Effects of water browning and visual predator cues on *Rana (Lithobates) clamitans* and *Rana (Lithobates) catesbiena* foraging behavior

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Abstract

Increased amounts of terrestrially derived dissolved organic carbon (DOC) are not only changing the color of waterways, but also the physical properties of freshwater habitats throughout the northeastern United States. These changes could be affecting the biodiversity, survivability, distribution, and foraging behavior of animals which inhabit these ecosystems. We investigated the effects of increased DOC levels on the foraging behavior of *L. clamitans* and *L. catesbeiana* tadpoles in the presence and absence of predators to evaluate not only the effects of water transparency on their foraging behavior, but also if visual predator cues affect foraging. To do so, we collected tadpoles of both species from lakes in the Upper Peninsula of Michigan and ran lab trials in aquariums filled with water prepared to different DOC levels with and without visual predator cues. Our results showed that the visual presence of predators resulted in a significant increase in *L. clamitans* tadpole foraging in both high and low DOC treatments. Also, our results showed that *L. clamitans* showed a significant preference for foraging at the surface in the absence of predators and a significant preference for foraging from the bottom of the water column when exposed to visual predator cues. *L. catesbeiana* showed no significant changes in foraging behavior across any treatment. These results suggest that visual cues, an area of little previous study in tadpoles, can influence tadpole foraging behavior. Furthermore, these findings suggest that increased amounts of terrestrially-derived DOC could facilitate the spread of species with high invasive potential, such as *L. catesbeiana,* within affected ecosystems across the country.

Introduction

Water "browning" has been occurring in lakes throughout the northeastern United States since the 1980's due to increased levels of terrestrially-derived dissolved organic matter (DOC) in conjunction with other factors including decreased acid precipitation, increased precipitation (causing increased erosion of terrestrial organic matter), changes in land use, and iron interactions (Williamson *et al*., 2015). Furthermore, increases in overall DOC and DOC interactions that increase color can cause changes in water conditions (Brothers *et al.,* 2014; Wehenmeyer *et al.*, 2014). Although the causes and future trends of this phenomenon are unclear (Graneli, 2012), studies have shown water browning to increase water temperature and thermal stratification, lead to oxygen depletion, alter vertical habitat and food distribution, and "switch lake metabolisms from autotrophy to heterotrophy" (Williamson *et al*., 2015), indicating the immense potential of this phenomenon to catalyze significant ecosystem changes. Water

browning can also have a "shading effect" on the water column, which can influence the behavior of grazers by reducing productivity of algae within that ecosystem (Williamson *et al.*, 1999).

Indicators species are often used to monitor the health of ecosystems, and amphibians have been hailed as a reliable indicator because they make use of both aquatic and terrestrial habitats, are relatively long lived, and use their skin for respiration (Welsh & Ollivier, 2000; Niemi & McDonald, 2004). Welsh and Ollivier (2000) found that an influx of fine sediments resulted in density reductions of amphibians which inhabited different microhabitats within affected streams, as compared to pristine streams. Amphibians also provide many ecosystem services, meaning that the loss or reduction of these populations can cause major changes to ecosystem dynamics (Hocking and Babbitt, 2014). In particular, many studies have investigated the importance of tadpoles as essential nutrient cyclers, detritivores, algal consumers, and sources of energy transfer within freshwater streams and riparian ecosystems (Ravenstel *et al*., 2004). For example, Seale (1980) found that tadpoles reduced the amount of suspended particles, increased nitrogen availability, reduced primary production rates, and influenced aquatic-terrestrial nutrient balances in a pond ecosystem.

Given the unique role of amphibians in ecosystems and the significant impacts of tadpole foraging on ecosystem dynamics, understanding the mechanisms that affect tadpole foraging is important to understanding ecosystem health. Optimal foraging theory has been used to describe decisions made by organisms in terms of energy gain; however the influence of predators on foraging is not always included in this model, even though their presence alone can cause a significant change in foraging behavior (Brown *et al*., 1999). Most research conducted on tadpole-predator responses have, to date, centered on the chemical cues related to foraging

behavior (Lima, 1998; Horat & Semlitsch 1994; Hettyey *et al.*, 2015). Not much is known about tadpole responses to visual predator stimulus because they have previously been described as having "poor vision" or little use for vision in benthic habitats (Stauffer & Semlitsch, 1993). However, Collier *et al.* (2008) showed leopard frog tadpoles to exhibit visual detection and response to predator cues. As such, additional research on the responses of tadpoles to visual cues is necessary. As more cases of water browning occur, increased DOC could decrease visibility within tadpole habitat and therefore negatively impact these potentially visual organisms' ability to forage or detect predation.

Although *L. catesbeiana* is a species native to the eastern United States, it has proven itself to have devastating invasive potential, especially within modified habitats (Fuller *et al.,* 2010). Bullfrog adults are indiscriminate predators and will eat essentially anything that they can fit in their mouths (Murphy, 2003), a trait which could prove devastating to populations of small endangered animals if their habitats are invaded by these predators. In addition, Kupferberg (1997) found that in California river systems, the presence of invasive bullfrog tadpoles caused as much as a 48% reduction in the population of other species native to those areas. Kruse and Francis (2011) also found that predators of different tadpole species, including large-mouth bass and other fish, preferentially choose to avoid eating bullfrog tadpoles when given a choice. Water browning, if shown to be detrimental to the foraging or predator detection of native tadpole species, might increase the susceptibility of habitats to bullfrog invasion, considering the decreased risk of predation and the natural invasive ability of bullfrogs.

In order to test the effect of increased DOC on tadpole foraging, we compared two species of anuran tadpoles native to our study area: *Rana (Lithobates) clamitans* (the green frog) and *Rana (Lithobates) catesbeiana* (the American bullfrog). We hypothesized that foraging would decrease

in all high DOC treatments for both species of tadpole, due to decreased visibility. We further hypothesized that the visual presence of predators would affect the foraging behavior of green frog tadpoles, but that bullfrog tadpoles would not be affected due to their low risk of predation.

Methods and Materials

Study Site

The study site for this research took place at the University of Notre Dame Environmental Research Center- East (UNDERC-EAST). The property crosses the state lines of Gogebic County, Michigan and Vilas County, Wisconsin and is located at an altitude between 1640ft and 1700ft above sea level. The lakes that were sampled for this study experience spring and fall turnover, as well as ice coverage during the winter. One lake (Long Lake) is an experimental lake that has had a curtain installed dividing the lake in half. East Long has an influx of nutrients and sediments while West Long has minimal inputs.

Field Sampling

Photosynthetically active radiation (PAR) data for the top of the water column was collected from three known High DOC lakes and three known Low DOC lakes (Table 1) as categorized by Kelly et *al*. (2014) and Sanchez (2016). Using a Li-Cor LI-1000 Data Logger set on channel 2, we took readings in W/m^2 above the water surface and at a depth of .25m. We took six readings from each lake (three replicates from two locations on the lake) near the edge of the littoral zone. Then, we divided the submerged reading by the reading above the surface to measure the percent penetration of PAR. Afterwards, we took the mean of all the low DOC readings and all the high DOC readings to get a single percent PAR penetration value for the littoral zones of the High and Low DOC lakes for this study.

Tadpoles of the species *Lithobates clamitans* and *Lithobates catesbiena* were collected from June 16th - July 14th, 2017 using both long-handled pasta strainers and seine net. Tadpoles were found in shallow areas of lakes, places with plenty of emergent grasses, detritus, and long inlets with 1-4 foot depths. Upon collection, tadpoles were brought back to the lab and kept in a 4-gallon aerated tank filled with water from their respective lakes. These tanks were kept in a room with direct sunlight for at least 24 hours to allow for adjustment to lab temperatures.

Lab Work and Experiments

Water was prepared according to the average light penetration in 90-gallon cattle tanks filled with a groundwater source and warmed to lab temperature (20-21° C). Like Weidel et al. (2017), we used commercially available concentrated terrestrial DOC (Super Hume) to mimic desired penetration of photosynthetically active radiation (PAR) of high and low DOC lakes. Low DOC water was prepared to a measurement of $53.1 \pm 1\%$ PAR at .25m compared to above the water surface and High DOC water was prepared to $26.7 \pm 1\%$. Tadpoles were then randomly placed in individual containers filled with 4 inches of prepared water of either high or low DOC concentrations and fasted for 24 hours prior to trial.

Non-predator experiments were run between the hours of 11:30 am and 6:00 pm. Tadpoles were chosen at random after the 24 hour fasting period for feeding trials. Trials were conducted in a 10-gallon plexiglass aquarium with a sheet of plexiglass fixed with clear epoxy that divided the tank in half (Figure 2). Before tadpoles were introduced, 3.5 gallons of water of the treatment which the tadpoles had been previously selected for and 10 crushed pellets of Hartz brand pet rabbit food were placed in each side of the tank. Approximately 50% of these crushed pellets sank to the bottom of the trial tank, while the other 50% remained floating on the surface of the

water. Next, one tadpole was placed in each side and allowed to habituate for two minutes. After two minutes, scan samples of each tadpole's activity were recorded every 20 seconds for 10 minutes according to the pre-determined activity codes in Table 1. Tadpoles were then weighed, their lengths were measured from tip of head to tip of tail, and their developmental stage was determined according to Gosner (1960). Within 24 hours after feeding trials, tadpoles were released to their original collection sites.

For predator experiments, the tadpole tank was surrounded from three sides with 10-gallon tanks that held one adult largemouth bass each (Figure 2b). Bass were placed in water of the same concentration as their associated tadpoles. This method was adapted from a similar experiment used by Dunlop-Hayden and Rehage (2011) to look at prey response to visual predator cues. All other procedures were identical to non-predator experiments.

Statistical Analyses

To analyze the data, we ran two 2-way ANOVA tests to determine whether the arcsine squareroot transformed proportion of time spent foraging for each tadpole differed across high/low DOC concentration or in predator/no predator conditions. In this case, an ANOVA was run for each species. We also ran a 2-way ANOVA to compare the transformed proportion of time spent foraging across high/low DOC treatments by species. This test was conducted on the no-predator data only. Two chi-squared tests were run, one for each species, to assess whether the preference for foraging location (top or bottom of trial tank) in the presence/absence of bass differed from theoretically expected values. Lastly, we ran Pearson's product-moment correlation tests to ensure that differences in collection date, weight, or tadpole development

stages did not significantly affect the foraging behavior of tadpoles in this study. All analysis was done using R (R Core Team, 2017).

Results

Feeding trials were run on 59 bullfrog and 62 green frog tadpoles, with each species being split equally between high and low water treatments with and without predators. A significant difference was detected in *L. clamitans* for the proportion of time spent foraging between the predator and non-predator treatments (df = 1, F=7.704, p = 0.00734; Figure 3). No significance was found for *L. catesbeiana* for proportion of time spent foraging across DOC levels (df =1, F=1.125, p= 0.294) or predator treatments (df= 1, F=0.076, p= 0.783).

L. clamitans showed a significant preference for foraging location within the water column across different predator treatments (X^2 (1, N = 56) = 25.755, p = 3.877×10^{-7}; Figure 4). *L. catesbeiana* showed no significant foraging preference in the absence or presence of visual predator cues (X^2 (1, N = 52) = 0.678, p = 0.41; Figure 5).

Discussion

This study showed little support for our hypothesis that tadpole species would decrease their foraging behavior in high DOC conditions. In fact, the only statistically different conditions for tadpole foraging frequency were for the predator and non-predator treatments of green frog tadpoles. Foraging was significantly higher for the predator treatment than for the non-predator condition. This lends support to our second hypothesis, that the visual presence of predators would affect the foraging behavior of green frog tadpoles. Indeed, a study by Bridges (2002) also reported an increase in foraging behavior in *Pseudacris tristeriata* tadpoles with the introduction of a predator, as we found in our data. Bridges hypothesized this to be an adaptive strategy used

by tadpoles to ultimately escape predation, as anuran metamorphosis requires a minimum size. Thus, in the presence of predators tadpoles might increase their foraging in order to more quickly reach the weight necessary to initiate their metamorphosis, and thus escape a predator-rich aquatic system. Peacor and Werner (2000) also found that larger *L. clamitans* tadpoles did not alter their behavior in the presence of a predator as much as smaller tadpoles did, further strengthening the theory that tadpoles might increase foraging in the presence of a predator to more quickly escape predation. However, other studies have found evidence of foraging behavior reductions in tadpoles in the presence of a predator (Peacor, 2002). This finding also correlated with an increase in the quality of foraging material, however, which offset the negative impacts of foraging activity reduction and resulted in a positive effect on tadpole growth in the presence of the predator. Bullfrog tadpoles showed no significant differences in foraging behavior among any of the treatments, which could have been due to their size. Werner and Anholt (1996) found that larger bullfrog tadpoles did not reduce their activity in the presence of a predator while smaller tadpoles did.

Furthermore, we found that in the absence of visual predator cues, *L. clamitans* showed a significant preference for foraging at the surface of the water column, while in the presence of a predator the tadpoles showed a significant preference for foraging from the bottom of the water column. Peacor and Werner (2000) discovered that in the presence of predators, *Rana (Lithobates) clamitans* and *Rana (Lithobates) catesbeiana* tadpoles changed their foraging habits and decreased their activity within the water column. However, another study by Anolt and Werner (1995) also discovered that predator presence and a decrease in food availability were correlated with increased movement and an increase in mortality of tadpoles. Notably, in our study *L. catesbeiana* were found to exhibit no such preference. This finding seems to indicate

that green frog tadpoles have indeed adopted an adaptive strategy to avoid predation by reducing the amount of time spent swimming throughout the water column without reducing foraging, while the "less tasty" bullfrog tadpoles have no such reservations in the presence of predators.

Although darker water treatments did not produce any significant changes in tadpole behavior for either species in this study, *L. clamitans* might be more likely to be affected by this phenomenon; since their behavioral strategy seems to be preferentially foraging from the bottom of the water column in the presence of predator visual cues, in browning lakes with shrinking littoral zones this could confine them to poor food sources. Williamson *et al.* (1999) described a shading effect of increased DOC in waterways that decreased primary productivity in darker water. Some fish species have already shown declines due to decreased food availability from increased levels of DOC (Craig *et al.,* 2015). This is not just due to the "shading effect" of increased DOC, but also to a decrease in the availability of warm and well-oxygenated habitat, a resource that tadpoles also rely on. If tadpoles do indeed respond to visual cues more than previous studies have suggested, then the results of this study certainly suggest a potential threat to their survivability with the well-documented water-browning occurring region-wide (Williamson *et al.,* 2015). Negative effects could also be incurred by predators within these habitats that rely on visual cues for predation, like bass (Crowl, 1989).

With many different threats to ecosystems, especially at a time when environmental regulations are being reduced, it is imperative to understand the important effects that anthropogenic change can have on ecosystems. Losses and reductions of amphibian populations have traditionally been monitored as an indicator of habitat degradation; however, some studies have found that amphibian populations may not be any more vulnerable than other species (Beebee & Griffiths, 2005). If this is indeed the case, then habitats experiencing amphibian declines may be in worse

condition than previously thought, compelling the need for a better understanding of how changes in water condition can affect the fitness of many different species.

Acknowledgements

References

Anholt, B. R., & Werner, E. E. (1995). Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology*, *76*(7), 2230-2234.

Beebee, T. J., & Griffiths, R. A. (2005). The amphibian decline crisis: a watershed for conservation biology?. *Biological Conservation*, *125*(3), 271-285.

Bridges, C. M. (2002). Tadpoles balance foraging and predator avoidance: effects of predation, pond drying, and hunger. *Journal of Herpetology*, *36*(4), 627-634.

Brothers, S., Köhler, J., Attermeyer, K., Grossart, H. P., Mehner, T., Meyer, N., Scharnweber, K., & Hilt, S. 2014. A feedback loop links brownification and anoxia in a temperate, shallow lake. Limnology and Oceanography, 59(4), 1388-1398.

Brown, J. S., Laundré, J. W., & Gurung, M. (1999). The ecology of fear: optimal foraging, game theory, and trophic interactions. *Journal of Mammalogy*, *80*(2), 385-399.

Collier, A., Bronk, C. C., Larson, B., & Taylor, S. (2008). The impact of predation stress by largemouth bass (Micropterus salmoides) on the growth and development of leopard frog tadpoles (Rana sphenocephala). Journal of Freshwater Ecology, 23(2), 281-289.

Craig, N., Jones, S. E., Weidel, B. C., & Solomon, C. T. (2015). Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, *60*(6), 2079-2089.

Crowl, T. A. (1989). Effects of crayfish size, orientation, and movement on the reactive distance of largemouth bass foraging in clear and turbid water. *Hydrobiologia*, *183*(2), 133-140.

Dunlop-Hayden, K. L., & Rehage, J. S. (2011). Antipredator behavior and cue recognition by multiple Everglades prey to a novel cichlid predator. *Behaviour*, *148*(7), 795-823.

Fuller, T. E., Pope, K. L., Ashton, D. T., & Welsh, H. H. (2011). Linking the distribution of an invasive amphibian (Rana catesbeiana) to habitat conditions in a managed river system in northern California. *Restoration Ecology*, *19*(201), 204-213.

Graneli, W. (2012). Brownification of lakes. In *Encyclopedia of Lakes and Reservoirs* (pp. 117-119). Springer Netherlands.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, *16*(3), 183-190.

Hettyey, A., Tóth, Z., Thonhauser, K. E., Frommen, J. G., Penn, D. J., & Van Buskirk, J. (2015). The relative importance of prey-borne and predator-borne chemical cues for inducible antipredator responses in tadpoles. Oecologia, 179(3), 699-710.

Hocking, D. J., & Babbitt, K. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, *9*(1), 1-17.

Horat, P., & Semlitsch, R. D. (1994). Effects of predation risk and hunger on the behaviour of two species of tadpoles. Behavioral Ecology and Sociobiology, 34(6), 393-401.

Lima, S. L. (1998). Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Advances in the Study of Behavior, 27, 215-290.

Kelly, P. T., Solomon, C. T., Weidel, B. C., & Jones, S. E. (2014). Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology*, *95*(5), 1236-1242.

Murphy, M. (2003). Introduced Species Summary Project. Retrieved July 23, 2017, from http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/Rana_catesbeiana.htm

Niemi, G. J., & McDonald, M. E. (2004). Application of ecological indicators. *Annu. Rev. Ecol. Evol. Syst.*, *35*, 89-111.

Pauley, T., & Lannoo, M. (n.d.). Rana Clamitans. Retrieved May 28, 2017, from http://amphibiaweb.org/species/5009

Peacor, S. D. (2002). Positive effect of predators on prey growth rate through induced modifications of prey behaviour. *Ecology Letters*, *5*(1), 77-85.

Peacor, S. D., & Werner, E. E. (2000). Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. *Ecology*, *81*(7), 1998-2010.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ranvestel, A. W., Lips, K. R., Pringle, C. M., Whiles, M. R., & Bixby, R. J. (2004). Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biology, 49(3), 274-285.

Sanchez, A (2016). Unpublished data.

Seale, D. B. (1980). Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology*, *61*(6), 1531-1550.

Stauffer, H. P., & Semlitsch, R. D. (1993). Effects of visual, chemical and tactile cues of fish on the behavioural responses of tadpoles. Animal Behaviour, 46(2), 355-364.

Weidel, B. C., Baglini, K., Jones, S. E., Kelly, P. T., Solomon, C. T., & Zwart, J. A. (2017). Light climate and dissolved organic carbon concentration influence species-specific changes in fish zooplanktivory. *Inland Waters*, *7*(2), 210-217.

Welsh, H. H., & Ollivier, L. M. (1998). Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications*, *8*(4), 1118-1132.

Werner, E. E., & Anholt, B. R. (1996). Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology*, *77*(1), 157-169.

Weyhenmeyer, G. A., Prairie, Y. T., & Tranvik, L. J. (2014). Browning of boreal freshwaters coupled to carbon-iron interactions along the aquatic continuum. PloS one, 9(2), e88104.

Williamson, C. E., Hargreaves, B. R., Orr, P. S., & Lovera, P. A. (1999). Does UV play a role in changes in predation and zooplankton community structure in acidified lakes?. Limnology and Oceanography, 44(3), 774-783.

Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brentrup, J. A., Knoll, L. B., & Moeller, R. E. (2015). Ecological consequences of long-term browning in lakes. *Scientific reports*, *5*.

Tables

Low DOC Concentrations	Bay (5.99mgC/L)	Crampton (5.49gC/L)	West Long (8.09mgC/L)
High DOC Concentrations	Morris (17.49mgC/L)	Hummingbird (25.9mgC/L)	East Long (Manipulated for high DOC)

Table 1: The above table presents DOC designations for studied Lakes. The concentrations and designations are taken from Craig et al. (2015) and Kelly et al. (2014)

Not Moving (NM)	Tadpole is resting on bottom of tank and generally inactive for a majority of the 20s period
Swimming Vertically (SV)	Tadpole is swimming vertically for a majority of the 20s period
Swimming Horizontally (SH)	Tadpole is swimming horizontally for most of the 20s period
Wall (WALL)	Tadpole is swimming against wall opening and closing mouth in a way that resembles a feeding attempt for most of the 20s period
Foraging from top of tank (FT)	Tadpole foraged from the water surface at any time during the 20s period
Foraging from bottom of tank (FB)	Tadpole foraged from the bottom of the tank during the 20s period (easily distinguished from NM by active swimming motion downward)

Table 2: The above table was used to designate tadpole activity types during 20 second scan sampling for this study.
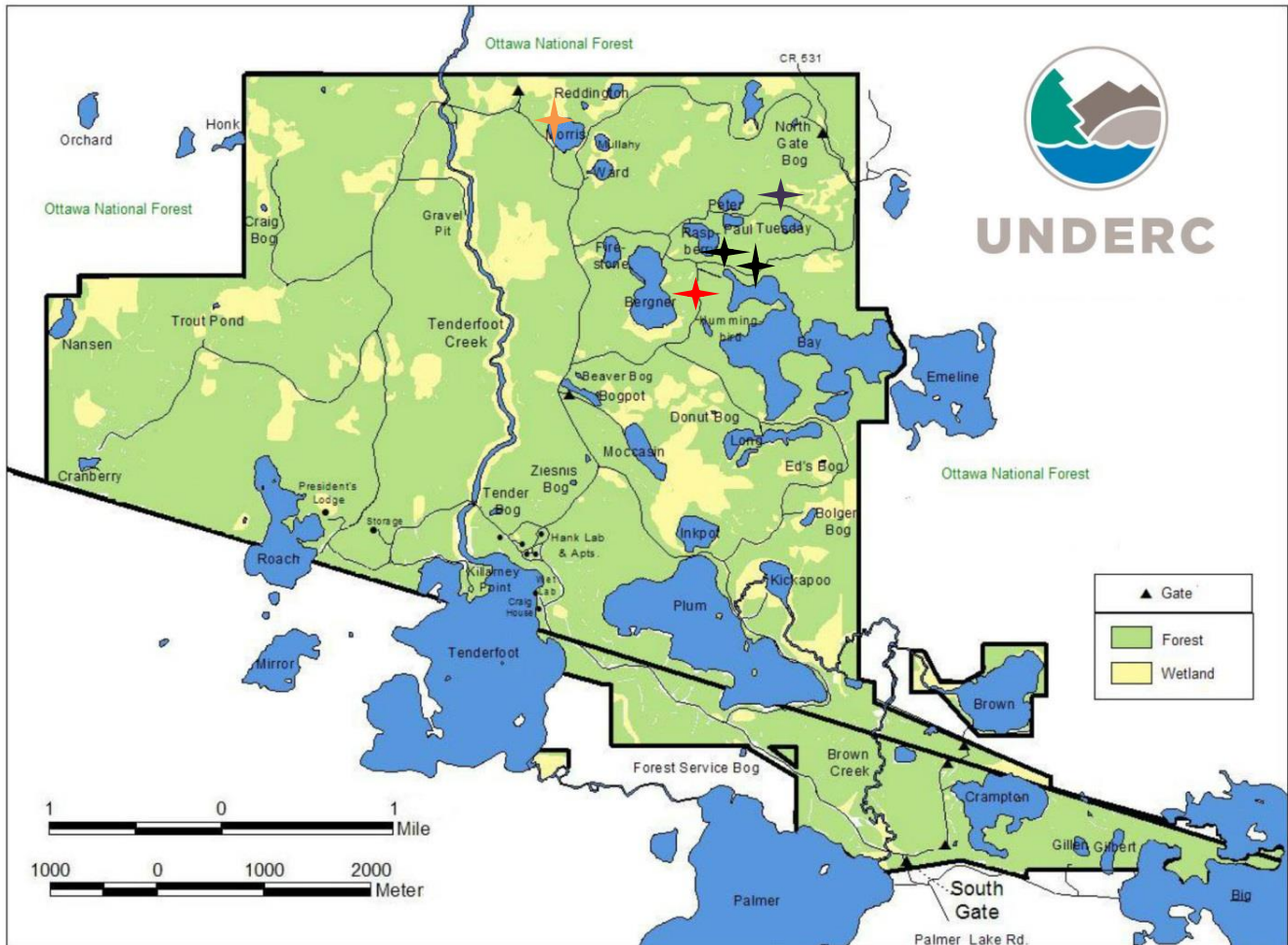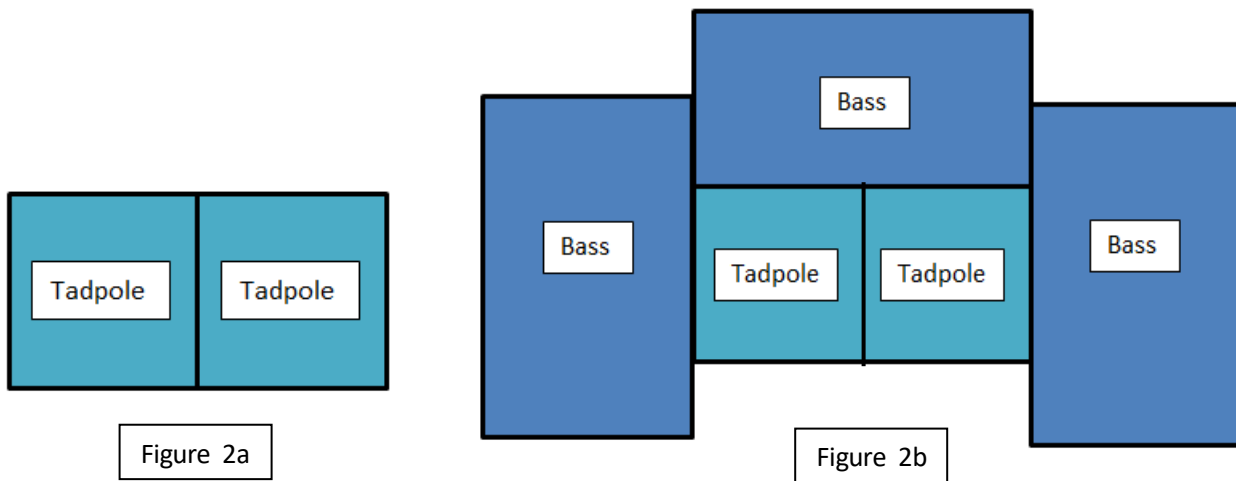
Figures

Figure 1: Above presents a map of UNDERC- East property. Collection sites for Bay Lake are indicated by the stars in black, Morris Lake in orange, Tuesday Lake in purple, and Humming Bird Lake in red.

Figures 2a & 2b: 2a: Above is a visual representation of the tadpole trial tank set-up for non-predator experiments, from an overhead view. The tank is a clear 10-gallon aquarium divided in half, with each side containing 3.5 gallons of treatment water. 2b: Above is a visual representation of the tadpole trial tank set up for predator experiments, from an overhead view. The center tank is a clear 10-gallon aquarium divided in half with each side containing 3.5 gallons of treatment water and the predator (bass) tanks containing 7.5 gallons of the same treatment water.
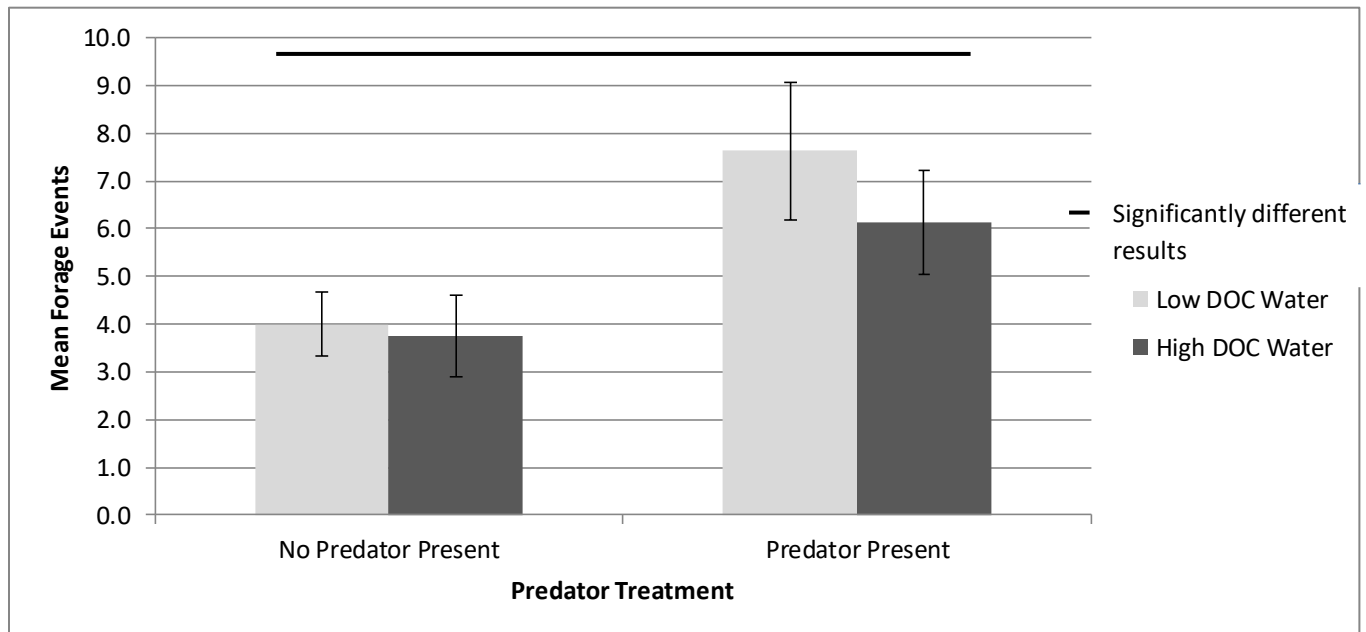

Figure 3: Mean foraging events by _L. clamitans_ among all trial conditions. A 2-way ANOVA determined that there was a significant difference between the foraging events of _L. catesbienna_ in predator and no-predator conditions, although there was no significant difference between DOC treatment types. Significance is indicated by the solid black line (df = 1, F= 7.704 p = 0.00734.). Error bars are represented by Standard Error.
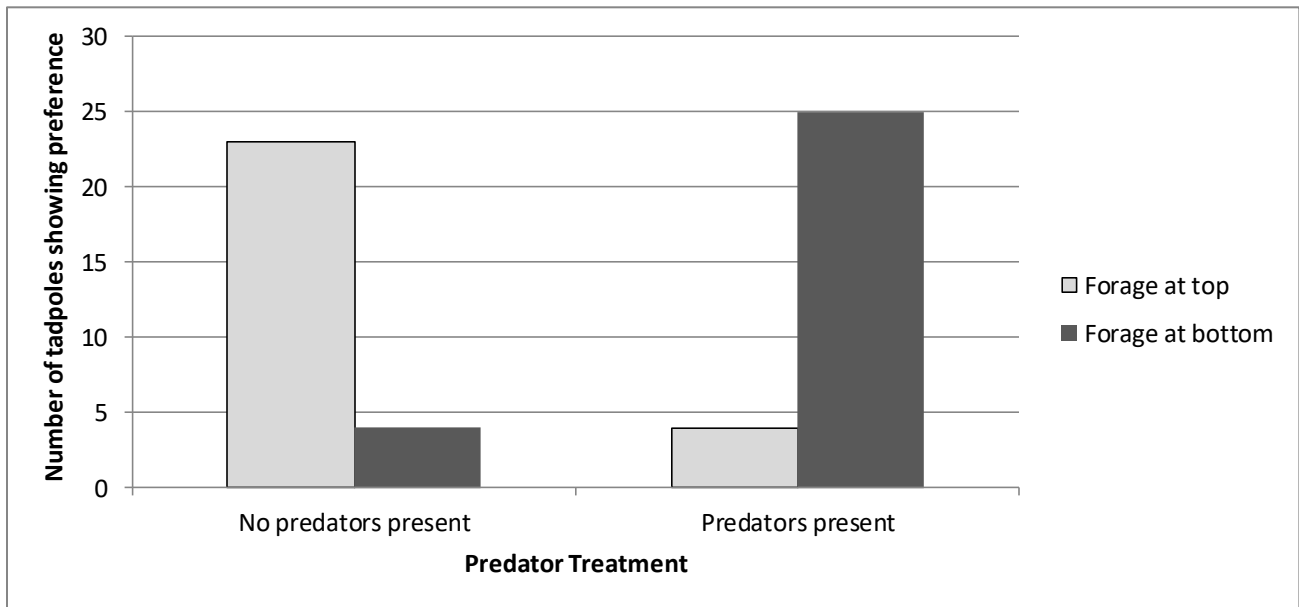
Figure 4: Number of *L. clamitans* tadpoles showing preference for foraging location within the water column. A Chi-squared test showed *L. clamitans* to display a significant preference for foraging location in the absence and presence of predators (X^2 (1, N = 56) = 25.755, p = 3.877×10^{-7}).
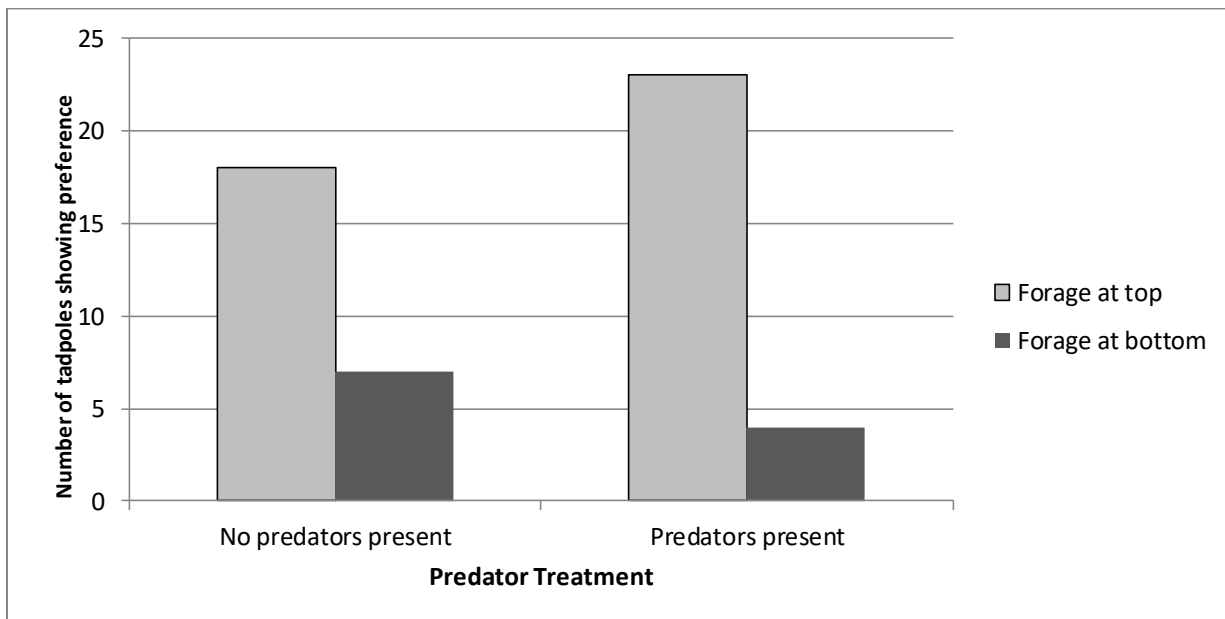

Figure 5: Number of *L. catesbeiana* tadpoles showing preference for foraging location within the water column among different predator treatments. A Chi-squared test showed that *L. catesbeiana* displayed no significant preference for foraging location in the absence and presence of predators (X^2 (1, N = 52) = 0.678, p = 0.41).