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Medium Term Control Methods for Cane Toads: Olfactory and Acoustic Attractants

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Cover images: photos of acoustic arena, y-maze and cane toad trap: James Cook University.

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Summary

Large populations of cane toads (*Bufo marinus*) occur in Queensland, the Northern Territory and New South Wales, and toad populations are still expanding west and south. In states where toads occur, and states toads will reach soon (i.e., Western Australia), there is significant public concern about toads and their impacts on natural ecosystem values, native wildlife and domestic pests. Methods of toad control involving gene technology or introduced or genetically engineered diseases are in the early development stage, and are not likely to be available in the short to medium term (5-10 years). Thus, low technology approaches to control, such as trapping, provide a temporary solution that may allow control of toads in localised areas, and empower the public to "do something". Recent mathematical modelling suggests that if traps are very efficient, cane toad control is possible using traps, or that traps might be useful as part of an integrated pest management scheme, incorporating several methods. Wire cage traps have been designed that trap toads with little by-catch.

The purpose of this study was to examine attractants that may enhance the effectiveness of trapping. We examined the possible effectiveness of scents as attractants using two techniques. We used a Y-maze to determine whether toads are attracted to the odours of other toads, food, and pond water, and performed cafeteria trials of food preferences to screen a variety of foods that seemed likely to attract toads. In Y-mazes, we exposed focal toads to the odours of male toads, female toads, dog food (Masterfoods™ My Dog™ Lamb Classic) and pond water. In the cafeteria trials we allowed toads to choose between minced beef, minced chicken, cat food and dog food. We also examined the effectiveness of acoustic cues, in the form of male toad mating calls, for attracting toads. We initially performed tests in a large circular arena to determine whether male and female toads were attracted to a toad mating call when they were not reproducing. We placed toads in the centre of the arena and exposed them to loud (67dB at 1 m) and soft (47 dB at 1 m) cane toad calls, and to 'pink' noise at the same volumes. We recorded the distance and direction the toads moved after 5 minutes of exposure to sound.

The results of the Y maze experiments indicated that toads do respond behaviourally to the scents of conspecifics and the single type of food we used in these trials, but their responses were not simple strong attraction of the sort that would have allowed us to design improved trapping methods. In the Y-maze, male toads were more likely to approach male toads than female toads when given a choice between males and females, but avoided male toads when given the choice between males and nothing. Female toads approached other females when given a choice between males and females, showed no preference when choosing between other toads of either sex and no stimulus, and avoided the dog food. Neither sex showed any response, positive or negative, to the scent of pond water. If the data for both sexes were considered together, both approached their own sex when given the choice between conspecifics of the same sex and the opposite sex, both avoided the dog food, and neither responded to water. Examining data only for trials in which responding toads (and stimulus toads, where applicable) were in reproductive condition as determined by dissection following trials, led to the same

conclusions, indicating that reproductive condition of toads did not affect their responses to odours.

In our cafeteria trials, toads did consume artificial foods when presented with them, but it appeared that consumption was initiated when toads responded to the presence of insects on the food and consumed food incidentally while attacking the insects. They may then have continued to feed on the artificial food, however, as in the Y-maze results, it did not appear that scent had a role in the initial attraction of toads to artificial foods.

Our results for behavioural responses of toads to olfactory cues indicate that toads can make behavioural decisions based on scents, but our data suggest that scent cues from other toads and dog food repel toads to various degrees, rather than attracting them. Much more extensive work, involving isolating components of scents and testing them to determine which if any components serve as attractants, is needed to dissect these responses in detail and determine whether any scent can serve as a strong attractant. Because some odours clearly repel toads, we would also suggest that future research should examine this in more detail, as it might be possible to use odours to disperse toads or repel them from selected locations, for example favourable breeding sites.

The results of our acoustic trials are much more promising for immediate application. In the acoustic arena, toads showed no tendency to go towards pink noise at any volume, but both males and females were attracted to toad calls. Males moved towards calls at both of the volumes we tested, while females moved towards calls only at the lower volume. These results suggest that toads use conspecific mating calls as a means of locating aquatic habitats, which they need to access regularly to avoid desiccation, at long distances. Female toads may not be attracted to loud calls because encountering males near water when females are not ready for reproduction can lead to unwanted mating attempts by the males.

The arena results suggested that trapping success can be enhanced using acoustic lures. We conducted a field trial over a three-month period, using traps built to the design developed by Frogwatch NT. We compared sets of two pairs of traps placed in several locations. All traps were equipped with fluorescent lights to attract insects. One pair of traps at each location had speakers mounted on them that played recorded toad calls all night, while the other did not. Speakers were switched between pairs of traps at a location on successive nights to eliminate any affect the exact positioning of traps might have had on capture rates. We found that capture rates were significantly higher in traps with playbacks; on average, traps with playbacks caught 3 times as many toads as those without. It is clear that the effectiveness of traps can be substantially increased by adding acoustic lures in the form of call playbacks to existing trap designs. We recommend that this should be adopted by toad trapping programs immediately, when practicable, following the design we used in our trapping study.

We also examined and described 100 locations selected by toads as shelter sites. The results of this should be useful in the design of traps that attract toads simply because they provide shelter, and would therefore not require maintenance, as do traps with light or sound attractants. They could also be used to modify the design of traps that are equipped with lures to increase their attractiveness to toads. Our results also suggest that simple modifications to built infrastructure could be made that would reduce the attractiveness of urban environments to toads. We found that toads prefer shaded burrows or hollows on moist soil, sand or leaf litter, with walls and sides of soil or earth. More than half (58% of 48) of toads in urban environments selected man-made shelters. Toads in bushland used mostly burrows (38% of 52) and hollow logs (33%). Closing small holes in concrete and putting mesh on openings of pipes and culverts should discourage toad use of many man-made shelters. Traps designed to attract toads should be 1000 – 10,000cm^3 in volume, with moistened earth or sand inside. Traps should be located in areas with 50 -100% shade, and placed well outside of streambeds.

Future Directions

We plan to carry out additional research in this area that will allow us to further enhance the success of toad traps.

Future studies will:

(1) Experimentally determine whether the effectiveness of acoustic attraction can be enhanced by changing aspects of the calls (loudness, dominant frequency, call intensity and call rate). We will conduct initial arena trials, following up any promising developments by conducting additional experimental studies in the field.

(2) Experiment with the location of the speaker relative to traps. Speakers in elevated locations may cause toads to search for the source, making movement and therefore trapping more likely. Because it appeared that female toads were not attracted to nearby calls at high volume, the effectiveness of acoustic lures for females may be greater if the speaker is not directly on the trap, but within a few metres, so that toads are attracted to the vicinity of the trap by sound and enter it in response to the light and insects.

(3) Experiment with sound used on different trap designs: larger traps, traps with ramps and trap doors in the top and traps equipped with UV lights. All such traps have been used with some success in non-experimental set-ups (G. Saywer, pers. comm.), but experiments would clearly distinguish effective from ineffective strategies.

(4) Carry out additional Y-maze trials using fractionated extracts from toad skin secretions and other body products. We are in the initial stages of collaborating with a group of researchers at the University of Queensland headed by Professor Robert Capon, who have been funded by the Queensland government to explore the chemical ecology of cane toads. They will carry out extractions of potential attractants and repellents from toads and toad venom, and provide them to us to test.

(5) Experiment with methods to modify the basic FrogWatch trap design to make it more intrinsically attractive to toads as a shelter site

(6) Design and test simple “shelter traps” with parameters suggested by our descriptions of toad shelters.

Introduction

Since its intentional introduction to Australia in the early 1930s as a biological control agent, the cane toad (*Bufo marinus*), has proven to be the most successful invasive amphibian in the world (Estoup et al. 2004). Over the past eighty years, cane toad populations have not only increased in density, but have expanded their intended range, and continue to spread (Estoup et al. 2004, Sutherst 1995). *B. marinus* eat native species (Werren and Trenerry 1993), compete with them for resources (Crossland 1998) and are toxic to individuals that prey upon them (Phillips et al. 2003). Thus, cane toads are likely to threaten the health of Australian ecosystems where they occur. In spite of this threat, there is presently no coordinated control program for *B. marinus* in Australia (Baskin 2002, CSIRO 2004).

One method to reduce the abundance of invasive species populations is broad-scale trapping. Trapping programs have been successful in controlling numerous species over a variety of habitats (Baskin 2002). When deployed correctly, traps can reduce the number of individuals in a population, decreasing their impact on native populations and ecosystem functions.

Recently, the Northern Territory Conservation Commission ran a contest to compare the efficacy of traps designed for cane toads. Wire cage traps with trap doors, lights attractive to insects (and possibly directly to toads) and trap doors preventing exit of trapped animals proved the most effective trap designs, catching toads and few, if any, non-target species. However, if trapping programs are to effectively control toads, they must trap a very high proportion of the population (25–40 % of the population, R. Thresher and H. McCallum, pers. comm.).

The purpose of our study was to examine possible methods to enhance trapping using olfactory and acoustic attractants. Adult amphibians are able to respond to olfactory cues, from food (e.g., Shinn and Dole 1979), from conspecifics (e.g., Waldman and Bishop 2004), and from predators (Flowers and Graves 1997). Thus, if particular odours are attractive to adult cane toads, they could potentially be used to attract individuals to traps, enhancing their trappability. Surprisingly, cane toad responses to the smells of conspecifics and food have not been studied, although reports that cane toads appear to find food using olfaction are common (e.g., Boland 2004).

Most anuran vocalisations are emitted by males and serve to attract females for mating (Ryan et al. 1981). Only male cane toads vocalise, and they only emit two types of vocalisations: a drawn-out trilling call that serves to attract females to males for mating, and a quiet, "chuckling" call that serves to notify a male that he has attempted to mate with another male and should release him. Possibly, neither of these would serve as a general toad attractant. However, many anurans do have a generalised tendency to respond to the mating calls of conspecifics with phonotaxis (Gerhardt 1994), so cane toad mating calls could serve as an attractant, luring toads to the vicinity of traps or causing them to enter traps. It is clear that cane toad calls must attract females who are ready to reproduce, however the degree to which they might be attractive to males and nonreproductive females had not been measured.

Our study experimentally examined olfactory attractants (conspecifics, food and water) as possible attractants for toads to traps. In addition, we determined whether toad mating calls were generally attractive to toads under experimental conditions and in the field when associated with traps. We also measured the characteristics of shelter sites selected by toads in the field, to determine their features in detail with three goals. First, to determine whether it may be possible to modify existing trap designs to make them more attractive to toads. Second, to provide the background information needed to design low-maintenance traps that simply provide very high quality shelter sites. Such traps would require no maintenance to recharge batteries of lights and sound generators, and so could be effectively deployed more remotely, and checked less frequently than existing trap designs. Third, to suggest modifications to existing and new man-made structures that can make them less attractive and useful to toads as shelter sites.

Methods

This study was conducted between March 2004 and April 2006, at the James Cook University Campus (19°15’42”S 146°48’34”E), the Billabong Sanctuary Wildlife Park south of Townsville (19°21’27” S 146°53’09” E), and Table Top Station on Hervey’s Range west of Townsville (19º22’53”S 146º28’25”E). Cane toads were collected by hand from these areas, and from suburban gardens around Townsville. Toads were held in buckets overnight, and kept in small groups (<10) in 1000 litre plastic cattle watering tanks covered with 80% shade cloth when they were not being used in experiments. Cattle tanks were supplied with a small amount of water, and kept on an angle so there were both dry and wet areas available to toads at all times. Toads were fed commercially obtained crickets and mealworms (Suppliers: Pisces) weekly if held for long enough to require feeding. Toads that were exposed to food trials were not fed for up to 48 hours before the trial.

After experiments, toads were killed, by freezing, for dissection. We recorded reproductive condition of toads: developmental stage of eggs in females, and testes size and colour, and nuptial pad colour for males. Female reproductive condition was scored on a scale of 0-4, with zero being ovaries fully regressed and 4 being highly distended ovaries packed with fully yolked eggs. For analysis, we considered females with ovaries scored 2, 3, or 4 to be in reproductive condition. All males we examined either had extremely regressed testes or testes greater than 10mm in length; we used this dichotomy to classify males as reproductively inactive or active.

Y-Maze Experiments

We constructed Y-mazes, which were 2.4m long, 30 cm wide, and 30 cm high, and were made of grey PVC sheeting with a removable, transparent Perspex lid (Figure 1). There was a holding area for the focal toad at the base of the Y. The central division of the arms was 140 cm from the base, and the separate sections of the arms were 86 cm in length. Stimulus samples were separated from the toad by baffles that allowed air to pass but prevented the toad from seeing the source of the stimulus. The mazes were supplied with a quiet Sirocco™ 125 mm fan that drew air through the maze from the samples to the focal toad at 2.3m^3/min, producing a mean air flow rate of 42.6 cm/sec or approximately 1.53 km/h, a very gentle breeze. The room was dimly illuminated by a 5-watt, red light globe. A video camera was positioned above the holding area such that the entire maze was visible, and the movements of toads were recorded on video tape.

Before each run of the Y maze experiment, to remove possible scent trails or lingering odours, the maze was washed using detergent, rinsed, wiped out with ethanol, and allowed to dry. Stimuli were then placed in their holding areas, the focal toad was placed into its holding area, and the trial began when the observer activated the fan, withdrew from the room, and shut the door. Responses of the focal toad were recorded for 1 hr after the observer withdrew. For analysis, the video tape was played back and the behaviour of the toad was timed using a stopwatch. We recorded the latency time until the toad entered an arm of the maze, the identity of the arm first entered and the time spent in that arm. The trial was terminated when the toad exited the first arm it had entered, or at the end of the hour, whichever came first. Most toads moved and made a choice in the maze within the period of observation.

Figure 1. One of the Y-mazes used in evaluating toad behavioural responses to odours. The video camera used for observation was mounted on the tripod visible at the right.

In the initial series of experiments (Table 1) each individual toad was tested with all stimuli on the same night; this avoided the possibility that individual differences in preferences might bias the results (Table

1). Stimuli were presented in random order, and the arm of the maze in which the stimulus was presented was also randomised. The maze was cleaned thoroughly, as detailed above, between trials. Animals were sacrificed by freezing and later dissected to determine their reproductive status; a freezer failure made it impossible to determine the reproductive status of 8 males and 14 females.

A separate series of trials were conducted with water as the stimulus in the maze. A petri dish of fresh pond water obtained from a still pool in Campus Creek on the campus of James Cook University was placed in the stimulus chamber of a randomly selected arm of the maze; the other was left empty. Trials were otherwise conducted as described above.

A series of preliminary trials were also conducted using toad scents extracted by grinding toad skin in dichloromethane, filtering the result, and evaporating the filtrate to near dryness. Samples placed on cotton buds did not appear to attract toads at rates greater than controls of dichloromethane on cotton buds. We did not pursue this line of work further because the results from our whole-animal trials made it clear that whole-animal odours did not exert strong attractant effects on toads, and we decided to concentrate on cafeteria trials to evaluate the attractiveness of different foods. Further work on toad scent extracts may produce positive results; we discuss this in the context of our results below.

Table 1. Stimuli to which toads (*Bufo marinus*) were exposed in a Y-maze. The initial series of trials included only the first five treatments; in this series all treatments were presented, in random order, to each focal animal. The pond water stimuli were presented at a later time to a different series of focal animals. Stimuli were allocated at random to the left and right arms of the Y maze.

One arm	Other arm
Empty	Empty*
Same sex	Empty
Opposite sex	Empty
Same sex	Opposite sex
Dog food**	Empty
Pond Water	Empty

*Control to determine if toads had a directional preference
**Dog food was Masterfoods™ My Dog™ Lamb Classic

Cafeteria Trial Experiments

To simultaneously determine whether toads consumed and showed any preferences among a variety of foods that might be used as attractants, we conducted a series of cafeteria trials. In a 1000 l cattle watering tank, toads that had not been fed for 48 h were exposed to equal, weighed amounts (50 +/- 2 g) of cat food (Masterfoods™ Dine Liver and Bacon™), dog food (Masterfoods™ My Dog™ Lamb Classic), minced chicken, minced beef and minced sardines in shallow foil containers. Control tanks contained equal amounts of each food, arranged in the same way, but with no toads, to control for mass loss of foods due to drying. Screen over the top of one half of the cattle tanks prevented the entry of most insects, although small ants sometimes reached the food. Chicken wire over the remaining tanks allowed large insects to enter. This part of the experiment was designed to determine whether toads were more likely to consume food containing insects than food without insects.

Food was placed in the tanks between 1900 and 2000 h. Toads were placed in the tanks at 2000 h and left there for 12 hours, until 0800 h the next morning. Toads were then removed from tanks and the remaining food in control and toad exposure tanks was weighed. Toads from these experiments were sacrificed by freezing and dissected so that we could determine whether they were consuming the food.

Acoustic Arena Trials

Acoustic trials were conducted in a 7.6 m diameter circular arena with thin plastic-coated sheet-metal walls 1.2 m high, and a natural, mowed grass substrate. Toads were exposed to toad calls played back at volumes of 67 dB(A) at 1 m and 47 dB(A) at 1 m, and to playbacks of “pink” noise (Bradbury and Verhencamp 1998) at both levels. Pink noise is a random noise signal in which the spectral density varies with the inverse of frequency, and is thought (Bradbury and Verhencamp 1998) to be the best simulation of environmental noise. Each toad was used in only one trial.

Dummy speakers (14 cm H x 8 cm W x 8 cm D paving bricks painted black), the same size, shape and colour as real speakers, were placed at 30° intervals around the arena. Before each trial, one randomly chosen brick was removed and replaced with a speaker (RealisticTM Minimus 0.6). These speakers were verified as providing adequate sound reproduction quality for

Figure 2. The arena in which acoustic trials were conducted. The cage in which toads were positioned at the start of trials is visible in the centre, in raised position. The dummy speakers were not in place.

frog calls by playing recorded calls through them, recording the playbacks, and comparing their sonograms to those from the original calls. The call played by the speaker was a continuous loop of the cane toad call from the CD "Australian Frog Calls of the Tropical NE", by D. Stewart. The focal toad was placed in the centre of the arena in a small wire cage, attached to a rope that ran through an overhead pulley. The observer switched on the speaker, retreated behind the wall of the arena, and lifted the cage confining the toad using the rope. After 5 min, the observer stood and illuminated the arena with a spotlight, and recorded the location of the toad in the arena.

Comparison of Traps With and Without Acoustic Attractants

This experiment was designed to compare the capture rates of pairs of traps, one pair with playbacks and one pair without, over an extended trapping season. We compared pairs of traps to increase capture rates and reduce the variance of capture rates. We used three-door traps purchased from Northern Territory FrogWatch (http://www.frogwatch.org.au/canetoads/default.cfm). This study was conducted between 10/02/2006 and 29/04/2006, at the James Cook University Campus (19°15'42"S 146°48'34"E), and the Billabong Sanctuary Wildlife Park south of Townsville (19°21'27" S 146°53'09" E). Trapping was conducted on 54 nights. Trapping nights spanned a wide range of weather conditions. On trapping nights, two pairs of traps were set at either or both of the Billabong Sanctuary and Campus Creek sites. The traps in each pair were approximately 50 m apart., and the two pairs of traps at each site were at least 250 m apart The playback equipment was swapped between pairs of traps on successive trapping nights at each site, to avoid any bias in capture rates that might have resulted from one pair of traps being in a location with higher toad densities. The Billabong Sanctuary and Campus Creek sites are approximately 17 km apart and are on opposite sides of Mt Stuart, so toad populations at the two sites should be relatively isolated from each other. The toad populations at both sites are large, which we verified by collecting toads at each of them for our acoustic experiments and other studies.

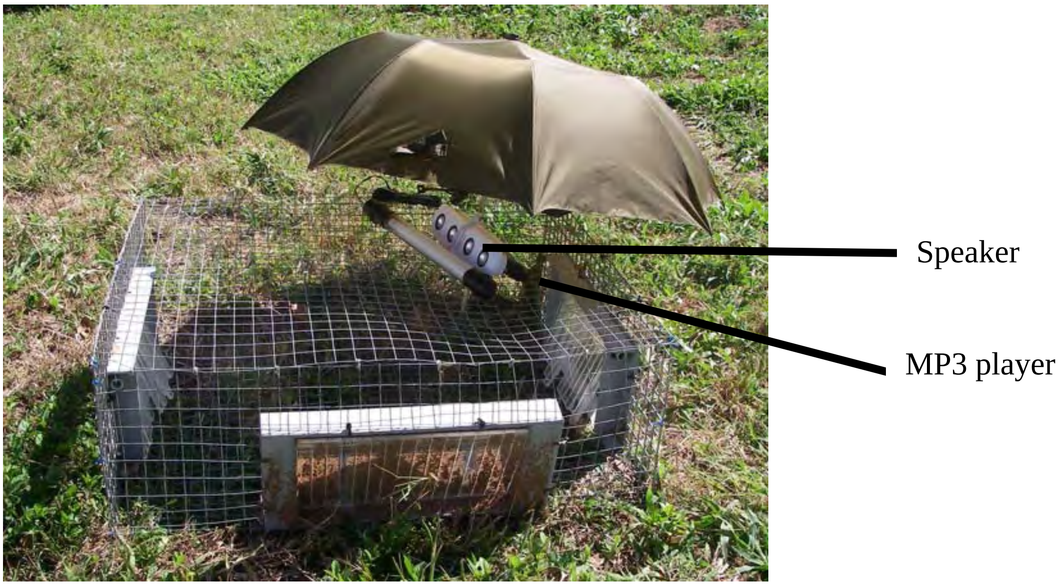


Figure 3. Toad trap showing location of speaker, trap doors, light and MP3 player. The umbrella protected the speaker and MP3 player from rain.

Traps with playbacks were equipped with a Compupal™ PS-20 portable 4-watt speaker attached to a Aerpro digital MP3 player positioned on top of the trap (Figure 3). An umbrella protected the speaker and MP3 player from rain. Traps without speakers were assembled in a similar fashion but without the speaker, MP3 player or umbrella. Umbrellas exposed about 70% of the trap to rain, so traps with speakers were no drier than traps without speakers. Because toads move very little during the day, it is unlikely that shade provided by the umbrella affected their decisions to enter traps.

All traps were equipped with 8-watt white fluorescent lights powered by rechargeable 12-volt gel-cell batteries. The MP3 player used 1 AAA battery. Typically, one battery lasted approximately 8 h, so calls (the same call used in the arena experiments, played in continuous repeat mode) were audible all night. Call volume from speakers on traps ranged from 58-72 dB(A) at 1 m.

Traps were checked and emptied of toads each morning. The sexes and numbers of toads in traps were recorded, and toads were removed from the area and disposed of by freezing. We obtained weather data (rainfall in the 24 hours to 9 AM on the day traps were emptied, and hours of sunshine, evaporation in mm, and minimum and maximum temperatures on the day traps were set) from the Bureau of Meteorology recording station at Townsville Airport for use in determining whether capture rates were affected by weather.

Description of Shelter Sites Selected by Toads

Toad shelter sites were surveyed during the late dry and early wet seasons between September and November 2005. One hundred sites were examined, 48 in urban environments and 52 in woodland. All woodland shelters were at Table Top Station. Urban sites were located in and around the JCU campus and in the residential suburbs of Townsville.

Shelters were located by attaching tracking spools (Figure 4) to toads captured while foraging or hydrating. Tracking spool packs were assembled by attaching a single quilting thread bobbin (no.10 or no. 8, Danfield™ Sewing Thread Specialists Australia), to a harness attached to the toad's waist. Spools were placed in heat-shrink tape with a small strip of corrugated plastic, and a length of dialysis tubing was run through a hollow section of the corrugated plastic by piercing the heat-shrink tape. The tracking unit was attached to the toad by tying the dialysis tubing around the toad's waist. Cane toads inflate

Figure 4. Cane toad with prototype version of spool tracking apparatus attached. In the final version, spool was oriented parallel to the long axis of the toad, with line paying out at posterior end, and was encased in heat-shrink tubing.

when handled, therefore the latex tubing was tied snugly, which leaves the pack firmly in place when the toad deflates. Once properly attached, the spool sits on the lower back of the animal with thread paying out from the centre of the posterior end of the spool. The free end of thread was tied to any nearby stationary object and marked with flagging tape to indicate the starting point for tracking the following day. The date of capture, ambient temperature and humidity, as well as the total number of animals tracked was recorded for each night.

Each morning, the thread was followed to find the diurnal shelter the toad had selected. Direction and distance travelled were recorded for each toad. When possible, the toad was removed from the shelter, its tracking pack removed, and its mass (to the nearest g, using a Pesola™ spring balance) and snout to urostyle length (to the nearest mm, using a ruler) were recorded. After measurements, toads were released in the nearest shaded area. When it was possible to do so, we measured the size of the shelter interior (length, width and height). When the interior could not be accessed, such as when shelters were under concrete sidewalks or large rocks, the entrance was measured and the internal size of the shelter was estimated by inserting a thin stick and probing to determine the shape and dimensions of the cavity. Materials that composed the shelter floor, walls and ceiling were recorded. Distance from capture point, distance to nearest water, % shade over shelter site, shelter type (i.e., animal burrow, hollow log, gap under sidewalk), and vegetation surrounding the shelter were also recorded.

When measurements of the shelter site were complete, a Thermochron iButton™ temperature and humidity logger was placed into the shelter in direct contact with the substrate where the toad was sheltering when found. The iButton™ simultaneously recorded temperature and humidity levels of the shelter every ten minutes from the time that it was deployed until it was collected. If any part of the shelter was disassembled or disturbed to remove the toad and take measurements, every effort was taken to replace all materials to a pre-disturbed state before deploying the data recorder.

Results

Y-Maze Experiments

A total of 80 toads (40 males and 40 females) were each exposed to five choices in the Y-maze during the initial series of trials, for a total of 400 hours of trials. The results are summarised in Table 2, which shows data for all toads combined and only for trials in which the focal (responding) toad (and the stimulus toad or toads, where these were used) were reproductively active.

Table 2. Responses of cane toads (*Bufo marinus*) in a Y-maze with various stimuli in arms, stimuli listed in columns labelled A & B below. Summaries for all toads and only for trials in which both focal and stimulus animals were reproductively active, as defined in the text.

Sex	Choice A	vs.	Choice B	Number that chose A	Number that chose B	χ^2	exact P	Number that did not choose
				All individuals tested				
Male	Left	vs.	Right	16	21	0.676	0.511	3
	Opposite sex	vs.	Nothing	16	23	1.256	0.337	1
	Same sex	vs.	Nothing	10	23	5.121	0.035*	7
	Same sex	vs.	Opposite sex	26	10	7.111	0.011*	4
	Food	vs.	Nothing	15	21	1.000	0.405	4
Female	Left	vs.	Right	17	19	0.111	0.868	4
	Opposite sex	vs.	Nothing	17	16	0.030	1.000	7
	Same sex	vs.	Nothing	16	15	0.032	1.000	9
	Same sex	vs.	Opposite sex	21	10	3.903	0.071†	9
	Food	vs.	Nothing	9	22	5.452	0.029*	9
Combined	Left	vs.	Right	33	40	0.671	0.483	7
	Opposite sex	vs.	Nothing	33	39	0.500	0.556	8
	Same sex	vs.	Nothing	26	38	2.250	0.169	16
	Same sex	vs.	Opposite sex	47	20	10.880	0.001*	13
	Food	vs.	Nothing	24	43	5.388	0.027*	13
				Only reproductively active individuals (both responding and stimulus, where applicable)				
Male	Left	vs.	Right	14	17	0.290	0.720	1
	Opposite sex	vs.	Nothing	7	12	1.316	0.359	1
	Same sex	vs.	Nothing	7	16	3.522	0.093†	5
	Same sex	vs.	Opposite sex	20	6	7.538	0.009*	4
	Food	vs.	Nothing	12	16	0.571	0.571	4
Female	Left	vs.	Right	9	15	1.500	0.308	2
	Opposite sex	vs.	Nothing	12	7	1.316	0.359	1
	Same sex	vs.	Nothing	8	12	0.800	0.503	3
	Same sex	vs.	Opposite sex	16	7	3.522	0.093†	2
	Food	vs.	Nothing	6	17	5.261	0.0347*	3
Combined	Left	vs.	Right	23	32	1.473	0.281	3
	Opposite sex	vs.	Nothing	19	19	0.000	1.000	2
	Same sex	vs.	Nothing	15	28	3.930	0.066†	8
	Same sex	vs.	Opposite sex	36	13	10.800	0.001*	6
	Food	vs.	Nothing	18	33	4.412	0.049*	7

*significant at α=0.05; †approaches significance

During the 1-h period of each trial, males were more likely to enter an arm of the Y-maze than were females. Males failed to choose in 19 of 200 trials, while females failed to choose in 38 of 200 trials (Table 2). To examine the effects of treatments as presented in Table 2, we initially performed chi-squared contingency tests of the null hypothesis that responses were similar across all five treatments for each of the six sets of five results. All of these showed that responses varied significantly among treatments (all $P \ll 0.05$). We therefore used separate chi-squared goodness-of-fit tests of the null hypothesis that toads entered either arm of the maze at equal frequencies to determine which treatments the focal toads showed preferences in. Neither sex showed any particular preference for the left or right arm of the maze (Table 2). When presented with the choice between a male toad and an empty arm, males chose to enter the empty arm significantly more often than they entered the arm containing a male, i.e. they avoided the odour of other males. In contrast, and with strong statistical significance, when presented with the choice between the odours of a male and a female, males strongly significantly chose to enter the arm of the maze containing a male (Table 2). Females responded differently to the odours of conspecifics. When presented with the choice between entering an arm of the maze containing another toad of either sex and an arm containing nothing, they chose either direction with almost exactly equal frequency (Table 2), strongly suggesting that they did not discriminate based on scent. However, when presented with the choice between an arm containing a male and one containing a female, they chose to enter the arm containing the female much more frequently. This result is on the borderline of statistical significance ($P=0.07$; Table 2). Females thus showed a tendency approaching significance to move toward females when given the choice between males and females; females were also significantly more likely to enter the empty arm than the one containing dog food (Table 2). When the data for males and females were combined, both sexes preferred to move towards their own sex, and both sexes avoided dog food.

In general (Table 2) the data only for trials in which reproductively active individuals were used show patterns of responses very similar to those for trials including individuals that were not reproductively active, or whose status could not be determined (see methods). Levels of statistical significance differ slightly because excluding data for individuals not known to be reproductively active decreased sample sizes, but the patterns are very similar. The only difference is that the pattern of preferring an empty arm of the maze over one containing an individual of the same sex when presented with this choice is stronger for reproductively active individuals when the data for both sexes are combined. These results suggest that reproductive status has little effect on the olfactory cues provided by toads and the responses of toads to these cues.

The data for the separate series of trials conducted examining possible attraction to pond water indicated that there was no significant tendency of males, females, or both sexes combined to enter the arm of the maze containing pond water more frequently than the empty control (Table 3).

Table 3. Responses of cane toads to pond water in a Y-maze.

Sex	A	B	Chose A	Chose B	No Choice	χ^2	Exact P
Males	Water	Empty	5	5	0	0.00	1.00
Females	Water	Empty	6	9	2	0.60	0.61
Total	Water	Empty	11	14	2	0.36	0.69

Cafeteria Trial Experiments

Food in both our open and closed control treatments lost mass (Figure 5). Mass loss in control treatments was likely due to a combination of drying and removal of food by insects. Interestingly, in the controls, most of the foods lost more mass in the closed tanks (exposed only to small ants) than in those open to large insects (beef, $t = -2.1$, $df = 25$, $p = 0.04$, chicken, $t = -1.2$, $df = 26$, $p = 0.26$, sardines, $t = -3.5$, $df = 20$, $p = 0.002$, cat food, $t = -2.9$, $df = 19$, $p = 0.008$, dog food, $t = -2.9$, $df = 26$, $p = 0.006$). This is probably because small ants, which we saw in large numbers, carried off portions of the food.

Toads consumed food they were offered in our cafeteria trials. We confirmed this by dissecting sacrificed animals; their guts contained material identifiable as cafeteria-trial food. Significantly more food of four of the five types disappeared from open tanks containing toads than from open control tanks, (Figure 5), however no food type disappeared at greater rates in closed tanks containing toads than it did in control tanks (Figure 5).

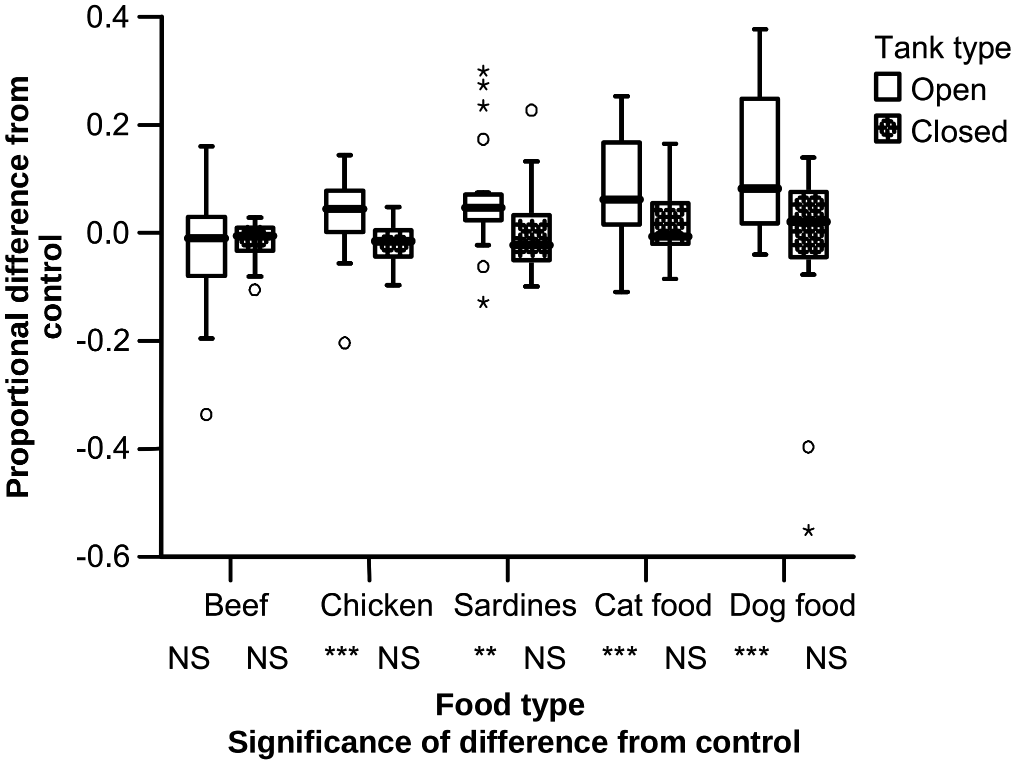


Figure 5. Food lost from tanks containing toads as a proportion of food lost from control tanks. Heavy lines indicate median values, bars are upper and lower quartiles, and points and asterisks are extreme values. Median values close to zero suggest that equal amounts of food disappeared from tanks with toads and those without toads. Positive median values mean more food disappeared from tanks containing toads. Significance of difference from 0 (toad tanks equal to control tanks) tested using exact Wilcoxon Signed Ranks tests in StatXact 4.0.

To further examine the effects of tank accessibility on food disappearance, we categorised the data for each food type in each tank containing a toad by whether the quantity of food that disappeared was less than or equal to, or was greater than, the mean amount that disappeared in the corresponding controls. These results appear in Table 4.

Table 4. Number of trials in which the amount of food that disappeared in a tank was either less than or equal to the mean for control tanks of that type on that date, or the amount of food that disappeared was greater than the mean for control tanks of that type on that date. Note that more open treatments with toads lose more food than controls and than closed treatments.

Food	Tank type	Toad present versus control	
		Less than or equal to	Greater than
Beef	Open	20	16
	Closed	27	21
Chicken	Open	12	24
	Closed	27	21
Sardines	Open	12	24
	Closed	28	20
Cat food	Open	10	26
	Closed	23	25
Dog food	Open	10	26
	Closed	21	27

It is clear from them that more food consistently disappeared when toads were present than in controls only when the tanks were open. A Zelen's test for homogeneity of odds-ratios indicated that the effect of tank type did not differ significantly among food types (ZE = 0.0004987, exact P = 0.5352, StatXact 4.0). Being in an open tank approximately doubled the odds that more food would disappear from a tank containing toads (maximum. likelihood estimate of the common odds ratio = 2.0, 95%CI 1.3 – 3.0). The fact that more food disappeared when toads were present only from open tanks, and that this effect did not differ significantly among food types, suggests that toads were initially attracted to food not by the properties of the food itself, including its scent, but by larger insects that were attracted to the food. Food consumption may have been incidental to insect consumption, or toads may have continued to consume food after being attracted to it by the presence of larger insects, but it did not appear that food by itself is an effective toad attractant.

Acoustic Arena Trials

We conducted a preliminary survey of the standing pattern of sound intensity in the arena by measuring sound pressure levels at distances of 1, 2, and 3 m from the centre of the enclosure on a radial grid arranged at 7.5 degree angles, while playing back pink noise at a constant volume of 67 dB(A) at 1 m from the speaker. The observer read the sound pressure level remotely while outside the arena. This demonstrated that reflections from the metal walls did not produce any inversions in the gradient of sound intensity; a toad travelling up gradients of sound pressure would move towards the speaker from any point in the arena.

When exposed to pink noise at either volume, both male and female toads showed no significant directional bias, moving at apparently random angles relative to the source of sound (Figures 6 and 7). Loud (67 dB at 1 m) calls may have attracted males; there was a marginally significant trend for male toads to move towards loud calls (Figure 8A). They did not appear to attract females (Figure 8B). However, both males and females were significantly attracted to low-volume (47 dB at 1 m) calls in our arena (Figure 9 A and B). This suggested that the attractiveness of traps might be enhanced by adding call playbacks to lure toads; we tested this using the trapping experiment discussed in the next section.

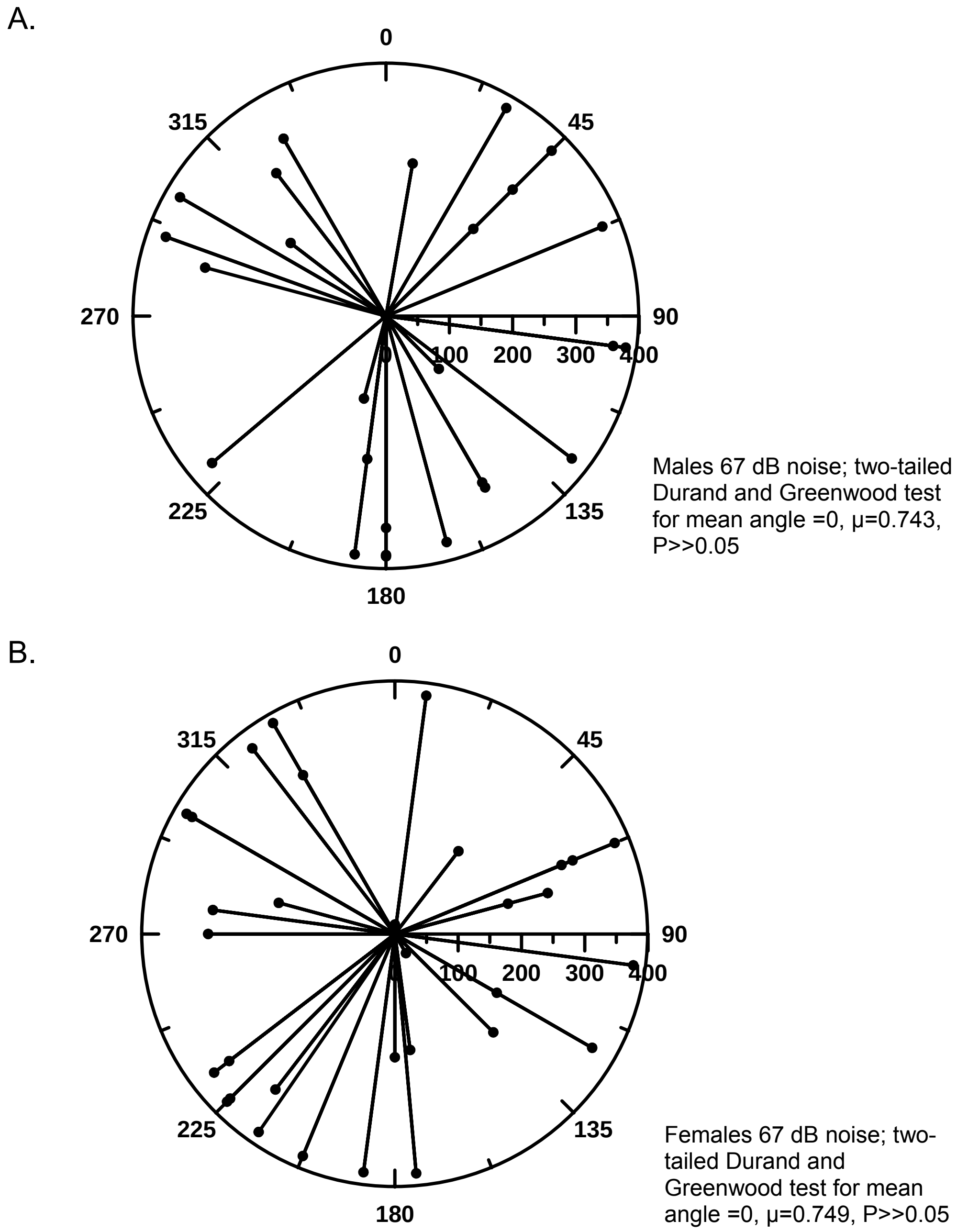
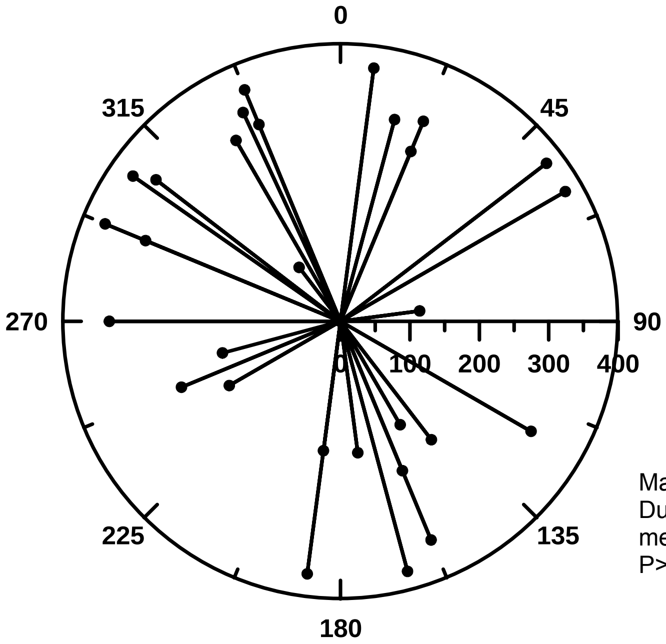


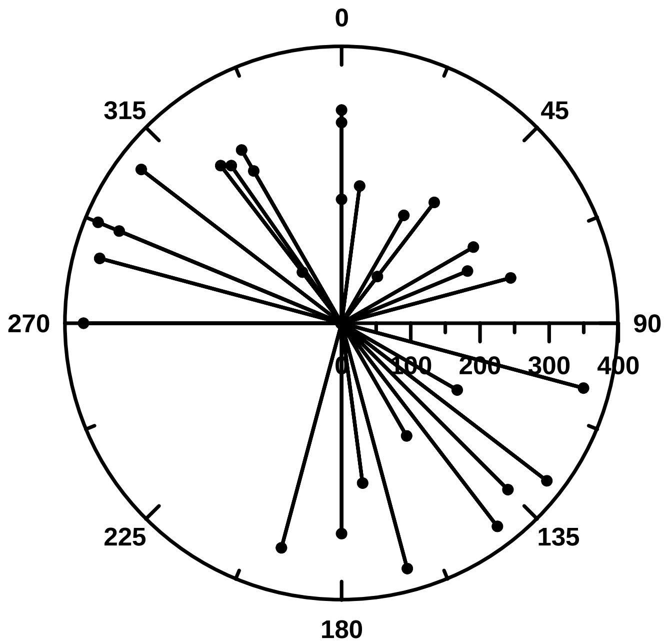
Figure 6. Toad responses to loud (67 dB(A) at 1 m) pink noise. Position of the speaker is normalised to zero. Points indicate positions of individual toads after 5 minutes.

A



Males 47 dB noise; two-tailed Durand and Greenwood test for mean angle =0, μ=0.794, P>>0.05

B



Females 47 dB noise; two-tailed Durand and Greenwood test for mean angle =0, μ=0.577, P>>0.05

Figure 7. Toad responses to quiet (47 dB(A) at 1 m) pink noise. Position of the speaker is normalised to zero. Points indicate positions of individual toads after 5 minutes.

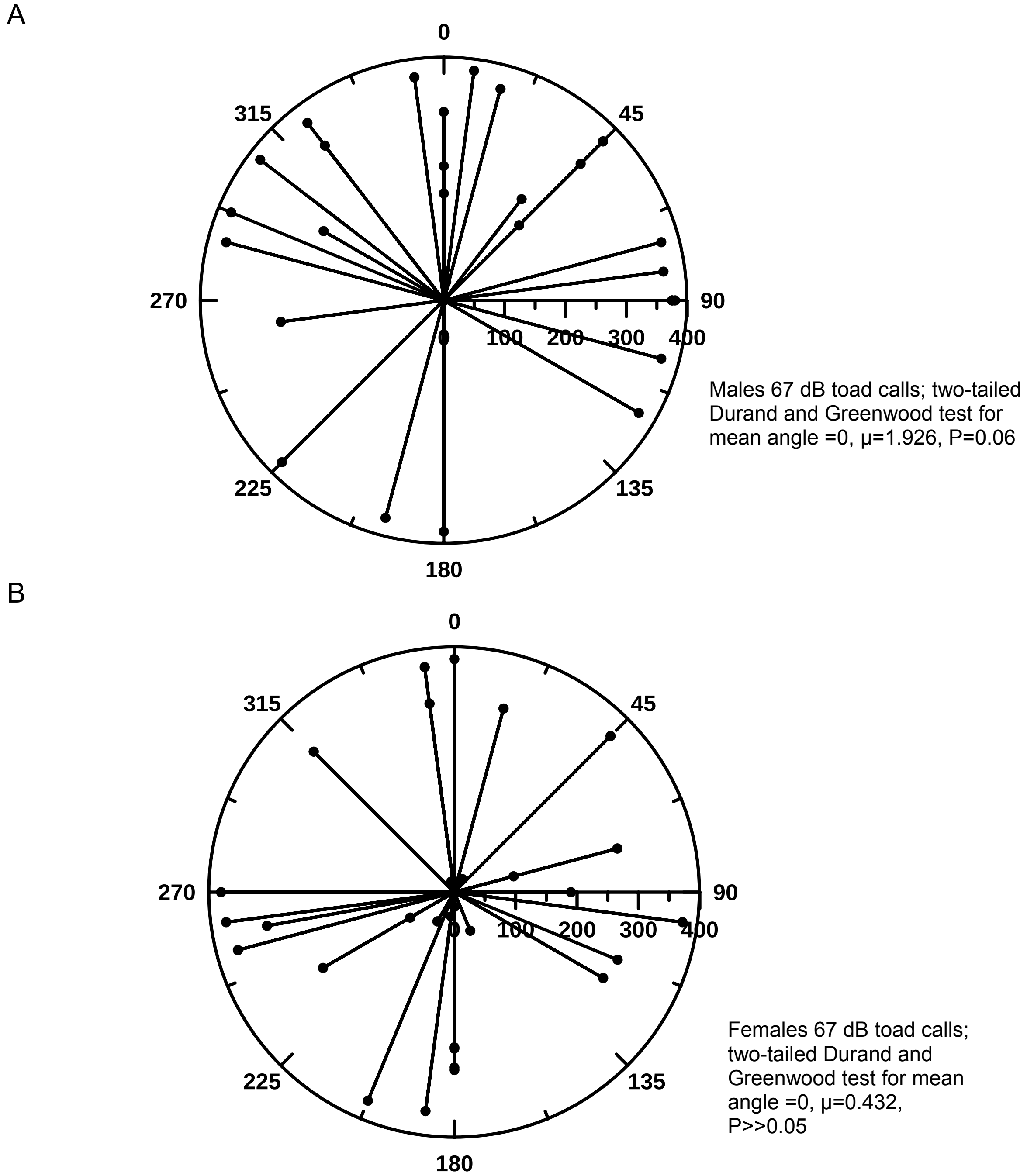
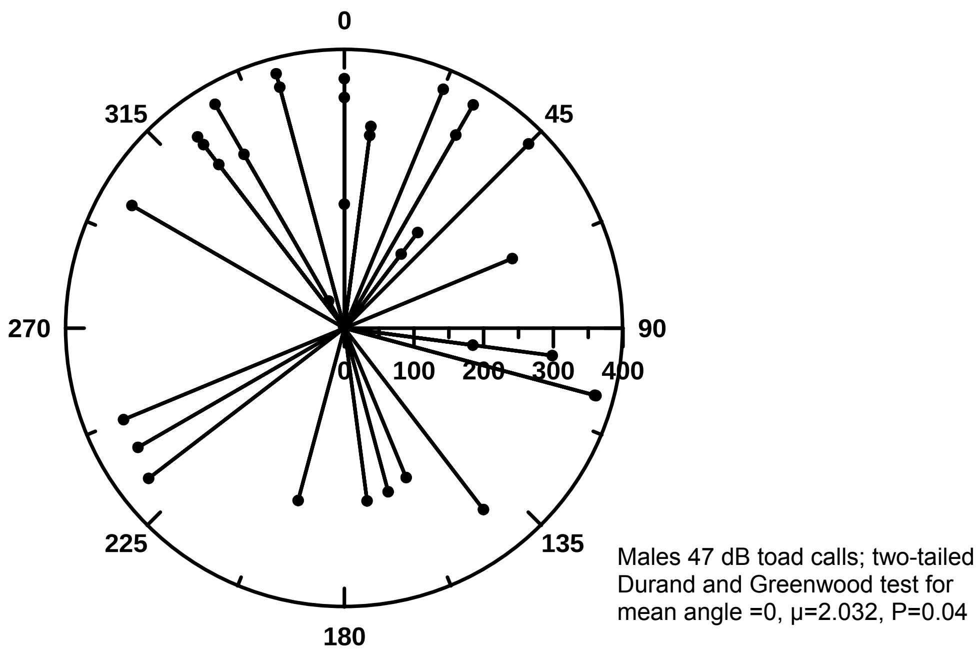


Figure 8. Toad responses to loud (67 dB(A) a 1 m) toad calls. A. Males, B. Females. Position of the speaker is normalised to zero. Points indicate positions of individual toads after 5 minutes.

A.



B

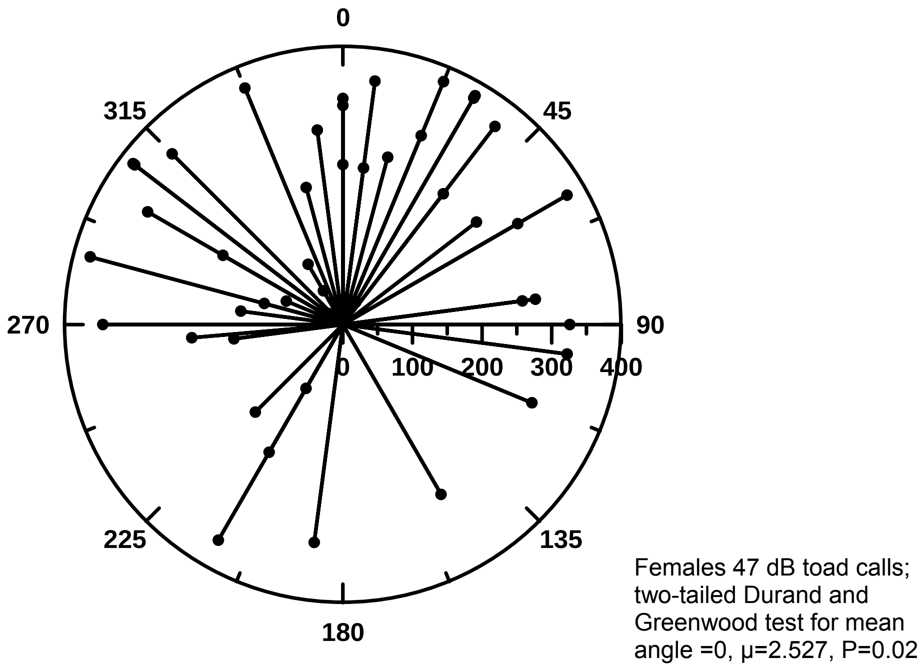


Figure 9. Toad responses to quiet (47 dB(A) at 1 m) toad calls. A. Males, B. Females. Position of the speaker is normalised to zero. Points indicate positions of individual toads after 5 minutes.

Comparison of Traps With and Without Acoustic Attractants

We trapped on 54 nights for a total of 292 trap-nights. We captured a total of 87 toads, 69 males and 18 females (Table 5). This is a low capture rate of only about 1.2 toads per night. Stepwise logistic regressions using weather variables to predict whether any toads were captured in traps indicated that catching or failing to catch toads on any night was not strongly affected by weather; no models were statistically significant. This result would probably differ if studies were carried out over a wider range of weather conditions; during the trapping study the Townsville area was experiencing an unusually prolonged and relatively constant wet period.

Table 5. Total numbers of male and female toads captured over 292 trap-nights of trapping effort in the Townsville region. Traps deployed in pairs, one with and one without an acoustic lure (toad call playback).

Sex	Trap type	
	Control	Playback
Male	17	52
Female	5	13

Table 6 summarises trapping success for all nights at each locality and the combined localities by sex and trap type. Initial analysis of these data using Zelen's test for homogeneity of odds ratios in StatXact 4.0 on the data for males and females on nights when toads were captured indicated no interaction between the effects of sex and locality on relative success rates of traps with and without playbacks (Zelen statistic = 0.5229, 1 d.f., exact P = 1.00). Subsequent analyses of 2 X 2 tables using Fisher's exact tests in StatXact 4.0

indicated no main effects of locality on relative success data combined across sexes (P = 0.740) or sex on relative success data combined across localities (P = 0.4859). A final binomial test on data combined across sexes and localities indicated that playback traps caught more toads than silent traps on significantly more than 50% of nights when toads were captured (32 of 39 nights, binomial P for playback total greater than silent total = 0.8205, 95% Blyth-Still-Casella confidence interval = 0.6758 to 0.9205).

Table 6. Trapping results by locality, trap type, and toad sex. Numbers are the numbers of nights at each location and for the combined locations on which no toads were captured, the number of toads captured in playback traps was less than or equal to the number caught in silent traps, and the number of toads captured in playback traps was greater than the number captured in silent traps.

Locality	Toad sex	Number of nights		
		No captures	Toads captured	
			Playback total ≤ silent total	Playback total > silent total
JCU campus	Male	28	2	11
	Female	34	2	5
	Combined	25	2	14
Billabong	Male	10	5	16
	Female	22	3	6
	Combined	8	5	18
Total	Male	38	7	27
	Female	56	5	11
	Combined	33	7	32

In spite of the low capture rates, significantly more toads were captured in traps with speakers playing toad calls (one-tailed Wilcoxon signed-ranks tests; females: $Z = 1.886$, $P < 0.05$; males, $Z = 3.889$, $P < 0.001$; both sexes combined: $Z = 4.144$, $P < 0.001$). More toads over all, more males, and more females were captured in traps with playbacks. To illustrate these results, we calculated the mean differences in numbers of toads captured per trap of each type, and used bootstrapping to estimate 95% confidence intervals for these differences (Figure 10).

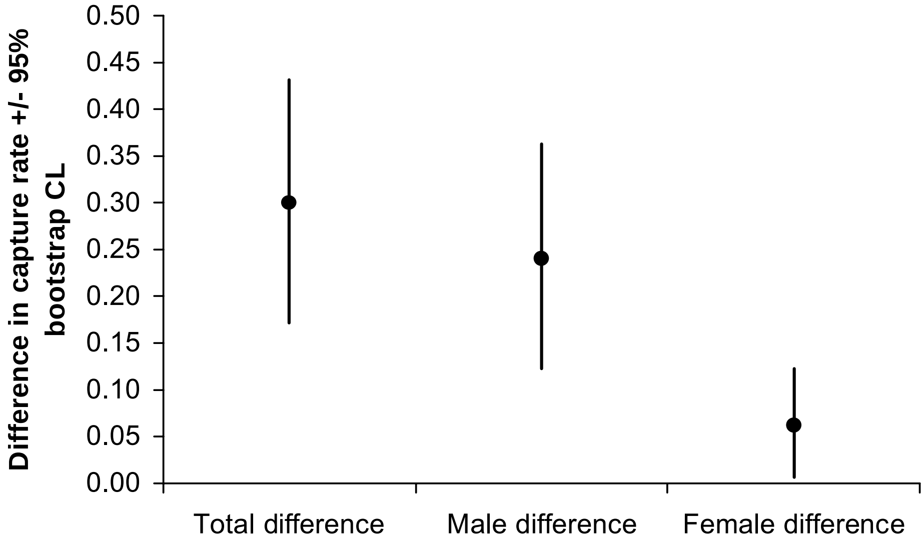


Figure 10. Mean differences in number of toads captured between pairs of traps with and without acoustic lures (toad call playbacks). Confidence limits calculated using 10,000 bootstrap resamplings of the data.

To make it easier to visualise the advantage that accrued to traps with playbacks, Figure 11 presents overall mean capture rates of all toads, males, and females for traps with and without playbacks. On average, traps with playbacks had capture rates (toads per trap-night) approximately three times as great as traps that only had lights (0.48 versus 0.15).

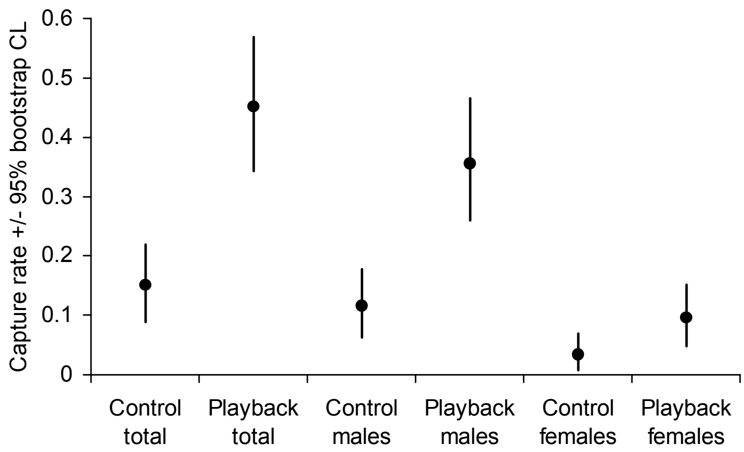


Figure 11. Mean capture rates per trap-night over the entire trapping study, +/- 95% confidence limits calculated from 10,000 bootstrap resamplings of the data.

Description of Shelter Sites Selected by Toads

We collected data on 100 shelter sites, 48 in urban areas and 52 in woodland. We obtained body sizes for 56 of the toads that occupied these sites, and measured temperature and humidity in 65 of the shelter sites. Cane toads use a variety of retreat sites made of numerous materials. Shelters included: hollow logs, cavities within living or dead vegetation, cavities under rocks, burrows in various soil types, open indentations in the substrate, and cavities created by man-made features (Figure 12).

Shelter site volumes were not significantly different among substrate types (one-way ANOVA on log_{10} transformed shelter site volumes: $F_{5,99} = 1.99$, p = 0.08, hollow logs were larger than other categories, so this ANOVA approached significance). There was also no significant correlation between the size of toads and the size of their shelter sites, and some shelter sites were very much larger than even the largest toads (n = 57, $r^2 = 0.08$, p = 0.3) In spite of this, toads preferred a relatively small range of shelter sizes between 1000 and 10,000 cm^3 (Figure 13).

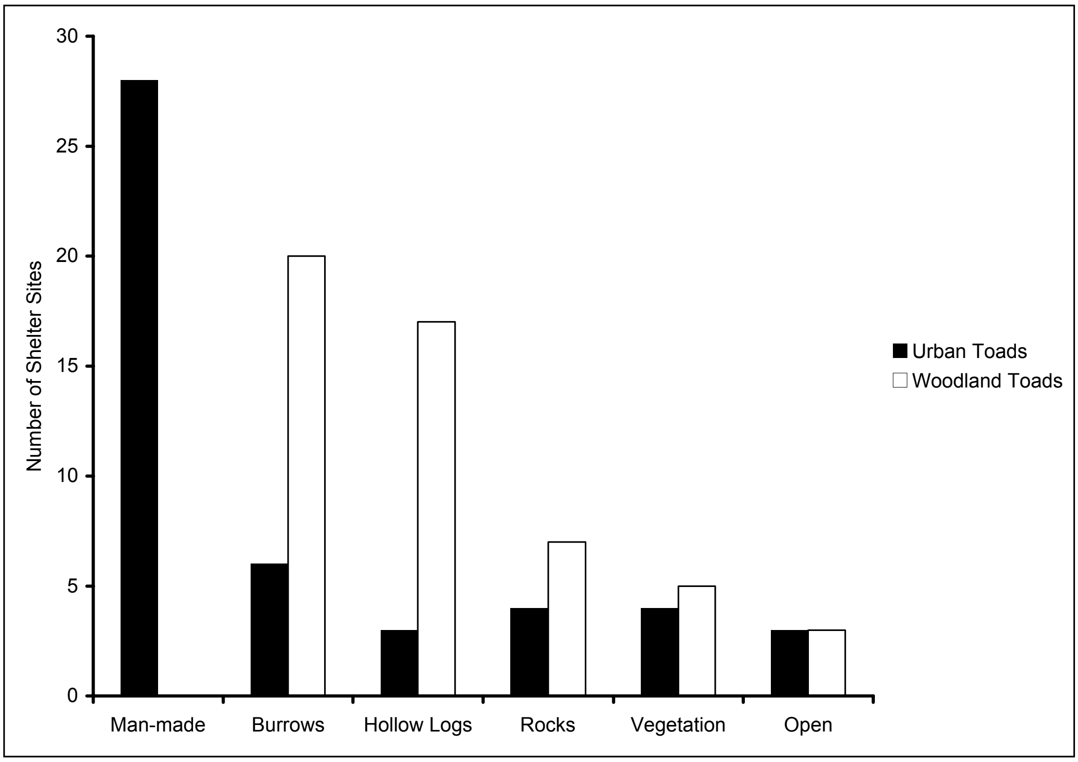


Figure 12. Frequency distribution of shelter types selected by toads from urban and woodland sites. Toads from woodland sites had no man-made features available.

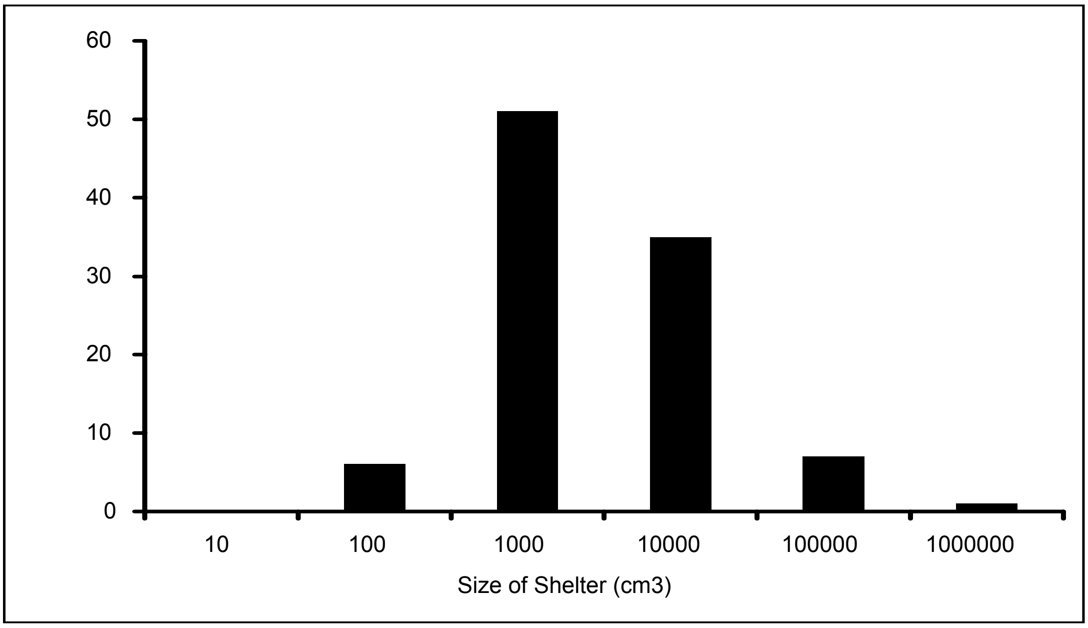


Figure 13. Numbers of shelters in a log series of sizes by volume. Number is the minimum volume of shelters included in each category. The modal shelter size is between 1000 and 10, 000 cm^3.

Toads occupied shelters with a relatively narrow range of mean temperatures (42 of 65 were between 26-30°C, Figure 14), and usually with high humidities (between 95 and 100%, Figure 15). Minimum, maximum and average temperature and humidity did not differ significantly between shelters in urban and woodland habitats (data log transformed: minimum temperature t-test, t= -1.7, df = 1, p = 0.09, maximum temperature t-test, t= -0.4, df = 1, p = 0.7, mean temperature t-test, t= -0.4, df = 1, p = 0.7, humidity t-test, t= -0.07, df = 1, p = 0.9) nor did those measurements vary significantly among shelter type (ANOVA $F_{5,64}$ = 1.7, p = 0.16).

There were, however, significant differences between the mean temperatures and humidities of shelters affected by different amounts of shade (data log transformed: mean temperature ANOVA $F_{4,64}$ = 5.2, p = 0.001, Table 7). Shelters with little to no shade typically had lower humidity levels and higher temperatures. Only 30% of toads used shelters with less than 50% shade (Figure 16).

Table 7. Means and standard deviations of microclimate measurements for shelters in various shade categories (for numbers of shelters in each category, see Figure 11).

Shelter category	Mean temperature (±SD) (°C)	Mean maximum temperature (±SD) (°C)	Mean minimum temperature (±SD) (°C)	Mean relative humidity (±SD) (%)	Mean maximum relative humidity (±SD) (%)	Mean minimum relative humidity (±SD) (%)
<10% shade	28.8±4.0	33.4±6.8	24.5±2.2	80.1±10.0	90.9±7.4	66.5±15.9
11-25% shade	29.0±3.3	33.8±6.9	24.8±2.2	76.4±16.4	87.3±10.4	63.3±25.2
25-50% shade	27.9±3.2	31.8±5.7	24.8±2.5	80.5±15.6	90.3±11.3	69.4±21.0
51-75% shade	25.2±0.9	26.1±1.2	24.1±1.3	99.5±4.1	100.0±3.3	96.1±6.6
>75% shade	25.0±1.9	24.6±1.8	23.7±2.5	95.2±4.8	98.7±9.6	91.1±7.2

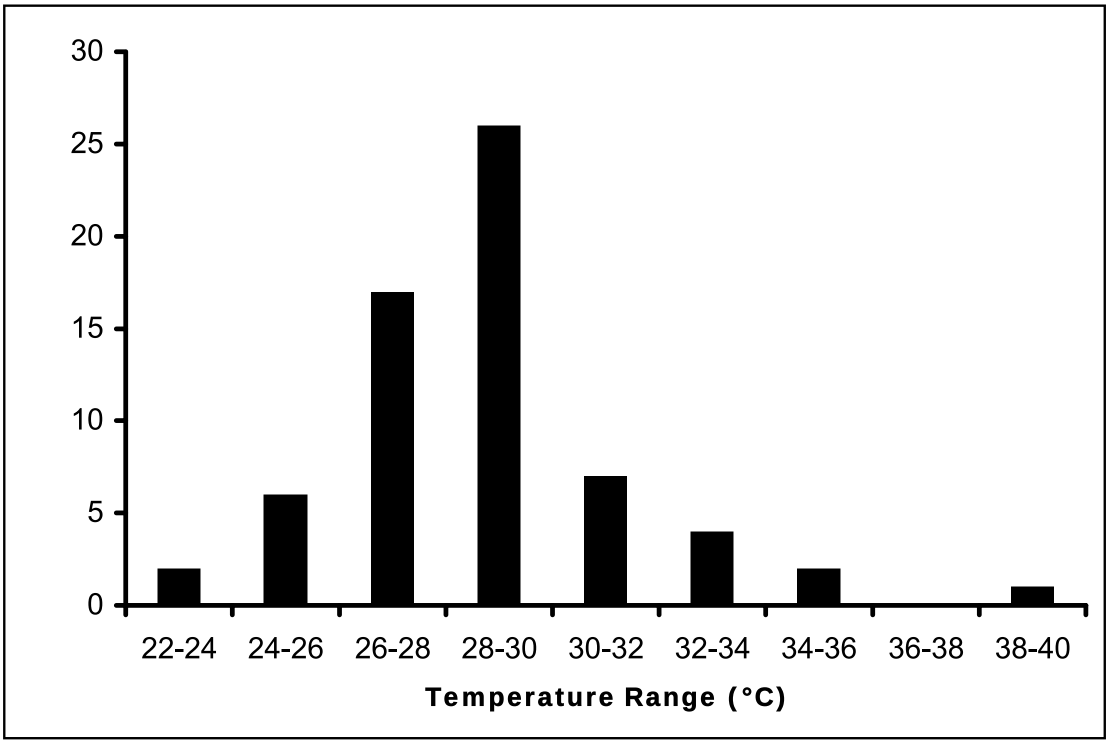


Figure 14. Numbers of toad shelters with mean temperatures in each category.

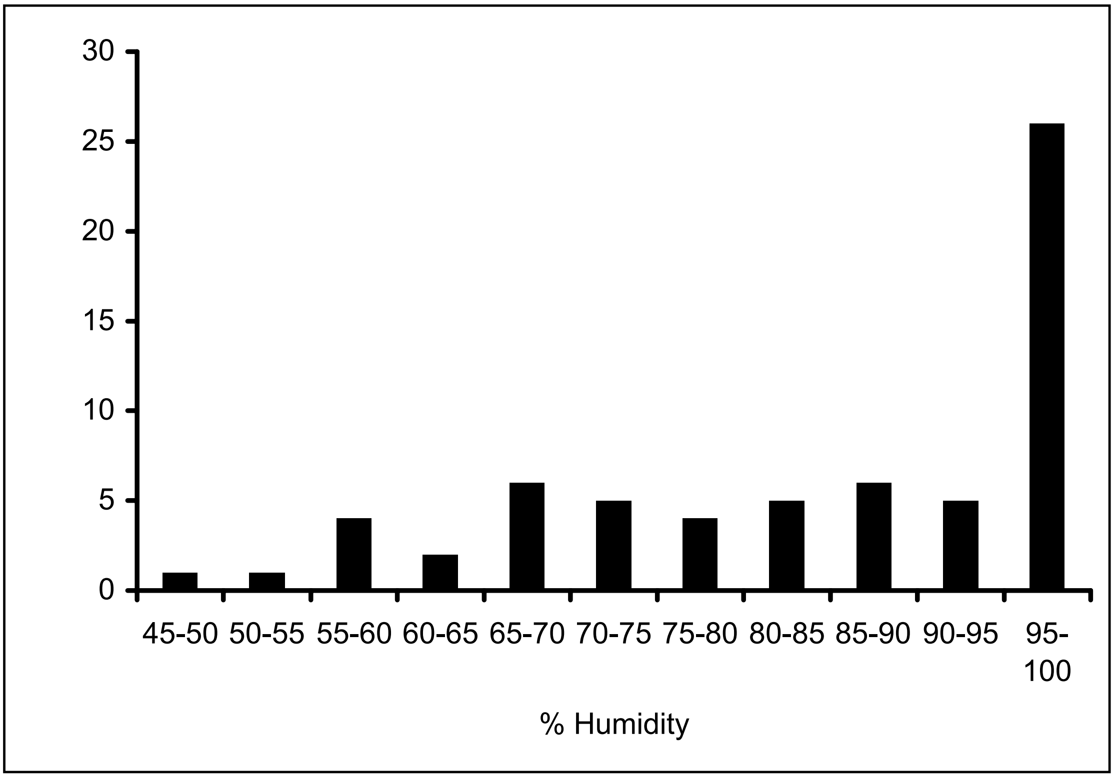


Figure 15. Numbers of toad shelters with mean relative humidity in each category.

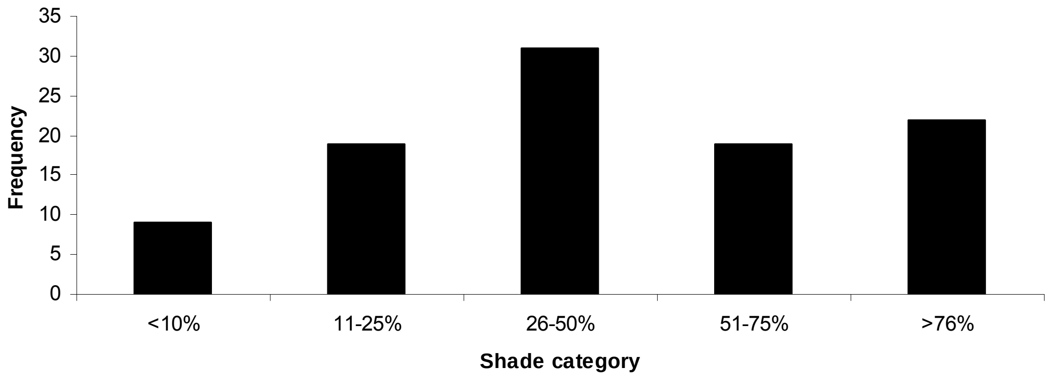


Figure 16. Numbers of cane toad shelter sites under different amounts of shade.

Shelter sites were composed of a variety of materials. Leaf litter was the most common feature of shelters. Soil, containing leaf litter, and sand with pebbles most commonly comprised the floors of shelters (Figure 17). The walls and roof of shelters were usually composed of soil (Figure 17). Man made shelters included cracks in concrete, piles of garden rubbish, building materials and drains and downpipes on buildings. The substrate in shelters was usually damp, even when substrate outside shelters appeared dry.

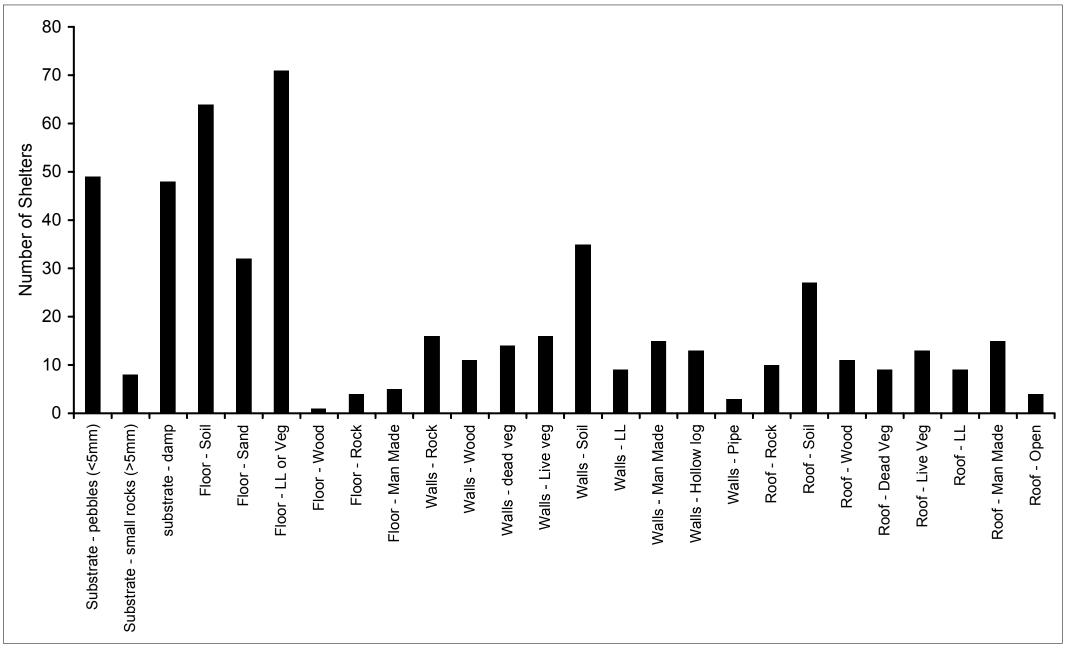


Figure 18. Numbers of cane toad shelters examined that incorporated each named material.

Sixty-three toads entered a streambed, either dry or with standing water present, at some point while being tracked. Only 9 of these toads (14%) used a shelter site in the streambed. Toads using streambeds during activity usually sheltered on the bank of the stream. Thirty-seven shelters were located on a bank above the streambed bottom, and 17 were on level terrain above the streambed. The average height above the streambed for shelters in which the toad had entered a waterway was 2.67m.

Discussion

Cane Toad Responses to Odours

Our Y-maze data clearly indicated that adult toads could respond to scents, using them to make decisions about movement. Although this has been shown for other species of anurans (Waldman and Bishop 2004), this is the first demonstration that cane toads have this ability. It may be possible to use their ability to orient themselves using olfactory cues to attract cane toads to traps, but our Y-maze trials revealed that whole-item odours emanating from food, pond water, and conspecifics of either sex do not exert simple attractive or repellent effects on cane toads. None of the stimuli we used attracted or repelled all toads. Toads moved towards their own sex, when given the choice between two toads of different sexes, but avoided (males), or did not respond (females) to their own sex when given the choice between a toad of their own sex and an empty arm. This suggests that there may be a combination of unequal-strength repellents and attractants at work, and detailed studies of the chemical composition of scents associated with males and females are required to investigate this further. We are planning further work in collaboration with Professor Rob Capon at the University of Queensland. His group has been funded by the Queensland government to investigate the chemical ecology of toads and the properties of toad venom. They will fractionate extracts from cane toads and we will evaluate each fraction separately in our Y mazes; this may separate the attractants from the repellents and lead to the development of a reliable scent lure.

The data for females and for both sexes combined indicated that toads avoided the odour of the dog food (Masterfoods™ My Dog™ Lamb Classic) we offered. This was surprising, because people regularly note that cane toads eat pet food (e.g., Australian Museum 2002). Our cafeteria trials suggest that toads may initially be attracted to insects attracted by the pet food, rather than being attracted directly by the scent (see below). It is not clear, however, why toads were repelled by the dog food. There are probably a wide range of formulations of pet foods, containing a wide range of natural and artificial ingredients. The same dog food disappeared in our cafeteria trials at the highest rate of any of the foods offered when toads were present, suggesting that it was the most attractive. It seems possible that naïve toads may avoid the odour of dog food simply because it is a strong and unfamiliar scent, but once they have become conditioned to associate it with food, through initial attraction to insects, they may be attracted to it. These results indicate that additional detailed work on the nature of the chemical repellents and attractants present in different potential foods could be productive.

Pond water was not attractive to cane toads in the mazes. While toads deprived of water do show increased activity and water-seeking behaviour (Jørgensen 1990), the toads in our experiments were not dehydrated. Our results indicate that water is unlikely to serve as an effective attractant for toads during the wet season when they are most active and experience little water stress (Schwarzkopf and Alford 1996). Previous studies, and our own present study of shelter sites (below), suggest that toads prefer shelter sites that provide high humidity. We plan to evaluate the attractiveness of water to partially dehydrated toads in future Y-maze trials.

Toad Responses to Food in Cafeteria Trials

We verified that some of the food that disappeared in cafeteria trials was eaten by toads by examining the gut contents of toads sacrificed at the end of these experiments. Only tanks with open mesh lids had significantly more food disappear than controls. In tanks with fine mesh lids, although dissected toads had some food in their guts, food did not disappear at significantly higher rates than in control tanks without toads. Thus, toads ate more food in tanks that were roofed with open mesh, allowing large insects to enter. This suggests that toads were initially attracted to insects that were attracted to the food. Perhaps after consuming food along with the insects, they learned to eat the food without the stimulus of the insects. This may account for reports that toads find and consume food by scent alone (Australian Museum 2002, Boland 2004). Our statistical analysis did not indicate that the range of foods we tried differed in attractiveness to toads, as would have been expected if toads were attracted by the scent of food, although it appeared that there were small differences among foods, with the dog food we used in this experiment disappearing at the highest relative rate. Our cafeteria-trial experiments did not provide strong evidence that toads are attracted to foods by scent, however, in conjunction with the results of the Y-maze experiments, it is clear that the scents of some foods can influence cane toad behaviour, so additional research in this area may be productive. Our results also suggest that placing small amounts of food in traps might serve as an attractant, possibly simply by attracting increased numbers of insects, and they suggest that Masterfoods™ My Dog™ Lamb Classic dog food may not be the best choice for this purpose, since its odour may repel toads at a distance.

Toad Responses to Acoustic Attractants

Toads moved towards some of the acoustic stimuli we presented in an arena. Male toads responded to both loud and quiet toad calls, while females responded most strongly to quiet calls. Neither sex responded to pink noise, indicating that they are attracted by the acoustic structure of toad calls, and not simply to any sound. These results suggested that phonotaxis by toads could be used to enhance trapping outcomes, by luring toads to traps using the sound of calling.

We experimentally tested the efficacy of using toad calls to enhance trapping success, and found that playbacks through speakers positioned atop traps tripled their capture rate. This suggests that sound can be used effectively to enhance the success of trapping efforts.

Many fewer females entered our traps than males (the proportion was similar in traps with and without call playbacks). This is probably because males are generally more prone to move and explore the habitat than are females (Schwarzkopf and Alford 2002). This tendency of males to move more than females was evident even in our Y-mazes, where females were less likely to enter an arm of the maze than were males (Table 2), and is evident when collecting toads by hand (pers. obs.). Because traps rely on active entry by toads, it is likely that more males will be captured in traps.

In our acoustic experiments in the arena, female toads responded more strongly to quiet calls than to loud calls. Males vigorously and tenaciously amplex with females that approach choruses (pers. obs.). We hypothesise that female toads may use the sound of choruses to orient to water, but may avoid entering a chorus, and experiencing the associated harassment by males, unless they are ready to breed. Future experiments should examine the effectiveness of speakers placed near traps, but not directly on them, as attractants for females. The sound of a "distant" chorus may be more attractive to females than when the speaker is directly on the trap. If it causes females to approach to within a few metres of the trap, they may then be induced to enter it by light and the presence of insects.

It is common in anurans for the calls of some individual males to be more attractive to females than those of others (Ryan 1991). Typically, low dominant frequency, long call duration, and high pulse rate are attractive to females (Ryan 1991). It may be possible to alter toad calls using a sound editing program to create "super attractive" calls. It is not clear how males might respond to such calls, so experimentation is necessary to ensure that the "new" calls would not attract females but repel males, for example.

Shelter Site Selection and Toad Shelter Trap Design

Toads used a very broad range of shelters of man-made and natural materials. There were, however, certain characteristics they favoured in shelter sites. Sites were typically between 1000 and 10,000 cm in size, between 26-30°C and with high humidity, and shaded by vegetation. They selected shelters with sand, soil or leaf litter floors, and walls and ceilings of earth or sand. These aspects of shelter site preferences could be exploited to construct traps, by using appropriately sized PVC pipe, covered with earth and with a floor of soil or sand inside, moistened and deployed in shady locations. PVC pipe has been used successfully as a method to capture tree frogs (Broughton et al. 2000), but has not been used, to our knowledge, for ground-dwelling frogs. Adding such shelters within existing traps, or using them to lead to the entrances of existing trap designs, might also enhance trap effectiveness. Future experimentation with such designs would be warranted.

Toads used man-made shelters with appropriate characteristics when they were active in urban areas. Barricading appropriate hollows (drains, culverts) with wire mesh, and making sure concrete slabs are in good repair, without underlying cavities, and that piles of gardening and building materials and debris are wrapped or in containers, with no gaps or cracks, would aid in reducing the attractiveness of these locations as shelters for cane toads.

Conclusions

This study has highlighted several directions that should lead to improvement of trapping outcomes for toads. It is clear that toads do use olfactory cues in the process of making decisions about movement, and we intend to further investigate the nature of the cues used and how toads respond to them, using purified extracts in collaboration with the Capon group at the University of Queensland. We did not find any simple olfactory cue that attracted all toads, however, and therefore cannot recommend anything that will immediately improve the effectiveness of trapping programs.

Toads did respond to food in our cafeteria trials, consuming the foods presented. Our results strongly suggested, at least initially, this consumption was accidental, as toads attacked insects attracted to the food. Although additional work with food scents seems warranted, at present it appears that placing some food or other substances in toad traps might be useful as a means of attracting insects, rather than attracting toads directly, however, because our Y-maze results indicated that the scents of food may repel naïve toads, we cannot recommend that this procedure be adopted before additional work is carried out to clarify the reactions of toads to food odours.

The use of playbacks of toad calls strongly enhanced the effectiveness of traps, and playback apparatus similar to that which we employed could be adopted immediately in programs currently underway. The total cost per trap of adding an inexpensive mp3 player and battery-operated speakers should be less than $70. We would suggest powering the acoustic luring equipment from the same gel-cell battery that most trap setups presently incorporate to illuminate a light to attract insects. A simple DC powered timer should be sourced that will turn off the light and playback during the day to conserve battery power.

Further studies refining the use of playbacks as attractants, and conducting experiments with various designs of shelter traps seem the most profitable directions to follow immediately. Other studies, such as using chemical or food attractants require more research to determine whether they are likely to be effective methods for enhancing trapping.

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Appendix I. Work contracted for and completed. Work to be undertaken from initial contract, with notes on work completed and changes and additions to work program dictated by results as they were acquired.

Stage 1. Examine Acoustic Attractants:

- Experimentally examine the aggregative responses of toads to playbacks of toad choruses.

  Work carried out between 15/3/2005 and 10/10//2005 demonstrated that toads of both sexes are attracted to conspecific mating calls. This made it unnecessary to carry out the next two components of the work as initially specified, since the main rationale for composing artificial calls was to design acoustic signals that would attract males, which we had believed would not be attracted to conspecific mating calls but in fact were.

- Compose artificial calls using sound analysis software, and performing playback experiments using these calls. Use the results of these experiments to design techniques for attracting males for trapping or hand capture and disposal.

  This work was omitted because the results of the first stage demonstrated that males were strongly attracted to unmodified conspecific mating calls.

- Design acoustic traps suitable for at least short-term unattended placement in the field at sites where toads must be controlled

  This work was completed, and traps were successfully tested and demonstrated to increase capture rates an average of threefold over traps with only lights as lures.

  Stage 1 was completed, and carried further than planned in the research contract, as we conducted an extended field trial that demonstrated a large increase in capture rate for traps with simple acoustic lures.

  The outcomes achieved from this work were:

  - We gained an understanding of the response of canetoads to acoustic signals
  - We designed traps that exploit the aggregative response of toads to lure them into a capture point
  - We field tested traps that exploit the aggregative response of toads to lure them into a capture

Stage 2. Examine Chemical Attractants:

- Extract toad skin secretions by various means

  We carried out extractions by grinding toad skin in dichloromethane, filtering the result, and evaporating the filtrate.

- Record movement towards control (distilled water, scent control) and test (fraction) samples in laboratory experiments

and

- Extract and test samples that elicit behavioural responses in field experiments, to determine whether the presence of the samples affects the rate of capture of traps

  Initial trials suggested that these crude extracts were not attractive to toads. Because our trials, intended to be preliminary, using whole-object and whole-animal scents as stimuli were far more time consuming than we had initially believed they would be, and we believed the results of those would be a necessary basis for determining what animals or objects to subject to more detailed analysis, we pursued those to a thorough completion at the expense of continuing along the path of examining extracts.

- Conduct trials under controlled laboratory conditions to determine whether toads recognize and consume artificial diets (such as dog food)

and

- Use extraction and fractionation to isolate and identify chemicals that elicit attraction and feeding responses, using protocols similar to those outlined above.

and

- Fractions that show promise as baits will be tested against other terrestrial frogs, birds, reptiles, and mammals to determine whether they attract cane toads specifically

  We conducted a large number of cafeteria-style trials under controlled conditions in outdoor enclosures. The results of these demonstrated that toads consumed artificial diets, but strongly suggested that they were initially attracted to these diets by the presence and activity of insects, rather than by their odours. In combination with the results of our Y-maze experiments, which indicated strongly that toads are actually repelled by the odour of the dog food we tested, it did not seem reasonable to continue with this aspect of the work as proposed.

The final work program pursued differed from that specified in the contract because the whole-sample trials were much more time and labour intensive than we had anticipated (each trial took 1 hour for taping behaviour, 1 hour for analysis of the tape, and at least one additional hour of setup time because the Y-mazes had to be thoroughly cleaned between runs, so our total of 420 trials took at least 1320 hours of data collection, plus the overheads of collecting toads, etc) and the results of these trials did not suggest that it was likely that a simple, strongly attractive scent would easily be found. Similarly, the results of our trials with food items indicated strongly that for a wide range of foods, cane toads were not initially attracted by scent, so these experiments did not identify any attractive substances to fractionate and test against toads and other species.

The outcomes of this work were, however as specified in the contract. We

- Gained an understanding whether there is an aggregative response of canetoads to chemical signals (e.g., sexual pheromones or olfactory cues from food)
- Cane toads did not respond in any simple, aggregative way to olfactory cues, and therefore we did not design traps that exploit the aggregative response of toads to lure them into a capture point or ensure that attractants are specific to cane toads and do not attract other animals

Stage 3. Combinations of acoustic & chemical attractants:

- We did not conduct field trials using a combination of acoustic and olfactory attractants, as we had not identified any olfactory attractants.

- Because we were unable to identify any olfactory attractants, we did not compare the effectiveness of chemical and acoustic lures.

  Instead of comparing the effectiveness of acoustic and chemical attractants, we carried out a field trapping study comparing the effectiveness of traps with light and acoustic lures and traps with the traditional light lure only. We found that trapping rates were increased by a factor of three times by acoustic lures. Because these are highly taxon-specific, they should offer an excellent means of increasing the effectiveness of traps without increasing bycatch. In our field trials, we caught no non-target species.

  The outcomes we achieved from this work are:

  - The demonstration that the addition of acoustic lures can increase capture rates threefold over traps with only lights as lures.

Stage 4. Characterising retreat sites used by toads in urban settings and in the bush, and developing plans for using this information to decrease local toad densities and enhance the success of toad traps.

- In addition to the three stages called for in the contract, we carried out additional work to examine how toads use retreat sites, since reducing the availability of retreat sites would be a useful means to reduce toad densities in the field, and increasing the attractiveness of traps as retreat sites could increase their success.

- The outcomes of this additional work were a much greater understanding of the characteristics that make locations suitable to toads as retreat sites, and a series of suggestions for altering artificial structures to decrease their attractiveness to toads and altering the design of toad traps to increase their effectiveness by making them more attractive as retreat sites.