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

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

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

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13 **Sex, Light, and Sound: Location and Combination of Multiple Attractants Affect**
14 **Probability of Cane Toad (*Rhinella marina*) capture**

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

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

37 **Introduction**

38 Invasive species cause negative economic, environmental, and social impacts
39 globally, and devising successful control strategies is a priority for conservation. While
40 genetic and biological control methods are under development for many exotic pests, traps
41 provide a rapid, economical, and effective strategy for local-scale management. Success of
42 trapping is influenced by behavioural (Greenslade, 1964) and physical factors (Beacham and
43 Krebs, 1980), and manipulation of these characteristics by trap modification helps target

44 specific demographic groups of the pest species. Modifications that increase capture
45 probability of the demographic groups important for population growth increase the impact
46 of each individual capture. For example, removal of ovigerous females from a population
47 eliminates not just mature adults, but future breeding potential as well (Thresher, 2007).

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

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

68 In general, adult cane toads immediately before they first reproduce are the ideal
69 target for trapping, because all their potential future offspring are removed from the
70 population, and adults have the highest survival rates (50%) of all life stages (Zug and Zug,
71 1979; Molloy and Henderson, 2006). It makes sense then, both in terms of effort and
72 biological impact, to target adults, especially immediately pre-reproductive adults, when
73 developing management strategies. However, targeting adult females is the most effective
74 management strategy for long-lived vertebrates, and specifically cane toads (Thresher, 2007).
75 Female toads can carry clutches of 5,000 to more than 35,000 eggs (Molloy and Henderson,
76 2006; Hagman and Shine, 2008; The State of Queensland DAFF, 2013). With a survival rate
77 from egg to adult of approximately 0.04%, there is a possibility of reducing the population by
78 at least 200 individuals by capturing only one adult female before reproduction (usually two
79 years old for female amphibians), whereas capturing a male toad reduces the adult population
80 directly by only one individual (Molloy and Henderson, 2006; Browne and Zippel, 2007).
81 Even if a significant portion of the male population were removed, one male cane toad can
82 fertilize multiple egg clutches, so recruitment rates may not change significantly, even with
83 the loss of multiple males. Therefore, culling of the female population is likely a more
84 effective way of reducing recruitment into the next generation.

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

89 **2. Methods**

90 ***2.1. Study site***

91 This study was conducted at James Cook University in Townsville, Australia
92 (19°19'47.74"S, 146°45'29.55"E). The campus is surrounded by poplar gum (*Eucalyptus*
93 *platyphylla*) woodland with a black spear grass (*Heteropogon contortus*) dominated
94 understory. Cane toads invaded the region in the 1940s (Alford et al., 2006) and have
95 established a self-sustaining population. Daily temperature and rainfall data was retrieved
96 from the Australian Government Bureau of Meteorology Oonoonba station 032057
97 (Commonwealth of Australia, 2013). Average daily temperature during this study was
98 22.5°C and average daily rainfall was 5.2 mm.

99 **2.2. Experiment**

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

109 *Rhinella marina* calls were produced using a speaker (Realistic™ Minimus 0.6)
110 housed inside a 36-cm long, 18.5-cm diameter PVC-pipe case, for waterproofing. This
111 housing sat upon another PVC holder, on the top of a cage (holder shown in Fig 1 on cage).
112 Sound was played using an Aerpro digital MP3 player equipped with a AAA battery, with the
113 volume set to 55 dB at 1 m (measured using a Lutron Electronic Enterprise SL-4013 sound-
114 pressure metre). Traps with lights were equipped with 8-W UV 'black' lights powered by

115 rechargeable 12-V gel-cell batteries. Solar panels may also be attached to traps for use in
116 remote areas lacking access to electricity.

117 Our intention was to create a high-energy call with a low frequency such as might be
118 produced by a large, healthy male, likely to be attractive to females (Gerhardt and Huber,
119 2002). Cane toad advertisement calls were recorded over five nights on an *M-Audio*
120 microtrack 24/96 portable digital recorder and *Sennheiser* ME66 microphone (Davis, 2008);
121 130 *R. marina* calls were recorded from 26 individual males. Calls were recorded in .wav
122 format and analysed using *Audacity 1.2.3* (Mazzoni, 2004) and *Raven Lite 1.0* (Bioacoustics
123 Research Program, 2003). Average call parameters from these recordings were 15 pulses/sec
124 with a dominant frequency of 601 Hz. These values were then used to create a modified call,
125 using *Audacity 1.2.3* (Mazzoni, 2004). The modified call had a pulse rate of 18 pulses/sec
126 and a dominant frequency of 496 Hz, which constituted a high pulse rate and relatively low
127 frequency that were just within naturally observed values (Davis, 2008).

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

138 Throughout the experimental period, the location of the acoustic attractant was
139 randomized in relation to time, and was either on top of or inside the trap. Each treatment

140 combination (acoustic attractants on top or inside traps and with or without UV lights) was
141 repeated three times. A control treatment without an acoustic attractant was not used because
142 of the strong positive effect these advertisement calls have on toad attraction, demonstrated
143 by previous studies both in an arena and in the field (Schwarzkopf and Alford, 2006;
144 Schwarzkopf and Alford, 2007).

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

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

160 ***2.3. Data analysis***

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

165 Significant Difference (HSD) tests were used to discriminate among groups within the
166 ANOVA. We normalised capture proportion distributions using an arc-sine transformation.
167 All tests were conducted using SPSS Statistics 20.0 (IBM, 2007) with a significance of $p \leq$
168 0.05. Post-hoc power tests were run on climate data and capture data using the program
169 G*Power 3.1.7 (Faul et al., 2009).

170 **3. Results**

171 In this study, 51% of males, 20% of females, and 12% of juveniles were trapped, and
172 sex significantly influenced cane toad capture probability (Fig 2); male toads were trapped
173 most frequently ($F_{2/24} = 18.9$, $p < 0.001$; Tukey HSD, M*F $p < 0.001$, M*J $p < 0.001$, F*J $p =$
174 0.46; Table 1). Traps with a light and an acoustic attractant had one half greater overall toad
175 capture probability (33% captured) compared to traps with acoustic attractants only (20%
176 captured; $F_{2/24} = 4.88$, $p = 0.04$; Table 1). This multiple-attractant configuration resulted in a
177 more than doubling of female capture probability from 12% to 28%, an increase of one
178 quarter for males from 43% to 56%, and a tripling of juvenile capture probability from 6% to
179 17% compared to traps with acoustic attractants only (Fig 3).

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

187 Acoustic attractant location was not a significant predictor of overall toad capture
188 probability ($F_{2/24} = 0.53$, $p = 0.47$; Table 1); acoustic attractant placement inside traps

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

202 **4. Discussion**

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

213 Although using a call as an attractant does attract toads (Schwarzkopf and Alford,
214 2007), the effectiveness of cage traps for cane toad capture is greater if both a light attractant
215 and a sound attractant are used (Hienton, 1974; Schwarzkopf and Alford, 2007; Davis, 2008).
216 Significantly more cane toads were trapped when the light attractant for insects was on than
217 when it was off in our study. Thus, capture probability was greater when a multi-lure
218 configuration was used than when an acoustic attractant alone was used. The additive
219 influence of several attractants may occur because toads with different internal states (e.g.,
220 hungry, ready to mate) approach the trap, and having several different attractant types
221 increases the likelihood of attracting any particular individual. Another reason the
222 combination of attractants might be additive is that the attractants likely operate at different
223 distance ranges. Possibly, the sound draws the toads close to the trap and then the light
224 attractant increases the likelihood they will enter the trap. Though the distance effect may
225 not be very strong in an enclosed arena such as was used here, this idea may be further tested
226 in future field studies. Combining a visual attractant with an olfactory attractant increased
227 invasive pine beetle capture rates, potentially due to effects at different spatial scales (Strom
228 et al., 1999).

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

238 toads was due to the light, and not to weather. At most, this small difference in weather
239 slightly diminished the observed effect of light on capture probability.

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

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

276

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282

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384 Native Populations. *Smithsonian Contributions to Zoology*, 284:1–54.

385 **Figure Captions:**

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

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

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

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

397 Figure 4. Mean (± 1 SE) percentage of male, female, and juvenile cane toads trapped per trial
398 in experiments with an acoustic attractant on top or inside of cage traps during 10-27 at
399 James Cook University, Townsville, QLD, AU. (* indicates significantly larger value at
400 $p < 0.05$)

401

402

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:

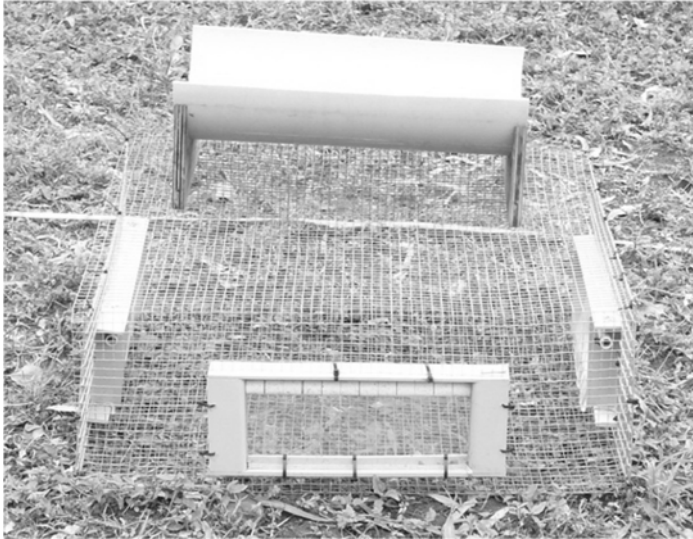


Fig 2:

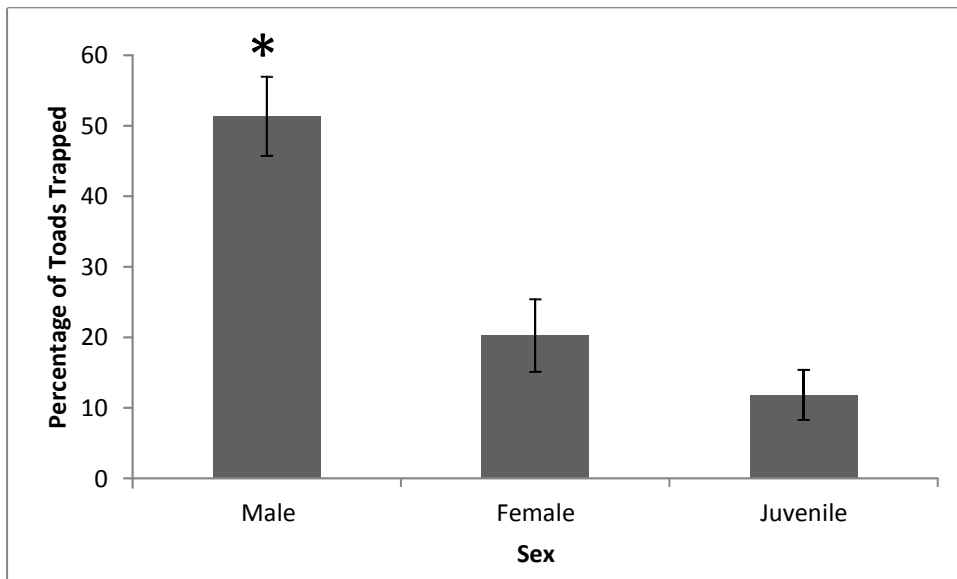


Fig 3:

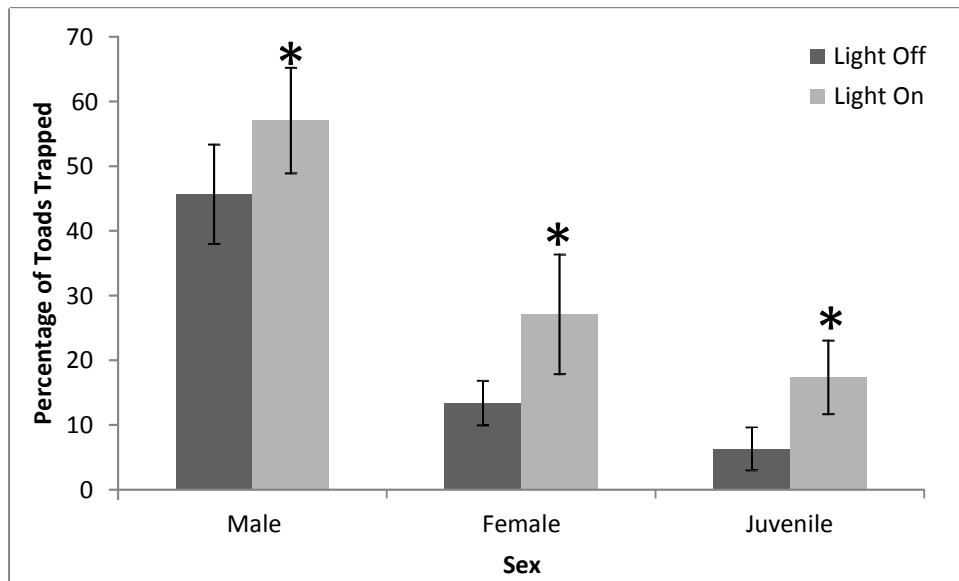


Fig 4:

