for this frog. It is possible that a drop in barometric pressure precedes a different atmospheric condition, such as the approach

of a cold front, which can influence calling behaviour. *Amietia quecketti*, however, does not show any significant reaction to a drop or spike in barometric pressure (Figure 4.9). In fact, like with humidity, *A. quecketti* has a number of ranges in which it prefers to call (Figure 4.8 and Figure 4.16). Again, temperature, barometric pressure and humidity are all linked, meaning that they influence one another and the effect of one on calling behaviour can show a similar result by another variable. Another possibility is that barometric pressure and humidity together might have a possible physiological effect on *A. quecketti*. It is well documented that barometric pressure and humidity can have a physiological effect on living organisms (Guedj and Weinberger, 1990; Ehara *et al.*, 2000). Early studies suggested that barometric pressure would influence physiological mechanisms as it plays such a big role in cellular structure (Cattell, 1936). It was also suggested that a drop in barometric pressure would decrease the body temperature (Gellhorn and Janus, 1936), an interesting thought for a frog that prefers calling at specific lower temperatures. However, no recent studies have been done to prove or disprove the above-mentioned studies and therefore it cannot be said for certain what physiological effect barometric pressure does or does not have.

6.2.6 Effect of ambient light on calling behaviour

Ambient light may have an effect on calling behaviour. Most amphibian species show some kind of response to the lunar cycle by increasing or decreasing calling intensity based on the moon phase (Grant *et al.*, 2012). Though there was a statistically significant effect of percentage moon illumination on the larger time scale, I rather looked at the effect on the shorter time scale, as there was no strong positive or negative correlation (Figure 4.3) on the larger time scale. The effect of percentage moon illumination on calling intensity was found to not be significant on the shorter time scale ($p > 0.05$). The easiest response to see is a response to the full moon or the new moon. The preference to call during moonlit nights or dark nights depends on the species and is usually related to avoiding predators, either by spotting predators first (moonlit nights) or preventing predators from spotting them (dark nights), as well as breeding synchronization (Grant *et al.*, 2012). *Amietia quecketti* appeared to call the most during new moon as well as full moon nights, though calling between these two phases was also observed (Figure 4.17). Mongooses have been spotted at the study site and so may be a possible predator for *A. quecketti* at night. It is possible that *A. quecketti*

employs both methods for predator avoidance (spotting predators and hiding from predators), or it is possible that the moon does not have any real effect on the calling behaviour of *A. quecketti*.

6.2.7 Non-significant atmospheric conditions

Though ambient temperature, maximum daytime temperature and percentage moon illumination were not found to be statistically significant, all these factors are still of ecological significance. A relationship can still be seen between ambient temperature and calling intensity in that calling intensity increases with a decrease in ambient temperature (Figure 4.4 and 4.11). Similarly, there are certain maximum daytime temperatures that result in higher calling intensities during the night (Figure 4.12). Looking at the percentage illumination, there are certain percentages where the frogs call more than others, specifically the new moon and the full moon. Knowing these relationships can be important for understanding the bigger picture of how atmospheric conditions affect *A. quecketti*.

6.3 Inter-male spacing

6.3.1 Presence of individuals at the study site

Certain species have designated “breeding ponds”, where males stay during the breeding season, as well as “resting ponds”, from which females migrate when they are ready to breed (Ringler *et al.*, 2009). The majority of frogs observed at the study site were males (Figure 5.1). Most males were also spotted a number of times, while most females were only spotted once or twice or maximally three times (Figure 5.1). It was also observed that males usually remained at the study site while females would be absent for a number of days before reappearing at Pond 1. Some of the other ponds had only female populations in which the numbers varied throughout the length of the experiment. It is possible that during the breeding season *A. quecketti* females have specific “resting ponds” from which they migrate to “breeding ponds” when they become gravid. I concluded that the site for this study was an active breeding pond as I observed pairs in amplexus as well as eggs and tadpoles.

6.3.2 Effect of individual's sex on spacing distances

In a lek breeding system, males aggregate at a specific site from which they advertise and compete for females to mate with (Sullivan, 1982; Klump and Gerhardt, 1992; Murphy and Gerhardt, 2002). In a lek breeding system, males defend small territories while female selection takes place (Balmford, 1991). Males of *A. queckettii* appeared to be a lot more tolerant with regard to nearest neighbours than the females, with males having closer nearest-neighbours than females (Figure 5.2). However, males were also a lot more specific with inter-male spacing distances than females, showing much less variation. In order to ensure that males are an optimal distance from one another they use amplitude thresholds of neighbours' calls to gauge a respectable distance (Brenowitz, 1989; Gerhardt *et al.*, 1989; Murphy and Floyd, 2005).

The other factor that could also contribute to the large difference in spacing distances between the two sexes is the migration of females. The females are the ones moving towards the males and choosing which males to mate with based on specific call properties (Balmford, 1991). The constant movement from and arriving at the "breeding pond" to finding the right male would explain the variation in female spacing distances. Males tend to clasp females and other objects that are roughly the right size and shape moving past them (Gerhardt *et al.*, 1994). For this reason, females would not want to sit too close to any males while waiting to hear from their ideal mate. This would explain why females tend to be rather far away from other individuals at times. Of course, as soon as they move towards a male they will get closer again which is another factor that can explain the large variation in distances.

6.3.3 Effect of vegetation on inter-male spacing distances

It is expected that calling sites with vegetation would be preferred as they provide shelter from predators, an important factor as the act of calling can attract predators (Ryan *et al.*, 1982; Given, 1988; Zuk and Kolluru, 1998). Frogs calling in a chorus specifically have an increased risk of predation (Ryan *et al.*, 1981). Males who sit under vegetation appear to allow other males to sit closer than males who sit out in the open (Figure 5.3). Hiding in

vegetation obviously provides a benefit to the individual, but this does not explain why inter-male spacing, and therefore male territory size decreases in vegetation. For certain species, vegetation increases sound attenuation and so larger territories are not needed in vegetation, as the calls do not reach that far (Roithmair, 1992). Literature in general does not offer many explanations, other than sound attenuation or perching sites, for the effect of vegetation on inter-male spacing (Telford and Dyson, 1988; Penna *et al.*, 2005). This, however, is not a likely explanation for *A. quecketti* as their calls are very loud and females are possibly attracted from other ponds, i.e. a shrub will not deter them in finding the calling male. *Amietia quecketti* males also do not perch on vegetation, making perching sites an unlikely advantage. A possible explanation could be that some of the males in the vegetation may be satellite males. Satellite males do not call at all, but readily share a larger, calling male's territory with the hope of intercepting females (Byrne and Roberts, 2004). It is possible that without vegetation cover, larger males will see the smaller ones and try and run off smaller satellite males, but under the cover of vegetation they are not seen by the calling males and become "invisible". Satellite males out in the open may also share a greater risk of predation without the greater benefit of attracting a female and so it pays to hide under vegetation. Certain females also prefer calls from more widely spaced males, indicating that if all males in the vegetation were to call it would not be as beneficial to them (Telford, 1985), which supports the theory of satellite males.

6.3.4 Site fidelity

Site fidelity seems to be common amongst species where males and females move to a "breeding pond" during a long breeding season (Pittman *et al.*, 2008; Ringler *et al.*, 2009). Certain males were observed to show a fair amount of site fidelity, returning to the same call site almost every night (Figure 5.4). Having constant recognisable neighbours that also show site fidelity most likely reduces the need for aggressive interactions, as these sites will most likely be an optimal distance away. This could be a selection pressure for site fidelity in prolonged breeders such as *A. quecketti* where inter-male spacing distances are important (Garcia-Rutledge and Narins, 2001). It did not appear as if any of the sites occupied the most by a single male showed a great advantage; two were in vegetation, but two were in the open and one was on a rock. A female was also observed showing site fidelity. This female was

seen for four nights in a row and all four nights she was perched on the same lily pad. The lily pad was near the edge of the pond (Figure 5.4), which makes it likely that it is where the female entered the pond. It is possible that the female was not yet gravid and was waiting for the right time to approach a calling male.

6.3.5 Effect of size on inter-male spacing distances

Certain call properties such as amplitude or dominant frequency can be used to determine the distance of a calling neighbour and so determine the spacing distance or territory size (Wilczynski and Brenowitz, 1988). Certain call aspects, such as dominant frequency, can be influenced by the size of an individual. The size of an individual, for example, can have a direct effect on dominant frequency. However, the size of a male is much more indicative of the outcome of an aggressive encounter than the size of its calling site/territory (Dyson and Passmore, 1992). This was evident in the results where there was no significant difference in inter-male spacing based on size ($p > 0.05$). There was still some variation, with medium sized frogs showing the largest average territories (Figure 5.5). The difference in size would account for the variation in spacing distances between the males. The fact that the smallest males had the largest inter-male spacing range (including outliers) is most likely because they are not yet big enough to defend a territory, with the result that they just stay further away from other calling males (Woolbright, 1983; Given 1988). They are also more inclined to be satellite males, which explain some of the shorter distances. The optimal inter-male spacing distance also falls within a very specific range, which is most likely why there is no statistically significant difference in territory size. Even accounting for the effect of body size on dominant frequency or amplitude, there is a specific range which allows the males to distance themselves optimally.

6.3.6 Calling intensity and inter-male spacing distances

Calling intensity increases as inter-male spacing distances decrease (Figure 5.6). Though it would seem that the two have a direct impact on one another, there is actually a third factor involved which affects both variables, namely chorus size. As chorus sizes increase, calling intensity will also increase, but as chorus sizes increase, inter-male spacing distances will

decrease (Gerhardt *et al.*, 1989). A possible explanation for this is based on female preference. Female preferences for specific call properties are only shown in smaller choruses as many properties become masked in the larger chorus (Telford *et al.*, 1989). In smaller choruses, males will actually call less as chorus size increases to allow for female preference in mate choice (Whitney and Krebs, 1975, Wollerman and Wiley, 2002). During optimal breeding times, however, as chorus size increases, female preference becomes less specific, so discrimination between males becomes less important while chorus participation becomes more important (Wollerman and Wiley, 2002). As chorus size increases, the average inter-male spacing distance will decrease and males will call closer to one another without becoming aggressive (Whitney and Krebs, 1975; Dyson and Passmore, 1992). This is most likely because the cost of aggressively keeping a larger territory outweighs the benefit of a larger territory in the presence of so many males. The end result of this is that there will be a negative correlation between calling intensity and inter-male spacing distances though the two may not have a direct effect on one another.

6.4 Conclusion

The first objective was to elucidate the context in which the different calls are produced by the males of *A. queckettii* by means of playback stimuli. *Amietia queckettii* was long considered a “common” frog with a limited vocal repertoire (Du Preez and Carruthers, 2009). This study has shown the opposite to be true. Not only does *A. queckettii* have a large and complex vocal repertoire, but the note types produced also appear to have specific meanings. I managed to postulate a purpose for five of the note types, excluding the two-tonal whine note, where the context in which it is produced is still not clear. The most unexpected result was that each note type is used differently in response to the same stimulus. It was also interesting to note how much the advertisement call (click note) differs from the rest of the calls produced. It would be interesting to determine in a future study why the advertisement call is so different. The results of this study indicate that communication in frogs is more complex than we think. All the note types (and their inferred meanings) have not yet been described, paving the way for future research in this species. It also raises questions on frog communication, such as why there is such a variety of note types, how did they evolve and how will continuous habitat encroachment affect them?

The second objective was to investigate the influence of six meteorological variables on the calling activity of *A. queckettii*. No one can escape the weather and all its variables and it is no different for frogs. In fact, it is expected that atmospheric conditions would have a profound effect on frogs, as they are so vulnerable during their entire life cycle. I found that of the six variables tested, water temperature, wind speed, humidity and barometric pressure showed a significant effect. The effect of wind speed is a surprise as literature suggests that wind speed should have a negative effect on call intensity, while it had a positive effect on *A. queckettii*. The effects of barometric pressure and humidity were also not as expected based on the literature and so while these effects are now known, the reasons for them are still vague. Future studies should definitely focus more on the effects of barometric pressure and humidity on frog behaviour in general, as it would appear that it is assumed that these factors only have one of two effects. It would also be beneficial to take a closer look at the effect of wind speed on sound propagation of frog calls at ground level. Finally, it is necessary that future studies focus more on the relationship between atmospheric conditions as well as the

effect of single conditions on calling behaviour. Climate change is a real problem and understanding how the current (and past) climate affects frogs can be a powerful tool for conservation.

The third, fourth, fifth and sixth objectives were to investigate whether spacing is influenced by sex, whether the absence or presence of vegetation in a male's territory affects inter-male spacing distance, whether the size of an individual affects inter-male spacing distance, and to examine the effect of male density (distance to nearest male) on the calling intensity of *A. quecketti*. The relationship between animals and their immediate environments are always important. Understanding what these (and other) frogs need to breed successfully is important for conservation as well as to just form a better understanding of a specific species. I found that spacing distance is indeed influenced by the sex of an individual, with females preferring their neighbours much further away than males. I also found that the absence or presence of vegetation definitely affects inter-male spacing distances, with males in/under vegetation having much closer neighbours than those outside. I also found that males, and sometimes females, show site fidelity and return to the same site each night. However, the absence or presence of vegetation did not affect site fidelity. Lastly, I found that calling intensity and inter-male spacing distance is negatively correlated. However, a third factor, chorus size, is what causes the correlation. The most interesting result obtained from this part of the study was that females migrate to a "breeding pond" as opposed to just staying in one pond with the males. It was also really interesting to see how much variation there is within inter-male spacing distances. This part of the study was done over only four weeks and it would be interesting to see the results of a study done over an entire breeding season, specifically aimed at females and how often they return to the "breeding pond". It would also be interesting to see how far the frogs, both male and female, travel to reach the "breeding ponds".

In conclusion, *Amietia quecketti* shows that no species should be taken for granted simply because it is often seen. Each species is complex and unique in its own way. Living in a world where so many amphibians are endangered, it becomes even more important to understand these creatures in the hope of saving them as well as highlighting their significance.

Reference style according to *African Zoology*.

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