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AN EXAMINATION OF CANE TOAD (*Rhinella marina*) BEHAVIOUR: HOW CAN WE USE THIS KNOWLEDGE TO REFINE TRAPPING REGIMES?

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ETHICS STATEMENT

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian code of practice for the care and use of animals for scientific purposes, 7th edition, 2004, and the Queensland Animal Care and Protection Act (2001). The study received approval from the James Cook University animal ethics committee (approval numbers A2275 and A2046), the Western Australia AEC (permit number: U232/2017), and the Northern Territory AEC (permit number 01/2275).

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72

ABSTRACT

Invasive species are of major concern to ecologists, because of their impacts on native fauna, communities, and ecosystems. Invasive species may alter the evolutionary pathways of native species by competitive exclusion, niche displacement, hybridisation, introgression, and predation, at times ultimately causing extinction. Further, the economic cost associated with invasive species, through losses in agriculture, forestry, and tourism, as well as the costs of preventing and controlling these species, are of major concern to land managers and governments. Specifically, the management of vertebrate invasive species is a crucial component of biosecurity, ecology, and land management. There are a range of control methods for invasive vertebrates, including hand-capture, trapping, baiting, shooting, and biological and genetic control methods. These control strategies vary in efficacy, depending on the life history and behaviour of the target species, the area over which removal occurs, and the method of delivery of the control. Understanding these factors assists with designing targeted control strategies, in which the chance of removal of each individual, or the impact of each capture, or both, is increased. The success of control methods for some invasive vertebrates has improved considerably over the last several decades, due to the ever-increasing body of research about the behaviour and life history of certain invasive species, and the refinement of control regimes in relation to new information.

The invasive capabilities and impacts of amphibians generally receive less attention than other invasive vertebrates; as such, control methods for invasive amphibians are rare. Some invasive amphibians are generalist feeders, have high reproductive rates, and attain large population sizes; however, specific behavioural and life history traits are varied, and are often unknown. Further, abiotic factors, such as atmospheric temperature and moisture, effect the behaviour and activity of many amphibians. Current control strategies for invasive amphibians (e.g., hand-capture, exclusion fencing, and habitat modification) are often non-targeted, under-researched, and ineffective.

Cane toads (*Rhinella marina*) are highly invasive anurans, native to south and central America. Their invaded range extends through many tropical areas worldwide, including Australia. Cane toad paratoid (shoulder) glands secrete powerful bufotoxins that are lethal to some native predators, and domestic pets. The impact pathways of cane toads on native species include poisoning after ingestion (both at larval and adult stages), and competition with other anurans. Further, the presence of cane toad tadpoles may affect growth rates of native tadpoles, while the presence of adults may affect calling behaviour of some native anurans.

Potential control strategies for cane toads within their invaded range include hand-capture, tadpole traps, and biological and genetic control methods. These strategies are often non-targeted (e.g., tadpole traps, biological and genetic control methods), have been ineffective at suppressing toads for long periods, on a large scale, and in some cases require extremely high effort (e.g., hand-capture events). Trapping adult individuals using a solar-powered light and acoustic lure that automatically plays a cane toad call to attract toads into traps may be a viable control method that is easily refined to increase captures by exploiting behavioural characteristics of the cane toad. The success of any control method is dependent on the ability to refine it by targeting specific demographics of the invasive population, and increasing the number of captures per unit effort spatially, and temporally.

Many control methods for cane toads are ineffective because they do not consider the activity patterns of toads in response to abiotic factors; however, understanding and exploiting these patterns could allay wasted effort. For example, land managers could augment captures by understanding the environmental conditions that drive activity, at different times of year, and focusing trapping effort on periods when toads are most active. I examined cane toad activity (numbers of captures) in response to several environmental variables (humidity, temperature, rainfall, wind speed, and moon luminosity) over eleven months of trapping. Captures were highest (i.e., toads were most active) in the wet season (Dec – Feb), and lowest in the dry season (Jun – Aug). In the wet season, wind speed and minimum temperature effected activity (toads were most active on warm, still nights), while rainfall was the strongest predictor of activity in the dry season. I suggest that land managers could allay wasted trapping effort by focussing on nights with conditions conducive to toad activity (e.g., wet nights during the dry season).

It is important to determine the area over which toads are attracted to the call used as a lure in traps (the active space of the call), to aid in trap placement and the design of large scale trapping regimes. A vocalisation's active space is the area within which a receiver responds to it, while its maximum extent occurs when a receiver stops responding. I mapped behavioural responses of male and female cane toads to advertisement calls by conducting experimental playbacks to quantify the active space of calls for both sexes, separately. Both sexes displayed positive phonotaxis 20 – 70 m from calls. Males also displayed positive phonotaxis 70 – 120 m from calls, whereas females' movement preferences were random >70 m from a call. Differences between male and female responses were likely driven by differences in their use of information provided by calls. I suggest that traps should be placed 140 m apart, such that a female toad can never be more than 70 from a trap, but effort is not wasted by 'over-trapping' in the target area.

Targeting reproductively active females is the best strategy for reducing recruitment into the next generation, and is a common control technique for vertebrate pests with high reproductive rates. Female cane toads can lay over 10 000 eggs per clutch, and should be targeted, however current control regimes do not focus on the removal of females. The lures used in adult cane toad traps play an advertisement call used by male toads to attract females. In many anurans, females select mates based on the structural parameters of advertisement calls (e.g., dominant frequency and pulse rate), therefore modifying the parameters of calls used as lures in cane toad traps, to create especially attractive calls, may augment gravid female captures. I altered the frequency and pulse rate of artificial calls used as lures, and conducted several trapping regimes in and around the Townsville region in northern Australia, to determine which calls were most attractive to gravid females. Overall, gravid females preferred a ‘combination’ call with a low dominant frequency, and high pulse rate (relative to the population median for these parameters). Approximately 91% of the females trapped using a low frequency and high pulse rate combination call were gravid, whereas in traps using a call with population median parameters only approximately 75% of captured females were gravid. Calls that indicated large-bodied males (low frequency) with high energy reserves (high pulse rate) are often attractive to female anurans, and were effective lures for gravid female toads in my study.

Often, advertisement calls differ among populations. In this case, the attractive ‘combination’ call I identified in the Townsville cane toad population may be less attractive to gravid females in other populations. I sampled calls from 4 cane toad populations across Australia (south east Queensland, north Queensland, Western Australia, and the Northern Territory), and constructed artificial vocalisations based on the median parameters of the sampled calls. I conducted trapping at each population, using calls tailored to each population, to determine which call was most attractive to gravid females in those populations. I created ‘median’ calls based on median call parameters of each population. I also manipulated the frequency and pulse rate of tailored calls from the population median by the same percentages as the altered parameters of the Townsville combination call (an artificially manipulated call with a low frequency and a high pulse rate, relative to the median values of these parameters in Townsville) to create a unique combination call for each population. Median calls, from Townsville or the local population, were always less attractive to females than combination calls. In south east Queensland, Western Australia, and the Northern Territory, there was no significant difference in mean nightly female captures between traps producing the Townsville combination call, and traps producing tailored combination calls for each population. In north Queensland, traps producing the Townsville combination call caught significantly fewer females than traps producing the tailored combination call for that region. I suggest that calls used as lures in traps should have tailored parameters derived from vocalisations in the area in which trapping occurs, to maximise gravid female captures.

Cane toad management strategies should increase the chance of removal of every individual, by exploiting behavioural characteristics, and by increasing the period over which removal occurs. The lures in cane toad traps start and stop automatically, and operate all night, thus managers need only be on-site to remove trapped toads. Conversely, 'toad-busting' hand-capture events require participants to be on-site to find and remove toads, and may therefore be less efficient, in terms of captures per person-hour, than trapping. I used capture-mark-recapture analysis to compare the efficacy of trapping, and hand capturing cane toads, over 10 weeks, in Townsville, Australia. I trapped 7.1% - 22.4% of the estimated population per week, and hand-captured 1.7% - 6% of the estimated population per week. Trapping was more efficient than hand-capture in my regime; overall, more toads were caught per trapping person-hour than per hand-capture hour. Traps attract toads and maximise the period over which removal occurs, thus the probability of removal for each toad was higher than by hand-capture. Also, many toads caught in traps were not encountered during active searches, and vice versa, so the use of both methods, together, may be beneficial. I conclude the thesis by placing my research into an applied context, and exploring future directions for cane toad management.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 3.

Chapter 4.

Chapter 5.

Chapter 6.

LIST OF FIGURES

Chapter 2.

Chapter 3.

Chapter 4.

Chapter 5.

Chapter 6.

CHAPTER 1. INTRODUCTION

The impact of invasive species on native fauna, communities, and ecosystems is widely recognised (Lodge 1993; Simberloff 1996; Ehrenfeld 2010), and invasive species are a significant component of global change (Vitousek et al. 1996). The current rate of introduction of species into new areas (both purposely and accidently) is unprecedented in history, as international commerce and human movement make it possible for species to cross previously impervious physical barriers (Mooney & Cleland 2001). As a result, invasive species may alter the evolutionary pathways of native species by competitive exclusion, niche displacement, hybridisation, introgression, predation, and potentially by causing extinction (Mooney & Cleland 2001; Clavero & García-Berthou 2005). Further, the economic cost associated with invasive species, through losses in agriculture, forestry, and tourism (Pimentel et al. 2005), as well as the costs of controlling these species (Lovell et al. 2006), is estimated to be several hundred billion dollars per year worldwide ($120 billion per year in the U.S alone; Pimentel et al. 2005). Currently, invasive species are of major concern for ecologists and land managers worldwide, due to their numerous negative impacts.

1.2. IMPACT PATHWAYS OF INVASIVE VERTEBRATES

Invasive vertebrates are a primary cause of environmental change *via* numerous direct, and indirect impact pathways. Often, a single invasive vertebrate species may alter the evolutionary pathway of numerous native species in several ways. In Australia, feral cats (*Felis catus*) affect native species primarily through predation (Denny & Dickman 2010; Doherty et al. 2016), as they consume at least 400 native vertebrate species (Doherty et al. 2015), and are the primary cause of extinction of 22 Australian endemic mammals (Woinarski et al. 2015; Doherty et al. 2016). Feral cats also transmit diseases fatal to some native species (e.g., *Toxoplasma gondii*; Bettiol et al. 2000), and compete with native carnivores, such as quolls (*Dasyurus* spp.), Tasmanian devils (*Sarcophilus harrisii*), raptors, and varanids (*Varanus* spp.), due to extensive dietary overlap (Pavey et al. 2008; Glen et al. 2011; Moseby et al. 2012). Feral cats have also indirectly affected multiple ecological processes, and have contributed to a deterioration in ecosystem function. For example, many recently extinct native mammal species created extensive excavations whilst foraging or constructing burrows; the loss of these species has led to landscapes with reduced water retention, fungal diversity, seed germination and seedling establishment (Fleming et al. 2014; Doherty et al. 2016).

Another example of an invasive vertebrate with numerous direct and indirect impact pathways is the brown tree snake (*Boiga irregularis*) on Guam. The population of this snake reached densities of 100 individuals per ha on the island, and dramatically reduced native bird, mammal, and lizard populations, primarily *via* predation (Pimental et al. 2005). The direct impact of this invasion was the extinction of ten of Guam's 12 native frugivorous bird species, (Savidge 1987; Wiles et al. 2003). Brown tree snakes are also indirectly responsible for a 61 – 92% decline in plant recruitment on Guam, because of cascading effects from excessive predation on birds that carry and deposit seeds from fruit bearing trees (Rogers et al. 2017). The success of these invasive vertebrates, exacerbated by the multiple ways they displace, consume, or out-compete native species, is primarily due to the behavioural and life history traits underpinning the establishment and spread of the species within its invaded range (Sakai et al. 2001). Research is continuing to examine these processes in numerous invasive species worldwide, the results of which create opportunities for development, and refinement, of targeted control regimes that disrupt these processes.

1.3. CONTROL STRATEGIES FOR INVASIVE VERTEBRATES

There are numerous control methods for invasive vertebrates, however the efficacy of these methods is extremely variable, depending on the life history and behaviour of the target species, the area over which removal occurs, and the method of delivery of the control strategy. For example, feral cats strongly avoid humans (Gosling et al. 2013), are opportunistic hunters that locate, stalk, and capture their prey primarily using visual and auditory cues (Bradshaw 1992), and often follow established routes around their home range for hunting (Recio & Seddon 2013). Therefore, methods that exploit these avoidance and hunting habits; such as shooting from a distance (Fisher et al. 2015), or trapping and baiting using aural or visual lures placed along a known hunting route (Fisher et al. 2015), are effective over small scales, in combination with other methods (Algar et al. 2013). However, these control methods may not be useful for removal of other invasive vertebrates (e.g., some species may be too small to shoot, or too numerous to justify manually removing single individuals).

The black rat (*Rattus rattus*), and Norway rat (*Rattus norvegicus*), are small rodents that have invaded many areas worldwide (Lowe et al. 2000). They are most commonly controlled using poison baits and traps that remove numerous individuals with minimal human effort per individual. Baiting success is dependent on bait uptake (Leung & Clark 2005), the type of bait and shape of the container containing the bait (Inglis 1996), availability of other food sources (Leung & Clark 2005), and the area over which baiting occurs (Innes et al. 1995). Using this knowledge to refine targeted baiting and trapping methods has led to eradication of rats on several offshore islands (Bell 2002; Burbidge & Morris 2002; Howald 2007). Overall, the success of control methods for some invasive vertebrates has improved drastically over the last several decades, due to the ever-increasing body of research about the behaviour and life history of certain invasive species, and the availability of new information to help refine control regimes.

1.4. INVASIVE AMPHIBIANS

The invasive capabilities and impacts of amphibians are often overlooked, and control regimes for many of these species are ineffective. Invasive amphibians tend to be generalist feeders, have high reproductive rates, and attain large population sizes (Pitt et al. 2005); however, behavioural and life history traits of particular species are often unknown. Further, abiotic factors, such as temperature, wind, and precipitation, also strongly influence behaviour of ectothermic species (Wells 2010). Control methods for invasive amphibians are often non-targeted, and consist of hand removal, trapping using unsustainable baits (e.g., food baits that require changing daily), exclusion fencing, or habitat modification (e.g., draining waterbodies). In Hawaii, coqui frogs (*Eleutherodactylus coqui*) not only affect native species (Beard & Pitt 2005), but also the Hawaiian floriculture and tourist industries, and real estate prices (Pitt et al. 2005). Mechanical controls such as hand-capture and habitat modification have been ineffective, as coqui population size and density (up to 50 000 frogs per ha) is too great, and the effort required to remove coqui in complex environments is not realistically achievable (Pitt et al. 2005). Reduction in bullfrog (*Rana catesbeiana*) densities were small and short-lived when control occurred using annual non-targeted funnel trapping and hand removal (Rosen & Schwalbe 1995), or by exclusion fencing followed by excavation of water holes (Banks et al. 2000). Like most invasive species, eradication of large populations of invasive amphibians is improbable, however, a better understanding of the life history, behaviour, and the effect of abiotic factors on activity of these species, may produce targeted, efficient and effective control regimes to suppress populations.

1.5. STUDY SPECIES: THE CANE TOAD (*Rhinella marina*)

The cane toad (*Rhinella marina*) is a highly invasive, vocalising anuran. It is native to Central and South America; however, its invaded range extends through tropical areas worldwide, and initial establishment and spread is facilitated by human movement (Lever 2001). In Australia, cane toads have spread through much of Queensland, northern New South Wales, and the Northern Territory, and have recently moved into the Kimberley region in northern Western Australia (Shine 2010). Cane toads attain high densities due to their high fecundity, generalist diet, and rapid development rates, particularly in tropical regions (Freeland 1986; Lever 2001). They also occupy a wide range of habitats (including degraded or urban sites; Zug & Zug 1979), and are well suited to the environmental and climatic conditions within their invaded range (Urban et al. 2007). The type, and magnitude, of impacts of cane toads on native fauna is extremely variable, given the scale of the cane toad invasion in Northern Australia, and the range of native species affected (Shine 2010).

Cane toad paratoid (shoulder) glands secrete powerful bufotoxins (Zug & Zug 1979) that occur in various forms through each life stage, but are strongest in eggs and adults (Hayes et al. 2009). Consequently, direct poisoning of predators post ingestion is a significant pathway by which toads impact native Australian fauna (Shine 2010). Ingestion of cane toad eggs, tadpoles, or metamorphs, is lethal for several species of native freshwater fish (Crossland & Alford 1998; Wilson 2005; Greenlees & Shine 2010), tadpoles (Crossland & Alford 1998; Crossland & Shine 2010), and recently metamorphosed native frogs (Greenlees et al. 2010). Ingestion of adult cane toads is lethal for freshwater crocodiles (*Crocodylus johnstoni*) (Letnic et al. 2008), varanid lizards (goannas) (Doody et al. 2009), blue-tongue lizards (*Tiliqua scincoides intermedia*) in some parts of Australia (Price-Rees et al. 2010), frog-eating snakes (Phillips et al. 2010), and quolls (*Dasyurus hallucatus*) (O'Donnell et al. 2010), among several other native species. Competition with native vertebrates is also common within the cane toad's invaded range, because toads consume many invertebrates (Freeland 1984), use retreat sites similar to those of many native species (Schwarzkopf & Alford 1996), and can quickly attain high population densities (Cameron & Cogger 1992). The presence of cane toad tadpoles can effect growth rates and survivorship of native tadpoles (Williamson 1999; Crossland et al. 2009), while some native frog species avoid retreat sites previously used by cane toads (Pizzato & Shine 2009). The presence of cane toad advertisement vocalisations may also affect the calling and mating behaviour of some native anurans (Bleach et al. 2015). Overall, the invasion of cane toads across northern Australia has negatively affected numerous native Australian species, and therefore developing an effective control method for cane toads is crucial to combat their environmental impact within their invaded range.

1.6. CONTROL METHODS FOR CANE TOADS

Managers and ecologists have implemented several control methods for cane toads, with minimal success on a large scale (Tingley et al. 2017). Hand removal of juvenile and adult toads *via* active searches (e.g., toad-busting events; Peacock 2007) may suppress toad abundances in the short-term, over a limited area (Somaweera & Shine 2012). However, the effort required to remove enough individuals to substantially suppress a toad population in a given area is substantial. Some of this effort may be wasted if active searches occur on nights with unfavourable weather conditions, when many toads are inactive (Schwarzkopf & Alford 2002). Further, focusing removal effort around water bodies may result in male-biased captures (Gonzalez-Bernal et al. 2015; Tingley et al. 2017). Tadpole trapping is another potential control method for cane toads in northern Australia, whereby funnel traps baited with bufotoxins attract and trap cane toad tadpoles. Pilot studies report high removal rates (Tingley et al. 2017), however the longer-term effect of tadpole trapping is unknown. For example, removal of some, but not all tadpoles, may artificially reduce intraspecific competition for resources within the water body, decreasing time to metamorphosis for the remaining tadpoles, and producing larger, fitter metamorphs (e.g., Adams & Pearl 2007; Dayton & Fitzgerald 2011). Biological and genetic control methods for cane toads are limited, are unsuccessful in their current form (e.g., Tingley et al. 2017), and may have unknown impacts on native species. Finally, trapping adult individuals using an acoustic lure that plays a cane toad call may be a viable control method that is easily refined to exploit behavioural characteristics of the cane toad (Tingley et al. 2017). The success of any control method is dependent on the ability to refine it by targeting specific demographics of the invasive population, and increasing the number of captures per unit effort spatially, and temporally.

1.7 THE CANE TOAD TRAP

The cane toad trapping unit consists of three main components: a wire trap, a lure that plays a cane toad call, and a solar panel to charge the lure. The trap is a wire-mesh box (1 m x 1 m x

0.25 m) equipped with ‘finger’ doors that open easily with pressure from outside the trap but prevent egress of trapped toads. The acoustic lure is placed inside the wire trap; it plays a cane toad sexual advertisement call (.WAV file) repeatedly through a waterproof speaker, and has a small light-emitting diode ‘black’ (UV) light that attracts insects. Toads are attracted close to the trap by the advertisement call, and enter the trap to get closer to the call, or to eat the insects attracted to the UV light (the combination of light and sound doubles the capture probability of females and triples juvenile captures compared to the vocalisation alone; Yeager et al. 2014). The lure has the capability to play any vocalisation, because the .WAV file played through the speaker is easily changed. A solar panel charges the batteries used to power the electronic components required to play the vocalisation and activate the UV light. The lure is inactive when it receives charge (during the day), and operates automatically when it stops receiving charge (at night). A cane toad trap can operate automatically at night for over 8 months without maintenance if the solar panel consistently receives enough charge during the day to power the lure for the proceeding night (B. Muller, pers. obs).

1.8. DESIGNING TARGETED TRAPPING REGIMES FOR CANE TOADS

Targeting reproductively active females is the best strategy for reducing recruitment into the next generation, and is a common control technique for vertebrate pests with high reproductive rates (Reidinger & Miller 2013). Female cane toads can lay upwards of 10 000 eggs per clutch (Zug & Zug 1979), and should be targeted. Removing one female cane toad also removes their potential future offspring, and may reduce the future population more than removing one male or juvenile (Lampo & De Leo 1998). Most cane toad control regimes, and indeed most control regimes for invasive amphibians (e.g. manual removal, tadpole trapping, exclusion fencing, biological control etc.), do not target reproductively active females, and therefore the average impact of each capture is comparatively low. However, the lure used in adult cane toad traps plays an advertisement call used by male toads to attract females (Schwarzkopf & Alford 2007). Many female anurans use information provided by advertisement vocalisations to choose mates (Wells & Schwarz 2007); the vocalisation's structural parameters (e.g., dominant frequency and pulse rate) indicate the calling individual's body size and energy reserves (Gerhardt 1994), and therefore influence the attractiveness of that vocalisation. Altering the vocalisation used to lure toads into traps may increase its attractiveness to female receivers, and in this case, reproductively active female toads could be targeted using traps.

Many control methods for cane toads are ineffective because they do not consider the behaviour, activity patterns, or life history traits of toads (e.g., Tingley et al. 2017). Understanding and exploiting these traits could allay wasted effort. For example, managers could augment captures by focusing trapping effort when toads are most active. Further, examining the distance over which cane toads respond to the advertisement vocalisation used as a lure could aid in trap placement and designing trapping regimes. Further, cane toads are a model system for the study of evolution during invasion; previous studies have identified geographic variation in call characteristics within their invaded range (Yasumiba et al. 2016). Quantifying geographic variation in behavioural responses to vocalisations is also critical, given trapping success is primarily dependent on the attractiveness of the acoustic lure. For example, an attractive call in North Queensland may not be as attractive to females in Western Australia, if there is geographic variation in female preferences for calls. Finally, examining the number of captures per unit effort for trapping, in comparison to other control strategies for adult cane toads, could result in more efficient control regimes that may include more than one control method. At present, data addressing these issues are scarce, but necessary to design a targeted, efficient, and effective trapping regime for cane toads in Australia.

1.9. THESIS STRUCTURE AND OVERVIEW

The primary aim of my thesis was to describe and quantify cane toad acoustic communication and behaviour to aid in designing and refining cane toad trapping methods. This goal extended beyond the scope of an intrinsically biological focus to also include questions addressing the design of effective and efficient trapping regimes. In chapter 2, I used cane toad traps, in combination with a new analytical technique, to thoroughly quantify toad activity in response to several environmental factors, across 11 months of trapping. These data allowed me to examine when toads were most active, and answered the question: when is the best time to trap toads?

In chapter 3, I defined the distance over which male and female cane toads responded to a vocalisation. These data quantified toad phonotaxis in response to a call, and identified traits that may facilitate the attraction of conspecifics to breeding aggregations in large numbers. Further, I identified the distances at which male and female toads stopped responding to the call used as a lure in cane toad traps, and therefore quantified the area over which traps attracted toads.

In chapter 4, I directly addressed methods to target reproductively active females using cane toad traps. I used traps equipped with lures that played calls with different parameters (volume, dominant frequency and pulse rate) to examine: i) which calls were more attractive to gravid female toads, and ii) whether I could increase the number of gravid female toads trapped by manipulating the call played by the lure.

Chapter 5 expanded on the theme of Chapter 4, whereby I examined whether female preferences for particular call parameters varied across northern Australia, and whether calls used as lures to target gravid females should be unique to the population in which trapping occurs, to maximise gravid females captures.

My final data chapter addressed two key points generally omitted from the cane toad control literature: i) the number of captures per unit effort (e.g., per person-hour) for a given control strategy, and ii) the percent of toads removed from a known population. Specifically, I quantified and compared effort for both trapping and manual hand removal. I also calculated the efficacy of both methods (i.e., the percent of toads removed from a known population) using capture/mark/recapture population estimates.

Chapter 2. Effects of environmental variables on invasive amphibian activity: using model selection on quantiles for counts

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2.1. ABSTRACT

Many different factors influence animal activity. Often, the value of an environmental variable may influence significantly the upper or lower tails of the activity distribution. For describing relationships with heterogeneous boundaries, quantile regressions predict a quantile of the conditional distribution of the dependent variable. A quantile count model extends linear quantile regression methods to discrete response variables, and is useful if activity is quantified by trapping, where there may be many tied (equal) values in the activity distribution, over a small range of discrete values. Additionally, different environmental variables in combination may have synergistic or antagonistic effects on activity, so examining their effects together, in a modelling framework, is a useful approach. Thus, model selection on quantile counts can be used to determine the relative importance of different variables in determining activity, across the entire distribution of capture results. I conducted model selection on quantile count models to describe the factors affecting activity (numbers of captures) of cane toads (*Rhinella marina*) in response to several environmental variables (humidity, temperature, rainfall, wind speed, and moon luminosity) over eleven months of trapping. Environmental effects on activity are understudied in this pest animal. In the dry season, model selection on quantile count models suggested that rainfall positively affected activity, especially near the lower tails of the activity distribution. In the wet season, wind speed limited activity near the maximum of the distribution, while minimum activity increased with minimum temperature. This statistical methodology allowed me to explore, in depth, how environmental factors influenced activity across the entire distribution, and is applicable to any survey or trapping regime, in which environmental variables affect activity.

2.2. INTRODUCTION

Animal activity is influenced by a complex web of factors (Tester & Figala 1990), including a range of environmental variables (Chamaillé-Jammes et al. 2007; Upham & Hafner 2013). Animal activity can vary widely in response to a variety of different environmental variables, but rather than determining the mean number of active animals, such variables may impose a limit on the maximum or minimum number of active animals. In such cases, it should be more appropriate to analyse particular portions of an activity distribution, rather than simply describing the rate of change of the mean, which may or may not change with the variable of interest. Examining the rate of change of the mean may underestimate, overestimate, or neglect changes at the minimum and maximum extents of a heterogeneous distribution (Terrell et al. 1996). Quantile regression is especially useful for examining distributions with heterogeneous variances (Koenker & Bassett 1978); a common characteristic of distributions in ecology (Cade & Noon 2003), including animal activity in relation to environmental variables (Johnson et al. 2014). Specifically, rates of change near the maximum (i.e., 0.95 quantile) or minimum (i.e., 0.05 quantile) of the distribution are often a better representation of the influence of the measured variable than the mean (Thomson et al. 1996; Cade et al. 1999). If, for example, a particular measured variable imposes a limit on activity, the organism’s response cannot increase to more than the upper limit set by that factor; however, it can be any value less than that, for example, if other, unmeasured, factors are also influencing activity (Cade & Noon 2003).

My motivating example was estimating effects of various environmental variables on cane toad (*Rhinella marina*) activity in northern Australia. Cane toads are large, nocturnal, terrestrial anurans originating from South America, whose invaded range includes many tropical and subtropical areas globally, including Australia. The physiological constraints on terrestrial amphibians (Tracy 1976), and experimental data on cane toads (e.g., Cohen & Alford 1996), suggest that seasonal variation in activity should be strongly associated with environmental moisture. Wind speed also affects desiccation rates, and activity, of anurans (Henzi et al. 1995), while locomotor performance and behaviour are strongly dependent on temperature in ectotherms (Huey & Stevenson 1979; Huey 1982). Finally, positive and negative effects of lunar cycles on amphibian biology have also been observed (Grant et al. 2012). These factors limit activity in other species; for example, several ectothermic species are inactive below certain temperatures (e.g., Lei & Booth 2014). Any combination of these environmental variables may impose a limit on the maximum or minimum activity of cane toads.

Trapping is a common method for measuring animal activity (e.g., Gibbons & Bennett 1974; Price 1977; Rowcliffe et al. 2014) and could be used to measure cane toad activity (Muller & Schwarzkopf 2017a, b). Cane toad traps for adults contain a lure that produces a cane toad advertisement call, and a light that attracts insects as a visual cue (Yeager et al. 2014). Trap efficacy depends primarily on activity; toads must be active to approach the lure, and enter the trap. Therefore, the number of toads trapped per night provides an estimate of toad activity on that night. However, if captures are low, or if the trap has limited capacity (i.e., the maximum number of animals capturable is constrained by trap size), trapping may result in a very small range of counts, with numerous tied (equal) count values. Indeed, previous studies report mean cane toad capture rates of approximately 1 – 6 individuals per trap per night, and it is uncommon to exceed 14 captures in a single night (although the maximum number of toads caught in a single trap to date was 31; Muller pers. obs.). In this case, conventional quantile regression analysis creates serious interpretation and inference issues, because the models assume a continuous dependent variable, rather than a discrete dependent variable (Cade & Dong 2008).

The quantile count model is a special implementation of conventional quantile regression, whereby the changes in quantiles of counts are estimated by making them continuous random variables and then back-transforming estimates to the discrete response without sacrificing model accuracy (Machado & Santos Silva 2005). Therefore, a quantile count model can be used to analyse trapping data, to examine the entire cane toad activity distribution in response to an environmental variable.

Multiple environmental factors may influence toad activity across various parts of the activity distribution and, thus, quantile regression modelling, as with any regression modelling, may require considering alternative models with various combinations of predictor variables. Model selection using differences in Akaike’s information criterion (AIC) is often used to select among alternative candidate models for analyses in ecology (Arnold 2010). Akaike’s information criterion is valuable where there are a range of variables that may be associated with a biological variable and the researcher is interested in which are most influential (Symonds & Moussalli 2010). A range of competing models containing various combinations of variables are analysed simultaneously and AIC ranks these models (Akaike 1974, 1998, Richards et al. 2011). When differences in AIC among models with various combinations of predictor variables are calculated with respect to a null model with just an intercept, then the comparison of differences in AIC is related to the proportionate reduction in variation of the phenomenon explained by each combination of variables (adjusted by the number of estimated parameters), given what was measured (Richards et al. 2011). Akaike’s information criterion is calculated using the number of fitted parameters (including the intercept) in the model and the likelihood associated with the maximum-likelihood estimate. The weighted sums of absolute deviations minimised in conventional quantile regression estimation are maximum likelihood estimates assuming an asymmetric double exponential distribution, providing the basis for computing AIC and other information criteria on quantile regression models (Koenker & Machado 1999; Yu & Moyeed 2001; Cade et al. 2005). Therefore, model selection on quantile count models can be used to determine which combination of variables affects toad activity across the entire response distribution.

I trapped cane toads over eleven months at one location while simultaneously collecting information on humidity, temperature, rainfall, wind speed and moon luminosity. I examined the distributions of toad captures using model selection on quantile count models (using every 5th quantile between $\tau = 0.05$ and $\tau = 0.95$) to examine which environmental variables affected toad activity at different parts of the activity distribution during different seasons. I suggest that model selection on quantile count models is applicable to any trapping regime for which several environmental variables affect the number of individuals captured, especially if those effects occur near the lower or upper tails of the distribution.

2.3. MATERIALS AND METHODS

Study site

The study occurred on Orpheus Island (18°36′46.0″ S, 146°29′25.2″ E) from 21 May 2013 to 28 March 2014, with the exception of 16 days in November 2013, 17 days in December 2013, 10 days in January 2014, and 9 days in February 2014. The island is approximately 23 km east of the Australian mainland and 120 km north of Townsville, Queensland. It is approximately 12 km long and is comprised primarily of dry woodlands, with rainforest patches.

Data collection

To catch toads, I used wire traps (1 × 1 × 0.25 m), equipped with doors that opened easily with pressure from outside, but prevented egress of trapped toads. The trap contained a lure that repeatedly played a cane toad advertisement call at night, and had a small LED black (UV) light that attracted insects. More detail on the trap and methodology is available in section 1.6 of this thesis, and in Yeager et al. (2014).

I used two traps for the study, at two trapping sites. Both trapping sites were located in open, grassy areas and had similar ambient light ($\bar{x}$ = 0.051 lx) and environmental noise ($\bar{x}$ = 32.5 dB) levels. I measured light and noise levels at each site on 15 randomly selected nights, at 22:00 h, using a lux meter (ATP DT-1300), and a C-weighted Lutron sound level meter (model: SL-4013). I placed the traps 400 m apart, such that the acoustic lure at one site could not be heard by toads at the other site (see Chapter 3; Muller et al. 2016). I removed, counted, and sexed trapped toads daily by visual inspection of coloration and skin texture (females are dark brown with a smooth bumpy dorsum, whereas males are lighter with a rough sandpapery dorsum). I placed a water bowl and PVC pipe for shelter in each trap. Toads were euthanised immediately after their removal from the traps, using an overdose (350 ppm) of buffered tricaine methanesulfonate (MS-222), and exposure was *via* submersion in water containing a sodium bicarbonate-buffered solution. Euthanising toads after capture may have reduced the number of toads available for capture on subsequent nights, but there was never a decrease in toad numbers that was not easily explained by weather (e.g., there were no consistent patterns in which nightly captures were low following a large capture event). Toads move nomadically (Schwarzkopf & Alford 2002), and the size of the toad population, and the island, probably facilitated constant immigration into the study area, and therefore, the number of toads available for local trapping was likely approximately constant.

I collected humidity, minimum temperature, and mean wind speed (recorded every half hour), and recorded total nightly rainfall, from the Australian Institute of Marine Science weather station on Orpheus Island (located approximately 300 m from the study site) for every night during the trapping period. I averaged half-hourly recordings across the 12-h period from 18:00 h to 6:00 h to calculate nightly averages. I characterised moon luminosity as the percent of the moon illuminated on each night (as measured from Townsville; approximately 79 km from the study site) during the trapping period (obtained from www.timeanddate.com).

Statistical Methods

I divided the trapping period into four seasons: the dry season (June – August), the pre-wet season (September – November), the wet season (December – February), and the post-wet season (March – May). I used captures for each trap from each night as replicates so each night had two measures of toad activity which were counts of captured toads. I used the quantile count model of Machado & Santos Silva (2005), where the discrete count response (y) is transformed to the continuous scale (jittered) for quantile estimates by adding a random uniform number between 0 and 1 to each count, $z = y + U[0, 1)$. I used an exponential count model, $Q_z(\tau|\mathbf{X}) = \tau + \exp(\mathbf{X}\boldsymbol{\beta}(\tau))$, estimated in its linear form by taking logarithms, for $\log(z - \tau)$ the $Q_{\log(z - \tau)}(\tau|\mathbf{X}) = \mathbf{X}\boldsymbol{\beta}(\tau)$, where **X** is the matrix of predictor variables and a column of 1's for the intercept. Estimates in the artificial continuous scale are then back-transformed with a ceiling function, $Q_y(\tau|\mathbf{X}) = \lceil \tau + \exp(\mathbf{X}\hat{\boldsymbol{\beta}}(\tau)) - 1 \rceil$, to recover the quantile estimates in the discrete random variable scale (counts y). My quantile count model had the typical multiplicative exponential form used with other parametric count models (Cade & Dong 2008) that ensures that all estimates are greater than or equal to zero. For each season, I estimated 5 candidate quantile count models with environmental predictors (humidity, minimum temperature, rainfall, wind speed, and moon luminosity) and one 'null' quantile count model with just an intercept. Estimates were implemented with the rq() function in the quantreg package for the R environment for statistical computing and graphics (Koenker 2015). Models were estimated for $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$. To integrate out the artificial noise introduced by jittering toad counts to a continuous variable ($z = y + U[0, 1]$), I estimated each model $m = 500$ times, using m random samples between 0 and 1 ($U[0, 1]$) and averaged the estimates (Machado & Santos Silva 2005; Cade & Dong 2008).

I calculated the AIC for each model, including a null model with just an intercept, for each of the $m = 500$ replications at every quantile for which models were estimated ($n = 9500$ AIC estimates across the entire distribution per candidate model). To calculate ΔAICs for each candidate model, I subtracted the AICs of each candidate model from the AICs of the null model for each of the $m = 500$ replications at every quantile for which models were estimated (Cade et al. 2017). Therefore, models with higher ΔAIC are better supported because the null model had no significant relationship with any predictor variable. I averaged across $m = 500$ replications by quantile to compute the average ΔAIC of each candidate model at $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$. This calculation disclosed the strength of the relationship between toad captures and each predictor variable across the entirety of the distribution in the continuous log-transformed scale of toad counts. I performed model selection for strong predictor variables by identifying models that had the highest ΔAIC at any quantile or were within 2 ΔAIC of the strongest model at any quantile (Burnham & Anderson 2004). Often, different models were strongest at different parts of the distribution. I then considered candidate models that included all possible combinations of the strong predictor variables, and a null model containing only an intercept to which candidate models were compared. I once again identified which models had high average ΔAICs across the entirety of the distribution, and selected the strongest model for further analysis.

After deciding on a reasonable set of predictor variables to include in my seasonal models, I estimated the models again incorporating the count of toads on the previous night as an additional predictor variable to account for 1st-order temporal autocorrelation in my estimates. I compared models with and without the lagged toad counts across quantiles with AIC, as before, to determine whether the 1st-order temporal autocorrelation improved my quantile estimates. Estimates of the 1st-order temporal autocorrelation parameter were also examined to determine whether they were sufficiently different from zero to justify their inclusion in the seasonal models.

Confidence intervals for parameter estimates made in the continuous log scale were estimated by integrating out the artificial noise introduced by the m = 500 random jitters to the continuous scale. I averaged estimates of confidence interval end points for parameters in the strongest model based on the quantile rank score test inversion approach in rq(), with weights based on a local bandwidth of quantiles to account for heterogeneity (Koenker & Machado 1999; Cade et al. 2005; Cade & Dong 2008). Other approaches to estimating confidence intervals for quantile count models based on estimating the asymptotic variance/covariance from averaging components across m simulations have been developed (Machado & Santos Silva 2005) and implemented in the Qtools package for R (Geraci 2016). However, the quantile rank score test inversion approach usually provides better confidence interval coverage and length at smaller to intermediate sample sizes than procedures based on the variance/covariance estimates as it neither requires estimating the density of observations near the quantile estimate of interest nor the direct computation of variances of parameter estimates. Properties of the quantile rank score test have been investigated in Koenker (1994) and Cade et al. (2006).

The confidence intervals for parameter estimates and AIC model selection statistics were all obtained in the continuous log scale, but interpretation of the model estimates were made in the discrete count scale. I back-transformed quantile estimates of the strongest model from the continuous log scale to the discrete count scale using the ceiling function (Machado & Santos Silva 2005, Cade & Dong 2008). In cases where the strongest model included more than one predictor variable, I calculated quantile estimates for each variable while holding all other variables included in the model at their median values. From these estimates, I examined the proportional changes in counts by calculating, as a percentage, the changes of estimated counts at particular quantiles, across a selected range of values of the predictor variable.

2.4. RESULTS

Traps were open for 91 nights in the dry season, 74 nights in the pre-wet season, 54 nights in the wet season, and 39 nights in the post-wet season (total of 516 effective trap nights, given 2 traps were open each night throughout the trapping period). I trapped 241 toads in the dry season, 387 toads in the pre-wet season, 490 toads in the wet season, and 167 toads in the post-wet season. Toads were most active in the wet season, and were least active in the dry season (Fig. 2-1).

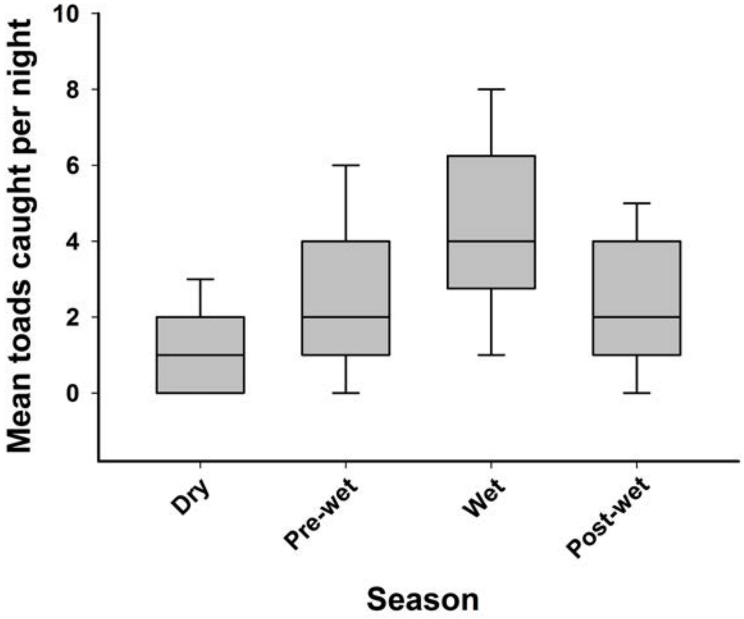


Figure 2-1. Seasonal variation in mean nightly cane toad captures on Orpheus Island, from 21 May 2013 to 28 March 2014.

Dry Season

In the dry season, the model including rainfall consistently had the highest average ΔAIC, across all quantiles (Fig. 2-2A). I did not include any other variables in a combination model with rainfall, because the ΔAIC of every other variable was < 2 at all quantiles ≥ 0.15 (Fig. 2-2A). The model that included a 1st-order temporal autocorrelation effect, in combination with rainfall, was slightly better supported across most of the distribution, but was particularly well supported at lower quantiles (Appendix S1: Fig. S1). In this model, rainfall had a positive effect on all quantiles ≥ 0.10 of the toad counts; the estimated partial effect was strongest near the minimum of the distribution (Fig. 2-3). The proportional changes in counts at quantiles ≥ 0.75 increased 60% - 67% as rainfall increased from 20 mm to 33 mm, however the greatest proportional increases (up to 200%) occurred at quantiles ≤ 0.25 as rainfall increased from 20 mm to 33 mm (Fig. 2-4). This indicated that rain events were the strongest driver of activity in the dry season. It may also indicate that generally inactive toads (represented by counts at quantiles ≤ 0.25) were most likely to be trapped during rain events when more than 20 mm fell per night, because the minimum activity (i.e., minimum captures) greatly increased when rainfall was > 20 mm.

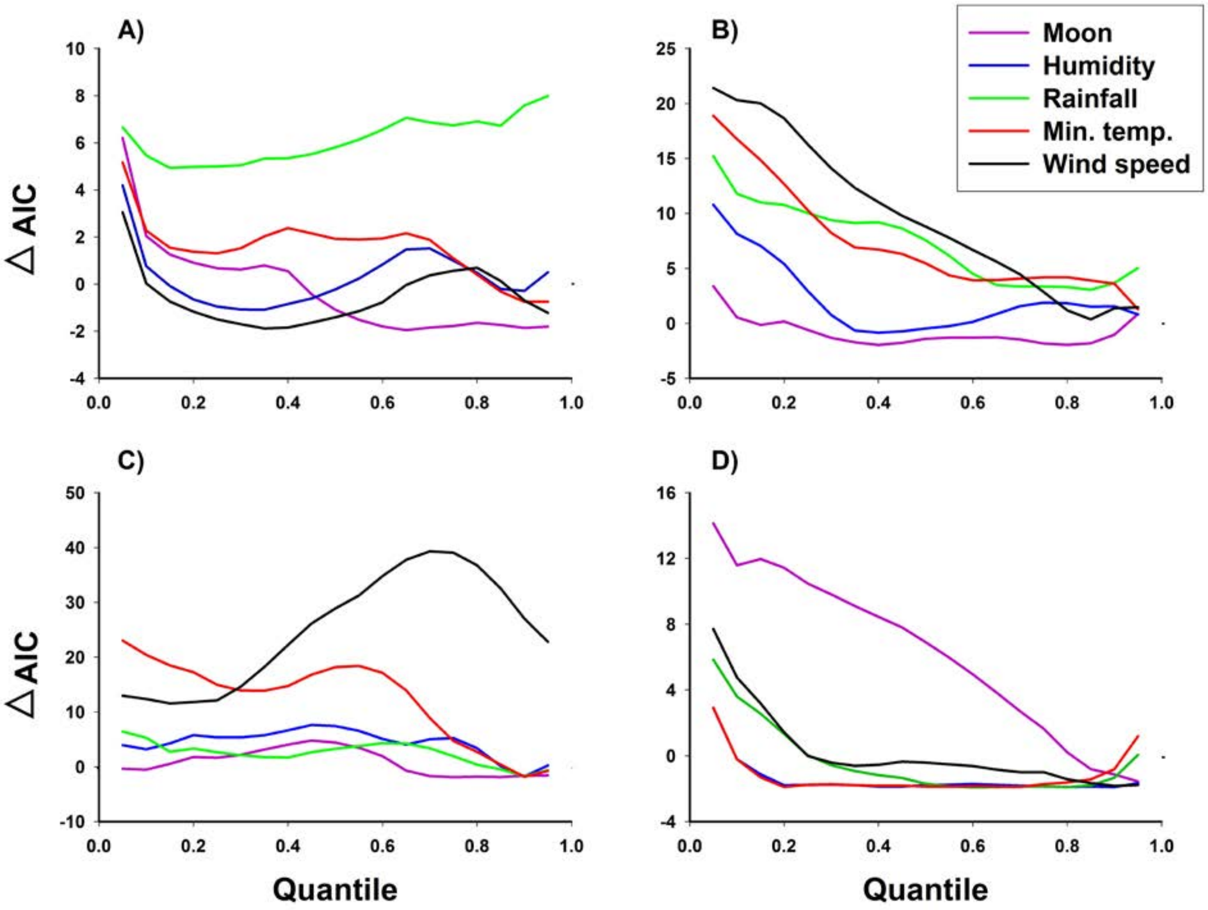


Figure 2-2. Change in average ΔAICs of candidate variable models in the dry (A), pre-wet (B), wet (C), and post-wet (D) seasons, on Orpheus Island, from 21 May 2013 to 28 March 2014, across $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$, for $m = 500$ replications of $z = y + U[0, 1)$. In the dry season (A), rainfall was the strongest predictor variable, at every quantile, therefore a combination model that included other variables was not estimated. In the pre-wet season (B), wind speed, minimum temperature and rainfall were all strong predictor variables at different points across the distribution. In the wet season (C), minimum temperature and wind speed were both strong predictors of activity. In the post-wet season (D), moon luminosity was the strongest predictor of activity, especially at lower quantiles.

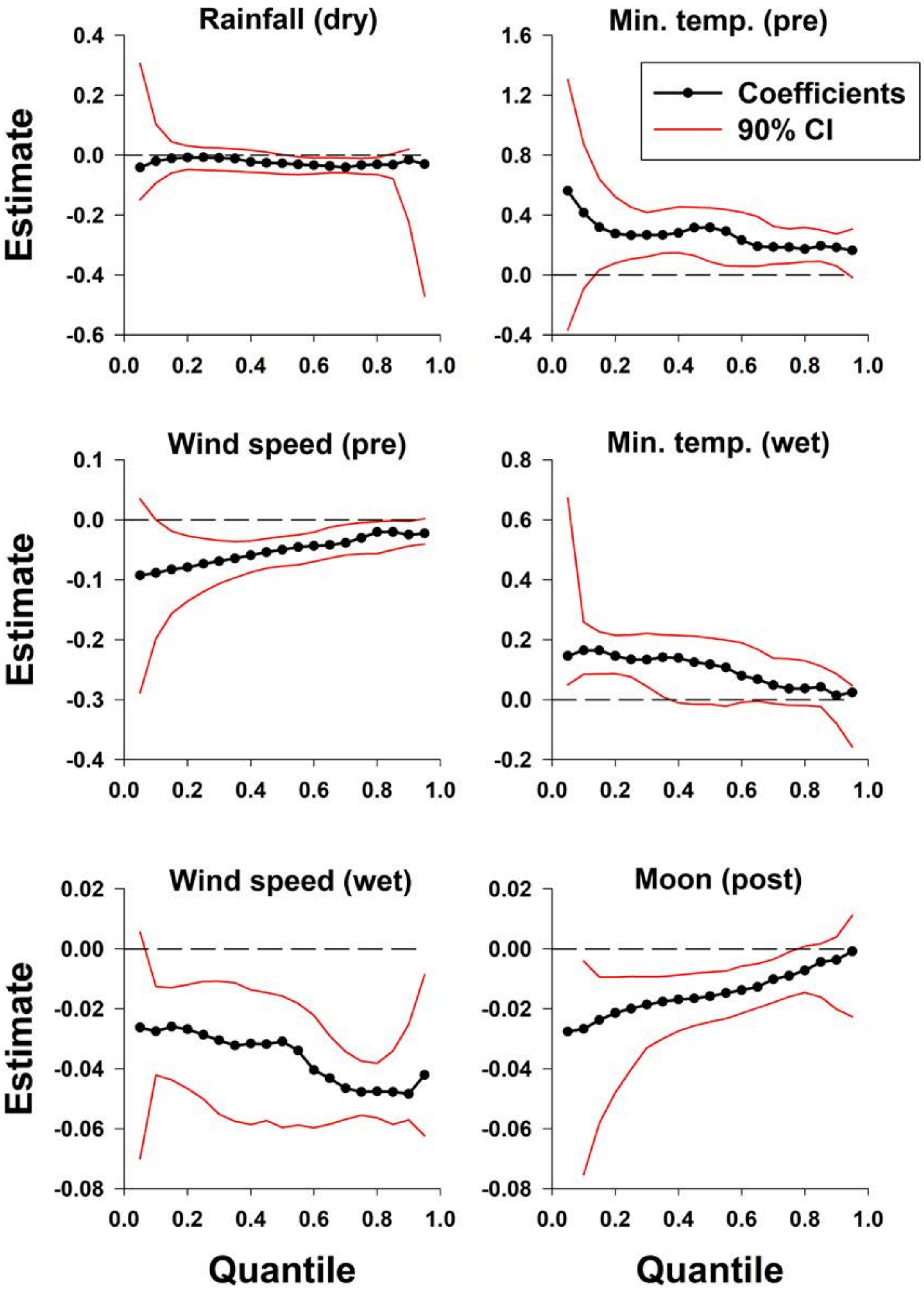

Rainfall (dry)
Min. temp. (pre)
Wind speed (pre)
Min. temp. (wet)
Wind speed (wet)
Moon (post)
Coefficients
90% CI
Estimate
Quantile
0.4
0.2
0.0
-0.2
-0.4
-0.6
1.6
1.2
0.8
0.4
0.0
-0.4
0.1
0.0
-0.1
-0.2
-0.3
-0.4
0.8
0.6
0.4
0.2
0.0
-0.2
0.02
0.00
-0.02
-0.04
-0.06
-0.08
0.0
0.2
0.4
0.6
0.8
1.0

Figure 2-3. Average of $m = 500$ parameter estimates of 90% confidence intervals (rank score test inversion) for quantile count models of trapped cane toads on Orpheus Island, from 21 May 2013 to 28 March 2014, where $z = y + U[0, 1)$ was randomised m times for the estimate of strongest model chosen from a selection of models containing various combinations of environmental variables. Shown are the rates of change of the number of toads trapped with the strongest environmental predictor variable(s) in each season, as identified by the quantile count model.

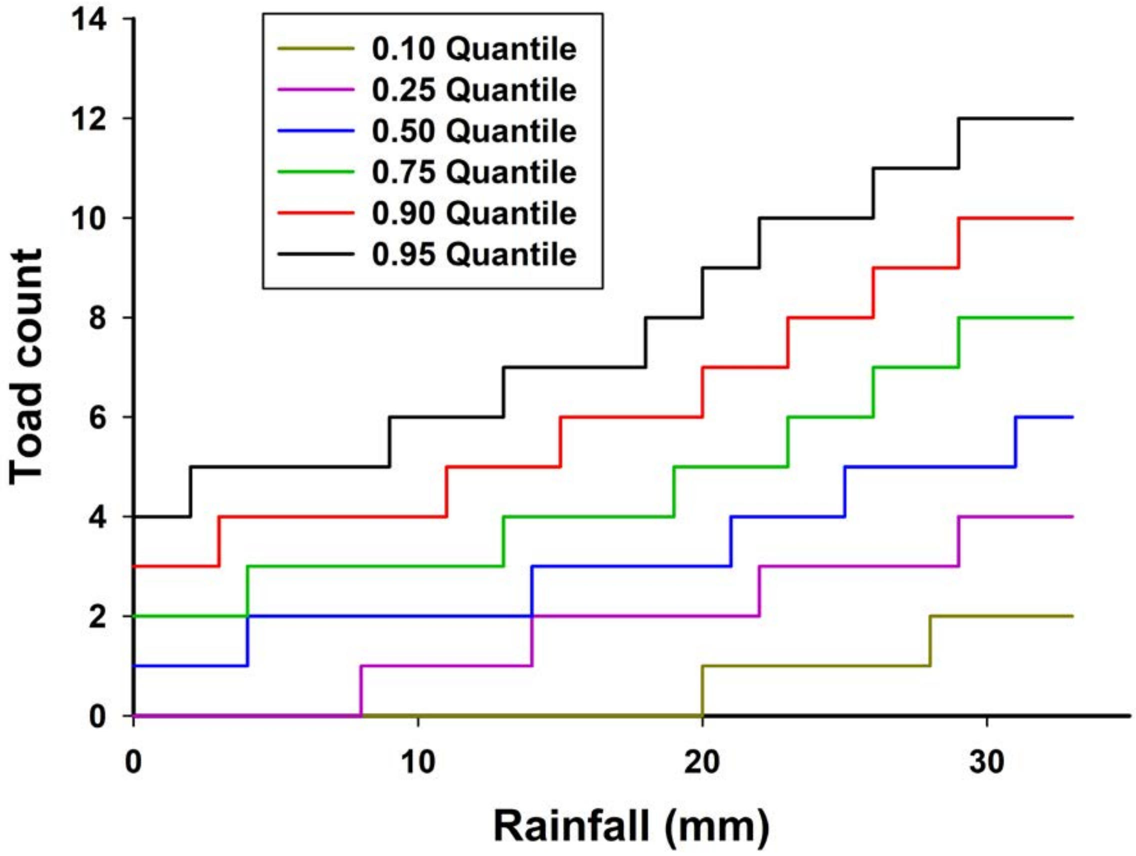


Figure 2-4. Estimated quantile count model for cane toad captures (n = 182) on Orpheus Island in the dry season (June – August 2013), as a function of rainfall and a 1st-order autocorrelation effect, estimated using a ceiling function. An average of estimates for *m* = 500 random jitterings for cane toad counts was used.

Pre-Wet Season

In the pre-wet season, wind speed, minimum temperature, and rainfall were all strong predictors of activity, at different points across the distribution (Fig. 2-2B). Models including various combinations of these variables were of similar strength, especially at higher quantiles (Fig. 2-5). I selected for further examination a model that included minimum temperature and wind speed, because this model was the strongest at all quantiles ≥ 0.20 (Fig. 2-5). The model that included a 1st-order temporal autocorrelation effect, in combination with minimum temperature and wind speed, was well supported, especially at lower quantiles (Appendix S1: Fig. S2). In this model, minimum temperature had a positive effect on all quantiles ≥ 0.10 of the toad counts, when wind speed and lagged toad counts were fixed at their respective median values (Fig. 2-3; Fig. 2-6A). Proportional increases in toad counts were largest (57% - 200%) at quantiles ≥ 0.50, when the minimum temperature increased from 22°C to 26°C. Proportional increases in toad counts when minimum temperature increased from 19°C to 22°C were considerably smaller, and only occurred at quantiles ≥ 0.75. This may indicate that many toads were inactive when the temperature was below 22°C; the highest chance of capture for these individuals was when temperatures were 22°C to 26°C. Conversely, wind speed had a negative effect on all quantiles ≥ 0.10 of the toad counts when minimum temperature was fixed at its median value (Fig. 2-3, Fig. 2-6B). Proportional changes in toad counts were largest when wind speed was below 25 km/h; counts at quantiles ≥ 0.50 decreased 38% - 67% when wind speed increased from 5 km/h to 25 km/h, and toad counts at quantiles ≤ 0.25 decreased to zero. The negative effect of wind tapered off when speed exceeded 25 km/h. The combination model suggests that toads are most active in the pre-wet season when the minimum temperature was above 22°C and wind speed was low.

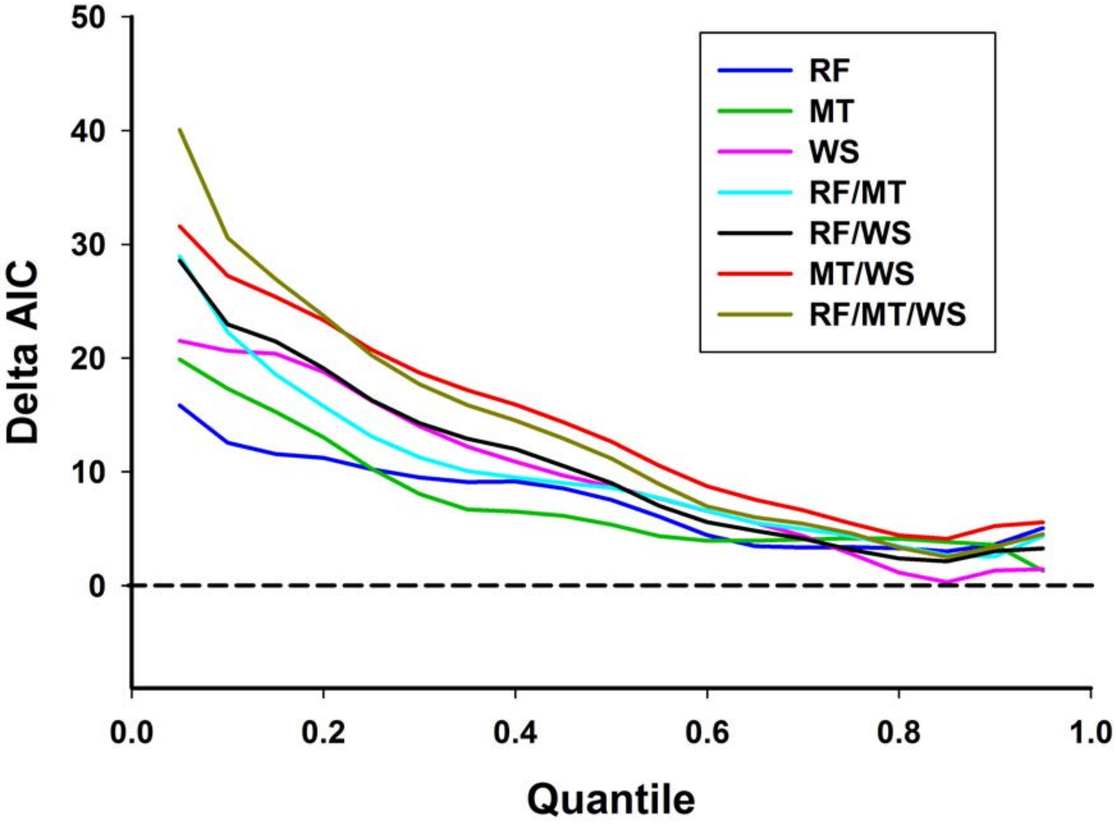
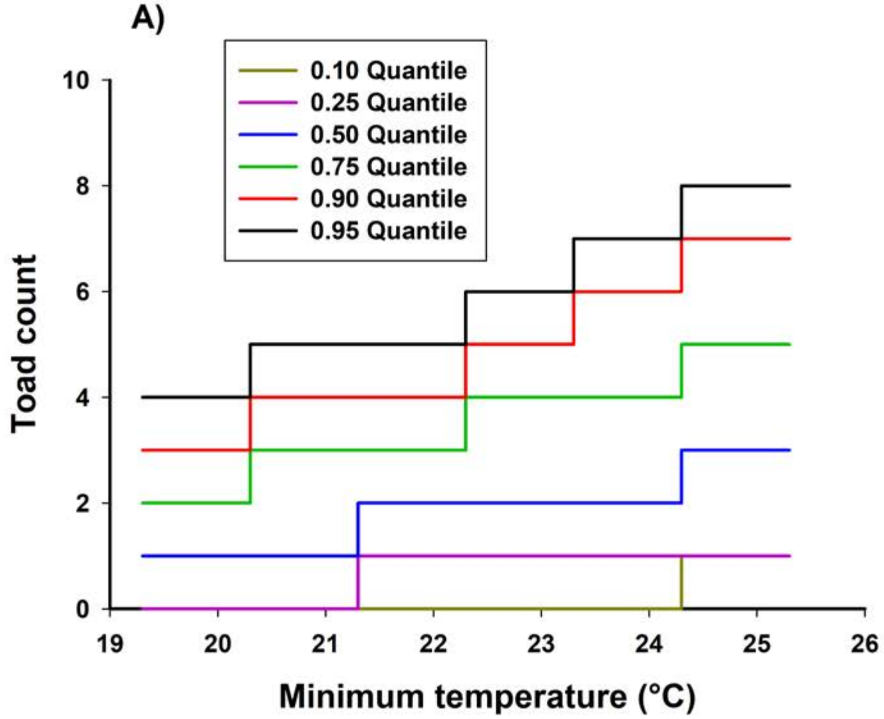


Figure 2-5. Change in average ΔAICs of models containing various combinations of rainfall, minimum temperature, and wind speed, across $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$, for $m = 500$ replications of $z = y + U[0, 1)$, on Orpheus Island, in the pre-wet season (Sep – Nov 2013). The relative strength of models containing individual environmental variables in the pre-wet season is shown in Fig. 2-2B. In the pre-wet season, a combination model containing minimum temperature and wind speed was strongest at all quantiles ≥ 0.20.


A)
0.10 Quantile
0.25 Quantile
0.50 Quantile
0.75 Quantile
0.90 Quantile
0.95 Quantile
Toad count
10
8
6
4
2
0
19
20
21
22
23
24
25
26
Minimum temperature (°C)

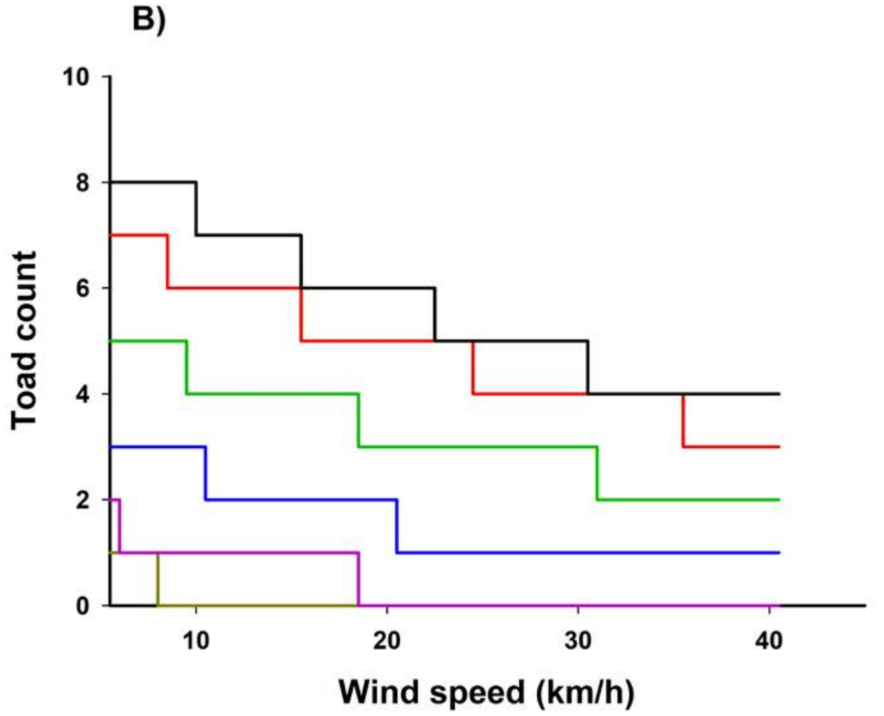

B)
Toad count
10
8
6
4
2
0
10
20
30
40
Wind speed (km/h)

Figure 2-6. Estimated quantile count model, including a 1st-order temporal autocorrelation effect, for cane toad captures (n = 148) on Orpheus Island in the pre-wet season (Sep – Nov 2013), as a function of minimum temperature, with wind speed and lagged toad counts fixed at their median values (A), and as a function of wind speed, with minimum temperature and lagged toad counts fixed at their median values (B), estimated using a ceiling function. An average of estimates for *m* = 500 random jitterings for cane toad counts was used.

Wet Season

In the wet season, minimum temperature and wind speed were the two candidate variable models that had the highest average ΔAIC, across all quantiles (Fig. 2-2C). Minimum temperature was the strongest predictor variable at quantiles ≤ 0.25, while wind speed was the strongest predictor near the middle and upper limits of the distribution. A model including both variables had considerable support across all of the distribution, especially at lower limits (Fig. 2-7). The model that included a 1st-order temporal autocorrelation, in combination with minimum temperature and wind speed, was never within 2 ΔAIC units of the selected model at any quantile, and was not considered further. Minimum temperature had a positive effect on all quantiles ≥ 0.10 of the toad counts, when wind speed was fixed at its median value; however, this effect was considerably stronger at lower quantiles (Fig. 2-3). The proportional changes in counts increased 67% - 200% at quantiles ≤ 0.5 when temperature increased from 24°C to 28°C; however, proportional changes in counts at higher quantiles were comparatively lower, across the same temperature range (Fig. 2-8A). The obvious interpretation is that even the lowest minimum temperatures in the wet season were warm enough to allow toad activity, however when temperatures were higher, the minimum activity (i.e., minimum captures) greatly increased. Wind speed had a negative effect on all quantiles ≥ 0.10 of the toad counts, when minimum temperature was fixed at its median value (Fig. 2-3). When τ ≥ 0.75, the proportional changes in counts decreased 42% - 45% as wind speed increased from 5km/h to 20 km/h (Fig. 2-8B). This indicated that wind may have limited toad activity in the wet season, given that the rate of change of toad counts was highest at quantiles near the maximum of the distribution. Overall, the model indicated that warm, still nights were most conducive to toad activity. While minimum temperatures were generally warm enough to facilitate high toad activity, wind speed constrained the maximum activity of toads, and may be the primary driver of activity in the wet season.

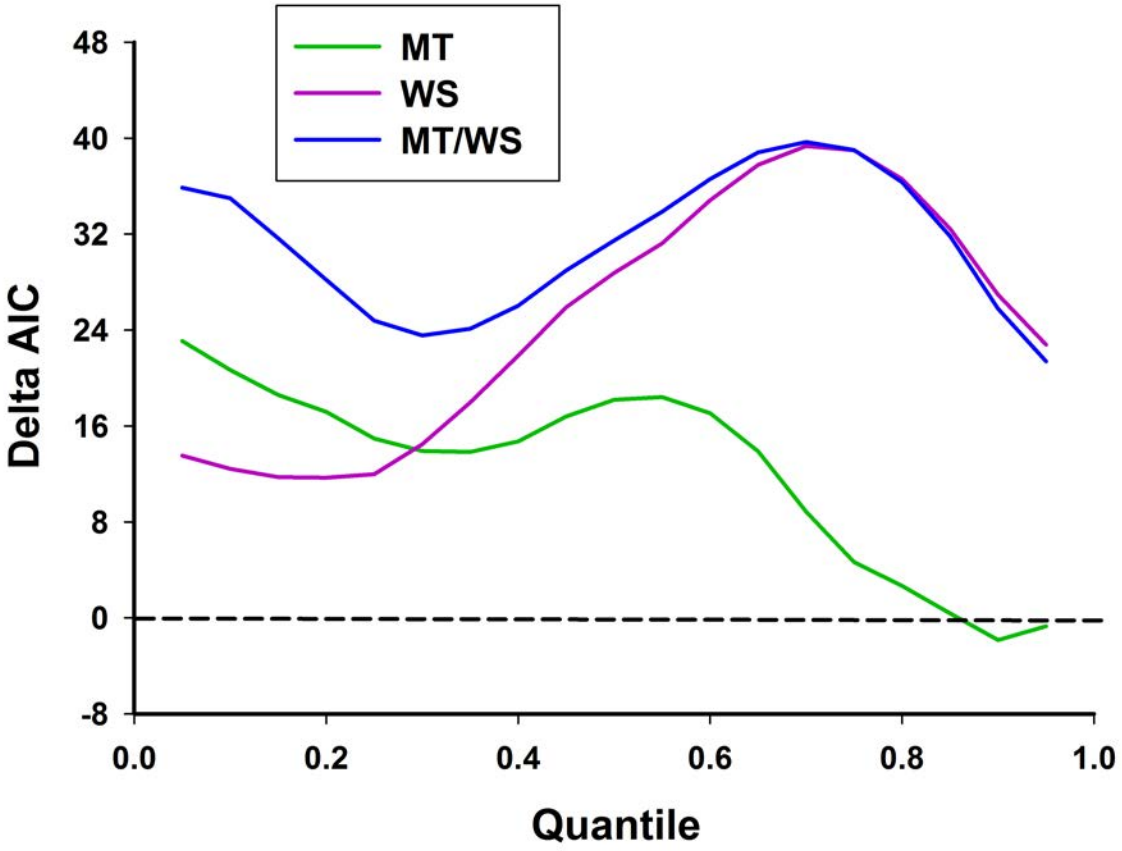


Figure 2-7. Change in average ΔAICs of models containing minimum temperature, wind speed, and a combination of both variables, across $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$, for $m = 500$ replications of $z = y + U[0, 1)$, on Orpheus Island, in the wet season (Dec 2013 – Feb 2014). The relative strength of models containing individual environmental variables in the wet season is shown in Fig. 2-2C. In the wet season, a combination model containing minimum temperature and wind speed was strongest at quantiles ≤ 0.70, and within 2 ΔAIC units of the strongest model at upper quantiles.

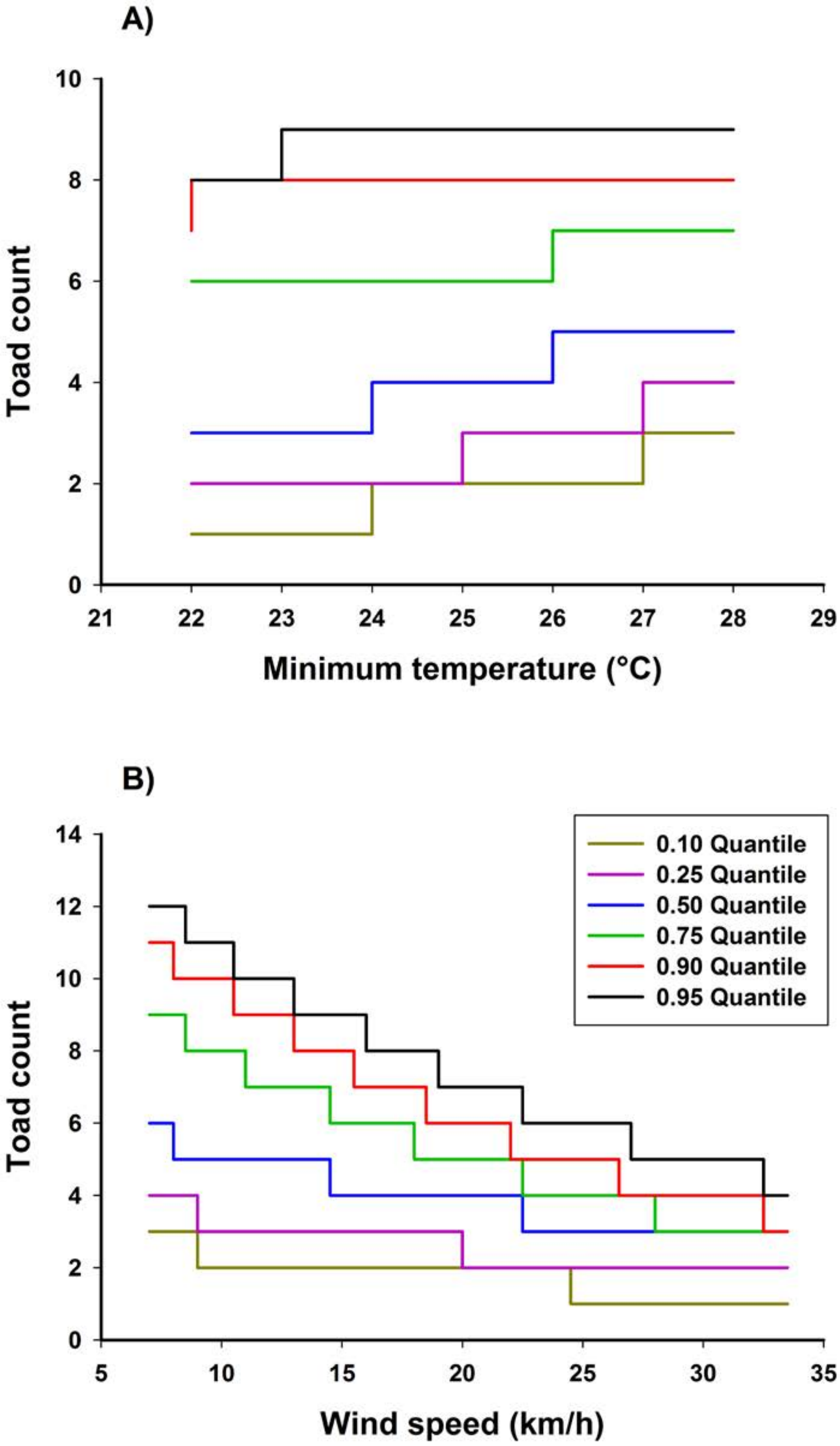

A)
Toad count
0
2
4
6
8
10
21
22
23
24
25
26
27
28
29
Minimum temperature (°C)
B)
Toad count
0
2
4
6
8
10
12
14
5
10
15
20
25
30
35
Wind speed (km/h)
0.10 Quantile
0.25 Quantile
0.50 Quantile
0.75 Quantile
0.90 Quantile
0.95 Quantile

Figure 2-8. Estimated quantile count model for cane toad captures (n = 108) on Orpheus Island in the wet season (Dec 2013 – Feb 2014), as a function of minimum temperature, with wind speed fixed at its median value (A), and as a function of wind speed, with minimum temperature fixed at its median value (B), estimated using a ceiling function. An average of estimates for m = 500 random jitterings for cane toad counts was used.

Post-Wet Season

In the post-wet season, moon luminosity was the strongest predictor of toad activity; the model including moon luminosity had the highest average ΔAIC for m = 500 replications of jittered toad counts, across most quantiles (Fig. 2-2D). This model was strongest at the lower limits of the distribution, and gradually weakened at higher quantiles. The model that included a 1st-order temporal autocorrelation, in combination with moon luminosity, was never within 2 ΔAIC units of the selected model at any quantile, and was not considered further. Although the negative effect of moon luminosity on toad activity was strong at quantiles ≤ 0.50 (Fig. 2-2D, Fig. 2-3), none of the models had an average ΔAIC > 2 at quantiles ≥ 0.80, indicating that none of the measured variables limited toad activity in the post-wet season. The proportional changes in counts decreased 67% - 200% (to zero in some cases) at quantiles ≤ 0.50 as moon luminosity increased from 0% to 52% (Fig. 2-9). The decrease in proportional changes in counts was not as rapid at moderate to high moon luminosities (≥ 52%), at quantiles where counts were above zero. This may indicate that most toads preferred dark conditions in the post-wet season, and were not active when moon luminosity was ≥ 52%; however, some toads were always active, regardless of moon luminosity.

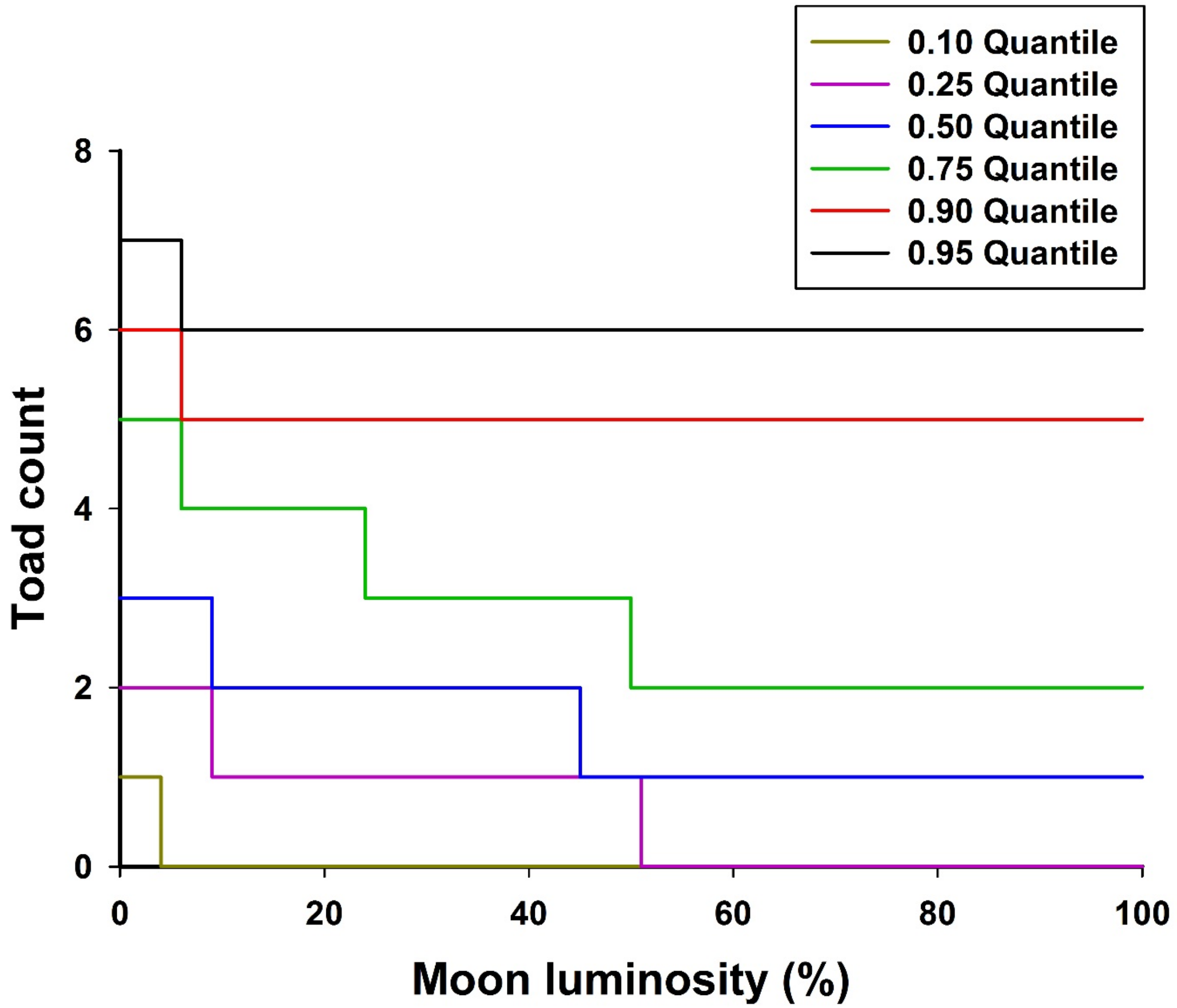


Figure 2-9. Estimated quantile count model for cane toad (n = 78) captures on Orpheus Island in the post-wet season (May 2013, March 2014), as a function of moon luminosity, estimated using a ceiling function. An average of estimates for *m* = 500 random jitterings for cane toad counts was used.

2.5. DISCUSSION

Overall, several different variables had synergistic and antagonistic effects on cane toad activity. Using my combination of statistical techniques, I detected the influence of environmental variables on both lower and upper bounds of toad activity. I also found that there was high seasonal variability in cane toad activity; toads were more active in the wet season (December – February) and less active in the dry season (June – August). Further, there was variability in the combinations of environmental variables that influenced toad activity, depending on the time of year. This may be because particular environmental variables were sufficient for minimum activity during certain seasons, but not others.

Results acquired using model selection on quantile count models were consistent with expectations based on the physiological requirements of cane toads. For example, rainfall was the strongest predictor of toad activity in the dry season, across all measured quantiles. Minimum toad activity increased up to 200% when rainfall exceeded 20 mm, suggesting that many toads may be generally inactive during the dry season, and only emerge from their burrows, forage, or search for mates, when rainfall is high. Cane toads emerge from their burrows more frequently (Seebacher & Alford 1999), and move longer distances (Schwarzkopf & Alford 2002) when there is more atmospheric and soil moisture, probably because moist conditions limit water loss *via* their permeable skin (Schwarzkopf & Alford 1996). The first-order temporal autocorrelation effect evident in the dry season indicated that activity on a given night partially predicted activity on the subsequent night. This could be interpreted as a lagged effect of rainfall, where soil moisture was comparatively high for several consecutive nights after rain, which created extended periods of favourable conditions for toad activity. Rainfall in the dry season was rare; therefore, the physiological cost of movement was generally high. Toad capture rates increased with rainfall, probably because the cost of movement (i.e., water loss) was lower than in dry periods (Schwarzkopf & Alford 2002).

In the wet season, wind speed appeared to limit toad activity (Fig. 2-8B). This may be because evaporative water loss rates increase when wind speed is high (Bentley & Yorio 1979); therefore, toads may reduce activity when wind exceeds a certain speed. High winds may also reduce insect activity (Holyoak et al. 1997), so toads may be less active for feeding, and the insect-attracting UV light lure may also be less attractive when it is windy (McGeachie 1989). Windy conditions may have also increased the excess attenuation of the call used to lure toads, and therefore reduced the distance the call carried (Larom et al. 1997). The strongest predictor model in the wet season also included minimum temperature, the effect of which was strongest at lower quantiles. Toad captures increased a great deal (67 – 200%) at lower quantiles (≤0.5), when minimum temperature increased 4°C (from 24° to 28°C), while captures at upper quantiles, across the same temperature range, remained relatively stable. This large increase in toad captures with a relatively small increase in ambient temperature indicates that minimum temperature in the wet season was well above the minimum threshold for toad activity, because many toads were active, regardless of temperature. The increase in minimum toad activity is consistent with the strong increase in toad locomotor performance from a preferred temperature of 24°C toward a thermal optimum of approximately 30°C (Kearney et al. 2008). The availability of temperatures conducive to high performance may have encouraged activity from even the most inactive toads, and greatly increased their chance of capture.

My toad activity models included various combinations of rainfall, minimum temperature, and wind speed in most seasons. However, in the post-wet season, moon luminosity appeared to influence toad activity, especially at lower quantiles. Activity in the post-wet season may occur because there is a need to feed after breeding in the wet season (Yasumiba et al. 2016). Toads strongly avoid light (Davis et al. 2015), but will feed under lighted conditions if there is food available (González-Bernal et al. 2011). I suggest some toads limited their activity as ambient light increased; however, bolder (or hungrier) individuals may have continued feeding despite the moonlight. Several studies report depressed nocturnal activity in amphibians due to moonlight, probably because amphibians avoid light, which may occur because there is an increase in their detectability to predators in lighter conditions (reviewed in Grant et al. 2012). It was surprising that the moonlight effects were only detectable in one season, and that the magnitude of reduction in activity appeared to vary across the moon luminosity spectrum. Possibly, the effects of moonlight were most detectable in this season because, after the wet season, toad activity was most strongly determined by foraging needs. Temperature and humidity were still high enough to encourage activity, so that an otherwise weak effect of moon luminosity, not detectable in other seasons, when other factors (such as reproduction or hydration) were affecting the toad's propensity to be active, then became influential.

One of the main strengths of quantile count models, and the quantile regression approach more generally, is that prediction intervals for future new observations are easily obtainable, without any of the parametric distributional assumptions (e.g., a normal error distribution) required for interpretation of prediction intervals obtained using ordinary least squares regression (Neter et al. 1996; Cade & Noon 2003). In quantile regression, the interval between 0.10 and 0.90 quantile regression estimated at any specified value of X is an 80% prediction interval for a single future observation of y (Cade & Noon 2003). For example, in the dry season, the 80% prediction interval increases from 0 – 4 toads when rainfall is 10 mm, to 1 – 8 toads when rainfall is 25 mm (Fig. 2-4). Conversely, in the wet season, the 80% prediction interval decreases from 2 – 10 toads when wind speed is 10 km/h to 1 – 5 toads when wind speed is 25 km/h (Fig. 2-8B). My quantile count models characterise the variability of prediction intervals for future toad counts reasonably, in each season, with few assumptions. An additional advantage of the quantile count model over traditional parametric count models is that it avoids having to select from among various parametric distributions (e.g., Poisson, negative binomial, and their zero-inflated counterparts).

Examining rates of change at various points across cane toad capture distribution models, using model selection, enabled me to more effectively examine the influence of several environmental factors across the entire distribution. The jittered quantile count model is particularly useful when the dependent variable includes many tied values, across a small range of values. Indeed, nightly numbers of toads captured often ranged between 0 and 5 (89% of the toad counts fell within this range). Thus, my jittered quantile count model allowed for interpretation of a discrete count response variable with many tied values, across an extremely limited range of values (Machado & Santos Silva 2005; Cade & Dong 2008). Finally, my model selection procedure allowed me to select strong predictor models at any quantile in the distribution to include in combination models, while simultaneously rejecting weak predictor models that may have otherwise added an uninformative parameter to the combination model (Arnold 2010). This method streamlined the model selection process and reduced the chance of misinterpretation of AIC results (see Arnold 2010).

Model selection on quantile count models was extremely effective at examining, in depth, the effect of environmental variables on cane toad trapping rates, and activity. This chapter provides a simple example of this methodology, using only five environmental variables. Future studies could incorporate a wider range of variables to better approximate the factors effecting activity, and counts. This methodology could also be used for standard quantile regressions, when the range of values is large, with few tied values, using a process similar to generalised linear modelling to obtain slope estimates at various quantiles across the distribution. The independent use of AIC model selection, and quantile count models, is not new; however, I have demonstrated that the use of both methods, simultaneously, can allow us to examine extensively the relationship between environmental variables and rates of capture in trapping and mark – recapture regimes, and also to determine which of these variables affect the study organism's activity.

Chapter 3. Defining the active space of cane toad (*Rhinella marina*) advertisement calls: males respond from greater distances

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3.1. ABSTRACT

Many animals produce advertisement vocalisations to attract mates. A vocalisation's active space is the area within which a receiver responds to it, while its maximum extent occurs when a receiver stops responding. I mapped behavioural responses of male and female cane toads (*Rhinella marina*) to advertisement calls, by conducting experimental playbacks to: (i) examine attenuation of a cane toad call, (ii) define the active space of these vocalisations, by measuring phonotaxis at different distances from the call, and (iii) quantify the active space of calls for both sexes, separately. The call was fully attenuated 120 – 130 m from its source. Both sexes displayed positive phonotaxis 20 – 70 m from calls. Males also displayed positive phonotaxis 70 – 120 m from calls, whereas females' movement preferences were random >70 m from a call. Differences between male and female responses were likely driven by differences in their use of information provided by calls.

3.2. INTRODUCTION

Vocalisations are crucial to conspecific communication in many organisms (Parris & McCarthy 2013). The area over which a vocalisation is recognised, and elicits a response from a conspecific receiver, is termed its active distance, or active space (Marten & Marler 1977; Bradbury & Verhencamp 2011). The active space of a call is determined, in part, by its propagation through the environment, and its rate of attenuation, which is the decay of a sound over distance from its origin (Charlton et al. 2012). Background ambient noise, and obstructions such as trees, long grass, or shrubs, decrease the active space of calls (Marten & Marler 1977). Interference at the location of the receiver causes the signal-to-noise ratio, which is the difference in sound pressure level between the call and the background noise, to decline with distance until it reaches zero (Vélez et al. 2013). Phonotaxis, usually measured by the direction and distance moved by focal animals when exposed to a vocalisation (Brenowitz & Rose 1999), is influenced by attenuation (Forrest 1994), but the signal-to-noise ratio at which responses to calls occur varies (Gerhardt & Klump 1988; Wollerman 1999). Variations in responses to calls may be determined by the hearing sensitivity of the receiver (Feng et al. 1976), but the information derived from calls (e.g., body size or condition of the vocalising individual; Gerhardt & Huber 2002) may also play a part in the tendency for phonotaxis.

Male anurans typically rely on vocalisations as a mechanism for attracting mates and maintaining distances between potential rivals during breeding choruses (Bee 2007; Gerhardt & Bee 2007; Swanson et al. 2007). For mating, the receiver of the call must identify the location of the calling individual(s) over the vocalisation’s active space (Gerhardt & Bee 2007): a call with a large active space reaches more potential mates (Penna & Moreno-Gomez 2014). The active space has not been determined for many anurans, but is relevant to descriptions of breeding ecology because it helps determine the distance over which individuals respond to calls to form breeding aggregations (Christie et al. 2010). In addition, females may use information derived from vocalisations to determine the fitness (size, energy levels, health) of potential mates (Gerhardt 1994). Thus, while males may move towards vocalisations to increase their mating probability (Ryan et al. 1981), female anurans may be more aurally sensitive to conspecific vocalisations than males, because of sexual differences in the auditory system (e.g., Wilczynski 1986). Alternatively, female anurans may hear a vocalisation from the same distance or further than males, but may be more selective about whether to approach it.

Cane toads (*Rhinella marina*) have successfully invaded tropical areas worldwide. Male cane toads produce an advertisement call designed to attract females, and often form choruses of calling individuals (Schwarzkopf & Alford 2007; Bowcock et al. 2008). Cane toad vocalisations vary among individuals in volume, frequency and pulse rate, which influences mate attraction (Yasumiba et al. 2015), but propagation and attenuation of these calls in natural environments, and the active space of these calls, have not been quantified. Understanding the active space of these calls is important to understanding where breeding aggregations form in the landscape, and how toads locate one another in unfamiliar landscapes. In this study, I determined the signal-to-noise ratio of cane toad calls of typical call volume by measuring their attenuation in relation to environmental sounds, using an artificial call played at a known volume and measurements of sound pressure levels at different distances from the call. In addition, I measured the active space of cane toad calls using a series of playback trials, and quantified toad movement vectors when they were exposed to a call (compared to a silent control) from a range of distances. Finally, I examined the movements of each sex in response to the call. Both male and female toads must locate appropriate breeding locations in a newly invaded area, but females must also locate potential mates and select them from a range of individuals in novel landscapes, at least in part using attraction to calling conspecifics. An understanding of these behaviours provides insights into the process of mate attraction in cane toads; an essential process that may facilitate invasion, and could be used to design targeted control regimes.

3.3 MATERIALS AND METHODS

Toad collection and husbandry

I hand-captured adult cane toads of both sexes on the James Cook University campus in Townsville, Queensland, Australia (19°19’47.74”S, 146°45’29.55”E) between May 2014 and May 2015. I temporarily placed toads into a 15-l bucket during each capture session, and afterwards placed them into a 1000 l cattle watering tank (diameter 4 m), where they remained overnight. The tank contained ample shelter (8 PVC pipes, each 15 cm D × 30 cm L) and water was available ad libitum. Fly screen and 85% UV block shade cloth were secured over the top of the tank to prevent escape, and the tank was located in a shady area. Toads remained in the tank for less than 24 h before use in experiments the following evening.

Determining the attenuation distance of a stimulus call

Trials were conducted on a large, mowed, open field (diameter 400 m) near the James Cook University Townsville campus (19°19’47.74”S, 146°45’29.55”E). The field was free of trees and other obstructions that may have caused attenuation of the call by factors other than distance (Marten & Marler 1977; Forrest 1994), or that would alter the movement vectors of toads. Ambient light during experimental trials was low (range: 0.001 – 1.1 lx) and dispersed uniformly across the field, and ambient noise was low (mean: 37 ± 0.6 dB). I placed a speaker (Digitech

Naval Comms AS3186, housed in waterproof speaker box) at the centre of a field and played the call (see below for characteristics of this call). The speaker automatically replayed the call on an indefinite loop, until it was manually switched off. The speaker cone was facing upwards, so the sound spread evenly across the field. I measured the propagation and attenuation of the call at various distances from the speaker: 1 m, 5 m, 10 m and every 10 m up to a distance of 200 m. I then turned the call off and measured ambient noise at the same distances. This process was repeated on 10 randomly selected nights over a three-month period, during which temperature (mean 23.5 ± 2.7°C), and wind speed (mean 8.6 ± 3.2 km/h) were similar. I chose nights using a random number generator that selected 10 numbers between 1 and 92, where 92 is the total number of days over the 3-month period I selected to obtain measurements (March, April and May 2015). All sound pressure measurements (Lutron sound level meter, model: SL-4013, C-weighted) were taken 5 cm above the ground, the approximate level from which toads hear the call. I measured sound pressure levels along a straight line originating at the speaker unit and extending away from the speaker. The direction of the line was randomly chosen from a range of 0 – 360° using a random number generator, with a line extending from directly in front of the speaker representing 0°.

Experimental design and vocalisation parameters

The stimulus call was a modified natural toad call, artificially manipulated to have the median call parameters calculated for several local toad populations (Yasumiba et al. 2015). The call lasted for 8 s, had a pulse rate of 15 pulses s^{-1}, a frequency of 600 Hz and a volume of 80 dB at 1 m (reproduced in high quality .WAV format and manipulated using Audacity 1.2.3). I created a loop of this call with a 2-s pause after the conclusion of the call before it automatically replayed indefinitely, until switched off.

Release and behavioural observations of toads

I conducted behavioural experiments in the same area in which I conducted attenuation trials. Nights used for behavioural observation were randomly chosen using a random number generator using all possible days over a year period. Toads were released individually at randomly selected distances (5, 10, 20, 50, 70, 100, 120, 130, 150 or 200 m) from the speaker, and at a randomly selected angle relative to the speaker as a central point (0 – 360°). I used a random number generator to select the distance and angle of release of all toads. I placed the toad beneath an upturned 20 l bucket at the selected location, began playing the call, and allowed the toad to habituate beneath the bucket for two minutes. I then carefully lifted the bucket, and stood to one side of the bucket (in relation to the speaker) to avoid inadvertently encouraging the toad to move towards or away from the call. I alternated the side of the bucket upon which I stood when each toad was released. I measured the initial movement vector of the toad (during the first 30 s after release, to the nearest 30°) and scored this as either moving towards or away from me (the observer), over 360°. After release, the toad was allowed to move freely for 10 minutes, which I observed from >10 m with the aid of night vision equipment (White Night NG111M, Metron). After 10 min, I marked the final location of the toad with a flag and removed the toad. I then measured the linear distance from the flag to the speaker and distance from the flag to the point of release (to 1 cm), as well as the direction moved by the toad with respect to the speaker in degrees (to the nearest 30°, with the position of the speaker at 0°). To quantify normal behaviour of toads when not exposed to a call, I also conducted trials in which the speaker was not playing a call (randomly interspersed amongst the sound trials). I did not conduct trials using a neutral noise, such as pink noise, because toads do not respond to such noise (Schwarzkopf & Alford 2007). Each toad was tested only once before being humanely euthanised, using an overdose (350 ppm) of buffered tricaine methanesulphonate (MS-222). Exposure was *via* submersion in water containing a sodium bicarbonate buffered solution. I recorded the sex and snout-urostyle length of each toad, and dissected female toads to determine stage of gravidity.

Statistical analysis

All statistical analyses were conducted using SPSS V22 (IBM), R Statistical Software (V. 3.1.2) and Oriana V4 (Kovach Computing Services). I calculated the signal-to-noise ratio by subtracting the ambient noise level from the sound pressure level of the call, at each distance from the speaker (1 m, 5 m, 10 m and every 10 m thereafter up to a maximum of 200 m from the speaker). The mean ambient noise level and sound pressure level at each distance was used, from the 10 nights of data collection. Initially, male and female movements were combined for analysis at each distance of release. To examine if the initial orientation of toad's bodies differed from random when the bucket was first removed, I used a series of Rayleigh Z-tests. I also determined whether the presence of an observer influenced the initial movement. If toads were selecting an angle randomly with respect to the observer, on average, half of the toads would move towards the observer, while the other half would move away. I compared the expected ratio to the actual movement vectors of the toad with respect to the observer, using a Chi-squared contingency test.

To determine if control toad 10-min movement vectors were random with respect to the speaker when it was off, I used Rayleigh Z-tests. To determine if toad 10-min movement vectors were towards the speaker when the call was playing, I normalised the position of the speaker to zero degrees, and used a series of two-tailed Durand and Greenwood V -tests, using an expected mean movement vector of zero degrees. Durand and Greenwood V -tests compare the direction of movement relative to a specific location, rather than relative to a random expectation (Zar

1984).

To determine the influence of sex on movement vectors and distance moved by toads when exposed to the call, I grouped release distances into four ranges (<10 m, 20 – 70 m, 70 – 120 m, >120 m) based on similar phonotaxis of toads in these categories. Creating larger categories with similar movement behaviour maximised sample sizes of each sex that could be compared at various distances from the call. I determined whether the movement vectors of male and female toads were significantly different from random within each distance category, using a series of Rayleigh Z-tests. When movement vectors were significantly different from the random expectation, I normalised speaker position to zero degrees and used a Durand and Greenwood V-test to determine if movement vectors were towards the speaker. I examined whether gravidity affected the movement vectors of females at each distance category by analysing gravid and non-gravid females separately, using a series of Rayleigh Z-tests, and where appropriate (i.e., when movement was non-random), I used Durand and Greenwood V-tests to determine if movement vectors were towards the speaker, when its position was normalised to zero degrees.

I examined whether toads moved further when they moved towards the call by correlating the distance moved by toads with the direction of their movement paths at each distance category (i.e., the circular-linear association), using Mardia's rank correlation co-efficient (Mardia 1976). I used this analysis when toads were released both when the speaker was off (controls) and on, and analysed male and female toads separately. I also examined whether the distances moved by toads when the speaker was off (control trials) differed significantly among release distances, using a one-way analysis of variance. Finally, I examined the possibility that one sex may move further than the other when a call was playing, using a one-way analysis of variance, comparing distance moved between males and females within each distance category from the speaker. I corrected *P*-values for multiple tests on the same data set where necessary, using Bonferroni corrections.

3.4. RESULTS

I tested 163 male and 149 female toads on 31 dry nights, for which the mean ambient temperature was 22.6°C, and mean wind speed was 9.2 km/h. The mean number of toads tested at each release distance was 31.2, and 73.8% of females (110 in total) were gravid at the time of the trial. Two female toads did not move for the duration of the 10-minute release period, and were excluded from analysis; all other individuals moved during this period.

Signal to noise ratios

Signal-to-noise ratios decreased as the inverse square of the distance from the speaker (Fig. 3-1), reaching 0.3 dB at 120 m from the speaker, and 0 dB at 130 m from the speaker. That is, at 130 m from the speaker, the sound pressure level from the speaker was equivalent to the ambient environmental noise.

Influence of observers on the initial direction of toad movement

I found no evidence that the presence of an observer influenced direction of toad movement; when the trial was initiated by lifting the bucket over the toad and when a call was playing, toads oriented randomly at all distances from the call (Table 3-1). Toads also oriented randomly at all distances at the initiation of control trials (Table 3-1). I conclude that observers did not influence the initial movements of toads upon their release from the bucket, given that the direction of movement of toads with respect to the observers was not significantly different from random ($\chi^2_1 = 0.321$, $P = 0.57$).

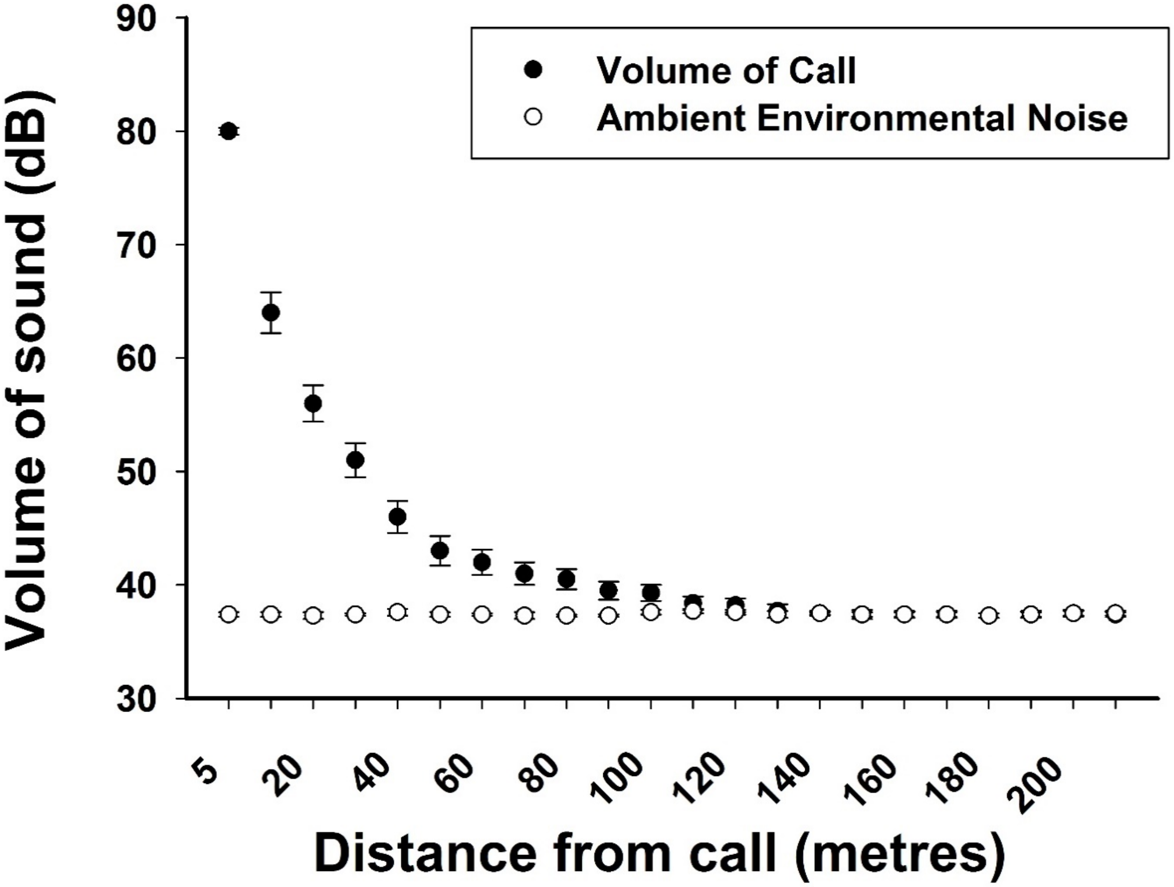


Figure 3-1. The mean attenuation of a cane toad (*Rhinella marina*) call over 200 m with respect to mean ambient environmental noise. The difference in sound pressure level between the two measurements is the signal-to-noise ratio. Points represent the mean ± SEM.

Table 3-1. The initial orientation of toads when first exposed to the trial was random, both when there was a call present, and during control trials, where there was no call. This table shows the results of several Rayleigh-Z tests examining if initial toad movement was random.

	Control		**Speaker On**	
Release distance	***Z***	***P-value***	***Z***	***P-value***
5m	2.69	0.065	0.32	0.733
10m	0.23	0.798	1.57	0.210
20m	1.67	0.187	1.21	0.301
50m	0.36	0.708	0.28	0.761
70m	0.42	0.666	0.01	0.995
100m	0.71	0.497	2.28	0.101
120m	0.31	0.740	1.43	0.243
130m	0.29	0.760	0.23	0.806
150m	0.73	0.495	0.06	0.944
200m	1.83	0.161	0.18	0.842

Did toads move towards the speaker when a call was played?

At all tested distances, the vectors of movement of toads in control trials (speaker off) were not significantly different from random expectations (Table 3-2). In contrast, when the speaker was playing a call, toads showed a strong preference for moving towards the speaker when released 20 – 70 m from it, but showed no preference when released less than 10 m from the speaker, or at distances greater than 70 m from the speaker (Table 3-2, Fig. 3-2).

Effect of sex on toad movement vectors

When released from within 10 m of the speaker playing a call, movement vectors of both male (*Z* = 0.37, *P* = 0.70) and female (*Z* = 1.53, *P* = 0.22) toads were not significantly different from random (Fig. 3-3A, B). When released from between 20 and 70 m from the speaker, male (*Z* = 13.36, *P* < 0.001) and female (*Z* = 3.96, *P* = 0.02) toads moved non-randomly, with their preferred vector of movement was oriented towards the call (males: *V* (expected mean value of 0°) = 0.553, *u* = 4.70, *P* < 0.001, females: *V* (expected mean value of 0°) = 0.398, *u* = 2.81, *P* = 0.002; Fig. 3-3C, D). When a call was playing, male toads released 70 – 120 m from the sound also moved non-randomly (*Z* = 3.57, *P* = 0.03) and towards the call (*V* = 0.35, *u* = 2.55, *P* = 0.005; Fig. 3-3E), whereas the movements of female toads released from more than 70 m away from the speaker were not significantly different from random (*Z* = 0.13, *P* = 0.88; Fig. 3-3F). The movement of both male (*Z* = 0.83, *P* = 0.44) and female (*Z* = 0.01, *P* = 0.99) toads was not significantly different from random when they were released from >120 m from a speaker playing a call (Fig. 3-3G, H).

The movement of non-gravid female toads was not significantly different from random when they were released within 10 m of the speaker (*Z* = 0.51, *P* = 0.66). However, movement of non-gravid females was non-random when released 20 – 70 m from the speaker, with their preferred vector of movement oriented towards the call (*V* (expected mean value of 0°) = 0.393, *u* = 1.669, *P* = 0.048). When non-gravid females were released 70 – 120 m away from the speaker, their movement paths were not significantly different from random (*Z* = 0.232, *P* = 0.801), movement paths of non-gravid females were also not significantly different from random when they were released from more than 120 m from the speaker (*Z* = 1.091, *P* = 0.395). Movements of gravid females were qualitatively similar to those of non-gravid females. Their preferred movement path was towards the call when released 20 – 70 m away from the speaker (*V* (expected mean value of 0°) = 0.4, *u* = 2.264, *P* = 0.011), while their movement paths were not significantly different from random when they were released within 10 m of the speaker (*Z* = 1.083, *P* = 0.35), 70 – 120 m from the speaker (*Z* = 0.687, *P* = 0.511), and more than 120 m from the speaker (*Z* = 0.766, *P* = 0.474). Because the movement preferences of gravid and non-gravid females were similar, gravidity did not appear to influence movement vectors in my study.

Table 3-2. Toads (sexes combined) were most attracted to calls between 20 and 70 m from the call. This table shows the results of several Rayleigh-Z tests testing if toad movement was random during control trials, and Durand and Greenwood V-tests, testing the hypothesis that toad movement was in the direction of the speaker at a range of distances away from the call, when the speaker was playing a call. The position of the speaker was normalised to an angle of 0°. Also indicated are the sample size (n), mean angle of movement ($\bar{x}$) and circular standard deviation (SD). P-values were corrected for multiple tests using the Bonferroni method, with significance accepted when $P < 0.005$ indicating that toads oriented directionally towards the speaker (significant results are indicated with asterisks). The letter 'c' appears in subscript next to several statistical parameters to signify that these results occurred under control conditions, when the speaker was off.

Release distance	**Control**					**Speaker On**					
	n_c	$\bar{x}_c$	**SD$_c$**	Z_c	P_c	n	$\bar{x}$	**SD**	V	u	P
5m	11	131.6°	90.1°	0.93	0.404	13	169.1°	101.9°	-2.02	-1.031	0.846
10m	18	262.2°	84.6°	0.31	0.739	16	56.2°	94.9°	0.141	0.798	0.215
20m	20	84.1°	80.3°	0.94	0.398	25	27.4°	62.0°	0.473	3.28	0.0004**
50m	13	195.0°	85.3°	1.46	0.234	21	3.1°	72.4°	0.456	2.95	0.001**
70m	11	186.2°	199.4°	0.14	0.872	16	19.3°	58.7°	0.528	2.892	0.002**
100m	15	173.8°	87.5°	0.18	0.842	25	29.0°	97.4°	0.206	1.459	0.073
120m	15	203.3°	78.2°	0.11	0.899	18	42.1°	109.6°	0.195	1.403	0.081
130m	14	175.7°	94.6°	0.11	0.898	15	169.3°	98.1°	-0.097	-0.413	0.657
150m	16	218.8°	93.8°	0.71	0.503	18	144.4°	100.7°	0.077	0.461	0.324
200m	10	245.1°	111.2°	0.23	0.611	23	70.4°	96.2°	0.132	0.895	0.187

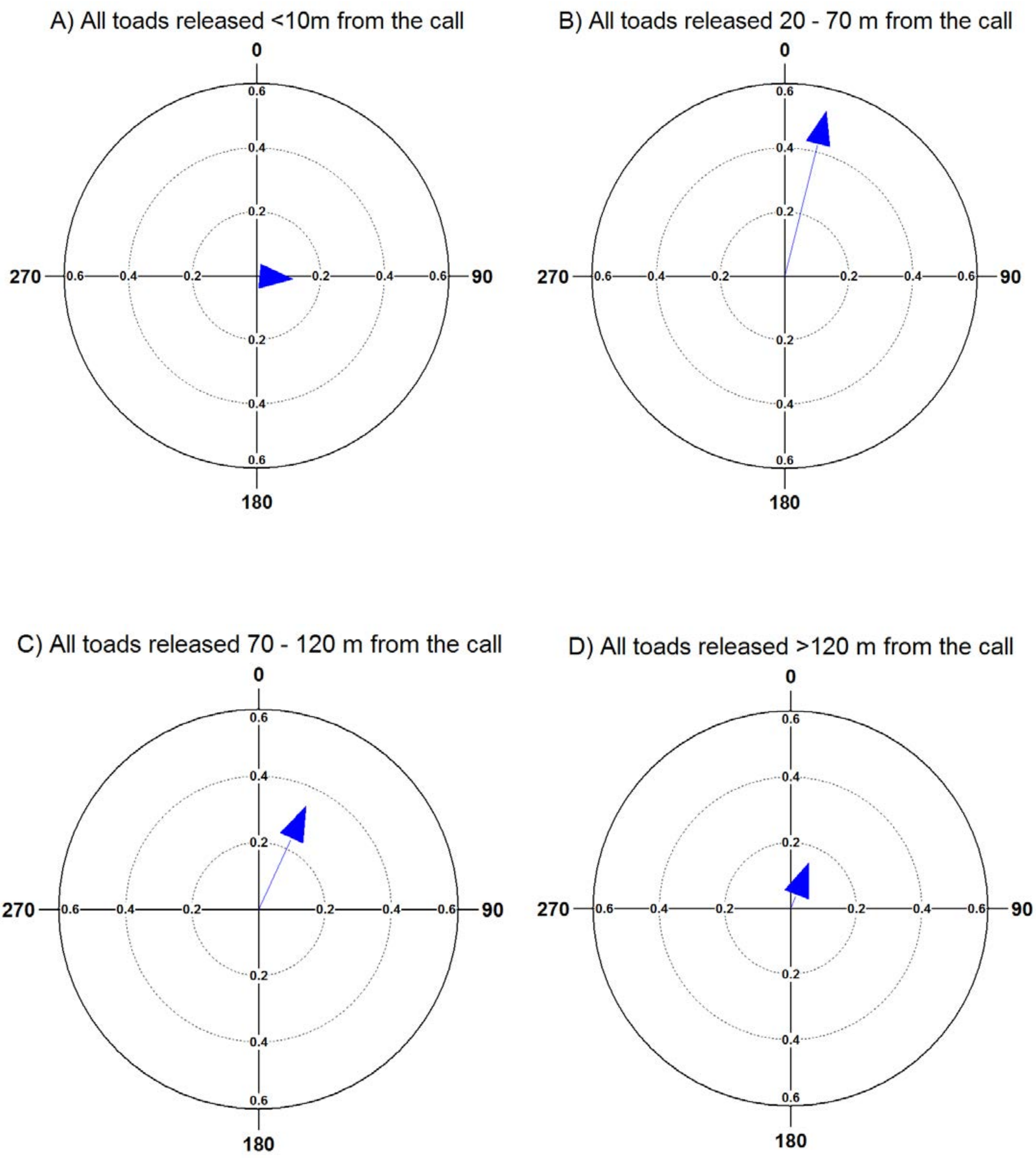
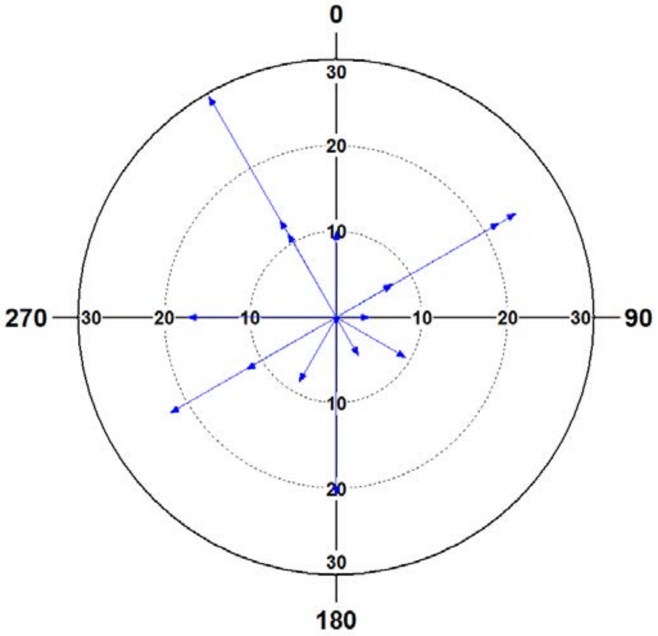
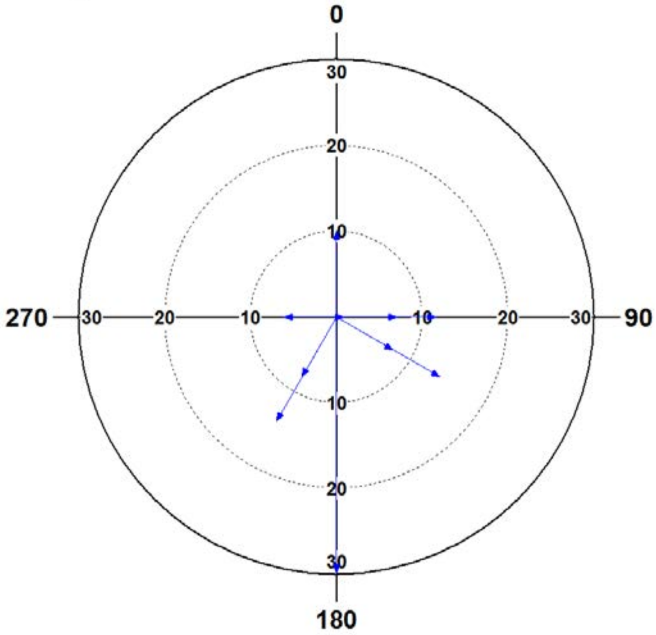

A) All toads released <10m from the call
B) All toads released 20 - 70 m from the call
C) All toads released 70 - 120 m from the call
D) All toads released >120 m from the call
0
90
180
270
0.2
0.4
0.6

Figure 3-2. The angle, and length of the mean vector for movement paths when toads were released from; (A) 120 m from the calling speaker, (B) 20 – 70 m from the calling speaker, (C) 70 – 120 m from the calling speaker, and (D) >120 m from the calling speaker. The angle of each arrow represents the mean direction of movement from that release distance, while the length of each arrow represents the concentration of angles around that mean direction (i.e., the longer the arrow, the more concentrated movement paths are around the mean).

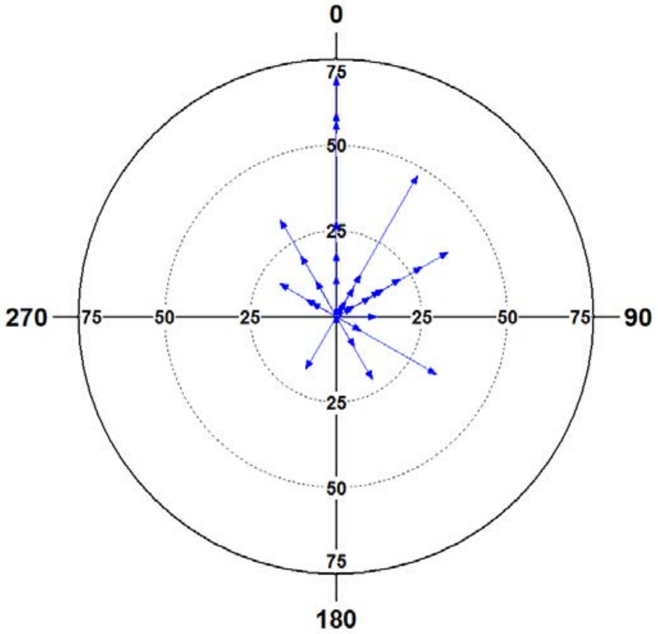
A) Males released <10 m from the call



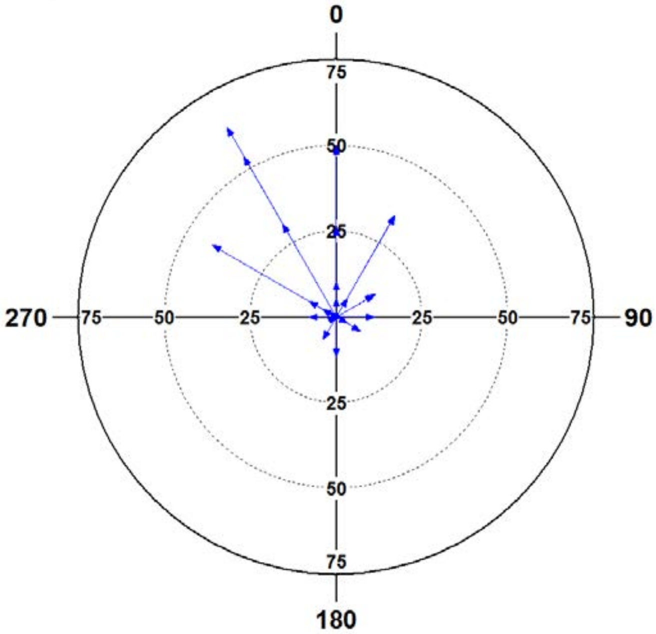
B) Females released <10 m from the call



C) Males released 20 - 70 m from the call



D) Females released 20 - 70 m from the call



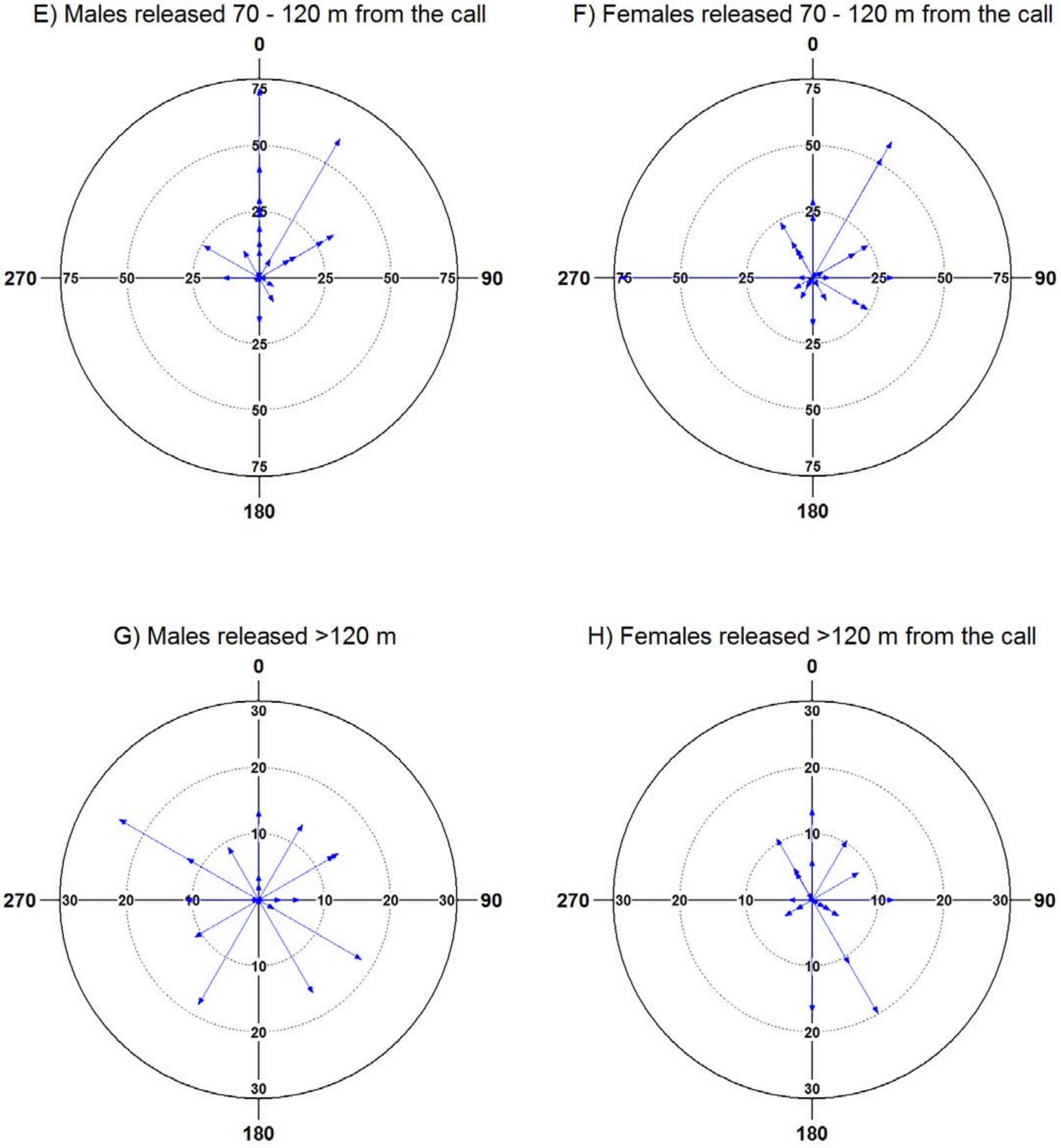

E) Males released 70 - 120 m from the call
0
90
180
270
25
50
75
F) Females released 70 - 120 m from the call
0
90
180
270
25
50
75
G) Males released >120 m
0
90
180
270
10
20
30
H) Females released >120 m from the call
0
90
180
270
10
20
30

Figure 3-3. Direction and distance moved by male (A, C, E and G) and female (B, D, F and H) toads exposed to advertisement calls when released up to 200 m away from the speaker. Arrow heads indicate the location of toads after 10 min: (A, B) 120 m from the sound of a cane toad call (vectors not significantly different from random). Full results reported in the text. Figures (C), (D), (E) and (F) have been rescaled due to the larger distance moved by toads when released at these distances.

Did toads move further when moving towards a call?

During the control experiments, in which no call was played, I found no significant differences in the distances moved by toads at any distance from the speaker ($F_{9,118} = 0.677$, $P = 0.728$). Furthermore, there was no significant correlation between the distance moved by toads and their path of movement, when released during the control experiments (Table 3-3). Conversely, when the call was playing, I found a significant correlation between the distance and direction of movement of male toads, when released 70 – 120 m from the speaker. At these release distances, male toads moved further when they were moving towards the call. There was no significant correlation between the distance and direction moved by male toads when released within 70 m of the speaker, or greater than 120 m from the speaker (Table 3-3). Female toads also moved further when they moved towards a call, when released 20 – 70 m away from the speaker; however, I found no significant correlation between movement distance and direction when female toads were released within 10 m of the call, or from greater than 70 m from the call (Table 3-3). Unlike movement direction, which differed between sexes, comparisons between males and females revealed no significant differences in movement distances between the sexes when the speaker was on, regardless of their category of release distance (<10 m: $F_{1,27} = 1.713$, $P = 0.202$, 20 – 70 m: $F_{1,52} = 1.266$, $P = 0.266$, 100 – 120 m: $F_{1,49} = 0.033$, $P = 0.856$, >120 m: $F_{1,39} = 1.240$, $P = 0.272$).

Overall, toads stopped responding to the call at 120 – 130 m from its origin, therefore the minimum signal-to-noise ratio required to elicit a behavioural response was 0 – 0.3 dB (Fig. 3-1). The strongest behavioural responses, in which both males and females moved towards the speaker, occurred at distances of 20 – 70 m from the call. Thus, the range of signal-to-noise ratio most likely to elicit a strong response from both sexes was 3.2 – 13.6 dB (Fig. 3-1).

Table 3-3. Results of Mardia's rank correlations examining the circular-linear relationship between the distance and direction moved by toads when exposed to a call played from different distances. Significantly correlated results are marked with asterisks, are Bonferroni adjusted (significance accepted when *P* < 0.013), and are representative of a model where toads moved further when they moved towards a call. Included are results from control experiments, when the speaker was off, also shown are mean distances moved by toads when released from each distance category.

	Circular – linear correlation							
	Control				**Speaker on**			
Release distance	**Males**		**Females**		**Males**		**Females**	
	$\bar{x}$	***P***	$\bar{x}$	***P***	$\bar{x}$	***P***	$\bar{x}$	***P***
<10m	12.0 ± 2.0 m	0.93	9.9 ± 2.5 m	0.40	16.1 ± 2.6 m	0.052	10.9 ± 2.4 m	0.400
20 – 70 m	7.9 ± 1.2 m	0.30	7.8 ± 1.5 m	0.98	20.1 ± 3.2 m	0.023	18.2 ± 3.7 m	0.012*
100 – 120 m	7.2 ± 1.5 m	0.98	8.4 ± 2.1 m	0.13	19.0 ± 3.5 m	0.005**	12.8 ± 3.6 m	0.054
>120 m	8.7 ± 2.0 m	0.17	6.9 ± 1.9 m	0.77	9.3 ± 1.5 m	0.920	7.1 ± 1.3 m	0.437

3.5. DISCUSSION

I determined the active space of cane toads in response to a recorded call in an open, low-noise environment. Both male and female toads actively moved towards the sound of a cane toad call when located 20 – 70 m from the speaker (Fig. 3-3C, D). Males continued responding to the call to up to 120 m, whereas female toads ceased moving towards the call when released from more than 70 m from the speaker (Fig. 3-3E, F). My results confirm that male toad advertisement calls attract both males and females, and demonstrate that calls were attractive to males over greater distances, i.e., the active space of calls is greater for males. Attraction to calls benefits females by allowing them to find and distinguish among potential mates, but males are most likely attracted to other factors signaled by calls, for example, a potential water source, or the presence of conspecifics for breeding (Swanson et al. 2007; Yasumiba et al. 2015). The active calling space of cane toads is remarkably large, and encompasses nearly the full range of the attenuation distance of the call (Fig. 3-1), indicating an auditory threshold (obtained *via* observations of phonotaxis) for both sexes of approximately 3.2 dB. This value is much lower than the auditory threshold described for other anurans (obtained *via* midbrain multi-unit recordings; e.g., Penna & Moreno-Gómez 2014), and indicates that cane toads may communicate more effectively than other frog species over large areas. The resulting active space of a cane toad vocalisation may, thus, encompass more individuals, and facilitate higher recruitment to breeding areas than at least some other species.

When released at distances less than 10 m from the speaker, the movements of both sexes of toads did not differ significantly from random. Toads were clearly capable of locating the speaker, and sometimes stood directly in contact with it (*n* = 2 individuals; B. Muller, pers. obs.), but most toads did not. Possibly, toads were searching for other aspects of the environment usually signalled by calls, for example, both sexes may have been searching for water or suitable breeding habitat indicated by the presence of a calling individual (Buxton et al. 2015).

As toads were released further from the call, a disparity in the behaviour of the sexes became apparent. Male toads moved towards the call when released up to 120 m away, and moved further when they moved towards the call when released 70 – 120 m away from it. In contrast, females appeared to move randomly at distances 70 – 120 m from the call, and there was no correlation between the distance and direction they moved at these release distances. Thus, in my experiments, female toads ceased to display positive phonotaxis at approximately 70 m from a call, while male toads continued responding. Degradation of the temporal structure of vocalisations, as a function of distance, occurs in other anurans (Ryan & Sullivan 1989). I did not measure temporal structure degradation in this experiment; however, the call structure may have degraded to a point where it was no longer attractive to female toads when it reached 70 m from its origin. Female cane toads prefer very specific call characteristics, but male toads are attracted to most calls (Yasumiba et al. 2015) and, therefore, may have continued to respond to degraded calls.

Both male and female toads moved randomly when released more than 130 m from the call. Equivalence of movement vectors in the control and sound trials at this great distance from calls suggests that there is a maximum distance at which cane toads can respond to calls, probably because at these distances they cannot hear the call over ambient environmental noise. Thus, the maximum extent of the active space of a cane toad call is close to the point at which the signal-to-noise ratio reaches zero.

My estimates of the active space of calls for toads are probably overestimates of propagation distances of toad calls in the wild, because my experimental area had very simple habitat structure, allowing effective propagation (Penna & Solis 1998). Habitat structure greatly influences the propagation of sound and its attenuation (Marten & Marler 1977), and thus the call may have propagated further in my trials than would be expected in woodland, urban, or rainforest habitats (Marten & Marler 1977) or over waterbodies (e.g., Penna & Solis 1998). Thus, in natural habitats, toads may stop responding to the advertisement call over shorter distances than those determined in my study, and my study provides an estimate of the maximum active distance of calls.

Future research could explore the apparently random movement of toads very close to calls, to determine why some individuals appeared to move away from the call at close range. For example, the call I used was not especially attractive to females; they prefer lower frequency calls (Yasumiba et al. 2015). It may be possible to manipulate the call to make it more attractive. Also, my study occurred in a relatively obstruction-free environment. Quantifying the attenuation rate, signal-to-noise ratio and active space of calls in natural habitats may help determine potential pathways for toad invasion and clarify speed and likelihood of spread across different habitats (e.g., Murphy et al. 2010).

My research demonstrates that toad calls have a large active space, a trait that may facilitate the attraction of conspecifics to breeding aggregations (Buxton et al. 2015). A large active space may attract many individuals, at relatively great distances from the chorus, and may increase the invasion speed of cane toads in their invaded range. Control programs could exploit the large active space of the call, for detection of breeding aggregations, and for appropriate spacing for call recording or acoustic traps (e.g., Schwarzkopf & Alford 2007).

Chapter 4: Success of capture of toads improved by manipulating acoustic characteristics of lures

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4.1. ABSTRACT

Management of invasive vertebrates is a crucial component of conservation. Trapping reproductive adults is often effective for control, and modification of traps may greatly increase their attractiveness to such individuals. Cane toads (*Rhinella marina*) are invasive, and males use advertisement vocalisations to attract reproductive females. In amphibians, including toads, specific structural parameters of calls (e.g., dominant frequency and pulse rate) may be attractive to females. Some cane toad traps use an artificial advertisement vocalisation to attract toads. I determined whether variation of the call's parameters (volume, dominant frequency and pulse rate) could increase the capture rate of gravid females. Overall, traps equipped with loud calls (80 dB at 1 m) caught significantly more toads, and proportionally more gravid females, than traps with quiet calls (60 dB at 1 m), and traps with low dominant frequency calls caught more gravid females than traps with median frequency calls. Traps with high pulse rate calls attracted more females than traps with low pulse rate calls. Approximately 91% of the females trapped using a low frequency and high pulse rate combination call were gravid, whereas in traps using a call with population median parameters only approximately 75% of captured females were gravid. Calls that indicated large-bodied males (low frequency) with high energy reserves (high pulse rate) are often attractive to female anurans, and were effective lures for female toads in my study. The design of future trapping regimes should account for behavioural preferences of the target sex.

4.2. INTRODUCTION

Invasive species cause negative environmental, economic, and social impacts worldwide (Pimentel et al. 2005), and devising management strategies for these species is a crucial component of conservation. The use of traps is a common, effective, and economical strategy for removing pests on a local scale (Nogales et al. 2004; El-Sayed et al. 2006). Behavioural (Greenslade 1964; Saunders et al. 1993) and physical (Beacham & Krebs 1980) factors influence the success of traps in the field. Manipulation of these factors, using modifications to the trap, may help target particular age classes or sexes such that the impact of each individual capture is increased. For example, the targeted removal of reproductively active females can reduce future breeding potential and therefore the number of new individuals entering the population in the future (Epsky et al. 1999).

The cane toad (*Rhinella marina*) is a highly invasive, vocalising anuran. It is a pest in tropical regions worldwide, including Australia, and is one of the 100 worst invasive alien species (Global Invasive Species Database 2016). Cane toad parotoid glands secrete powerful bufotoxins that cause post-ingestion poisoning and mortality of native predators (Letnic et al. 2008; Doody et al. 2009; Shine 2010) and domestic pets (Reeves 2004). Toads may also reduce the nocturnal activity levels of some native frogs (reviewed in Shine 2010). Biological and genetic control methods for cane toads are limited and have not yielded a significant reduction in cane toad numbers (Shanmuganathan et al. 2010; Tingley et al. 2017). Trapping adult toads is a control method that can be implemented in a variety of regions (Tingley et al. 2017), and therefore modifications that increase its efficacy should be explored (Miller 2006).

For vertebrates with high reproductive output, removing females is an effective strategy, as the removal of a single female from the population also removes their potential future offspring. A female cane toad may lay upwards of 10 000 eggs per clutch (Zug & Zug

1979); therefore removing one adult female prior to reproduction may potentially reduce the future population more than removing one male or juvenile (Lampo & de Leo 1998). Conversely, removing several males is unlikely to significantly reduce population numbers, or recruitment into the next generation, because a single male can fertilise multiple clutches (Browne & Zippel 2007). Targeting reproductively active female toads is the best strategy for reducing recruitment into the next generation (Thresher 2007) and is often the primary method of population reduction and management for vertebrate pests.

Most anurans, including cane toads, use vocalisations for mate attraction (Wells & Schwarz 2007). Female anurans use information provided by advertisement vocalisations to choose males (Gerhardt 1994): the vocalisation's structural parameters influence its attractiveness and therefore phonotaxis by female receivers. Female anurans often prefer energetic calls with a high pulse rate, which indicates that the calling individual is investing a lot of energy in the vocalisation (Wells & Taigen 1992). Pulse rate, however, is not always an important criterion for mate choice in anurans (e.g., Meuche 2013). Advertisement calls with a low dominant frequency are indicative of large body size (Gerhardt 1991; Felton et al. 2006; Richardson et al. 2010), and are preferred by females of many anuran species (Ryan 1983; Gerhardt 1991), although not always (Arak 1988). Traps for adult cane toads include a lure, which plays a male advertisement call, to attract conspecifics (Schwarzkopf & Alford 2007). It is reasonable that a louder call should be more attractive, and yield higher trap captures, because the call can be heard from further away; however, quiet calls have been more attractive to female toads than loud calls in an experimental arena using speakers (not traps: Schwarzkopf & Alford 2007). Therefore, lures that produce different call volumes should be tested, in addition to lures that produce different call frequencies and pulse rates.

The aim of this study was to determine if alteration of particular call characteristics (call volume, pulse rate and dominant frequency) influences the capture rate of adult, reproductively active female toads. Modifying the volume, frequency and pulse rate of the vocalisation used in cane toad traps may influence the attractiveness of the call, and enhance our ability to remove gravid female toads from invasive populations.

4.3. MATERIALS AND METHODS

The trapping unit

To catch toads, I used a trap consisting of a wire-mesh box (1 m × 1 m × 0.25 m) equipped with doors that opened easily with pressure from outside of the trap, but preventing egress of trapped toads. I placed an acoustic lure inside each trap, which played one of several possible cane toad advertisement calls (.WAV files) on repeat at night and had a small light-emitting diode ‘black’ (UV) light that attracted insects. I modified the call parameters by changing the .WAV file played by the lure. More details on the trap and methodology are available in Yeager *et al*. (2014), and in section 1.7 of this thesis.

Determining the relative attractiveness of loud and quiet calls

The study was performed on Orpheus Island (18° 36' 46.0" S, 146° 29' 25.2" E) from 6 May 2013 to 31 March 2014, with the exception of 17 days in December 2013. The island is within the Palm Island Group on the Great Barrier Reef, about 23 km east of the Australian mainland coast and 120 km north of Townsville, Queensland. It is approximately 12 km long and is composed primarily of dry woodlands with dry rainforest patches. Data collection occurred around the Orpheus Island Research Station.

I chose four trapping sites, each approximately 10 m from the research station's primary footpath, and placed one trap at each site. All trapping sites were located in open, grassy areas, and had similar ambient light ($\bar{x}$ = 0.057 ± 0.03 lx) and environmental and anthropogenic noise ($\bar{x}$ = 38.6 ± 0.5 dB) levels. I measured light and noise levels at each site on 10 randomly selected nights, at 21:00 h, using a lux meter (ATP DT-1300, Leicestershire, UK) and a C-weighted Lutron sound level meter (model SL-4013, Taipei, Taiwan). The trapping sites were 400 m apart, such that the advertisement call at one site could not be heard by toads at any other site (Chapter 3; Muller et al. 2016). Prior to the experiment commencing, I adjusted the call volume of the lures in a soundproof studio, using a C-weighted Lutron sound level meter (model SL-4013). Two of the lures produced a loud call (maximum 80 dB at 1 m); the other two lures produced a quiet call (maximum 60 dB at 1 m). I used the same, artificially manipulated advertisement call in all four lures (except that it was loud in some and quieter in others). Its characteristics were the median call parameters of 26 sampled cane toads from Townsville, Australia (frequency 600 Hz, pulse rate 15 pulses s^{-1}; Yasumiba et al. 2015). All four lures were activated nightly, such that all four calls played simultaneously on any given night. Lures activated automatically at twilight (at approximately 18:00 – 19:00 h) and stopped the following morning at sunrise (lures were active for approximately 10 − 12 h per night, depending on season). All lures started and stopped automatically at the same time each night. To avoid confounding trap site and call characteristics in my measure of trap success, I rotated the four lures among sites every 4 days, such that over 16 days every call was played at every site, and there was a different call playing at every site every night. A water bowl and polyvinyl chloride pipe for shelter were placed within each trap. I removed and counted trapped toads daily. Toads were euthanised immediately after their removal from the traps, using an overdose (350 ppm) of tricainemethanesulfonate (MS-222) *via* submersion in water containing a sodium bicarbonate buffered solution. I recorded mass to the nearest 1 g using a Pesola spring scale, and recorded snout − urostyle length (SUL) to the nearest 1 mm using a clear plastic ruler. I visually determined the sex of trapped toads (males have rough textured, light brown to yellow skin; females have dark brown, comparatively smooth skin), and dissected female toads post-euthanasia to determine whether they were gravid.

Determining the relative attractiveness of various call frequencies and pulse rates

I trapped cane toads at a freshwater creek, at James Cook University in Townsville, Australia (19° 19′ 47.74″ S, 146° 45′ 29.55″ E), from 9 May 2014 to 4 March 2015. The creek traversed a woodland habitat, composed mainly of popular gum (*Eucalyptus platyphylla*), with an understorey of black spear grass (*Heteropogon contortus*). I selected four discrete pools of water (located 300 – 400 m from one another) along the predominantly dry creek bed as trapping sites; each trap was 10 m from the water's edge. Each trapping site had similar ambient light ($\bar{x}$ = 0.094 ± 0.08 lx) and noise ($\bar{x}$ = 37.8 ± 0.9 dB) levels. I recorded ambient light and noise using the same methodology as previous experiments.

I placed one trap at each site; each lure had a call volume of 80 dB at 1 m. I artificially manipulated either the frequency or the pulse rate of the median call (using Audacity V 1.2.3), such that each of the four lures played a unique vocalisation that had at least one parameter at a median value, every night, at each site. The median values for call parameters were defined by median values for this population of toads, determined by another study (600 Hz, 15 pulses s^{-1}; Yasumiba et al. 2015). Vocalisations included a low frequency call (500 Hz, 15 pulses s^{-1}), a high pulse rate call (600 Hz, 18 pulses s^{-1}) and a low pulse rate call (600 Hz, 13 pulses s^{-1}), as well as the median call. ‘Low’ and ‘high’ values were within values determined for this population but at the extremes of the distribution. The structure of the trapping regime was the same as the previous experiment; all lures ran simultaneously, lures activated and stopped automatically at the same time every night (lures operated for approximately 10 – 12 trap hours per trap per night, depending on season), and I rotated lures every 4 days, but there was a lure and trap combination playing at each site every night. I removed and euthanised trapped toads daily, before recording mass and SUL, visually determining sex, and establishing which females were gravid at the time of capture by dissection.

Determining the attractiveness of a call combining low frequency and high pulse rate

I trapped toads between 7 October 2015 and 23 February 2016 around the James Cook University Creek, Townsville. I used three traps, two of which had lures playing the same median and low frequency calls already described. I introduced a ‘combination’ call in the third lure which had both a low frequency (500 Hz) and a high pulse rate (18 pulses s^{-1}). I randomly excluded one of the previous trapping sites from the experiment, using a random number generator. The procedure for activating and rotating lures, and processing and measuring trapped toads, was the same as in previous experiments.

Statistical analysis

Determining the relative attractiveness of loud and quiet calls

There were a high proportion of nights on which no toads were captured, creating zero-inflated distributions; I therefore used a square-root transform to normalise the distributions (Maindonald & Braun 2007). I examined if the mean number of females captured nightly differed among the four lures, using a one-way ANOVA, and identified significant differences in the attractiveness to females of each lure using a Tukey's HSD (honest significant difference) post-hoc test. I then combined data from trapping units of the same volume and examined if captures of gravid females differed significantly between loud and quiet calls, using a two-tailed *t* test. I examined if there was a significant difference between the mean nightly capture rates of the two sexes, using a two-tailed *t* test for each call volume. Finally, I determined if body mass of trapped females varied significantly between the two call volumes, using a two-tailed *t* test, and repeated this statistical procedure to examine if the SUL of trapped females varied between call volumes.

Determining the relative attractiveness of various call frequencies and pulse rates

I used a square-root transformation to normalise the distributions of numbers of trapped toads, and identified if the mean number of females trapped varied significantly with call type, using a one-way ANOVA in combination with Tukey's HSD test. I determined if the mean number of gravid females trapped varied significantly with call type, using a one-way ANOVA. I determined if the mean proportion of captured males and females differed among call types, using a one-way ANOVA, and used the Holm − Sidak method for pairwise comparisons (Seaman 1991). Finally, I examined whether body mass of trapped female toads varied with call type, using a one-way ANOVA, in conjunction with a Tukey's HSD test for pairwise comparisons. I repeated this methodology to examine if the SUL of trapped females varied with call type.

Determining the attractiveness of a call combining low frequency and high pulse rate

I examined if call type influenced mean nightly female captures, mean nightly gravid female captures, the sex ratio of trapped toads, and the body mass and SUL of trapped females, using the same methodology used to determine the attractiveness of various frequencies and pulse rates. Once again, I used a square-root transformation prior to analysis. I used R (R Core Team, V 3.1.2) for all statistical analysis.

4.4. RESULTS

The total number of toads caught in the different trapping sessions was extremely variable due to location (the size of the Orpheus Island and Townsville populations were probably different), season, and length of the trapping regime. I compared mean nightly captures among traps, within trapping sessions, to control for these effects, and have reported the total number of toads trapped in each session.

Determining the relative attractiveness of loud and quiet calls

Traps that produced loud calls caught 1314 toads (1038 males, 276 females), and traps that produced quiet calls caught 338 toads (264 males, 74 females). There was a significant difference in the mean number of females caught per night among the four trapping units; significantly more females were caught in traps that produced loud calls (Fig. 4-1). In addition, significantly more gravid females were caught in traps that produced a loud call, relative to a quiet call ($t = -9.17$, df = 547, $P < 0.001$), but the proportion of trapped females that were gravid did not differ between call types (loud, 76.5% gravid; quiet, 75.9% gravid). Both loud ($t = 13.6$, $P < 0.001$) and quiet ($t = -9.13$, $P < 0.001$) calls attracted more males than females into traps. There was no significant difference in the mass (mean 117.09 ± 2.89 g, $t = 0.30$, df = 1060, $P = 0.38$) or SUL (mean 112.05 ± 2.43 mm, $t = -0.04$, df = 1060, $P = 0.48$) of trapped females between call types.

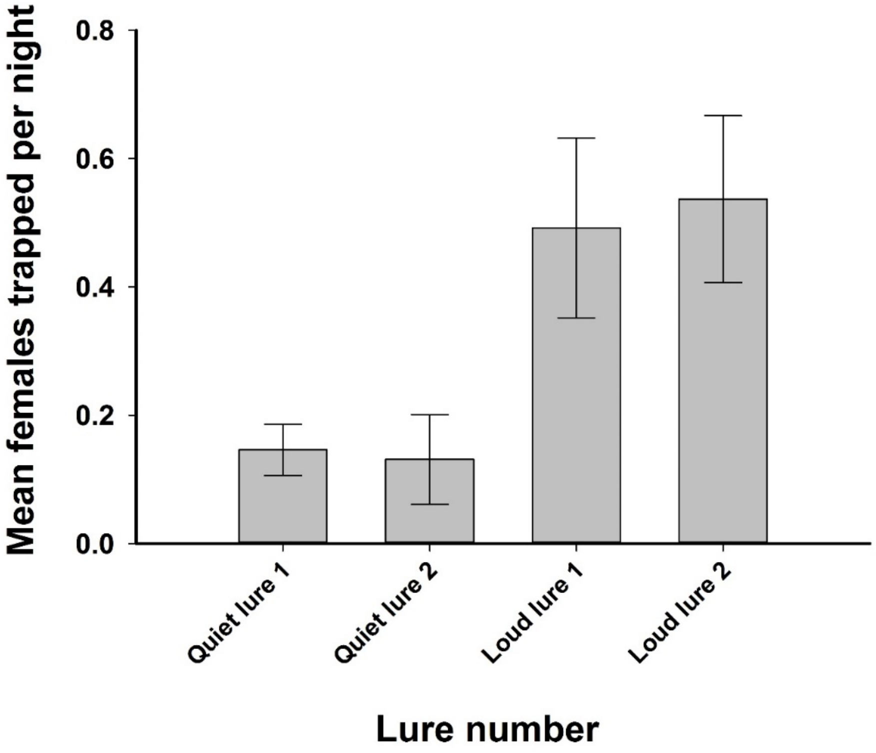

Figure 4-1. Comparison of mean nightly female captures among four lures on Orpheus Island, between 16 May 2013 and 31 March 2014. Pairwise comparisons show no significant differences between lures of the same volume (L1 *versus* L2, $q = 0.64$, $P = 0.97$; Q1 *versus* Q2, $q = 0.44$, $P = 0.99$); however, there are significant differences between lures of different volumes (L1 *versus* Q1, $q = 10.39$, $P < 0.001$; L1 *versus* Q2, $q = 10.84$, $P < 0.001$; L2 *versus* Q1, $q = 11.03$, $P < 0.001$; L2 *versus* Q2, $q = 11.47$, $P < 0.001$). L1, loud trap 1 (80 dB at 1 m); L2, loud trap 2 (80 dB at 1 m); Q1, quiet trap 1 (60 dB at 1 m); Q2, quiet trap 2 (60 dB at 1 m).

Determining the relative attractiveness of various call frequencies and pulse rates

Traps producing a median call caught 620 toads (492 males, 128 females), traps producing a low frequency call caught 673 toads (357 males, 316 females), traps producing a high pulse rate call caught 507 toads (337 males, 170 females) and, finally, traps that produced a low pulse rate call caught 472 toads (308 males, 164 females). The mean number of females trapped per night varied significantly with call type ($F_{3,469} = 38.27$, $P < 0.001$; Fig. 4-2), as did the mean number of gravid females trapped per night ($F_{3,469} = 31.60$, $P < 0.001$). The majority of trapped females were gravid; however, the proportion of trapped females that were gravid varied among call types (Fig. 4-3). The trap that produced a low frequency call caught significantly more gravid females than any other trap (Tukey's HSD: $P < 0.001$ for all comparisons), and the trap that produced a high pulse rate call caught significantly more gravid females than the trap that produced a median call (Tukey's HSD: $P = 0.008$). There was no significant difference in mean nightly captures of gravid females between median and low pulse rate calls (Tukey's HSD: $P = 0.091$), or between high pulse rate and low pulse rate calls (Tukey's HSD: $P = 0.772$).

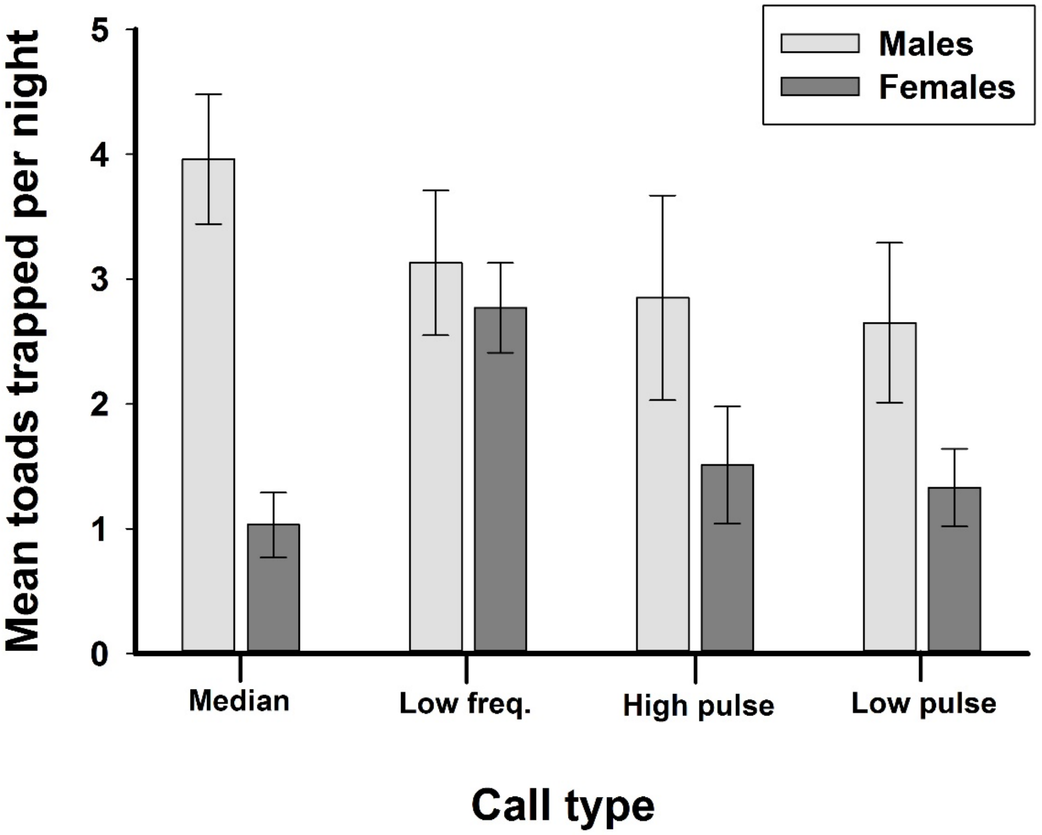

Figure 4-2. Comparison of mean nightly female captures in traps from 9 May 2014 to 4 March 2015, at James Cook University, Queensland, Australia, using median (600 Hz, 15 pulses s^{-1}), low frequency (500 Hz, 15 pulses s^{-1}), high pulse rate (600 Hz, 18 pulses s^{-1}) and low pulse rate (600 Hz, 13 pulses s^{-1}) advertisement calls as lures. I rotated lures between trap locations, with one lure playing at each location each trap night. Pairwise comparisons show that traps playing low frequency calls caught significantly more females per night than traps playing any other call (low frequency *versus* median; $q = 14.19$, $P < 0.001$; low frequency *versus* low pulse, $q = 11.76$, $P < 0.001$; low frequency *versus* high pulse, $q = 9.88$, $P < 0.001$), while traps playing the high pulse rate call caught significantly more females per night than traps playing the median call ($q = 3.91$, $P = 0.029$). There was no significant difference in mean nightly captures of females between median and low pulse rate calls ($q = 2.57$, $P = 0.266$) or between high pulse rate and low pulse rate calls ($q = 1.46$, $P = 0.733$).

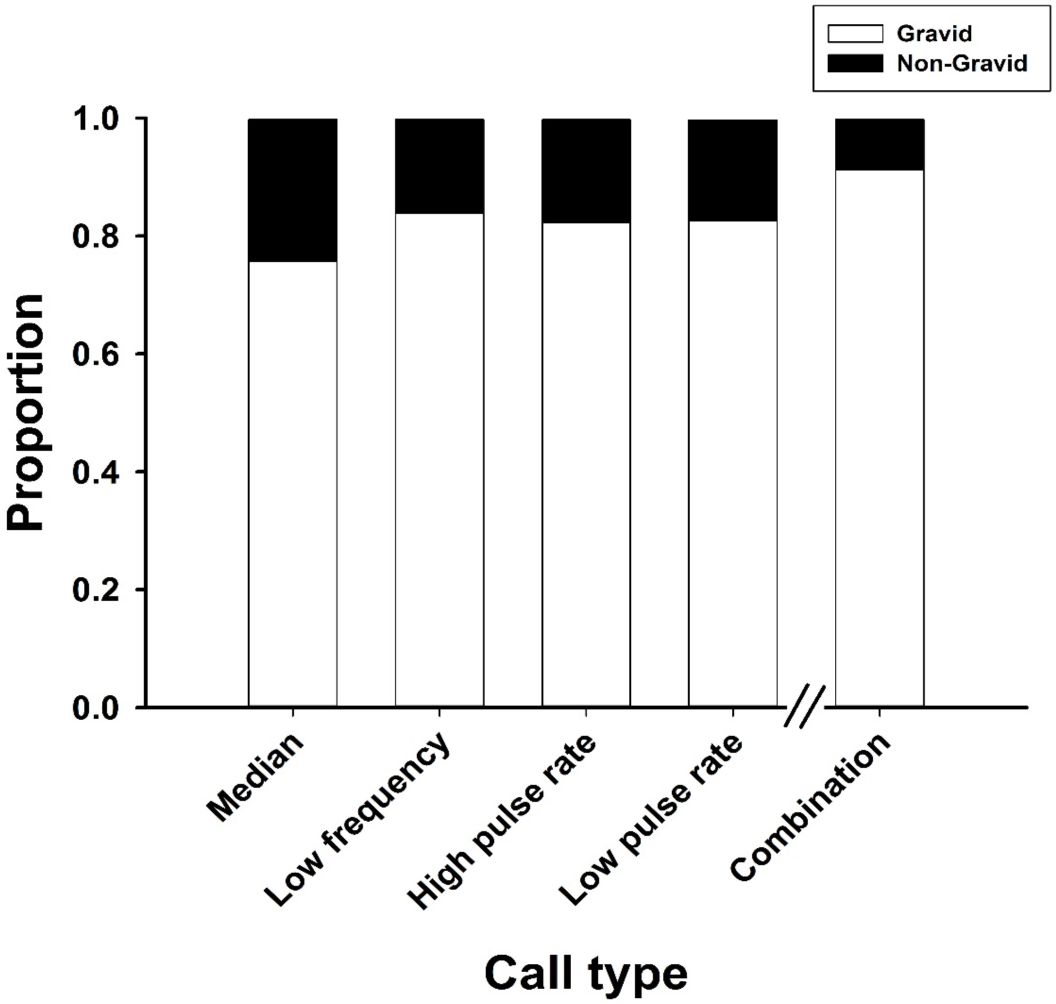

Figure 4-3. Proportion of gravid females trapped using median (600 Hz, 15 pulses s^{-1}), low frequency (500 Hz, 15 pulses s^{-1}), high pulse rate (600 Hz, 18 pulses s^{-1}), low pulse rate (600 Hz, 13 pulses s^{-1}) and combination (500 Hz, 18 pulses s^{-1}) advertisement calls as lures. I rotated lures between trap locations, with one lure playing at each location each trap night. I collected data over two trapping periods. All calls except the combination call were used in the first trapping period. The graph presents results for these calls during the first trapping period. The combination, low frequency and median calls were used in the second trapping period; however, the graph only presents results for the combination call for this trapping period. The distinction between the two trapping periods is represented by an axis break. There was no significant difference in the proportion of gravid females caught using median and low frequency calls between the two trapping periods.

Overall, there was a significant difference in the proportion of each sex captured in response to different call types during the trapping period ($F_{3,469} = 9.84$, $P < 0.001$); lures producing median ($t = 13.13$, $P < 0.001$), high pulse rate ($t = 5.57$, $P < 0.001$) and low pulse rate ($t = 5.95$, $P < 0.001$) calls attracted a significantly higher proportion of males than females (Fig. 4-2). There was no difference in the proportions of males and females captured in traps producing a low frequency call ($t = 1.54$, $P = 0.12$; Fig. 4-2). There was a significant difference in the mass of female toads trapped using various call types ($F_{3,469} = 3.68$, $P = 0.012$). Females trapped using a low frequency call were significantly heavier than females trapped using a median call ($q = 4.52$, $P = 0.011$; Table 4-1). There was no significant difference among call types ($F_{3,469} = 1.68$, $P = 0.17$) in the SUL of trapped females.

Table 4-1. The mean mass and SUL (snout-urostyle length) of female toads trapped at James Cook University, Townsville, Australia, using calls with different parameters as lures. Trial 2 occurred between the 9 May 2014 and 4 March 2015, and trial 3 occurred between 7 October 2015 and 23 February 2016.

M = Call with median parameters (600 Hz, 15 pulses s^{-1}), LF: Call with lower than median frequency (500 Hz, 15 pulses s^{-1}), HP: Call with higher than median pulse rate (600 Hz, 18 pulses s^{-1}), LP: Call with lower than median pulse rate (600 Hz, 13 pulses s^{1}), Combo: Call with lower than median frequency and higher than median pulse rate (500 Hz, 18 pulses s^{-1}).

Call type	**Mean mass**	**Mean SUL**
M	98 ± 3 g	98 ± 3 mm
LF	117 ± 10 g	107 ± 2 mm
HP	110 ± 11 g	102 ± 3 mm
LP	104 ± 3 g	100 ± 3 mm
Combo.	116 ± 5 g	108 ± 2 mm

Determining the attractiveness of a call combining low frequency and high pulse rate

Traps producing the median call caught 991 toads (783 males, 208 females), traps producing a low frequency call caught 803 toads (411 males, 392 females) and, finally, traps producing a low frequency and high pulse rate combination call caught 821 toads (424 males, 397 females). The mean number of females trapped per night varied significantly with call type ($F_{2,417} = 29.27$, $P < 0.001$; Fig. 4-4). Significantly fewer females were caught in traps playing the median call compared to the low frequency call ($q = 8.88$, $P < 0.001$) and the combination call ($q = 8.90$, $P < 0.001$); however, there was no difference in mean nightly female captures between the low frequency and combination calls ($q = 0.03$, $P = 0.99$). The majority of trapped females were gravid; however, the proportion of trapped females that were gravid varied among call types; the traps producing the high frequency and low pulse rate combination call caught the highest proportion of gravid females (Fig. 4-3). Significantly fewer gravid females were caught in traps playing a median call compared to the other two call types ($F_{2,417} = 33.31$, $P < 0.001$). Overall, there was no significant difference in the proportions of each sex captured during the trapping period ($F_{3,417} = 0.177$, $P < 0.84$); also, there was no difference in the proportion of males and females captured in traps that produced a low frequency call ($t = 0.63$, $P = 0.53$) or the combination call ($t = 0.80$, $P = 0.42$), whereas traps producing a median call captured significantly more males than females ($t = 8.08$, $P < 0.001$). There was a significant difference in the mass of female toads trapped using various call types ($F_{2,417} = 7.28$, $P < 0.001$). Females trapped using the median call were significantly lighter than those trapped using the low frequency call (Holm − Sidak $q = 4.86$, $P = 0.003$) and the combination call (Holm − Sidak $q = 4.40$, $P = 0.008$; Table 4-1). There was a significant difference in the SUL of females trapped using different call types ($F_{2,417} = 8.17$, $P < 0.001$). Females trapped using the median call were significantly smaller than those trapped using the low frequency ($q = 4.64$, $P = 0.005$) and combination calls ($q = 5.16$, $P = 0.002$). There was no significant difference in the mean SUL of females caught using the low frequency and combination calls ($q = 0.57$, $P = 0.92$; Table 4-1).

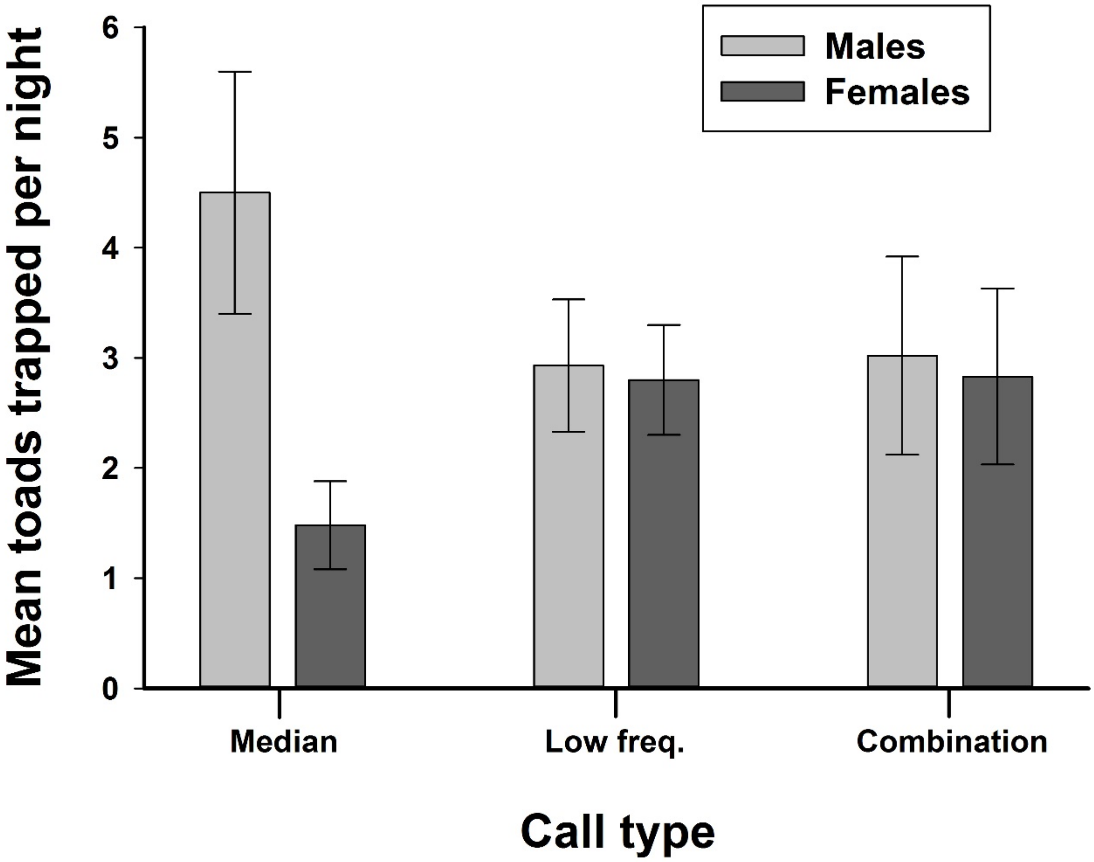

Figure 4-4. Comparison of the mean nightly female captures per night from 7 October 2015 to 23 February 2016, at James Cook University, Queensland, Australia, using median (600 Hz, 15 pulses s^{-1}), low frequency (500 Hz, 15 pulses s^{-1}) and combination (500 Hz, 18 pulses s^{-1}) advertisement calls as lures.

4.5. DISCUSSION

In my experiments, female toads discriminated strongly among calls; therefore, I could manipulate the proportion of females trapped by changing the calls used as lures. Conversely, male captures were stable when I manipulated the calls to attract more females, which is advantageous for cane toad control because, while I increased female trapping rates, I did not sacrifice captures of males. When I compared loud and quiet calls, significantly more females were trapped using a call with a volume of 80 dB at 1 m compared to the quieter call (volume

60 dB at 1 m), probably because the loud call could be heard over a larger area by more toads. However, both loud and quiet calls (with median parameters) captured more males than females. When I compared calls with different parameters, traps producing low dominant frequency calls caught significantly more female toads than traps producing median calls (Fig. 4-2). The low frequency call was a more effective lure than high or low pulse rate calls, suggesting that female toads were attracted to low frequency calls. Calls that included one extreme (non-median) parameter (e.g. low frequency, high pulse rate or low pulse rate) were more effective lures for trapping a higher proportion of gravid females than the median call. The combination call, in which both parameters were extreme, caught the highest proportion of gravid females overall.

Vocalisation parameters are often indicative of the body size and fitness of the calling individual; for example, male body size influences dominant call frequency in cane toads (Yasumiba et al. 2015). In my experiments, females displayed a strong preference for low frequency calls, which indicate a large male toad; female cane toads seem to prefer the largest male available, which is slightly smaller than themselves (Yasumiba et al. 2015). This preference may explain why female toads attracted by the median call were slightly smaller than those attracted by the modified calls. Calling anurans also often prefer high energy and therefore high pulse rate calls (Wells & Taigen 1992). My results suggest that a high pulse rate call was a more effective lure for females than a call with median or low pulse rate; however, it was not as effective as a low frequency call (Fig. 4-2). There was no significant difference in mean nightly female captures between traps producing the combination call (low frequency and high pulse rate) and the low frequency call; however, a higher proportion of the females trapped using the combination call were gravid.

Previous, non-targeted management strategies for cane toads were apparently unsuccessful at long-term population size reduction (Peacock 2007; Tingley et al. 2017). A targeted management strategy that focuses on the removal of gravid females may be more successful at achieving this goal, at least locally (Lampo & de Leo 1998; Thresher 2007). The majority of females that were trapped during my experiments were gravid; however, the proportion of trapped females that were gravid varied among call types. Traps that produced the median call caught the lowest proportion of gravid females, while calls with extreme pulse rates or a low dominant frequency were more effective lures, suggesting these call types were more attractive to gravid females. The combination call caught the highest proportion of gravid females: 91.2% of females trapped using this call were gravid, whereas approximately 85% of females trapped using the next most attractive call (low frequency) were gravid. There was no significant difference in the numbers of gravid females trapped between these two calls; however, 28 more gravid females were trapped using the combination call than the low frequency call, over the same trapping period. Therefore, the trap that produced the combination call removed many more potential future offspring, possibly because this vocalisation represents a large male with a high energy call and therefore was more attractive to gravid females than any of the other calls. A trapping regime using a call with multiple attractive parameters may augment gravid female captures, which is predicted to be a successful population size reduction strategy for vertebrates (Lampo & de Leo 1998; Thresher 2007).

The most effective control strategy explored in my experiments was a combination call, because the proportion of gravid females captured was highest in this treatment. However, I only used a few variations of the median call, out of many possible combinations of frequency and pulse rate within the natural acoustic range of a cane toad vocalisation. Gravid female cane toads, like some other species of anuran (Klump & Gerhardt 1987), may be attracted to calls with parameters outside the natural range of the vocalisation (super-stimuli). Using multiple calls simultaneously, emulating a breeding chorus, increases the transmission distance of calls (Rehberg-Besler et al. 2016), and these strategies could also be explored as methods for increasing capture rates of gravid females. Combinations of several attractive calls played consecutively by a single lure may also increase gravid female captures, because a range of male toads, of varying sizes and fitness, could be represented by the various calls. In this case, individual female toads may have a higher chance of identifying an attractive mate. Finally, I based the vocalisations used in my experiments on the median call characteristics of the Townsville population, but there is geographical variation in the vocalisation parameters of cane toads (Yasumiba 2016). Therefore, a call attractive to females in Townsville may not be equally attractive to females in other areas.

My results show that small modifications to the acoustic attractant used in cane toad lures can strongly affect the number of gravid female toads caught, and therefore the number of new individuals entering the population. Large-scale eradication of cane toads from mainland Australia using traps is probably not possible; however, eradication of island populations could be achievable if the trapping regime was correctly designed and implemented. Future management strategies should explore further call modifications, based on the median call parameters of the population from which toads are being trapped, to achieve maximum success from acoustic adult cane toad traps.

Chapter 5: Geographic variation in calling of invasive cane toads (*Rhinella marina*): refining calls used as lures in traps

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5.1. ABSTRACT

Many anurans use advertisement calls to attract mates. Specific structural parameters of these vocalisations (e.g., dominant frequency and pulse rate) provide information about the body size, condition, and energetic capabilities of the caller, and females may select mates using this information. Often, advertisement calls differ among populations. In this case, a call attractive in one population may be less attractive in others. Cane toads (*Rhinella marina*) are highly invasive, vocalising anurans. In a single location, I demonstrated they can be successfully lured using an artificially manipulated advertisement call with a dominant frequency lower than the population median, and a pulse rate higher than the population median (around Townsville in the north Queensland tropics). In this study, I sampled calls from 4 cane toad populations across Australia (south east Queensland, north Queensland, Western Australia, and the Northern Territory), and constructed artificial vocalisations based on the median parameters of the sampled calls. I conducted trapping for 16-nights at each population, and compared capture success in traps playing one of 4 possible calls: the population's median call, a call that was lower in frequency and higher in pulse rate than the population median (local combination call), a Townsville median call, and a Townsville enhanced combination call. In all locations, the median Townsville call, and the specific median call for that location, were less attractive than the combination calls. In south east Queensland, Western Australia, and the Northern Territory, there was no significant difference in mean nightly female captures among traps producing the local combination call or Townsville combination call. In north Queensland, however, the local combination call was significantly more attractive to females than the Townsville combination call. Calls used as lures in traps should have unique parameters derived from vocalisations in the local area, to maximise captures of females.

5.2. INTRODUCTION

Anuran vocalisations attract mates (Wells & Schwartz 2007), and are, therefore, examples of sexually selected characters (Gerhardt & Huber 2002; Wells 2007). Vocalisation structural parameters, such as dominant frequency and pulse rate, are often correlated with aspects of male quality, and females select mates based on these parameters (Martin 1972; Wells 2007). For example, female anurans often prefer energetic calls with a high pulse rate (compared to the median value of the population), indicating that the calling male is investing a lot of energy in the vocalisation (Wells & Taigen 1992). Similarly, advertisement calls with low dominant frequency are indicative of large body size (compared to the median; Gerhardt 1991; Felton et al. 2006), and are preferred by females of some anurans (e.g., Ryan 1983; Gerhardt 1991). In some species, there is signaling variation among populations (Pröhl 2007; Klymus et al. 2012; Baraquet et al. 2014), such that median values of call parameters differ among populations. Variation among populations in vocal signals has been attributed to morphological, environmental, and genetic factors, including differences in body size and climate (Pröhl 2007; Klymus et al. 2012), as well as local female preferences (Yasumiba et al. 2016). When there is geographic variation, a call attractive to females in one population may not also be attractive in other populations.

Cane toads (*Rhinella marina*) are highly invasive, vocalisaing anurans. They are pests in tropical regions worldwide, including Australia, and are among the 100 worst invasive alien species (IUCN Global Invasive Species Database). In and around Townsville in northern Australia, female cane toads prefer calls with a lower frequency, and a higher pulse rate than the population median (henceforth the Townsville combination call; Chapter 3; Muller & Schwarzkopf 2017a). This call is likely representative of a large male toad investing substantial energy into the call, and is derived from calls recorded locally (Yasumiba et al. 2015). Even after controlling for body size and temperature, there is, however, signal variation among cane toad populations in Australia (Yasumiba et al. 2016); potentially driven by divergence in female choice, allocation of metabolic or energetic resources, or heterogeneity in environmental conditions (other than temperature) among sites (Yasumiba et al. 2016). In this case, an attractive call in the Townsville region may be less attractive in other populations, if the median dominant frequency or pulse rate of calls in these areas is different, or if females have different preferences for the magnitude of these parameters.

Management strategies for invasive cane toads have generally been unsuccessful (reviewed in Tingley et al. 2017). A successful management strategy should have a relatively high removal rate of gravid female toads, across all populations. Traps for adult cane toads include a lure, which plays an artificially manipulated male advertisement call, to target female toads (Chapter 4; Muller & Schwarzkopf 2017a). At present, lures play only the Townsville combination call, which is most attractive to gravid females in the Townsville region (Chapter 4; Muller & Schwarzkopf 2017a), but may not be as attractive in other populations. To avoid wasting trapping effort, the most attractive call for gravid females, tailored to local populations, should be used in lures.

I sampled male cane toad calls in four populations across northern Australia (south east Queensland (SEQ), north Queensland (NQ), the Northern Territory (NT), and Western Australia (WA)). I constructed unique calls for each population, the dominant frequency and pulse rate of which were derived from vocalisations from those populations. I trapped cane toads using these calls as lures, over 16-night trapping periods at each population, to determine which call was most attractive to gravid female toads, in each population.

5.3. METHODS

Study populations

I sampled calls, and trapped toads, around water bodies in Withcott in south-eastern Queensland (SEQ; 27°32'34.0"S 152°01'32.5"E), in Cairns in north-eastern Queensland (NQ; 16°50'45.6"S 145°41'40.6"E), near Palmerston in the Northern Territory (NT; 12°28'59.6"S 130°58'00.8"E), and near Kununurra in Western Australia (WA; 15°46'02.9"S 128°36'43.3"E; map of study populations in Appendix S3). I sampled calls and completed trapping regimes in SEQ from 7 December 2016 – 30 December 2016, NQ from 11 January 2017 – 29 January 2017, WA from 27 October 2017 – 14 November 2017, and NT from 12 November 2017 – 31 November 2017.

Recording of cane toad advertisement calls

I recorded cane toad calls using a Marantz PMD 661 compact digital audio recorder (D&M Professional, Itasca, USA), equipped with a NTG3 shotgun microphone (RØDE, Australia). I recorded consecutive advertisement calls from each male in .WAV sound format with 96 kHz sample rate and 24 bit-resolution with manual level adjustment. Immediately following each recording, I measured the body temperature of the calling individual, to an accuracy of 0.1 °C, using a digital non-contact infrared thermometer (QM-7221, DIGITECH, Australia), before I hand-captured it. I recorded mass to the nearest 1 g using a Pesola spring scale, and recorded snout urostyle length (SUL) to the nearest 1 mm, using a transparent plastic ruler. I then marked and released captured vocalising individuals, because removal of these individuals may have affected captures in the subsequent trapping regimes. I used a unique identification toe-clip code for each calling toad captured, removing the most distal phalanx from a predetermined series of digits (Schmidt & Schwarzkopf 2010). Tissue regrowth was minimal during my short surveys; therefore, toe clipping was a reliable means of identifying individuals (Luddecke & Amezquita 1999). I used single-use gloves and stainless-steel scissors sterilised using antiseptic (Bactine^{TM}) for toe clipping to minimise infection. The scissors were sterilised between the processing of each toad in the field, and were thoroughly cleaned and sterilised after use each night. I noted most of the previously recorded and marked toads calling during subsequent recording surveys, in locations similar to where they were captured; therefore, I concluded that marking these toads did not negatively effect calling behaviour.

Acoustic analysis

I used Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, USA) and Audacity 1.2.3 to measure dominant frequency and pulse rate of 90 recorded advertisement calls from SEQ (31 individuals; 2.9 ± 0.6 calls per individual), 95 calls from NQ (29 individuals; 3.3 ± 0.5 calls per individual), 82 calls from NT (32 individuals; 2.6 ± 0.3 calls per individual), and 102 calls from WA (31 individuals; 3.2 ± 0.9 calls per individual). I downsampled the sampling rate of each call from 96 kHz to 44.1 kHz using r8brain v1.9, to reduce frequency grid spacing from 93.8 Hz to 43.1 Hz in Raven Pro. I measured the dominant frequency of each call using Raven Pro's spectrogram function (1024 points fast-Fourier transform [FFT], overlap 75%, Hamming's sampling window with a frequency resolution of 56 Hz). I calculated the average pulse rate (number of pulses per second) of each call by dividing the number of pulses in the entire call by the call duration (in seconds). I calculated the median dominant frequency and pulse rate for each individual, and subsequently calculated the overall median dominant frequency and pulse rate for each study population.

The trapping unit

To catch toads, I used a trap consisting of a wire-mesh box (1 m x 1 m x 0.25 m), equipped with doors that opened easily with pressure from outside of the trap, but prevented egress of trapped toads. I placed an acoustic lure inside each trap, which played one of several possible cane toad advertisement calls (.WAV files) on repeat at night, and had a small LED 'black'

(UV) light that attracted insects. I modified the call parameters by changing the .WAV file played by the lure. More detail on the trap and methodology is available in Yeager et al. (2014), and in section 1.7 of this thesis.

Trapping sites

I chose trapping sites close to the same waterbodies at which I sampled cane toad vocalisations, in each of the four study populations across northern Australia. I chose four trapping sites at each study population, each approximately 10 m from the water's edge, and placed one trap at each trapping site. All trapping sites were located in open, grassy areas, and had similar ambient light ($\bar{x}$ = 0.045 ± 0.01 lx) and environmental and anthropogenic noise ($\bar{x}$ = 34.9 ± 0.7 dB) levels. I measured light and noise levels at each trapping site on 6 randomly selected nights, at 21:00 h, using a lux meter (ATP DT-1300, Leicestershire, UK) and a C-weighted Lutron sound level meter (model SL-4013, Taipei, Taiwan). Trapping sites were always more than 300 m apart, such that the advertisement call played by the lure at one site could not be heard by toads at any other site (Chapter 3; Muller at al. 2016).

Trapping regimes

I conducted a 16-day trapping regime, using four traps each time, at each study population across northern Australia. Each of the four lures produced a unique, artificially manipulated advertisement vocalisation (dominant frequency and pulse rate were manipulated using Audacity 1.2.3), and each lure had a call volume of 80 dB at 1 m (calibrated in a soundproof studio using using a C-weighted Lutron sound level meter [model SL-4013]). No other call parameters were manipulated. One vocalisation used in the lures was engineered so it had median values for call parameters in Townsville (TSV median; 600 Hz, 15 pulses s^{-1}), determined by sampling calls across several Townsville cane toad populations (Yasumiba et al. 2015). Another vocalisation had a dominant frequency approximately 17% lower than the TSV median, and a pulse rate approximately 20% higher than the TSV median (TSV combination; 500 Hz, 18 pulses s^{-1}); this combination of call parameters was most attractive to gravid female toads in Townsville in similar experiments (Chapter 4; Muller and Schwarzkopf 2017a). Another vocalisation had median values for call parameters at the study population where trapping occurred (location *X* median), and was determined by sampling calls in that population (see above). Another vocalisation had a dominant frequency approximately 17% lower than the study population median, and a pulse rate approximately 20% higher than the study population median (location *X* combination), to increase and decrease median parameter values by the same amounts as values attractive to Townsville toads. I used the TSV median and TSV combination calls at every study population, however the parameters of the other two calls used at each study population were unique to that population, determined by the sample of toad calls.

All four lures were activated nightly, such that all four calls played simultaneously on any given night, although too far apart to be heard at once by a single toad (see Chapter 3; Muller et al. 2016). Lures activated automatically at twilight (at approximately 18:00 – 19:00 h) and stopped the following morning at sunrise (lures were active for approximately 10 h per night). All lures started and stopped automatically at the same time each night. To avoid confounding trapping site effects and call characteristic effects in my measure of trap success, I rotated the four lures among sites daily, such that over 16 days every call was played at every site for four nights, and there was a different call playing at every site every night. A water bowl and PVC pipe for shelter were placed within each trap. I removed and counted trapped toads daily. Toads were euthanised immediately after their removal from the traps, using an overdose (350 ppm) of tricainemethanesulfonate (MS-222) *via* submersion in water containing a sodium bicarbonate buffered solution. I recorded mass of trapped toads to the nearest 1 g, and recorded snout − urostyle length (SUL) to the nearest 1 mm. I visually determined the sex of trapped toads (males have rough textured, light brown to yellow skin;

females have dark brown, comparatively smooth skin), and dissected female toads post-euthanasia to determine whether they were gravid. I repeated the process or trap placement and rotation, euthanised trapped toads, and dissected females, at every study site.

Statistical analysis

Calls

I determined if the mean dominant frequency of cane toad vocalisations varied significantly among my study populations, using a one-way ANOVA in combination with a Tukey's HSD post-hoc test. I repeated this analysis to determine if call pulse rate varied significantly among populations.

Trapping

Because I was interested in the effect of different calls within each population, and not the overall effect of these calls among populations, I performed identical, but separate, statistical analyses for each study population. I used a square-root transformation to normalise the distributions of numbers of trapped toads (Maindonald & Braun 2007), and identified if the mean number of females trapped varied significantly with call type, using a one-way ANOVA in combination with Tukey's HSD test for pairwise comparisons. I determined if the mean number of gravid females trapped varied significantly with call type, using a one-way ANOVA with Tukey’s HSD test. Finally, I examined whether body mass of trapped female toads varied significantly with call type, using a one-way ANOVA, in conjunction with a Tukey's HSD test for pairwise comparisons. I repeated this methodology to examine if the SUL of trapped females varied with call type.

5.4. RESULTS

Calls

Dominant frequency did not differ significantly among populations ($F_{3,\,119} = 1.93$, $P = 0.13$; Table 5-1), although the Queensland populations had slightly higher median dominant frequency than the Northern Territory and Western Australia populations. Pulse rate varied significantly among populations ($F_{3,\,119} = 45.6$, $P < 0.001$; Table 5-1); the mean pulse rate in SEQ was lower than all other populations ($P < 0.001$ for all comparisons).

Table 5-1. Mean (± SD) body sizes, body temperatures, and median call parameters of vocalising male cane toads across four study sites in northern Australia. Also shown are parameter values for the combination calls used for each population. The Townsville median call (dominant frequency; 600 Hz, pulse rate: 15 pulses s^{-1}) and a Townsville combination call (dominant frequency: 500 Hz, pulse rate: 18 pulses s^{-1}) were also used in these trapping regimes.

SEQ = south east Queensland; NQ = northern Queensland; NT = Northern Territory; WA = Western Australia

Variable	**SEQ (N = 31)**	**NQ (N = 29)**	**WA (N = 31)**	**NT (N = 32)**
SUL (mm)	96.4 ± 1.6	101.6 ± 1.8	118.1 ± 1.6	109.8 ± 1.5
Mass (g)	97.48 ± 1.9	104.4 ± 1.9	147.3 ± 5.1	134.9 ± 4.7
Body temperature (°C)	23.1 ± 0.5	28.4 ± 0.2	30.8 ± 1.2	29.4 ± 0.5
Median pulse rate (pulses s^{-1})	15.0	18.0	18.0	17.0
Median dominant frequency (Hz)	667.0	658.0	604.0	621.0
Combination pulse rate (pulses s^{-1})	18.0	21.0	21.0	20.0
Combination dominant frequency (Hz)	554.0	546.0	501.0	515.0

South East Queensland

The mean number of females trapped per night varied significantly with call type ($F_{3, 60}$ = 4.83, P = 0.004; Fig. 5-1; Table 5-2), as did the mean number of gravid females trapped per night ($F_{3, 60}$ = 4.38, P = 0.007). Traps that produced a SEQ combination call caught significantly more gravid females than the traps that produced a TSV median call (Tukey's HSD: P = 0.01), or a SEQ median call (Tukey's HSD: P = 0.03). However, there was no significant difference in mean nightly captures of gravid females between SEQ combination, and TSV combination calls (Tukey's HSD: P = 0.55). There was no significant difference in mean nightly captures of gravid females between any other calls (TSV combination vs. TSV median; Tukey's HSD: P = 0.24; TSV combination vs. SEQ median; Tuckey's HSD: P = 0.43, SEQ median vs. TSV median; Tukey's HSD: P = 0.98). There was no significant difference in the mass ($F_{3, 42}$ = 2.09, P = 0.09) or SUL ($F_{3, 42}$ = 1.51, P = 0.22) of trapped females among call types.

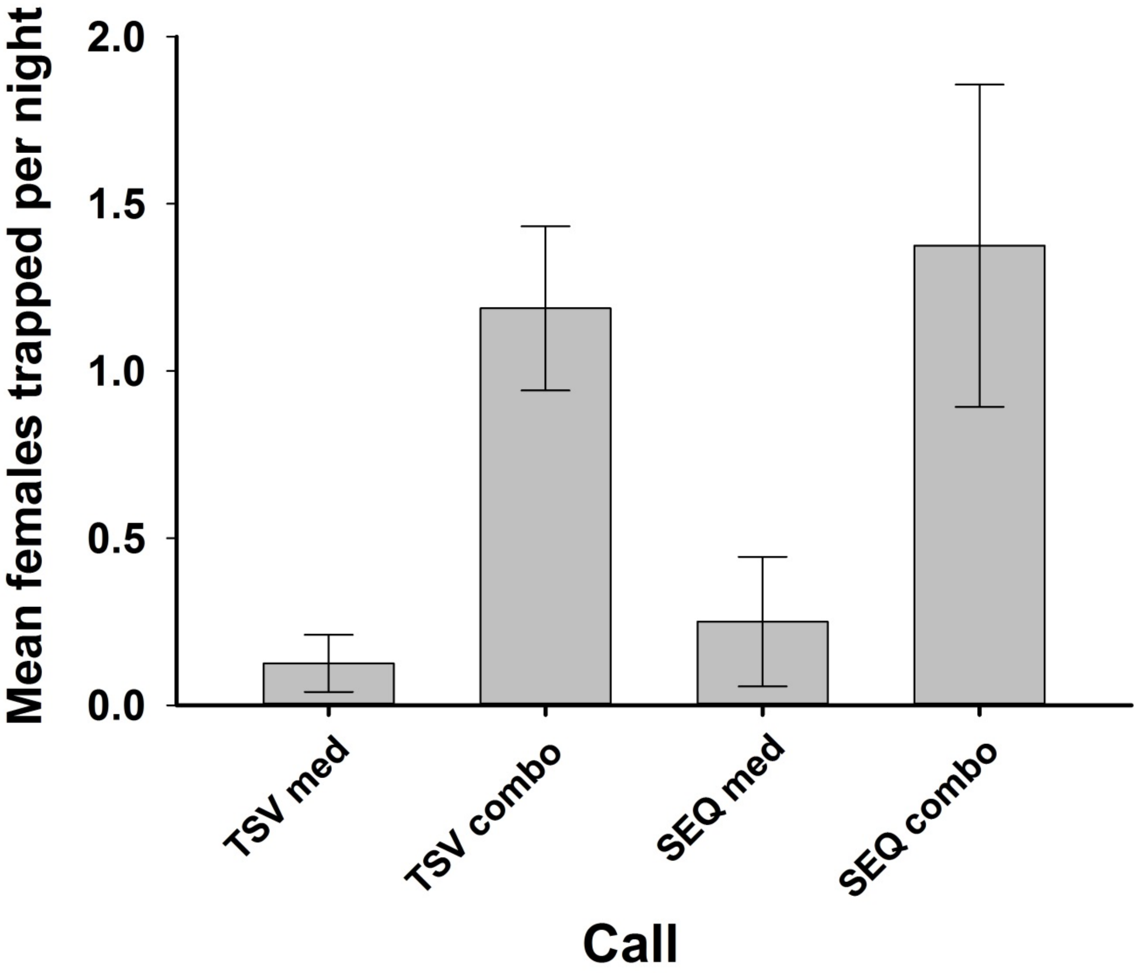

Figure 5-1. Comparison of mean nightly female cane toad captures in traps from 14 Dec to 30 Dec 2016, at several water bodies around Withcott, south east Queensland. Traps producing the SEQ combination call caught significantly more females than traps producing calls with median parameters (SEQ combo vs TSV med, $q = 4.31$, $P = 0.02$; SEQ combo vs SEQ med, $q = 3.87$, $P = 0.04$), however there was no significant difference in mean nightly captures of females between the TSV combination and the median calls (TSV combo vs TSV med, $q = 3.66$, $P = 0.06$; TSV combo vs SEQ med, $q = 3.23$, $P = 0.11$). There was no significant difference in mean nightly captures between the two combination calls (TSV combo vs. SEQ combo, $q = 0.65$, $P = 0.97$), or between the two median calls (TSV med vs. SEQ med, $q = 0.43$, $P = 0.99$).

Table 5-2. Number of cane toads caught in traps producing various calls as lures, across four study sites in northern Australia. ‘TSV median’ and ‘TSV combo.’ calls were used at each study site, while other calls were tailored to each population, based on the specific call parameters of toads within each population.

Median = calls with parameters (dominant frequency and pulse rate) set at the population median; Combo. = calls with low dominant frequency, and high pulse rate, based on each population’s median parameters.

TSV = Townsville; SEQ = south east Queensland; NQ = northern Queensland; NT = Northern Territory; WA = Western Australia

Population	**Call**	**Males**	**Females**	**Total**
SEQ	TSV median	14	2	16
	TSV combo.	25	19	44
	SEQ median	23	4	27
	SEQ combo.	30	22	52
NQ	TSV median	16	1	17
	TSV combo.	21	9	30
	NQ median	15	2	17
	NQ combo.	19	23	42
WA	TSV median	15	2	17
	TSV combo.	25	21	46
	WA median	23	3	26
	WA combo.	22	24	46
NT	TSV median	21	5	26
	TSV combo.	21	20	41
	NT median	25	3	28
	NT combo.	23	19	42

North Queensland

The mean number of females trapped per night varied significantly with call type ($F_{3,\,60}$ = 18.78, $P < 0.001$; Fig. 5-2; Table 5-2), as did the mean number of gravid females trapped per night ($F_{3,\,60}$ = 19.42, $P < 0.001$). Traps producing an NQ combination call caught significantly more gravid females than traps producing a TSV median call (Tukey's HSD: $P < 0.001$), an NQ median call (Tukey's HSD: $P < 0.001$), and a TSV combination call (Tukey's HSD: $P = 0.001$). There was no significant difference in mean nightly captures of gravid females between any other calls (TSV combination vs. TSV median; Tukey's HSD: $P = 0.051$; TSV combination vs. NQ median; Tuckey's HSD: $P = 0.051$, NQ median vs. TSV median; Tukey's HSD: $P = 0.99$). There was no significant difference in the mass ($F_{3,\,28}$ = 1.50, $P = 0.24$) or SUL ($F_{3,\,28}$ = 1.45, $P = 0.25$) of trapped females among call types.

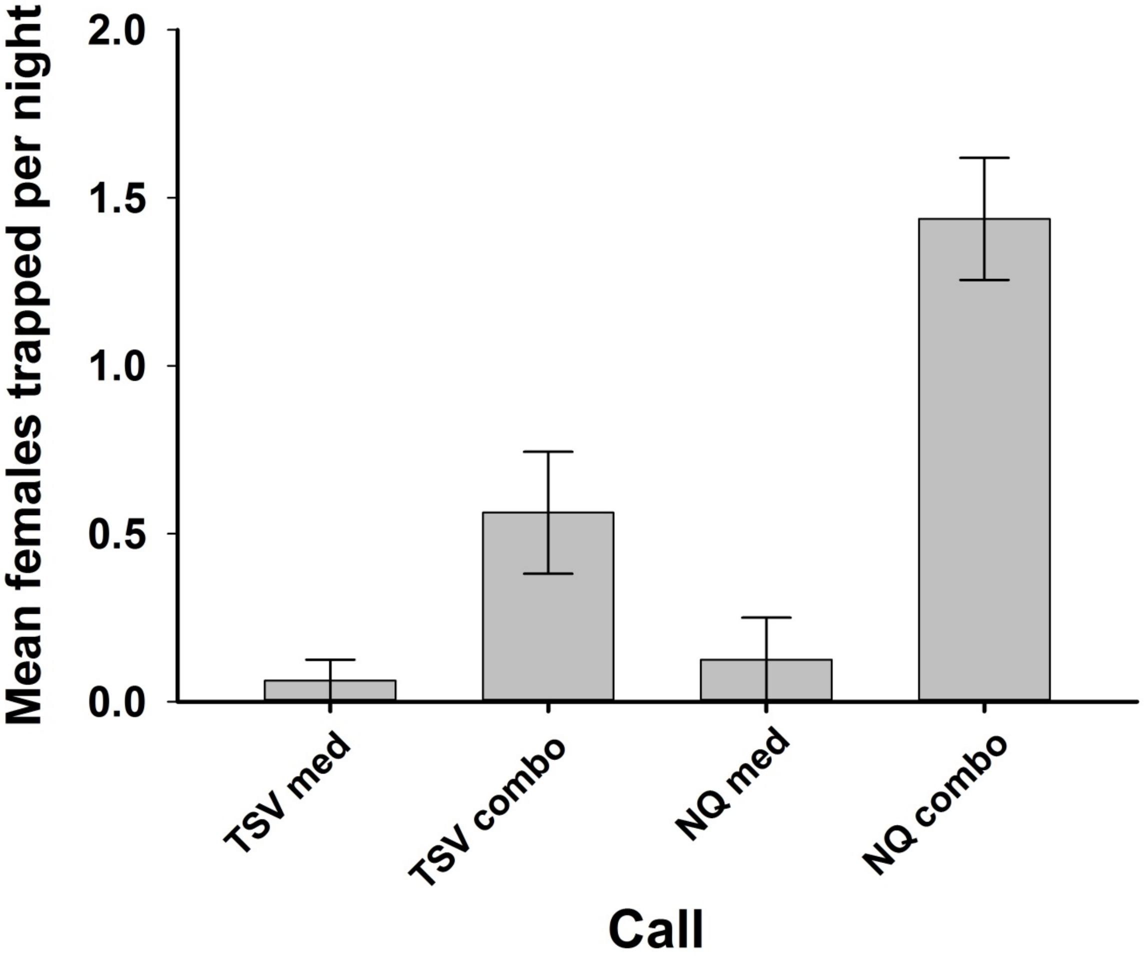

Figure 5-2. Comparison of mean nightly female cane toad captures in traps from 14 Jan to 30 Jan 2017, at several water bodies around Carins, north Queensland. Traps playing the NQ combination call caught significantly more females than traps playing any other call (NQ combo vs TSV med, q = 9.40, P < 0.01; NQ combo vs NQ med, q = 8.97, P < 0.01, NQ combo vs TSV combo, q = 5.98, P < 0.01). There was no significant difference in mean nightly captures of females between the TSV combination and the median calls (TSV combo vs TSV med, q = 3.42, P = 0.09; TSV combo vs NQ med, q = 2.99, P = 0.16). There was no significant difference in mean nightly captures between the two median calls (TSV med vs NQ med, q = 0.43, P = 0.99).

Western Australia

The mean number of females trapped per night varied significantly with call type ($F_{3,\,60}$ = 17.09, $P < 0.001$; Fig. 5-3; Table 5-2), as did the mean number of gravid females trapped per night ($F_{3,\,60}$ = 16.39, $P < 0.001$). Traps producing a WA combination call caught significantly more gravid females per night than traps producing a TSV median call (Tukey's HSD: $P <$ 0.001), and a WA median call (Tukey's HSD: $P < 0.001$), however there was no significant difference in mean nightly gravid female captures between a WA combination call and a TSV combination call (Tukey's HSD: $P = 0.26$). Traps producing a TSV combination call caught significantly more gravid females per night than traps producing a WA median call (Tukey's HSD: $P = 0.002$), or a TSV median call (Tukey's HSD: $P = 0.002$). There was no significant difference in mean gravid female captures per night between the TSV median and WA median calls (Tukey's HSD: $P = 0.99$). There was no significant difference in the mass ($F_{3,\,46}$ = 1.30, $P = 0.29$) or SUL ($F_{3,\,46}$ = 0.93, $P = 0.43$) of trapped females among call types.

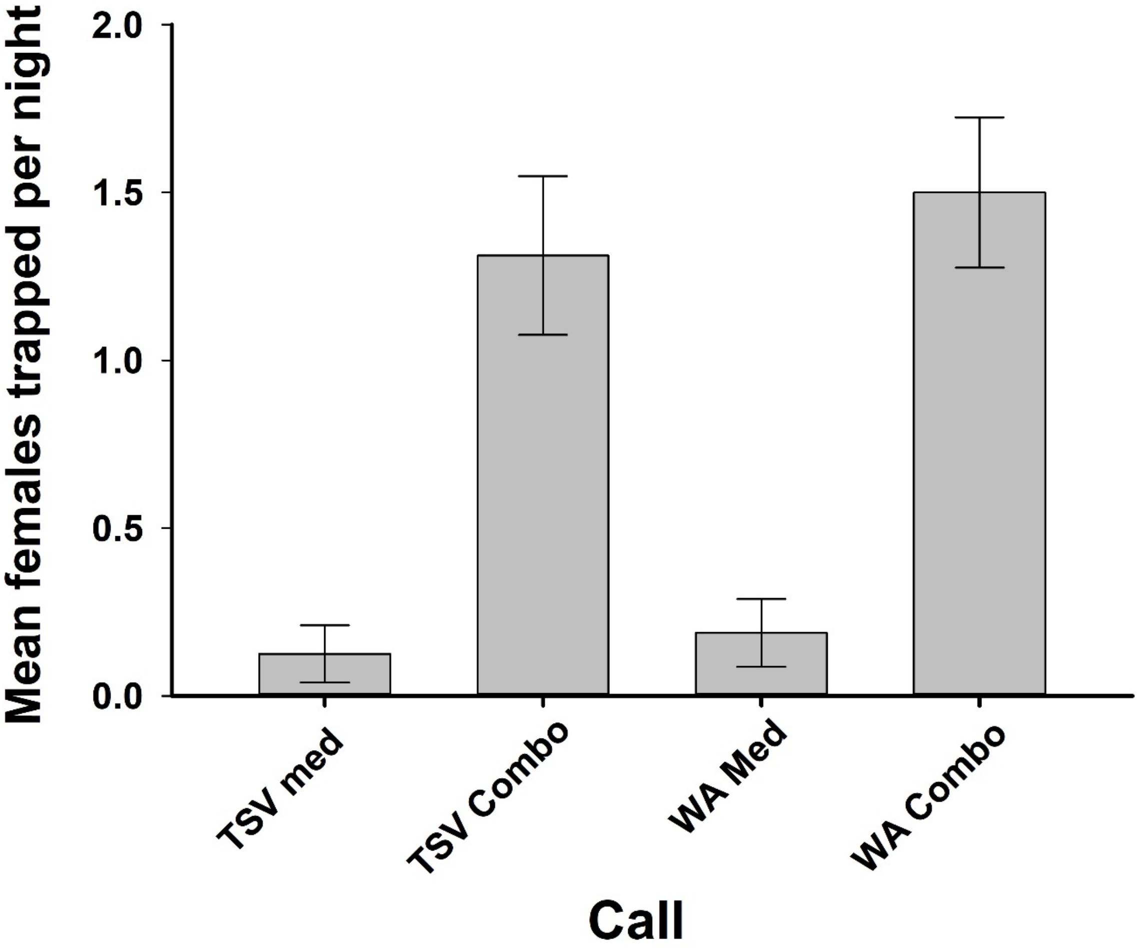

Figure 5-3. Comparison of mean nightly female cane toad captures in traps from 30 Oct to 14 Nov 2017, at several water bodies around Kununurra, Western Australia. Traps producing the WA combination call caught significantly more females than traps producing median calls (WA combo vs TSV med, *P* < 0.001; WA combo vs WA med, *P* < 0.001). There was no significant difference in mean nightly captures of females between the WA combination and the TSV combination call (WA combo vs TSV combo, *P* = 0.87). Traps producing the TSV combination call caught significantly more females than traps producing median calls (TSV combo vs TSV med, *P* < 0.001; TSV combo vs WA med, *P* < 0.001). There was no significant difference in mean nightly captures between the two median calls (TSV med vs WA med, *P* = 0.99).

Northern Territory

The mean number of females trapped per night varied significantly with call type ($F_{3,\,60}$ = 7.01, $P < 0.001$; Fig. 5-4; Table 5-2), as did the mean number of gravid females trapped per night ($F_{3,\,60}$ = 7.22, $P < 0.001$). Traps producing an NT combination call caught significantly more gravid females per night than traps producing a TSV median call (Tukey’s HSD: $P = 0.009$), and an NT median call (Tukey’s HSD: $P = 0.004$), however there was no significant difference in mean nightly gravid female captures between an NT combination call and a TSV combination call (Tukey’s HSD: $P = 0.99$). Traps producing a TSV combination call caught significantly more gravid females per night than traps producing an NT median call (Tukey’s HSD: $P = 0.009$), or a TSV median call (Tukey’s HSD: $P = 0.017$). There was no significant difference in mean gravid female captures per night between the TSV median and NT median calls (Tukey’s HSD: $P = 0.99$). There was no significant difference in the mass ($F_{3,\,44}$ = 0.22, $P = 0.88$) or SUL ($F_{3,\,44}$ = 0.05, $P = 0.99$) of trapped females among call types.

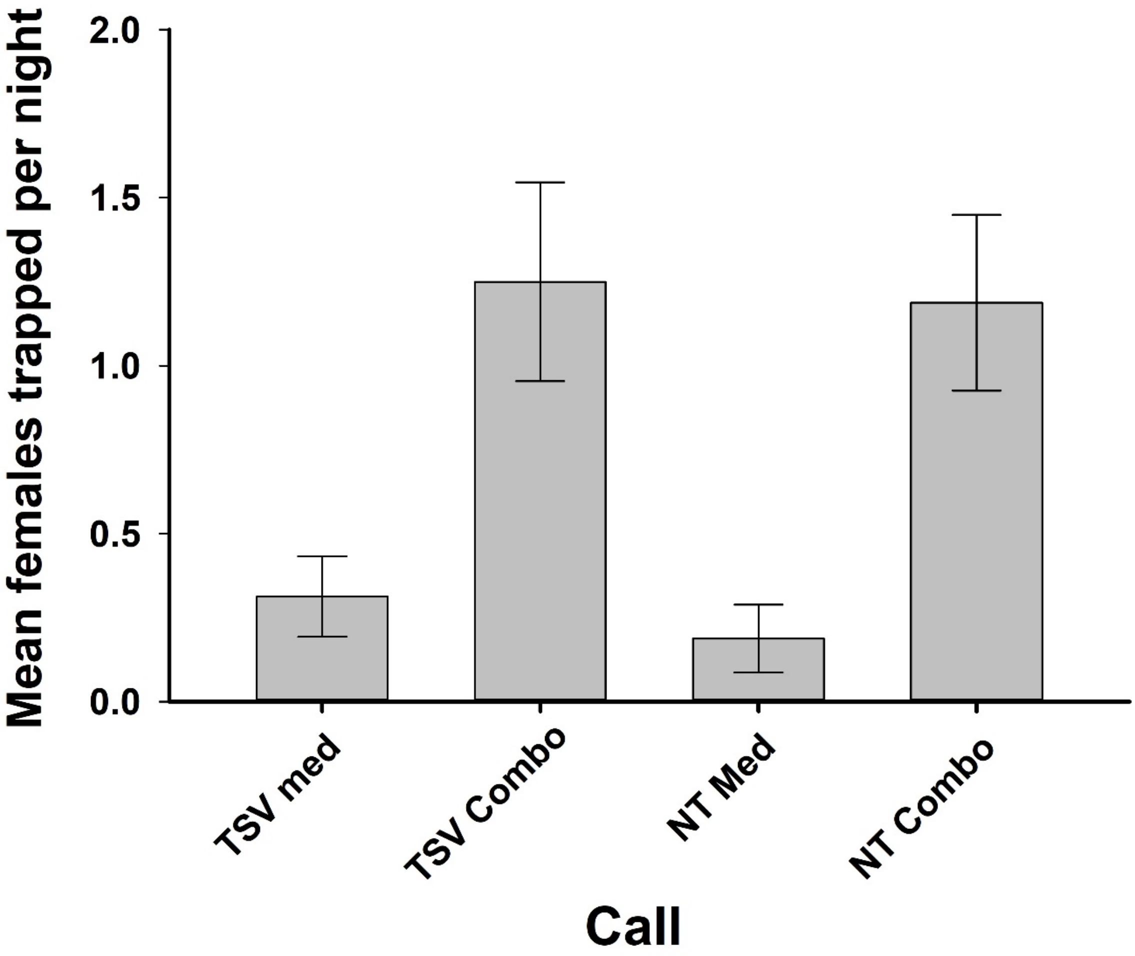

Figure 5-4. Comparison of mean nightly female cane toad captures in traps from 16 Nov to 31 Nov 2017, at several water bodies around Palmerston, Northern Territory. Traps producing the NT combination call caught significantly more females than traps producing median calls (NT combo vs TSV med, *P* = 0.025; NT combo vs NT med, *P* = 0.007). There was no significant difference in mean nightly captures of females between the NT combination and the TSV combination call (NT combo vs TSV combo, *P* = 0.99). Traps producing the TSV combination call caught significantly more females than traps producing median calls (TSV combo vs TSV med, *P* = 0.014; TSV combo vs NT med, *P* = 0.004). There was no significant difference in mean nightly captures between the two median calls (TSV med vs NT med, *P* = 0.98).

5.5. DISCUSSION

There was variation in the pulse rates of cane toad vocalisations among populations across northern Australia, however the median dominant frequency of advertisement calls did not vary significantly among populations, when calls were not adjusted for body size or temperature. Female toads in all populations selected strongly for calls with a lower-than-median frequency, and a higher-than-median pulse rate (i.e., for combination calls). In south east Queensland, Northern Territory, and Western Australian populations, there was no significant difference in mean nightly female captures between traps producing the Townsville combination call, and traps producing the specific combination call for that population. Conversely, females in Cairns selected strongly for the north Queensland combination call, over the Townsville combination call. Traps that produced calls with median parameters caught significantly fewer females in every population, regardless of location.

There is geographic variation in cane toad call parameters across northern Australia, however females did not appear to discriminate between a unique combination call derived from the population from which they were trapped, and a Townsville combination call, in most populations. The dominant frequencies of all combination calls were relatively similar, and the difference between calls may have been insufficient to elicit differing responses from females. However, similar to patterns in Townsville (Chapter 4; Muller & Schwarzkopf 2017a), females selected against calls with median parameters, and instead preferred calls representative of a larger-than-median male toad investing considerable energy in the call. This preference was apparent in all populations, and may indicate that there is strong sexual selection for particular call parameters, regardless of the location or age of the population.

Female captures were highest in traps producing combination calls, and lowest in traps producing median calls. In most populations, female captures were not significantly different between the tailored combination call for that population, and the Townsville combination call. The dominant frequency of the Townsville combination call was similar to the dominant frequencies of the Western Australia and Northern Territory combination calls (Table 5-1). In these populations, combination calls were equally attractive, while the corresponding median calls were equally unattractive. In south east Queensland, the pulse rates of the tailored combination call and Townsville combination call were the same (18 pulses s^{-1}; Table 5-1), and both calls were attractive, while median calls with lower pulse rates were unattractive. However, slightly fewer females were trapped using the Townsville combination call than the south east Queensland combination call (Fig. 5-1). This may be because the dominant frequency of the Townsville combination call was 25% (167 Hz) lower than the population median, and may fall outside the natural range of calls in this population. In this case, some females may have been deterred, given the apparently extremely large body size indicated by this call (female toads apparently prefer males slightly smaller than themselves; Yasumiba et al. 2015). In north Queensland, the Townsville combination call was significantly less attractive than the north Queensland combination call, probably because the median pulse rate in this population was the same as the pulse rate of the Townsville combination call. Further, the dominant frequency of the Townsville combination call was 24% (158 Hz) lower than the population median, and, as with south east Queensland, may have fallen outside the natural range of cane toad calls in this population (Table 5-1). These results may indicate that any call with a sufficiently high pulse rate, or a sufficiently low frequency, or both, will be more attractive to females than calls with parameters closer to median values, as long as the values of these parameters fall within the natural range of calls in the target population.

Call pulse rate varied significantly among populations. The median pulse rate of calls in the SEQ population was substantially lower than that of the northern populations. The pulse rate of anuran calls is correlated with body temperature in many species (Castellano et al. 1999), including cane toads (Yasumiba 2015). Nightly temperatures were substantially lower at the SEQ site than at the three northern sites, which probably caused the lower pulse rate in SEQ. Dominant frequency did not vary significantly among populations in my study, however; some variation was evident (Table 5-1). Yasumiba et al. (2016) reported variation in dominant frequency among cane toad populations in Australia, and suggested that dominant frequency is strongly selected within populations. It is important to note that Yasumiba et al. (2016) adjusted call parameters for body size and temperature before comparisons, which I did not. Unadjusted, the NT and WA combination calls had similar dominant frequencies to the Townsville combination call, but higher pulse rates. In this case, I expect females to prefer higher pulse rate calls (Wells & Taigen 1992; Muller & Schwarzkopf 2017a). However, there was no significant difference in the mean nightly female captures between traps producing the Townsville combination call, and traps producing the unique combination calls in these populations, even though the pulse rate of the Townsville call was considerably lower. This may indicate that dominant frequency is more important to female toads than pulse rate, within the natural range of a toad calls. I did not examine female preferences for call duration, pulse length, or inter-pulse interval during this study, but doing so may be worthwhile because these parameters also vary among populations (Yasumiba et al. 2016).

Patterns of call variation identified during this study were different from those uncovered in a previous study (Yasumiba et al. 2016), for the same, or similar, populations. This is likely due to their adjustment of call parameters to accommodate body size and temperature. I was interested in determining the effect of call parameters on capture rates, specifically relative to absolute values of these parameters in the population at the time of measurement. Further studies should adjust the population values I obtained for body size and temperature to compare more directly with previous studies.

Using the Townsville combination call in all cane toad traps in northern Australia was fairly effective, but may be a less-than-optimal management strategy in the medium- to long-term. There was no statistically significant difference in female captures among the Townsville combination call and tailored combination calls in south east Queensland and Western Australia, although slightly fewer females were trapped using the Townsville combination call than the tailored combination call in these populations (Figs. 5-1 & 5-3). However, in Cairns in northern Queensland, traps producing the Townsville combination call caught significantly fewer females than traps producing the north Queensland combination call. In addition, my trapping regimes were relatively short (16 nights), and occurred during the wet season when toads are most active (Chapter 2; Muller at al. 2018). Longer trapping regimes in drier periods using these calls may more clearly elucidate female preferences, and may identify if female toads are more, or less, selective about approaching specific calls when resources are limited. Further, longer trapping regimes, with larger sample sizes, may illuminate subtle differences in the attractiveness of calls. Finally, body size and condition, and ambient temperature, drastically effect the parameters of a cane toad vocalisation (Yasumiba et al. 2016), and may change temporally. In the long term, ongoing call analysis may be useful to determine if male vocalisations change in specific populations, and if it is efficacious to alter the call used as a lure accordingly. Without this information, I recommend a management strategy that includes utilising specialised attraction calls based modified from the call parameters of the population in which trapping occurs, specifically by lowering the frequency and increasing the pulse rate.

Chapter 6. Relative effectiveness of trapping and hand-capture for controlling invasive cane toads (*Rhinella marina*)

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6.1. ABSTRACT

Management of invasive vertebrates is a crucial component of conservation. Management strategies should increase the chance of removal of every individual, by exploiting behavioural characteristics, and by increasing the period over which removal occurs. For example, traps can operate automatically over long periods, and often include attractants to increase captures. Management strategies for the invasive cane toad (*Rhinella marina*) in Australia include hand-capture, and trapping adult individuals (toads are attracted to an acoustic lure, and to insects attracted to a light, also on the lure). I used capture-mark-recapture analysis to compare the efficacy of trapping, and hand capturing cane toads, over 10 weeks, in Townsville, Australia. I trapped 7.1% - 22.4% of the estimated population per week, and hand-captured 1.7% - 6% of the estimated population per week. Trapping was more efficient than hand-capture in my regime; overall, more toads were caught per trapping person-hour than per hand-capture hour. Traps attract toads and maximise the period over which removal occurs, thus the probability of removal for each toad was higher than by hand-capture. Because hand-capture and trapping seemed to remove different toads, a combination of these methods may work well.

6.2. INTRODUCTION

Invasive vertebrates cause negative environmental, social, and economic impacts worldwide (Pimentel et al. 2005), and are a leading cause of species decline and extinction (Clavero & Garca-Berthou 2005). Management strategies are diverse, and their success is extremely variable, depending on the population size and biological characteristics of the target species, the scale over which removal occurs, and the cost and effort associated with implementing the regime (Eiswerth & Johnson 2002). Effective management strategies should maximise the probability of removing individuals, by exploiting behavioural characteristics (Bravener & McLaughlin 2013), increasing the intensity of removal, targeting a specific demographic if possible (Hastings et al. 2006), and maximising the period over which removal occurs. Trapping, for example, is one of the most successful control strategies for pest species (Nogales et al. 2004; El-Sayed et al. 2006), because traps operate for long periods, and generally use attractants that vastly improve removal rates (Alam & Hasanuzzaman 2016).

The cane toad (*Rhinella marina*) is a highly invasive anuran. Its range extends through tropical areas worldwide, including Australia (Lever 2001). Cane toad parotoid glands secrete powerful bufotoxins that are poisonous to native predators (e.g., Bowcock et al. 2009; Shine 2010), and domestic pets (Reeves 2004). Cane toads may also reduce the nocturnal activity of native anurans (Greenlees et al. 2007), and may contribute to biodiversity loss within their invaded range (Shine 2010). Managers have implemented several control methods for cane toads (Tingley et al. 2017), with minimal success on a large scale; biological and genetic control methods are limited, and, in their current form, have been unsuccessful (e.g., Tingley et al. 2017). ‘Toad-busting’ events, in which members of the community remove toads by hand, are also common; however, these events have apparently not initiated population decline (Peacock 2007). Trapping adult toads removes multiple individuals and may be less labour intensive than other control methods, especially hand-capture; however, the relative efficiency of trapping versus hand-capture has not been quantified.

Cane toad management strategies should augment the probability of removal of each toad in the population, by exploiting behavioural characteristics, and maximising the period over which removal can occur. A trap for adults includes a lure, which produces a cane toad advertisement vocalisation, and an LED ultra-violet light, to which insects are attracted, and from which toads are not repelled (Schwarzkopf and Alford 2007; Schwarzkopf and Forbes 2010; Davis et al. 2015). The advertisement call attracts both sexes to the area surrounding the trap, and toads enter the wire trap to get closer to the call, or to consume the insects attracted by the light. The combination of light and sound doubles the capture probability of females, triples juvenile captures, and increases male captures by 25% compared to the vocalisation alone (Yeager et al. 2014). The solar-powered lure operates automatically at night, therefore trapping can occur nightly, over an extended period, with relatively low effort. Most trap hours do not require participants to be on site, other than initial trap placement, and removal of trapped toads. Hand-capture, especially large scale ‘toad-busting’ events, requires extensive, consistent community involvement, and is extremely labour intensive, where every search hour requires participants to be on site (e.g., Peacock 2007). Cane toad traps may be more efficient than hand-capture for toad removal, but this remains to be tested.

I estimated the proportion of the cane toad population I trapped, and hand-captured, in the same area, over a ten-week period, using population estimates obtained *via* capture-mark-recapture analysis. I also estimated labour, for both methods, and compared the number of toads captured per person-hour, to compare the efficiency of both methods.

6.3. MATERIALS AND METHODS

The Trap

The trapping unit consisted of a wire trap, acoustic lure, and solar panel to power the lure. The wire trap was a cage 1 m x 1 m x 0.25 m, with mesh sides, with trap doors on three of the four sides. The trap doors consisted of a series of narrow (2 cm wide), transparent plastic strips hinged at the top, which open easily with pressure from outside of the trap. A small ledge at the bottom of each door prevented the fingers from being opened from within the trap, preventing egress of trapped toads.

I placed one acoustic lure in each wire trap. At night, the lure activated an LED light, and repeatedly played a cane toad advertisement call (reproduced in high quality .WAV format and manipulated using Audacity 1.2.3) through the speaker. The duration of each call was 8 seconds, followed by a 2-second pause, before repeating, creating an infinite loop. The volume of the call was 80 dB at 1 m, it had a pulse rate of 15 pulses s^{-1} and a frequency of 500 Hz. A call with these parameters is equally attractive to male and female toads in the study area; other call types attract more males than females (Muller & Schwarzkopf 2017a). A solar panel charged the lure's batteries during the day; the lure automatically activated when charge from the solar panel stopped (that is, once the sun retreated), and de-activated when it received charge the following morning. Full details about the trap are available in section 1.7 of this thesis.

Capture-mark-recapture regime

The capture-mark-recapture regime occurred in a freshwater creek bed, adjacent to James Cook University, in Townsville, Australia (19°19'47.74"S, 146°45'29.55"E), in the dry season from 20 July 2016 to 28 September 2016. The creek bed traversed a woodland habitat, comprised mainly of popular gum (*Eucalyptus platyphylla*), with an understory of black spear grass (*Heteropogon contortus*). The creek bed had two small bodies of freshwater (located approximately 290 m apart), that I used as trapping sites, and as focal points for hand-capture active searches. Traps were operational for approximately 11 - 12 hours each night, for 70 consecutive nights.

I placed one trap at each site, approximately 10 m from the water's edge. I checked the traps daily, visually determined sex (males have rough-textured, light brown to yellow skin; females have dark brown, comparatively smooth skin), marked and released trapped toads, and recorded recaptures. I released all toads after capture, regardless of their capture record. I also performed active searches for toads, which commenced 30 minutes after sunset, every second night over the period of the trapping regime (n = 35 nights). There were two circular active search sites, the boundaries of which were 120 m from the traps, with the trap at the centre of each site (area for each site = 45,238.9 m^2). Toads are attracted to a call from up to 120 m (Chapter 3; Muller et al. 2016); therefore, the toads that were available for hand-capture in an active search area were also likely to hear the acoustic lure in the trap. I disabled lures before and during the active searches, such that no toads were artificially encouraged into the search sites by the call produced by the lure. Six observers searched both sites simultaneously for 30 minutes (three observers searched each site; 90 minutes effective search time per site), and placed all hand-captured toads in a 30 l bucket. A search time of 30 minutes was sufficient for three observers to thoroughly search each site, based on several pilot studies at the same sites prior to the trial commencing. Cane toads are uniformly active after dark, until first light, and apparently do not have peak activity periods in terms of movement (Schwarzkopf and Alford 2002). Therefore, the likelihood of encountering toads should not have changed had I searched later at night. After the active searches, I visually sexed, identified, marked, and released all toads at the exact location from which the observers removed them. I reactivated each lure after the active searches were complete (i.e., approximately 1 hour after sunset).

I used a unique identification code for each toad captured during the capture-mark-recapture regime, derived from toe-clipping; a process where the most distal phalanx is removed from a digit (Phillott et al. 2007). Tissue regrowth was minimal during my frequent surveys; therefore, toe clipping was a reliable means of identifying individuals (Luddecke and Amezquita 1999). I used single-use gloves and stainless steel scissors sterilised using antiseptic (Bactine^{TM}) for toe clipping to minimise infection. The scissors were sterilised between the processing of each toad in the field, and were thoroughly cleaned and sterilised after use each night.

Statistical analysis

I combined capture-mark-recapture data from trapping and hand-capture for analysis. I divided the study into trapping periods of 7 trap nights, and 3 – 4 active search nights each (one week of capture-mark-recapture). There were 10 trapping periods in the study. An individual was ‘captured’ in a trapping period if it was caught at least once during that period, by either capture method. I estimated the total number of individuals to ever inhabit the area (during my study), as well as determining effective abundances for each trapping period, using an open population Cormack-Jolly-Seber model (Cormack 1964; Jolly 1965; Seber 1965), in which both capture probability and survival were time-dependant. I estimated abundances for each trapping period based on the cumulative number of marked individuals re-captured in all previous trapping periods. I assumed that: (1) marked individuals did not lose their marks, nor were the marks misidentified; (2) every individual present in the study population had the same chance of capture, whether it was marked or unmarked; (3) every marked individual had the same probability of surviving from one trapping period to the next; and (4) sampling periods were instantaneous relative to the interval between capture sessions.

I determined if there was a significant difference in the number of individuals caught by each capture method, using a two-tailed *t* test, and examined if there was a significant difference in the sex ratio of captured toads, using two-tailed *t* tests for each capture method. I also determined if any differences in captures between the two methods were influenced by minimum temperature, or rainfall (data available at http://www.bom.gov.au/climate/data/stations/), by comparing conditions on nights when active searches occurred, to nights with no active searches, using a two tailed *t* test for each variable. Minimum temperature and rainfall strongly influence toad activity in the dry season (Chapter 2; Muller et al. 2018).

Adult toads are typically nocturnal, and both lures began calling at sunset, and ceased calling at sunrise, every night. I calculated the total number of hours for which traps were operational, for each week during the trapping regime, by determining night length (available at https://www.timeanddate.com/sun/australia/townsville). I quantified trapping labour, by calculating the number of person-hours used per week to set up and maintain traps, remove toads, and travel between sites, and calculated the mean number of toads trapped per trapping person-hour, for each week of the regime. I also calculated the total number of person-hours used per week for hand-capture, including search time, toad disposal, and travel between sites, and calculated the mean number of toads trapped per hand-capture hour, for each week of the regime. I calculated mean person-hourly captures using only individuals that had not been caught previously (i.e., new captures), to replicate a regime where trapped or hand-captured individuals, or both, were removed from the population. Finally, I determined whether there was a significant difference in the mean number of new individuals caught per person-hour, between the two removal methods, using a Mann-Whitney *U* test. I used R (R core Team V.3.1.2) for all statistical analysis, and the package RMark (Laake 2013) for capture-mark-recapture analysis.

6.4. RESULTS

I had 615 captures of 363 different individuals, using both capture techniques. I was likely sampling from the same population, because 40.2% of individuals caught more than once were encountered at both sites. I therefore combined data for both sites to estimate overall population size. The model estimated an effective population size of 533 ± 32.4 individuals. I estimated effective captures for each method by eliminating recaptures, and including only the first capture event for each individual (i.e., my estimates assumed a realistic control regime, in which toads were removed from the population after capture, rather than released). I trapped 49.3% ± 3.1% (263 individuals) of the estimated population over the entire capture-mark-recapture regime, and hand-captured 18.8% ± 1.2% (100 individuals) of the estimated population, over the same period. Given these estimates were calculated using an open population model, which allowed for immigration, emigration, births, and deaths, it is likely that the effective population size is applicable over an unknown, larger area, rather than an exact measure of the population within the boundaries of the study site. Traps caught significantly more toads, over the entirety of the trial (t = 3.92, df = 9.47, P = 0.003, Table 6-1). The number of toads caught per week was extremely variable, but traps caught more toads than hand-capture in every trapping period (Fig. 6-1). There was no significant difference in the sex ratio of trapped (t = 0.68, df = 17.38, P = 0.51), or hand-captured toads (t = -0.28, df = 16.6, P = 0.79). There was no significant difference in weather conditions between nights on which I searched actively and nights when active searches did not occur (minimum temperature; t = 0.45, df = 69.9, P = 0.65; rainfall; t = -0.007, df = 65.2, P = 0.99).

Table 6-1. Estimated population size for 10 weeks, and approximate proportion of that population trapped, and hand-captured, and standard errors, based on population estimates.

Week	Estimated abundance	Proportion trapped	Proportion hand-captured
1	-	-	-
2	419.8 ± 94.9	22.4% ± 4.4%	4.8% ± 0.4%
3	465.4 ± 38.9	12.7% ± 1.6%	1.7% ± 0.2%
4	465.4 ± 38.9	15.3% ± 1.2%	1.9% ± 0.2%
5	325.5 ± 29.1	15.1% ± 4.9%	3.7% ± 0.3%
6	325.5 ± 29.1	8.6% ± 0.7%	4.6% ± 0.4%
7	325.5 ± 29.1	7.1% ± 0.6%	5.8% ± 0.5%
8	218.6 ± 37.1	11% ± 1.6%	5.0% ± 0.7%
9	232.7 ± 39.1	13.8% ± 2.0%	6.0% ± 0.8%
10	-	-	-

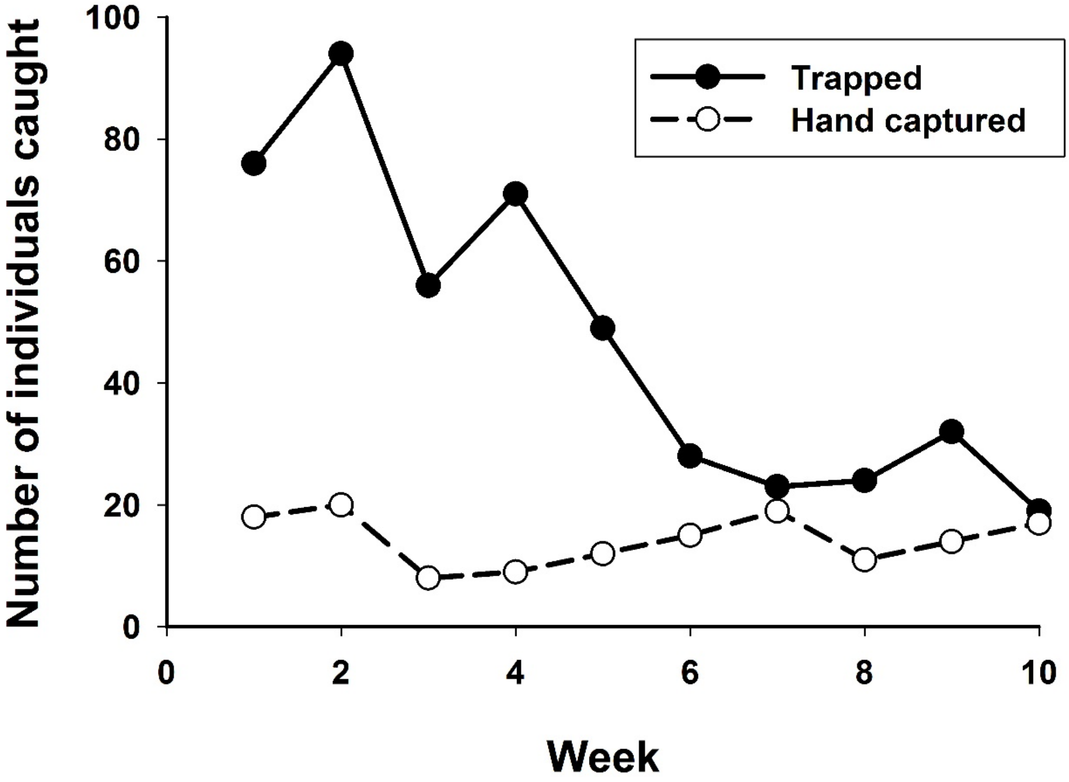

Figure 6-1. The total number of individual toads caught per week, using trapping and hand-capture, over the course of the capture-mark-recapture regime that occurred at the James Cook University, Townsville, between 20 July 2016 and 28 September 2016.

Hand-capture was more labour intensive than trapping in my regime; I spent 140 person-hours actively searching for toads (including active search, toad removal, and travel time) over the course of the regime, and 72 person-hours checking, maintaining and travelling between traps, over the same period (938 total trap hours). Significantly more toads were caught per trapping person-hour than per hand-capture hour (Mann-Whitney *U* test, $W = 9$, $P = 0.002$, Table 6-2), over the course of the regime. Overall, an average of 1.05 toads were caught per hand-capture hour, while an average of 3.98 toads were trapped per trapping person-hour.

Approximately 92.8% of the toads caught in traps were not caught during active searches, while 69% of the toads caught by hand were not trapped. Overall, 55.1% of individuals were only captured once over the entire trapping regime, while 28.4% were captured twice, and 9.9% were captured 3 times. There were no individuals caught in more than 5 trapping periods. The number of new individuals trapped (i.e., individuals that were not caught previously) decreased sharply after the first 4 weeks of the regime (Fig. 6-2A), as did the number of individuals that were hand-captured (Fig. 6-2B). The majority of toads caught by both methods after week 5 of the regime were recaptures (Fig. 6-2A, B).

Table 6-2. Mean number of toads caught per trap hour, per trap person-hour, and per hand-capture hour, for each week during the trapping regime, where trapping person-hours include time spent travelling to, maintaining, and removing toads from traps. Table shows mean hourly captures of toads that were not caught previously, that is, capture numbers are applicable to a regime where trapped/hand-caught individuals are removed from the population.

Week	Mean toads trapped per trap hour	Mean toads trapped per person-hour	Mean toads caught per hand-capture hour
1	0.77	9.5	1.5
2	0.84	11.71	0.94
3	0.51	6.86	0.25
4	0.43	5.86	0.25
5	0.18	2.43	0.13
6	0.06	0.86	0.17
7	0.04	0.57	0.08
8	0.03	0.43	0.19
9	0.09	1.14	0.17
10	0.03	0.43	0.06

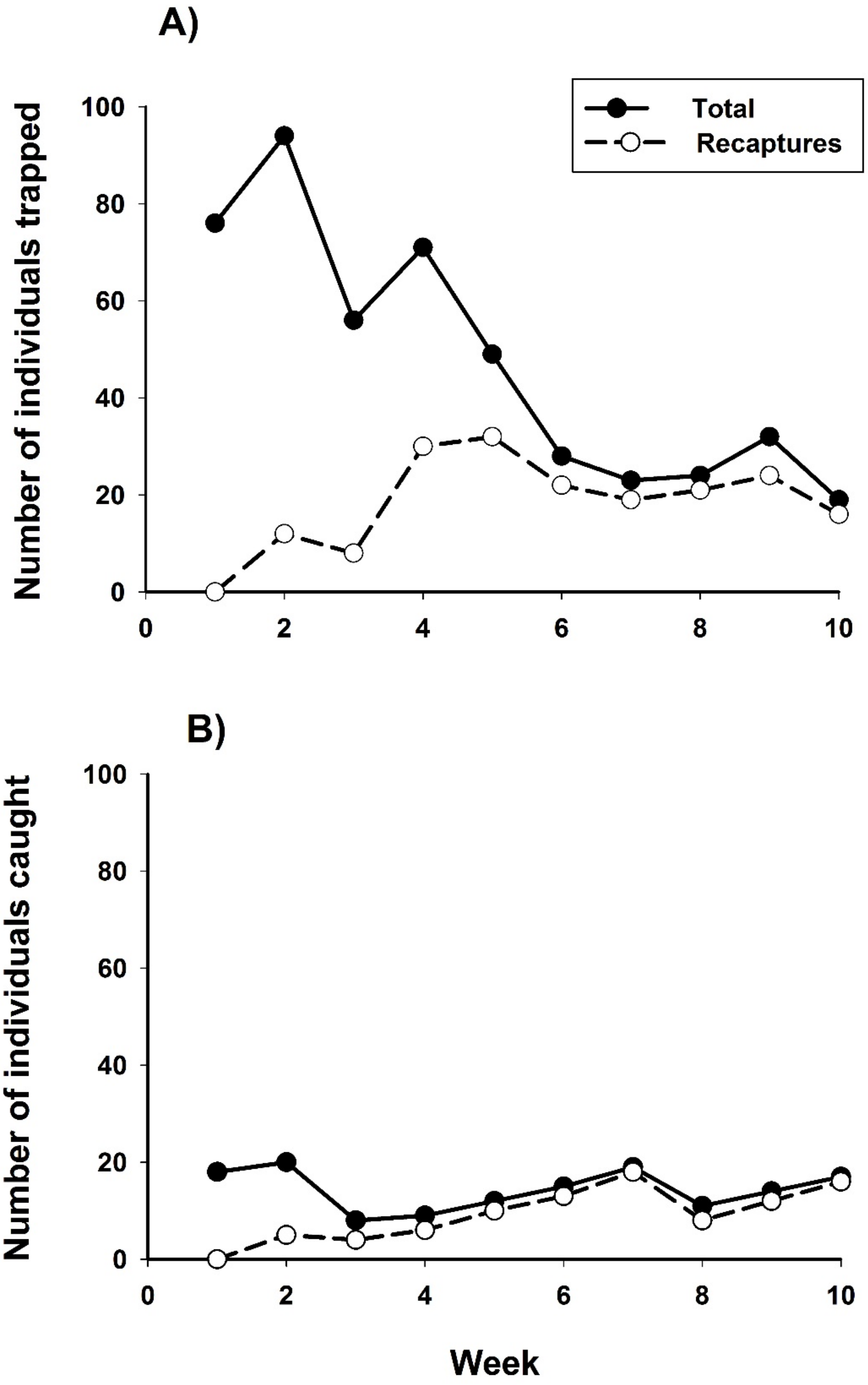

Figure 6-2. The total number of individuals, and the number of recaptures, caught per week, using trapping (A) and hand-capture (B), over the course of the capture-mark-recapture regime that occurred at the James Cook University, Townsville, between 20 July 2016 and 28 September 2016.

6.5. DISCUSSION

I caught more toads using traps than using hand-capture over a similar area, therefore traps are at least potentially a viable method for cane toad removal on a small scale. Traps would have removed approximately 49.3% of the population over the duration of the capture-mark-recapture regime, whereas approximately 18.8% of the population would have been removed over the same area in the same period using hand-capture for 1.5 person-hours every second day. The disparity in captures between methods was not due to weather conditions, probably because active searches occurred frequently and sampled more-or-less the same weather conditions as did trapping. Both sexes had a roughly equal chance of capture using either method. I caught more toads per trapping person-hour than per hand-capture person-hour, however, I caught more toads per hour by hand than with traps, if total trap hours are considered (Table 6-2). Thus, a high intensity toad-busting event, conducted over a large area and involving many people and search-hours, may be more effective than a trapping regime, but such an event will require significantly higher effort expenditure (both more hours and people) than my active searches.

Trapping adult toads was more effective than hand-capture in my regime, because the traps were operational all night, every night, for the entire study period, while only 3 person-hours every second night were devoted to hand-capture. Overall, toads were much more likely to encounter a trap than be hand-captured, because there was a total of approximately 938 trap hours, but only 105 hand-capture person-hours (not including travel and processing time). More toads were caught per hour by hand than with traps, however, trapping was considerably more efficient, because traps operate automatically (other than time spent setting them and removing toads from traps), therefore more toads were caught per person-hour when trapping than when capturing toads by hand (Table 6-2). Even if active searches had occurred nightly, the number of hand-captures would still have been less than the number of toads trapped when person-hours are compared (assuming a linear increase in toad captures with search time, as occurred in my regime). Further, the lures in the traps may have attracted toads from locations that were inaccessible by hand, such as burrows (Seebacher & Alford 1999; Schwarzkopf & Alford 2002); such that the removal of these toads without traps was unlikely.

Traps were also more labour efficient than hand-capture; 100% of the hand-capture hours were person-hours, such that the operators had to be physically on-site to remove toads, while 6.68% (72 hours) of the trap hours were person-hours, including daily processing and travel time (approximately 7 hours per week), and installation and removal of traps (approximately 2 hours). To remove as many toads by hand as I did with traps, I would have had to spend approximately 531 hours (53.1 hours per week) performing hand-capture, assuming the relationship between search time and number of captures remained approximately constant, as it was in my regime. My study was conducted in the dry season, and the chance of encountering toads during hand-capture events may be significantly higher in the wet season, because toads are more active than during dry periods (Schwarzkopf & Alford 2002; Brown et al. 2011; Yasumiba et al. 2016; Muller et al. 2018). In this case, trapping effectiveness should also increase, but traps have a maximum capacity of approximately 30 toads (B. Muller; pers. obs.), whereas in hand-capture events it is possible to remove more than 30 toads in a single night when population density is high, although even hand-capture is limited by handling time. Setting more traps at times when toads are abundant should increase captures with a relatively small increase in labour required, although increasing the number of traps used in a regime will also increase set-up and maintenance costs. On the other hand, every increased hour of hand-capture is an hour of extra labour. Implementing large-scale hand collecting (toad-busting) events during the wet season may be more economically feasible, because, if the labour is free, collecting occurs over a larger area than the area reached by traps, unless a very large number of traps were set. Trapping is probably a specialised activity for which volunteers may be less appropriate than hand-capture.

Cane toads are nomadic, and can move long distances quickly if the environmental conditions are appropriate (e.g., Schwarzkopf and Alford 2002). I replicated a realistic, intensive, ‘toad-busting’ hand-capture event (Boulter et al. 2006), in which searchers moved through the area systematically, rather than remaining in the same location for an extended period. In this case, toads moving through the area have a lower probability of being captured, because the active search event was also moving (i.e., if a toad moved through the area before or after the active search event, it wasn’t caught). In my study, the majority of toads (55.1%) were only caught once, possibly because they were transient, and were only in the area for a short time. In this case, transient toads only had a small chance of removal as they passed through the area, and were more likely to encounter a trap than be hand-caught. In general, hand-capture events move in sweeps through areas, removing the toads present at the time people are present, whereas traps have a lengthier presence in a single area, removing toads that move towards them.

In my trapping regime, the number of toads trapped decreased markedly over time (Fig. 6-1). Toads are long lived, so mortality probably didn't cause the decline in captures I observed in traps. The decline may have occurred because: i) toads became trap-shy (e.g. McGregor and Moseby 2014; Mali et al. 2012; Weggie et al. 2004), or ii) toads gradually left the area, but were not immediately replaced by immigrating toads. The number of toads trapped decreased after the first 4 weeks, while the number of hand-captured toads was low but remained relatively stable, in comparison (Fig. 6-1 and 6-2B). The overall decline in numbers of toads captured in traps, but not by hand, appears to support the hypothesis that many toads became trap-shy after capture, but remained in the area. The number of individuals that had not been trapped previously also decreased, however, as did the hand-captures of those individuals. Furthermore, if toads left the area, one would expect the captures of recaptured toads to also decrease, however the number of recaptures was generally stable for both methods after the fourth week of the regime (Fig. 6-2). The overall decrease in the number of toads trapped was probably caused by trap-shyness, coupled with a decrease in activity caused by seasonal changes in temperature and rainfall (Seebacher and Alford 1999). Of course, normal trapping regimes do not release toads, so trap shyness is not a concern when trapping for removal.

The activity of toads in my study population was probably typical of, if not less than, other populations across Australia, because my study occurred at a time of year when toad activity was low (the dry season: Schwarzkopf and Alford 2002; Chapter 2; Muller et al. 2018), in a location far from the invasion front, where toads are least likely to move long distances (Alford et al. 2009). My capture rate (i.e. the number of toads caught per unit area, per person, within a specified period) should, therefore, be comparable to other regimes across Australia, because the effectiveness of these removal methods is dependent on toad activity. Indeed, the hand-capture rate of cane toads in my regime was somewhat comparable to other studies, if the search area of those studies is re-scaled to the area of my active searches (e.g. Somaweera and Shine 2012). The total number of hand-captures reported from previous toad-busting events (Somaweera and Shine 2012) is much higher than in my study, because these events were larger and involved many more participants, and therefore more labour, than my study. Of course, changes to my hand-capture regime (e.g., longer, more frequent active searches, undertaken by more people, over a larger area) may have augmented the total number of individuals caught, however implementing any of these changes would also result in a great increase in labour.

The number of hours spent trapping and capturing toads by hand in my particular study was circumscribed by the nature of my study. I placed the traps close together (290 m apart), and there were only two, and my hand-capture regime was designed to cover more-or-less the same area as that ‘covered’ by the sound of the trap. The fact that I had only two traps, and that they were close together, meant that the time spent setting and checking traps was limited. Similarly, the area searched for toads was circumscribed by the small area of attraction of these two traps, and the (short) time required to thoroughly search that area and remove all the toads. Also, I sampled for a set period of weeks in the dry season, and used one population of toads. All these parameters could influence my comparison of the capture success of the two methods. It is important to note, however, that the time for which traps can remove toads is always many more hours than the hours required to check the traps, and therefore, if both methods catch toads, the labour required per toad will always be less with traps.

In my regime, 69% of toads caught by hand were not trapped, and the majority of toads that were trapped were not encountered during active searches (92.8%). This may indicate that that some toads may be bolder, or more likely to enter a trap, than others (e.g., Carter et al. 2012), or, some toads may not be attracted to traps, and thus hand-capture is their only chance of removal. Therefore, future regimes could include trapping, in combination with hand-capture events, to maximise toad captures, because the use of only one method may limit the number of toads that are available for removal. Labour would obviously be high for hand-capture events, however the efficiency of these events could be improved by only searching when toads are most active, in areas where population density is high. Furthermore, examining personality traits of toads in relation to trap capture could refine current and future control methods, given cane toads exhibit a range of personality traits within their invaded range (Gonzalez-Bernal et al. 2014). Ultimately, eradication of cane toads on mainland Australia, using only trapping or hand-capture, is impossible, given the current population size, and rate of expansion (Phillips et al. 2006; Dall 2011). Even so, a well-designed management strategy may suppress populations on islands, or suppress population size in areas in which toads are already established.

CHAPTER 7. DISCUSSION

For many invasive vertebrates, trapping is an effective control method at small-to-medium geographic scales (Burbidge & Morris 2002; Howald 2007; Zuberogoitia et al. 2010; Algar et al. 2013). Trap success has been improved by using and understanding the behaviour of the target species to increase captures per unit effort, for example by targeting specific demographics by customising the trap, or the lure used to attract individuals. Trapping cane toads in Australia using lures that produce a cane toad advertisement call may be a feasible method for population suppression. Over the course of several long-term trapping sessions during my research for my thesis, I demonstrated that cane toad traps can consistently remove a substantial number of toads, across various locations within their invaded range. Further, I demonstrated that gravid female toads can be targeted by altering the vocalisations used as lures, and I have identified the at least one process that could be used to manufacture attractive calls for female toads across Australia. I have also identified the conditions under which toads are most active (and therefore available to be trapped), estimated a method for trap placement that maximises captures and simultaneously minimises trap use, and calculated the weekly effort (in person-hours) required to undertake trapping, in comparison to hand-capture.

The overall aim of my thesis was to quantify cane toad acoustic communication, activity, and behaviour, and use these results to refine cane toad trapping methodology. My data chapters each address a separate component of this aim, and together provide a comprehensive examination of cane toad behaviour, within the context of trapping adults. I presented my second chapter as a statistical methods paper (Chapter 2; Muller et al. 2018); demonstrating high variability in cane toad activity, and trap captures, caused by variation in environmental conditions. Although variation in activity driven by small increases or decreases of a particular environmental variable at a particular time of year is interesting from an ecological and statistical viewpoint, land managers operating cane toad traps should draw broader conclusions from these results. For example, mean nightly cane toad captures were 4 times higher in the wet season (December – February) than the dry season (June – August). Trapping during wet periods may yield more captures, however the net impact of these captures may not be as high as during dry periods; toads move further when it is wet (Schwarzkopf & Alford 2002), therefore individuals captured in the wet season may be immediately replaced by immigrating individuals. In my third chapter (Chapter 3; Muller et al. 2016), I demonstrated that calls from lures attract males from further than females (males respond from up to 120 m, while females respond from up to 70 m). Successful management strategies should target females, therefore land managers should place traps approximately 140 m apart, to maximise the probability of attracting females without leaving spatial ‘sound gaps’ between traps.

To refine the trap to target gravid female toads, I conducted several trapping programs using different calls as lures. In my fourth chapter (Chapter 4; Muller & Schwarzkopf 2017a), I identified that females preferred ‘combination’ calls with a relatively low frequency and high pulse rate, compared to the population median in Townsville. This preference was also apparent in 4 other populations across northern Australia; females in these populations also preferred combination calls, and in some cases preferred calls with parameters altered relative to the median dominant frequency and pulse rate within that population (Chapter 5). In my experiments, there was variation in call parameters among populations, and variation in female responses to particular calls among populations. For example, the Townsville combination call was significantly less attractive to gravid females in the Cairns north Queensland population (NQ) than was the NQ combination call. Land managers should consider performing acoustic analysis within target populations, prior to trapping, to determine the call parameters that are most attractive in that population. Finally, in my sixth chapter (Chapter 6; Muller & Schwarzkopf 2017b), I determined a given amount of effort invested in trapping yielded a capture rate 4 times higher than the same amount effort invested in hand-capture, over the same target area. The most interesting result in that chapter was that traps caught toads that were not encountered during hand-capture events, and vice versa. In future management strategies, hand-capture events should be used to complement trapping regimes, to increase the chance of removal of each toad in the population, or effort should be invested to determine and mitigate the factors causing some toads to avoid traps.

7.2. FUTURE DIRECTIONS

The focus of my thesis revolved primarily around increasing cane toad captures by refining the lure, rather than refining the wire cage trap. The trap is about as effective can be expected; the doors are as wide as possible to allow maximum opportunity for entry, without causing interference with other doors inside the trap when open, and without compromising the structural integrity of the trap. Further, the trap is large enough to accommodate approximately 30 toads, but small enough to be easily transported and erected in the field by a single person. However, trap shyness or avoidance is common in trapping regimes for many invasive species (e.g., Reed et al. 2011), and may also occur in cane toads (Chapter 6; Muller and Schwarzkopf 2017b). The sides of the trap act as a barrier to the lure, so toads may approach the trap, but be unwilling (or unable) to enter it (B. Muller pers. obs.). There is no estimate for the number of toads that approached the trapping unit, but did not enter it, however I expect that at least some of the population are attracted to the lure but are not removed, due to trap avoidance. Trapping in a small (7.6 m diameter) arena, with a known number of toads suggested as much as 50% of individuals may be trap shy, although such measures were not intended to estimate this. The solution may be to remove the wire trap completely, and incorporate an automatic method of killing animals when they approach the lure (without bycatch). This design would allow toads to approach the lure, without the trap acting as a physical barrier. This technology would rely upon a system that can differentiate toads from native animals such as frogs, and a method of euthanasia for toads, with no adverse effects on the surrounding habitat. Research to develop such a system is continuing.

A primary advantage of the cane toad lure is that the call it plays is easily changed. To date, I have used only variations of a cane toad call, however any sound can be played by the lure, as long as the sound file is in .WAV format. Therefore, this technology has a wide scope for targeting and attracting various vocalising anurans. Indeed, preliminary data indicates that introduced red toads (*Schismaderma carens)*, in South Africa, and bullfrogs (*Rana catesbeiana*) in the U.S., are attracted to the lure when their respective advertisement calls are played. Further, acoustic lures could be effective for trapping any species that vocalises, or hunts using auditory cues from prey (e.g., feral cats in Australia; Fisher et al. 2015). Obviously, the trap itself may require customisation, depending on the target species. For example, whereas bullfrogs and red toads are large, coqui (*Eleutherodactylus coqui*) are very small, and would fit through gaps in the wire of the current cane toad cage trap. The obvious implementation of this technology is to attract and remove invasive species, however it could also be used as a tool for sentry systems, fauna surveys, or capture-mark-recapture studies.

Finally, further research about the ecology and behaviour of cane toads within their invaded range is imperative to design and refine new management strategies. Understanding boldness in toads could aid in producing traps that reduce trap- and lure-shyness, while exploiting learned preferences for food sources, or breeding habitat, could provide other options to lure toads. Further examination of mating behaviour is also required, specifically, understanding the conditions required for breeding choruses to form. Studies examining chorus formation have found only weak influences of physical parameters of the environment (e.g., surface area or depth of the waterbody, presence of aquatic vegetation, proximity to surrounding waterbodies), and weather conditions (air and water temperature, atmospheric moisture, wind speed), on breeding in toads. Understanding the factors promoting chorus formation should allow management strategies targeted at reducing the attractiveness of potential chorusing and breeding sites.

7.3. CONCLUSIONS

The cane toad trap is probably the most efficient and cost-effective removal method currently available. However, complete eradication of cane toads from mainland Australia, using only traps, is impossible, due to the abundance of toads on the mainland, and their nomadic movement habits (trapping opportunity is limited if toads move through a management area quickly; Schwarzkopf & Alford 2002). Cane toad traps should be most effective on offshore islands, with few water bodies, and to which immigration of new individuals is low. It may be possible to eradicate toad populations on these islands using intensive trapping regimes and hand-capture events, and by tightly controlling immigration of new individuals. Further, traps could be used on islands with no cane toads; for example, traps could be strategically placed near ports and airports to capture any newly introduced toads that escape from quarantined areas or procedures. In this case, trapping should be especially efficient because the probability of a toad encountering a trap may be much higher than an active search encountering that toad (see chapter 6; Muller & Schwarzkopf 2017b). Traps may also be useful for controlling small isolated cane toad populations on the mainland, where immigration is also low. In this case, the lure should encourage toads to enter and remain in the immediate area surrounding the trap, even if some do not enter the trap itself. This ‘concentration’ of toads in the trapping area will make other methods of control (e.g. hand capture) more efficient by reducing the area over which they occur, and increasing the chances of encountering toads within the trapping area.

Although eradication of toads from the Australian mainland is currently impossible, future control methods may be more effective. Genetic or biological control methods may have a higher chance of instigating large-scale population decline, as they have in other species (e.g. Saunders et al. 2010), especially if a large proportion of the population is infected or genetically altered. The use of trapping, and hand-capture, in combination, could maximise the initial number of toads that can be infected and re-released, and greatly increase the efficiency of biological or genetic control methods.

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APPENDIX S1: CHAPTER 2. Muller BJ, Cade BS & Schwarzkopf L. 2018. *Ecosphere*.

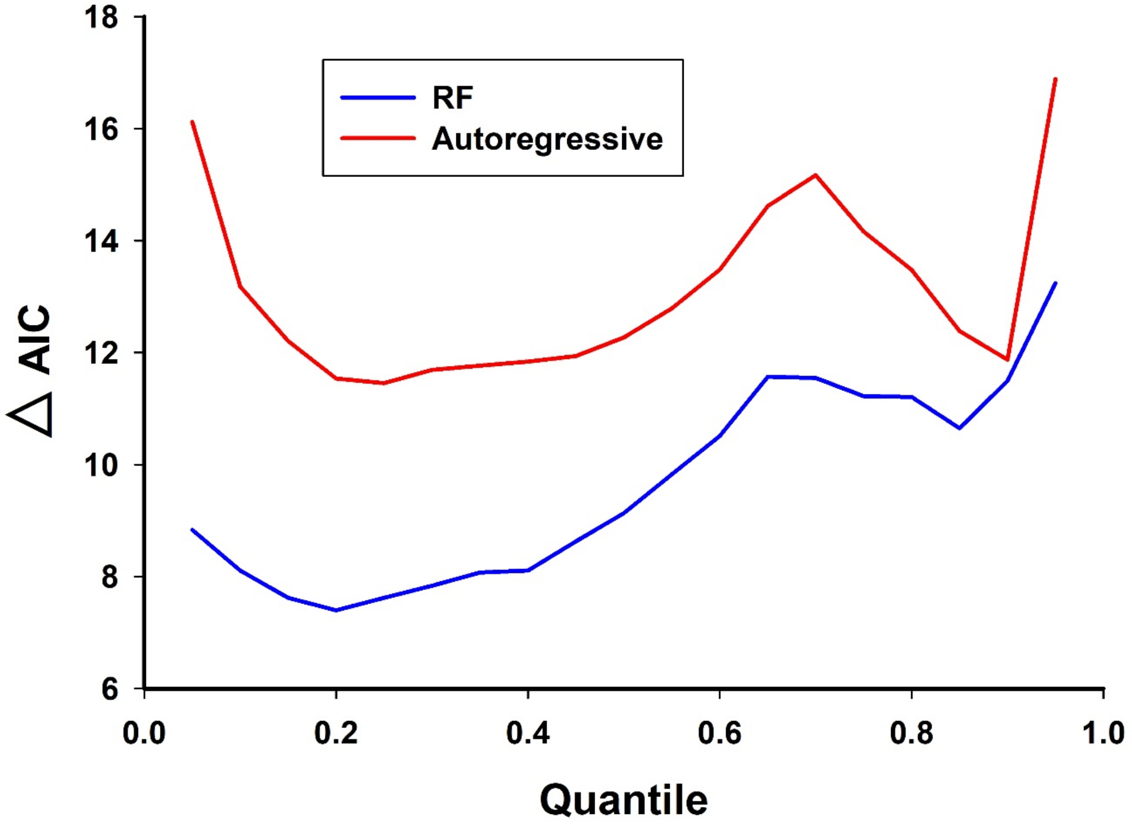

Figure S1. Change in average ΔAICs of the strongest environmental predictor model (rainfall), and a model including rainfall and a 1st-order temporal autocorrelation effect in the dry season (June – August 2013), on Orpheus Island, across $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$, for $m = 500$ replications of $z = y + U[0, 1)$. The model that included an autocorrelation effect was better supported, especially at lower quantiles.

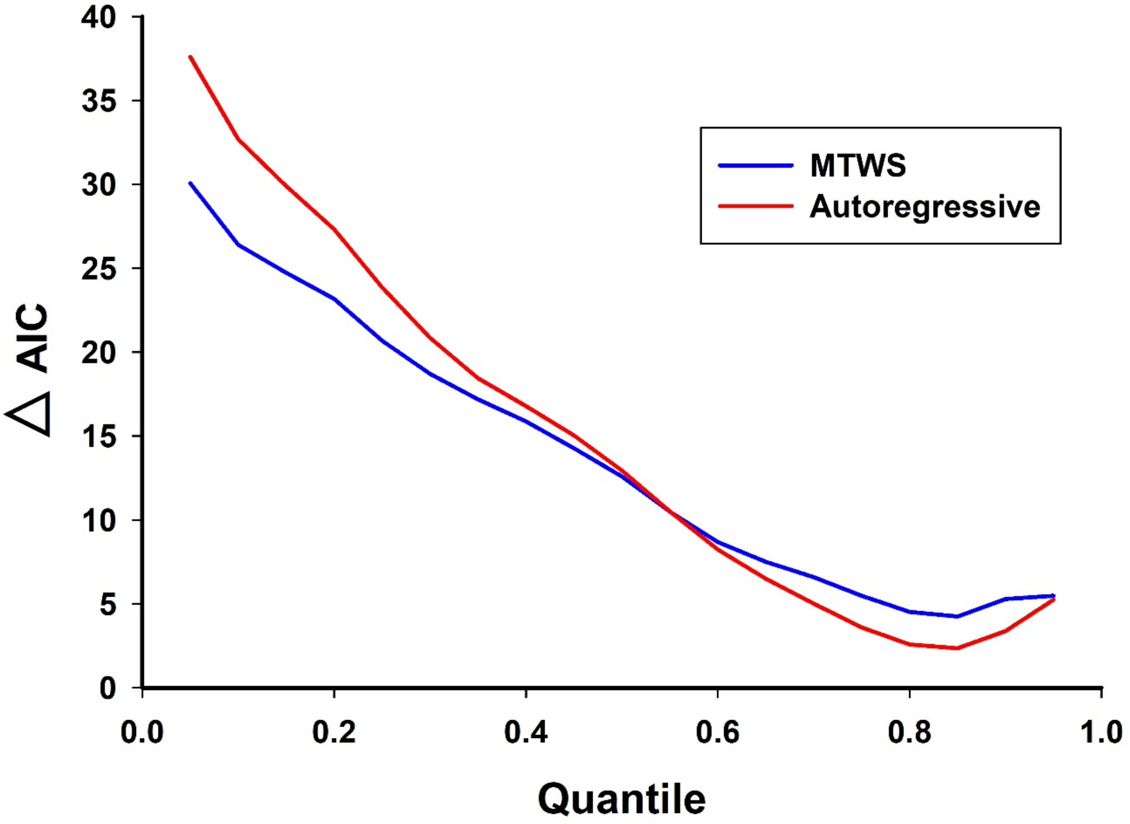

Figure S2. Change in average ΔAICs of the strongest environmental predictor model (minimum temperature and wind speed), and a model including minimum temperature, wind speed, and a 1^{st}-order temporal autocorrelation effect in the pre-wet season (September – November 2013), on Orpheus Island, across $\tau \in \{0.05, 0.10, 0.15, \ldots, 0.95\}$, for $m = 500$ replications of $z = y + U[0, 1)$. The model that included an autocorrelation effect was better supported at lower quantiles, and was generally within 2ΔAIC units of the environmental predictor model at higher quantiles.

APPENDIX S2: CHAPTER 2. Muller BJ, Cade BS & Schwarzkopf L. 2018. *Ecosphere*.

```
###This code is for the wet season data set only
###AIC model selection of quantile count models.
###Note that by using a null model with just an intercept as the reference
model to compare AIC for candidate models with predictor variables, the
subtraction for computing delta AIC values implies that models with higher
delta AIC are better supported.

library(quantreg)

###set up for tau = 0.05, 0.10, ... 0.95.

###First order temporal autoregressive
###To get Date and Time column in date/time format Lag 1 dates and toads
done in Excel.  Lag toads and previous date for first record taken from
last record of pre Wet Season data file.

WetSeason$date <- as.character(WetSeason$Date)
WetSeason$date <- strptime(WetSeason$date,"%d/%m/%Y")

WetSeason$lagdate <- as.character(WetSeason$lagdate)
WetSeason$lagdate <- strptime(WetSeason$lagdate,"%d/%m/%Y")

WetSeason$difdays <- WetSeason$date - WetSeason$lagdate
WetSeason$difdays <- as.numeric(WetSeason$difdays)

###We have skipped the steps to identify reasonable variables to include in
combination models, and identification of the most reasonable combination
model. In this case, separate models for each variable must be estimated.
After selection of variables, separate candidate models must be estimated
for each combination of those variables (e.g. if there are 3 variables, 7
candidate models must be estimated, including models containing only one
variable). The following process must be repeated 3 times, the first to
identify which individual variables are most likely to affect activity, the
second to estimate the most reasonable combination of the candidate
variables, and the third to compare the most reasonable combination model
to a model containing an autoregressive effect.

###Compute AIC for null and models MT + WS and MT + WS + lagToads
###MT = minimum temperature, WS = wind speed
taus<- c(1:19/20)
m <- 500
numtaus<- length(taus)

toads.lag.aic <-matrix(0,nrow=m*numtaus,ncol=4)

###There are three models, a null model with just an intercept and the
###models with MT + WS and MT + WS + lagToads.

###Randomly jitter the counts with uniform [0, 1) and then take logs.
###Jittered values - tau <=0.0 are given log(0.00001)

###We have eliminated large lags of 20 and 31 days from estimates

colnames(toads.lag.aic)<- c("tau","AIC.null","AIC.mtws","AIC.mtws.lag")

for(i in 1:m){
      WetSeason$toads.jit<- WetSeason$Toads + runif(length(WetSeason
```

```
      $Toads),min=0,max=0.999999)

      for (j in 1:numtaus){
            WetSeason$toads.jittau <-WetSeason$toads.jit - taus[j]
            WetSeason$toads.logjittau[WetSeason$toads.jittau<=0.0] <-
            log(0.00001)
            WetSeason$toads.logjittau[WetSeason$toads.jittau>0.0] <- log
            (WetSeason$toads.jittau[WetSeason$toads.jittau>0.0])

###Estimate null and two candidate models (exponential)

      fit.null<- rq(toads.logjittau ~ 1, data=WetSeason[WetSeason
      $difdays<5,],tau=taus[j])

      fit.mtws<- rq(toads.logjittau ~ MT + WS,data=WetSeason[WetSeason
      $difdays<5,]tau=taus[j])

      fit.mtws.lag<- rq(toads.logjittau ~ MT + WS +
      lagToads,data=WetSeason[WetSeason$difdays<5,],tau=taus[j])

      toads.lag.aic[j + (i-1)*numtaus,]<-c(fit.null$tau,AIC(fit.null)
      [1],AIC(fit.mtws)[1],AIC(fit.mtws.lag)[1],use.names=F)
      }}

###Compute delta AIC (from null model) for each of i = 1 to 500 m
replications by quantile before averaging across m replications.

toads.lag.aic <- as.data.frame(toads.lag.aic)


toads.lag.aic$d.AIC.mtws <- toads.lag.aic$AIC.null - toads.lag.aic$AIC.mtws
toads.lag.aic$d.AIC.mtws.lag <- toads.lag.aic$AIC.null -
toads.lag.aic$AIC.mtws.lag

###Now to average across m replications by quantile

toads.lag.aic.avg <- matrix(0,nrow=numtaus,ncol=6)

for (i in 1:numtaus){
toads.lag.aic.avg[i,] <-
apply(toads.lag.aic[toads.lag.aic[,1]==taus[i],],2,mean)
}

colnames(toads.lag.aic.avg)<-
c("tau","AIC.null","AIC.mtws","AIC.mtws.lag","d.AIC.mtws",
"d.AIC.mtws.lag")


toads.lag.aic.avg <- as.data.frame(toads.lag.aic.avg)




####To estimate coefficients and confidence intervals for a selected model
with lagged counts (not linear in day difference since nearly all equal 1).
Used this form with simple lagged effect.



taus<- c(1:19/20)
m <- 500
```

```
numtaus<- length(taus)

###Set up matrix for tau + intercept + 3 covariates and their lower and
upper confidence interval endpoints.

toads.mtws <-matrix(0,nrow=m*numtaus,ncol=13)

###Randomly jitter the counts with uniform [0, 1) and then take logs.
###Jittered values - tau <=0.0 are given log(0.00001)

colnames(toads.mtws)<-
c("tau","Intercept","Lwr90.intcpt","Upr90.intcp","MT","Lwr90.mt","Upr90.mt"
,"WS","Lwr90.ws","Upr90.ws","LagToads","Lwr90.lagtoads","Upr90.lagtoads")

for(i in 1:m){
      WetSeason$toads.jit<- WetSeason$Toads + runif(length  (WetSeason
      $Toads),min=0,max=0.999999)

      for (j in 1:numtaus){
            WetSeason$toads.jittau <-WetSeason$toads.jit - taus[j]
            WetSeason$toads.logjittau[WetSeason$toads.jittau<=0.0]
            <- log(0.00001)
            WetSeason$toads.logjittau[WetSeason$toads.jittau>0.0] <-
            log(WetSeason$toads.jittau[WetSeason$toads.jittau>0.0])


      fit.mws<- rq(toads.logjittau ~ MT + WS + lagToads
      ,data=WetSeason[WetSeason$difdays<5,],tau=taus[j])

      rqfit <- summary(fit.mws,se="rank",iid=F,alpha=0.10)

      toads.mtws[j + (i-1)*numtaus,]<-c(rqfit$tau,rqfit$coef
      [1,1],rqfit $coef[1,2],rqfit$coef[1,3],rqfit$coef[2,1],rqfit$coef
      [2,2],rqfit $coef[2,3],rqfit$coef[3,1],rqfit$coef[3,2],rqfit$coef
      [3,3],rqfit $coef[4,1],rqfit$coef[4,2],rqfit$coef[4,3],
      use.names=F)
      }}

###Now to average across estimates and CI endpoints by tau in the
continuous linear scale.

toads.mtws.avg <- matrix(0,nrow=numtaus,ncol=13)

for (i in 1:numtaus){
toads.mtws.avg[i,] <- apply(toads.mtws[toads.mtws[,1]==taus[i],],2,mean)
}

colnames(toads.mtws.avg)<-
c("tau","Intercept","Lwr90.intcpt","Upr90.intcp","MT","Lwr90.mt","Upr90.mt"
,"WS","Lwr90.ws","Upr90.ws","Lagtoads","Lwr90.lagtoads","Upr90.lagtoads")


toads.mtws.avg <- as.data.frame(toads.mtws.avg)

###For MT we restricted CI to 0.10-0.95 because lower limits for 0.05
become huge.

plot(toads.mtws.avg$tau,toads.mtws.avg$MT,type="n",cex=0.75,pch=16,col="bla
ck",,xlim=c(0,1),ylim=c(-0.20,0.30), ylab="Estimate",
xlab="Quantile",main="MT")
```

```
abline(h=0,xaxs="i",yaxs="i",xaxt="n",yaxt="n")

polygon(c(toads.mtws.avg$tau[2:19],rev(toads.mtws.avg$tau[2:19])),c(toads.mtws.avg$Lwr90.mt[2:19],rev(toads.mtws.avg$Upr90.mt[2:19])),col="grey",border="grey")

points(toads.mtws.avg$tau,toads.mtws.avg$MT,type="b",cex=0.75,pch=1,col="black",xlim=c(0,1),ylim=c(-0.20,0.30),ylab="",xlab="")


###For WS we restricted CI to 0.10-0.95 because lower or upper limits for more extreme tau become huge.

plot(toads.mtws.avg$tau,toads.mtws.avg$WS,type="n",cex=0.75,pch=16,col="black",,xlim=c(0,1),ylim=c(-0.1,0.05), ylab="Estimate", xlab="Quantile",main="WS")

abline(h=0,xaxs="i",yaxs="i",xaxt="n",yaxt="n")

polygon(c(toads.mtws.avg$tau[2:19],rev(toads.mtws.avg$tau[2:19])),c(toads.mtws.avg$Lwr90.ws[2:19],rev(toads.mtws.avg$Upr90.ws[2:19])), col="grey",border="grey")

points(toads.mtws.avg$tau,toads.mtws.avg$WS,type="b",cex=0.75,pch=1,col="black",xlim=c(0,1),ylim=c(-0.1,0.05),ylab="",xlab="")

###For Lagtoads we restricted CI to 0.10-0.90 because lower or upper limits for more extreme tau become huge.

plot(toads.mtws.avg$tau,toads.mtws.avg$Lagtoads,type="n",cex=0.75,pch=16,col="black",,xlim=c(0,1),ylim=c(-0.1,0.1), ylab="Estimate", xlab="Quantile",main="Lag 1 count")

abline(h=0,xaxs="i",yaxs="i",xaxt="n",yaxt="n")

polygon(c(toads.mtws.avg$tau[2:18],rev(toads.mtws.avg$tau[2:18])),c(toads.mtws.avg$Lwr90.lagtoads[2:18],rev(toads.mtws.avg$Upr90.lagtoads[2:18])), col="grey",border="grey")

points(toads.mtws.avg$tau,toads.mtws.avg$Lagtoads,type="b",cex=0.75,pch=1,col="black",xlim=c(0,1),ylim=c(-0.1,0.1),ylab="",xlab="")
```

APPENDIX S3: CHAPTER 5.

Figure S3. I sampled calls and trapped cane toads around waterbodies near Withcott (SEQ), Cairns (NQ), Palmerston (WA), and Kununurra (WA). Townsville is also indicated; Townsville calls were used at each study site.