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Improving Short-term Removal Methods of the Introduced
Pest Species the Cane Toad, *Chaunus [Bufo] marinus*, in
North-eastern Australia

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Submitted for the Degree of Master's of Science

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I declare that no other persons contributed to this thesis other than myself and my supervisors, Ross Alford and Lin Schwarzkopf. I did not receive any financial help or editorial assistance. I also declare that all procedures reported in this thesis were conducted under the approval of JCU Animal Ethics Committee, approval number A1166.

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Abstract

The cane toad's (*Chaunus [Bufo] marinus*) introduction in 1935 into Australia has had tremendous consequences for the native wildlife which inhabit the area of invasion. Much effort has recently been concentrated on minimizing the impact of the toad, especially along the invasion front in the Northern Territory and Western Australia. Efforts have focused on implementing short-term management solutions while long-term solutions are being developed. Trapping has proved useful in the Northern Territory, and there is strong interest in improving the efficiency of this technique. Current traps use fluorescent lights which improve trapping success, but the mechanism of this has not been documented, and the efficacy of alternative light sources has not been investigated. I investigated the reactions of toads to four different light sources (incandescent, white fluorescent, yellow fluorescent and ultraviolet lights). My results indicate that toads are not attracted to any of these light sources, and white fluorescent and incandescent lights actively repel toads. This suggests that the attractiveness of lighted traps is due to the insects that are attracted by lights, which the cane toads feed upon. Yellow fluorescent and ultraviolet lights neither attract nor repel toads; given this result, and the fact that toads are apparently attracted to traps by the insects that lights attract, it is likely that using UV lights on traps would increase their effectiveness. Previous research indicated that both male and female cane toads are attracted to conspecific mating calls, and that playing back toad calls in the vicinity of traps can improve their efficiency. In other species, it has been established that manipulating certain aspects of calls can produce "superstimuli", calls that are more attractive than any natural call. Playbacks of a superstimulus might provide even greater efficiency increases for toad traps. I recorded and analyzed the calls of toads in nature to

determine the mean and range of dominant frequency, pulse rate, and call length. I evaluated the responses of toads to manipulated calls with characteristics just outside the natural range for dominant frequency (high and low) and pulse rate (high and low). Both male and female toads were more attracted to calls with high pulse rates than they were to natural calls. The responses of females to manipulated calls also depended on their reproductive status. Based on my findings, it appears that the efficiency of traps could be substantially improved by using ultraviolet lights instead of white fluorescent lights, and by adding playbacks of high pulse rate advertisement calls. Validating these suggestions will require field experiments, which should be undertaken as soon as possible.

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Chapter 1

Introduction

1.1 Background

Biological control (the intentional introduction of an organism or virus that is intended to control a pest species) has been useful for certain pest species. One of the first documented introductions of a biological control agent occurred in 1889 in California (Caltagirone 1981). An Australian beetle was successfully introduced to control an orange crop pest and paved the way for further biological control (Howarth 1991). The use of biological control agents can reduce the need to use potentially harmful pesticides, and thus introductions can lead to long-term economic and public health benefits (Greathead 1971; Rao, Ghani et al. 1971; DeBach 1974; Caltagirone 1981; Cock 1985; Samways 1988). Another success story for biological control is the moth *Cactoblastis cactorum* which originates in Argentina, and was used to control the highly noxious prickly pear cactus (*Opuntia stricta*) in Australia (Dodd 1940). This same moth, however, now threatens many native *Opuntia* spp. in the U.S. and Mexico due to an accidental introduction (Stiling 2002). The negative impact of *C. cactorum* in some locations highlights the need for caution when using biological control agents.

In many cases control agents have done more harm than good. For example, non-target species have become endangered or even driven to extinction by biological control agents (Howarth 1991). The mongoose *Herpestes auropunctatus* was introduced into the Caribbean to control introduced rats, but caused the extinction of 7 reptile species (Julien 1987). Similarly, in Hawaii the release of a predatory snail to control an exotic African snail species led to the extinction of several endemic Hawaiian

snail species (Funasaki, Lai et al. 1988). While introduced biological controls can be very helpful in reducing the effects of pest species, their environmental impacts need to be considered and evaluated prior to release (Howarth 1991).

The cane toad *Bufo marinus* was first described by Linnaeus in 1758; however has recently has been placed into the genus *Chaunus* (Frost, Grant et al. 2006) but there is ongoing debate on what genus the cane toad should be placed (another suggestion is *Rhinella* or to keep it in the genus *Bufo*; (Pramuk 2006; Pramuk, Robertson et al. 2008). The cane toad was introduced into Australia in 1935 as a biological control for two sugar cane pests: the greyback beetle, *Lepiderma albohirtum*, and the frenchi beetle, *L. frenchi* (Froggatt 1936). Although originally intended as a biological control for destructive pests of sugar cane, it was soon obvious that the cane toad was ineffective at controlling the two beetle species, despite beetles generally being part of its native diet (Zug and Zug 1979; Evans and Lampo 1996). As early as 1936, less than one year after its introduction, speculation that the cane toad might turn into a major pest began to arise (Froggatt 1936).

Researchers soon became aware that the toads were threatening native Australian fauna (Covacevich and Archer 1975). *Chaunus marinus* has both direct and indirect effects on native species (Ewel, O'Dowd et al. 1999). The cane toad is toxic to many predators in all its life stages (Crossland 1997; Crossland and Alford 1998; Letnic, Webb et al. 2008; Hagman, Phillips et al. 2009; Halliday, Venables et al. 2009; Hayes, Crossland et al. 2009) One of the most dramatic effects of *C. marinus* is that ingesting or even attacking it is fatal to many native predators, especially varanid lizards (Phillips, Brown et al. 2004; Phillips and Shine 2006; Phillips and Shine 2006) and elapid snakes (Phillips, Brown et al. 2003; Webb, Shine et al. 2005).

Many other native predators experience serious declines due to the invasion of the cane toad. It is strongly suspected that the introduction of the toad is leading to the drastic decline of the northern quoll, *Dasyurus hallucatus*. The carnivorous marsupial succumbs almost instantly after attempting to ingest a cane toad (Burnett 1993; Braithwaite and Griffiths 1994). Cane toads are fatal to most anurophagous predators such as tiger snakes, red-bellied black snakes (Phillips, Brown et al. 2004; ICUN 2006; Phillips and Shine 2006; Phillips and Shine 2006; Phillips and Shine 2006), death adders (Webb, Shine et al. 2005), dingoes (Catling, Hertog et al. 1999), goannas, freshwater crocodiles (McLeod 2004; Letnic, Webb et al. 2008), and microchiropteran bats (ICUN 2006). Predatory native tadpoles also suffer lower survival rates when cane toads are present due to the toxicity of the *C. marinus* tadpoles (Crossland 2000; Punzo and Lindstrom 2001). Cane toads also eat native vertebrates such as snakes, frogs, and planigales (Covacevich and Archer 1975; Shine and Covacevich 1983) and even rainbow bee-eaters, *Merops ornatus* (Boland 2004), and reduce the densities of native invertebrates more than do native frogs (Greenlees, Brown et al. 2006). Small arboreal and terrestrial reptiles have also declined due to toad invasions, probably due to competition causing a reduction in food sources (Catling, Hertog et al. 1999).

Originally introduced into a few areas of northeastern Queensland in 1935, the cane toad's distribution has increased at an alarming rate and recent models suggest they may be capable of spreading at an even faster rate (Urban, Phillips et al. 2007). By 1974 the toad's distribution stretched from the Queensland/New South Wales border to the Gulf of Carpentaria, occupying 584,000 square kilometres. (Sabath, Boughton et al. 1981) The area of distribution expanded at the rate of about 25 kilometres per year for many years (Easteal, Vanbeurden et al. 1985). As of 2005, the cane toad had expanded its distribution into the top end of the Northern Territory and has caused major issues

for the fauna in sensitive ecosystems within Kakadu National Park and continues to do so in other areas with its expansion (Land Protection 2006; Sawyer 2006). Toads are projected to colonize the majority of Australia, by 2030 (Figure 1.1), with the only exceptions being areas in the extreme dry interior and higher elevations (Sutherst, Floyd et al. 1996).

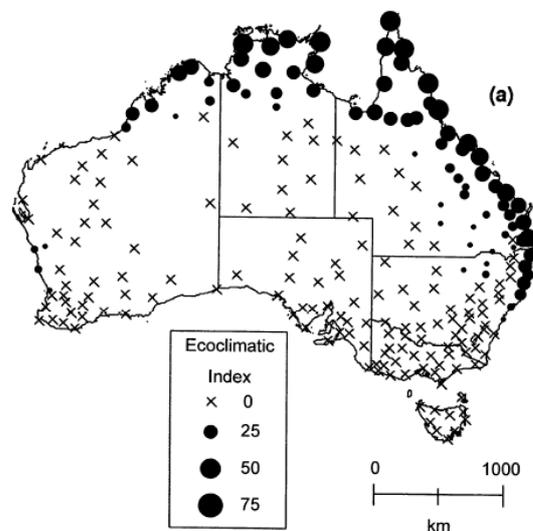


Figure 1.1. Projected distribution of *Chaunus marinus* in Australia for the year 2030 (from Sutherst et al. 1996 using CLIMEX simulations). The climactic favorableness of each location for permanent colonization is proportional to the area of the circles.

Long-term and short-term management of current *C. marinus* populations is extremely desirable in Australia (Lampo and De Leo 1998; Commonwealth Scientific and Industrial Research Organisation (CSIRO) 2006; Land Protection 2006; Tyler 2006), but the control of this species is still in early stages. *Chaunus marinus* was listed in a April 2005 as a “key threatening process” under the Australian Government’s Environment Protection and Biodiversity Conservation Act, 1999 (Threatened Species Scientific Committee 2005) from pressures from the World Wildlife Fund (WWF) in 2004 (World Wildlife Fund (WWF) Australia 2008) even though not having been declared a pest in Queensland as of 2008 (Department of Primary Fisheries 2008). The

cane toad is an extremely successful invader, hence total eradication is quite unlikely (Lampo and De Leo 1998) but through control efforts in affected areas it may be possible to reduce its impact on native wildlife and ecosystems (Lampo and De Leo 1998; Lever 2001; Tyler 2006; Hagman and Shine 2008).

As early as 1983, possible long-term control methods have been proposed for the reduction of toad densities throughout its introduced range (Tyler 2006). Research conducted on possible management strategies such as: iridoviruses (frog oedema virus complex; Whittington, Kearns et al. 1997); bacterial pathogens (*Aeromonas hydrophila*); natural pathogens and parasite research in Venezuela; recombinant viruses (Hazell, Nott et al. 2003), Genetically Modified Organisms (GMO's); non-disseminating agents (Robinson, Hyatt et al. 2006); sterile male development (Mahoney 2004); daughterless toads (The University of Queensland 2003), and other possible toad pathogens is still in its early stages. While some of these methods may prove very useful as management tools in the future, their development is slow and studies will have to be carried out for at least 10 years (especially in the case of viruses and pathogens). These are very costly, in terms of both time and money, and are likely to have limited success (Tyler 2006).

Short-term control methods should be implemented as soon as possible in ecologically sensitive areas until more permanent, long-term control methods are available (Schwarzkopf and Alford 2007). Manual control, by hunting or trapping, could help to prevent or delay the toads' spread throughout Australia (Sawyer 2006; Schwarzkopf and Alford 2007). This method may even be a useful tool in temporarily eradicating or drastically reducing local populations in ecologically sensitive areas (Burnett 1997). There is some debate on whether trapping can slow the cane toad's expansion or even serve as an adequate population control method (Peacock 2007). If

trapping success can be improved, it might prove more useful in managing certain populations. Trapping other vertebrate pests have proven very useful for population reduction; for example, trapping has been used as a control method for brown tree snakes (*Boiga irregularis*) in Guam (in this case, mice were used as bait; (Engeman, Linnell et al. 1998; Engeman, Vice et al. 2000; Johnston, Savarie et al. 2002). Trapping cane toads has been trialed in the Northern Territory with some success (Sawyer 2006) and research has recently turned to improving trapping success by using lures (Schwarzkopf and Alford 2007).

It has been suggested that the best management strategy for reduction of the population is to reduce adult survival (because larval survival is density dependent; Alford, Cohen et al. 1995). Therefore, trapping should target adults (Lampo and De Leo 1998). Cane toads are highly successful breeders in many Australian habitats (Easteal 1981) and are very opportunistic feeders (Covacevich and Archer 1975; Shine and Covacevich 1983) suggesting that breeding calls or food may be useful as lures. Successful trapping that removes a large portion of the adult population could have dramatic implications for wildlife and ecosystems in northern Australia.

Male anurans produce advertisement calls during the breeding season to attract females for mating. The anuran advertisement call is a very energy-expensive activity (Ryan 1988) and conservation of energy appears to be a major factor shaping call characteristics (Schwartz 1994). Females use these calls to choose their mates, so sexual selection also shapes call characteristics (Blair 1958). For this reason, anuran mating calls are species-specific. Call characteristics can vary among individuals of the same species with body size (generally the larger the body size the lower the frequency and the slower the repetition rate of the call), and the call also varies with the ambient temperature (frequency often increases with temperature; Blair 1958). Recent research

has indicated that both male and female *C. marinus* are attracted to conspecific advertisement calls (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007).

Male fitness plays an important role in call characteristics (Blair 1958). Certain call characteristics convey male fitness to the female during mate selection (Gerhardt 1991). There is potential to manipulate calls so that they embody desirable traits, and can be used as lures (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007). Past research has examined which characteristics of calls are attractive to females. Female anurans of many species prefer longer mating calls (Sullivan 1983; Sullivan 1987; Gerhardt 1991; Lopez and Narins 1991; Sullivan and Hinshaw 1992; Cherry 1993; Wagner and Sullivan 1995; Bee 2008; Marquez, Bosch et al. 2008), but dominant frequency (the sound frequency that is most prominent in the call sound) varies in importance among species. In some species females prefer calls with lower dominant frequencies (Straughan 1975; Gerhardt 1982; Jennions, Backwell et al. 1995; Howard and Young 1998; Bee, Perrill et al. 2000), whereas in other species dominant frequency does not appear to play a role in male selection (Sullivan 1983; Sullivan 1987; Lopez and Narins 1991; Sullivan and Hinshaw 1992; Wagner and Sullivan 1995; Witte, Ryan et al. 2001). Pulse repetition rate is also an important characteristic for female choice in some species (Straughan 1975; Cherry 1993; Gerhardt and Watson 1995; Wollerman 1998; Bee 2008). It would be beneficial to examine these characteristics of cane toad advertisement calls more closely, to investigate the possibility that manipulation of one or more of these traits can increase call attractiveness.

Anecdotal evidence suggests that cane toads aggregate around artificial light sources to eat insects attracted by the lights (Crawford and Jones 1933; Sawyer 2006). Anurans are capable of distinguishing different light colors, and also show preferences for different light qualities (Muntz 1962; Marangio 1975). In tropical Australia cane

toads aggregate near lights at night, and artificial lights form a part of at least two commercial trap designs {Sawyer, 2006 #125; Schwarzkopf, 2006 #139; Schwarzkopf, 2007 #8}. The mechanism of attraction has not been investigated; toads might be attracted to the light itself, or might be attracted to the insects that are attracted by light sources. It is also possible that, since they are primarily nocturnal, toads are actually repelled by lights but the repulsion is outweighed by attraction to insects. Because an objective of trap design is to maximize the number of toads caught per unit trapping effort, it is worth investigating the effects of a variety of light sources on toad behaviour, in isolation from possible effects caused by insects. If one type of light is more attractive (or less repulsive) than others, using it could increase trapping success.

1.2 Thesis Outline

Are cane toads attracted to lights?

In Chapter 2 I investigate the effects of a variety of light sources on movements by cane toads in a Y-maze. This concludes with recommendations regarding the most effective light source to use with existing traps to maximize trap success.

What are the call characteristics of cane toad populations in north Queensland?

In Chapter 3 I present baseline data and analyses of recordings of natural cane toad mating calls collected in North Queensland. These data were used as a baseline for the call manipulations described in Chapter 4.

Can manipulation of a cane toad call make the call more attractive to cane toads?

In Chapter 4 I examine the effects of manipulations of five different call characteristics on the attractiveness of calls to male and female cane toads in an experimental arena.

In Chapter 5 I discuss the overall implications and utility of this research in relation to cane toad control in Australia. Trapping regimes are discussed and recommendations for management are made.

Chapter 2

Lights as Lures for Cane Toads: Implications for Improving Short-term Removal of an Invasive Species

2.1 Introduction

The introduction of *Chaunus marinus* into Australia in 1935 (Froggatt 1936) led to some serious threats to native Australian fauna. Drastic population declines have been observed in snakes (Elapidae; Phillips, Brown et al. 2003; Phillips, Brown et al. 2004; Webb, Shine et al. 2005; ICUN 2006; Phillips and Shine 2006; Phillips and Shine 2006; Phillips and Shine 2006), monitor lizards (*Varanus sp.*; Phillips, Brown et al. 2004; Phillips and Shine 2006; Phillips and Shine 2006), dingos (Catling, Hertog et al. 1999), freshwater crocodiles (*Crocodylus johnstoni*; Mcleod 2004; Letnic, Webb et al. 2008), bats (ICUN 2006). Other groups also have some suggested population effects from cane toad introduction such as planigales (Covacevich and Archer 1975) and rainbow bee-eaters, *Merops ornatus* (Boland 2004).

Efforts have been undertaken to develop management strategies for the cane toad to minimise its impact on native fauna (Lampo and De Leo 1998; Commonwealth Scientific and Industrial Research Organisation (CSIRO) 2006; Land Protection 2006; Sawyer 2006; Schwarzkopf and Alford 2006; Tyler 2006; Department of Primary Fisheries 2008). Manual removal using trapping has been trialed in the Northern Territory using cage traps with lights on them as lures (Sawyer 2006). Acoustic attractants, in the form of cane toad mating calls, have also been investigated (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007) to potentially increase the success of traps. Traps with improved success should aid in reducing toad numbers

in ecologically sensitive areas, while efforts are continued to find an appropriate long-term solution to reduce the Australian toad population. To maximise trapping success, it is important that every aspect regarding potential attractants for traps should be investigated thoroughly.

There is anecdotal evidence that cane toads aggregate around artificial light sources, predated on the insects attracted by the lights (Crawford and Jones 1933; Sawyer 2006). Lights have been used previously on traps to capture cane toads (Sawyer 2006; Schwarzkopf and Alford 2006), however, no experimental research has demonstrated whether lights attract or repel toads. Anurans are capable of distinguishing between light colors and can display preferences for different light qualities (Muntz 1962). It is possible that toads are attracted to light itself, but it is more likely that toads are attracted to the insect fauna that is attracted by the lights. The cane toad is a nocturnal animal; hence it could be possible that lights repel toads, instead of attracting them. It is essential to examine the effects of different lights on toad behaviour to determine the most appropriate light source for toad traps. Using the light source that is most attractive (or least repulsive) to toads should increase trapping success.

To determine how lights influence the attraction of toads in the absence of insects, I measured the responses of toads to four different light sources (white incandescent, white fluorescent, ultraviolet and yellow anti-insect) in a laboratory y-maze. The behaviour of the toads in response to the different light sources was compared to their behaviour toward darkness, the lights were also paired against each with other to further compare their attractive/repulsive effects on toads.

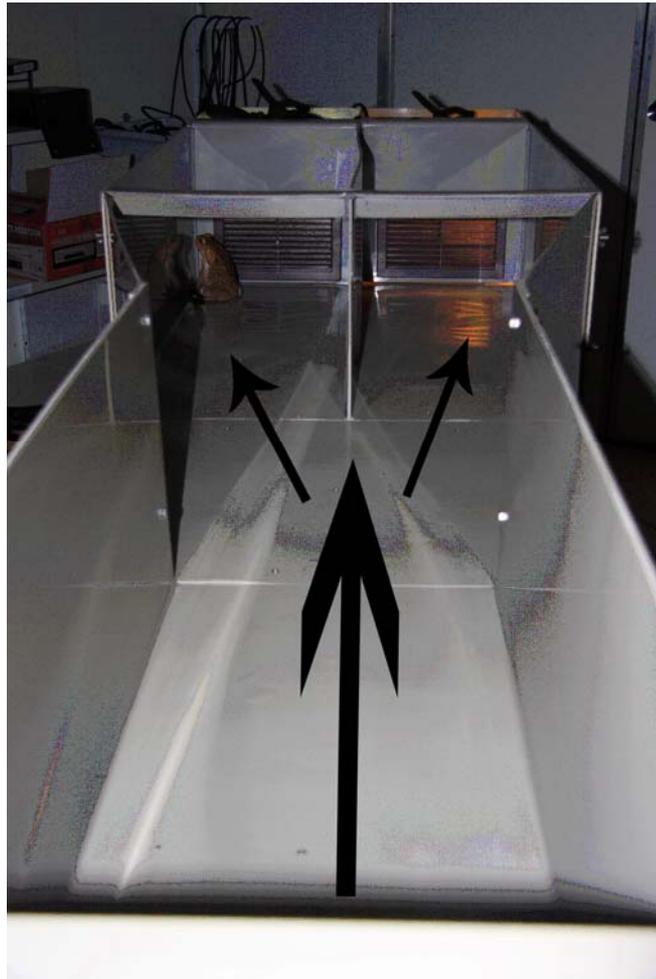
2.2 Methods:

2.2.1 Experimental Subjects:

Cane toads (*Chaunus marinus*) were collected either the night before or the night of experimental trials. The toads were collected around the “Freshwater Compound” at the James Cook University campus in Townsville, Queensland (19°15'42”S; 146°48'38”E). All trials were conducted between 7 November 2007 and 12 December 2007. Sixty-three toads were used in the experiments (40 females and 23 males). All experimental trials took place in a laboratory setting at James Cook University.

2.2.2 Lights:

To test the attractiveness of lights to cane toads, four different kinds of widely-available lights that could easily be incorporated into existing cane toad traps were selected. The four light types were: 60w clear incandescent globes (Phillips D7 250-260), 20w white fluorescent spiral tubes (Phillips “Tornado” cool daylight), 20w ultraviolet fluorescent spiral tubes (Nelson “party” Ultra Violet), and 20w yellow fluorescent spiral tubes (Nelson “Anti-Insect”). Each light was placed in an ARLEC heavy duty worklight (230-240Vac 50Hz, 60w max) inside one arm of a Y-shaped maze (dimensions: 2.4m long, 30cm high, 30cm wide, central division 140cm long, divided “Y” areas 86cm long; Figure 2.1). The entire maze was covered with clear plastic sheeting to prevent the toads from escaping during the trials. The end of the maze contained a vent that allowed light to show through, but prevented the toads from reacting to any other visual stimulus inside the room.



A.



B.

Figure 2.1 The Y-Maze. A) The Y-maze in its entirety shown; B) shows a cane toad after choosing one of the sides of the maze.

2.2.3 *Trials:*

To begin each trial, a toad was placed into the base of the Y-maze. Each trial lasted 5 minutes, during preliminary trials toads had made a choice well within 5 minute time frame. Each toad was exposed to seven light conditions: four trials in which each light type (see above list), was paired with darkness (no ambient light in the room), and presented in a random order, and, two trials in which 2 light types were paired, and one with no light stimulus. In each trial, the arm (left or right) of the Y-maze in which each stimulus was placed was also chosen randomly (by using a generated random number table). Although each toad was presented with only two of the six possible paired combinations of light sources (Table 2.1), all pairs were presented to approximately equal numbers of toads during the trials; the particular pairs presented to each individual were chosen at random.

Trials were conducted inside a closed room which the experimenter left for the duration of the trial. The behaviour of the toads was scored as having selected an arm of the maze at the end of 5 minutes. The trial was terminated after 5 minutes even if a toad had not yet selected an arm. After each trial, the Y-maze was thoroughly cleaned with a mild detergent solution and dried with clean towels. The toads were held in a clean 20 litre bucket with a lid between each trial.

Table 2.1 Experimental Trials. Each toad was presented with all seven of the below trials in differing orders. Trials 5 and 6 consisted of two of the lights paired together, then the remaining two lights were paired; all paired combinations were used in equal numbers of times in all experimental trials.

Treatment	60w clear incandescent bulb	20w white fluorescent spiral	20w ultra violet fluorescent spiral	20w yellow fluorescent spiral	No light
1	X				X
2		X			X
3			X		X
4				X	X
5 (one pair)	X	X	X	X	
6 (one pair)	X	X	X	X	
7					XX

2.2.4 Data Analysis

To determine whether toads discriminated between light sources and darkness Chi-squared goodness-of-fit tests were used with the null hypothesis being that each arm was entered with equal frequency. These tests were carried out using a Bonferroni-adjusted (for four tests) alpha level of 0.0125.

2.3 Results

When given the choice between the normal incandescent light and no light, toads were more attracted to the dark side of the Y-maze ($X^2 = 6.65$, $df = 1$, $p = 0.01$; Figure 2.2). Similarly, when given the choice between white fluorescent and no light, significantly more toads also selected darkness ($X^2 = 9.98$, $df = 1$, $p = 0.001$). Toads

also showed a similar trend (although not statistically significant) in preference for darkness when choosing between the ultraviolet (UV) light and the yellow “anti-insect” light paired with darkness (UV light $X^2 = 0.09$, $df = 1$, $p = 0.76$; $X^2 = 0.69$, $df = 1$, yellow “anti-insect” $p = 0.40$; Figure 2.2).

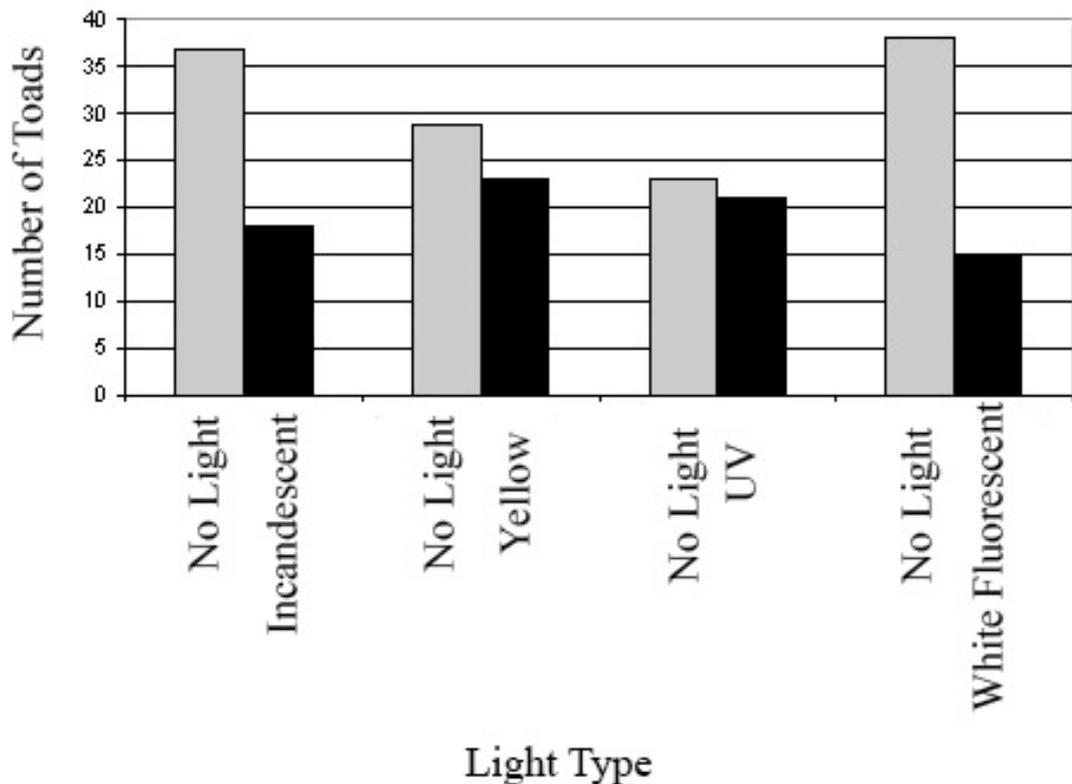


Figure 2.2. Light versus no light choice in Y-maze experiments. Number of toads entering the Y-maze containing the stimulus or control; gray bars indicate a ‘No light’ choice; the black bars indicate a light choice (for the four light choices: normal 60w, UV, white fluorescent and yellow fluorescent lights).

Chi-squared tests for paired light sources were carried out at Bonferroni-adjusted (for six tests) alpha levels of 0.0083. Toads did not significantly prefer either light source in any of the pairs (incandescent paired with UV, $X^2 = 0.07$, $df = 1$, $p = 0.78$; incandescent paired with white fluorescent, $X^2 = 0.22$, $df = 1$, $p = 0.63$; yellow “anti-insect” paired with UV, $X^2 = 2.00$, $df = 1$, $p = 0.15$; yellow “anti-insect” paired

with white fluorescent $\chi^2 = 1.00$, $df = 1$, $p = 0.31$; UV paired with white fluorescent $\chi^2 = 1.31$, $df = 1$, $p = 0.25$; incandescent paired with yellow “anti-insect” $\chi^2 = 5.4$, $df = 1$, $p = 0.039$; Figure 2.3). The pairing of incandescent and yellow “anti-insect” was an interesting result because when compared previously with darkness, there was a significant avoidance of incandescent light but not of yellow light. The reason for the result of toads preferring incandescent more than yellow “anti-insect” when paired together is unclear. The trend of avoidance in yellow “anti-insect” was still apparent, however insignificant. The strongest trend occurred between the UV light and yellow fluorescent light (Figure 2.3), with toads appearing to prefer the UV, however this relationship was not statistically significant. Another potential trend is that toads were always observed to prefer UV, or avoid it less strongly, than any other light source.

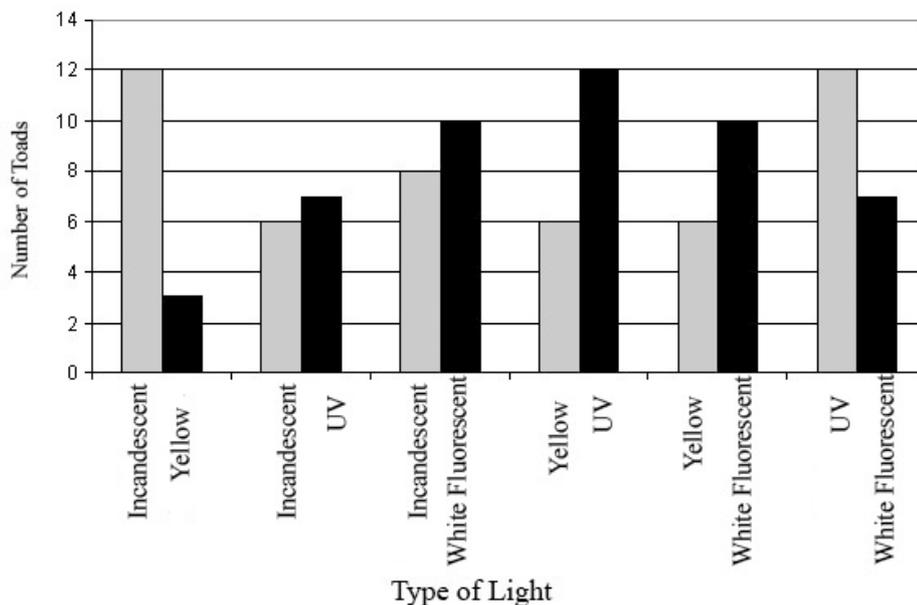


Figure 2.3 Pairwise comparisons between different light sources. The graph shows the number of toads that chose the side of the y-maze when two lights were shown simultaneously in the Y-maze experiments. The groups of bars in the graph indicate pairs of lights that were shown together in the Y-maze, each light is shown grouped with each other light.

Because there appeared to be a trends in preference for UV, a series of chi-squared tests in which I compared one light source against all other light sources, with their data aggregated, were carried out. These tests were Bonferroni-adjusted (for four tests) alpha level of 0.0125. When compared to all the other light types combined, toads did not show significant preference for, or discrimination against, the UV light ($X^2 = 2.88$, $df = 1$, $p = 0.089$), normal incandescent light ($X^2 = 0.78$, $df = 1$, $p = 0.376$), nor the white fluorescent light ($X^2 = 0.018$, $df = 1$, $p = 0.890$), although preference of toads to the UV light as opposed to the other light sources remained suggestive. However, they significantly avoided the yellow “anti-insect” light choosing this light less often than the other light types ($X^2 = 7.36$, $df = 1$, $p = 0.006$; Figure 2.4).

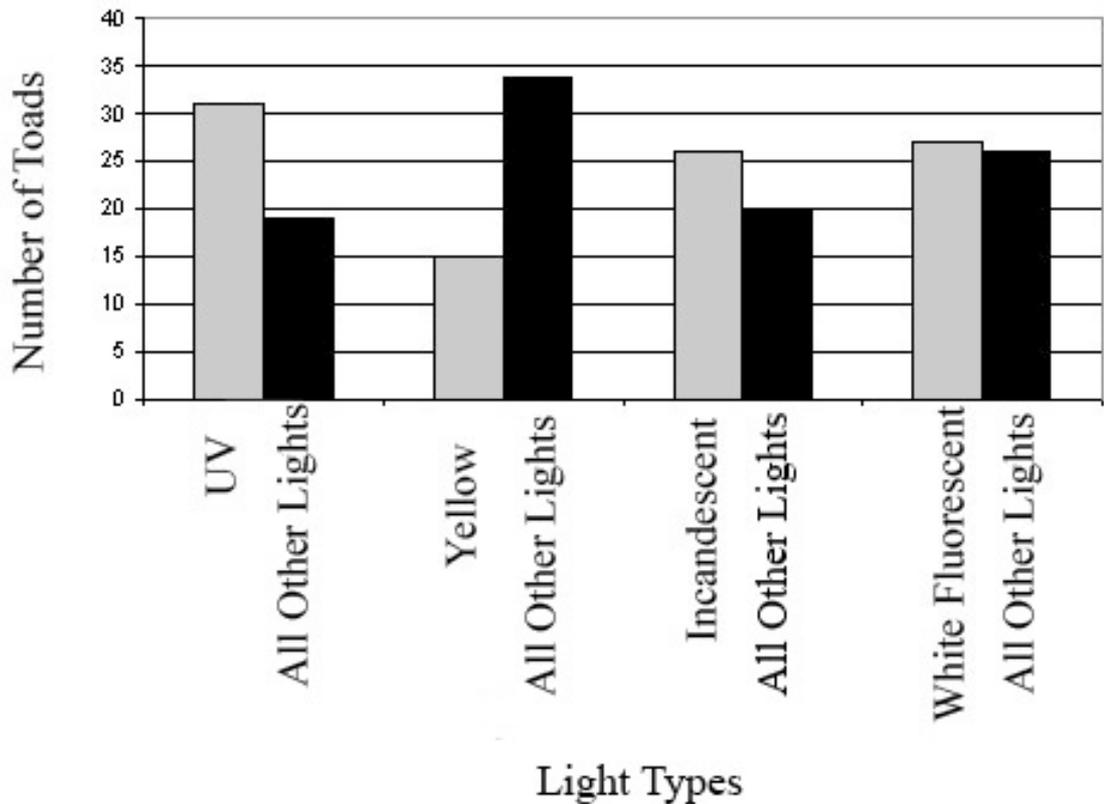


Figure 2.4 Each light paired against all other lights. Gray bars indicate each target light; the black indicate all other lights as paired against the target light. This graph indicates the number of toads which chose the side of the y-maze that contained the lights.

2.4 Discussion

When given the choice between any of the lights used in this experiment and darkness, toads preferred either the darkness (compared to incandescent and fluorescent lights), or did not distinguish between that light type and darkness (in the case of UV light and yellow light), however, no light type actually attracted the toads (Figure 2.2). When different light sources were examined in relation to each other, the yellow “anti-insect” light was the most “repulsive” of all the lights (Figure 2.4). Of the other three lights, UV was the least repulsive (Figures 2.2 and 2.3). Since none of the lights were

chosen by toads more than the no-light condition, lights themselves cannot be considered attractive to toads. These experiments demonstrate that toads are not attracted to lights *per se*. Instead, the apparent attraction to lights observed when traps are equipped with lights, or anecdotally reported around human habitation, is likely to be due to an attraction to the insects that are attracted to the lights (Hienton 1974).

Anurans are able to discriminate between different light wavelengths (Muntz 1962; Muntz 1962). Studies on *Rana pipiens* showed that frogs can discriminate certain colors and show stronger positive phototaxis to the blue light wavelengths than to other colors (Muntz 1962). Ultraviolet wavelengths are near the blue light wavelengths of the spectrum (figure 2.5). Cane toads may be able to distinguish (and prefer) shorter wavelengths, which may explain why UV wavelengths do not repel toads as the other, longer wavelength lights did.

Currently, commercially available cane toad traps are equipped with white fluorescent lights, and there is evidence that these traps catch toads (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007). It is likely that the presence of insects around these lights is sufficient to overcome their repulsive effects. However, the observations from this experiment suggest that the white fluorescent lights (or incandescent lights) for traps may not maximise trap success. In fact, they may actually repel toads that would otherwise be attracted to the insects the lights have attracted. My experiments suggest that UV lights may be less likely to repel toads from traps. The results concerning the yellow “anti-insect” light are not as clear. When paired with darkness it did not significantly differ from the no light condition, however when paired with incandescent light it appears that there might be a trend for yellow “anti-insect” light to repel toads. In any case, the benefit of having lights on traps is most likely due to their attractiveness to insects. Hence, using yellow “anti-insect” lights on traps may

be counter-productive; although they do not repel toads (when paired with darkness), they also may attract fewer insects (Hinton 1974). Thus, the light type that is probably the most beneficial for attracting insects and not repelling toads from traps, is the UV or “black” light. UV lights failed to repel toads both when paired with darkness and when paired with other lights. There is also evidence that UV lights are among the most successful lights for attracting insects (Hinton 1974). Many entomologists use UV lights for insect collection (Mitchell 1979; Schauff 1986; Waringer 2003; Bishop, Bellis et al. 2006). The efficiency of these lights when deployed on traps should be tested in field trials comparing the capture success of traps equipped with white fluorescent lights (which are now commonly used) and UV lights.

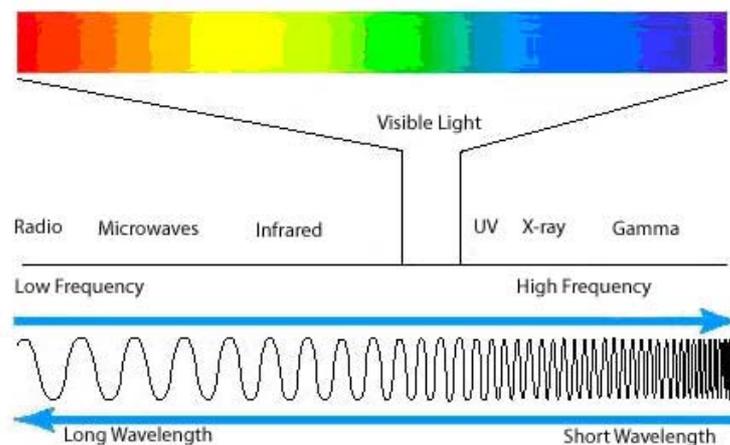


Figure 2.5 Light Spectrum (Pollution Issues 2008). Ultraviolet light and the blue lights are both shorter wavelengths and therefore toads may be able to discriminate between them and other light wavelengths.

To further improve the effectiveness of toad traps further research analyzing the effectiveness of other potential lures (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007) and combinations of lures is recommended. In addition to modified light

sources, as suggested here, other lures such as modified mating calls, pheromones, etc. should be evaluated to further increase trapping success.

Chapter 3

Cane Toad (*Chaunus marinus*) Advertisement Calls: An Analysis from North Queensland, Australia

3.1 Introduction

Advertisement calls occur in virtually all anurans, but most of what is known about anuran advertisement calls comes from studies from relatively few taxa: *Alytes sp.* (Lea, Dyson et al. 2001), *Bufo sp.* (Howard 1988; Cherry 1993; Howard and Young 1998; Castellano, Rosso et al. 2004), *Epipedobates sp.*, (Hodl, Amezcuita et al. 2004), *Hyla sp.*, (Gerhardt 1982; Gerhardt 1991; Gerhardt and Watson 1995; Brenowitz and Rose 1999; Burmeister and Wilczynski 2000; Bee 2008), *Hyperolius sp.* (Jennions, Backwell et al. 1995), *Physalaemus sp.* (Bernal, Rand et al. 2007), and *Rana sp.* (Brzoska 1982; Bee, Perrill et al. 2000; Boatright-Horowitz, Horowitz et al. 2000). Descriptions of call characteristics for individual populations of anurans provide important information about factors used in mate attraction (Gerhardt 1982; Gerhardt 1991; Jennions, Backwell et al. 1995; Howard and Young 1998; Boatright-Horowitz, Horowitz et al. 2000; Hodl, Amezcuita et al. 2004). Although the call characteristics of some *Bufo* species have been described, there is surprisingly little information (Jaeger 1976; Pettigrew and Carlile 1984; Orchinik, Licht et al. 1988; Bowcock, Brown et al. 2008) about the call characteristics of one of the most notorious members of this group, the cane toad *Chaunus marinus*. Cane toads have been widely introduced in tropical areas of the world, and are declared among the 100 worst invasive species (ICUN 2006). Given the widespread nature of this species, there is potential for the evolution of

divergent call characteristics (Blair 1958). Descriptions of the call characteristics of individual populations are required for comparison among populations. In addition, there is considerable need for management of cane toads (Lampo and De Leo 1998; Department of Primary Fisheries 2008; World Wildlife Fund (WWF) Australia 2008).

Recent research has indicated that both male and female toads are attracted to their own advertisement calls (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007) but there is no formal description of calls for Australian *Chaunus marinus* in the literature. In fact, it appears that there is also no published description of the cane toad call in its natural range. This study describes toad advertisement calls in North Queensland and compares them to the calls of other Bufonids with the aim of gaining an insight into the variability within this population. Understanding the call variability will lend clues to which call characteristic may play important rolls during mate attraction, and if those particular call characteristics can be manipulated to produce a highly attractive call. Calls that are highly attractive for sexual selection might prove useful to use as lure on cane toad traps, aiding in the short-term control of this species. The goal of this research is to apply this understanding of call characteristics to find an acoustic lure which increases trap attractiveness and thus the effectiveness of *C. marinus* short-term management techniques.

3.2 Methods

Cane toad advertisement calls were recorded during the breeding season (December 2007- February 2008) at breeding ponds located along Herveys Range Road near Townsville, Queensland, Australia (site A 19.32153°S, 146.61243°E; site B 19.31535°S, 145.58803°E; site C 19.339305°S, 146.50723°E). Over a period of 5 nights,

130 *C. marinus* calls were recorded from 26 individual calling males (average of 5 calls per male) using an *M-Audio* microtrack 24/96 portable digital recorder and *Sennheiser* ME66 microphone. After recording, the toads were captured then euthanised by freezing. Each call was recorded with the microphone held 1 metre from the calling toad. Calls were recorded in .wav format and analysed using *Audacity 1.2.3* (Mazzoni 2004) and *Raven Lite 1.0* (Maher, Mills et al. 2006).

Each of the following call characteristics were measured for each recorded advertisement call: call length (length of each call from start to finish); pulse rate (number of pulses per second); time between calls (period of time from the end of one call to the beginning of the next call); and dominant frequency (the frequency between the highest and the lowest frequency contained in each call).

The analysis of characteristics of natural toad calls was performed using the descriptive statistics module in SPSS v15.

3.3 Results

Call length (n=130) had a mean of 8.16 seconds, median of 7.7 seconds, mode of 5.91 seconds, standard deviation of 2.71, minimum of 2.37 seconds and maximum of 16.63 seconds (Figure 3.1). Pulse rate (n=130) had a mean of 15.28 pulses per second (p/sec), median and mode of 15 p/sec, standard deviation of 0.577, minimum of 14 p/sec and maximum of 17 p/sec (Figure 3.2). Time between calls (n=100) had a mean of 12.11 seconds, median of 8.66 seconds, mode of 4.22 seconds, standard deviation of 9.84 seconds, minimum of 3.09 seconds and maximum of 47.66 seconds (Figure 3.3). Sample size differed in time between calls due to individual toads ending their calling with no additional call to measure between. Dominant frequency (n=130) had a mean

of 613.71Hz, median of 615.87Hz, mode of 614Hz, standard deviation of 22.75Hz, minimum of 524.88Hz and maximum of 753.55Hz (Figure 3.4).

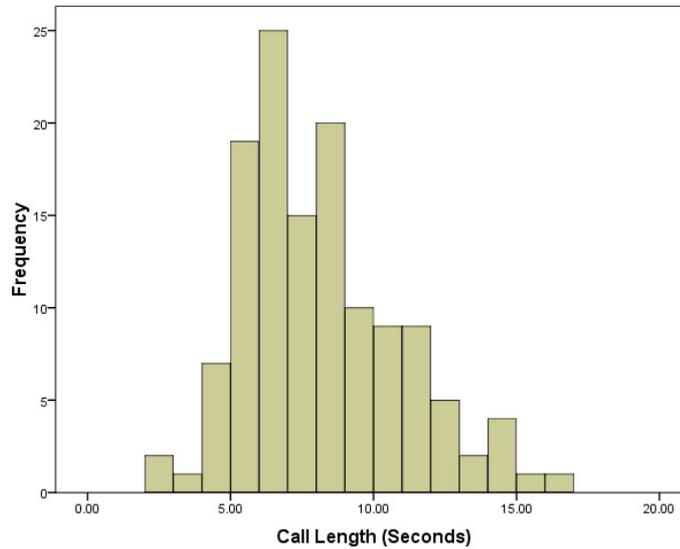


Figure 3.1 Frequency Distribution of Call Length (seconds) for *Chaunus marinus*. Call length (n=130) had a mean of 8.16 seconds, median of 7.7 seconds, mode of 5.91 seconds, standard deviation of 2.71, minimum of 2.37 seconds and maximum of 16.63 seconds.

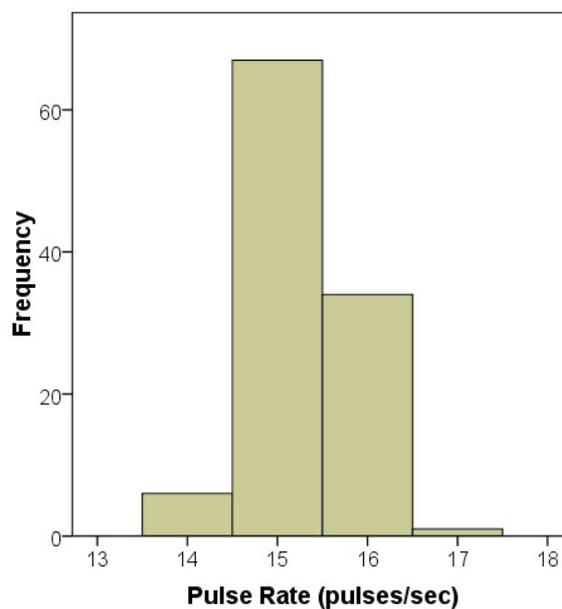


Figure 3.2 Frequency Distribution of Pulse Rate (p/sec) for *Chaunus marinus*. Pulse rate (n=130) had a mean of 15.28 pulses per second (p/sec), median and mode of 15 p/sec, standard deviation of 0.557, minimum of 14 p/sec and maximum of 17 p/sec.

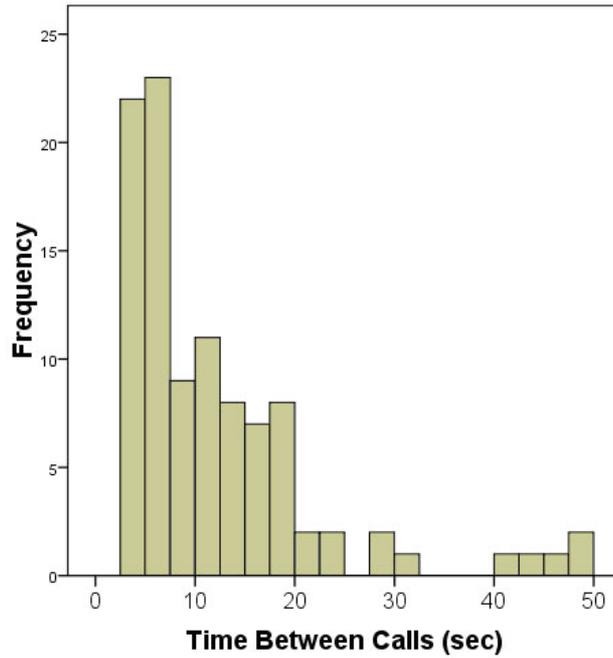


Figure 3.3 Frequency Distribution of Time between Calls (seconds) for *Chaunus marinus*. Time between calls (n=100) had a mean of 12.11 seconds, median of 8.66 seconds, mode of 4.22 seconds, standard deviation of 9.84 seconds, minimum of 3.09 seconds and maximum of 47.66 seconds.

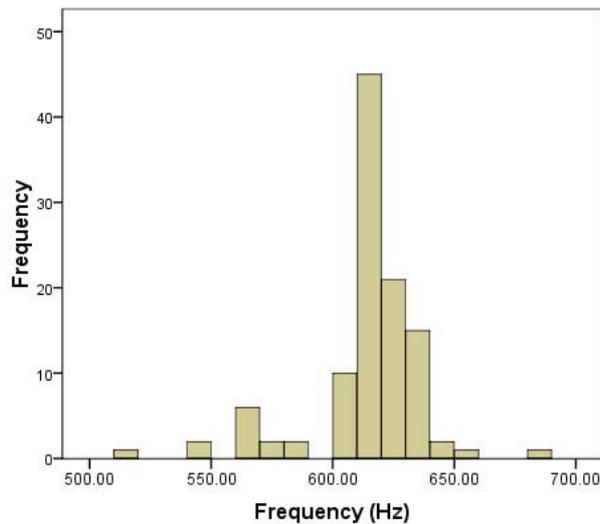


Figure 3.4. Frequency Distribution of Dominant Frequency (Hz) for *Chaunus marinus*. Dominant frequency (n=130) had a mean of 613.71Hz, median of 615.87Hz, mode of 614Hz, standard deviation of 22.75Hz, minimum of 524.88Hz and maximum of 753.55Hz.

When comparing the calls of *C. marinus* with *B. valliceps* and *B. americanus*, the results are not drastically different. Dominant frequency recorded for all three species were similar (Tables 3.1). The variation in pulse rate of the *C. marinus* population was the same as in *B. valliceps*, but was higher in *B. americanus*. Call length varied similarly in *C. marinus* and *B. americanus*, but was higher in *B. valliceps* (Table 3.2).

Table 3.1 Comparison of call characteristics between *Chaunus marinus*, *Bufo valliceps* and *Bufo americanus*. Call characteristics for *B. valliceps* (Wagner & Sullivan 1995) and *B. americanus* (Howard & Young 1998) compared to *C. marinus*. All figures are mean +/- one standard deviation.

	<i>Chaunus marinus</i>	<i>Bufo valliceps</i>	<i>Bufo americanus</i> (4 separate years of data)			
Call Length (seconds)	8.16 ± 2.17	3.15 ± 1.15	6.6 ± 1.4	7.2 ± 1.5	7.8 ± 2	8.4 ± 2.2
Dominate Frequency (Hz)	651.19 ± 35.87	1480 ± 0.08	1797 ± 92	1770 ± 98	1841 ± 97	1745 ± 125
Pulse Rate (Pulses per second)	15.43 ± 0.658	35.9 ± 1.52	46.8 ± 4	47.2 ± 4.8	39.2 ± 2.8	33.2 ± 2.8

Table 3.2 Comparison of variation of call characteristics in *Chaunus marinus*, *Bufo valliceps*, and *Bufo americanus*. Coefficients of variance listed for each call characteristic.

	<i>C. marinus</i>	<i>B. valliceps</i>	<i>B. americanus</i>
Call Length	0.27	0.37	0.21-0.26
Dominant Freq	0.06	0	0.05-0.07
Pulse Rate	0.04	0.04	0.07-0.10

3.4 Discussion

The data analysed the variation within all of the recorded calls, including the within-individual as well as among-individual variation. The data collected from this *Chaunus marinus* population has about the same amount of variation of other recorded *Bufo* species in North America (Sullivan and Wagner 1988), thus pseudoreplication of calls is not likely an issue. Pulse rate appears to be the recognition mechanism for different species of Bufonids (Sullivan and Wagner 1988). There was also variation among males within each of the three species (Sullivan and Wagner 1988; Howard and Young 1998) as with what was seen in the recording of *Chaunus marinus*. *Bufo valliceps* and *Bufo americanus* both have female-choice-dominated mating systems (Sullivan and Wagner 1988; Howard and Young 1998) and the differences among males are likely due to male competition for females. *Chaunus marinus* has similar levels of variation in calls, and is likely operating under the same female-choice mating system (Howard 1988; Howard and Young 1998) leading to call competition between males.

By understanding the baseline data for *C. marinus* call characteristics, it will be possible to evaluate the effectiveness of particular call characteristics as potential

attractants, which may prove valuable in finding effective acoustic lures to improve trapping efforts.. Without the baseline data, it would be impossible indicate which variables should be manipulated, and to what degree. Manipulation of important call characteristics could find a “superstimulus” quality that will be highly attractive to cane toads. The two main characteristics of anuran calls that often vary, and are important in mate selection, are pulse rate (Straughan 1975; Cherry 1993; Gerhardt and Watson 1995; Howard and Young 1998; Wollerman 1998; Bee 2008) and dominant frequency (Straughan 1975; Gerhardt 1982; Gerhardt 1991; Lopez and Narins 1991; Jennions, Backwell et al. 1995; Wagner and Sullivan 1995; Howard and Young 1998; Bee, Perrill et al. 2000; Witte, Farris et al. 2005). Dominant frequency recorded for all three species were similar and *C. marinus* has the same amount of variation in pulse rate as *B. valliceps*, which indicated that the pulse rate and dominant frequency might play a role in sexual selection for the cane toad as well. Call length might also be important in sexual selection (Bee 2008) for *C. marinus* due to the amount of variation.

This research describes the means and ranges of call characteristics for cane toads, and it is highly recommended that these calls be used to further research toad traps through experimental trials using cane toad calls as trap attractants. By varying call characteristics within and outside the limits of the population distribution, it might be possible to create a “superattractive” call (Arak 1983; Ramer, Jenssen et al. 1983; Sullivan and Wagner 1988; Schwartz, Buchanan et al. 2001). Previous research indicated that toads respond strongly to playbacks of their own, unmodified mate attraction calls (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007). Both male and female toads respond to mate attraction calls of male toads. However, according to studies of sexual selection in other frogs, if calls are modified, for example by increasing pulse rate and lowering dominant frequency (Bernal, Rand et al. 2007), it

is difficult to say whether male toads will be attracted. Males in other species (*Leptodactylus albilabris*) responded to call playbacks by altering their own call with a change of frequency (Lopez, Narins et al. 1988). *Bufo calamita*'s behavioural response to playback calls depended on which call was played; males swam away when a low-frequency call was played, but when a high-frequency call was played the males attacked the speaker (Arak 1983). In an experiment with *Rana clamitans*, the responses of males to high-frequency and low-frequency calls depended on the size of the male; large males responded antagonistically to the low-frequency call while small males would give weak or no response; a high-frequency call evoked strong agonistic vocalizations from small males, large males responded by a decrease in the rate of calling (Ramer, Jenssen et al. 1983).

The observations of this research serve as a basis for experimental manipulations of call characteristics to evaluate the attractiveness of manipulated calls to male and female toads; the goal of which is to find an attractive lure to improve trapping success.

Chapter 4

Putting Sexual Selection to Good Use: Experimental Evaluation of the Attractiveness of Natural and Modified Mating Calls to Invasive Anurans

4.1 Introduction

The introduction of cane toads (*Chaunus marinus*) into northern Australia has had a significant negative effect on the native fauna (Froggatt 1936; Covacevich and Archer 1975; Crossland 1997; Crossland and Alford 1998; Ewel, O'Dowd et al. 1999; Phillips, Brown et al. 2003; Boland 2004; Phillips, Brown et al. 2004; Webb, Shine et al. 2005; Phillips and Shine 2006a; Phillips and Shine 2006b), and they are continuing to threaten native species (Letnic, Webb et al. 2008). Thus far, management efforts have been limited to trapping of adults (Sawyer 2006) while research continues into less labour intensive solutions (Commonwealth Scientific and Industrial Research Organisation (CSIRO) 2006; Land Protection 2006; Tyler 2006).

Recent research suggests that trapping success can be improved by using lures (Schwarzkopf and Alford 2006). Food odors did not prove unambiguously attractive to toads (Schwarzkopf and Alford 2006), but playbacks of mating calls improved trap effectiveness three-fold (Schwarzkopf and Alford 2007). Strong attractiveness of mating calls to females is not surprising; as these calls have evolved to attract them (Ryan 1988). More puzzling, but useful in a trapping context, was the observation that male toads were also strongly attracted to calls (Schwarzkopf and Alford 2007).

Studies of sexual selection in anurans suggest that calls can be modified to form a superstimulus to females (Gerhardt 1982; Gerhardt and Watson 1995). Typically,

calls with lower dominant frequencies and higher pulse rate calls are more attractive to female anurans (Gerhardt 1982; Gerhardt and Watson 1995). However, it is not clear whether modified calls would attract males, or, if modified calls did attract males, which modifications would be most attractive to them. It is even conceivable that modified calls might repel males, for example if they sound like large males (Gerhardt 1982).

I measured the attractiveness to male and female cane toads, in an experimental arena, of a series of experimentally modified calls. I engineered calls to be just outside the natural ranges and measured their attractiveness by observing how closely toads approached speakers producing the calls. The toads were also evaluated for their sexual condition at the time of the trial to examine any effects this may have on call attraction. The goal of this research is to evaluate the attractiveness of different call characteristics of cane toads to both male and female cane toads, with the hopes of combining the calls with traps to improve trapping success.

4.2 Methods

These experiments were conducted on the James Cook University campus (19° 15'42"S; 146°48'38"E) between 29 February and 6 June 2008. *Chaunus marinus* individuals were collected from the area around the James Cook University campus in Townsville, Queensland. The toads were used in experiments on the night of capture, or were held in 1000 litre plastic cattle watering tanks overnight, and tested on the night following capture. Holding tanks were placed at an angle with a small amount of water in the bottom, to allow toads' access to both water and dry conditions. The tanks were covered with 80% shade cloth. After being used for experiments, toads were euthanised

by freezing and kept for dissections (see below). A total of 37 toads (19 males and 18 females) were used in experimental trials.

4.2.1 Call Recordings

Recordings of calling male cane toads were made in the Townsville region during the mating season (December 2007 - February 2008; Chapter 3). Each toad was recorded using a portable digital audio recorder *M-Audio* microtrack 24/96), equipped with a *Sennheiser* ME66 microphone, while calling in the wild. Recordings were made in the Townsville area at several different breeding ponds (along Herveys Range Road: Site A 19.32153°S, 146.61243°E; Site B 19.31535°S, 145.58803°E; Site C 19.339305°S, 146.50723°E). Each toad call was recorded, and sound pressure readings taken with the microphone or sound pressure meter (CE Digitech™ Model QM1588) held 1 meter away from the calling toad. Toad temperatures (to the nearest °C, with an infrared thermometer), snout to vent length (to the nearest mm with calipers) and mass (to the nearest .05 g with a spring balance, Pesola™) were recorded. I measured call length, frequency, pulse rate and the time between calls on each call recorded (Chapter 3).

4.2.2 Call Manipulation

A pre-recorded *Chaunus marinus* call was obtained from the CD *Frog Calls of the Tropical Northeast* (Stewart 1998), the same toad call that was used in previous experiments (Schwarzkopf and Alford 2007). The call was copied directly from the CD in 44,000 samples per second .wav format. A “normal” call was created using this

original call from the *Frog Calls of the Tropical Northeast* CD that matched the mean or median values of each call characteristic (call length, frequency, pulse rate and time between calls) based on values measured from recorded natural calls (Chapter 3). I then modified calls to be higher frequency, lower frequency, higher pulse rate and lower pulse rate than natural calls recorded in Chapter 3. Digital modifications were performed using the Cool Edit 96 (Johnston 1996) and Audacity 2006 (Mazzoni 2004) computer software programs.

The normal call (Norm) had a fundamental frequency of 601Hz, 15 pulses per second, was 8 seconds long and was 4.2 seconds between looped calls, looped for 5 minutes (Figure 4.1). All other calls were created using this “normal” call, such that all other call characteristics remained identical, except the manipulated parameter. In the high frequency (HF) call the fundamental frequency was altered from 601Hz to 696Hz. All other parameters were the same as the normal call (Figure 4.2). In the low frequency (LF) call the fundamental frequency was altered from 601Hz to 496Hz. All other parameters were the same as the normal call (Figure 4.3). In the high pulse (HP) call the pulse rate was altered from 15 to 18 pulses per second. All other parameters are the same as the normal call (Figure 4.4). In the low pulse (LP) call the pulse rate was modified from 15 to 13 pulses per second. All other parameters are the same as the normal call (Figure 4.5).

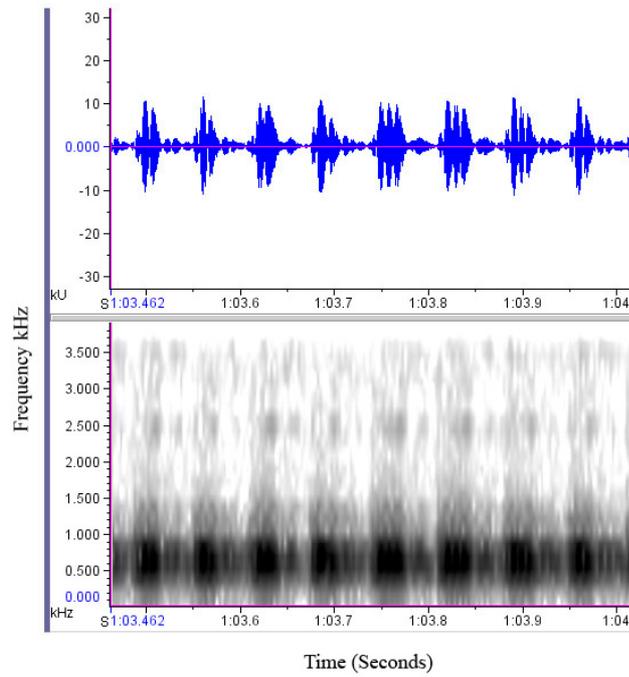


Figure 4.1 Standardised normal *Chaunus marinus* mating call. Call based on mean and median characteristics of *C. marinus* calls recorded around Townsville. The fundamental frequency of 601Hz, 15 pulses per second, was 8 seconds long and was 4.2 seconds between looped calls, looped for 5 minutes.

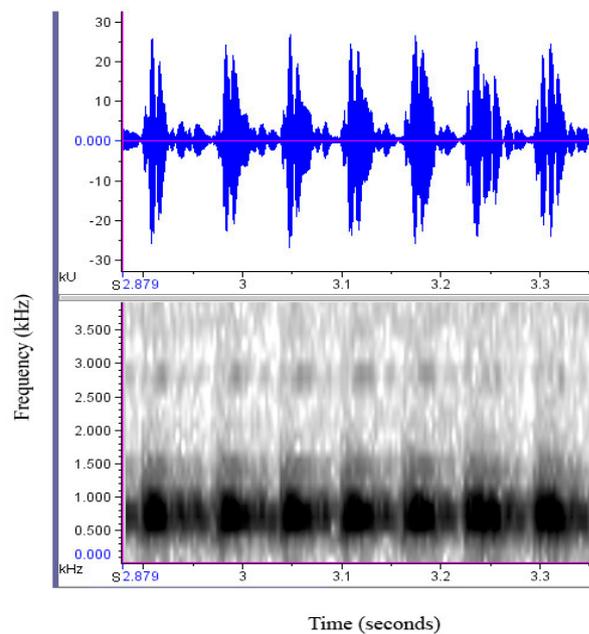


Figure 4.2 Experimentally modified high-frequency *Chaunus marinus* call. The frequency was modified from 601Hz to 696Hz.

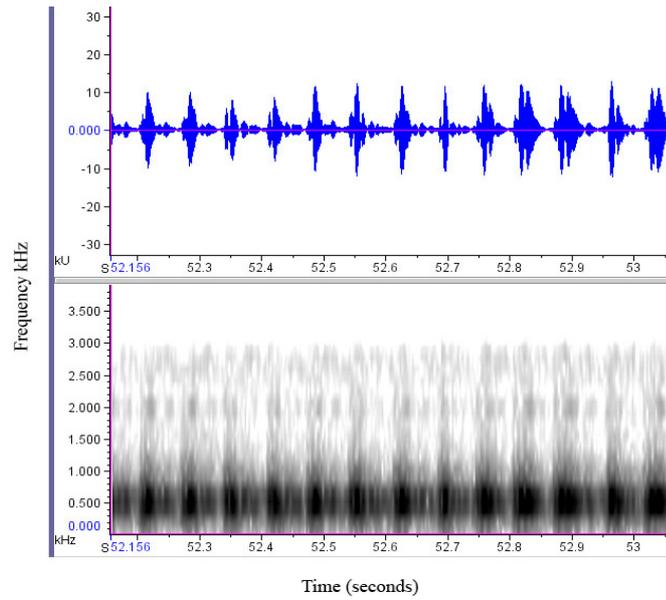


Figure 4.3 Experimentally modified low-frequency *Chaunus marinus* call. The frequency was modified 601Hz to 496Hz.

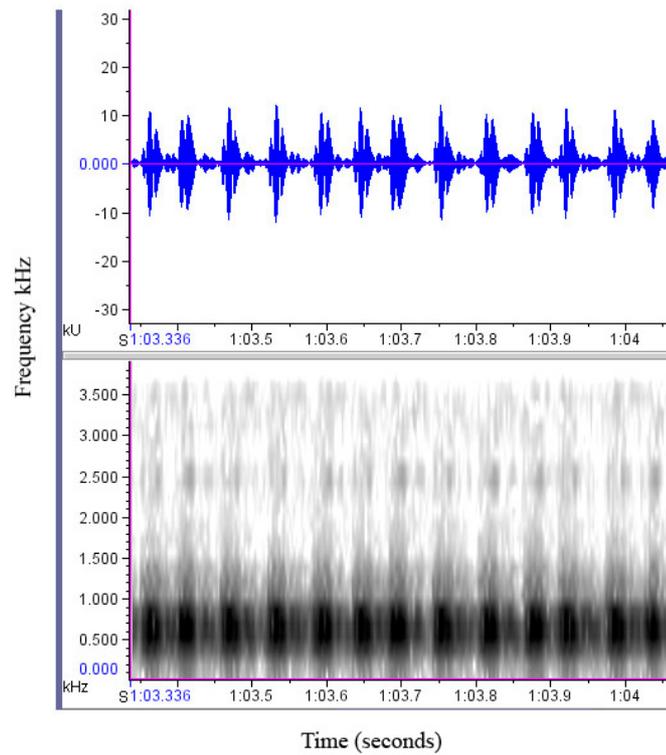


Figure 4.4 Experimentally modified high pulse rate *Chaunus marinus* call. The pulse rate was modified from 15 p/sec to 18 p/sec.

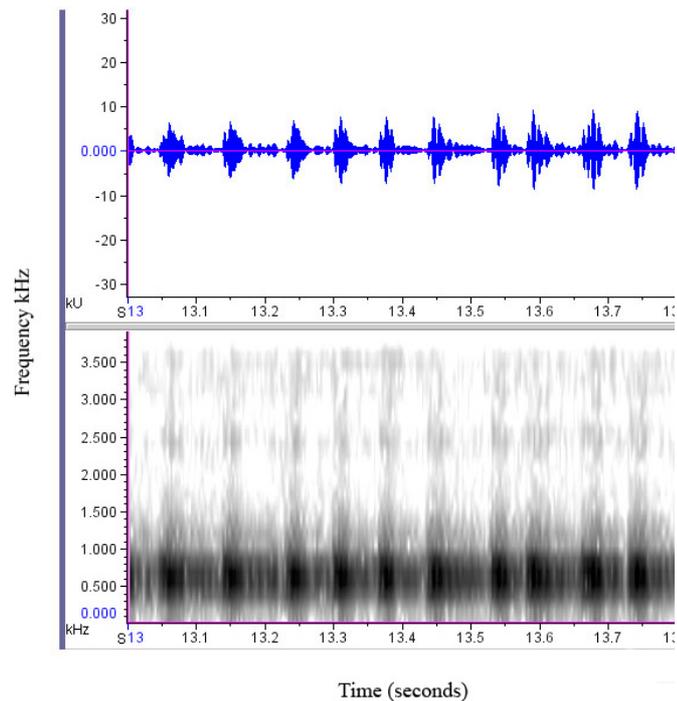


Figure 4.5 Experimentally modified low pulse rate *Chaunus marinus* call. The pulse rate was modified from 15 p/sec to 13 p/sec.

I tested the attractiveness to toads of the five call modifications in an acoustic arena located in the James Cook University “Freshwater Research Compound”. The arena was 7.6m in diameter, with a 1.2m high wall constructed of plastic-coated sheet metal. Latex sound insulating foam (Dunlop, 2.5mm) was attached to the side of the arena using contact cement (Dap & Wellwood Contact Cement) to reduce any sound reverberations from the metal siding.

Sound pressure meter readings from a speaker (Realistic™ Minimus 0.6) playing sounds at 60 decibels (measured at 1 metre from the speaker) were taken from inside the arena at 1 metre intervals to ensure that as distance from speakers increased, sound pressure decreased predictably. A map of these pressure levels (Figure 4.6) indicated that the speaker was indeed the loudest point in the arena, with SPL decreasing in all directions away from the speaker. A video recording camera (AVC

301 B/W CCD) was mounted on a large tripod 4 m above the arena, and 7 infrared spotlights (CCD Camera Infrared Day/Night N28, 56 LED) provided light to allow observation of the toads' behaviour on a remote video monitor (Teac CT M342H) throughout experiments. Twelve paving bricks were painted black and placed around the arena at approximately 22.5° intervals, to simulate the appearance of speakers.

Before starting each trial, a randomly selected paving brick in one of 4 quadrants of the arena was removed, and one speaker connected to an mp3 player (Beat™ Digital MP3 Player) was placed in that location. The speaker was turned on and played a randomly selected toad call type at 60 decibels (1 metre from the speaker). Each trial commenced when a small, metal, bottomless cage containing a toad, placed in the centre of the arena, was raised using a rope-and-pulley system, from outside of the arena. Toads were observed until they were motionless inside the cage, and then the cage was raised.

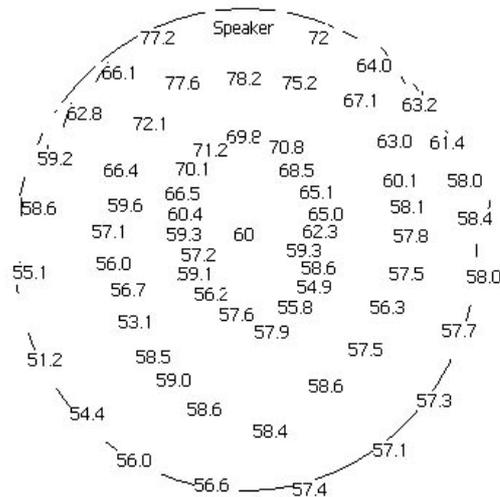


Figure 4.6 Sound Pressure Readings. Sound pressure readings were taken at 1m intervals from inside the arena at ground level to ensure that there were no substantial sound reverberations inside the arena.

Every call type was tested on each of 37 toads. The control for this experiment was a no sound (NS) condition, in which all other variables remained the same, except there was no sound being played for the toads. Previous experiments had demonstrated that toads did not respond to white or “pink” noise controls (Schwarzkopf and Alford 2007). Every toad experienced all 6 treatments in random order, one stimulus after another with a waiting period of 5 minutes between trials (toads placed in a holding bucket) while the arena was set up for the next experiment. The speaker placement was also randomized to one of the four quadrants of the arena for every trial.

Each trial was allowed to proceed for 5 minutes. The toads’ movements were recorded by tracing their paths onto a clear plastic overhead projection transparency film (Xerox Ink Jet Transparencies, 40L91334) placed directly onto the monitor. This provided an accurate record of the toads’ movements inside the arena. After each five-minute trial, the toad was retrieved, placed into a 20 L holding bucket and the arena was set with the new speaker position and new sound stimulus for the next experimental treatment.

The transparencies were scanned (Hewlett Packard PCS 1315) into digital format and analyzed using the *Image Tool* program (UTHSCSA 2002). Each toad’s closest position to the speaker for each call type was determined, and then the closest the toad got to the speaker in the No Sound control was determined. The distance from the speaker to the toad when there was a sound playing was subtracted from the distance from that toad to the speaker in the No Sound controls. This converted the measure of absolute distance from the speaker to the toad to a distance relative to the distance when there was no sound. This relative distance measure was necessary to control for differences in overall movement tendency of different toads. Differences among distances were analyzed using the SPSS Statistical software package V. 14.

The toads from these trials were euthanised and dissected and their reproductive condition was examined. Right testis length (in millimeters) in males and egg development (on a scale from 0 to 4; 0 = no egg development to 4 = mature egg development) in females were measured for each toad to establish reproductive condition.

I examined relative nearest distance to speaker, total distance moved, and straightness of path (total displacement divided by total distance moved) for males and females in different reproductive states.

4.3 Results

Examining the difference between the toad's distance to the speaker in the no sound treatment compared to the toad's distance to the speaker in the sound treatments, there was no significant difference between the responses of males and females to each call type examined independently (HF: $t = -1.895$, $df = 18$, $p = 0.074$; HP: $t = -0.693$, $df = 21$, $p = 0.496$; LF: $t = -1.114$, $df = 19$, $p = 0.279$; LP: $t = -0.388$, $df = 19$, $p = 0.702$; Norm: $t = 0.098$, $df = 21$, $p = .923$). Because the effect of sex on distance to speaker was non-significant, both sexes were combined for the remainder of the analysis.

During control treatments (when no sound was played) there was a significant bias in directionality of toads, they were much more likely to go to the south of the arena ($t = -2.431$, $df = 28$, $p = 0.022$, figure 4.7). Because of this bias I only analyzed responses to calls when the speaker was placed on the opposite (unattractive) side of the arena (HF $n = 20$, HP $n = 23$, LF $n = 21$, LP $n = 21$, Norm $n = 23$) to err on the side of caution.

Because toads had different propensities to move, influencing the distance they approached the location of the speaker in each trial, I subtracted the minimum distance each toad reached from the location of the speaker in each trial during the no-sound treatment from the minimum distance to the speaker for each call type, as explained in the methods section. Toads approached the speaker more closely when calls were played than during the no sound control (all mean relative approach distances were positive, Figure 4.8). I Bonferroni adjusted significance levels to account for multiple tests (alpha level for each of the four tests was 0.0125). When the high pulse rate calls were played toads came significantly closer to the speaker position than in the control (no sound) treatment (HP: $t = 2.853$, $df = 22$, $p = 0.009$), whereas high frequency calls (HF: $t = 1.956$, $df = 19$, $p = 0.065$), low frequency calls, low pulse rate calls and normal calls were not significantly attractive in these trials (LF: $t = 2.417$, $df = 20$, $p = 0.025$, LP: $t = 0.833$, $df = 20$, $p = 0.414$; and Norm; $t = 1.203$, $df = 22$, $p = 0.242$), although the low frequency calls closely approached the adjusted cutoff for significance.

So that I could use a multivariate repeated-measures analysis, I used all the movement data (including toad movements towards the “attractive” side of the arena) to analyse the effect of sexual condition on responses to toad calls. The response was the vector of responses for each individual in all trials in which sound was played. Excluding data for which the speaker was on the more attractive side of the arena resulted in almost all individuals having missing values, since the speaker was relocated randomly between trials. Females were lumped into two categories, those with eggs and those without. Females with eggs showed a trend to approach the speaker more closely than those without, however this trend was not significant using a multivariate repeated-measures analysis (Wilk’s Lambda, $F=0.940$, $df = 4, 11$, $p= 0.477$). The females with eggs moved further in the arena than those without eggs (Wilk’s Lambda,

F= 15.446, df = 4, 10 p< 0.0001). Females with eggs also moved in a straighter line than did those without (Wilk's Lambda, F= 4.895, df=4, 10, p= 0.019).

There was no correlation between male testis size and relative distance to speaker for any call type (Wilk's Lambda, F= 0.963, df = 4,10 p= 0.984), between testis size and total distance moved (Wilk's Lambda, F= 0.896, df = 4,10 p= 0.911), or between testis size and straightness (Wilk's Lambda, F= 0.721, df=4,10 p= 0.568).

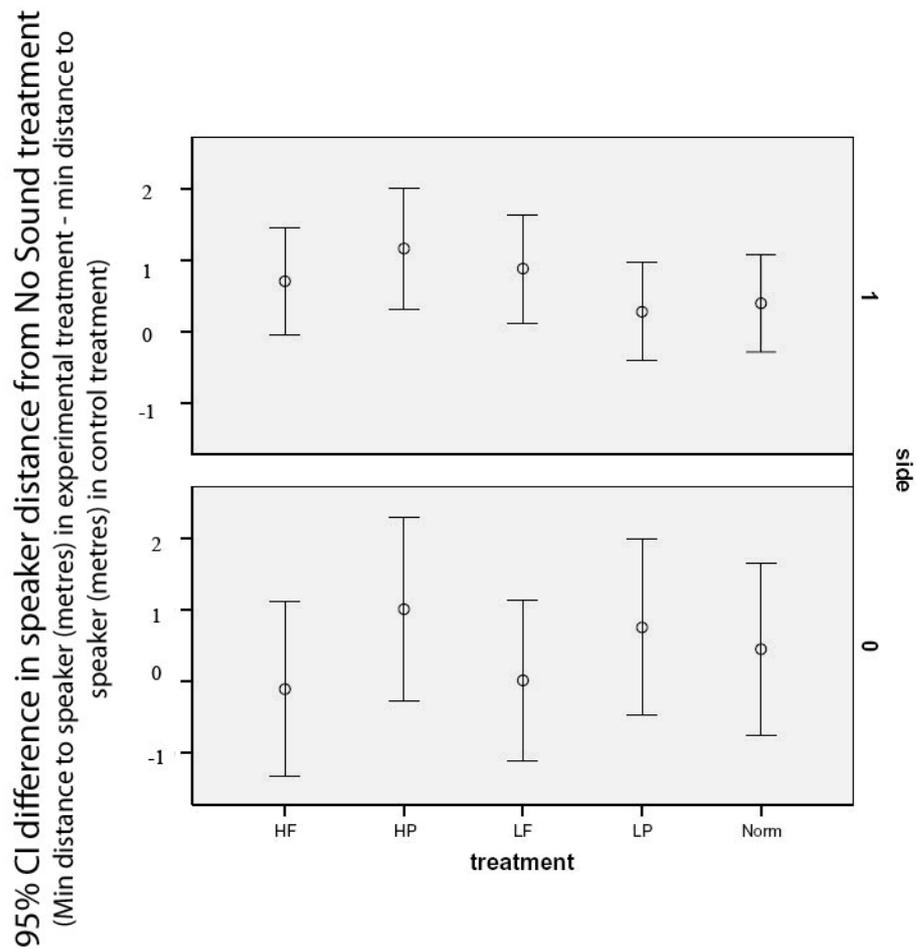


Figure 4.7. Directional Bias in the Acoustic Arena. This graph illustrates the directional bias found in the NS (control) treatments versus the experimental sound trials. The responses of toads to the sound treatments when the speaker was placed in Side 0 of the arena are closer than the responses in Side 1 of the speaker. In the NS control trial, toads had a bias for entering into Side 0, which affected the sound trials located on that side of the arena.

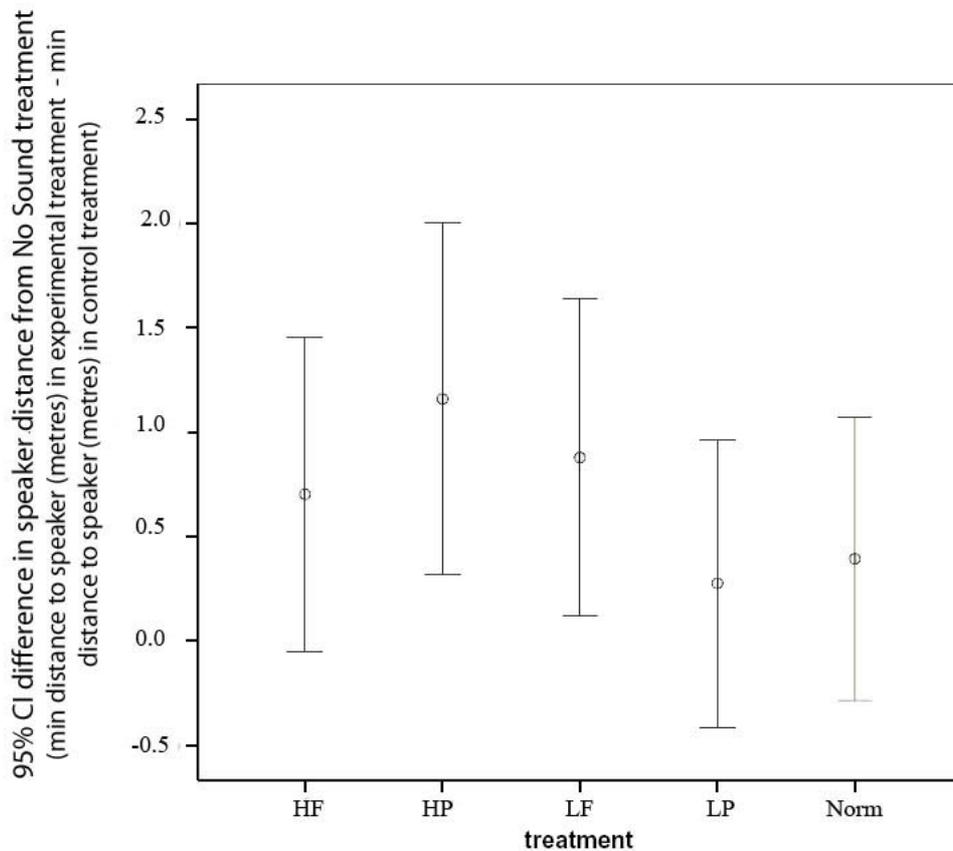


Figure 4.8. Mean minimum relative distance to speaker: difference between Experimental Treatments and Control Treatments. This graph illustrates the attractiveness of the manipulated *C. marinus* advertisement calls. The minimum distance to the speaker in the HP condition was significantly closer than in the NS conditions; however all of the means are above 0 indicating that all calls are possibly attractive to cane toads.

4.4 Discussion

Compared to no sound, both male and female toads were significantly attracted to high pulse rate calls, and showed a tendency for attraction to high pulse rate calls, although not significant following Bonferroni correction. These results are consistent with studies on other anuran species, which often indicate that high pulse rate and low dominant frequency are attractive characteristics (Straughan 1975; Gerhardt 1982;

Gerhardt 1991; Lopez and Narins 1991; Jennions, Backwell et al. 1995; Wagner and Sullivan 1995; Howard and Young 1998; Wollerman 1998; Bee, Perrill et al. 2000; Witte, Ryan et al. 2001; Bee 2008). In addition, although it did not approach significance following Bonferroni correction, the high frequency call did appear to show a trend of being more attractive than no sound. On average, toads approached the speakers more closely when there was sound than without but, surprisingly, the normal call was not significantly more attractive on average than the no sound control, even though the trend was present. The result in which a normal call is no more attractive than no sound contrasts with results demonstrating that a pre-recorded cane toad call was attractive to toads, both in the arena and in traps (Schwarzkopf and Alford 2006; Schwarzkopf and Alford 2007). It is important, however, to remember that my “normal” call was modified using mean characteristics from natural calls, whereas Schwarzkopf and Alford (2007) used the ‘natural’ call from a frog call recording (Stewart 1998). The call used by Schwarzkopf and Alford (2007) had a dominant frequency of 660Hz, 16 pulses/sec, and a length of 6.7 seconds long, which has a higher frequency and a higher pulse rate than my “normal” call. Thus, the “normal call” used by Schwarzkopf and Alford (2007) may have been somewhat more attractive than the one I used.

The inherent bias in movement direction in the arena was probably due to light from a newly installed street light located within 200m of the arena. Although the arena was not brightly lit, one side was just perceptibly lighter than the other, and it was this side the toads avoided. My other experiments (Chapter 2) demonstrated that toads prefer darkness over light, and these experiments inadvertently demonstrated the same phenomenon. By removing from the main analysis all trials conducted on the attractive “dark” side of the arena, I ensured that estimates of call attractiveness were

conservative. This conservative approach may also explain the lack of significance of the “normal” call in these experiments. Perhaps ‘normal’ toad calls are not as attractive if toads have to move into a lighter area to locate them.

While sexual condition didn’t appear to have any bearing on male response to calls, females did behave differently when they were gravid. Females had a tendency to move closer to the speaker when gravid, and they moved more overall and in straighter patterns. In a previous study female *Alytes muletensis* varied in their choice of frequency calls when in different reproductive states (Lea, Dyson et al. 2001) and another study found that the closer to having mature egg a female *Physalaemus pustulosus* become less choosy about call preference (Lynch, Rand et al. 2005). Reproductive condition may influence response to calls in females (Lea, Dyson et al. 2001; Lynch, Rand et al. 2005).

These results may be very useful in improving the success of trapping for *C. marinus*. Further field studies should use these sounds on traps, to test their attractiveness (Schwarzkopf and Alford 2006). High pulse rate or low frequency calls may improve trapping success. Because both these call types were attractive to toads, it would be important to determine the attractiveness of combining these features (for example creating a high pulse rate, low frequency call) for it is possible that in combination they might produce more attractive call than each on their own.

Few studies have examined the attractiveness of mating calls to males in anurans (Arak 1983; Ramer, Jenssen et al. 1983; Lopez, Narins et al. 1988; Bee, Perrill et al. 2000; Boatright-Horowitz, Horowitz et al. 2000; Burmeister and Wilczynski 2000) but features of calls may be especially designed to provide information to males. In a study using dominant frequency, researchers were able to evoke a call response in male *Leptodactylus albilabris* (Lopez, Narins et al. 1988); but in other experiments

researchers have evoked an aggressive response in *Rana clamitans* (Ramer, Jenssen et al. 1983) and *Bufo calamita* (Arak 1983) using calls. This “aggressive” response (to a low frequency call in these cases) may attract large males but not small males. Male size is likely to effect call attraction (Arak 1983; Ramer, Jenssen et al. 1983; Lopez, Narins et al. 1988). In addition, the attractiveness of these calls to males should be examined further, to determine which call characteristics are most attractive to which males, and why.

Chapter 5

Conclusions

For many pest vertebrates, trapping provides the most feasible measure for control (Fritts, Scott et al. 1989; Shivik 1998; Spurr and O'Connor 1999; Shivik, Wright et al. 2000; Savarie, Shivik et al. 2001; Johnston, Savarie et al. 2002; Murphy and Fechney 2003). For example, management of pest stoats *Mustella erminea* in New Zealand has focused on trapping with lures to improve trapping success (Spurr and O'Connor 1999; Murphy and Fechney 2003). Lures have also helped improve trapping success for the brown tree snake, *Boiga irregularis*, in Guam (Fritts, Scott et al. 1989; Shivik 1998; Shivik, Wright et al. 2000; Savarie, Shivik et al. 2001; Johnston, Savarie et al. 2002). At present, trapping cane toads is the most realistic, efficient, economic, and feasible method for control and management (Lever 2001; Schwarzkopf and Alford 2007). My study indicates that change from the use of white fluorescent light to ultraviolet light and the use of modified calls could increase the attractiveness of traps to toads. While the difference between UV and white fluorescent weren't statistically significant, there was a trend for toads to prefer UV over white fluorescent light. Any improvements to trap success are extremely valuable when the ultimate goal is to maximize capture per unit effort.

5.1 Implications for Management

My observations strongly suggest that trapping success for cane toads could be improved by using a UV light instead of the white fluorescent light that is presently common on traps constructed and sold by Frogwatch (Sawyer 2006) and Paul Baker

(2008, pers. comm.) in the Northern Territory. My results suggest that in addition to using UV lights on traps, using any toad call has the potential to increase trapping success (all calls showed a trend for potential attractiveness, although only the high-pulse rate call was significantly more attractive). Utilizing high pulse rate mating calls on traps may increase toad capture rates even further. The outdoor, arena trials were conducted during the wet season (February) through to the dry season months (June) in Townsville. It is possible that some toad behaviour is seasonal in nature and it would be beneficial for further trials that are conducted at other times of the year: throughout the wet season when toads are most active and breeding takes place, and in the dry season when toads are more likely to aggregate around remaining water holes for moisture. Using a combination of improved traps and targeted trapping effort could substantially reduce the investment of money and resources required to control cane toads via trapping.

5.2 Directions for Further Research

Although my experiments suggest that various lures might be successful at enhancing traps, experiments using these lures on traps should be conducted to ensure that call enhancements, and changes in the types of lights used on traps, do increase trap success compared to traditional trapping protocols. Using unmodified mating calls as lures greatly improved trap success in previous field studies (Schwarzkopf and Alford 2007). Similar trials should be carried out using UV lights and the modified cane toad advertisement calls. Calls can be played from mp3 players attached to portable amplified speakers and powered by gel cell batteries. In remote areas, a solar panel and a timer attached to the speaker should provide power and minimize trap maintenance,

allowing traps to be cleared every few days. Trapped toads can be disposed of by humane means, for example by chilling, then freezing them (Sustainable Futures 2008). The optimum use of traps with light and sound lures is probably in relatively remote, ecologically sensitive areas where toads are having a negative impact on specific endemic species (Schwarzkopf and Alford 2007). Traps equipped only with UV lights might be better for deployment in urban areas, where continuous playing of toad calls might be regarded as noise pollution.

Documenting the natural variability of toad calls in north Queensland has shed light on some of the call characteristics that play important rolls for male and female attraction. In this study high pulse rate calls were strongly attractive and low frequency calls showed a marginal effect to both males and females, and further study should consider what roles combinations of these call characteristics may play in attraction. It may be possible to generate a call that is highly attractive to both sexes by, for example, combining low frequency and high pulse rate in a single call. It is also highly likely that a call that is a “superstimulus” for females (i.e. low frequency) may be repulsive to small males and therefore not be suitable for reducing male populations. My data indicated that gravid females behave differently towards calls than non-gravid females; therefore calls that serve as a “superstimulus” for gravid females might not have the same attraction for non-gravid females. Further research may find that trapping protocols need to utilize gender-specific and possibly size-specific toad calls in a given area to effectively control an adequate proportion of the toad population.

The attraction of males to calls in general suggests that further research into this phenomenon might offer insights into the role of calls as signals as well as mate attractants. Limited research and primary literature is available regarding whether mating calls have a purpose outside of attracting females; in particular, few studies have

examined the behavioural responses of males to conspecific calls. It appears that both males and females obtain information about males through their advertisement calls. Some research has suggested that males may even alter their own calls in response to other males (Lopez, Narins et al. 1988). Other males have physically encountered call back speakers in response to low frequency calls that simulate calls from large males (Arak 1983; Ramer, Jenssen et al. 1983). Additional research could examine in more detail which characteristics of male calls elicit responses of various types from conspecific males.

The impact toads will have on presently toad-free areas that are ecologically important will not be realized until the toads invade those areas. Data from many taxa indicate that toads are likely to continue to have devastating effects on native Australian fauna (Burnett 1993; Phillips, Brown et al. 2003; Boland 2004; ICUN 2006; Letnic, Webb et al. 2008). The results of my research can potentially enhance the practicality of localized control of cane toads in northern Australia. This brings the probability for successfully and selectively reducing the impact of this noxious pest within closer reach.

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