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Behavioral Response of Adult and Larval Wood Frogs (Lithobates sylvaticus) to a Common Road De-Icer, NaCl

Dylan Jones
Montclair State University

Abstract

Amphibians are highly vulnerable to aquatic pollutants. Due to the permeability of their skin and their aquatic larval stages, pollutants are easily absorbed into the body, which can have adverse effects on performance, survival, and fitness. This has prompted research on how environmental pollutants affect amphibian populations, especially road deicers such as sodium chloride (NaCl). Elevated NaCl can have a negative physiological impact on both adult and larval stages of amphibians, leading to reduced breeding success, morphological abnormalities, and even mortality. However, less is known about the behavioral responses of adults and especially larval amphibians to increased environmental salinity. Earlier studies suggested that adult wood frogs did not show any behavioral responses to varying salinity with short-term (10 min) exposure, while larvae had not been assessed. In this study, the behavioral responses of both adult and larval wood frogs, *Lithobates sylvaticus*, to increased salinity were studied via salinity choice trials where a control (aged tap water) and a designated salt solution were placed on opposite sides of a binary arena for 3,600 seconds. Adults spent less time in NaCl solutions with increasing salinity. The threshold for response was approximately 0.17 M (slightly hyperosmotic to internal osmotic concentrations). For tadpoles, time spent in salt solutions did not change as salinity increased (to a maximum of 0.25 M NaCl), but these results were confounded by mixing between the control and the salt solutions. There were no behavioral differences in tadpole activity level (number of moves between chambers) as salinity increased. Since increased salinity has been associated with decreased fitness, behavioral avoidance of high salinity and preference for lower saline systems could be advantageous for wood frogs. Adults could potentially select breeding

sites with lower solute levels that would be beneficial to egg masses and offspring. However, this study suggests that tadpoles in a high solute habitat may not change their activity level, potentially leading to inability to select microhabitats within a system. In addition, although adult wood frogs did respond to increasing salinity, they did so slower than previously assessed species, potentially making them more susceptible to habitat degradation. This study furthers the understanding of how amphibian populations respond to salinity influxes in the wild and will help to promote better conservation efforts for species vulnerable to salt pollution.

MONTCLAIR STATE UNIVERSITY

Behavioral Response of Adult and Larval Wood Frogs (*Lithobates sylvaticus*) to a Common Road De-Icer, NaCl

By Dylan Jones

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College of Science and Mathematics

Department of Biology and Molecular Biology

Thesis Committee:

[redacted] Dr. Lisa Hazard

[redacted] Dr. Kirsten Monsen-Collar

[redacted] Dr. Scott Kight

Behavioral Response of Adult and Larval Wood Frogs (*Lithobates sylvaticus*) to a Common Road De-Icer, NaCl

A THESIS

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By

DYLAN JONES

Montclair State University Montclair, NJ

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By Dylan Gage Jones

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Table of Contents

Chapter Title	Page
Abstract	1
Thesis Signature	3
Title Page	4
Copyright Page	5
Acknowledgements	6
Table of Contents	7
List of Figures and Tables	9
Introduction	10
Materials and Methods	13
Animal Collection	13
Adult Husbandry	13
Tadpole Husbandry	14
Sodium Chloride Solution	14
Adult Response Trials	14
Adult Mass	15
Tadpole Response Trials	15
Statistical Analysis	16

Results	17
Adult	17
Tadpole	18
Discussion	19
Adult Behavior	19
Adult Mass	20
Tadpole Behavior	21
Conclusion	22
Figures	25
Literature Cited	36

<u>**Figures**</u>

Figure 1 - Total time spent by adult wood frogs in each of six NaCl concentrations during binary choice trials for 3,600 secs.

Figure 2 - Nominal logistic regression curve for adult wood frogs representing the effect of salt concentration on proportion of individuals showing salt tolerance.

Figure 3 - Total time spent by adult wood frogs in each of six NaCl concentrations during binary choice trials for first 600 sec of 3,600 sec trials.

Figure 4 - Mass change across five different NaCl concentrations.

Figure 5 - Preliminary data for total time individual tadpoles spent in salt solution for 3,600 trials (0.0 vs 0.0 and 0.0 vs 0.15M NaCl)

Figure 6 - Preliminary data for number of times individual tadpoles moved between solution and control for 3,600 sec trials (0.0 vs 0.0 and 0.0 vs 0.15M NaCl).

Figure 7 - Total time individual tadpoles spent in salt solution for 3,600 sec trials.

Figure 8 - Number of times individual tadpoles moved between solution and control for 3,600 sec trials.

Figure 9 - Adult wood frog choice trial arena

Figure 10 - Tadpole behavioral trial arena

Figure 11 - Adult wood frog mass trial arena

Introduction

Chemical de-icers such as sodium chloride (NaCl) have been applied to public roads since the 1950s (Transportation-Research-Bored 1991). The state of New Jersey has 68 storage facilities with capacity to store an accumulative 228,000 tons of salt for use on state Department of Transportation-controlled roads (Transportation-Research-Bored 1991). The application of road de-icers helps improve public safety by reducing ice and snow buildup(NJDOT 2017); however, much of these pollutants is mobilized and has the potential to negatively affect the local terrestrial and aquatic ecosystems (Transportation-Research-Bored 1991). The accumulation of NaCl within the runoff leads to increased saline levels in fresh water systems (Miller, Joyce et al. 1996, Norrström and Jacks 1998, Mayer, Rochfort et al. 1999, Trombulak and Frissell 2000, Williams, Williams et al. 2000, Evans and Frick 2001, McDaniel and Borton 2002, Riitters and Wickham 2003, Kaushal, Groffman et al. 2005, Karraker, Gibbs et al. 2008, Corsi, Graczyk et al. 2010, Cañedo-Argüelles, Hawkins et al. 2016, Milotic, Milotic et al. 2017)

Vernal pools are seasonal bodies of water that fill within the spring and usually by late summer have completely dried (Trombulak and Frissell 2000, Ruth 2003, Thunqvist 2004, Karraker, Gibbs et al. 2008, Corsi, Graczyk et al. 2010). These pools are usually closed aquatic systems, having few outlets, so salinity influxes can increase conductivity over time (Banta 1914, Turtle 2000, Karraker, Gibbs et al. 2008). This has the potential to affect salt-sensitive species that reside within these habitats, especially obligate vernal pool breeding species that are dependent on these habitats for successful reproduction.

Wood frogs, *Lithobates sylvaticus*, have a large distribution, being found as far south as Alabama, throughout most of the central to north mid-west, central to north east United States, and throughout Canada and Alaska (Trombulak and Frissell 2000, Ruth 2003, Thunqvist 2004, Karraker, Gibbs et al. 2008, Corsi, Graczyk et al. 2010). Wood frog breeding varies with geographic distribution due to climatic factors such as temperature and precipitation (The-IUCN-Red-List-of-Threatened-Species. 2012), but northern populations emerge in early spring, with New Jersey populations typically emerging between late February-mid-March. Males emerge first, travel to breeding pools, and are later followed by females (Banta 1914). Females usually lay a single egg mass containing 200-300 individual eggs. Egg masses are often laid in group clusters within a pond (Banta 1914, Turtle 2000), and there is no parental care after egg deposition (Banta 1914, Seigel 1983). This species of frog is notable for its ability to tolerate sub-freezing temperatures, as low as -3C^{o}, through metabolite (urea and glucose) accumulation within the extracellular fluid, which prevents crystallization within the cell at freezing temperatures (Banta 1914).

Although freeze tolerance has been documented in wood frogs, salt tolerance has not. With permeable skin, water and salt loss and gain occur cutaneously and can lead to osmotic imbalances (Costanzo, Lee et al. 1993). In the wild, wood frogs naturally occur within hypoosmotic, not hyperosmotic, environments (Hillyard, Viborg et al. 2007). They thus have mechanisms of water and salt regulation appropriate to low solute habitats. Passive uptake of water and loss of salts is countered by excretion of dilute urine and active uptake of salts across the skin to maintain homeostasis. Exposure to high saline environments could therefore interfere with maintenance of homeostasis (Karraker, Gibbs et al. 2008, Langhans, Peterson et al. 2009), because the frogs may lack sufficient mechanisms for coping with passive water loss and salt gain, leading to solute imbalances. This ionic imbalance can be energetically taxing for amphibians with the potential of developmental abnormalities and even death, especially in eggs and tadpoles (Victor E. Hall 1949, Feder and Burggren 1992, Hillman 2009). This sensitivity to salt makes adult selection of appropriate breeding sites critical. Several amphibian species have exhibited behavioral aversion to elevated salinities in laboratory trials (Sanzo and Hecnar 2006, Karraker, Gibbs et al. 2008, Langhans, Peterson et al. 2009) however, in short-term (600 second) laboratory choice trials, adult wood frogs surprisingly showed a lack of behavioral aversion to salinities as high as 0.5 M NaCl (roughly seawater) (Gonzalez-Abreu 2011, Kwasek 2011, Lindstrom Hasko 2013, Zilinskis 2015, Krolik 2017). If this behavior persists under natural conditions, wood frogs could potentially breed in unsuitable habitats, thus exposing egg masses and larvae to harm.

Wood frogs may have a delayed response or greater tolerance to increase salinity than other species. Additional studies are needed to determine whether wood frogs show behavioral aversion to increased salinity if they are given longer to assess their environment. Also, while wood frog tadpoles are known to show habitat preferences for temperature, substrate, and other factors (Koelmel 2011), research in salinity preference has not been investigated. Wainscot found that bullfrog tadpoles did not avoid NaCl in concentrations as high as 18,500 ppm (approximately 0.25 M NaCl) (McDiarmid and Altig 1999). This suggests that wood frog tadpoles also may not avoid harmful salinity levels, despite the known negative physiological effects of high salinity on tadpoles (Wainscott 1997).

Our first question for this study was whether adult wood frogs show salinity aversion if given longer time periods than previously assessed. I hypothesized that as salinity increases, time spent in saline solution would decrease. The second question of this study was whether wood frog tadpoles show salinity aversion. I hypothesized that both tadpoles and adult wood frogs would show NaCl aversion at concentrations that exceed internal osmotic concentrations when given a choice between salty and control solutions.

Methods

Animal Collection

All individuals were recruited from the New Jersey School of Conservation in Sandyston, NJ from the same vernal pool. The pool was located at 41.22027° N, -74.74903° W in a wooded area and isolated from any major road system pollutants. Animals were transported to Montclair State University by car in a 10 L bucket. Adult male wood frogs were collected in March 2017 using a dip net, and recently hatched tadpoles were collected in April 2017 using a bucket and dip nets.

Adult husbandry

Adults were kept in 40-60 l glass terraria in small groups between 3-8. Moistened coconut bark (3-5 cm deep) and peat moss (3-5 cm deep) were provided as the substrate. Curved pieces of 1 cm plastic mesh, measuring 15-25 cm in length and 5-8 cm in width allowed frogs access to water bowls filled with aged or dechlorinated tap water. Food (live crickets) was supplied every 2-3 days. Lighting was provided with ZooMed™ 10.0 ultraviolet lighting at 12L:12D. Room temperature ranged from 21.7-24.4 °C. Individuals were toe clipped with unique clip sequences for identification.

Tadpole husbandry

Tadpoles were kept in aged tap water in 1000 mL containers, in an incubator at 12L:12D with a constant 15°C temperature to approximate pond temperature. Each container housed 10-15 tadpoles. Pelleted fish food for herbivorous fish was crushed and fed to tadpoles every 2-3 days; water was changed immediately before as feeding or as needed.

Sodium Chloride Solution

Saline solutions were produced with aged tap water and NaCl. A stock solution of 1M sodium chloride was made and then diluted to 0.05, 0.1, 0.15, 0.2 or 0.25 M. Solutions were stored in the tadpole incubator to ensure temperature equalization.

Adult Response Trials:

Individual adult frogs were placed in a 20 L terrarium with two 200 mL plastic trays. One tray contained 200 ml of aged tap water (control), while the other contained a test solution (0.0, 0.05, 0.1, 0.15, 0.2 or 0.25 M NaCl) (Figure 9). Frogs were placed in the test solution at the beginning of the trial. Behavioral activity of subjects was recorded from above for 3,600 seconds using an iPhone 5 camera and the video function of the built-in Photo app to minimize human interference. Mass of each subject was measured to the nearest 0.01 g pre- and post-trial using an electronic digital balance. Location of NaCl vs. control (left or right) was randomized for each trial using the RND function in Microsoft Excel. Forty-eight adult wood frogs were used; each was tested once (n = 8 per concentration). Temperature ranged from 21.7-24.4 °C. Terraria were thoroughly rinsed with hot water and allowed to air dry between trials, and frogs were returned to their home cages. Time spent by each animal in the control and salt solutions was determined from recorded video. Frogs were considered to be on the test solution side if more than half of the body was positioned on solution side and was considered out of the solution if more than half was on the control side. Additionally, the number of times the animal moved from one solution to the other was recorded.

Adult Mass Trials:

Measuring mass change is a simple way to evaluate osmotic gain or loss of water. However, interpretation of mass change during choice trials is complicated by the fact that animals spend time in two different salinities. Therefore, to evaluate the effect of salinity on water flux, frogs were exposed to a single salinity for one hour and mass change was recorded. Individual frogs were placed in 500 ml plastic deli cups that contained 100 ml of NaCl solution (0.0, 0.05, 0.1, or 0.2 M NaCl) (Figure 11). Trial duration was for 1 hour. Frogs were weighed pre and post-trial to the nearest 0.01 g. Thirty individuals were each tested twice, at two different concentrations, with selection of the solution tested being random but not repeating. Fourteen of 60 trials were removed from analysis due to feces release. This resulted in 7-10 individual trials per treatment group, N = 46.

Tadpole Response Trials:

Individual tadpoles were placed in the center connector of a dual 10 cm petri dish chamber (Carolina Biological Supply #746618) containing 75 mL of control solution on one side and 75 mL of saline solution (0.0, 0.05, 0.1, 0.15, 0.2 or 0.25 M NaCl) on the other side (Figure 10). Location of NaCl vs. control (left or right) was randomized using an excel random number generator. Tadpole activity was recorded from above for 3,600 seconds using an iPhone 5 camera and the video function of the built-in Photos app to minimize human interference. Mass was measured immediately after the trial. Initial weight was not measured in order to reduce stress on the tadpoles. Two rounds of testing were conducted. In the first round, 50 randomly selected tadpoles were used for 0.15 M NaCl and control binary choice trials with a total of 100 individuals. For the second round, 20 tadpoles were randomly selected to be tested at each of the 6 concentrations, for a total of 120 individuals. Tadpoles were tested between stages 26-36 of development (Sanzo and Hecnar 2006, Karraker, Gibbs et al. 2008, Langhans, Peterson et al. 2009). Trials were performed at 15 °C. Time spent in the control and sodium chloride sides of the arena were determined from recorded video. A conservative designation of tadpoles being located in the salt solution was applied, with tadpoles only being considered in solution if the entire body (excluding the tail) was within the dish designated as the test side. Tadpoles were considered to be outside the test solution if they were located in the center connector or the control solution side. Rate of mixing between the two sides of the test arena was initially evaluated visually in mock trials, using tap water colored with food coloring. No mixing was observed after one hour. Rate of mixing was evaluated after trials were completed by taking 100 µl subsamples from several points in each dish at the beginning and end of mock trials (0.0 vs. 0.25 M NaCl) and measuring chloride using a chloridometer (Labconco Digital Chloridometer, Model: 442-5000).

Statistical analysis:

Statistical tests were performed with JMP 13.0 (SAS Institute); a P value of less than 0.05 was considered to be statistically significant. ANOVAs were used to test for differences in means for both adult and larval stage behavioral trials and adult mass trials. Where ANOVA results were significant, Student's t-tests were performed to test for differences among means.

Results

Adult:

Adult wood frogs spent less time in the test solution as salinity increased (Figure 1; ANOVA: $F_{5,42} = 3.0190$, $P = 0.0204$) In particular, the highest concentration (0.25 M NaCl) differed from the lowest three (0.0, 0.05, and 0.1 M), and the second highest concentration (0.2 M) differed from 0.0 and 0.1 M (Student's t-tests, Figure 1).

In prior studies on other species, time was bimodally distributed (Gosner 1960). Additionally, activity level varied between individuals and across species, such that in a control trial (0.0 vs. 0.0 M, with the starting side being defined as the "test" side) some animals stayed on the test side while others paced constantly and spent approximately half of the trial on each side of the arena. Therefore, a standardized method for evaluating salinity tolerance was developed for comparisons across studies. A new binary variable, tolerance, was defined using the control trial as a baseline. For an individual trial, if the time spent in the test solution was greater than half of the average time spent in the test solution in the 0.0 M vs. 0.0 M trial, the animal was said to show tolerance of the solution. Otherwise it was said to show aversion to the solution. In the current study, frogs spent an average of 2828 seconds at 0.0 M, so frogs that spent more than 1414 seconds in the test solutions were defined as showing tolerance. A nominal logistic regression analysis (Figure 2) of the effect of salt concentration on the proportion of individuals showing salt tolerance found that as salinity increased, tolerance decreased

($X^2 = 11.7769$; P = 0.0006). An EC_{50} (concentration at which 50% of individuals showed aversion) was determined to be approximately 0.169 M NaCl (95% C.I. = 0.1157 – 0.2658 M NaCl).

The results of these 60-minute trials differed from the results found in earlier 10-minute trials (Gonzalez-Abreu 2011, Kwasek 2011, Lindstrom Hasko 2013, Zilinskis 2015, Krolik 2017), showing a decrease in time spent with increasing salinity. To evaluate whether the length of the trial was the reason for the difference between studies, further analysis was done on a subset of the data from this study, restricted to the initial 600 seconds of each trial (Figure 3). There were no significant differences among concentrations (ANOVA: $F_{5,42} = 0.6116$, $P = 0.6915$).

In dehydration trials, mass change differed across salinities (Figure 4; ANOVA: $F_{4,39} = 2.8283$, $P = 0.0375$). Frogs gained mass in 0.0 and 0.1 M NaCl and lost mass in 0.2 M NaCl, and the mean mass at 0.2M NaCl significantly differed from control and 0.1 M NaCl (Student's t-tests, Figure 4).

Tadpoles:

In the first round of choice trials for tadpoles, there was no effect of salinity on time spent on the high salinity side of the arena (Figure 5; t-test: $t = 0.0016$, $P = 0.9987$); however, activity level (number of times moved between concentrations) was lower for tadpoles choosing between 0.0 M and 0.15 M NaCl than for the control (0 vs. 0 M) (Figure 6; t-test: $t = 4.2380$, $P = <0.0001$). In the second round of trials, testing an expanded range of salinities (0.0-0.25 M), there was also no effect of salinity on the amount of time spent on the high salinity side of the arena (Figure 7; ANOVA: $F_{5,114} = 0.5272$, $P = 0.7553$); however rapid and complete mixing of the solutions were observed using a chloridometer, which determined tadpole choice trials to be inconclusive. In addition, activity level (total times moved between salinities) differed among trials (ANOVA: $F_{5,114} = 3.0664$, $P = 0.0124$), but there were no differences between means (Student's t-tests) (Figure 8).

Discussion

Adult Behavior

When adult wood frogs were tested at varying solutions of NaCl for one hour trials, wood frogs spent significantly less time in higher, hyperosmotic solutions than in lower hypoosmotic, solutions (Figure 1). EC_{50} for wood frog aversion was 0.17 M NaCl (Figure 2). This concentration is slightly hyperosmotic, meaning solute concentrations in the aqueous solution are greater than internal concentrations. Although there was no significant difference between time spent at 0.05 M and 0.2 M NaCl (Figure 1), time spent at 0.0 M and 0.1 M were shown to be significantly different from 0.2 M NaCl. This overlap could be due to small sample size, as only 8 individuals were tested at each concentration. However, 0.0 M, 0.05 M, and 0.1 M were significantly different from 0.25 M NaCl, thus showing a notable trend of decreasing time with increasing NaCl solution. This behavior suggests that adult wood frogs are capable of detecting and actively avoiding NaCl concentrations that are physiologically harmful. Although this is contradictory to prior studies of adult wood frog behavior that found no aversion to concentrations as high as 0.5 M NaCl, the present study demonstrated that wood frogs did not avoid saline solutions until after the first 600 seconds of the trial (Figure 3). The first 600 seconds of observations in the present study align with previous findings. I propose that previous studies did not conduct trials long enough to elicit an aversion response, thus resulting in no significant difference among concentrations. The current study is consistent with several other laboratory studies that have shown that various native New Jersey amphibians, such as spotted salamanders and spring peepers, contain the ability to detect and avoid elevated NaCl levels (Koelmel 2011); however, unlike other species tested, wood frogs took significantly longer to respond. Other amphibian species typically responded before 600 seconds (Gonzalez-Abreu 2011, Kwasek 2011, Lindstrom Hasko 2013, Zilinskis 2015, Krolik 2017).

Adult Mass

If physiological NaCl concentrations are lower than environmental concentrations, a frog should experience cutaneous water loss (Gonzalez-Abreu 2011, Kwasek 2011, Lindstrom Hasko 2013, Zilinskis 2015, Krolik 2017), so we further tested mass change at different salinities (Figure 4). In hyperosmotic solutions compared to internal osmotic concentrations, body mass at the end of a trial was lower than at the beginning. This further supports the supposition that high levels of salinity can result in potential harm or discomfort for wood frogs. Although the highest solution tested was 0.2 M NaCl, there were significant differences in mass change between solutions 0.0 M NaCl, 0.1 M NaCl and 0.2 M NaCl. Frogs had the highest mass gain in 0.1 M NaCl when compared to other salt concentrations. This may be due to internal concentration becoming hyperosmotic to the solution through active cutaneous salt transport followed by passive water uptake; however, both 0.0 M NaCl and 0.1 M NaCl were both significantly different than 0.2M.

Tadpole Behavior

Although behavioral aversion was recorded in adult wood frogs, results for larval wood frogs differed from adult trials (Figure 7). In both sets of choice trials, there was no effect of salinity on time. However, the rate of mixing between solutions on each side of the test arena resulted in homogenization of the two solutions before the end of the 3,600 second trials. Since full homogenization occurred during trial time, the results are inconclusive and it remains unclear whether tadpoles did not make a choice, or were unable to make a choice, due to mixing of the two test solutions.

Although solutions in the test arena mixed, activity level in response to elevated salinity could still be evaluated. Animals might be expected to move more frequently in noxious environments as they attempted to find more suitable habitat. Assuming complete mixing of solutions, tadpoles were exposed to salinities ranging from 0.0 to 0.125 M NaCl. In the first experiment, tadpoles in 0.15 M NaCl had more activity than in the control trial (Figure 6), while in the second experiment activity level for 0.15M NaCl was lower than control. There was a significant difference between activity level for the second experiment covering a broader range of salinities; however, there was no notable trend when graphing this data (Figure 8). Although behavioral choice trials were inconclusive, the lack of overall differences in activity suggests that tadpoles did not change their behavior in response to salinity. This research aligns with the findings of Wainscott (Feder and Burggren 1992, Hillman 2009)where bullfrog tadpoles (*Lithobates catesbeianus*) did not avoid NaCl at saline levels of approximately 0.25 M NaCl.

Conclusion

These findings provide some insight into the impact road de-icers may have on amphibian communities. Although the physiological impact of NaCl has been well-studied (Wainscott 1997), many investigations did not examine behavior. In naturally occurring populations, behavior plays a key role in the ability for an organism to select for hospitable habitat (Turtle 2000, Sanzo and Hecnar 2006, Karraker, Gibbs et al. 2008, Langhans, Peterson et al. 2009, Milotic, Milotic et al. 2017). If an amphibian can detect toxins, it could initiate a response to avoid these toxins. The current study suggests that adult wood frogs can detect and avoid elevated saline levels, specifically NaCl. This could mean that adult wood frogs select against vernal pools that have reached toxic salinity concentrations. Although it appears that adult wood frogs can discriminate against elevated salinity, larvae do not appear to select against toxic levels of NaCl. Unlike other studies that have provided evidence that tadpoles will select microhabitats based off of substrate and temperature within a vernal pool (McDiarmid and Altig 1999, Hillyard, Viborg et al. 2007, Hillman 2009), salinity preference was not exhibited by tadpoles in the present study, suggesting that wood frog tadpoles may not select microhabitats based solely on salinity. It remains unclear why wood frog tadpoles in our studies did not change their behavior when emerged in physiologically dangerous levels of sodium chloride. In *Rana catesbeiana*, the outer layer of the larval skin does not function the same as adult skin, contributing very little to ionic and water exchange (Stebbins and Cohen 1997, McDiarmid and Altig 1999). It has also been shown that tadpoles possess a lateral line that could be capable of Na+ detection; however, chemical detection has not been fully investigated and may be lacking (Hillman 2009). The exchange between water and ions occurs almost solely through the gills (Feder and Burggren 1992). Gills may not contain the sensory neurons needed for detection of dangerous solute levels, and it is not until the outer epithelial layer of skin is shed during metamorphosis and the basal layer becomes the outer layer that epithelial sodium channels become active (Alvarado and Moody 1970). In addition, studies have also shown that Ranid (now referred to as Lithobates) tadpoles can regulate osmotic concentrations only at low levels, when internal concentrations are hyperosmotic to external concentrations, but become conformers beyond this point and lack the capability to actively transport ions (Robinson and Heintzelman 1987, Feder and Burggren 1992, Hillman 2009). Although it has been noted that in the short-term tadpoles can regulate ion and water exchange hormonally (Feder and Burggren 1992, Hillman 2009), this research could suggest that tadpoles have a severely reduced ability to manage and detect salinity influxes in the long term.

If adults do not choose a breeding site that will remain low in salinity throughout the larval developmental period, it could result in tadpole abnormalities and die-offs. It remains unclear however if adults will select for hospitable habitat. Adults had an EC_{50} of 0.17 M NaCl. A concentration of 0.17 M NaCl is known to have detrimental effects on the development and longevity of larvae and egg masses (LC_{50} < 0.05M NaCl) (Feder and Burggren 1992). With egg masses having no ability to relocate or solute regulate, they are extremely vulnerable to positionality within a vernal pool (Sanzo and Hecnar 2006, Langhans, Peterson et al. 2009). In addition, if larvae do not contain the ability to select microhabitats within a system, they too are highly dependent upon parental locality choice (Turtle 2000). Further research needs to be conducted on how adults behave in the wild as research has shown that wood frogs usually return and breed at the vernal pool they developed in, and no research has determined whether they will shift breeding site loyalty based on salinity toxicity. Comparison between road side and undisturbed populations should be investigated to see if there are behavioral and tolerance differences among populations that have and have not been exposed to road de-icers as tolerance overtime has been demonstrated for other toxins (Sanzo and Hecnar 2006, Karraker, Gibbs et al. 2008, Langhans, Peterson et al. 2009). In addition, experiments should be designed to test tadpole choice behavior in a system that prevents mixing, as well as incorporating other factors, such as being within a group and under low lighting to determine if lack of behavioral change is due to other stressors. I hope that through further investigation that this research can reduce amphibian population declines related to road disturbance and pollution.

Figures

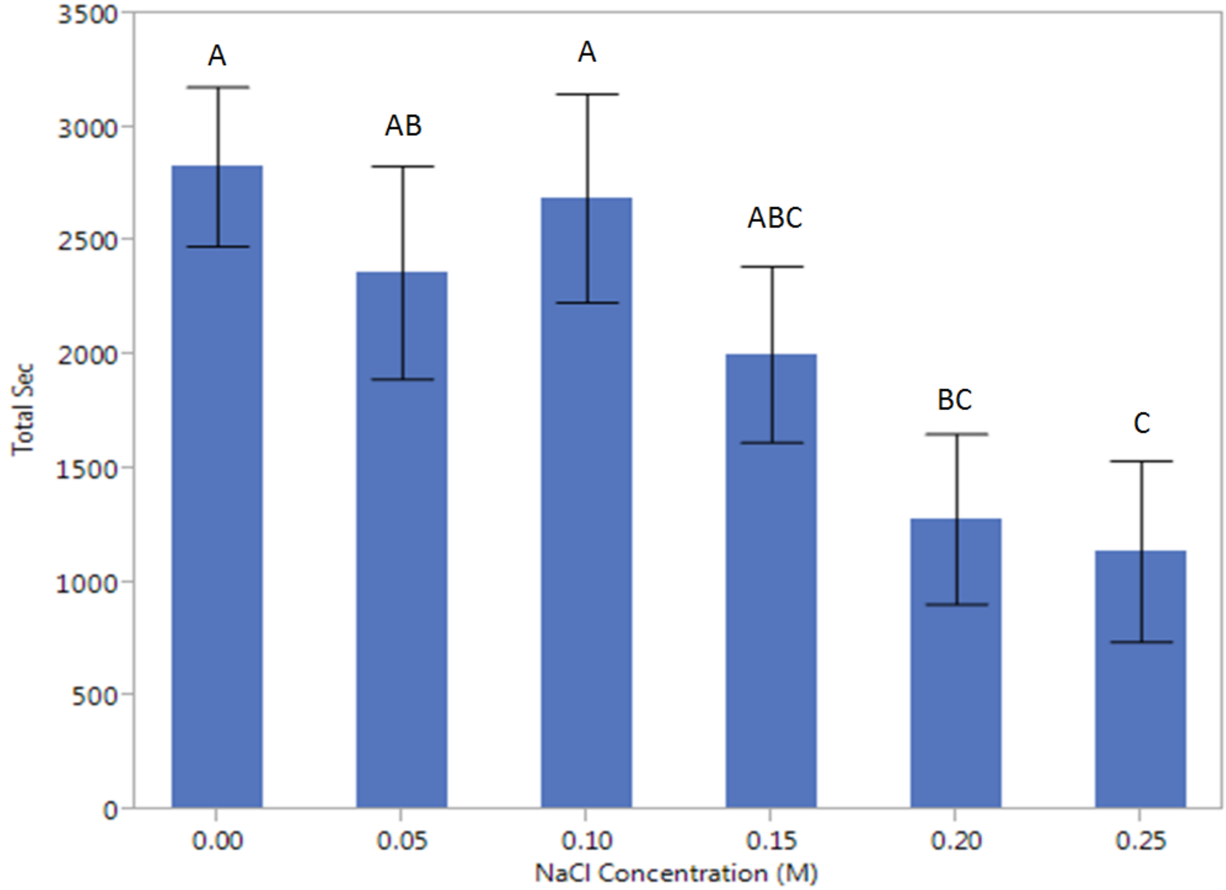

Figure 1. Total time spent by adult wood frogs in each of six NaCl concentrations during binary choice trials (mean ± S.E.) for 3,600 sec. ANOVA: $F_{5,42} = 3.0190$, $P = 0.0204$ and Student's t-tests. Concentrations that share the same letter did not differ. N = 48.

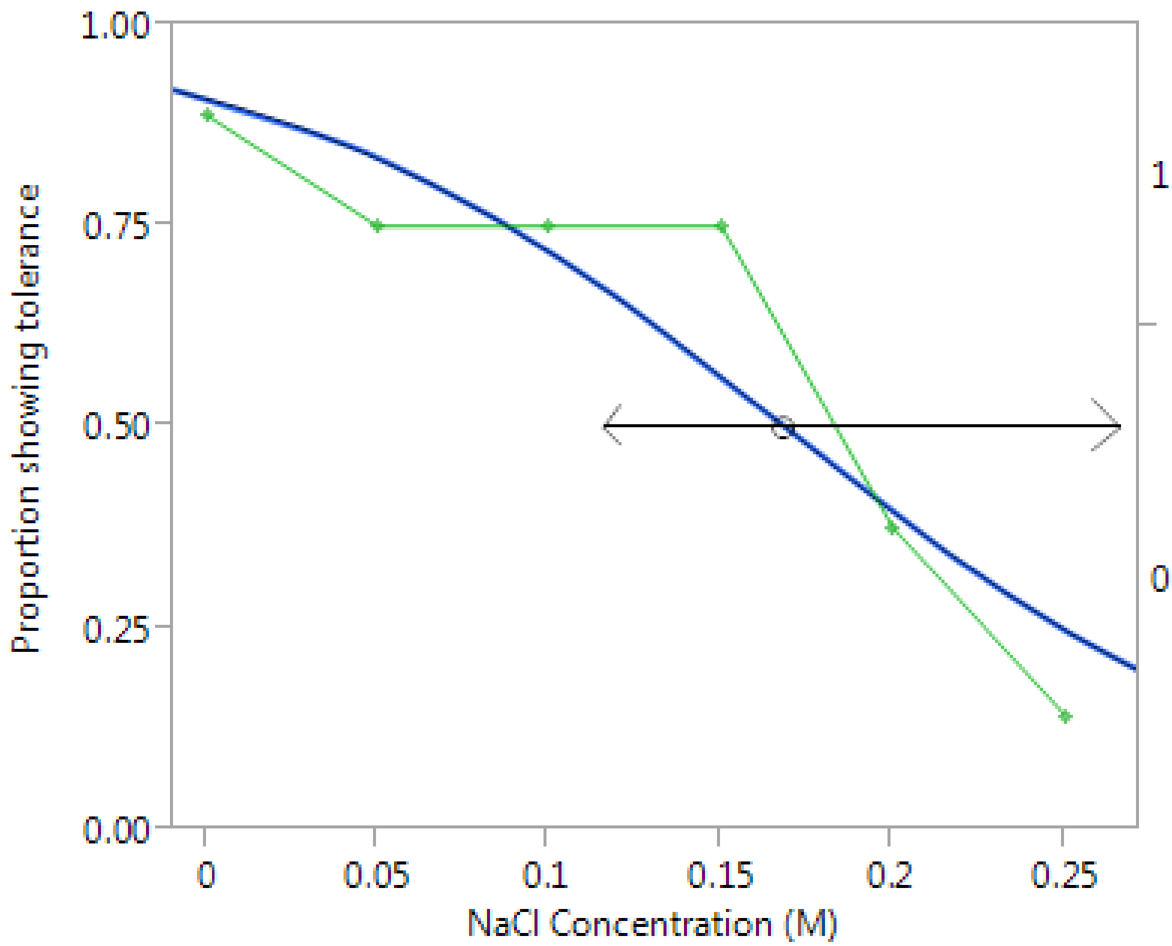

Figure 2. Salinity tolerance of adult wood frogs. Nominal logistic regression curve of the effect of salt concentration on proportion of individuals showing salt tolerance (X^2 = 11.7769; P = 0.0006). EC_{50} (concentration at which 50% of individuals showed aversion) was 0.169 M (95% C.I. 0.116-0.266). An individual was defined as showing tolerance if it spent more than 1414 sec in the test solution (half of the average time for individuals in a 0 M vs. 0 M trial).

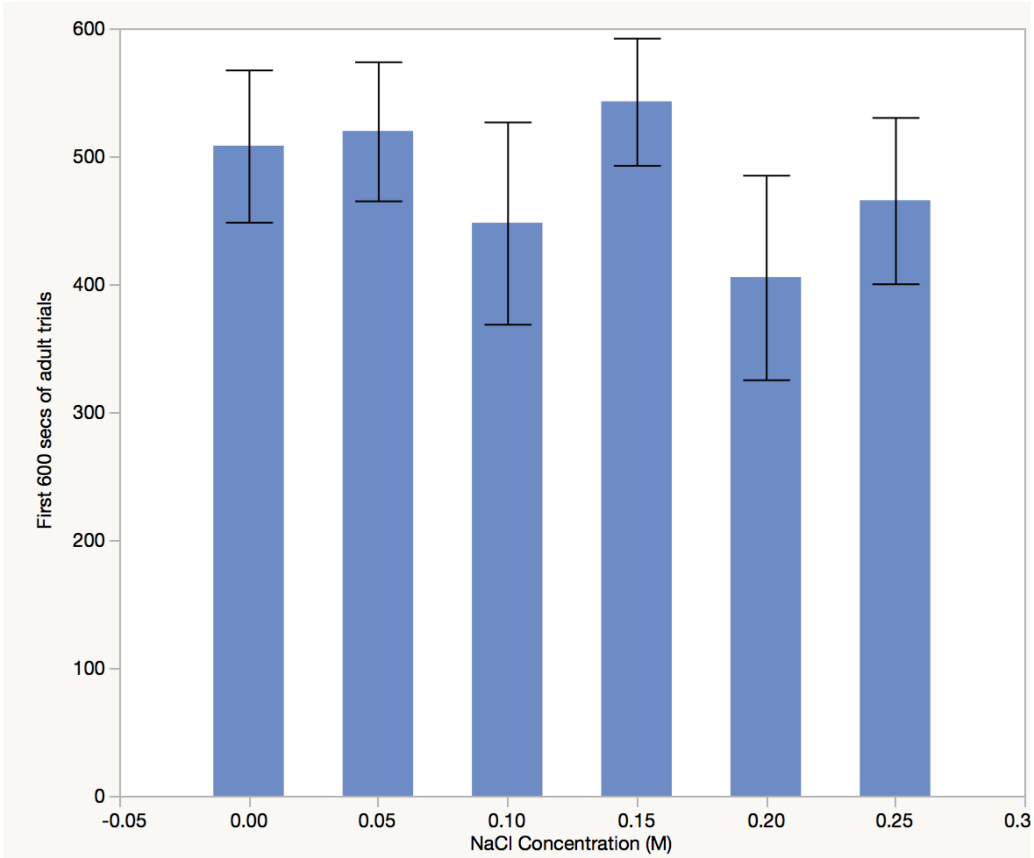

Figure 3. Total time spent by adult wood frogs in each of six NaCl concentrations during binary choice trials for the first 600 sec of 3,600 sec trials (mean ± S.E.). ANOVA: $F_{5,42} = 0.6116$, $P = 0.6915$.

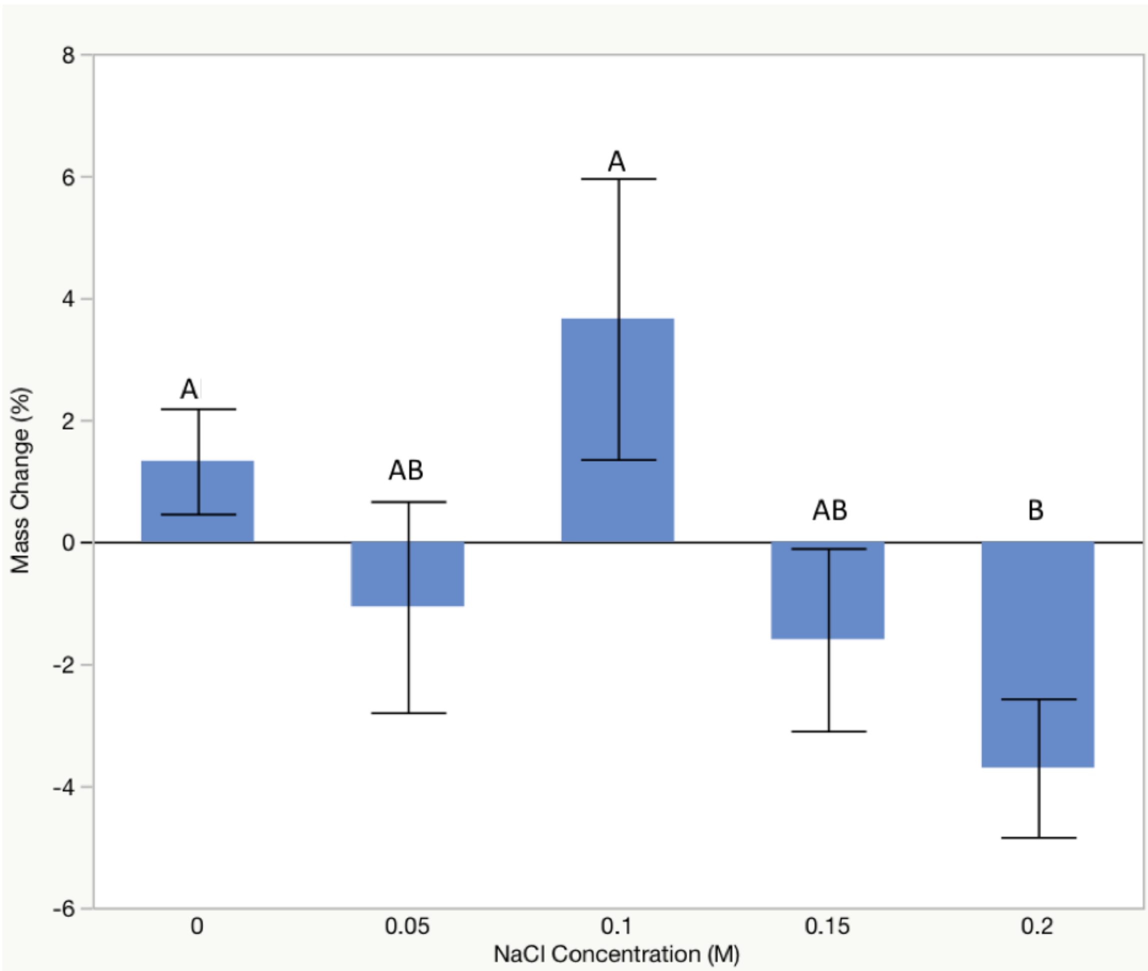

Figure 4. Mass change across five different NaCl concentrations for adult wood frogs (mean ± S.E.). ANOVA: $F_{4,39}$ = 2.8283, P = 0.0375. Mass change that share the same letter did not differ (Student's t-tests). N = 48

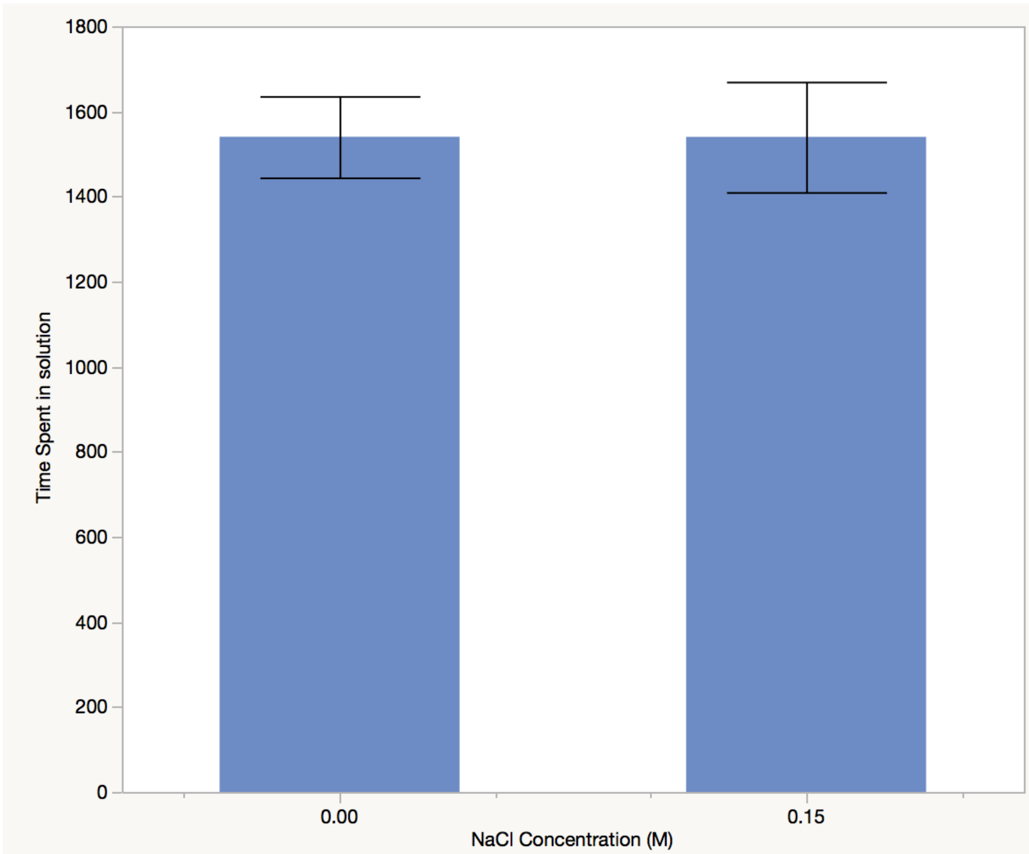

Figure 5. Total time individual tadpoles spent in salt solution for 3,600 sec trials in the first set of trials. t-test: $t = 0.0016$, $P = 0.9987$. N = 50 per trial.

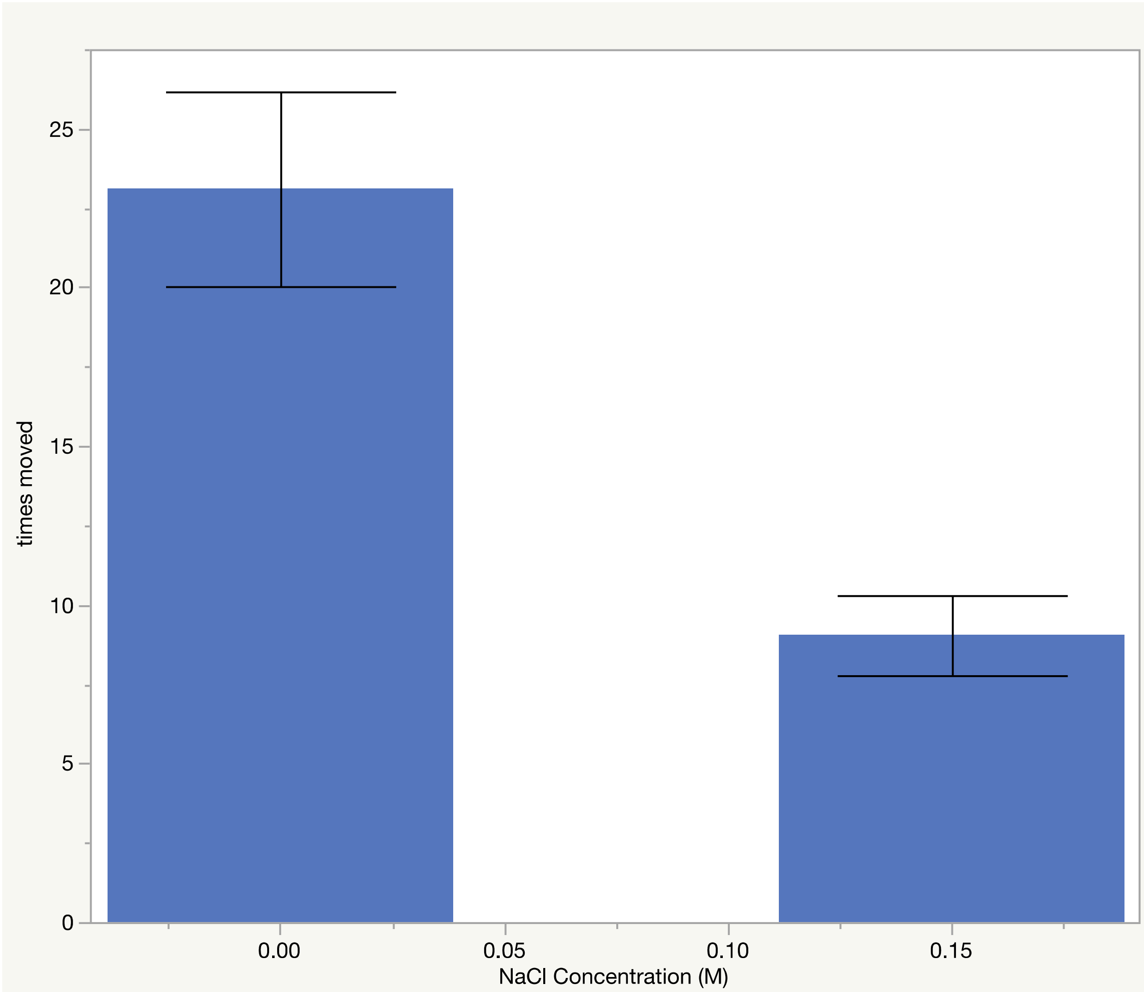

Figure 6. Number of times individual tadpoles moved between salt solution and control for 3,600 sec trials. t-test: $t = 4.2380$, P = <0.0001. N = 50

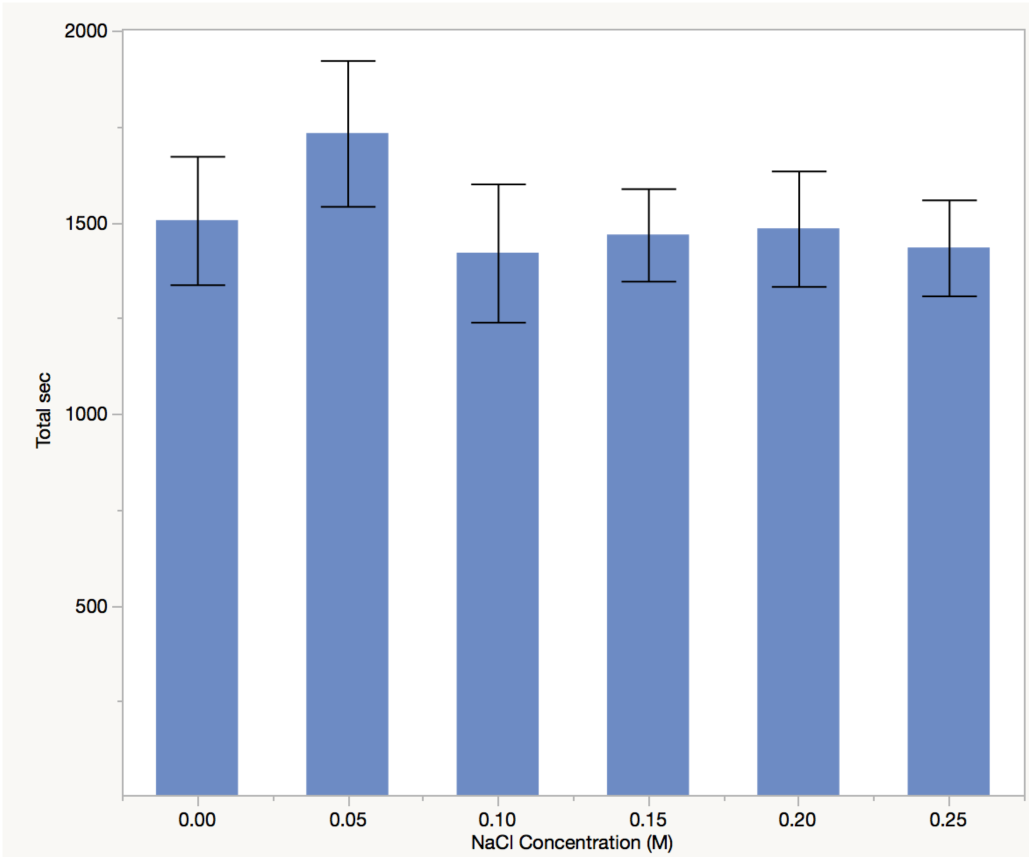

Figure 7. Total time individual tadpoles spent in salt solution for 3,600 sec trials at a broad range of concentrations. ANOVA: $F_{5,114} = 0.5272$, $P = 0.7553$. N = 20

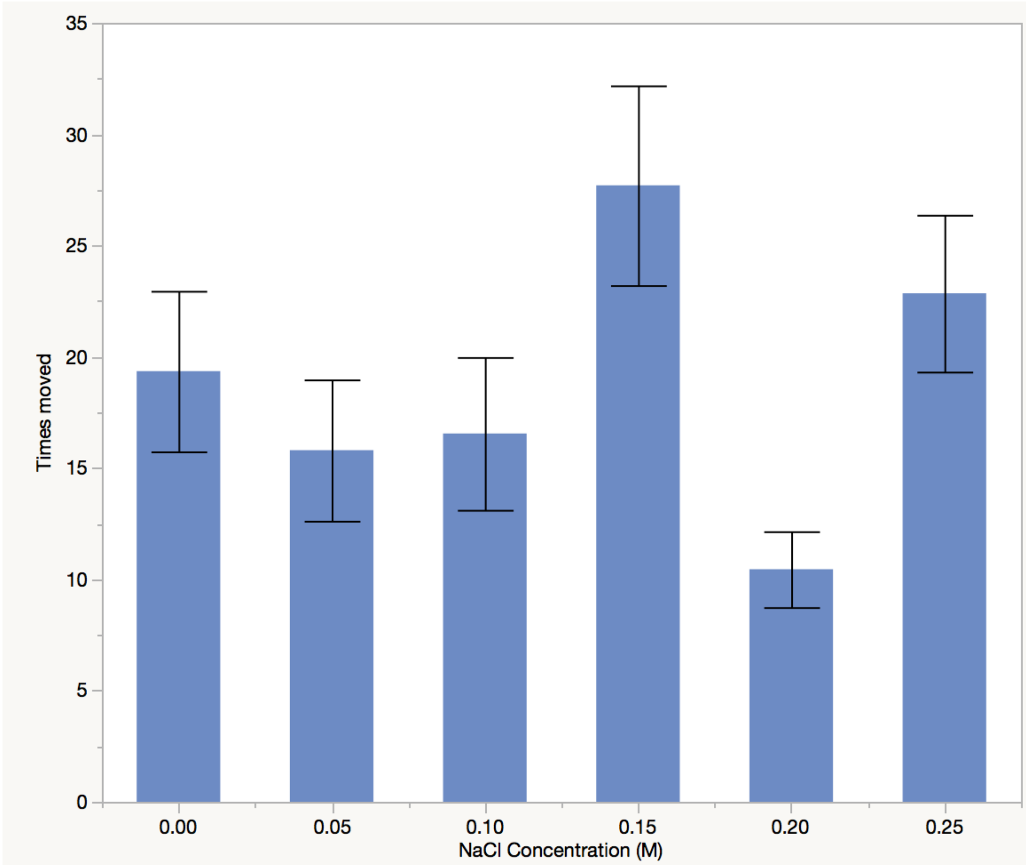

Figure 8. Number of times individual tadpoles moved between solution and control for 3,600 sec trials. ANOVA: $F_{5,114} = 3.0664$, $P = 0.0124$. N = 20 per concentration.

Figure 9. Adult wood frog Binary Choice Trial. 20 L arena with two trays containing 200 mL of either saline or control solution.

Figure 10. Wood frog tadpole choice trial arena. Dual 10 cm petri dishes with center connector.

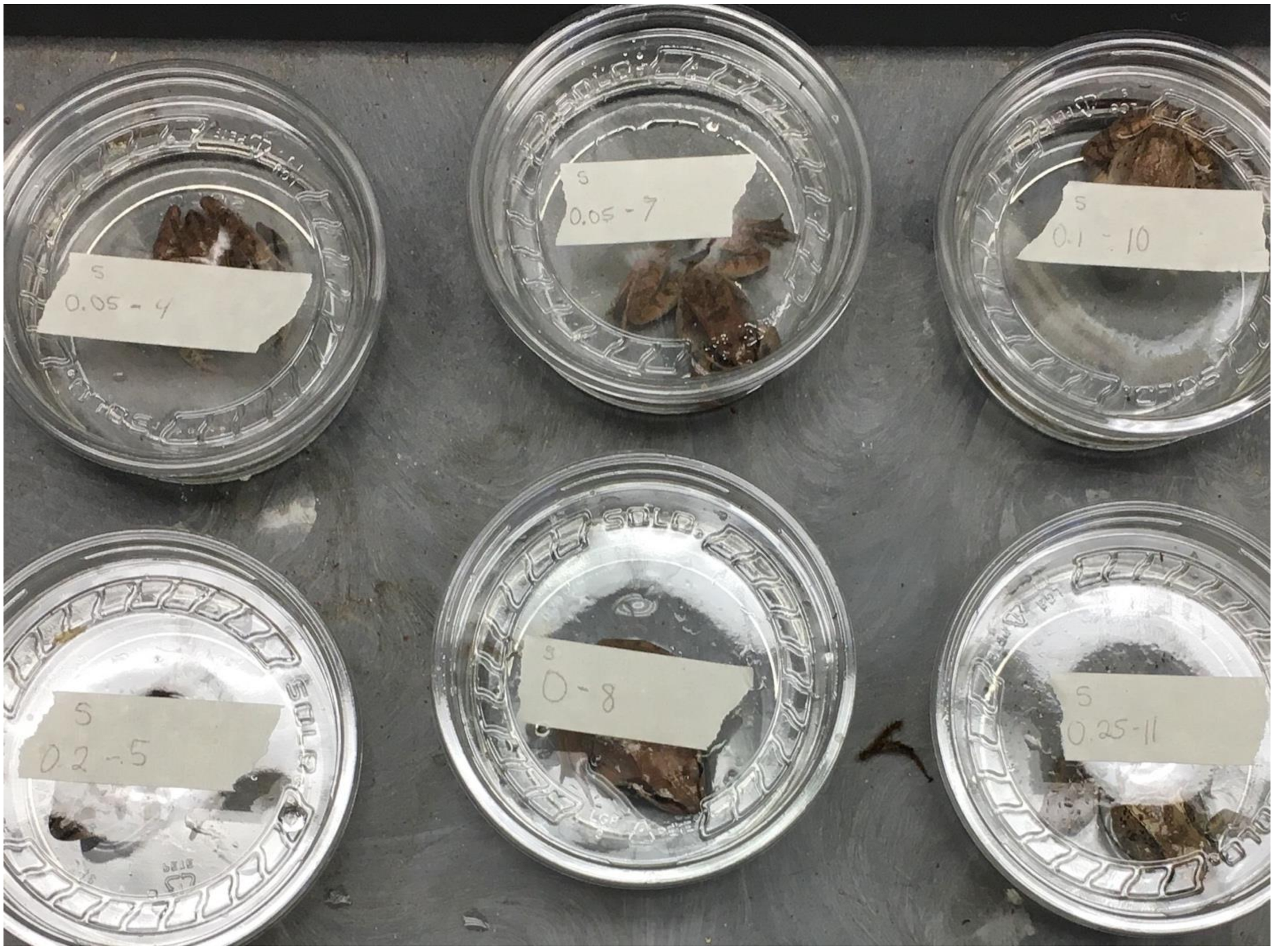

Figure 11. Wood frog mass trial chambers. 500 ml circular container.

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