Environmental Analysis of the Declination of Blanchard's Cricket Frogs (*Acris blanchardi*)

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Abstract: Blanchard's Cricket frog has been experiencing declining populations in its northern most regions. 33 frogs were divided into three separate treatment groups (wet, dry, normal) depending on soil hydration so that the effect on their foraging habits and well-being could be observed. ¾ oz of water was added to the normal treatment group, 1.5 oz of water was added to the wet treatment group, and no water was added to the dry treatment group, maintaining a constant soil hydration for all three groups (dry=20, wet=90, normal=60). No dramatic weight change patterns were experienced in any of the three test groups, meanwhile the dry treatment group spent the most time in the water petri dish and therefore the least amount of time foraging in the substrate. Generalized linear mixed models and a linear regression model were used to quantify the data and partially gave us the results we hypothesized. While no correlation between time spent in petri dish and weight change was established the hypothesis that the dry treatment group would spend the majority of its time in the petri dish was correct.

Keywords: Soil Hydration, Blanchard's Cricket Frog, Space Use

Introduction

In the northern regions of the US, like Ohio and the Dakotas, Blanchard's cricket frog (*Acris blanchardi*), has been experiencing a decline in population (Beauclerc et al. 2010; Gray and Brown, 2005; Gray et al. 2005; Lannoo, 1998; Lehtinen and Skinner, 2006). While there are many theories regarding the cause of the decrease in the northern populations of Blanchard's cricket frog, currently, there have been no studies establishing a causal relationship between potential abiotic and biotic factors and reductions in population. To date, most studies only present results on the current status of Blanchard's cricket frog populations. For example, Lehtinen and Witter (2014), provided data on the extinction rates of Blanchard's cricket frog in different habitats across Ohio, but information on the status of other populations and the results of attempted conservation efforts are lacking. In turn, there is a large gap in what scientists know about the causes and consequences of the decline of Blanchard's cricket frog.

One of the reasons for this lapse in conservation efforts is that documenting the presence of a cryptic species, like Blanchard's cricket frog, is difficult and makes predicting its occupancy in any given habitat problematic. Often the presence of such a cryptic species is overlooked, resulting in a pseudo-absence, which biases population and occupancy estimates (MacKenzie et al. 2002). This is especially true for transient habitats, like wetland sites with short hydroperiods-one of the main breeding sites of Blanchard's cricket frog, where species presence is highly dependent on site conditions. In addition to altering population estimates, wetland hydroperiod can greatly alter the ecology of amphibians that have a wide span of populace and breed in temporary, as well as permanent, ecosystems (Gray et al., 2005). Due to having such widespread populations and dwelling in all sorts of hydrological conditions, Blanchard's cricket frogs are considered a sentinel species for understanding how changes in climate may influence anurans (Gordon et al. 2016).

One possible cause for the decline in Blanchard's cricket frog populations is pollution. Research has proposed that the era of pesticides, somewhere around the 1950s, corresponds to when cricket frog populations began to decline (Knutson et al. 2000). In support of this, Russell et al. (2000) found that organic pollutants from the environment persisted in the tissues of cricket frogs, which could be contributing to the loss of northern cricket frogs. Meanwhile, through the use of simulation-based modeling, McCallum (2010) hypothesized that in Arkansas the reproductive rate of Blanchard's cricket frog will decrease from 33-94% by 2011 due to climate change.

Yet pesticide use in the United States is ubiquitous while Blanchard's cricket frog is predominantly declining in the northern part of its range, suggesting that temperature or changes in precipitation patterns, may be contributing to their decline. Swanson and Burdick (2010) have already declared that Blanchard's cricket frog has a poor freezing tolerance and survives overwintering by finding habitats in the ground, or "terrestrial hibernacula". Meanwhile, data was provided that stated from -1.5 to -2.5 degrees Celsius Blanchard's cricket frog had an 80% survival rate yet experienced more losses when experienced in 24 hour sessions (Swanson and Burdick, 2010). Also, how cricket frogs cope with winter conditions, and how winter conditions subsequently affect behavior in the spring is unknown.

Ralin and Rogers (1972) stated that out of all the hylids in North America, *Acris crepitans* is one of the most aquatic, making it more suitable to wetter soil levels. They then go on to state that they do not show an increase in tolerance or size to body water loss with decreased rainfall, yet they do exhibit an increase in per cent body water. Therefore subjecting the cricket frog to hydration levels anywhere <15% would be cruel. Walvoord (2003) addressed the impacts of jump distance from varying hydration levels and temperatures. Walvoord later displayed that a frog's jump distance remained unaltered when jumping at 85% hydration and 15 degrees and 30 degrees Celsius compared to 95% hydration and 30 degrees Celsius, yet when decreasing from 85% hydration to 75% hydration, the 85% hydration frog jumped significantly better.

We intend to explore this gap in what is known about Blanchard's cricket frog population declines by examining how the cricket frog copes with varying soil hydration levels. Anurans begin to aestivate, or go into dormancy enduring extreme conditions, especially when temperatures become too dry. Since it is apparent that as conditions cool and warm the soil hydration with fluctuate as will the cricket frog's per cent body water.

I will accomplish this by subjecting wild caught Blanchard's cricket frogs to a different combination of soil hydration levels. While frogs are enduring these different environmental conditions. Krynak et al. (2015) declared that an anuran's environmental conditions have an impact on its immune response. If it becomes apparent that the cricket frogs are gradually becoming less adapted to surviving in less suitable conditions, the data gathered will determine the combination of environmental conditions at which cricket frogs are able to maintain a healthy level of activity (e.g. jump distance) before they begin to slow down, and hopefully why they have become more susceptible to varying northern conditions.

Methods

Wild cricket frogs were collected from the Oklahoma State University's Experimental Pond in Stillwater, OK and divided into three treatment groups and housed individually for this study-normal, wet, and dry substrate. The cricket frogs were housed in small plastic bins with coco bark and sphagnum moss, and water dishes filled with 50 mL of dechlorinated water. Also, they were fed 10-15 flightless fruit flies (Drosophila hydei and Drosophila melanogaster) every 2 days, which were coated in a vitamin and calcium powder for their required nutrients. The normal hydration group, received ¾ oz water added to their coco bark every other day, mimicking the water frogs typically receive under laboratory conditions. Meanwhile, another group (i.e. the wet group) inhabited extremely wet conditions and had 1.5 oz of water added to their coco bark every other day. The last treatment group, the dry group, did not receive any water added to their coco bark during the experiment. Frogs were exposes to these conditions for two weeks.

I kept track of each frog's weight and space use for two weeks while they were exposed to the soil hydration treatments described above. Frogs were weighed immediately prior to the start of the experiment and t the end of the experiment, then the change in weight was calculated. To keep track of frog space use, I recorded whether the frog was in its water dish or on land once a day. I ended up with results that showed that the frogs in the dry treatment group spent the most time in their water dishes while the wet and normal treatment frogs alternated.

The sample consisted of 33 wild-caught juvenile/adult Blanchard's cricket frogs, 11 per treatment group, which had no prior lab experience. I then used linear regression model and generalized linear mixed models (LM and GLMM, respectively) to determine if frog weight change and the proportion of time spent in their water dish was influenced by the soil hydration treatments. In the GLMM, individual frog was treated as a random effect

I predict that hydration will play a factor in the cricket frog's space use in that if a frog is subjected to a non-ideal moisture level, their eating habits, and in turn, their weight will reduce. Given that frogs are sensitive ectotherms and their physiological functions are determined by ambient temperature and humidity, I predict that the frogs housed in the drier conditions will spend more time in the water than frogs in wetter treatments. While metabolism is not directly related to overall well-being, it does influence a frog's activity level. Therefore, Blanchard's cricket frog will more than likely experience a greater decrease in body size, weight, and overall well-being while enduring the extremely dry conditions.

Results

I found that frogs in the dry treatment groups spent more time in their water dish than the wet and normal test groups (Treatment: $p < 0.0001$; Figure 1), but soil hydration treatment did not influence the amount that frogs changed their weight over the course of the experiment (Treatment: $p = 0.32$; Figure 2).

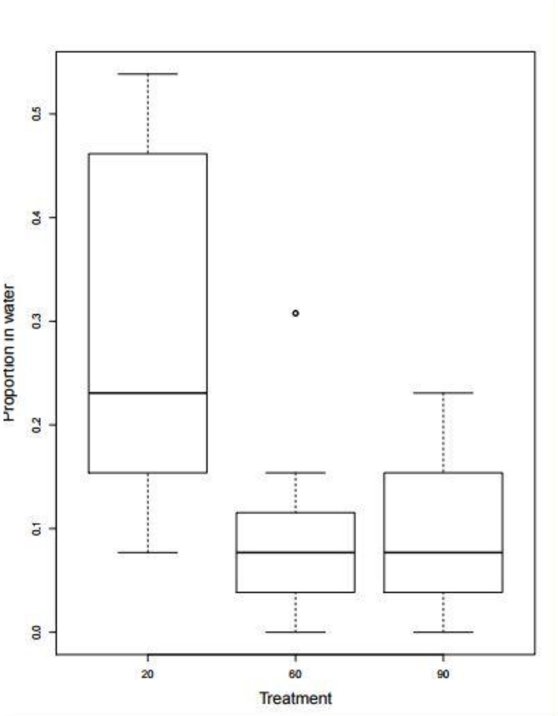

Figure 2 - The influence of frog soil hydration on the proportion of time a frog spent in the water (dry soil treatment = 20, normal soil treatment = 60, wet soil treatment = 90). The dry treatment group had the highest probability that on any given day a subject was likely to be present in the water dish ($p < 0.05$*).*

Discussion

This finding supports my hypothesis in that the space use of the dry treatment group would be affected due to their increased time spent in the petri water dishes. While the treatments did not span long enough for a relationship between treatment group and weight gain to be formed, a pattern regarding the normal test group's weight formed. The pattern displayed that as the trials continued, the normal test group experienced the least amount of weight differentiation, with the wet and dry test group experiencing the most change in weight.

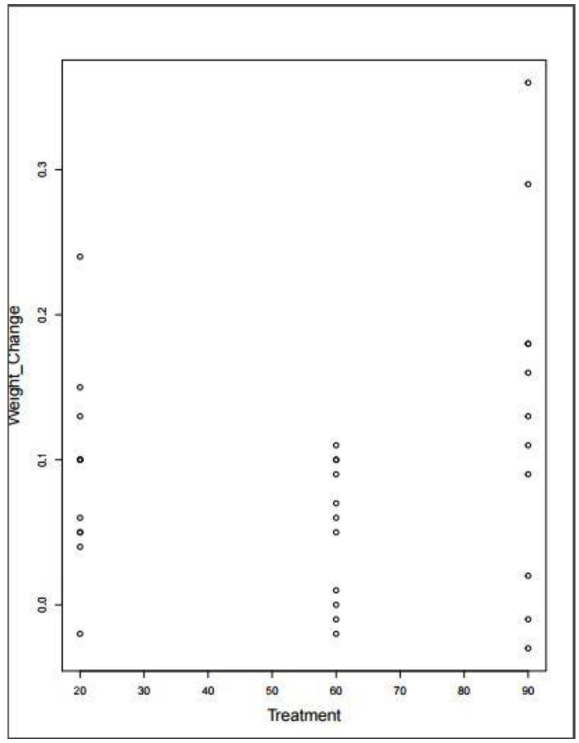

Figure 1 - The influence of soil hydration treatment (dry = 20, normal = 60, wet = 90) on the change in frog weight (g) over the course of two weeks. Treatments did not significantly influence the amount of weight change frogs had during the experiment.

Along with the GLMM, a linear regression model was used to find a connection between the treatment group and the weight change by individual ($p > 0.05$). The data resulting from the algorithm showed that there was no direct correlation between treatment group and weight change, but when examining the data (Figure 2), it is apparent that the normal test group experienced the most centralized weight change. Therefore working against the original hypothesis that the more time spent in a water dish would drastically impact foraging habits and impact weight change as a result.

While the data shows no correspondence with weight change and time spent in the water dish, it does show that the drier the soil hydration levels, the more likely the subject is to be found in their water dish. Assumptions can be made referring to the constantly changing climate due to pollutants and global temperature changes, but when weight change is used as a reference for overall well-being, no connection can be made. Therefore the algorithm has provided insight on the changing habits of Blanchard's cricket frog when associated with changes in soil water content, but until a connection can be made to frog well-being, no claims can be made on the declination of the norther-most populations of Blanchard's cricket frog.

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