The Effect of Time Spent in the Lab on the Behavioral Flexibility of Blanchard's Cricket Frogs

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Abstract: To gain knowledge about animals and repopulate declining species, researchers often conduct husbandry programs that involve housing wild-caught animals in laboratories. Housing animals in labs for long periods may affect their behavioral flexibility, or their ability to change their behavior to adapt to changes in their environment. We measured and compared the behavioral flexibilities of wild-caught Blanchard's cricket frogs, or *Acris blanchardi*, which spent a long time (40 days) and a short time in the lab (13 days). To measure behavioral flexibility, we taught frogs to go to one end of a two-arm maze. Once the frogs learned this, we switched which door was correct and measured how quickly the frogs could learn to go to the newly correct door in a process called serial reversal learning. We switched which door was correct three times. We found that frogs that spent more time in the lab before testing were better at learning the maze and showed some evidence of behavioral flexibility. Frogs that spent little time in the lab showed no evidence of behavioral flexibility. Frogs that spent more time in the lab before testing may have been more habituated to lab conditions and thus less stressed when placed in the maze. Keeping wild-caught frogs in the lab for a longer time before release may increase their chances of survival upon reintroduction to the wild, since they will have enough time to overcome the initial stress of being in a new environment and have better behavioral flexibility when released.

Keywords: Behavior, Flexibility, Serial Reversal, Frog

Introduction

Amphibian populations are rapidly declining and will most likely continue to do so (Stuart et al. 2004) due in part to climate change (Pounds et al. 1999, Kiesecker et al. 2001), disease (Daszak et al. 2003), and several other combinations of factors. Among the species in danger of extinction is Blanchard's cricket frog (*Acris blanchardi)* (McCallum 2010), which is semi-aquatic and hibernates terrestrially (Irwin et al. 1999). Its range includes the Midwest, Great Plains, and parts of the Appalachians (Gamble et al. 2008). This range is decreasing substantially along with the frog's population numbers (Lehtinen and Skinner 2006), making Blanchard's cricket frog an ideal species for conservation efforts (Beauclerc et al. 2010).

Researchers are trying to slow the decline of amphibian populations through animal husbandry programs under the Amphibian Conservation Action Plan. In the Amphibian Conservation Action Plan, the International Union for Conservation of Nature (IUCN) supports captive breeding with intent for supplementation of wild populations. Captive breeding may be one of the Union's only options for repopulation since habitat conservation is not sufficient to prevent species loss, especially in the case of extremely endangered species.

However, scientists know little about the behavioral flexibility, or the ability to change behaviors according to environmental changes, that lab-habituated

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Figure 1 - The two-arm learning maze is used to assess the behavioral flexibility of cricket frogs (1.5'x1' center chamber, 1'x0.5' arms). Photo credit: Rebecca Atherton

individuals possess upon reintroduction to the wild. Higher behavioral flexibility upon reintroduction to the wild is likely to increase the likelihood of the animal's survival, since the animal uses behavioral flexibility to adapt to a new environment and its rules. Therefore, if amphibians had lower behavioral flexibilities after a longer lab stay, captive husbandry would be an ineffective way to slow the decline of wild populations. Additionally, research projects that require keeping frogs in the lab for long periods would harm their test subjects' chances of survival upon release.

We compared the behavioral flexibility of Blanchard's cricket frogs at the beginning of a lab stay to the behavioral flexibility of Blanchard's cricket frogs a few weeks into a lab stay to see how the length of the stay would affect their flexibilities. The results of this study could predict if research and husbandry programs with long lab stays damage a wild-caught frog's chances of survival after release more than projects with short lab stays. We used serial reversal learning, or learning how to complete a task differently each time the rule shifts, as an indicator of behavioral flexibility (Bond 2007, Ghahremani et al. 2010, Liu et al. 2016). Liu et al. (2016) used a two-arm maze to measure the serial reversal learning abilities of poison frogs, and we applied their methods to Blanchard's cricket frogs.

We hypothesized that frogs that spent less time in the lab before testing would have higher behavioral flexibilities than frogs that spent more time in the lab before testing. Animals that live in complex social structures tend to have more behavioral flexibility than animals that live in simple ones (Day et al. 1999, Jones 2005, Bond 2007), which suggests that animals which live in environments with more stimuli may have higher degrees of behavioral flexibility than those that live in environments with less stimuli. We predicted that as the frogs habituated to the isolated and constant lab conditions, they would lose some of their ability to adapt their behaviors to environmental changes as they would in the wild.

Methods

Materials

We used 20 mature Blanchard's cricket frogs caught by hand (Licht 1974) in February 2017 at Oklahoma State University's Aquatic Ecology Research Station in Stillwater, OK. We began testing the first 10 frogs (the short lab stay group) 13 days after collection. We began testing the second 10 frogs (the long lab stay group) 40 days after collection. All living conditions of the frogs in the lab mimicked those of their natural habitat as closely as possible. We housed each frog in an individual container with moist, mossy substrate, at about 79°F, with humidity around 30%, and fed each frog approximately 15 *Drosophilia hydei* every other day. Individual housing limited the

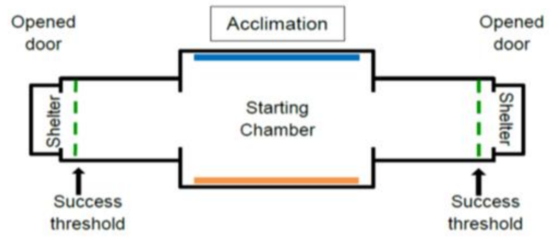


Figure 2 - During the Acclimation trials, both doors are open.

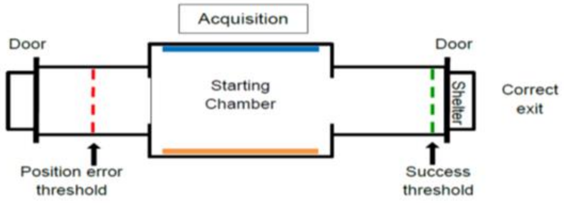


Figure 3 - During the Acquisition trials, one door is designated correct.

social interaction and stimuli of the frogs so that we could more accurately predict how these factors affected the behavioral flexibility of the two groups.

The two-arm maze, serial reversal techniques, and statistical analysis used were nearly identical to those used by Liu et al. (2016). The maze was constructed of a 1.5 foot by 1 foot central box, with two identical 1 foot by 0.5 foot arms attached on opposite sides of the box (Figure 1). At the far end of each arm, we placed doors that could be opened or blocked from the outside, depending on a frog's stage of trials. The inside of the arena was completely white except for two different visual cues, one on each side of the inside of the main box, parallel to the arms. To motivate the frogs to exit the maze and seek shelter, we heated the maze to approximately 100°F and lowered its humidity to about 20% using two heat lamps above the arena and a heating pad underneath it. We wiped down the interior of the maze after each trial so that the frogs could not associate olfactory cues from other frogs with the correct end of the maze. The roof of the maze was removable screen so that we could easily place the frogs in the maze and so that they could not escape. We painted the outside of the maze white so that the researcher could not see the frog's position in the maze and so that the frog could not see anything outside of the maze to orient itself. We put a camera 1.5 m over the maze to tape the trials. A researcher sat outside the maze during each trial to record the results and open the correct door at the trial's completion.

We completed all four stages of trials listed below for the short lab stay frogs before we began the four stages of trials for the long lab stay frogs.

To determine if treatment, sex, or size influenced the likelihood that a frog reached criterion, we compared the Akaike's Information Criterion scores corrected for small sample sizes (AICc) of two binomial generalized linear models that did or did not have treatment, sex, or size as a fixed effect.

Procedure

Stage 1. Acclimation

Before running any learning trials, we placed the frogs individually in the maze with both doors open to acclimate the frogs to the maze (Figure 2). We transported the frogs to the maze using a closed fist and placed them in the center of the starting chamber facing a random direction. Each frog ran one trial per day for two days. Between every trial and at the end of each day, we wiped the maze interior with alcohol for every trial stage.

Stage 2. Acquisition

For these trials, we designated one door as correct (Figure 3). The correct door had a shelter behind it while the incorrect door had a heavy block behind it. The doors appeared identical from inside the maze. We placed one frog in the maze at a time and oriented them randomly as before. Each frog underwent an average of three trials per day, with an approximately 50 minute wait between trials for an individual frog.

Figure 4 - A frog reaches the threshold for success. Photo credit: Rebecca Atherton

The frogs could perform one of three behaviors during Acquisition and Reversal trials: successful trials without error (in less than two and a half minutes after being placed in the maze, the frog approached within 0.5 cm of the correct door without going more than halfway down the wrong arm beforehand and making a position error); successful trials with error (in less than 2 and a half minutes after being placed in the maze, the frog approached within 0.5 cm of the correct door but went more than halfway down the wrong arm beforehand and made a position error); and unsuccessful trials (in less than two and a half minutes after being placed in the maze, the frog never reached within 0.5 cm of the door) (Liu et al. 2016). When a frog was successful, we immediately opened the correct door and allowed the frog to enter the shelter (Figure 4). When a frog was unsuccessful, we opened the correct door at the end of the two and a half minute trial time and gently guided the frog towards the exposed shelter. When a frog left the maze and entered the shelter, we used the shelter to replace the frog in its original container.

To minimize the likelihood of confounding variables affecting the data, we used the results of the first acquisition trial for each frog in the short lab stay group to find the probability of an untrained frog randomly completing the maze once without error. One out of the ten frogs from the short lab stay group successfully completed the maze without error on its first Acquisition trial; the random probability of success was 0.1. We used a binomial test to set the learning criterion, or the percentage of trials a frog must complete successfully to have learned the skill and be able to move on to the next stage, as 83% or five out of six consecutive successful trials ($p = 5.5 * 10^{-5}$). We used this criterion during the Acquisition and Reversal stages for both testing groups. If a frog could not reach the criterion for Acquisition within thirty trials, its trials were finished.

Stage 3. Reversal

Whenever a frog met the learning criterion, we changed which door was correct and switched the block and the shelters accordingly (Figures 5, 6, and 7). Every time an individual reached criterion, we switched which door was correct until the frog had completed three Reversals. If a frog could not reach criterion for a Reversal within twenty trials, we stopped its trials. We noted how many trials a frog took to meet criterion across the Acquisition and

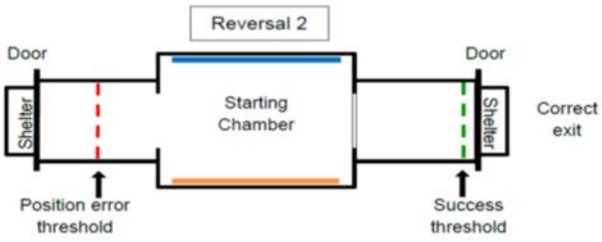


Figure 8 - During the Reversal 1 trials, the correct door was the reverse of the correct door from Acquisition.

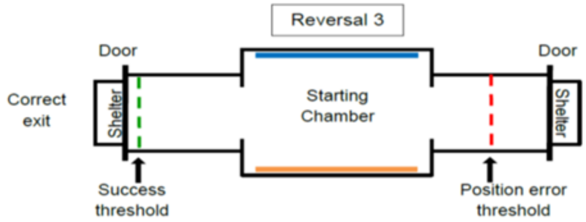


Figure 8 - During the Reversal 2 trials, the correct door was the reverse of the correct door from Reversal 1.

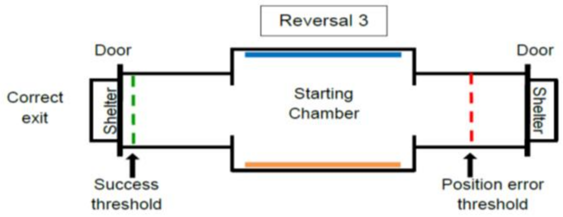


Figure 6 - During the Reversal 3 trials, the correct door was the reverse of the correct door from Reversal 2.

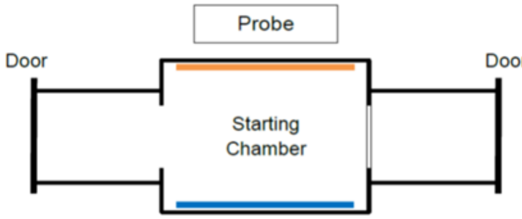


Figure 6 - During the Probe trials, both doors were blocked and the visual cues were reversed from their positions in all other trials.

three Reversals, and observed whether this number decreased across reversals using a paired value t-test. Next, we compared the average p-values and significance levels of the t-tests for each group to see if one group learned the task and changed its behavior across Reversals more effectively than the other group.

Stage 4. Probe Trials

To verify that the frogs navigated the maze using the visual cues provided and not other cues that were undetectable to us, we performed two four-minute probe trials for each frog, one after the frog met criterion in Acquisition and one after it met criterion on the third Reversal. For these trials, we blocked both doors from the outside and switched the patterns on the inside walls while leaving all other possible cues intact (Figure 8). If the frog went to the door deemed correct by the visual cues (visually correct) and not the door that was correct in previous trials (previously correct), it had responded to the provided visual cue. We compared the time spent in each arm using a paired-values t-test to see if the frogs navigated the maze using the provided visual cues or other unknown cues.

Results

One of the ten short stay frogs reached criterion for Acquisition. It could not reach criterion on the first Reversal. This frog reached the criterion for Acquisition within five trials. Four of the ten long stay frogs reached criterion for Acquisition. One of these four reached criterion on the first Reversal. This frog reached criterion for all three Reversals. The successful long stay

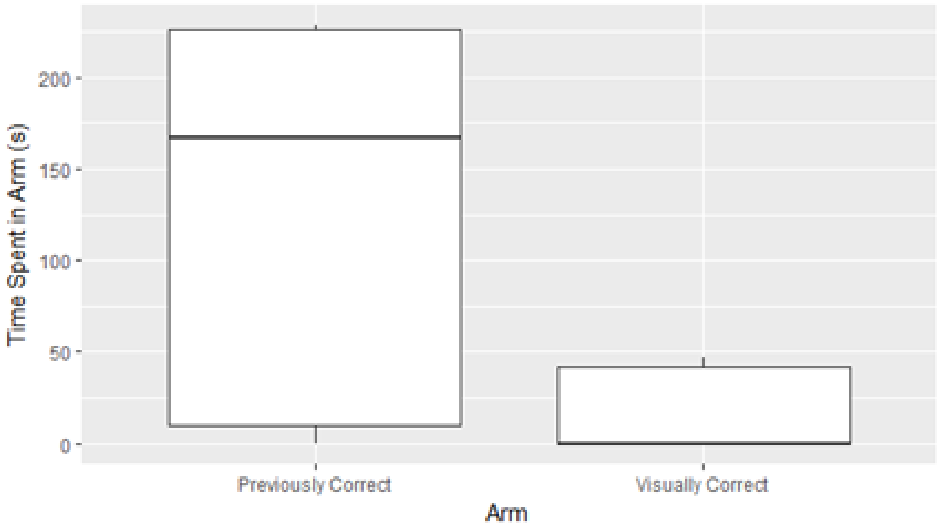


Figure 10 - The Probe trials showed that the frogs did not rely on the provided visual cues to navigate the maze.

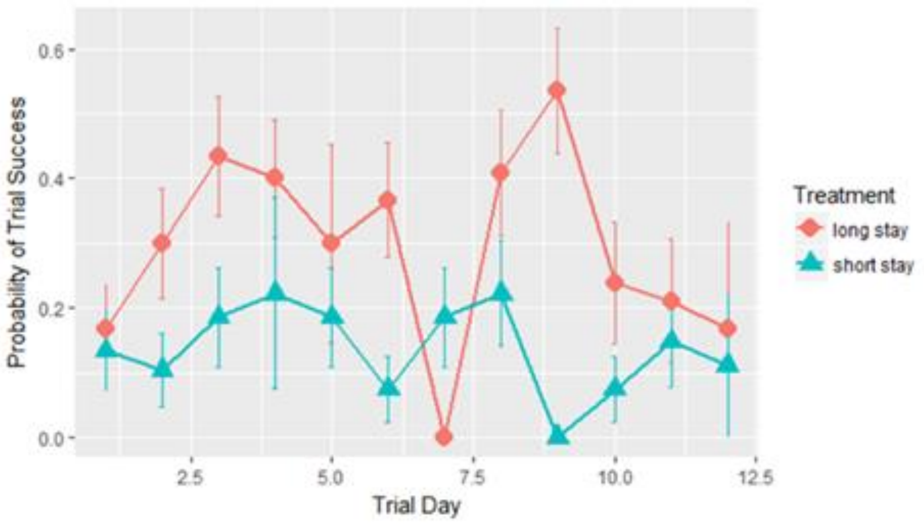


Figure 9 - For any given trial, a frog from the long lab stay group was more likely to be successful than a frog from the short lab stay group.

treatment frogs took an average of 22.5 trials to reach criterion during Acquisition.

The paired t-tests for the first Probe Trial showed that the frogs spent less time in the arm that was visually correct that in the arm that was previously correct and thus did not use the visual cues to navigate the maze ($t_4 = -1.9836$, $p = 0.12$) (Figure 9).

Only one frog successfully completed multiple reversals. This long stay frog did show evidence of serial reversal learning as it took fewer trials on average to learn the correct door across reversals (Acquisition- 16 trials to criterion; Reversal 1- 6 trials to criterion; Reversal 2- 11 trials to criterion; Reversal 3- 6 trials to criterion).

We compared the Akaike's Information Criterion scores corrected for small sample sizes of two binomial generalized linear tests to determine whether sex or size influenced a frog's likelihood of reaching criterion for Acquisition (1= reached criterion; 0= did not reach criterion). While larger frogs were slightly more likely to be successful, the difference was insignificant (Table 1). Sex also had no effect of a frog's likelihood of reaching criterion (Tables 2 and 4). The models with higher AICc scores were considered to be more supported, so size and sex did not have an effect.

We also used Akaike's Information Crierion to compare two binomial generalized linear tests to examine if

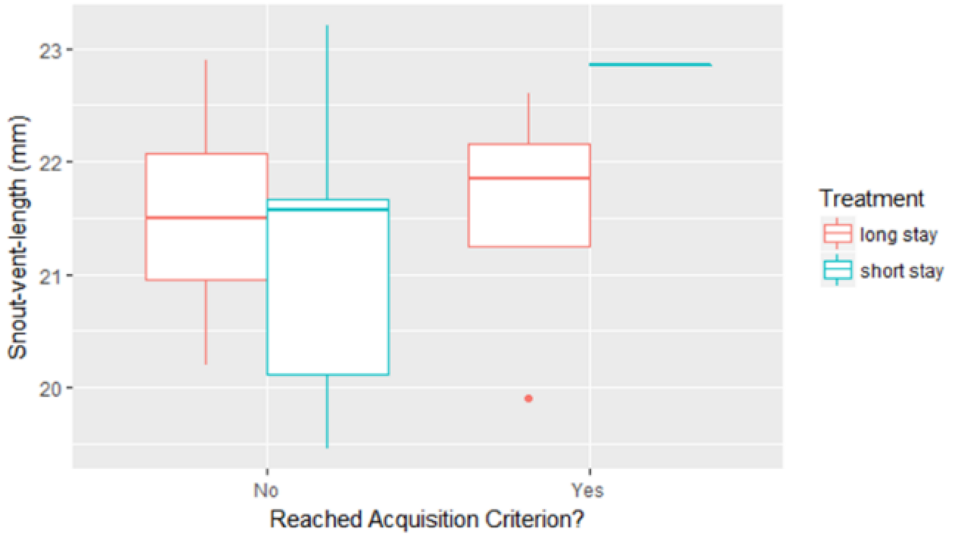


Figure 11 - A frog's size did not significantly affect its likelihood to reach criterion during the Acquisition trials.

treatment influenced whether the frogs reached criterion for Acquisition (Table 3). The model for the group with the lower AICc score was more supported, so treatment did have an effect. For any given trial, a frog from the long lab stay group was more likely to have successfully oriented the maze than a frog from the short lab stay group (Figure 10).

Table 2 - The model selection results for the effect of size on the likelihood of a frog reaching criterion show that size has no effect.

Table 1	dAICc	df	weight
m.0	0.0	1	0.74
m.size	2.1	2	0.26

Table 3 - The model selection results for the effect of sex on the likelihood of a frog reaching criterion show that sex has no significant effect.

Table 2	dAICc	df	weight
m.0	0.0	1	0.84
m.sex	3.3	3	0.16

Table 1 - The model selection results for the effect of treatment on the likelihood of a frog reaching criterion show that treatment has an effect.

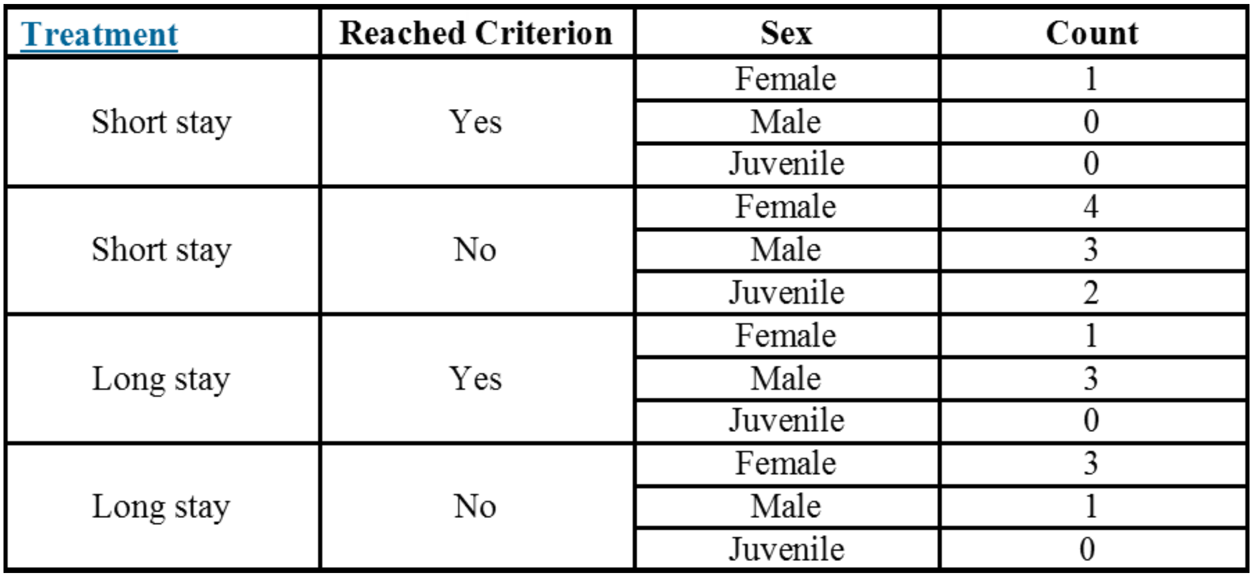
Table 3	dAICc	df	weight
m.treatment	0.0	2	0.64
m.0	1.1	1	0.36

Discussion

One likely explanation for the poor display of learning and behavioral flexibility of the short lab stay frogs is high stress levels. At the time of their trials, these frogs were likely still stressed from their capture in the wild and placement in the lab whereas the long stay lab group frogs were probably more habituated to lab conditions at the time of their trials. Since increased behavioral flexibility might lead to increased chances of survival in the wild, the long stay frogs may be more likely to readapt to their natural environment when released than the short stay frogs.

It would be interesting to test behavioral flexibility over a longer period spent in the lab, for example, test groups at one day after capture, one week, two weeks, one month, six months, and a year. We might discover if there is a specific length of time to keep frogs in the lab when using them for research to optimize their behavioral flexibilities and thus chances of survival upon release. There may be a balance between enough lab habituation to lower stress levels but not so much consistency in lab conditions and food

Table 4 - The distribution of sexes in each treatment that reached criterion was used to see if sex had an effect on a frog's likelihood of reaching criterion.



Treatment	Reached Criterion	Sex	Count
Short stay	Yes	Female	1
		Male	0
		Juvenile	0
Short stay	No	Female	4
		Male	3
		Juvenile	2
Long stay	Yes	Female	1
		Male	3
		Juvenile	0
Long stay	No	Female	3
		Male	1
		Juvenile	0

availability that the frogs cannot survive on their own.

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