

Exploratory behavior of a native anuran species with high invasive potential

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Abstract Exploratory behavior can be a key component of survival in novel or changing environments, ultimately determining population establishment. While many studies have investigated the behavior of wild animals in response to novel food items or objects, our understanding of how they explore novel environments is limited. Here, we examine how experience affects the foraging behavior of a species with high invasive potential. In particular, we investigate the movement and behavior of cane toads as a function of experience in a novel environment, and how the presence of food modulates exploration. Cane toads, from a population in their native range, were repeatedly tested in a large, naturalistic arena with or without food present. Both groups exhibited significant but different changes in exploratory behavior. While toads in an environment without food reduced exploratory behavior over trials, those with food present increased both food intake per trial and the directness of their paths to food, resulting in fewer approaches to food patches over time. Our results suggest that cane toads learn patch location and provide preliminary evidence suggesting toads use spatial memory, not associative learning, to locate food. In sum, we show that with experience, cane

toads alter their behavior to increase foraging efficiency. This study emphasizes the role of learning in foraging in cane toads, a characteristic that may have facilitated their success as invaders.

Keywords *Rhinella marina* · *Bufo marinus* · Spatial learning · Exploration · Foraging success · Resource tracking

Introduction

Gathering and using information from surrounding areas is an important evolutionary strategy for organisms that live in changing environments (Dall et al. 2005). Active exploration provides information about potential resources and risk factors and may be particularly beneficial when animals encounter novel environments (Russell et al. 2010). Exploratory behaviors can have a direct impact on the fitness of an organism, facilitating identification of available resources and predator avoidance. Brown rats (*Rattus norvegicus*), for instance, released into unfamiliar rat-free environments show random search patterns moderated by returning to known areas, leading to efficient resource exploration that results in a rapid increase in range size and ultimately successful colonization (Russell et al. 2010). Similar exploratory movements that increase search efficiency have been described in insects when displaced from their burrows and nests (Hoffmann 1983; Reynolds et al. 2007a) or when searching for new food sources (Reynolds et al. 2007b). Exploratory behavior, paired with spatial learning, allows individuals to gather and store information about their environment.

Exploration and spatial learning have been documented in a wide range of taxa (e.g., Cheng and Jeffery 2017; Pritchard and Healy 2017; mammals: Hayes et al. 1953;

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Mead 1957; Poirier and Hussey 1982; Poucet et al. 1986; Deecke 2006; Drea 2006; birds: Sol 2002; fish: Warburton 1990; reptiles: Paulissen 2008; insects: Durier and Rivault 2001; Schultheiss et al. 2015). There is, however, little evidence of anuran spatial learning, and even less of anuran exploratory behavior. Limited evidence for reptile and amphibian learning has been proposed to be due to a failure to elicit natural behaviors from these animals, rather than lack of ability in these taxa (Suboski 1992). Consistently, studies based on natural behaviors using structures similar to those found in the species' natural environment have revealed spatial learning in some anuran species (Bilbo et al. 2000; Dall'Antonia and Sinsch 2001; Daneri et al. 2011; Fontanet and Horta 1989; Lüddecke 2003; Stynoski 2009; Pašunokis et al. 2016). While studies on this topic are increasing, the use of spatial learning in exploration and navigation of novel environments has received little attention in anurans.

In this study, we investigate exploratory behavior in native cane toads (*Rhinella marina*, formerly *Bufo marinus*), and how such behavior modulates foraging success in a novel environment. Cane toads are large [snout-vent length (SVL): 9–20 cm], terrestrial anurans native to Middle and South America. They are opportunistic feeders that often colonize urban areas. Early observations suggest that individuals have the ability to quickly learn novel food resources and locations (Alexander 1965). Cane toads are a highly successful invasive species (Phillips et al. 2007; Van Bocxlaer et al. 2010); their distribution includes well-established invasive populations across the world (Lever 2001; Lindstrom et al. 2013; McCann et al. 2014). Recently, cane toads in invasive populations in Australia were translocated to novel versus familiar sites, revealing differential use of daytime shelters (Pettit et al. 2017). Toads translocated to unfamiliar sites used more superficial, less hidden shelters during the day, while toads translocated to familiar sites sheltered in deeper, less accessible day burrows. The behavioral or cognitive mechanisms underlying these differences in habitat use beg investigation.

Here, we conduct an in-depth study of the exploratory behavior of native cane toads in unfamiliar environments to evaluate their response in areas with and without food present. In particular, we examine how exploratory and foraging behavior change as experience with a novel environment increases, providing preliminary insights about the cues the toads use to recall food location. Ultimately, we investigate the hypothesis that anuran species with high invasive potential, such as the cane toad, learn information about resources when exploring a novel environment.

Methods

Study site and species

Cane toads were collected and tested when they were not breeding during the rainy season (June–August 2011), near the town of Gamboa, Panama (9°07.0'N, 79°41.9'W), at a facility of the Smithsonian Tropical Research Institute. Twenty toads were tested, 18 males and two females (SVL = 114.8 ± 11.3 mm, mass = 145.0 ± 45.6 g). Cane toads are not as abundant in their native habitat as in their invasive range and not enough males were found during the study period to use individuals of only one sex. Females tested were within the size range of males and did not show evidence of egg production (i.e., enlarged abdomen but not due to lung inflation). Once captured, toads were housed individually for the duration of the testing period. Following a feeding regime established in our long-term captive colony of *R. marina*, toads were fed ten mealworms over the course of their first 4 days in captivity and then food deprived for 3 days to standardize hunger levels prior to testing. This feeding regime mimics cane toad feeding behavior in the wild (Zug and Zug 1979). Animals were toe-clipped prior to release to avoid retesting. All animal handling and experimental procedures were reviewed and approved by the Smithsonian Tropical Research Institute IACUC (#2011-0616-2014-09) and Texas Tech University IACUC (#09023-5). Collection permits were also approved by the Panamanian authorities (Autoridad Nacional del Ambiente, ANAM#SE/A-50-11).

Experimental arena design

The experimental arena was based on a traditional maze design (Hall 1934; Walsh and Cummins 1976; Olton et al. 1977; Bilbo et al. 2000), modified to represent an ecologically relevant environment that cane toads might encounter in nature (Fig. 1a). Cane toads are frequently found in human environments and opportunistically feed from a wide variety of anthropogenic food sources (Alexander 1965), including areas resembling our experimental arena. We used a circular arena (244 cm diameter × 76 cm high) with 3–5 cm of cleaned and dried leaf litter and forest soil. To create spatial complexity, cement block formations (20 cm deep × 15.25 cm high × 44.5 cm long) were randomly placed at equal spacing along the margin of the arena (Fig. 1a). Toads could easily hop on top of the blocks and frequently used them during the trials. Six smooth plastic bowls (6 cm tall × 15 cm opening diameter) were placed in a randomized block design, with three bowls on the substrate and three on blocks. The height of these bowls was selected based on pilot studies such that cane toads had to approach within 3 cm to detect mealworms inside. In our pilot studies, we offered the

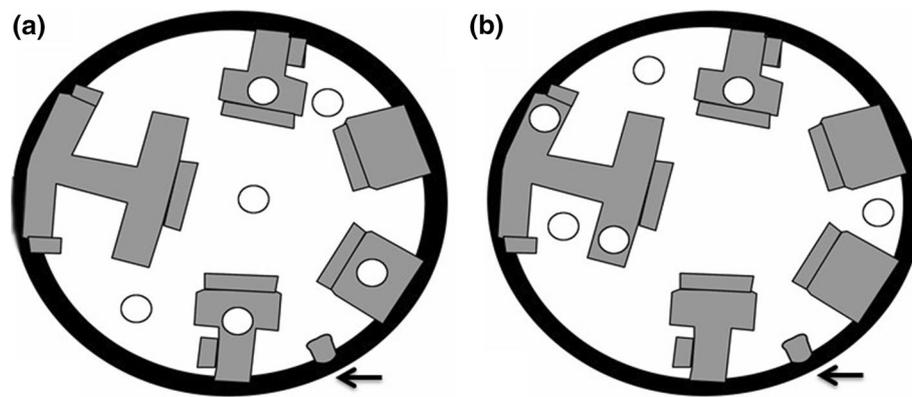


Fig. 1 Experimental arena used to examine exploration and foraging behavior in cane toads in a novel environment. Cinderblock formations are marked by the shaded area and food bowls by circles. Arena illustrates **a** the location of the bowls in trials 0–5, and **b** the “new”

locations of the bowls used in trial 6 to examine the cues associated finding food. Toads were introduced into the arena inside a small pot indicated by the arrow

toads identical bowls with and without a single mealworm reward. We found that for the bowl type we chose for the experiment, toads could not determine whether the bowl was empty or had prey until approaching within 3 cm of the bowl. As such, we are confident that the toads were unable to detect any prey sensory cues until they came in close proximity (< 3 cm) to the bowl. Infrared lights (Clover electronics model # IR010) and a high-resolution outdoor security camera (Supercircuits model # PC88WR) were placed 167 cm above the arena. All trials were video-recorded to an external DVR box (Supercircuits model #DMR80U) and imported into Ethovision XT 8.0 for analysis. Unless otherwise stated, all variables were calculated using Ethovision’s analysis functions. All statistical analyses were conducted using SPSS 19.0.

Experimental procedure

Toads were randomly assigned to one of two treatment groups: (1) “Food”, in which we placed one mealworm in each of the six bowls in the arena; and (2) “No-Food”, in which the arena contained empty bowls. This second treatment allowed us to quantify baseline behaviors when food was absent and assess potential effects of habituation and changes in motivation over time. Toads began each trial from a standardized location along the edge of the arena, and were tested during known foraging times (20:00–02:00 h, Zug and Zug 1979). We recorded the number of mealworms eaten and bowls from which they ate at the end of each trial. Toads are visually driven predators (Ewert 1980, 2004) and not known to use chemical cues when foraging (Martof 1962). Indeed, cane toads do not respond to non-moving mealworms even in close proximity (0.2–1 m; Bernal *pers. observ*). In addition, to minimize the potential use of

chemical cues, we sprayed the arena with water and mixed the substrate after each trial.

Toads were individually introduced to the arena by placing them in a small ceramic pot with the opening pointing away from the wall of the arena. They were then given 60 min to explore and encounter food bowls. Toads from both treatments were tested once in the unfamiliar arena and then five additional times to evaluate exploratory and foraging behavior in the now-familiar environment. If a toad in the food treatment did not eat a mealworm during the first trial, we conducted up to five trials (every other day). If a toad did not eat by the fifth trial it was not tested further. Toads in the no-food group were fed one mealworm in their housing bin every other day after trials were completed.

Changes in exploration with increased experience

To examine how cane toads modify movement in the arena as they increase experience in this environment, we quantified the time they spent moving, the total length of their path, the time they spent at the margins (outer 25% of the arena), latency to leave the pot in which they were introduced to the arena, and time it took to encounter a bowl. Proximity to the margins was determined given that spending time away from walls is associated with foraging in environments perceived as safe (Simon et al. 1993). To also address intention to move to a different area, we quantified the number of escape attempts during each trial. Escape attempts were defined as jumps toward the exterior walls of the arena. In general a toad would orient its body to face the wall of the arena and jump toward it (Supplemental video 1). In most cases, toads tried this repeatedly, each time bouncing back to the bottom of arena. Toads in this experiment were unsuccessful at escaping due to the height of the walls of the arena. As toads become familiar with the environment, we

predicted a shift from time spent exploring to time spent on other activities such as foraging. We recorded the latency to leave the origin and the number of escape attempts directly from videos, while all other variables were obtained using Ethovision XT 8.0. We used Friedman tests to examine changes in exploratory behavior across trials. Highly correlated variables ($\rho \geq 0.90$) were removed from the analysis based on biological relevance to prevent overlapping interpretations. The number of escape attempts was scored as a count variable and square-root-transformed before analysis (McDonald 2009).

Changes in foraging behavior with increased experience

To determine how foraging behavior changes with experience, we quantified bowl encounters during each trial. We scored an encounter when a toad physically contacted a bowl or came within 3 cm and stopped moving for more than 2 s. Pilot tests indicated that within this distance, toads can see inside the bowl and detect food. The total number of bowl encounters and the number of unique bowl encounters were also scored for each trial.

If cane toads were learning the spatial location of food in the novel environment, we predicted that toads in the treatment with food in the arena would increase both the number of unique bowls visited during a trial, and the overall number of bowl encounters. Four additional variables served as proxies for foraging behavior: time until first mealworm eaten, path length to first mealworm eaten, tortuosity (curvature) of said path, and total number of mealworms eaten per trial. Path length to first mealworm eaten was calculated using point coordinate data exported from Ethovision XT 8.0, and both time to eat first mealworm and number of mealworms eaten were quantified directly from the video. Tortuosity was calculated following Benhamou (2004) by dividing the length of the path to first mealworm eaten by the Euclidean distance between the starting and ending points of this path. We expected that as experience with food locations increased, toads would travel more direct paths to the food bowls and take less time to find food, eating a greater number of mealworms overall.

Following our analysis of exploratory behavior, Friedman tests were used to analyze the variables related to foraging. Count variables (total and unique bowl encounters, mealworms eaten) were square-root-transformed. Highly correlated variables were excluded a priori from the analysis, keeping those that provided greater biological insights to this study.

To estimate the tendency of the toads to follow regular routes, we estimated sequence predictability using a determinism index (DET) following Ayers et al. (2015). This index is a modified version of DET from recurrence quantification analysis where it is used as a standard metric for

quantifying sequential behavior. Broadly speaking, the DET measures predictability in a sequence of events by comparing the number and length of events in which an organism returns to an area that it has previously visited. In the case of the toads in this study, DET represents the proportion of revisited bowls that were visited in the same continuous order in several parts of the bowl visitation sequence. This index has been used successfully to examine foraging behavior of bumblebees (Ayers et al. 2015) and more recently investigate inter-individual variability in their foraging behavior (Klein et al. 2017). We quantified DETs to examine bowl visitation sequences within the same trial and bowl visitation visits between consecutive trials by the same toad. DETs were calculated including perpendicular diagonals. This index ranges from zero (the toad never repeats the same bowl visitation sequence) to one (the toad always repeats the same bowl visitation sequence).

The role of spatial and visual cues involved in learning

To investigate the cues cane toads use to locate food, after the fifth trial, toads were tested in a final probe trial in which the bowls were moved to new locations that did not previously contain bowls (Fig. 1b). We recorded whether each toad first visited a location that previously held a bowl or a new bowl location. We thus investigated whether toads located food using spatial learning, returning to the location of a past food encounter, or through association with a particular food cue (the food bowl). We used binomial probabilities to contrast the number of new versus old bowl locations first visited by the toads against random expectations for each treatment (Zar 1996).

Results

Eighty-five percent of toads (17 out of 20) ate at least one mealworm or encountered a bowl during the first trial. In the treatment with food in the arena, 70% of toads ate during the first trial, with one toad each eating on the second and fifth trials. One toad failed to eat during five trials and was removed from the experiment. When first confronted with the novel arena, there were no significant differences in exploratory behavior between the two treatment groups (food vs no-food treatments, Table 1).

Exploratory behavior and experience

Latency to leave the origin was highly variable both across and within individuals ($CV_{\text{within indiv}} = 0.66$; $CV_{\text{among indiv}} = 1.11$). Some toads quickly hopped out of their release pot into open space and began exploring. Others, however, waited up to 1 min to move into the open space.

Table 1 Cane toad behavior during the first time in the novel arena (trial 0)

Variable	No-food		Food		<i>p</i> *
	Mean	SE	Mean	SE	
Time spent moving (s)	1255.86	132.62	1296.96	126.67	0.99
Total path length (cm)	8113.76	735.10	8940.98	975.41	0.31
Time spent in margins (s)	2906.01	269.50	3226.12	53.12	0.44
Latency to leave origin (s)	96.29	11.54	57.72	16.46	0.20
Time to find 1st bowl (s)	217.22	83.48	231.34	80.21	0.22
Total bowl encounters	9.6	1.05	10.00	1.96	0.86
Unique bowl encounters	3.50	0.40	3.44	0.41	0.84
Number of escape attempts	7.9	2.14	10.89	2.66	0.18

* *t* test bootstrapped 1000 times at $\alpha = 0.05$

Indeed about 9% of the toads waited more than 2 min to leave the origin. The variation in latency to leave the origin, however, was not related to the treatment ($p > 0.05$ for all trials). After departing the pot, some toads would turn their body slightly and appear to look around before heading out.

The time toads spent moving and the total length of their paths were highly correlated ($\rho = 0.94$, $p < 0.001$). Given that the total path length covered by the toad reflects the locomotor behavior of this often sedentary species better than the time spent moving, we excluded this latter variable from the analysis. In both groups, there were significant differences across trials in the length of the path traveled (Fig. 2a; No-food: $\chi^2 = 29.14$, $p < 0.001$; Food: $\chi^2 = 23.89$, $p < 0.001$) showing a decrease in the distance traveled with increased exposure to the arena. All individuals decreased the number of escape attempts per trial, with 75% of individuals ceasing this behavior by the final trial (Fig. 2b; No-food: $\chi^2 = 20.29$, $p = 0.001$; Food: $\chi^2 = 22.95$, $p < 0.001$). The time the toads spent at the margins of the arena also changed significantly across trials in both groups (Fig. 2c; No-food: $\chi^2 = 21.03$, $p < 0.001$; Food: $\chi^2 = 19.27$, $p < 0.01$). Though individuals in both groups decreased the amount of time spent in the margin, toads in the food group showed a more rapid decline than toads without food in the arena (*t* test bootstrapped 1000 times: trial 2: $t = -0.325$, $p = 0.749$; trial 3: $t = -2.55$, $p = 0.025$; trial 4: $t = -2.90$, $p = 0.012$; trial 5: $t = -3.24$, $p = 0.01$; trial 6: $t = -2.19$, $p < 0.043$). In general, by the end of the trials, toads in both groups had shorter paths (Figs. 3, 4). Toads traveled shorter distances with increased experience in the arena but latency to leave the origin and time to encounter the initial bowl did not change significantly across trials for either group (No-food: $\chi^2 = 8.42$, $p = 0.135$; Food: $\chi^2 = 2.7$, $p = 0.746$ and No-food: $\chi^2 = 4.34$, $p = 0.50$; Food: $\chi^2 = 6.71$, $p = 0.243$, respectively).

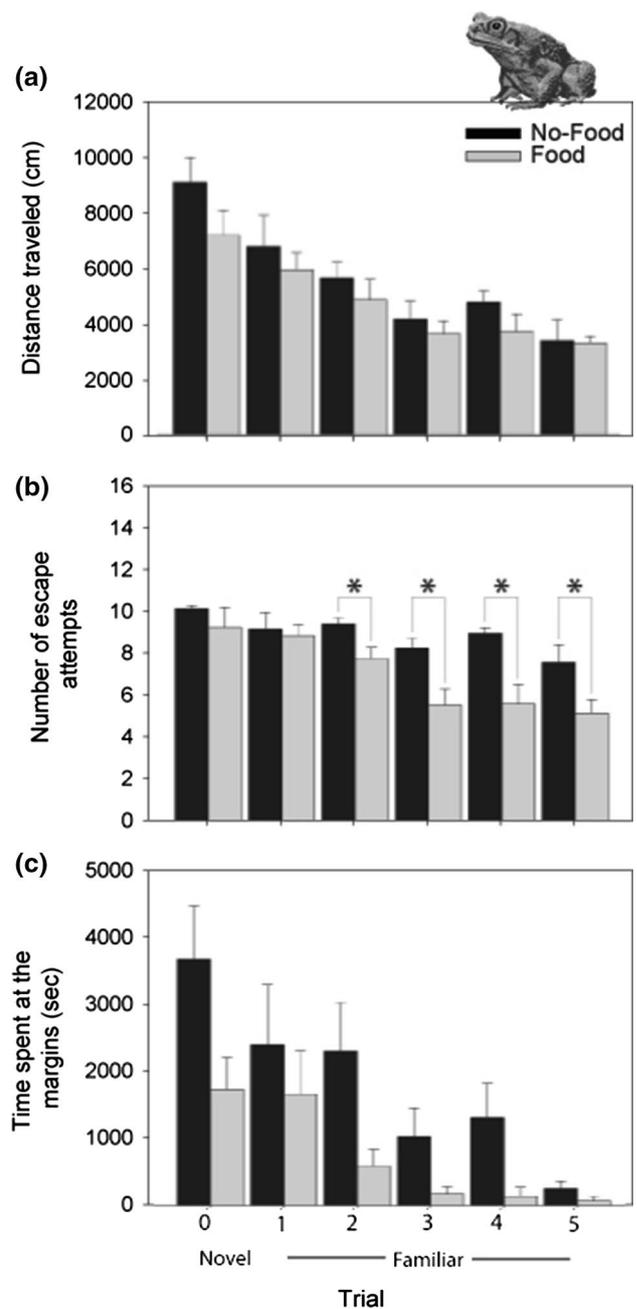
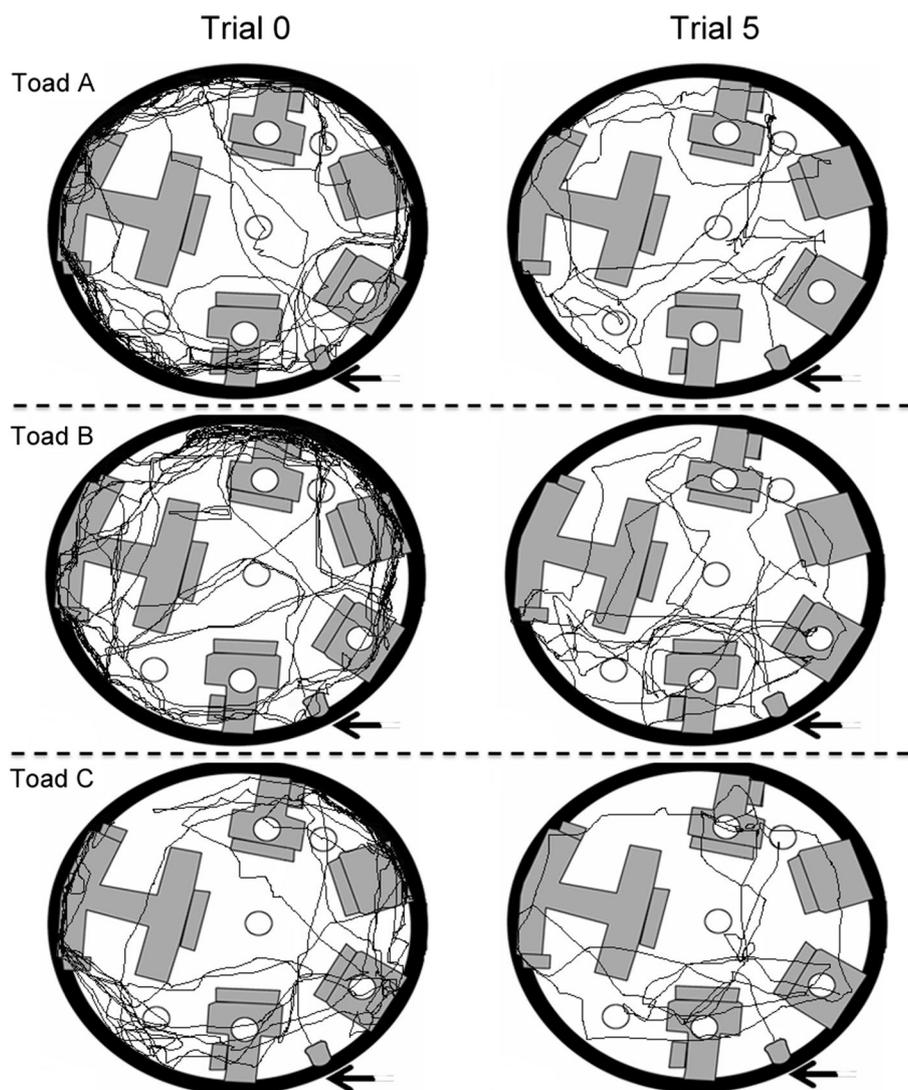


Fig. 2 Changes in locomotor behavior between cane toads in the Food (gray bars) and No-food (black bars) treatments. **a** Total path length covered, **b** time spent in the margin of the arena, and **c** number of escape attempts. * $p < 0.05$

Foraging behavior and experience

Time to eat and path length to the first bowl eaten from were significantly correlated ($\rho = 0.97$, $p < 0.001$). We excluded path length to the food bowl from further analysis, given that tortuosity of the path is a more robust way to describe animal movements (Benhamou 2004). There was a significant

Fig. 3 Paths followed by toads randomly selected from the Food treatment during their first (0) and last (5) trial. Toads were introduced into the arena inside a small pot indicated by the arrow



increase in the total number of mealworms eaten as trials progressed ($\chi^2 = 15.81$, $p < 0.01$). There were, however, no significant differences in the time to find a mealworm each trial ($\chi^2 = 8.30$, $p = 0.14$). Toads did not find mealworms faster as trials progressed, but there was a trend toward more direct paths to food over time (Fig. 5; $\chi^2 = 9.81$, $p = 0.08$). Seven out of the nine toads had more straight paths to the first mealworm they ate during their last trial compared to the first trial.

The total number of bowls and the number of unique bowls visited by the toads changed significantly across trials for the no-food treatment (total: $\chi^2 = 22.43$, $p = 0.001$; unique: $\chi^2 = 11.08$, $p = 0.05$), but not for the treatment with food (total: $\chi^2 = 6.91$, $p = 0.32$; unique: $\chi^2 = 4.00$, $p = 0.55$). As trials progressed, the no-food group encountered fewer bowls, while the food group approached a slightly higher number of bowls (Fig. 6a). The difference between unique bowl encounters for both groups increased,

with toads in the treatment without food approaching significantly fewer unique bowls than toads in the arena with food during the last two trials of the experiment (Fig. 6b; trial 5: $t = 2.33$, $p < 0.05$; trial 6: $t = 2.20$, $p < 0.05$). Toads in the food group, however, revisited bowls at which they had fed and were now empty throughout all trials. That is, there was not a tendency to visit empty bowls less frequently as the trials progressed.

For all trials, the determinism index (DET) of the bowl visitation sequence had extremely low values that approached zero (DET = 0.02) indicating low repetition in the order in which the toads visited the bowls. In addition, there was no difference in DETs between Food (0.02 ± 0.04) and No-food treatment (0.03 ± 0.01). The same was true for bowl visitation sequences in consecutive trials with the DET indicating low sequence repeatability (DET = 0.08) and lack of difference between treatments.

Fig. 4 Paths followed by toads randomly selected from the No-food treatment during their first (0) and last (5) trial. Toads were introduced into the arena inside a small pot indicated by the arrow

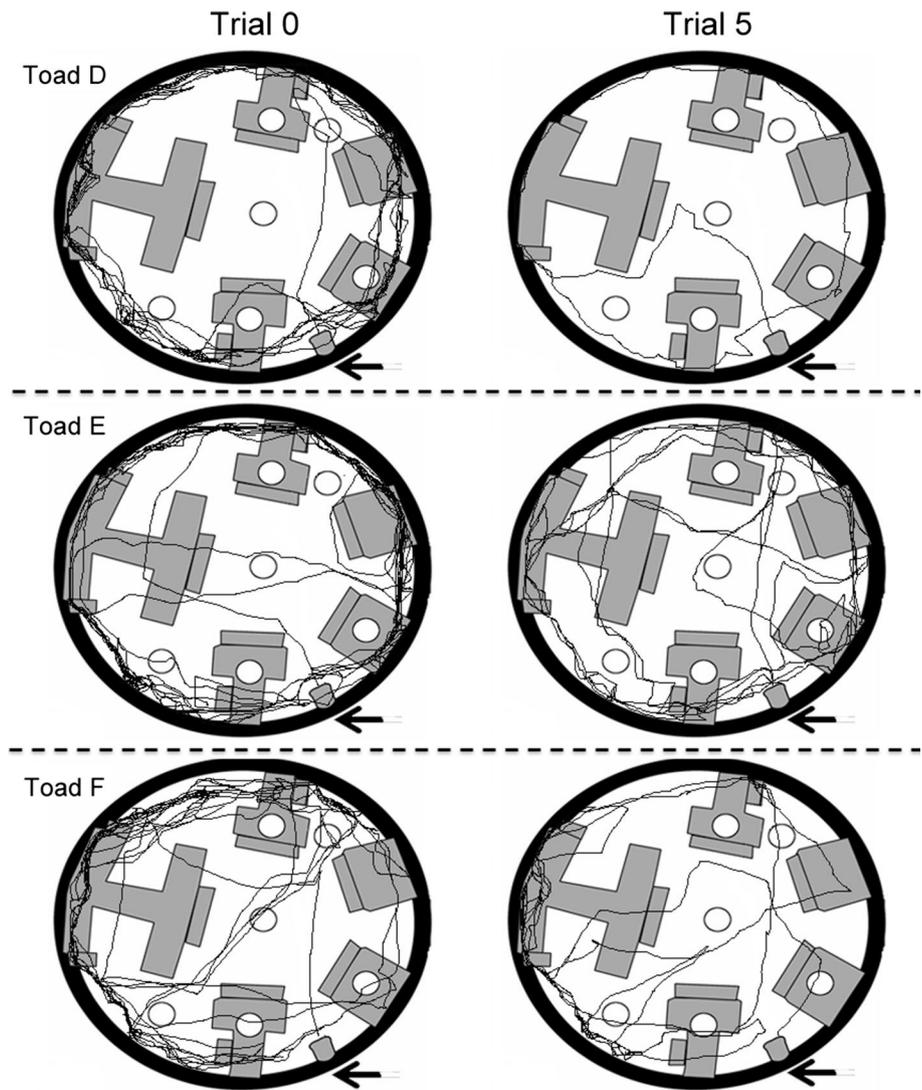
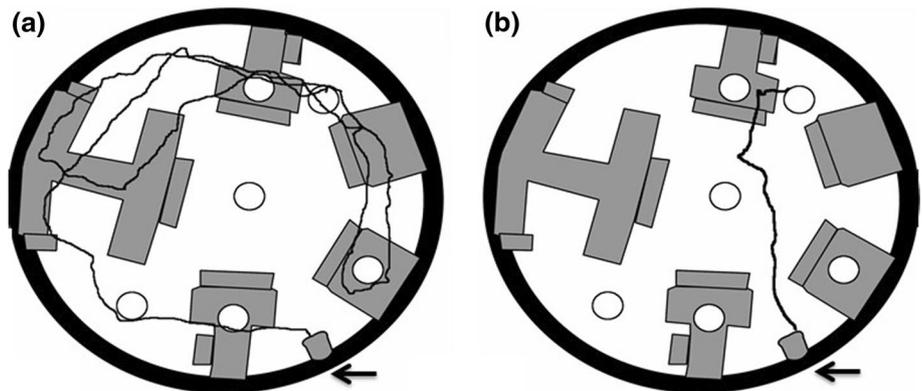


Fig. 5 Representative path followed by a randomly chosen toad to eat the first mealworm during the first (a) and fifth (b) trial of the experiment. Toads were introduced into the arena inside a small pot indicated by the arrow



Use of spatial and visual cues to locate bowls

To explore which cues toads used to find food locations, in the sixth and final trial, we moved the bowls to new

locations in the arena and scored whether the toads first visited old or new bowl locations (Fig. 1a vs b). Toads in the no-food group visited old and new bowl locations at near equal numbers (Binomial probability, $p = 0.21$).

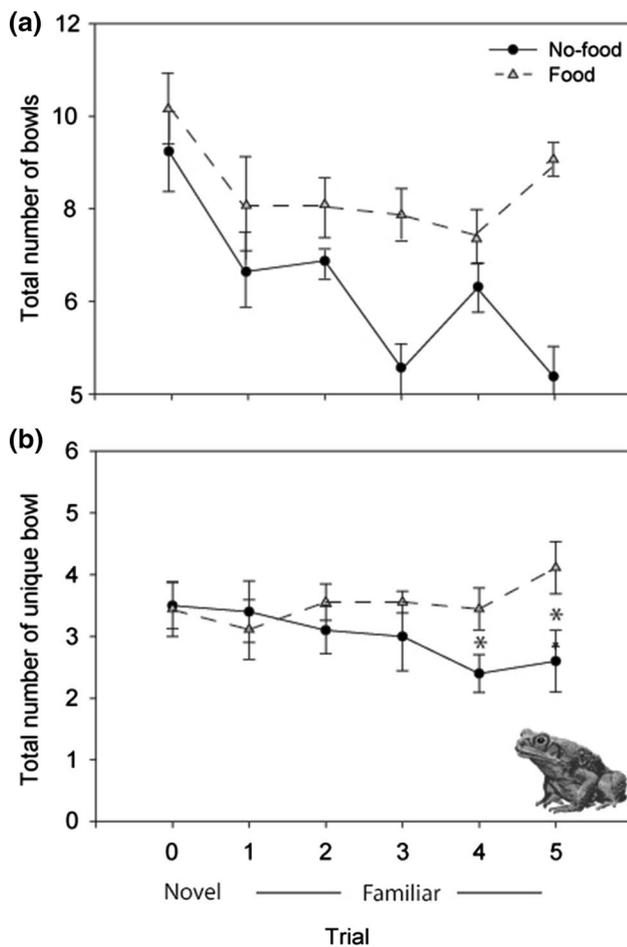


Fig. 6 Changes in total (a) and unique (b) number of bowls visited by cane toads across trials. * $p < 0.05$

In contrast, there was a non-significant trend for toads in the food group to visit the bowl's previous location first (Binomial probability, $p = 0.07$). While this result is tentative given the small sample size, it suggests toads use spatial memory, rather than associative learning of cues associated with the food bowls, to find resources. Consistent with this idea, the majority of toads in the food group (71.4%) remained at the previous location of a bowl for an extended period of time (10–40 s), raising and extending their forelimbs, and changing the orientation of their bodies. The toads then moved in the close vicinity of the former location of the food bowl before continuing on to different areas of the arena. In one instance, a toad encountered two new bowls first and returned to an old bowl location where it reoriented its body multiple times as if it was looking around.

Discussion

Our results indicate that cane toads modify their exploratory and foraging behaviors as a result of experience with an environment. The toads in this experiment behaved similarly to rats colonizing a new environment, decreasing exploration over time and staying close to known areas (Russell et al. 2010). Toads in both treatments began the experiment exhibiting similar behaviors; individuals explored the arena by repeatedly circling the perimeter and trying to escape. Over subsequent trials, individuals shifted their behavior based on presence or absence of food. When food was present, toads found it quickly, progressively spending more time foraging than exploring or attempting escape. In contrast, toads in areas without food continued to explore and attempt to escape, only ceasing escape attempts by the final trial. As the arena became more familiar, cane toads from both treatments decreased wall-hugging behavior (thigmotaxis). Our results suggest that toads remembered information about the arena from previous trials. This behavior is consistent with anecdotal reports of cane toads foraging in urban environments (Alexander 1965; Krakauer 1968).

Cane toads foraged more successfully as they spent more time in the arena, increasing the number of mealworms consumed per trial. Contrary to our predictions, however, toads did not significantly decrease the amount of time to find food as experience with the arena increased. This finding does not align with studies of exploration in other species (e.g., Punzo and Bottrell 2001; Mettke-Hofmann et al. 2002; Russell et al. 2010). For example, as *Colostethus palmatus* became more familiar with an environment individuals gradually spent less time searching for caves (Lüddecke 2003). Consistent with our study, however, toxic fire-bellied toads, *Bombina orientalis*, learn to navigate a complex maze more accurately but not more quickly with increased experience (Brattstrom 1990). Similarly, the water search behavior of *Epidalea (Bufo) calamita* changes, with experience leading to more accurate choices, but not decreasing the time needed for the choice (Dáll Antonia and Sinsch 2001). A lack of reduction of the time taken to perform the behavior, even in the presence of changes with experience, may explain why previous studies report inconclusive results about learning in anurans (e.g., McGill 1960; Grubb 1976; Suboski 1992). It is possible that unpalatable animals such as toads have not experienced strong selection for rapid foraging, since they have no significant predation risk. We found that in cane toads, learning was better described by increased encounter success than by changes in search duration.

When food was available, toads approached fewer total bowls while visiting more unique bowls as trials progressed, suggesting that toads learned bowls do not replenish food within a trial. While this behavioral pattern is consistent with foraging behaviors at nonrenewable patches, with

individuals remembering patches previously visited and subsequently visit patches that are least depleted, toads in the food group revisited empty bowls from which they had previously eaten even in the last trials. Resource tracking has been described in a variety of mammals and birds (Devenport et al. 1998; Zamora et al. 2012) but not in anurans, so it is unclear whether toads have the ability to remember previously visited feeding patches. Since cane toads often feed on insects with patchy distributions (Zug and Zug 1979) that vary in renewal rates, resource tracking would be particularly beneficial to them. The ability to recall depleted patches would allow toads to preferentially forage in new patches, increasing their foraging efficiency (Gibson et al. 2006). Further studies that directly examine resource tracking in anurans would provide valuable insights about their foraging adaptations, exploring cognitive aspects that have been previously ignored.

When examining cues used by cane toads to learn the location of food, our results suggest individuals remember the location of food in the arena rather than specific features associated with it (e.g., bowl). The high confidence internal (93%) showing preferential attraction to the old bowl location combined with repeated observations of toads seeming to search for missing bowls support the hypothesis that cane toads rely more heavily on location than specific associative cues to find food. The behavior of the toads is similar to the responses of chipmunks (*Tamias minimus*) and golden-mantled ground squirrels (*Spermophilus lateralis*) presented with an equivalent challenge in which feeding stands replete with seeds were shifted to a randomly selected location (Devenport et al. 1994). In both cases, the toads and sciurids, individuals preferentially approached where the food patch originally was located suggesting they used memories of spatial relations to locate the resources. Although spatial learning in amphibians has been largely unstudied (Crane and Mathis 2011), many species use fixed general landmarks to navigate their territory (e.g., Sinsch 2006); a similar mechanism could be used by cane toads to locate areas where food had been found before.

Cane toads are highly successful invaders. Several possible traits have been proposed as facilitating their range expansion, including a semi-terrestrial niche, presence of parotid glands, and large body size (Alexander 1965; Van Bocxlaer et al. 2010). The exploratory behavior of native cane toads in a novel environment suggests that this species also exhibits behaviors that promote successful strategies in new habitats. We propose that cane toad exploratory behavior and spatial learning ability are contributing factors to its success as an invasive species. This interpretation is consistent with the findings from a recent study in which cane toads in Australia were translocated to novel

sites where, despite changing the type of day shelters used, the toads did not suffer from reduced feeding rates or experience lower body condition (Pettit et al. 2017). Learning the location of resources could increase the probability that a population of cane toads is successfully established at a new location, a critical phase of the invasion process after arrival (Davis 2009). Once the population is established, however, the adaptive value of spatial learning to track resources like food and shelter is expected to decrease as individuals spread to colonize new areas. Examination of the spatial learning ability of cane toads at populations in different stages of the invasion process could provide valuable insights about how ability to track the location of resources changes with invasion phase.

Extending this type of study to other anuran species, and directly comparing native and invasive populations, could provide a framework to further investigate the role of exploration and learning in successful colonization of novel habitats in this group. For example, the leaf litter toad (*Rhinella alata*), a sympatric congeneric species that is not invasive, was less likely to eat a novel prey item (mealworms) and the few individuals that did eat it were unable to locate this food in an arena equivalent to the one used with cane toads in this study (Miller et al. unpub data). Further studies that investigate the contrast between native and invasive populations can provide valuable insights about the traits that promote invasive potential. For instance, cane toads from introduced populations are less neophobic than toads from native populations (Candler and Bernal 2015). Although exploration paired with learning and behavioral flexibility increase invasion success in birds (Sol 2002; Sol et al. 2005), the role of behavior in invasive ability has received less attention in anurans.

Overall, this study suggests that spatial learning in cane toads increases foraging efficiency in a new environment. Our results also suggest the use of spatial memory, rather than food-associated cues, in cane toad foraging. Finally, this study brings to light the role of behavior in successfully exploiting novel areas in anurans, an ability that may allow some species to survive in changing environments or colonize new ones.

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