Sex, Light, and Sound: Location and Combination of Multiple Attractants Affect Probability of Cane Toad (Rhinella marina) Capture

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Abstract

Invasive species management is a critical issue worldwide, but mitigation strategies are slow to develop, and invader populations often expand too rapidly for eradication to be feasible. Thus, reduction in numbers of individuals is the most heavily used management strategy for invasive pests. While long-term biocontrol agents may take years or decades to develop, simple trap modifications can increase capture of targeted demographic groups, such as ovigerous females. The present study identifies the effectiveness of trap modification and use of multiple attractants to capture the invasive cane toad (Rhinella marina). Cane toad traps typically use lights to attract insect prey. Studies suggest that adding a male cane toad advertisement call to attract toads by phonotaxis may be effective. The aims of this study were to determine whether (i) female capture efficiency was influenced by attractants in the same manner as male and juvenile captures, (ii) an acoustic attractant alone (without a light attractant) was sufficient to attract toads, and (iii) the location of an acoustic attractant (inside or on top of the trap) influenced trap success. Male toads were captured more frequently than females and juveniles; combining light and acoustic attractants increased toad capture; and placing the acoustic attractant inside the trap increased the capture of female cane toads. Removal of adult, ovigerous females is a promising strategy to slow population growth of invasive species. Our results suggest that using a sound attractant inside the trap with a UV light is most effective in targeting that particular cane toad cohort.

Keywords

Rhinella marina, Cane toad, Trap, Invasive, Acoustic attractant, Light attractant, Capture, Management, Mating

Disciplines

Animal Sciences | Environmental Sciences

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Invasive species management is a critical issue worldwide, but mitigation strategies are slow to develop, and invader populations often expand too rapidly for eradication to be feasible. Thus, reduction in numbers of individuals is the most heavily used management strategy for invasive pests. While long-term biocontrol agents may take years or decades to develop, simple trap modifications can increase capture of targeted demographic groups, such as ovigerous females. The present study identifies the effectiveness of trap modification and use of multiple-attractants to capture the invasive cane toad (*Rhinella marina)*. Cane toad traps typically use lights to attract insect prey. Studies suggest that adding a male cane toad advertisement call to attract toads by phonotaxis may be effective. The aims of this study were to determine whether (i) female capture efficiency was influenced by attractants in the same manner as male and juvenile captures, (ii) an acoustic attractant alone (without a light attractant) was sufficient to attract toads, and (iii) the location of an acoustic attractant (inside or on top of the trap) influenced trap success. Male toads were captured more frequently than females and juveniles; combining light and acoustic attractants increased toad capture; and placing the acoustic attractant inside the trap increased capture of female cane toads. Removal of adult, ovigerous females is a promising strategy to slow population growth of invasive species. Our results suggest that using a sound attractant inside the trap with a UV light is most effective in targeting that particular cane toad cohort.

Keywords: *Rhinella marina*; cane toad; trap; invasive; acoustic attractant; light attractant; capture; management; mating

Introduction

Invasive species cause negative economic, environmental, and social impacts globally, and devising successful control strategies is a priority for conservation. While genetic and biological control methods are under development for many exotic pests, traps provide a rapid, economical, and effective strategy for local-scale management. Success of trapping is influenced by behavioural (Greenslade, 1964) and physical factors (Beacham and Krebs, 1980), and manipulation of these characteristics by trap modification helps target specific demographic groups of the pest species. Modifications that increase capture probability of the demographic groups important for population growth increase the impact of each individual capture. For example, removal of ovigerous females from a population eliminates not just mature adults, but future breeding potential as well (Thresher, 2007).

A highly invasive pest established in over 20 countries is the cane toad (*Rhinella marina*), one of the top 100 most destructive invasive species in the world (GISD, 2005). Cane toads compete with native anurans for resources, spread parasites, and cause injury and mortality to many native predators and prey species (Taylor and Edwards, 2005; Bowcock et al., 2009; Garg et al., 2009; Crossland and Shine, 2010). In addition, cane toads are considered unappealing and cause mortality and morbidity of pets (Reeves, 2004). Though research into biological control methods continues, none have been approved for environmental application; exclusion fencing, trapping, and physical removal are the only control methods implemented thus far (Schwarzkopf and Alford, 2007; The State of Queensland DAFF, 2013). Cage traps have been suggested as the most effective and least labour-intensive strategy for controlling toads at present (Lampo and De Leo, 1998; Taylor and Edwards, 2005; Miller, 2006).

Commercial cane toad cage traps use white fluorescent lights to attract insects as prey for the toads (Hienton, 1974; Schwarzkopf and Alford, 2007), but improvements have been made on the original model (Sawyer, 2006). For example, UV lights are more effective toad attractants because they attract insects but do not deter toads, as do white fluorescent lights (Davis, 2008; Schwarzkopf and Forbes, 2010). In addition, an acoustic attractant consisting of a cane toad advertisement call in association with the trap tripled capture rates in a field trapping study (Schwarzkopf and Alford, 2007), but the effect of the attractant's location relative to the trap has not been determined.

In general, adult cane toads immediately before they first reproduce are the ideal target for trapping, because all their potential future offspring are removed from the population, and adults have the highest survival rates (50%) of all life stages (Zug and Zug, 1979; Molloy and Henderson, 2006). It makes sense then, both in terms of effort and biological impact, to target adults, especially immediately pre-reproductive adults, when developing management strategies. However, targeting adult females is the most effective management strategy for long-lived vertebrates, and specifically cane toads (Thresher, 2007). Female toads can carry clutches of 5,000 to more than 35,000 eggs (Molloy and Henderson, 2006; Hagman and Shine, 2008; The State of Queensland DAFF, 2013). With a survival rate from egg to adult of approximately 0.04%, there is a possibility of reducing the population by at least 200 individuals by capturing only one adult female before reproduction (usually two years old for female amphibians), whereas capturing a male toad reduces the adult population directly by only one individual (Molloy and Henderson, 2006; Browne and Zippel, 2007). Even if a significant portion of the male population were removed, one male cane toad can fertilize multiple egg clutches, so recruitment rates may not change significantly, even with the loss of multiple males. Therefore, culling of the female population is likely a more effective way of reducing recruitment into the next generation.

The aims of this study were to determine whether (i) female capture efficiency was influenced by attractants in the same manner as male and juvenile captures, (ii) an acoustic attractant alone (without a light attractant) was sufficient to attract toads, and (iii) the location of an acoustic attractant influenced trap success.

2. Methods

2.1. Study site

This study was conducted at James Cook University in Townsville, Australia (19˚19’47.74”S, 146˚45’29.55”E). The campus is surrounded by poplar gum (*Eucalyptus platyphylla)* woodland with a black spear grass (*Heteropogon contortus)* dominated understory. Cane toads invaded the region in the 1940s (Alford et al., 2006) and have established a self-sustaining population. Daily temperature and rainfall data was retrieved from the Australian Government Bureau of Meteorology Oonoonba station 032057 (Commonwealth of Australia, 2013). Average daily temperature during this study was 22.5°C and average daily rainfall was 5.2 mm.

2.2. Experiment

A 7.6 m diameter circular, sheet-metal arena was set up on a lawn within a chain-link fenced compound near the Biological Sciences building. The walls of the arena stood 1.5 m above ground to ensure that toads could not escape, and sound-absorbent quilted cotton on the walls reduced echoes and external auditory stimuli (Davis, 2008; Schwarzkopf and Forbes, 2010). A cage trap, purchased from Northern Territory FrogWatch (http://www.frogwatch.org.au), was placed in the centre of the arena. The metal cage trap, with dimensions 72 x 66.5 x 26 cm, had three clear plastic “finger” doors (Fig 1). This experimental facility has been used in previous cane toad behavioural studies with similar objectives to test the effectiveness of trap modifications (Schwarzkopf and Alford, 2006).

Rhinella marina calls were produced using a speaker (Realistic^{TM} Minimus 0.6) housed inside a 36-cm long, 18.5-cm diameter PVC-pipe case, for waterproofing. This housing sat upon another PVC holder, on the top of a cage (holder shown in Fig 1 on cage). Sound was played using an Aerpro digital MP3 player equipped with a AAA battery, with the volume set to 55 dB at 1 m (measured using a Lutron Electronic Enterprise SL-4013 sound-pressure metre). Traps with lights were equipped with 8-W UV 'black' lights powered by rechargeable 12-V gel-cell batteries. Solar panels may also be attached to traps for use in remote areas lacking access to electricity.

Our intention was to create a high-energy call with a low frequency such as might be produced by a large, healthy male, likely to be attractive to females (Gerhardt and Huber, 2002). Cane toad advertisement calls were recorded over five nights on an *M-Audio* microtrack 24/96 portable digital recorder and *Sennheiser* ME66 microphone (Davis, 2008); 130 *R. marina* calls were recorded from 26 individual males. Calls were recorded in .wav format and analysed using *Audacity 1.2.3* (Mazzoni, 2004) and *Raven Lite 1.0* (Bioacoustics Research Program, 2003). Average call parameters from these recordings were 15 pulses/sec with a dominant frequency of 601 Hz. These values were then used to create a modified call, using *Audacity 1.2.3* (Mazzoni, 2004). The modified call had a pulse rate of 18 pulses/sec and a dominant frequency of 496 Hz, which constituted a high pulse rate and relatively low frequency that were just within naturally observed values (Davis, 2008).

The experiment was conducted 10-27 November 2010. Trials with acoustic attractants and no light attractants ran from 10-16 November 2010, while trials with both acoustic attractants and light attractants ran from 18-27 November 2010. The light attractant consisted of a UV light placed on top of the trap, facing the front. Because the different light treatments were conducted at slightly different times, there is some chance that differences in capture rates associated with our light treatment may have been influenced by the timing of the two treatments. Any differences in capture attributed to timing would likely be due to differences in factors found to influence toad activity, such as season, weather conditions, and night time temperatures (Seebacher and Alford, 1999). Using a Mann-Whitney U-test, we determined whether temperature and rainfall between our two time periods differed significantly.

Throughout the experimental period, the location of the acoustic attractant was randomized in relation to time, and was either on top of or inside the trap. Each treatment combination (acoustic attractants on top or inside traps and with or without UV lights) was repeated three times. A control treatment without an acoustic attractant was not used because of the strong positive effect these advertisement calls have on toad attraction, demonstrated by previous studies both in an arena and in the field (Schwarzkopf and Alford, 2006; Schwarzkopf and Alford, 2007).

Cane toads used in the experiments were collected within 2 h of use in trap trials. Toads were located by actively searching gardens and roads around the James Cook University campus at dusk. Toads were captured by hand and placed into a 20-L bucket, until 30 toads comprising equal proportions of males, females, and juveniles were collected. Toads <90 mm snout-urostyle length (SUL) were defined as juveniles (Alford et al., 2009). Each trial consisted nominally of 10 males, 10 females, and 10 juveniles, based on field classifications using external visual features such as skin texture and colour, but sex classification was later corrected using post-trial dissection to confirm sex and life stage. Classifications of sex determined from dissections were used in analyses. For each trial, the attractants were activated, and then all toads were released from the same location into the arena.

At 09:00 the following morning, we determined which toads were trapped. All toads were euthanised and dissected. SUL was measured to the nearest 0.5 mm using a caliper in order to validate age classifications (adult ≥90 mm vs. juvenile <90 mm). We determined sex visually using internal anatomy to validate field categorization.

2.3. Data analysis

The influence of sex (male, female, and juvenile), acoustic attractant location, and light attractant presence (and interactions among factors) on cane toad capture proportion were assessed using a 3-way analysis of variance (ANOVA). Each trap night was used as a sample unit, providing three sample units per light/acoustic/sex group. Tukey's Honestly

Significant Difference (HSD) tests were used to discriminate among groups within the ANOVA. We normalised capture proportion distributions using an arc-sine transformation. All tests were conducted using SPSS Statistics 20.0 (IBM, 2007) with a significance of $p \leq 0.05$. Post-hoc power tests were run on climate data and capture data using the program G*Power 3.1.7 (Faul et al., 2009).

3. Results

In this study, 51% of males, 20% of females, and 12% of juveniles were trapped, and sex significantly influenced cane toad capture probability (Fig 2); male toads were trapped most frequently ($F_{2/24}$= 18.9, p< 0.001; Tukey HSD, M*F p< 0.001, M*J p< 0.001, F*J p= 0.46; Table 1). Traps with a light and an acoustic attractant had one half greater overall toad capture probability (33% captured) compared to traps with acoustic attractants only (20% captured; $F_{2/24}$= 4.88, p= 0.04; Table 1). This multiple-attractant configuration resulted in a more than doubling of female capture probability from 12% to 28%, an increase of one quarter for males from 43% to 56%, and a tripling of juvenile capture probability from 6% to 17% compared to traps with acoustic attractants only (Fig 3).

Temperature and rainfall between our two trapping time periods (with "light" and "no light" treatments) were not significantly different ($U_{9,6}$ = 41, p= 0.11; $U_{9,6}$ = 17.5, p= 0.29), indicating that temporal differences probably did not affect toad behaviour in this experiment. A slightly higher mean rainfall was observed in the first time period associated with the "no light" treatment. Our statistical power (1-β) to detect between period differences in temperature and rainfall was low (0.18 and 0.47, respectively), but we suggest these effects were not biologically significant.

Acoustic attractant location was not a significant predictor of overall toad capture probability ($F_{2/24}$= 0.53, p= 0.47; Table 1); acoustic attractant placement inside traps increased female capture probability only, and this sex-by-acoustic attractant interaction was significant ($F_{2/24}$= 4.86, p= 0.02; Table 1). The effect of acoustic attractant location was dependent on sex. Female capture probability nearly tripled from 11% to 31% when the acoustic attractant was placed inside the trap (Fig 4). There was no significant sex-by-light interaction, indicating that light did not affect capture probability of males, females and juveniles differently ($F_{2/24}$= 0.29, p= 0.75; Table 1). No significant light-by-acoustic attractant interaction exists either, indicating that these two attractants have separate effects on capture probability ($F_{1/24}$= 0.50 p= 0.49; Table 1). Along with these results, the 3-way sex-by-light-by-acoustic attractant interaction was not significant, indicating all sexes were equally affected by multiple attractant combinations ($F_{2/24}$= 0.37, p= 0.70; Table 1). Post-hoc power analysis (Observed Power, SPSS) revealed that statistical power to detect differences between acoustic placements was low, but that power to detect differences between light and sex was adequate (Table 1).

4. Discussion

Adult male toads were the group most likely to be captured in traps across all trap treatments. Males investigate traps more frequently than do females (pers. obs.) and are more likely to move, in general (Schwarzkopf and Alford, 2007), increasing the probability of randomly encountering a trap, and therefore entering it. We do not know why males are attracted to calls, but they may engage in sexual parasitism, or “satellite male behaviour,” in which silent males sit near calling males to benefit from females that are attracted, a behaviour which occurs in anurans (Forester and Lykens, 1986). Compared to females, males were not sensitive to changes in acoustic attractant position. Capture probability of males was reduced when lights were not used, but capture probability of both females and juveniles was also reduced, maintaining the males’ standing as most likely to be captured.

Although using a call as an attractant does attract toads (Schwarzkopf and Alford, 2007), the effectiveness of cage traps for cane toad capture is greater if both a light attractant and a sound attractant are used (Hienton, 1974; Schwarzkopf and Alford, 2007; Davis, 2008). Significantly more cane toads were trapped when the light attractant for insects was on than when it was off in our study. Thus, capture probability was greater when a multi-lure configuration was used than when an acoustic attractant alone was used. The additive influence of several attractants may occur because toads with different internal states (e.g., hungry, ready to mate) approach the trap, and having several different attractant types increases the likelihood of attracting any particular individual. Another reason the combination of attractants might be additive is that the attractants likely operate at different distance ranges. Possibly, the sound draws the toads close to the trap and then the light attractant increases the likelihood they will enter the trap. Though the distance effect may not be very strong in an enclosed arena such as was used here, this idea may be further tested in future field studies. Combining a visual attractant with an olfactory attractant increased invasive pine beetle capture rates, potentially due to effects at different spatial scales (Strom et al., 1999).

We compared "light" and "no light" treatments at slightly different times, 2 days apart, and the earlier time period, without the light, had slightly, but not statistically significantly higher mean rainfall than the light treatment period. Due to a small sample size, our statistical power to detect differences in rainfall and temperature between the two experimental periods ("no light" and "light") was low. Rainfall increases soil moisture, which is positively correlated with cane toad activity (Seebacher and Alford, 1999; Schwarzkopf and Alford, 2002), which may have increased the random chance of toads entering the trap. However, given that light significantly *increased* capture probability during a treatment period with *lower* rainfall, we suggest that the increased trappability of toads was due to the light, and not to weather. At most, this small difference in weather slightly diminished the observed effect of light on capture probability.

Female toad capture success was further increased when the acoustic attractant was placed inside the trap rather than on top, while male and juvenile captures were not significantly affected by location of the lure. Because females respond to male attraction calls in order to mate, females were more likely than males to go directly to the source of the call (Gerhardt and Huber, 2002). By moving the acoustic attractant inside the trap, the likelihood of females entering the trap tripled. Approximately 9% of adult female cane toads captured in this study contained fully developed (stage 5) eggs; all adult females contained eggs at some stage of development. Applying the survival rates mentioned in the introduction of this paper, placing the acoustic attractant inside the trap could result in the removal of 600 potential toads (from the removal of three adult females) for every 200 potential toads removed (from one adult female) using the attractant on top of the trap. This estimate is conservative, as it uses the smallest clutch size of only 5,000 eggs and assumes that a given female would only produce one clutch. If the higher 35,000 egg estimate is used, the effect is much larger, showing a removal of 4,200 potential toads with the suggested trap modification versus 1,400 potential toads without this modification. However, with a current cane toad population size over 200 million in Australia (Dall, 2011), and a rapid population growth rate characteristic of most invasive species (Urban et al., 2007), these impacts are not likely to be enough to control the invasion of this species. Trapping is a promising strategy at least for short-term local-scale management (Taylor and Edwards, 2005), and these smaller goals seem even more attainable by tripling the previous trapping impact. With no true eradication strategies yet developed, improving our trapping capabilities is the best management practice that can be implemented at this time.

Based on the results of the present and previous studies, the recommended attractant equipment for a cage trap is a UV light attractant for insect prey, with an acoustic attractant placed inside the trap. As cane toads are capable of breeding year-round (The State of Queensland DAFF, 2013), acoustic attractants should be effective throughout the year and are not limited to a specified season. Even using only advertisement calls as attractants, however, more males were trapped than females and juveniles. To maximize trapping efficacy, trap engineering could be directed toward even further increasing capture rates of females. Equipping traps with an acoustic attractant inside as opposed to on top of the cage tripled female capture probability, revealing a trap modification that could contribute to female subpopulation reduction. Field tests with associated estimates of population effects will be needed to support trends seen in this study. If field studies corroborate our results, these findings could be applied to the control of other species, as cane toads demonstrate the importance of considering behavioural differences between sexes when developing trapping methods.

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Figure Captions:

Table 1. Summary of analysis of variance results for cane toad capture probabilities in experiments with a UV light off or on and an acoustic attractant on top or inside of cage traps during 10-27 November 2010 at James Cook University, Townsville, QLD, AU.

Figure 1. Cage trap, with dimensions 72cm x 66.5cm x 26cm, with speaker holder on top, used in experimental trials in Townsville, QLD, AU.

Figure 2. Mean (± 1 SE) percentage of male, female, and juvenile cane toads trapped per trial in experiments during 10-27 November 2010 at James Cook University, Townsville, QLD, AU. (* indicates significantly larger value at $p<0.05$)

Figure 3. Mean (± 1 SE) percentage of male, female, and juvenile cane toads trapped per trial in experiments with a UV light off or on during 10-27 November 2010 at James Cook University, Townsville, QLD, AU. (* indicates significantly larger value at $p<0.05$)

Figure 4. Mean (± 1 SE) percentage of male, female, and juvenile cane toads trapped per trial in experiments with an acoustic attractant on top or inside of cage traps during 10-27 at James Cook University, Townsville, QLD, AU. (* indicates significantly larger value at $p<0.05$)

Table 1:

Source of Variation	df	F-value	p-value	Observed power[a]
Intercept	1	196.2	<0.001	1.0
Light	1/24	4.88	0.04	0.56
Acoustic	1/24	0.53	0.47	0.11
Sex	2/24	18.9	<0.001	1.0
Light * Acoustic	1/24	0.50	0.49	0.10
Light * Sex	2/24	0.29	0.75	0.09
Acoustic * Sex	2/24	4.86	0.02	0.75
Light * Acoustic * Sex	2/24	0.37	0.70	0.10

[a] calculated using alpha = 0.05

Fig 1:

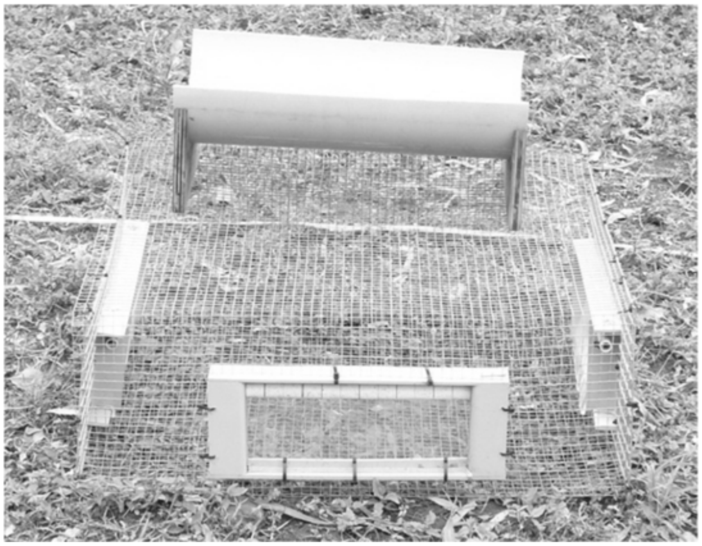
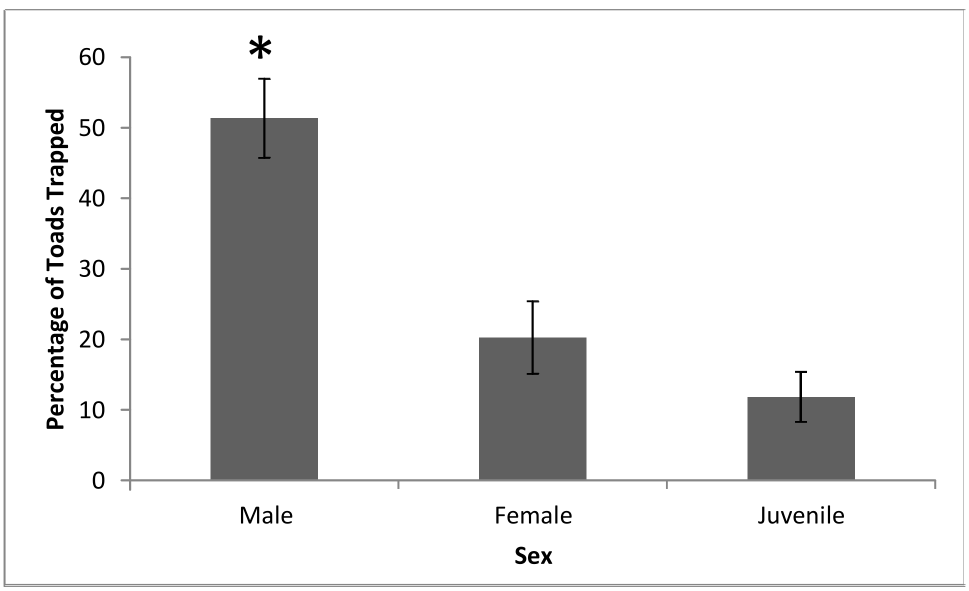


Fig 2:



*
60
50
40
30
20
10
0
Percentage of Toads Trapped
Male
Female
Juvenile
Sex

Fig 3:

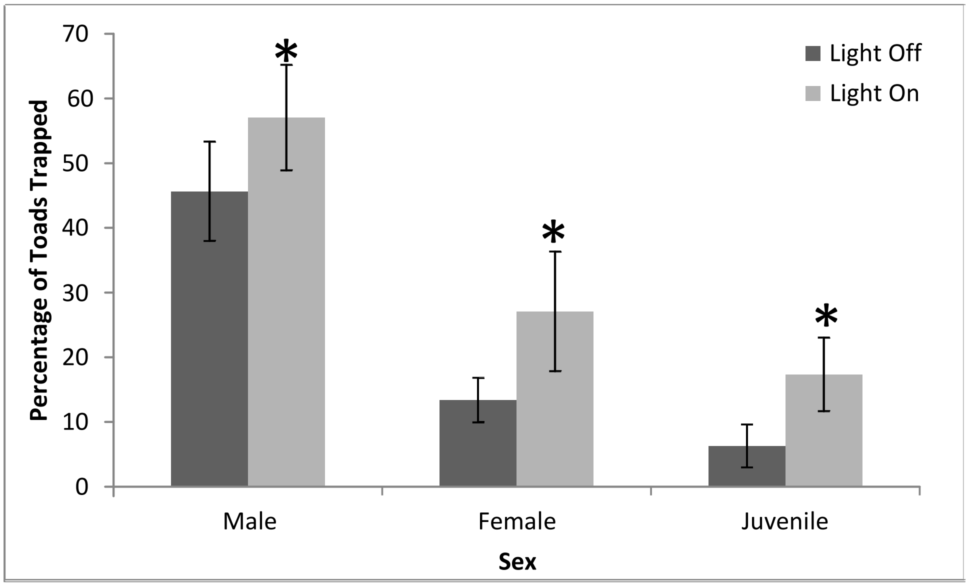
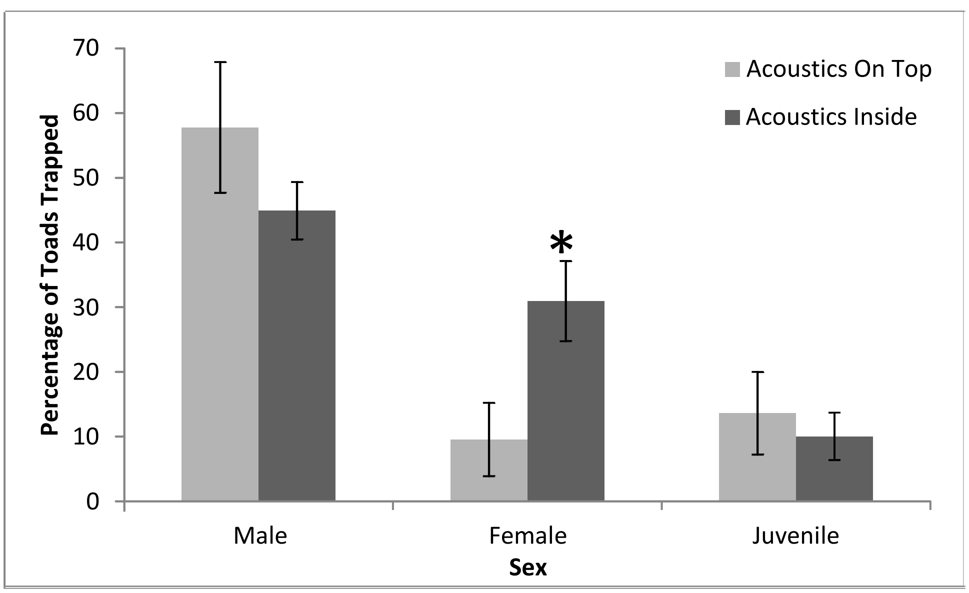

70
60
50
40
30
20
10
0
Percentage of Toads Trapped
*
*
*
Light Off
Light On
Male
Female
Juvenile
Sex

Fig 4:


70
60
50
40
30
20
10
0
Percentage of Toads Trapped
*
Acoustics On Top
Acoustics Inside
Male
Female
Juvenile
Sex