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Estimating the Total Biomass of Breeding Amphibians Bat Lake, Algonquin Provincial Park, Over the 2018 Season.

Abstract

Bat Lake is a fishless lake in Algonquin Park that has been identified as a breeding hotspot for amphibians. As these animals migrate seasonally to this habitat, their activity and oviposition have a proportional effect on ecosystem functions within the habitat. Here, we estimate the biomass of migratory amphibians breeding in Bat Lake. Surveys of both adult, and juvenile amphibians were performed using a continuous drift fence that surrounded the lake. Visual surveys were conducted on egg masses in the lake. Biomass was calculated for four common species of amphibians, amounting to approximately 10kg. As *Ambystoma maculatum* accounted for the largest proportion of amphibian biomass, further analysis was preformed to estimate egg oviposition and juvenile emergence biomass. The total weight of ova deposited into Bat Lake was 2.3 kg, and the total mass of metamorphs emerging from Bat Lake in autumn was 1.5 kg. These estimates are likely related to the import and export of nutrients into the habitat via *Am. maculatum*, and show that there is a net positive import into the lake, although further modelling work is needed. Given that Bat Lake is assumed to be a nutrient poor habitat due to its acidic nature and presence of carnivorous plants, influxes of biomass and the subsequent activity from adult and juvenile amphibians may be functionally important within the habitat and can likely contribute positively to ecosystem productivity. Using these estimates, future studies will be able to further investigate amphibian productivity, nutrient stock, and energy flow at the site.

Introduction

Declining amphibian biodiversity and abundance is a growing worldwide concern. The reasons behind these declines are multifarious. Habitat degradation, climate change, emerging infectious diseases and introduction of invasive species have all been attributed to these losses (Brito, D., 2008). Due to these declines and changes in communities, the importance of understanding the role of amphibians within ecosystems has become incredibly valuable (Hopkins, W.A., 2007, Browne, C.L., *et al*, 2009, DeAngelis, D.L., 1980).

Amphibians have been shown to play an important role in ecosystem function (Milankovich, J., R., *et al*, 2016, Davic, R., D., *et al* 2004). They assist in facilitating the flow of nutrients and energy and are important components within trophic structures. Due to their biphasic nature, these animals inhabit both aquatic and terrestrial habitats during different life stages- terrestrial as adults and aquatic as larva (Tiegs, S. D., 2016). Within these habitats' amphibians facilitate different functions.

In terrestrial habitats, adult amphibians are important regulators of invertebrates, and by extension invertebrate function in mineral cycling and microbiological communities (Semlitsch, R.D., *et al*, 2014, Best, M.L., *et al*, 2014). The fossorial activity of amphibians, namely salamanders, facilitates important soil disruption and abiotic mineral movement (Best, M.L., *et al*, 2014, Lovich, J.E., *et al*, 2018, Semlitsch, R.D., *et al*, 1982). Amphibians also serve as prey to higher trophic-level animals like birds and snakes (Wilson, J.D., *et al*, 2011).

Adult amphibians deposit egg masses during the breeding season within aquatic habitats such as vernal pools, lakes or ponds. Over time, the eggs that escape predation become aquatic juveniles (Caut, S., 2013, Rugenski, A. T., *et al*, 2012 and Schmidt, K., 2017). Larval caudatans are carnivorous, feeding on anything that they can fit into their small mouths, including fellow amphibians. Similar to their adult counterparts, caudatans exert top-down control of nutrients through invertebrate community predation (Hocking, D. J., *et al*, 2014). Anuran larva are omnivorous, primarily filter feeding on periphyton, phytoplankton, and zooplankton (Seale, D. B., 1980 Costa, Z. J., *et al*, 2013). As the larvae inhabit these aquatic habitats, they disturb algae and leaf litter which results in an increase in free nutrient particles such as carbon, and increased levels of primary productivity (Rugenski, A. T, *et al*, 2015, Iwai, N., *et al*, 2007, Seale, D. B., 1980). Metamorphosis signals a shift in activity to inhabit terrestrial habitats (Semlitsch, R.D., *et al*, 2014). This output of nutrients from the lake provides low quantity, high quality nutrients (low C:N and C:P ratios) into terrestrial systems (Fritz, K. A., *et al*, 2018). This seasonal flux of nutrients has been hypothesized to be a key driver in productivity in nutrient poor aquatic habitats such as vernal pools and bogs (Davis, C.L, *et al*, 2018, Regester, K. J., *et al*, 2006).

Bat Lake is a three-hectare large bog-like lake within Algonquin Provincial Park. Due to the presence of carnivorous plants, it possesses an unusually high level of acidity (pH 4.2). It maintains no connectivity to other watersheds and lacks higher vertebrates, such as fish. Therefore, it can be assumed to be a relatively nutrient poor site (Brooks, R.J., *et al*, 2003, Cunnington, D.C., *et al*, 2000). The fishless state of the lake, however, provides an ideal breeding habitat for amphibians due to decreased levels of predation, mimicking that of vernal pools (Brooks, R.J., *et al*, 2003). Over the past decade this site has been home to various amphibian studies and has been observed to hold a high magnitude of amphibians. Due to these unique traits, the lake provides an ideal site to observe the effect pond breeding amphibians may have on the functionality of aquatic habitats.

In order to further understand the function of amphibians within ecosystems such as Bat Lake, researchers use measures such as biomass. (Lovich, J., E., 2018, Welsh, Jr., H., H., *et al*, 1998). Biomass is used to inform ecologists on the amount of standing nutrient stock within an ecosystem, the relative community composition and the health of these communities (Lovich, J.E., *et al* 2018, Atkinson C *et al*, 2017).

This study focuses on four species of amphibians that breed at Bat Lake: two species of anurans; *Rana sylvaticus* (Wood frog), and *Anaxyrus americanus* (American toad), and two species of caudata; *Ambystoma maculatum* (Spotted salamander) and *Ambystoma laterale* (Blue-spotted salamander). The study aims to estimate the total biomass of adult amphibians that bred at Bat Lake during the 2018 spring season. Additionally, the total input biomass of *Am. maculatum* eggs that were deposited and biomass outputs from juveniles that left the site at the end of the summer will be estimated. Using these estimates, future studies will be able to further investigate amphibian productivity, nutrient stock/transfer, and energy flow at the site.

Methods

Study Site

This study was conducted at Bat Lake located within Algonquin Park, Ontario. The park was established in 1898 and is located within central Ontario, in an area of high elevation (300 metres to 600 metres) (The Friends of Algonquin Park, 2018). The lake is relatively undisturbed and is home to an interpretive public trail. The lake is approximately three hectares in area. Bat Lake is acidic (pH 4.2) and fishless providing an ideal habitat for breeding amphibians that mimics that of vernal pools (Brooks, R.J, *et al*, 2003, Cunnington, D.C., *et al* 2000). The surrounding area of the lake consists of old growth boreal forest. The forest is made up of a variety of conifers including white pine (*Pinus strobus*), red spruce, black spruce (*Picea mariana*), and balsam fir (*Abies balsamea*).

<u>Terrestrial Surveys</u>

Drift Fence

In order to monitor the breeding population of amphibians at Bat Lake, a drift fence was installed surrounding the lake during the previous summer (August 2017; Scott, D.E., *et al*, 2013, Gibbons, J.W., *et al*, 2013). This fence was equipped with both pitfall traps and funnel traps in order to assist with the capture of individuals at the fence (Strain, G.F., *et al*, 2009). The fence is constructed from 46cm tall aluminum flashing, supported by wooden stakes that are buried 6-8 cm into the ground and varies in distance from the lake between 2 to 40 metres. The total length of the fence was approximately 1000 metres (figure 1). There are two discontinuities in the fence both 3 to 4 meters in width, where a public trail intersects. At these junctions, the fence ends with pitfall traps, one on each side of the fence and an elongated piece of aluminum flashing placed perpendicular to the fence, to assist in amphibian capture (figure 2). The installation of funnel traps occurred at the start of the 2018 breeding season. Along the fence, two funnel traps were placed every 20 metres, on both the lake-side and the forest-side of the fence to assist with monitoring the direction of migration (Homan, R.N., 2018). In addition, each funnel trap had a corresponding plastic bin in order to deal with overcrowding in the traps.

Breeding Adults

Surveys of the fence occurred twice daily, at night (21:30 to 01:00) and in the morning (09:00 to 13:00) over the course of the 2018 breeding season (May to June). Night surveys consisted of active capture of individuals found along or near the fence. Many of the species breeding at the lake are nocturnal and are therefore more active at this time, assisting in their capture. Individuals caught in the night survey were placed into the plastic overfill bins, along with moist cover in order to combat desiccation. Morning surveys required another full survey of the fence and the processing of captured individuals. Processing consisted of identification of species, sex and gravidity in all individuals in addition to direction of migration (towards or away from the lake). Mass measurements of select individuals and species were also taken. Mass was measured using a spring-scale Pesola© spring scale (Feusisberg, Switzerland) (±1.0g). Although Bat Lake is home to a host of different amphibian species, this survey focused on the capture of *L. sylvaticus*, *An. americanus*, *Am. maculatum* and *Am. laterale*. Other amphibians that also breed within Bat Lake were not included due to low capture rates.

Metamorphosed Juvenile Emergence

Juvenile *Am. maculatum* migration from the lake was monitored using the drift fence over their emergence period; late summer/early fall (17 August to 07 October). These survey methods were identical to those of the breeding season except that only one survey was conducted each day (9:00 to 12:00). Night surveys were forgone because juveniles are far smaller than adults and therefore difficult to spot at night. In addition, juveniles leaving the fence do not migrate in the same numbers as adults during the breeding season, allowing for the researchers to record morphometric measurements for all individuals, rather than a select few (as was done for the adults).

<u>Aquatic Surveys</u>

Egg Mass Surveys

Egg mass surveys required a researcher to conduct a visual survey along the perimeter of the lake (figure 1), recording the number of egg masses that were spotted using a mechanical tally clicker. Only *Am. maculatum* egg masses were recorded. On days of limited visibility (e.g., increased cloud cover and high winds) egg mass surveys were not conducted to avoid miscounts. *Am. macultum* deposition and egg incubation occur over one month. Therefore, as the daily egg mass counts begin to plateau after one month, researchers were able to assume this peak was the total amount of clutches deposited.

Clutch Size Estimates

A selection of egg masses was removed at random from the site. The egg masses were held in plastic Tupperware bins to avoid excess desiccation of the embryos. To count the total number embryos within an egg mass, two planes of glass were used to flatten an individual mass and allow for each individual embryo to be clearly visible. Visual counts of each clutch were then preformed and recorded. Egg masses were returned to the lake the following day.

<u>Analysis</u>

Biomass Calculations for Adults and Metamorphs

Biomass calculations for adult amphibians and metamorphic *Am. maculatum* were performed using R statistical software (R version 3.2.1). Total population counts for breeding amphibians were obtained using species and sex data collected. Using a statistical simulation, the total number of amphibians within each population were sampled from the corresponding mass data collected. The simulation was conducted 1000X to provide upper and lower confidence intervals for the biomass estimates, and the average biomass over the simulations was taken.

Mass for *Ambystoma laterale* was not measured at the fence. Female mass measures for Jefferson-Blue spotted hybrids were used (Hossie, T. *unpublished*). These amphibians are larger than that of pure blue spotted salamanders and therefore may provide an overestimate of mass. In order to account for the size difference between males and females, for the male measurements, the total mass was multiplied by the relative fraction of difference between the two sexes (0.9195) as specified in De Lisle, S. P., *et al* (2013).

Data for female *Am. maculatum* migrating towards the lake lacked a significant amount of measures (n=16). Mass measures for females migrating away from the site (non-gravid) were used. In order to account for the difference in biomass deposited at the lake (in the form of egg masses), the average mass difference between the gravid and non-gravid individuals was added onto the lake side masses (+6.35g).

Biomass Calculations for Deposited Egg Masses

Biomass estimates for egg masses were calculated using egg mass counts and clutch size. These measures provided a total estimate for individual embryos deposited. In order to estimate individual embryo mass, each embryo was assumed to have an approximate diameter of 2.7mm (Bruce, R. C., 2003) and a density equivalent to water.

In order to compare the deposited egg mass data to current literature values, data from Cunnington, 2000 was used. Cunnington measured ash free dry mass (AFDM) of *Am. maculatum* egg masses at Algonquin park; (mean +/- SD, n=13): 0.60 +/- 0.30g. The total number of egg mass counts was multiplied by this value in order to provide AFDM estimate.

Bat Lake Area Calculations

In order to compare the adult biomass measure to the literature, terrestrial biomass density was calculated. Using data from the literature (table 1), it is predicted that the majority of adult amphibians remain within a 100m radius of the lake. Using ArcGIS software total area for a 100m radius surrounding the lake were calculated. Total area was then used to calculate the density of biomass terrestrially.

Results

Adult Amphibian Biomass Estimates

In total 6636 individual captures were recorded at the drift fence migrating towards the lake. Of these captures 983 individuals had mass measurements taken.

In total, adult amphibian biomass was estimated to be 108.9 kg (LCI:107.2; UCI:110.6). Of this total, 70% of the biomass was held by *Am. maculatum* (n=4276, biomass= 76.5 kg, LCI:76.0; UCI:77.0), *Anaxyrus americanus* held 14% of the biomass (n=124, biomass= 14.5kg, LCI:13.8; UCI:15.1), *Ambystoma laterale* held 13% of the total biomass (n=1515, biomass=12.9kg, LCI:12.7; UCI:13.1), and *Rana sylvaticus* made up approximately 5% (n= 643, biomass=5.0kg, LCI:4.7; UCI:5.3) (table 2). As seen in figure 3, three of the four species showed that females made up a greater proportion of the biomass.

A 100m radius surrounding Bat lake is approximately 9.5ha in area. Therefore, the average density of adult amphibians measured is approximately 11.5 kg/ha (figure 4).

Ambystoma maculatum Life Stage Biomass Estimates

Am. maculatum metamorphs had a total of 1559 captures of which 1544 mass measurements were acquired. Biomass measures for juveniles was 1.6kg (n=1559, LCI:1.4; UCI:1.5).

3642 egg masses were recorded to have been deposited into Bat Lake. 201(n) egg masses were collected in order to estimate clutch size. The average egg mass had 60.0+/- 27.8 embryos. In total there were approximately 218597 embryos deposited into Bat Lake amounting to 2.3kg of biomass. AFDM for egg masses was calculated to be approximately 2.2kg (figure 5).

Discussion

Adult Amphibian Biomass

Biomass measure

In total 108.9kg of biomass were calculated for Bat Lake (table 1/figure 3). Terrestrially, the 4 pond-breeding amphibian species can be estimated to make up approximately 11.5kg/ha within a 100m radius of the study site (figure 4). In comparison to current literature (table 3), this is the second highest measure for terrestrial amphibian biomass density, the highest being that of Petranka, J. W., 2001, which measured a total of 16.5kg/ha for 5 amphibian species. This measure is also approximately 10 times larger than that of Burton and Likens (1975), which had concluded their measure of biomass to be greater or equal to bird and small mammal biomass densities. Although there are no current publications on these densities within Algonquin Park, the magnitude of this biomass suggests that amphibians make up a large proportion of vertebrate biomass at the site.

Although the biomass is shown to be a significant calculation in comparison to current literature, this is a conservative measure of terrestrial biomass surrounding Bat Lake due to incomplete sampling of amphibious species and the total terrestrial populations of the 4 species of study. In this study, 4 amphibians were surveyed and sampled, however, in total 10 different species of amphibians have been observed at the study site. This includes an additional 4 species of anurans; *Lithobates clamitans* (Green frog), *Lithobates catesbeianus* (American bullfrog), *Hyla versicolor* (gray treefrog), and *Pseudacris crucifer* (spring peeper) and two species of caudatans; *Plethodon cinereus* (eastern red-backed salamander) and *Notophthalmus viridescens* (eastern newt). These amphibians, although not included, presumably maintain a similar function in nutrient cycling and energy transfer within and surrounding Bat Lake seasonally. With the inclusion of these species, the total biomass of amphibians is predicted to be higher than that of our estimate.

The use of a continuous drift fence allowed for a complete population census of the 2018 breeding season and is thusly represented in the biomass estimate. However, this measure is not descriptive of the entire adult population biomass of the four amphibians measured in this study. Amphibians at colder climates are more environmentally constrained as a result of decreased temperature and growing season and have been shown to favor biennial breeding (Morrison, C., *et al*, 2003). *Am. maculatum* through long term studies are shown to return to breeding sites at intervals of two to three years (Husting, E. L., 1965). This suggests that not all individuals bred at the lake over the 2018 season, suggesting that the total terrestrial biomass density of amphibians is greater than that estimated by the 2018 biomass measure.

Importance of amphibians at Bat Lake

The biomass measures of the four amphibian species at Bat Lake provided informative data on the community composition at the site. Figure 3 exemplifies the unique abundance of these amphibians. Caudatan biomass is shown to be in the highest proportion, making up approximately 83% of the total biomass. Pond-breeding adult amphibian community composition is related to the juvenile recruitment and breeding activity at the site (Semlitsch, R. D., *et al*, 1996). This is influenced by a number of factors including length of hydroperiod, environmental variation, and density of competitors and predators (Semlitsch, R. D., *et al*, 1996,). In vernal pools caudatans have been shown to be influential in the relative composition of amphibian communities (Mourin, P.J., 1981, Walls S. C., 2001). Salamander larvae are voracious carnivores and without the presence of fish they could take on a similar role to that of predatory fish and therefore exert top-down effects on community composition (Semlitsch, R.

D., *et al*, 1996). Further elucidation on the long-term trends of amphibian recruitment at the lake are needed to provide a conclusive explanation on the Bat Lake community.

In addition to a unique community composition, the biomass measures also highlight a large female bias for three of the four species surveyed (figure 3). The discrepancy in mass can be explained in part by the sexual dimorphism between the species that favours larger females due to their increased oviposition capacity (Kupfer, A. 2007). Additionally, the biomass measures calculated are representative of gravid females. Ultimately, the biomass bias is due to the increased number of females within the population. Amphibian populations are often reported as being strongly male biased (Petranka, J. W., 1998). One paper that studied the population of Desmognath salamanders concluded that a male bias is a result of increased female mortality within aquatic habitats (Organ, J. I., 1961). Husting E. L. (1965) proposed that the increased bias in males for *Am. maculatum* may be due to bigger body size in females leading to increased predation. Bat Lake however provides a different narrative; the sex ratio for females is higher than males, exemplified by the *Am. maculatum* population, which shows that there are 1.8 females for every male. Similar female biases were seen in *Am. laterale* and *An. americanus.* Although there is evidence that in some environments this bias does occur, there is currently no clear mechanism behind this change in bias and requires further research in order to explain.

Biomass measures at the site provide information on the relative health and physical make-up of individuals. In comparing densities and total biomass of the Bat Lake estimates with that of the literature, the individual amphibians at Bat Lake have a higher ratio of biomass per individual. Although this could be due to the differences in type of taxa measured, this could also suggest that the amphibians at Bat Lake may follow Bergmann's rule. Bergmann's rule hypothesizes that at higher latitudes, animals will shift to increase overall body size (Blackburn,

T. M., *et al*, 1999). Since Bat Lake occurs at a higher latitude and altitude than the current biomass estimates, one would see the average mass per individual appearing to be larger than that of more southerly estimates if this was true. In a paper by Powell, J. S. V. (2015), the biomass for 3624 *Am. maculatum* was 47kg. These measures represent a lower proportional mass to the populations at Bat Lake (table 1; *Am. maculatum* 4281 individuals, 76.5kg). Our estimates for *Am. maculatum* could support Bergmann's rule. Bergmann's rule, and whether it applies to amphibians is a controversial topic. Although there have been studies in the past that have shown evidence both for and against Bergmann's rule in amphibians, many of these studies that found no evidence for the rule, used measures of length rather than biomass to asses size differences (Semlitsch, R. D., *et al*, 2016, Ray, C., 1960, Adams, D. C., *et al*, 2008, Ashton, K. G., 2002). As suggested in a paper by Blackburn, T. M. (1999), mass may serve as a better measurement for this relationship. Increased taxa specific work is needed to clarify the relationship between size and climate in amphibians.

<u>*Ambysotma maculatum* Life Stage Biomass Estimates</u>

Nutrient cycling in Am. maculatum

The input of biomass via egg masses provided a measure of approximately 2.2kg (AFDM) or 2.3 kg (wet mass). The AFDM measure is three times higher than the current highest measure within the literature done by Regester, K. J., *et al*, (2006), that estimated a total of 0.8kg (AFDM) input maximum within a pond in Illinois. The large measure may be due to the size of Bat Lake. Deposition by *Am. maculatum* has been shown to increase exponentially with the size of the breeding site (Regester, K. J., *et al*, 2006).

As seen in figure 5, 1.7 kg of juveniles were measured to leave the study site at the end of the season. This represents a high-quality output of biomass (Fritz, K. A., *et al*, 2018). Many of these juveniles will serve as a high nutrient prey source to higher trophic terrestrial animals such as snakes (Regester, K. J., *et al*, 2006). This biomass represents an export of nutrients accumulated within the aquatic habitat being transferred to the terrestrial surroundings (Regester, K. J., *et al*, 2006).

Using the estimates of egg mass and metamorphic juveniles, *Am. maculatum* was shown to contribute a net input of 0.8kg into Bat Lake over the 2018 season. This net deposition of mass shows an exchange of energy from terrestrial to aquatic habitats. Bat Lake similar to a vernal pool, is not fed from a watershed. Therefore, all nutrients come from migrating fauna or through natural deposition. As such, this yearly deposition of biomass provides the lake with a seasonal flux of nutrients (Davic, R. D., et *al*, 2004). This measure is representative of only one of the eight known amphibians that breed at Bat Lake, suggesting that the total input into the lake by amphibians is far greater than this estimate.

Importance of Am. maculatum biomass

Over the 2018 breeding season, it was estimated that 219 598 embryos were deposited into the lake. Of this, 1559 individuals were recorded to emerge from the lake in the fall. This represents a mortality rate of approximately 99.2% within the lake. In comparison to literature, this rate of mortality is very high and is only seen in cases where breeding ponds dried up prior to amphibian metamorphosis. Survival in ambystomatid larvae is influenced by climate, habitat and hydroperiod (Shoop, C.R. 1974). Since Bat Lake is permanent, it is hypothesized that this high degree of mortality could be a result of predation by both conspecifics, as well as other amphibians and invertebrates at the site. A paper by Wilber H. M., (1972), showed that survival of *Am. maculatum,* in comparison to other ambystomatid salamanders, such as *Am. laterale,* favored a trade off in number of total surviving individuals for increased fitness of survivors through outcompeting/predating conspecifics. Additionally, it was shown that predation and competition by other amphibians including *Am. laterale* and *R. sylvaticus* rates vary based on changes in density and priority effects. Future work at the site may be able to inform researchers on the effects in which yearly changes in larval community structure, weather, and habitat have on metamorphic survival.

Within the literature, metamorph biomass has been used to quantify productivity within aquatic habitats (Gibbons, J. W., *et al*, 2006). However, this would prove to be an extremely conservative measure with our study as a large portion of larvae within the lake experienced mortality. The relative growth and activity of these amphibians within the lake requires further work in order to properly understand their function in productivity within the habitat.

Conclusions

Bat Lake, due to its fishless status, provides a unique habitat for amphibian. Using biomass estimates, it is shown that amphibians hold a great deal of biomass surrounding the lake. Data from the *Am. maculatum* suggests that amphibians breeding at the site provide a net input of biomass into the lake yearly. Although this study was able to provide a great deal of information on the population of amphibians breeding at Bat Lake, it also highlights the lack of current literature on amphibian function and community composition within aquatic and terrestrial habitats. In a paper published in 2018 (Bar-On, Y. M.) that set out to measure overall biomass distributions of all taxa on the earth, amphibian biomass was not included due to lack of sufficient data and an assumption that this measure of biomass could be assumed to be negligible. Studies such as Bar-On (2018) exemplify our current lack of knowledge with regard to amphibians and their role in ecosystem function. With the growing concern over mass amphibian extinctions, studies such as this one that work to quantify and understand the function of amphibians within habitats have become increasingly important.

Appendix

Table 1: Home ranges of 4 species of pond breeding amphibians collected from the literature.

Species	Home Range (m^2)	Reference
Blue Spotted Salamander *Ambystoma laterale*	67	Ryan, K. J., Calhoun, A. J. (2014). Post breeding habitat use of the rare, pure-diploid blue-spotted salamander (*Ambystoma laterale*). Journal of Herpetology, 48(4), 556-566.
Spotted Salamander *Ambystoma maculatum*	69	Madison, D. M. 1997. The emigration of radio-implanted spotted salamanders, *Ambystoma maculatum*. Journal of Herpetology 31:542–51.
	40	Rittenhouse, T. A. G. and R. D. Semlitsch. 2006. Grasslands as movement barriers for a forest-associated salamander: migration behavior of adult and juvenile salamanders at a distinct habitat edge. Biological Conservation 131:14–22.
American Toad *Anaxyrus americanus*	N/A	N/A
Wood Frog *Rana sylvaticus*	110	Rittenhouse, T. A., Semlitsch, R. D. (2007). Distribution of amphibians in terrestrial habitat surrounding wetlands. Wetlands, 27(1), 153-161.
	65.1	Bellis, E. D. (1965). Home range and movements of the wood frog in a northern bog. Ecology, 46(1-2), 90-98.
Amphibians	100	Powell, J. S. V., Babbitt, K. J. (2015). Despite buffers, experimental forest clear-cuts impact amphibian body size and biomass. PloS one, 10(11), e0143505.
	93* (radius from wetland)	Rittenhouse, T. A., Semlitsch, R. D. (2007). Distribution of amphibians in terrestrial habitat surrounding wetlands. Wetlands, 27(1), 153-161.

*this represents the 50% isopleth average from both salamanders and frogs.

Table 2: Biomass measures for 4 pond breeding amphibians; two species of anurans; *Lithobates sylvaticus* (Wood frog), and *Anaxyrus americanus* (American toad), and two species of caudata; *Ambystoma maculatum* (Spotted salamander) and *Ambystoma laterale* (Blue-spotted salamander). All measures were taken over the 2018 breeding season with one exception; *Am. laterale* mass measures were taken from a data collected by Hossie, T (*unpublished*) and De Lisle, S. P., Rowe, L. (2013).

Species	Sex	Total Captures	Total Mass Measurements	Average Mass (g)	Calculated Biomass (kg)
Salamander *Caudata*					
Blue Spotted Salamander *Ambystoma laterale*	Female	963	48	8.6	8.3 (UCI:8.4, LCI:8.2)
	Male	580	48	7.9	4.6 (UCI:4.7, LCI:4.5)
Spotted Salamander *Ambystoma maculatum*	Female	2694	362	19.8	58.7 (UCI:59.0, LCI:58.4)
	Male	1587	198	11.3	17.9 (UCI:18.1, LCI:17.7)
Frog *Anura*					
American Toad *Anaxyrus americanus*	Female	164	88	72.4	11.9 (UCI:12.4, LCI:11.4)
	Male	61	22	42.8	2.6 (UCI:2.8, LCI:2.4)
Wood Frog *Rana sylvaticus*	Female	201	44	13	2.6 (UCI:2.9, LCI:2.4)
	Male	402	173	6	2.4 (UCI:2.5, LCI:2.4)
Total Amphibian Biomass (kg)					108.9 (LCI:107.2; UCI:110.6)

Table 3: A comparison of biomass calculations and methodologies for amphibians within North America.

Species	Site	Method	Water	Area Sampled	Population Estimate	Biomass Estimate
Adult Amphibians						
Ambystoma maculatum	Maine	Continuous drift fence	T	$700km^2$ (11 vernal pools)	3642	47kg[1]
Rana sylvaticus	Maine	Continuous drift fence	T	$700km^2$ (11 vernal pools)	6521	64kg[1]
Plethodon serratus	Missouri	40 10m*10m transects	N/A	5 ha	7300 to 12900 individuals/ha	5 to 10 kg/ha[3]
Caudata (5 species)	North Carolina	2 30m*30m transects	P	N/A	18486 individuals/ha	16.5 kg/ha[4]
Caudata (3 species)	New Hampshire	Mixed	N/A	N/A	29500 individuals/ha	1.7 kg/ha[5]
R.sylvatica	North Michigan	Continuous drift fence	P	0.22ha	$0.39/m^2$	$0.20gms/m^2$ [6]
P.albagula	New Hampshire	12 100*100m transects	N/A	N/A	N/A	$75.67mg/m^2$ [7]

Water Classification: Permanent (P), Temporary (T) pond(s)

1. Powell, J. S. V., Babbitt, K. J. (2015). Despite buffers, experimental forest clear-cuts impact amphibian body size and biomass. PloS one, 10(11), e0143505.
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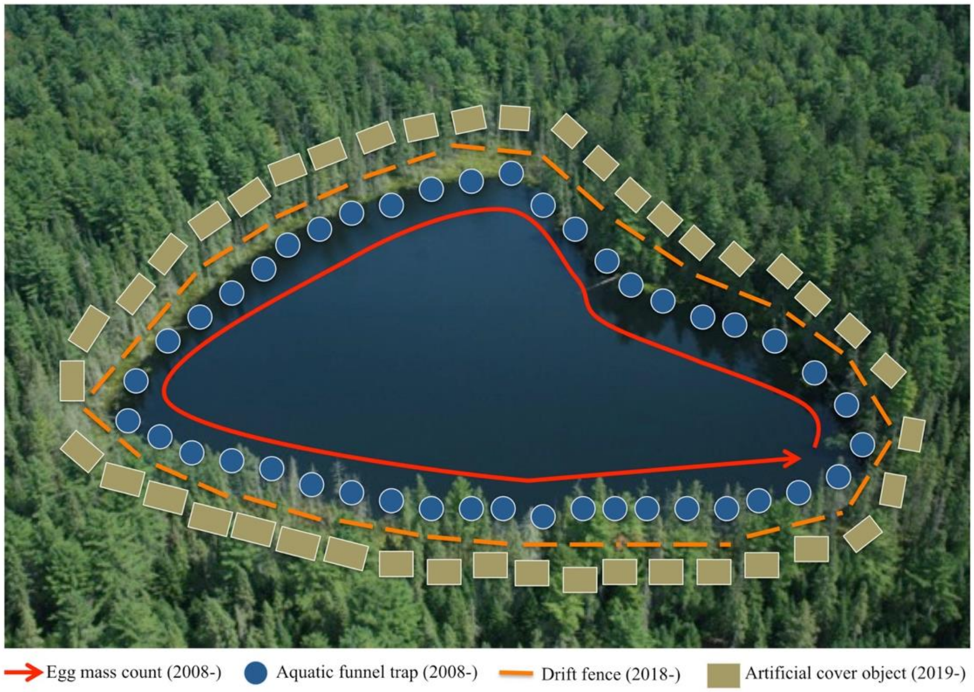

Figure 1: Conceptual representation of the three techniques used at Bat Lake to monitor six amphibian species populations (egg mass counts, aquatic funnel trapping, drift fence, artificial cover). Egg mass counts, and drift fence methods were used in this study. Graphic courtesy of Patrick Moldowan, 2018.

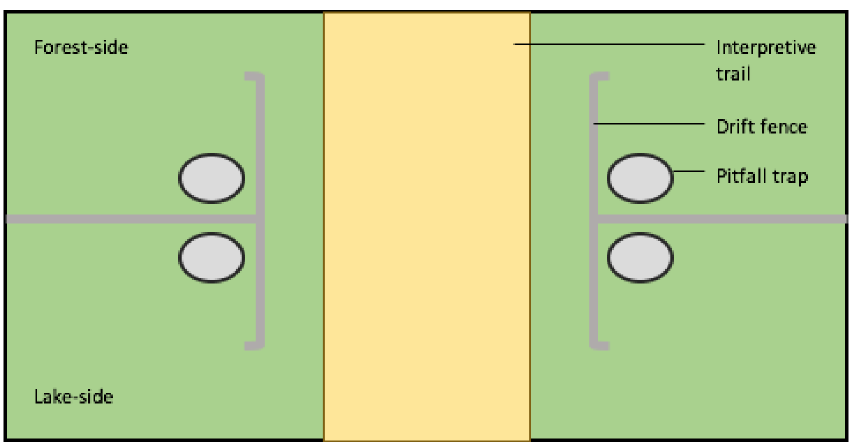

Figure 2: Graphic representation of the drift fence occurring at the discontinuities where the interpretive trail intersects. Pitfall traps and drift fence modifications were performed early in the 2018 breeding season.

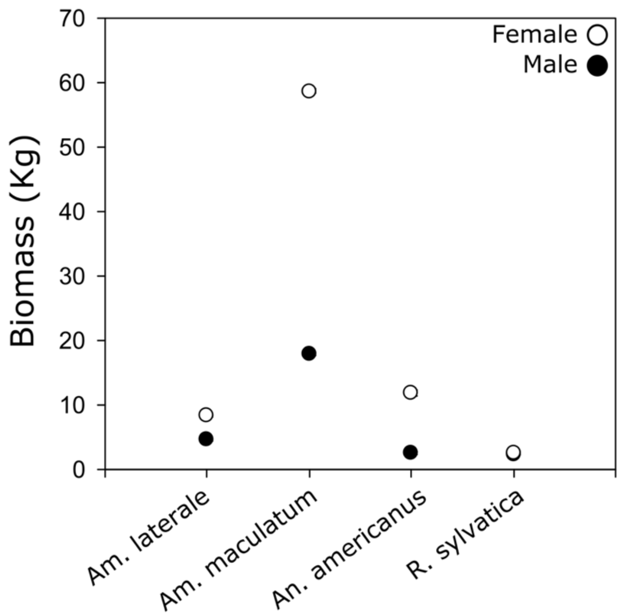

Figure 3: Biomass calculations shown for adult males and females of four species. Total biomass for all species measured was 108.9 kg (107.2, 110.6). 95% confidence intervals are also displayed however due to scale, are not visible.

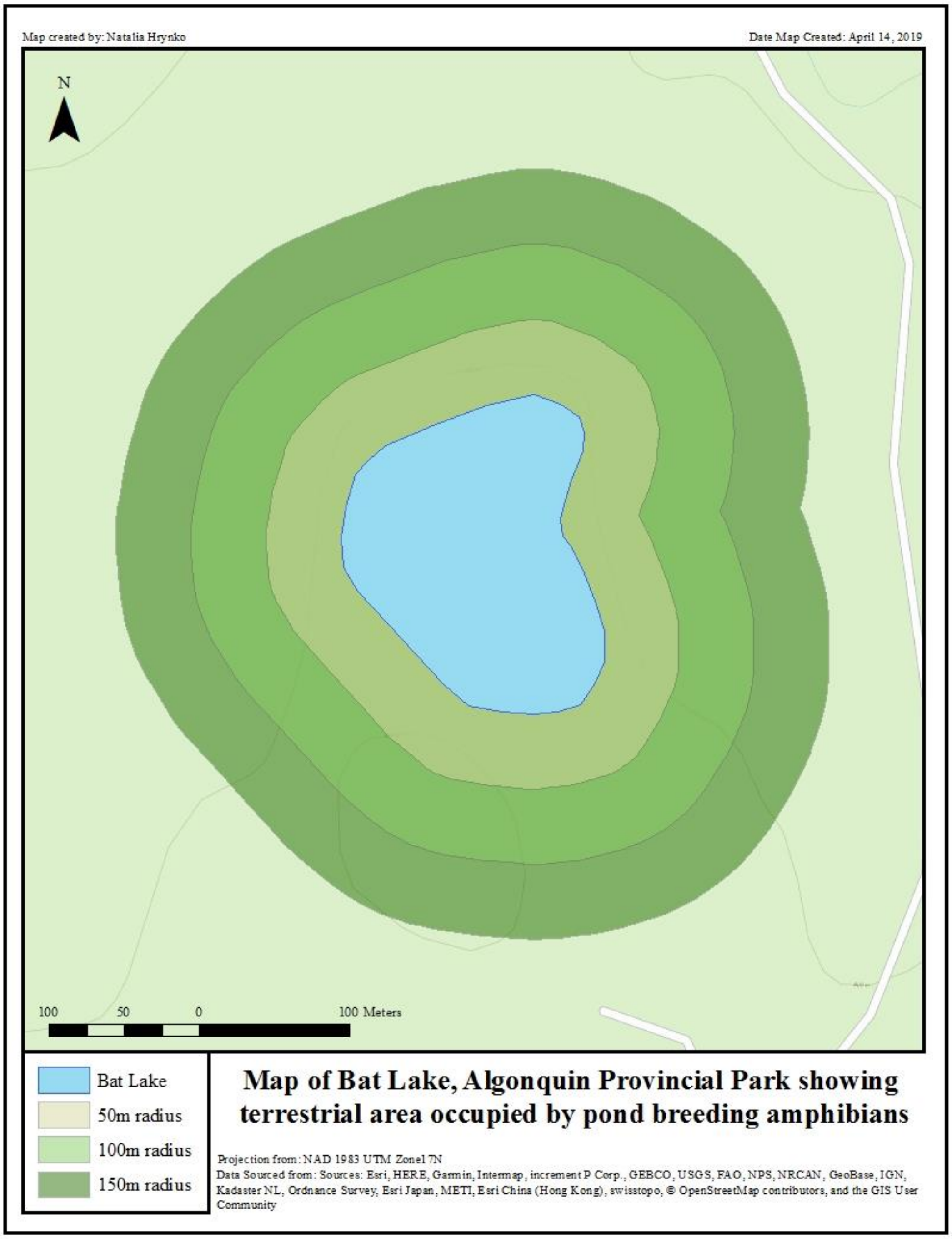

Figure 4: Map of Bat Lake with a radius of 50m, 100m and 150m, in which amphibians are predicted to remain in relation to their breeding site. The total area for each of these respective areas is 3.96ha, 9.48ha and 16.6ha. The density of biomass of the adults that came to breed at Bat Lake over the 2018 seasons within each of these radiuses is respectively 27.5kg/ha, 11.5kg/ha and 6.7kg/ha.

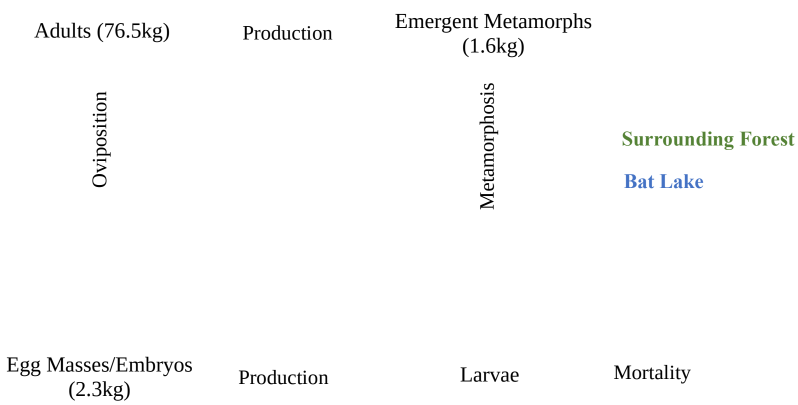

Figure 5: Nutrient cycling of *Am. maculatum* at Bat Lake, Algonquin Park, during 2018 quantified using biomass. Modified figure from Regester J. W., (2006).

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