

Investigation of Behavioral Change in Amphibians as a Result of Anthropogenic Disturbance

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Abstract

In a world where amphibians cannot escape the influence of humans, the effects that humans have on amphibians must be understood if the losses in amphibian diversity are to be curtailed. Amphibians are experiencing a worldwide decline for a variety of reasons; some of the biggest factors that contribute to their decline are habitat loss or fragmentation, disease, and climate change. The question under study in this experiment is whether anthropogenic disturbance affects the predatory success rate and chorus behavior of amphibians in the north woods of Michigan. The hypothesis for the analysis of the predatory success rate is that anthropogenic stimulus will reduce the amphibians' predatory success. This study was conducted by capturing amphibians and running experimental trials introducing anthropogenic stimulus in a laboratory setting. No effect was found from either the natural stimulus or the anthropogenic stimulus on amphibians' predatory success. The hypothesis for the analysis of the chorus behavior is that anthropogenic stimulus will stop the chorus and result in an increased time for the chorus to begin again. This study was conducted at night by locating vernal ponds and lakes with robust frog choruses and introducing anthropogenic stimulus to those environments. It was found that anthropogenic stimulus differentially affected amphibians chorusing in vernal ponds and lakes. If anthropogenic disturbance interferes with key aspects of amphibian life such as hunting or chorusing, then humans could be directly contributing to the steep decline of a model organism.

Introduction

The effect of anthropogenic noise on wildlife is not a well-studied phenomenon in most organisms, but amphibians have proven to be a model organism that yields fruitful results for

studies of this disturbance. Anthropogenic disturbance in the form of vehicular interference near amphibian habitat has been shown to negatively affect amphibian species richness and distribution when the road is found within one kilometer of amphibian habitat (Cosentino 2014). Anthropogenic noise also leads to an increase in male frogs' vocalization frequency when anthropogenic disturbance occurs in their habitat, which is energetically taxing for the frogs and can negatively affect their fitness over time (Lukanov et al. 2014). This physiologically-taxing behavior is likely a result of the fact that a frog's vocalizations do not travel as great a distance when anthropogenic interference is present in their habitat, potentially forcing the frog to increase their vocalization frequency in order to achieve successful communication with conspecifics (Parris 2013). Vocalization in amphibians is used by males to advertise mating opportunities to females, maintain territorial boundaries, attract other males to a breeding aggregation, or to facilitate interspecific recognition (Emerson and Boyd 1999). Understanding the greater biological role that anthropogenic noise plays in affecting amphibians, a model organism, could lead to greater cognizance of the effects that humans have on natural populations of wildlife.

This investigation sought to answer the question of whether there is a change in behavior of amphibians in the aftermath of exposure to anthropogenic noise. The two behaviors that were studied were predatory success rate and chorus behavior. Predatory success rate was assessed using two amphibian species, *Lithobates sylvaticus* (Wood Frog) and *Bufo americanus* (American Toad). *L. sylvaticus* prefers moist forest habitat (Harding 1997). Adults can only be found in water during the breeding season (Harding 1997). Subjects may be found in leaf litter or in felled trees (Harding 1997). *L. sylvaticus* is sensitive to habitat fragmentation, especially when the factor affecting their habitat is a road (Homan et al. 2010, Gibbs 1998). Roads

contribute greatly to mortality rates of amphibians, especially on roads that are not frequently traveled (Sutherland et al. 2010, Orłowski 2007). This is due to the fact that roads that do not have high traffic flow have a greater number of amphibians around them (Sutherland et al. 2010). Amphibians have been found to use roads to facilitate sit-and-wait hunting because the insects are easily located on the road (Sutherland et al. 2010). *L. sylvaticus*' adult diet consists mainly of insects, arthropods, and gastropods; but other terrestrial invertebrates may also be consumed (Harding 1997). Wood Frogs aggregate for mating in the spring in a variety of habitats including vernal pools, swamps, and streams (Harding 1997). Their breeding aggregation lasts only for a short time, ranging from less than a week to two weeks (Harding 1997).

B. americanus inhabit prairies, meadows, marshes, agricultural areas, forests (particularly those with canopy gaps), and forest edges (Harding 1997, Guerry and Hunter 2002). *B. americanus* shows affinity for a wider range of habitats when compared to *L. sylvaticus*, and a comparatively greater tolerance for edge habitats (Homan et al. 2010). *B. americanus* is extremely tolerant, thriving on a wide variety of food sources and habitats, even in the presence of human stressors (Swihart et al. 2003). A study conducted on various amphibian and rodent species in Indiana documenting their response to habitat loss and fragmentation found *B. americanus* to be one of the species under study with higher niche scores, meaning this species displays a pronounced ability to fill a multitude of niches (Swihart et al. 2003). Their diet mainly consists of a variety of insects, arachnids, centipedes, millipedes, snails, slugs, and earthworms (Harding 1997). Mating occurs in flooded fields, ditches, ponds, marshes, and slow-moving streams (Harding 1997). Breeding aggregations lasts for approximately ten to fourteen

days beginning in late April or late May, depending on temperature conditions in the area (Harding 1997).

The second behavior under study, chorus behavior, was assessed on an observational basis in the field. *Lithobates taipehensis* (Taipei tree frog) increased call frequency immediately after anthropogenic disturbance (airplane and motorcycle noise) was introduced (Sun and Narins 2005). Researchers hypothesized this increase in calling may be due to the fact that background noise of other species in the area is typically reduced after disturbance; therefore allowing *L. taipehensis*' calls to be heard by conspecifics with less auditory interference (Sun and Narins 2005). Increasing call frequency in the aftermath of anthropogenic disturbance will deplete the energy reserves of the amphibian and require it to forage more frequently as a result (Kaiser et al. 2010). Increased foraging activity removes the individual amphibian from the chorus (Kaiser et al. 2010) and could reduce mating opportunities for that individual.

The study system being used to investigate this behavioral question consists of both field observation and laboratory experimentation. In the field, *L. clamitans*, *P. crucifer*, *H. versicolor*, and *L. catesbeianus* choruses were observed in vernal ponds and lakes. Field observations focused on observation of chorus behavior in response to natural and anthropogenic stimulus. In the laboratory, specimens of *L. sylvaticus* and *B. americanus* were exposed to natural stimulus (leaf rustling) and anthropogenic noise (recording of vehicular noise and horn honking) and the variables of focus were predatory success rate and latency time prior to foraging. Predatory success rate was quantified as the number of worms consumed in the time given. This study system is ideal because it gives consideration to the fact that the frogs may behave differently under the stressful conditions of being confined in the laboratory. The field observations provide an opportunity to observe the frogs in an unaltered environment in order to observe behavioral

response to anthropogenic stimulus. The specific hypothesis being tested is when individuals of the species *L. sylvaticus* and *B. americanus* are exposed to anthropogenic noise, then these individuals will exhibit reduced predatory success in the form of fewer worms consumed in the time given with an increase in latency time prior to successful consumption of prey and a cessation of chorus vocalization with an increased time to rejoin the chorus.

Methods

Laboratory Experimentation

Prey (wax worms) for the subjects were acquired from bait shops in order to ensure all of the prey was the same species. Each subject was given five wax worms per treatment. The worms had to be alive in order to test the predatory success rate of the amphibian specimens as amphibians prefer prey that is mobile. Fifteen *L. sylvaticus* and sixteen *B. americanus* were tested. The specimens were housed with similarly sized individuals of the same species in order to prevent mortality of smaller individuals. Subjects were temporarily housed in small carriers with clear sides and aerated lids. The carriers were kept near a window in order to ensure the subjects continued with the day and night duration times that they are accustomed to. All specimens were caught by hand and released within forty-eight hours. A site was only used for amphibian collection once, because subjects were never tagged prior to release. After a period of acclimation that was at least twenty-four hours, the subjects were transferred to a larger clear aquarium (43 x 22 x 20 cm) for the trials. Each trial was conducted with only one subject in the tank at a time. Each subject was administered both treatments; each treatment administration lasted 10 seconds. The frog was placed into the aquarium with no substrate present on the floor of the aquarium. This was done in order to prevent the subject from hiding under leaf litter or

other natural debris that may be present. There was an acclimation period lasting five minutes in order to allow the subject to acclimate to the aquarium. At the conclusion of the acclimation period, five wax worms were disbursed on the floor of the aquarium. A random number generator was used to determine which trial would be administered to the subject first. An even number meant that the control treatment occurred first, whereas an odd number signified the anthropogenic treatment would be administered first. The stimulus was administered immediately after the addition of the worms. The control treatment was ten seconds of leaf litter rustling by hand. The anthropogenic treatment was ten seconds of a recording of a vehicle with the engine running and the horn honking intermittently. The subject was then observed for ten minutes. Trials were recorded in order to facilitate behavior scoring after data collection. The number of worms consumed was noted during this observation period.

Behavior was scored using *JWatcher* (Blumstein and Daniel 2007). The behaviors that were scored included eating, motionless sitting, moving, looking at a worm, looking outside of the aquarium, and being out of sight. Though there was no substrate present on the bottom of the aquarium, an amphibian could achieve being out of sight by sitting in certain corners of the tank or by being in line with the top of the tank closest to the camera.

Statistical analysis of the laboratory portion was carried out using two paired t-tests, a Shapiro-Wilk test, and a one-way analysis of variance (ANOVA). For the paired t-test carried out on wood frog feeding data, the variables were the number of feeding occurrences in the control trials and the number of feeding occurrences in the anthropogenic trials. The paired t-test carried out on American toad feeding data compared the number of feeding occurrences in the control trials and the number of feeding occurrences in the anthropogenic trials. For the one-way ANOVA, the independent variable was species. The dependent variable was the differences in

the latency time (control subtracted from anthropogenic). There were a total of eight subjects that had latency values for each trial type, four toads and four frogs.

Field Observation

Vernal ponds and lakes around the University of Notre Dame Environmental Research Center (UNDERC) property were chosen based on their proximity to roads. If the vehicle could be parked alongside the vernal pond or lake, it was included in the study. Upon arrival to the study site, the vehicle was turned off and experimenters were silent for five minutes. This allowed the chorus to begin again, if the arrival of the vehicle had caused it to cease. During this acclimation period, conditions such as temperature and weather were noted. After the acclimation period, the treatments were administered. The order of the treatment administration was determined using a random number generator. If the number generated for that site was even, the first treatment administered was the control treatment. If the number generated for that site was odd, the first treatment administered was the anthropogenic treatment. The control treatment consisted of natural disturbance; such as leaf rustling, branch beating, or noisy disturbance of vegetation present on the ground. The anthropogenic treatment consisted of the vehicle engine being activated and the horn being honked intermittently. Both treatments were administered for five seconds. After the introduction of the stimulus, the time that passed before the chorus began again was noted for each species present; as each amphibian species rejoined the chorus, the time elapsed since the end of the treatment stimulus was noted. This procedure was carried out in twenty-five habitats; thirteen vernal ponds and twelve lakes. Data were analyzed using a two-way ANOVA test. The independent variables were habitats (lakes versus vernal ponds) and treatments (control and anthropogenic), and time to rejoin the chorus (in seconds) was the dependent variable.

Results

Predatory Success

The one-way ANOVA test did not find the differences in latency data to be statistically significant ($F_{1,6} = 4.14$, $P = 0.08$). This result supports the null hypothesis and rejects the alternative hypothesis. Analysis of the latency period for each species revealed American toads to have a lower average latency period than wood frogs (Figure 1). American toads consumed more worms on average during the control treatment, but this was not statistically significant ($t_{14} = -1.52$, $P = 0.15$; Figure 2). Wood frogs consumed the same average amount of worms regardless of the treatment presented ($t_{14} = 0.22$, $P = 0.83$; Figure 3). Overall, the data set was found to be highly non-normal by the Shapiro-Wilk test ($W = 0.7930$, $P = 0.0029$).

Chorus Behavior

Analysis of the relationship between the habitats, treatments, and time to rejoin the chorus found habitats to be a statistically significant variable ($F_{1,108} = 3.83$, $P = 0.05$), which supports the alternative hypothesis and rejects the null hypothesis. This indicates there is a different response to the treatments depending on which habitat they are administered in. The effect from the treatments was not statistically significant ($F_{1,108} = 0.98$, $P = 0.32$). No statistically significant effect was found for the interaction between habitats and treatments ($F_{1,108} = 0.09$, $P = 0.75$). These results support the null hypothesis. In both vernal ponds and lakes, the control treatment led to a longer time to rejoin the chorus than the anthropogenic treatment (Figure 4). The control treatment at vernal ponds led to the greatest time to rejoin the chorus, an average of 70.8 seconds ($SE = 19.13$, Figure 4).

Discussion

This study addressed whether anthropogenic stimulus affects predatory and chorusing behavior of amphibians. The hypothesis for the predatory success study was that the anthropogenic stimulus would negatively affect the amphibians' predatory success. There was no significant relationship between administration of either of the treatments and the amphibians' predatory success. There are different explanations for why each species did not show any change as a result of the administration of the treatments.

No studies could be found where *B. americanus*' behavior is altered as a direct result of the introduction of anthropogenic noise. One possible explanation for why the American toads in this study displayed a statistically not significant but still noticeable response to the anthropogenic noise could be due to the fact that the amphibians on the UNDERC property are not used to high densities of vehicles in their habitats (Figure 2). Most studies that are conducted on the effects of traffic noise on amphibians are conducted on habitats near highways with, for example, ~18300 vehicles per day driving past the habitat (Vargas-Salinas et al. 2014). It is possible that the toads were displaying a response to the anthropogenic noise as a result of not being accustomed to frequent vehicular disruption in their environment. Although the American toads displayed a response to the anthropogenic noise, they consumed more worms on average than the wood frogs. This could be due to the fact that the toads have less strict habitat requirements than wood frogs and, as a result, were less stressed in the aquarium environment. American toads are habitat generalists, which may convey an increased capability for handling human-induced change (Swihart et al. 2003). The first seven trials of the experiment were conducted with leaf litter on the bottom of the aquarium in order to reduce stress to the animals. The leaf litter was eliminated after those trials because many of the wood frogs began hiding

underneath the leaves for significant portions of the trial. This observation supports the idea that the wood frogs were uncomfortable.

In contrast to *B. americanus*, *L. sylvaticus* is extremely restricted in its preferred habitat range. Habitat types include lakes, ponds, and wetlands for breeding, and grasslands or woodlands for terrestrial life as adults (Reeves 2006). Given that wood frogs are stricter in their habitat requirements, it could be that the wood frogs were too stressed in the lab environment to behave normally. Wood frogs displayed lower average worm consumption than American toads (Figure 3). There were instances in the video recordings where wood frogs were visually stressed, jumping erratically around the tank and then sitting still for long periods of time. This behavior puts the camouflage of the wood frog's markings to use, when used in the wild, as it can cause a predator to be unable to relocate the frog after the erratic movement (Harding 1997). This behavior suggests that the frogs were not comfortable in the environment, despite the acclimation periods, and could explain why they did not consume as many worms as the toads. Wood frogs were likely being more vigilant and searching for a potential predator, rather than searching for their next meal.

L. sylvaticus displayed no response to either treatment; subjects consumed the same average number of worms for the control and the anthropogenic treatment. It is possible that the wood frogs did not respond to the control treatment (leaf rustling) because the carriers that the amphibians were housed in prior trial administration were kept near to the experimental area. The frogs could have become accustomed not to the order of the trials, as that was randomized, but to the fact that the treatments happened on a regular basis. Amphibians can become desensitized to stimulus that may signal a predator if it is repetitively presented without ill effects for the frog (Mira et al. 1993). Similar to *B. americanus*, it is hypothesized that the reason the

wood frogs did not show any effects as a result of exposure to the anthropogenic treatment is due to the fact that amphibians have been found to not uniformly respond to the presentation of anthropogenic stimulus (Price et al. 2007).

Although neither species was found to be significantly responsive to the introduction of the stimulus, there were differences in the average number of worms consumed for each species. For toads, the average number of worms consumed during the control trials was 1.25 worms (SE = 0.38); for the anthropogenic treatment the number consumed was 1.19 (SE = 0.41, Figure 2). For wood frogs, the average number of worms consumed during the control treatment was 0.8 worms (SE = 0.29); for the anthropogenic treatment the number was also 0.8 (SE = 0.28, Figure 3). These differences in average worm consumption are logical given that wood frogs are more sensitive than American toads to habitat change. Being captured and moved into a new environment likely induced more stress on the wood frogs and, as a result, they ate less worms overall. In the case of the toad subjects, stress was likely not as attributable to the environment as much as it was to the presence of the anthropogenic treatment. These different responses to the environment change and the anthropogenic stimulus indicate differences in importance of these stressors for these species; wood frogs appear more sensitive to environment change, while American toads appear more sensitive to anthropogenic disturbance.

In trying to elucidate whether anthropogenic noise has an effect on the chorus behavior of amphibians, it was noted that the only variable that responded significantly to the different treatment types was habitats (vernal ponds versus lakes). Choruses at vernal ponds took a longer time span for all species to rejoin the chorus. This is likely due to the fact that vernal ponds are extremely sensitive, ephemeral habitats. Vernal ponds are characterized by having surface water isolation, periodic drying, small size, shallow depth, and ability to support a distinct biological

community (Brown and Jung 2005). The species supported by vernal ponds are typically species that are easily preyed upon in more permanent waters, so they seek refuge in water that is temporary and lacks predators (Brown and Jung 2005). Vernal ponds and the species contained within them are sensitive to human disturbance, because these species typically spend the majority of their lives in a 1000 feet radius from the pools (Brown and Jung 2005). The habitat surrounding vernal ponds could be used as a means of hiding during a potential predatory approach. Areas around vernal ponds typically feature an abundance of coarse woody material, leaf litter, and 50% canopy cover (Calhoun et al. 2014). These surrounding forest features provide ideal habitat for amphibians to temporarily leave the edge of the pool in the event that they are presented with a potentially threatening stimulus, and hide in the adjacent habitat until they no longer feel threatened. The forest structure surrounding lakes usually does not feature such a dense cover of leaf litter, as the types of trees that promote dense leaf litter, such as sugar maple, need deep moist soils in order to experience optimal growth. When the water table is too near to the surface, sugar maples can experience stunted growth and can easily be knocked over by wind (McIsaac 2012). The pervasive presence of herbaceous plants around lakes may not serve as a complement to the frog's attempts at using cryptic coloration to hide as effectively as leaf litter does. Around lakes, amphibians may flee to avoid predation instead of sitting still.

Amphibians that live on lakes have the capability to thrive in an environment with large predators present. It is possible that the amphibians in lakes did not take as long to rejoin the chorus because they are accustomed and adapted to living in a habitat where the threat of predation is high. This assumes that the treatment noises will make the amphibians feel threatened or in danger.

One idea that could be tested in further studies of the effects of anthropogenic noise on amphibians could look into the potential that amphibians in lakes can hear predators approaching from farther away due to the fact that sound travels across water easily. This may lead amphibians in lakes to be less sensitive to auditory cues that could potentially signal an incoming predator.

Amphibians are strong model organisms for learning about systems such as the circulatory system or processes like metamorphosis, but a method for using them as ecological health indicators has yet to be discovered. The variance in response to anthropogenic stimulus between species is great and could fluctuate from individual to individual depending on how easily threatened the individual is. Factors such as how frequently that particular amphibian encounters humans or predators could lead to the individual having a higher or lower tolerance for auditory cues in the immediate environment. Amphibian populations are declining quickly worldwide, whether that decline is occurring as a result of effects from anthropogenic noise disturbance is inconclusive given the fact that not many studies have researched the effects of anthropogenic noise on amphibian populations.

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Figures

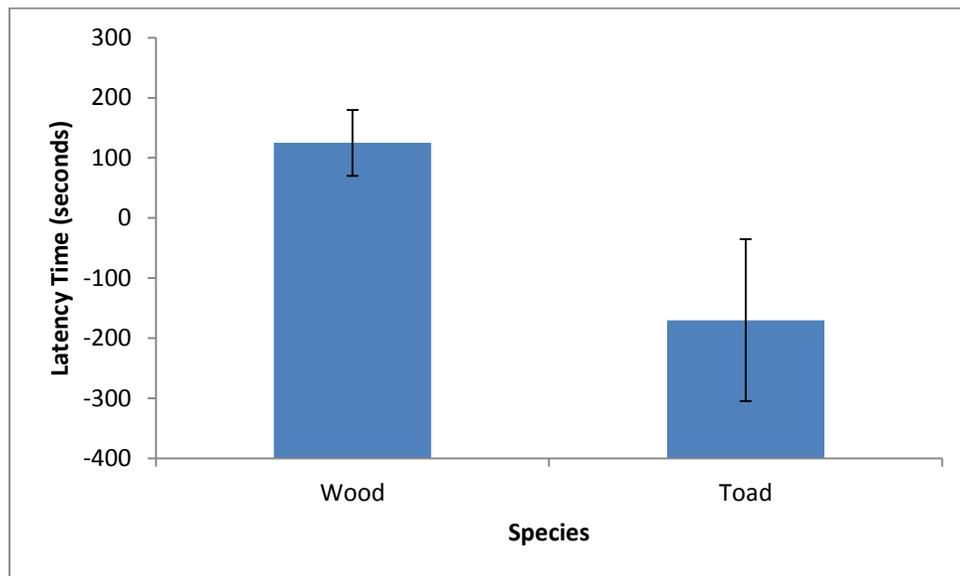


Figure 1: The relationship between species and average latency time. Latency time was calculated by subtracting the control latency time from the anthropogenic latency time for four different frogs and four different toads. A one-way ANOVA was run on the species and the latency time differences. The data was found to be statistically insignificant, supporting the null hypothesis that the latency time will not be changed as a result of exposure to the treatments ($F_{1,6} = 4.14, P = 0.08$). Toads have an average latency time of -170 seconds (SE = 134.60). Wood frogs have an average latency time of 125 seconds (SE = 54.83).

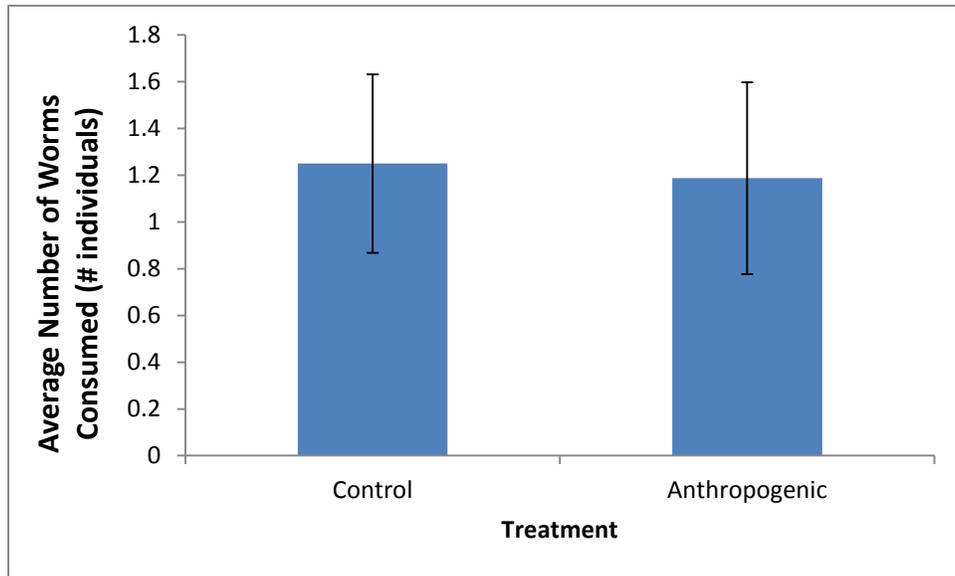


Figure 2: The relationship between treatment type and the average number of worms consumed by *Bufo americanus*. The average number of worms consumed during control trials was 1.25 (SE = 0.38). During anthropogenic trials the average number of worms consumed was 1.19 (SE = 0.41). Running a paired t-test on this data revealed the data to be statistically insignificant, supporting the null hypothesis that the anthropogenic treatment would not affect the predatory success rate of the amphibians ($t_{14} = -1.52$, $P = 0.15$).

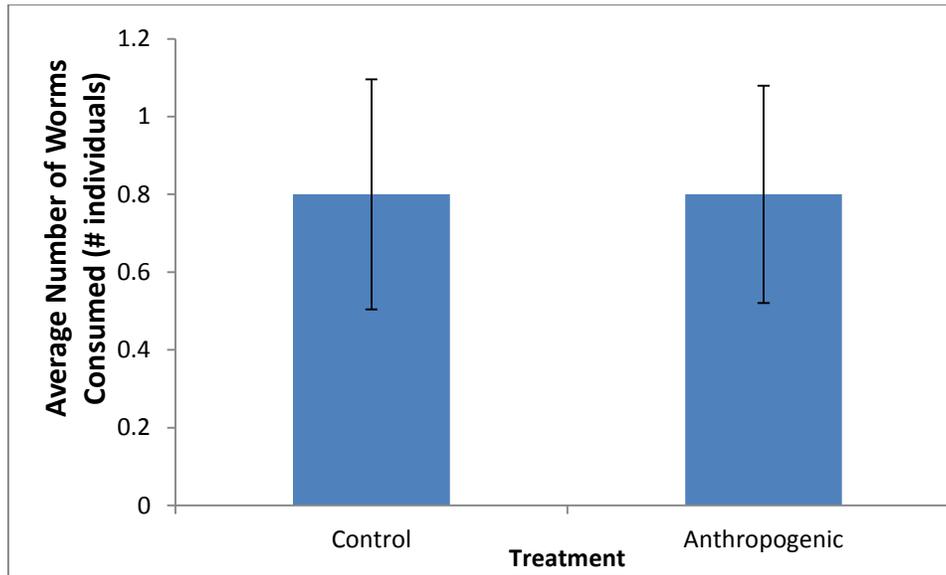


Figure 3: The relationship between treatment type and the average number of worms consumed by *Rana sylvaticus*. The average number of worms consumed during control trials was 0.8 (SE = 0.29). The average number of worms consumed during anthropogenic trials was 0.8 (SE = 0.28). Running a paired t-test on this data revealed the data to be statistically insignificant, supporting the null hypothesis that the anthropogenic treatment would not affect the predatory success rate of the amphibians ($t_{14} = 0.22$, $P = 0.83$).

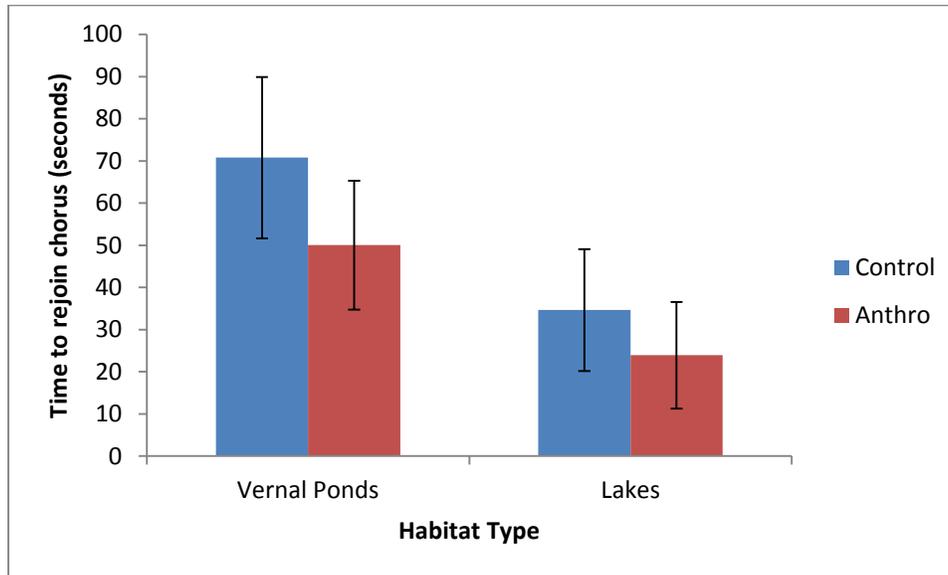


Figure 4: The relationship between habitat and the time it took the chorus to return after each treatment. Habitat was a significant variable for the chorus behavior experiment, indicating that the anthropogenic stimulus exerts an effect on the habitats ($F_{1, 108} = 3.83$, $P = 0.05$). At vernal ponds, the chorus took a longer time, on average, to return to pre-stimulus levels when compared to lakes. The chorus took 70.8 seconds ($SE = 19.13$) to recover after control treatments and 50.03 seconds ($SE = 15.26$) to recover from anthropogenic stimulus at vernal ponds. At lakes, the chorus took a longer time, on average, to return to pre-stimulus levels after the control treatment. The chorus recovery time from the control treatment was 34.65 seconds ($SE = 14.44$) at lakes, for the anthropogenic treatment it was 23.92 seconds ($SE = 12.64$).