Comparing Recognition of Predator Kairomones in Vernal Pool and Lake Tadpoles

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Recognition of predators and subsequent response by a prey organism is important for the organism's survival. Predator recognition and learning has been well studied in anuran tadpoles, but the instinctive response without the use of conspecific alarms cues has not been well examined. Two of the more common species of anurans in the Great Lakes region, the *Bufo americanus* and *Rana clamitans,* were used for this experiment. To measure the fear response in tadpoles, I looked at the change in activity level between these two species when three different predator kairomone types (control, vernal pool, and lake) were introduced. The vernal pool predator kairomones used were diving beetles (family: Dytiscidae) and *Ambystoma* salamander tadpole. The lake predator kairomones used were bluegill and smallmouth bass (*Lepomis macrochirus* and *Micropterus dolomieu*). The *Bufo americanus* tadpoles were collected from a small vernal pool while the *Rana clamitans* tadpoles were collected from a lake where fish predators were present. When the kairomones were introduced to tadpoles, the decrease in movement was significantly larger for the lake predator kairomones vs. both the control and vernal pool kairomones. The decrease in activity for the two species for the kairomone types was not significantly different. These results imply the tadpoles respond differently to different types of predators and that *Bufo americanus* tadpoles recognize lake predator kairomones without being pre-exposed to them. These results can help understand predator-prey dynamics in aquatic ecosystems.

Introduction

For a species to survive, they have to be able to recognize predators so they can adequately respond to those predators. Many animals such as mice have instinctive fear responses to the odor of predatory species, an example being bobcats (Kondoh et al. 2016). Recognition of these odors, or chemical cues, the predator produces (kairomones) can produce behavioral or physiological responses in an organism. These responses may help the organism to survive but could be a waste of energy if the predator is not a threat nor present in the environment (Gonzalo et al. 2009). Prey may also need to differentiate between different predators and respond differently to each type of predator (Relyea, 2001). Prey recognition and response to predator kairomones has been studied through the use of anuran tadpoles in the past. In many of these studies they looked at how tadpoles could learn to recognize the chemical cues of predators or even non-predators (Mirza et al. 2006; Gonzalo et al., 2009; Kishida & Nishimura, 2004;

Calsbeek & Kuchta, 2011). Tadpoles often decrease their activity as a defense mechanism and this is used to measure their reaction to the predator kairomones (Gonzalo et al., 2009; Mirza et al., 2006). These studies used tadpole alarm cues along with the predator kairomones instead of studying the instinctive response to the kairomone alone. The alarm cue would be created by having sacrificial tadpoles which would either be blended up or fed directly to the predator (Relyea, 2001; Kishida & Nishimura, 2004; Mirza et al. 2006; Gonzalo et al., 2009).

Tadpoles can live in a variety of habitats, even in the same geographic area, because their eggs can be laid in different bodies of water such as vernal pools and lakes. Different species of tadpoles can often be found in the different bodies of water. Two of the more common species of frogs in the Wisconsin and Upper Peninsula of Michigan are *Bufo americanus* (American toad) and *Rana clamitans* (green frog) (Wisconsin DNR). Both the *Bufo* and *Rana* can lay their eggs in either vernal pools or lakes. *Bufo* tadpoles are much smaller than *Rana* tadpoles, so they are able to live in areas with less water such as small vernal pools. *Rana* tadpoles can sometimes overwinter as tadpoles (Gray et al. 2016), so they often need bodies of water that do not fully freeze in the winter, such as lakes.

These different bodies of water would have different predator composition, specifically fish do not live in vernal ponds because they eventually dry up (Bourgeau-Chavez et al. 2016). Some vernal pool predators include diving beetles (family: Dytiscidae), dragonfly larvae, and *Ambystoma* salamander larvae (Relyea, 2001). Lake predators most often include fish and some common ones in Northern Wisconsin and Upper Peninsula include *Lepomis macrochirus* (bluegill) and *Micropterus dolomieu* (smallmouth bass). Bluegill have been seen to decrease the survivorship of *Bufo americanus* tadpoles (Smith et al. 2016). In one study *Rana clamitans* tadpoles were morphologically different based on the types of predators which dominated the

body of water (Johnson et al. 2015). The tadpoles in the fish-dominated ponds moved faster and had longer tails and shorter bodies than the tadpoles in the invertebrate-dominated pond (Johnson et al. 2015). This study though did not look at how a tadpole would react if it switched ponds. In another study tadpoles were found to either change their behavior or morphology based on either the predator introduced or the species of tadpole (Relyea, 2001). This study shows that tadpoles can differentiate between predator species without having been exposed to direct predation by the predator. This study fed the predators tadpoles, so the reaction could be to the alarm cues created by the tadpoles. This raises the question, if tadpoles would instinctively react to predator kairomones without being exposed to alarm cues or previously exposed to the predator. If the tadpoles do not react instinctively, then young tadpoles would be at a much higher risk for predation until they associate the alarm cue to the predator scent. I hypothesize that if tadpoles from both vernal pools and permanent bodies of water are introduced to kairomones from predators of permanent bodies of water and vernal pools, then they will respond similarly though decreasing their activity.

Materials and Methods

Study Area

This experiment was conducted at the University of Notre Dame Enviromental Research Center (UNDERC), which is located in Vilas County, Wisconsin and Gogebic County of the Upper Peninsula of Michigan. This research facility has many lakes as well as vernal pools which provides a great habitat for many species of larval amphibians.

Experimental Procedure – Collection and Containment

For this experiment, we collected *Bufo americanus* (American toad) tadpoles from a shallow vernal pool in a clearing near the Gravel Pit (Location 1 on Figure 1). *Rana clamitans* (green frog) tadpoles were collected from a pool which is connected to Bay Lake (Location 2 on Figure 1). These tadpoles were separated by species and placed in an aerated clear, plastic, 3.5 gallon aquarium (47.5 cm x 21 cm x 17 cm). The aquarium water was treated tap water kept between 17-23º C and the tadpoles were kept on a photoperiod of around 12 hours of light and 12 hours of dark. The aquarium contained rocks for shelter and also a rectangular piece of Styrofoam so metamorphosized tadpoles would be able to seek refuge. These tadpoles consumed a diet of alfalfa pellets, fish flakes, and floating reptile pellets and fed this diet ad libitum (Mirza et al, 2006). The tadpoles collected were all at or past Gosner stage 25 (Gosner, 1960) and thus were free swimming and feeding. The tadpoles stayed in this aquarium until their trials. After the tadpole finished a trial, it was placed in a different tank to keep it separated from the tadpoles which had not yet undergone trials. After all the tadpoles of one species had finished their trials, they were released back where they were captured.

Experimental Procedure – Kairomone Solution

For this experiment, we created 4 different predator kairomone solutions along with a control solution (treated tap water). Two of the four solutions would be for predators which inhabit vernal pools and the other would be for predators found in permanent bodies of water. The vernal pool predators used were diving beetles (family Dytiscidae) and *Ambystoma* salamander larvae. Two different species of fish (*Lepomis macrochirus* and *Micropterus dolomieu*) were used as the permanent body of water predators. The vernal pool predators were both collected from a vernal pool on UNDERC property and both fish were caught in Bay Lake. To create the kairomone solution each predator was placed in its own aquarium for three days

(Gonzalo et al, 2009). These aquariums were filled with either treated tap or distilled water and aerated. These predators, like the tadpoles, were fed ad libitum. To create similar concentrations of predator kairomones, we placed predators in tanks according to their size. The fish were placed in 5-gallon tanks (51 cm x 26.6 cm x 32 cm) while the diving beetle and salamander tadpole were placed in containers (23 cm x 13.5 cm x 16 cm). After the three days, the water was frozen in the small containers and then eventually separated into 15 ml centrifuge tubes with 10 ml of the predator kairomone in each (Gonzalo et al., 2009).

Experimental Procedure – Trials

To measure tadpoles' reactions to predator kairomones, we monitored their activity, which often decreases as a defense mechanism (Gonzalo et al., 2009; Mirza et al., 2006). Tadpole movement was measured by placing individual tadpoles in a 2 L container (26 cm x 8.5 cm x 17 cm) with treated tap water. Each round of trials used five tadpoles and each tadpole was randomly assigned either the control (treated tap water) or one of the four predator kairomones. The tadpoles acclimated for an hour before the actual trial period began which consisted of a 6-minute pre-stimulus and a 6-minute post stimulus period. During the pre-stimulus period, I measured the time the tadpole spent actively moving. After the 6-minutes elapsed, I added 10 ml of kairomone using a pipette and let the solution flow down the side of the tank so as not to disturb the tadpole (Mirza et al., 2006). After the stimulus was added, I recorded the time spent moving during another 6-minute period. Once the tadpole completed the trial it was placed into another aquarium separate from the other tadpoles. This process was repeated for each of the five tadpoles. For each species this whole process was repeated five times so 25 tadpoles of each species were used in the experiment.

Data Analysis

To compare tadpole response to the predator kairomones, I used the difference between the seconds of activity during post-stimulus and pre-stimulus periods. A positive difference meant that there was increased activity level and a negative difference implied a decrease in activity level when the stimulus was added. I used a 2-way ANOVA using MYSTAT 12 to compare change in tadpole activity across the different species and predator kairomones which were split into three groups (control, vernal pool predators, and lake predators).

Results

A total of 50 trials were run, 25 with *Bufo* and 25 with *Rana.* For the control, ten trials were run, half with *Bufo* and half with *Rana*. For the lake predator kairomones, 20 trials were run, half with *Bufo* and half with *Rana*. For the vernal pond predator kairomones, 20 trials were run, half with *Bufo* and half with *Rana*. The difference between the movement in species trends toward significance with *Bufo* having greater decrease in movement after the stimulus as compared to *Rana* (df = 1, F = 1.065817, p = 0.307533, Figure 2). The difference between kairomone types was significant assuming an alpha level of 0.1 because of small sample size and natural variation (df = 2, F = 2.640081, p = 0.082637, Figure 3). Using a Krusckal-Walis Test, lake kairomones were seen to cause a greater decrease in movement versus the control (df = 1, p = 0.038666, n = 30). Then using a one-way ANOVA, lake kairomones had a greater decrease in activity than the vernal pool kairomones (df = 1, F = 3.662893, p = .063191, n = 40). The interaction between species and kairomone was not significant (df = 2, F = 0.325217, p = 0.724092, Figure 4). A similar two-way ANOVA was also run, but instead of there being three kairomone types the five different kairomones were used (Figure 5). None of the results from this test was significant: difference between kairomones (df = 4, F = 1.585515, p = 0.196890), difference between species

(df = 1, F = 1.250480, p = 0.270132), and the interaction between species and kairomone (df = 4, F = 0.299616, p = 0.876457).

Discussion

The results from the trials disprove my hypothesis that if tadpoles from both vernal pools and permanent bodies of water are introduced to kairomones from predators of permanent bodies of water and vernal pools, then they will respond similarly though decreased activity level. The tadpoles decreased their movement more for the lake predators than for vernal pool predators. The *Bufo* tadpoles were from a shallow vernal pool with no fish, while the *Rana* tadpoles came from the same lake as both of the fish, but both reacted similarly to the lake predator kairomones. Since the *Bufo* reacted similarly to the *Rana*, they must have an instinctive fear response to fish without the need of an alarm cue (*Lepomis* and *Micropterus*). *Lepomis* can significantly impact the survivorship of *Bufo americanus* tadpoles, so being able to recognize this predator would be important for survival (Smith et al. 2016). Toads often prefer to breed in small pools of water where there would be minimal predation especially by fish (Pereyra et al. 2011). The number of shallow pools available each year may change depending on the amount of precipitation, so toads may have to lay their eggs in permanent bodies of water where they would need to be able to recognize fish as predators. Since the *Rana* tadpoles were collected from an area where they were most likely exposed to and possibly preyed upon by the two species of fish, it cannot be determined from this data if they also have an instinctive fear response. For the instinctive fear response to be tested in *Rana*, eggs would need to be collected so they are naïve to the predator scents.

The result of a greater decrease in movement with lake predators versus vernal pool predators does not necessarily mean that the tadpoles do not view the vernal pool predators threat. The lack

of movement may be a better defense mechanism versus fish than diving beetles and salamander larvae. In one study, *Bufo americanus* tadpoles were found not to change their activity in the presence of a predatory newt, but did change their morphology by developing shallower tails (Relyea, 2001). The shallower tail fin is associated with increased development, so the tadpoles may be trying to reach a stage where they are no longer preyed upon by the newt (Relyea, 2002). In the 2001 Relyea study, *Rana clamintans* tadpoles did not change their activity in the presence of a predatory salamander larvae, but they did change their morphology by increasing body width and length. This increase in body size is a better defense for salamander larvae because becoming larger would make them more difficult to eat. Certain fish such as *Lepomis* and *Micropterus* hunt by sight, so decreasing movement would help tadpoles to not be seen by the fish and thus help increase survivorship (Werner & Hall, 1974; Sweka, 1999). *Rana clamitans* tadpoles though do not decrease their movement for all predatory fish such as the mudminnows (Relyea, 2001). This difference between species of fish could be due to the difference in size of the fish. In one study, *Rana clamitans* were found to change their morphology based on whether they were raised in a fish-dominated pond or an invertebrate-dominated pond (Johnson et al. 2015). The tadpoles raised in the fish-dominated pond changed their morphology to be able to swim faster, while those in the invertabrate-dominated pond grew larger in body size. This study again shows that escaping predation is more beneficial against fish than with invertebrates where it is more beneficial to be larger and thus unable to be eaten.

The result that *Bufo* trends toward greater decrease in movement than *Rana* has been observed in other studies. In the 2001 Relyea study, *Bufo americanus* were found to be the most active of the sampled tadpoles, while *Rana clamitans* were the least active. This observance may seem contradictive to my results, but for the trials, the greater movement in the pre-stimulus period,

the greater the difference between the two periods. Also during the trials I noticed the *Bufo* tadpoles would spend more time moving than *Rana* tadpoles. *Bufo* tadpoles may move more because their metamorphosis time is shorter than that of the *Rana*, so they need to gain as much nutrients as possible to grow in a small period of time. The decrease in movement to the stimulus in *Bufo* compared to *Rana* could be due to *Bufo* tadpoles preferring to change their behavior instead of their morphology. Since *Rana* can overwinter as tadpoles and have a longer metamorphosis period, allocating resources to change morphology would be more beneficial in the long run.

By just looking at the mean values in Figure 4, there appears to be differences in change in activity level between species for each kairomone type, but the sample size for each kairomone type (five or ten) is relatively small causing a high standard error. By increasing the sample size, the difference between each tadpole species for each kairomone type may become significant. Also if the sample size is increased the difference between each of the five kairomones may also become significant (Figure 5). Looking at each kairomone by itself would help to see how the tadpoles react to each predator species individually. Something I noticed for the *Bufo* tadpoles during the trial was the observance of two different movement types. One being rapid movement which appeared to be the tadpole trying to escape and the other appeared to be foraging movement. One thing which could be looked at is the effect predator kairomones has on tadpole foraging. Also for this experiment, decreasing activity was used as the indicator of predator recognition, but this may change depending on the predator. Red-eyed tree frogs in the presence of predatory shrimp, increased their activity because being motionless was not advantageous (Warkentin, 1999). Fleeing and hiding could be other defense mechanisms induced by different predator kairomones.

Understanding the predator-prey dynamic of tadpoles and their vast array of predators can help understand more complex predator-prey relationships. Also, further study can continue to look at the genetic component of tadpoles' recognition of predator kairomones and their subsequent response. The response can help to understand how tadpoles value survivorship versus resource allocation. Anuran tadpoles are often near the bottom of the food chain in many fresh water aquatic systems, so better understanding of their relationship with predators can help the overall understanding of the food web.

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Figures

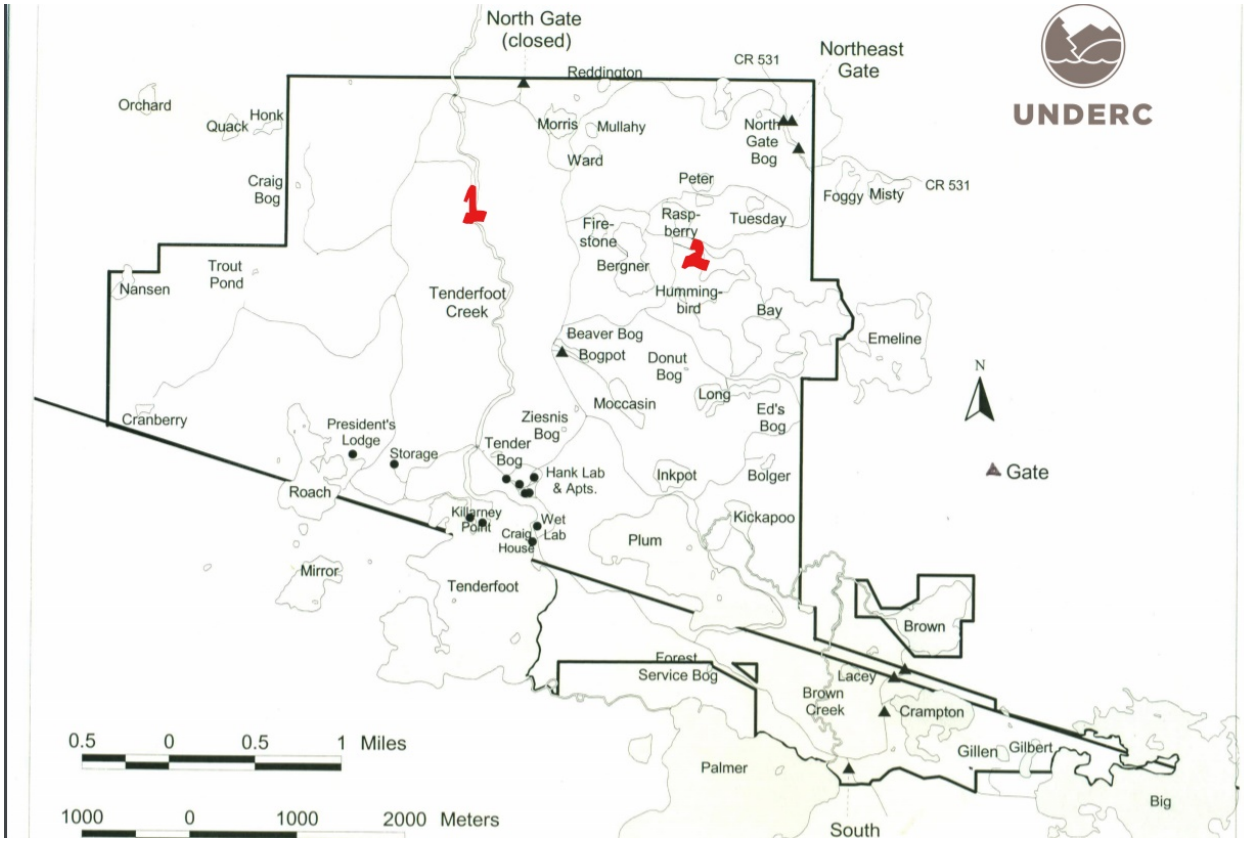

Figure 1. Map of UNDERC property with the location were the tadpoles were collected: 1-*Bufo americanus*, 2-*Rana clamitans*.

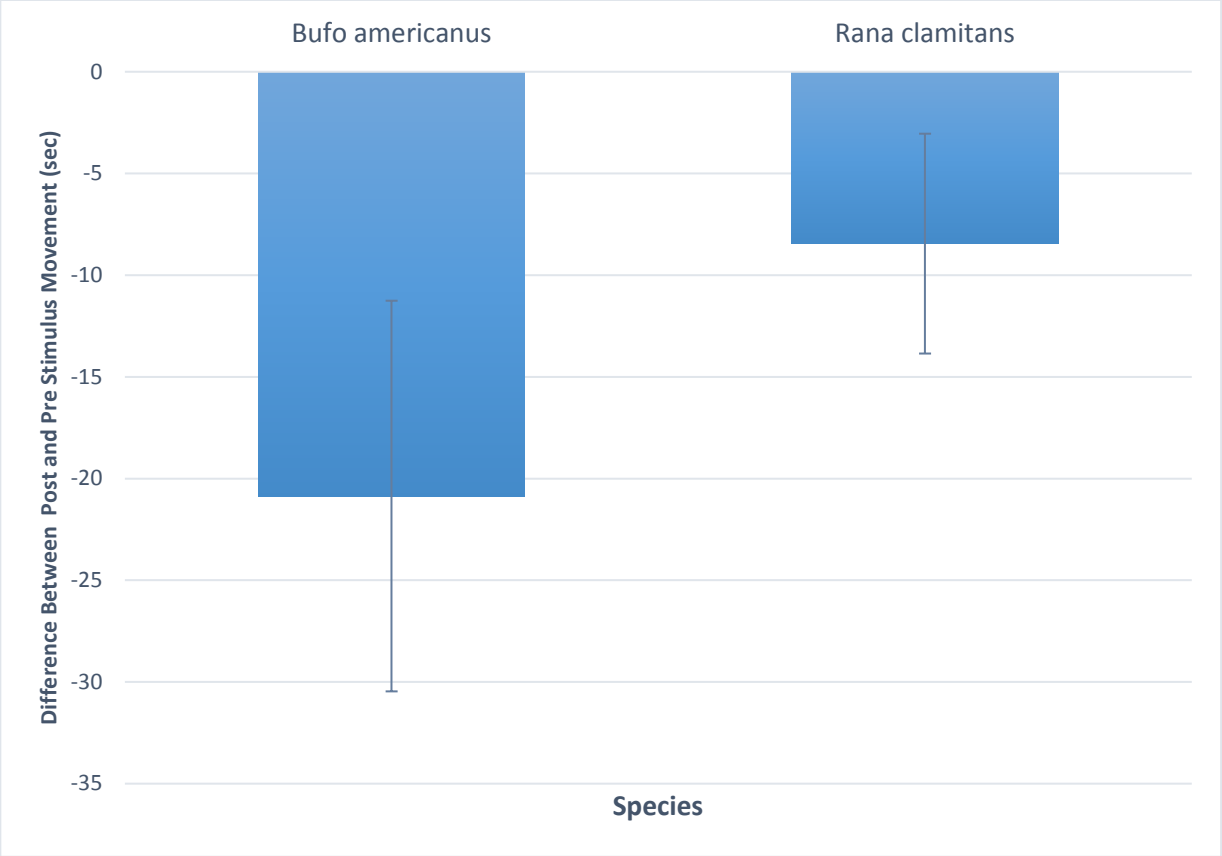

Figure 2. An ANOVA was used to compare the difference between post and pre stimulus movement between *Bufo* and *Rana*, which trends toward significance (df = 1, F = 1.065817, p = 0.307533, n = 50).

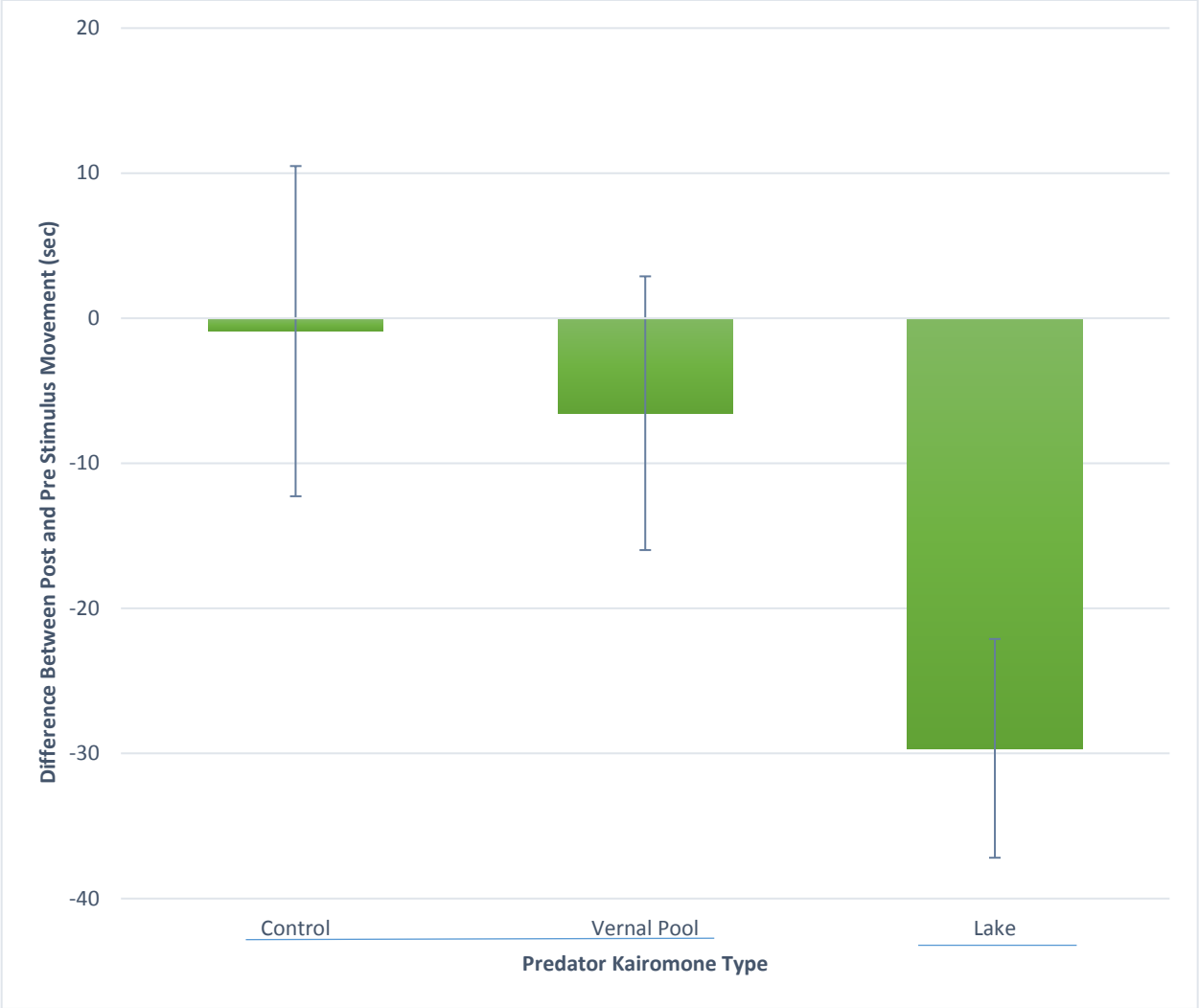

Figure 3. An ANOVA was used to compare the difference between post and pre stimulus movement between the three kairomone types: Control, Vernal Pool, and Lake and was found to be significant (df = 2, F = 2.640081, p = 0.082637, n = 50). A Krusckal-Walis Test was used to compare the Lake kairomone type from the Control and was found to be significantly different (df = 1, p = 0.038666, n = 30). An ANOVA was used to compare the Lake Kairomone type to the Vernal Pool type and was found to be significantly different (df = 1, F = 3.662893, p = .063191, n = 40).

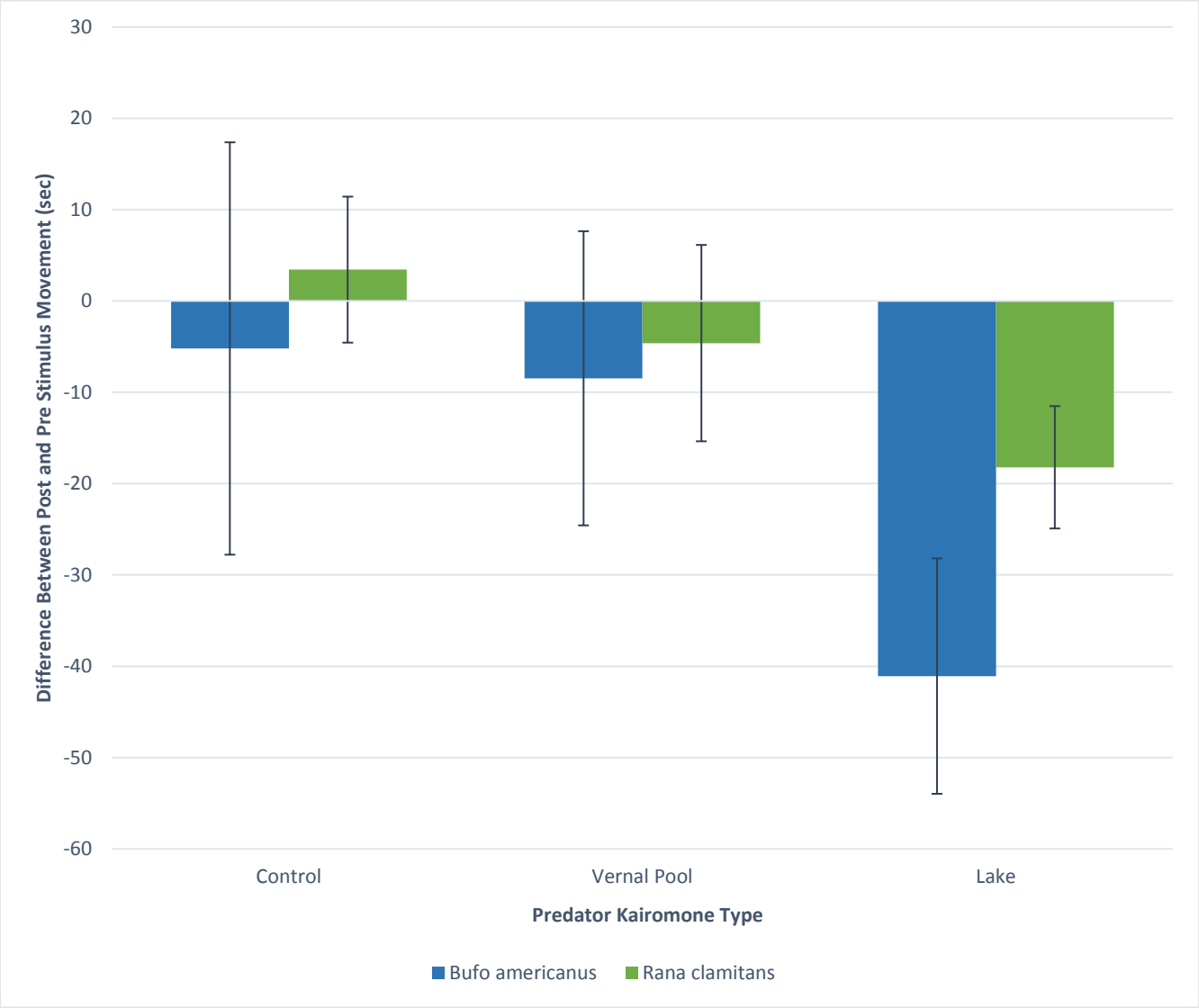

Figure 4. A 2-way ANOVA was used to compare the difference between post and pre stimulus movement between both species and kairomone type, which was found to be not significant (df = 2, F = 0.325217, p = 0.724092, n = 50).

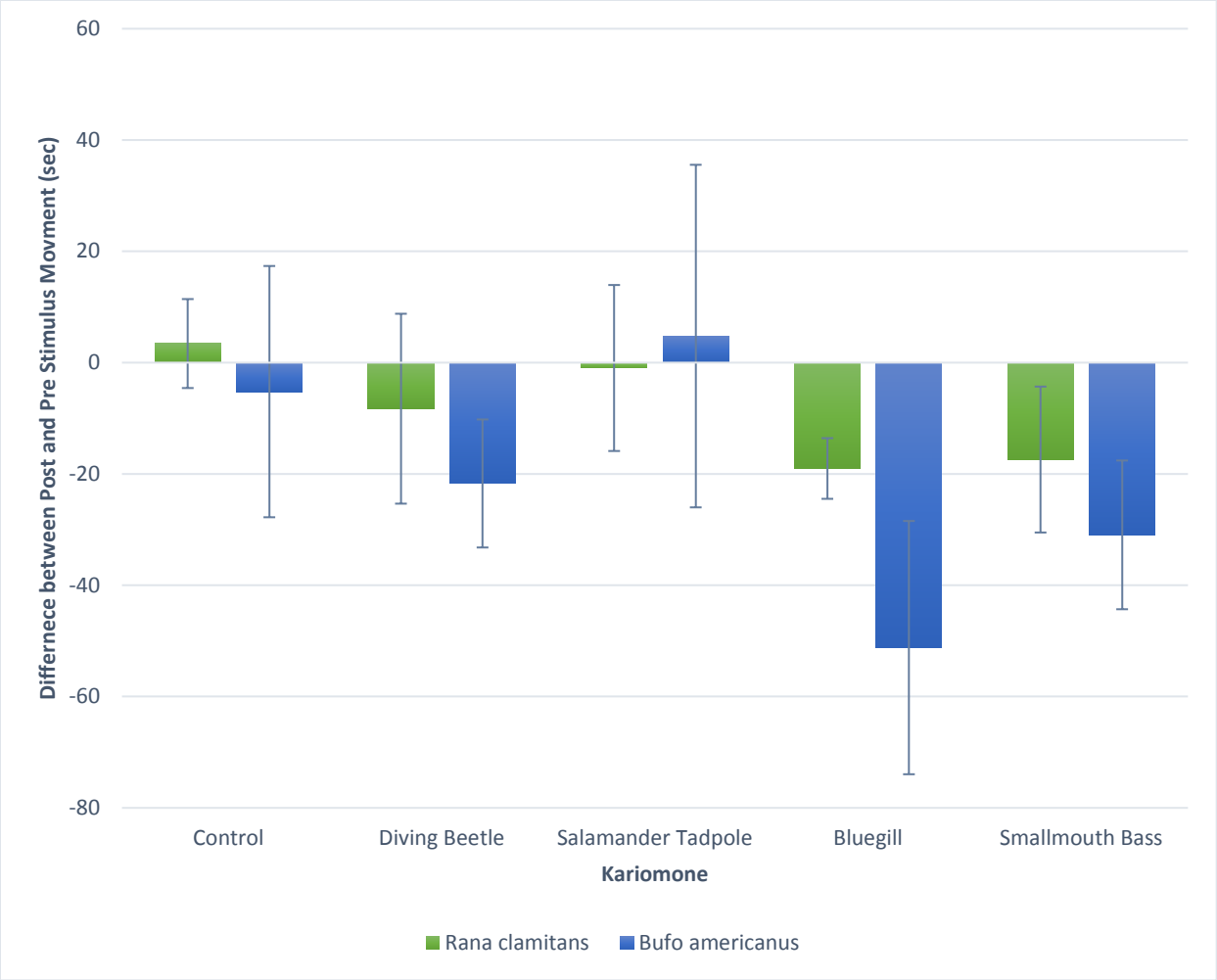

Figure 5. A 2-way ANOVA was used to compare the difference between post and pre stimulus movement between each predator kairomone for both species of tadpoles. None of the results were significant: between tadpole species (df = 1, F = 1.250480, p = 0.270132, n = 50), between predator kairomones (df = 4, F = 1.585515, p = 0.196890, n = 50), or the interaction between tadpole species and predator kairomones (df = 4, F = 0.299616, p = 0.876457, n = 50).