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The transfer of neurotoxic BMAA from cyanobacterial blooms in Province Lake, NH food chain

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Abstract

Cyanobacteria blooms are an increasing problem worldwide. Current studies are focusing on the cyanotoxins produced, their transfer across the food web, bioaccumulations, and the resulting effects on humans and wildlife. In the summer of 2019, the body of a five-week-old Common Loon was found on Province Lake, New Hampshire. As cyanobacterial blooms had been observed on the lake, we investigated whether cyanotoxins could be a possible underlying cause for the death of the chick, and possible sources of transfer. A necropsy was performed to learn whether and at what levels the neurotoxin BMAA was detectable in the loon. Samples of lake water, phytoplankton, zooplankton, and fish were also collected from Province Lake and tested for BMAA using the ELISA technique. BMAA was found to be present at all trophic levels in the lake with a 44.8-fold biomagnification from phytoplankton to zooplankton and biodilution higher up in the food chain leading to the loon. While the loon's immediate cause of death was determined to be attack-related trauma, sublethal concentrations of BMAA may have impacted the bird's survivability and contributed indirectly to its death. The detection of BMAA in loon lung tissue raises the question of whether, in addition to transfer via the food chain, inhaled aerosolized cyanobacteria could be another route of exposure to cyanotoxins for both animals and humans.

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Introduction

Cyanobacteria are photosynthetic bacteria found in aquatic environments worldwide. Assisted by human activities, cyanobacterial blooms are an increasing problem in nutrient-rich water bodies (Zurawell 2001). Several bloom-forming species of cyanobacteria are known to produce neurotoxins and hepatotoxins affecting both wildlife and humans. One such neurotoxin is β-N-methylamino-L-alanine (BMAA), a non-protein amino acid produced by a majority of cyanobacteria (Cox et al. 2005). It was first discovered in the seeds of the Cycad tree (*Cycas micronesica*) that houses the symbiotic *Nostoc* cyanobacteria. The water-soluble toxin exists both in free form and in protein-bound form and can be released from its bound state by acid hydrolysis (Murch et al. 2004, Jonasson et al. 2008). Although BMAA in the free unbound state is highly toxic, it is not known whether the protein-bound toxin can be transferred from prey to predator during feeding or what the biological effects of bound BMAA might be. Long-term dietary exposure to BMAA is a risk factor for the neurodegenerative disease amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC), previously observed in the Chamorro people of Guam, who consumed flying foxes that fed on cycad seeds (Cox et al. 2003, 2016). Not much is known about the effects of the cyanotoxin in wildlife such as fish and birds. BMAA from epiphytic Stigonematales cyanobacteria growing on aquatic *Hydrilla verticillata* plants has been linked with the neurological disease avian vacuolar myelinopathy (AVM) in wetland birds in the southeastern United States (Bidigare et al. 2009).

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Cyanobacterial toxins such as BMAA may be transferred through food webs, sometimes biomagnifying while biodiluting in others (Cox et al. 2003, Brand et al. 2010, Zguna et al. 2019). Over the past decade, Province Lake, New Hampshire has experienced repeated cyanobacterial blooms in summer months. Blooms in New Hampshire lakes are commonly dominated by the genera *Gloeotrichia*, *Merismopedia*, *Anabaena*, *Aphanizomenon*, *Oscillatoria*, *Coelospharium*, *Lyngba*, and *Microcystis* (FB Environmental Associates 2014). In July 2019, a pair of Common Loons on Province Lake successfully hatched a single chick, prompting delighted locals to affectionately name it Luna (Province Lake Association 2019). The body of the chick that grew up during an active cyanobacterial bloom was found dead on the lake 33 days later, having died of an unknown cause (Samuelson 2020). The death of the loon raised questions as to whether cyanotoxins may have been a contributing factor, which this study sought to investigate. The first step was to determine whether cyanotoxins were present in phytoplankton of Province Lake. Secondly, if present in the phytoplankton (cyanobacteria), were the toxins also present in the food chain that led to the loons? Thirdly, did the toxin biomagnify as it moved up the food chain, i.e. become more concentrated in the tissues of organisms in higher trophic levels? Finally, were the cyanotoxins detectable in the deceased loon, and if so, were they at levels that might have caused impairment?

Methods

Study Site – Province Lake is a shallow lake primarily in southern Carroll County, NH and also in Maine, with a mean depth of 2.8 m and a maximum depth of 4.9 m. Covering 4.1 km^2, it is bordered by three towns: Wakefield and Effingham, New Hampshire and South Parsonsfield, Maine (Fig. 1). It has a low flushing rate as it requires roughly one year to replace the

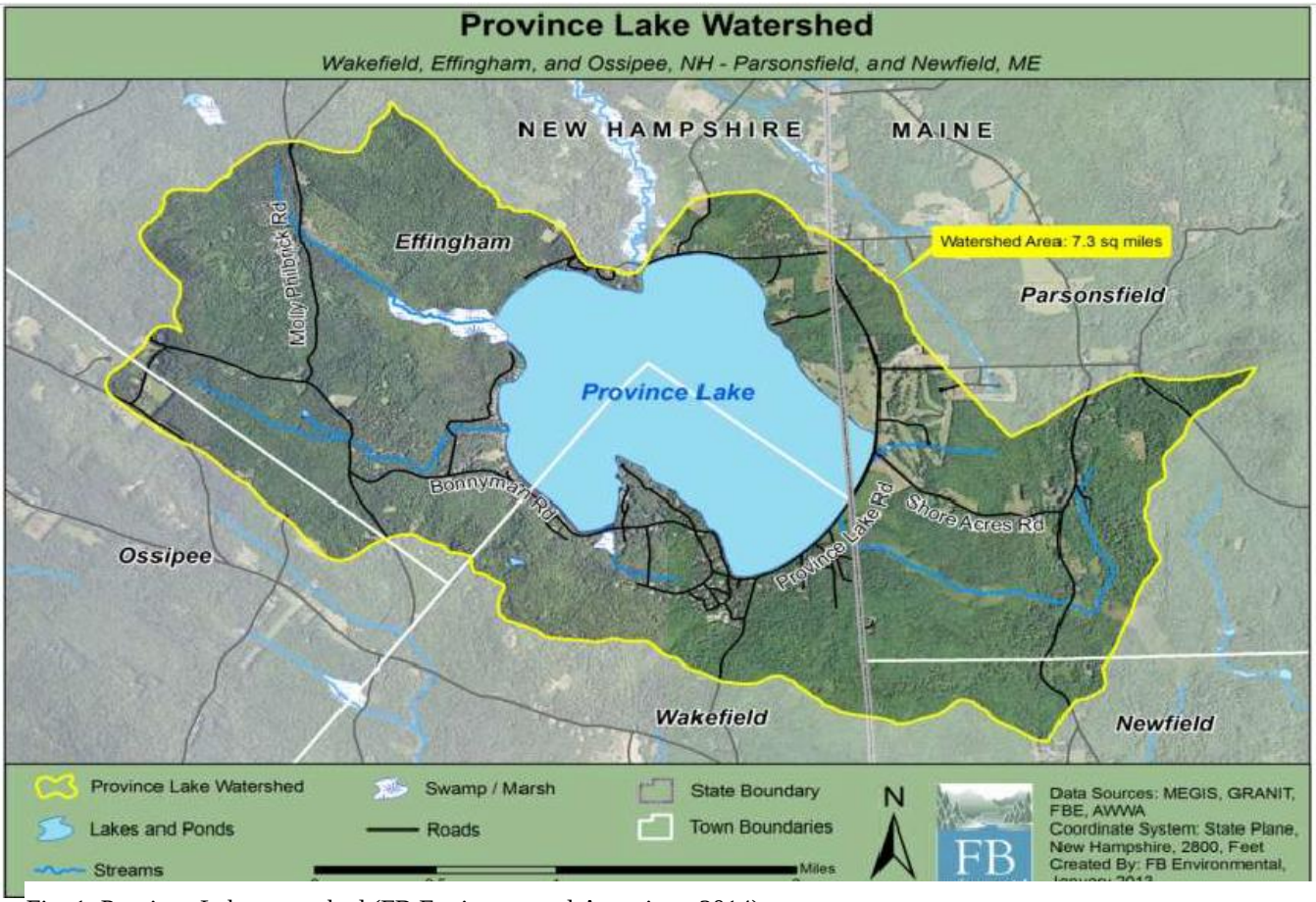

Fig. 1: Province Lake watershed (FB Environmental Associates 2014).

volume of water in the lake basin. It is classified as a mesotrophic lake with elevated phosphorus concentrations that have been rising over three decades of water quality monitoring (FB Environmental Associates 2014). The lake has experienced repeated cyanobacterial blooms in summer months, and the loon chick in the current study hatched and grew up during an active cyanobacterial bloom (Samuelson 2020).

Field Collection – Collection and analysis of whole lake water (WLW), phytoplankton and zooplankton samples were carried out by the UNH Field Lake Ecology class under the direction of Drs. A. Baker and J. Haney. Sampling occurred on 10 September 2019 between 15:30-17:00. Vertically integrated WLW samples were collected with a transparent polyethylene integrated tube (IT) (University of New Hampshire, Center for Freshwater Biology, Durham, NH) submerged to a depth of 3 m (U.S. EPA 2017). A concrete weight was fixed to the 6 m-long tube, which had a diameter of 19 mm. WLW was poured through a 50 µm filter funnel and into a graduated cylinder to obtain <50 µm water containing small, non-bloom forming picocyanobacteria.

Plankton were collected using a 14.7 cm diameter tow net with 50 µm Nitex mesh. The pre-rinsed net was lowered to a depth of 3 m and then slowly raised to the surface. A pocket ZAPPR (Lim-Tech, North Andover, MA) was used to separate the plankton samples into phytoplankton and zooplankton using light-induced phototaxis. Following separation, the zooplankton and then the phytoplankton were collected on 50 µm Nitex mesh and frozen until used for toxin analysis.

One Pumpkinseed Sunfish (*Lepomis gibbosus*) was collected from Province Lake on 19 August 2019 and five smaller sunfish (genus *Lepomis*) on 10 September 2019; the fish were frozen whole until tissue extraction and toxin analysis. Frozen samples of loon lung and liver were obtained from Dr. Mark Pokras, Tufts University, where the necropsy had been performed on the loon in December 2019.

Fish Dissection – The fish were dissected to extract two muscle and two liver tissue samples from the Pumpkinseed Sunfish, and two muscle tissue samples per specimen from the smaller sunfish. These samples were stored at -20°C until toxin analysis.

Toxin Extraction and Analysis – Toxin analysis was carried out on the WLW and plankton samples ~~as described by Veitch (2019).~~ Plankton samples were placed into 2 mL microcentrifuge tubes together with 1 mL of Milli-Q water. Toxins were released from cells in plankton and fractionated water samples by three rapid Freeze-Thaw-Vortex-Sonicate (FTVS) cycles. Samples were frozen at -20°C, thawed in a 40°C warm water bath, vortexed for 1 min at 1,500 rpm using the LP Vortex Mixer (ThermoFisher Scientific, Waltham MA), and sonicated for 3 min in the Ultrasonic Bath CPX/CPXH series (ThermoFisher Scientific, Waltham MA). Plankton samples were then centrifuged for 10 min at 10,000 rpm, after which the supernatants were transferred to new 2 mL microcentrifuge tubes. WLW samples were concentrated approximately 20 times and the plankton sample supernatants approximately five times in the Savant SPD1010 SpeedVac (ThermoFisher Scientific, Waltham MA) to increase toxin detection. These concentrate samples were stored frozen at -20°C until toxin analysis.

For the fish and loon tissue toxin extractions, two samples were taken from each tissue and macerated in the microcentrifuge with an electric homogenizer and Teflon pestle. Weighed tissue subsamples (~0.05 g) were mixed with 1 mL Milli-Q water in 2 mL microcentrifuge tubes and stored at -20°C. To release the toxins from the tissue, samples were frozen, thawed in a warm water bath (40°C), vortexed for 1 min at 1,500 rpm and sonicated for 3 min in a warm water bath. This was repeated twice more for a total of three FTVS cycles. Samples were centrifuged for 10 minutes at 10,000 rpm, after which the supernatants were transferred to new 2 mL microcentrifuge tubes and frozen. Frozen supernatants were concentrated in a SpeedVac for ~6 hr and diluted to 0.3 mL with Milli-Q

water. The samples were then stored frozen until toxin analysis.

The concentration of free BMAA was quantified using an enzyme-linked immunosorbent assay (ELISA) (Eurofins Abraxis). Samples were thawed and centrifuged at 10,000 rpm for 1 min 30 s before running the ELISA to minimize interference from suspended particles. Absorbances from the completed ELISA were measured using the 800TS Microplate Reader and Gen 5 Microplate Reader and Imager Software (BioTek, Winooski VT) with the primary wavelength set to 450 nm and the reference wavelength set to 630 nm. BMAA concentrations were determined from standard curves fitted with a four-parameter logistic (4-PL) equation.

Wet-to-Dry Weight Conversion – For the wet-to-dry weight conversions, weighed subsamples (0.05 to 0.7 g) of wet tissue were dried overnight in a 60°C oven and then reweighed. The fraction of mass lost for each sample was recorded as the correction factor for wet-to-dry weight conversion (Table 1). All BMAA concentrations in organisms are calculated per unit dry weight (DW).

Table 1: Wet-to-dry weight conversion factors. SF, sunfish; PSF, pumpkinseed sunfish; CL, common loon.

Species	Tissue Type	Fraction of Mass Lost	Multiply Factor
SF	Muscle	0.74	0.26
PSF	Muscle	0.79	0.21
PSF	Liver	0.77	0.23
CL	Liver	0.74	0.26
CL	Lung	0.85	0.15

Results

Toxin Concentrations – *Anabaena/ Dolichospermum* were identified as the dominant cyanobacteria in the 2019 Province Lake blooms (NHDES 2019). BMAA was detected in whole lake water (0.57 ± 0.20 ng L^{-1}) and the <50 µm fraction (0.36 ± 0.08 ng L^{-1}) (Table 2). A large proportion (63%) of BMAA in the lake water was found within the "edible" (<50 µm) fraction of phytoplankton that can be consumed by zooplankton. Furthermore, BMAA was detected at all trophic levels of the food chain (Table 3). The greatest increase in concentration occurred between the phytoplankton (0.12, 0.02–0.22 µg g^{-1} DW) and zooplankton (5.38, 4.79–5.96 µg g^{-1} DW) levels. A lower concentration of BMAA was found in muscle tissue of Pumpkinseed Sunfish (3.27, 3.08–3.45 µg g^{-1} DW) than of smaller sunfish (4.12 ± 0.27 µg g^{-1} DW). Differences in BMAA levels were observed between tissues as well as between organisms (Fig. 2). The Pumpkinseed Sunfish had a higher BMAA concentration in its liver (4.41, 4.35–4.46 µg g^{-1} DW) than in its muscle, while the Common Loon had a higher concentration in its lung (3.84, 3.44–4.24 µg g^{-1} DW) than in its liver (3.00, 2.78–3.23 µg g^{-1} DW).

Table 2: Mean BMAA concentrations in WLW and <50 water. WLW, whole lake water; <50; water fraction containing phytoplankton smaller than 50 µm.

Sample Type	BMAA Mean ± SE (ng L^{-1})
WLW	0.57 ± 0.20
<50	0.36 ± 0.08

Table 3: BMAA conc. Phyto, phytoplankton. Zoo, zooplankton; SF, sunfish; PSF, pumpkinseed sunfish; CL, common loon.

Species	Tissue	BMAA Mean (Min–Max) (µg g^{-1} DW)	Species Average BMAA Mean (Min–Max) (µg g^{-1} DW)
Phyto	Whole cell	0.12 (0.02–0.22)	0.12 (0.02–0.22)
Zoo	Whole body	5.38 (4.79–5.96)	5.38 (4.79–5.96)
SF	Muscle	4.12 ± 0.27 SE	4.12 ± 0.27*
PSF	Muscle	3.27 (3.08–3.45)	-
PSF	Liver	4.41 (4.35–4.46)	3.84 (3.08–4.46)
CL	Lung	3.84 (3.44–4.24)	-
CL	Liver	3.00 (2.78–3.23)	3.42 (2.78–4.24)

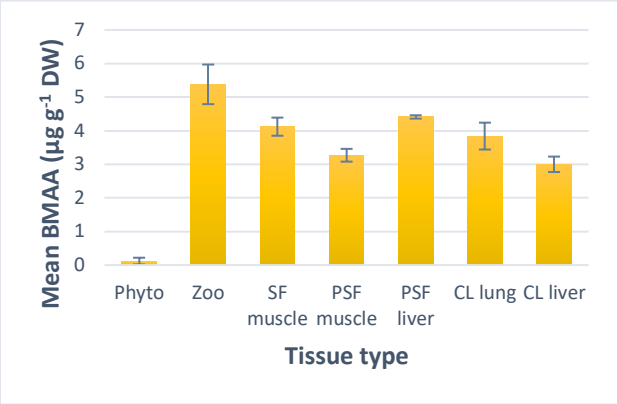

Fig. 2: Mean BMAA concentration in each species or tissue. Error bars indicate ± 1 standard error for SF muscle; range for all other tissues. Zoo, zooplankton; SF, sunfish; PSF, pumpkinseed sunfish; CL, common loon.

Bioaccumulation – BMAA concentrations were averaged for each organism (Table 3) in order to calculate their biomagnification factor (BMF), or the BMAA concentration in one trophic level divided by the BMAA concentration in the trophic level below it:

$$\text{BMF} = ([\text{BMAA}]\ \text{TL}_n) / ([\text{BMAA}]\ \text{TL}_{n-1})$$

where TL denotes trophic level. A BMF > 1 indicates biomagnification while a BMF < 1 indicates biodilution.

The highest BMF of 44.8 was found in zooplankton. In all higher organisms, biodilution of BMAA occurred as the toxin was more concentrated in their diet than in their own tissues and the BMF was less than one (Fig. 3).

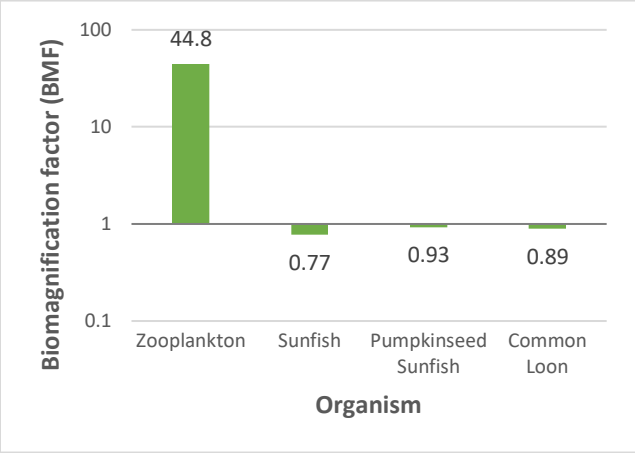

Fig. 3: Biomagnification factor (BMF) at each trophic level are on a logarithmic scale to clearly display either biomagnification (BMF > 1) or biodilution (BMF < 1).

Discussion

BMAA in Food Webs – BMAA has been identified in aquatic as well as terrestrial ecosystems around the world, especially in water bodies where cyanobacterial blooms occur. There is mixed evidence for its biomagnification through various food webs: Cox et al. (2003) suggested biomagnification in the Guamanian terrestrial food web while Zguna et al. (2019) found no support for biomagnification in the brackish Baltic Sea food web. The distribution of BMAA in South Florida marine food webs did not follow a typical biomagnification pattern because some organisms near the base of the food web showed higher BMAA concentrations than higher predators (Brand et al. 2010). Furthermore, higher levels of BMAA were detected in organisms that fed on the benthos rather than on plankton, indicating limitations of assuming a simplified single food chain. BMAA may biomagnify in some marine ecosystems, as it has been detected together with mercury in apex predators, i.e. sharks. Interestingly, the highest BMAA level was found in bonnethead sharks that form a link to the benthic food chain (Mondo et al. 2012, Hammerschlag et al. 2016).

The pattern of BMAA transfer observed in the Province Lake food chain corresponds to the findings of Brand et al. (2010) in Florida's marine food web. However, a comparison of the sunfish data in the present study with a fish from Brand et al.'s study in the same *Centrarchidae* family reveals a much higher concentration of total BMAA in the Largemouth bass (*Micropterus salmoides*) than in the Province Lake sunfish: 1040 µg g^{-1} in the head and 2317 µg g^{-1} in the tail (est. dry weight) (2010). This substantial difference may result from differing levels of the cyanotoxin in the overall food webs and/or Largemouth bass generally occupying a trophic level higher than sunfish. There are also methodological differences in the two studies, as Brand et al. analysed total BMAA using LC/MS/MS while the ELISA method used in the present study detected only free BMAA.

The ELISA provides an effective, quick and relatively inexpensive way to screen samples for BMAA but it can only be used to evaluate free

BMAA, since the acid hydrolysis necessary to extract protein-bound BMAA from tissues would interfere with the procedure (Clausi et al. 2016). The concentration of free BMAA found in Province Lake phytoplankton (0.12, 0.02–0.22 µg g^{-1} DW) is comparable to that previously found in an axenic culture of *Nostoc* cyanobacteria (0.3 µg g^{-1} DW) (Cox et al. 2003). Studies that assessed protein-bound as well as free BMAA detected higher concentrations in cyanobacteria by two orders of magnitude; generally a greater proportion of BMAA exists in the protein-bound than in the free form *in vivo* (Murch et al. 2004, Cox et al. 2005). The concentration of BMAA detected here in zooplankton (5.38, 4.79–5.96 µg g^{-1} DW) is also similar to the range of values reported for zooplankton in a study of a marine ecosystem (~7.5-9.5 µg g^{-1} dry mass), although the latter study examined total BMAA (Zguna et al. 2019).

The finding that a substantial portion of BMAA in the lake was in the edible (<50 µm) fraction of phytoplankton indicates the toxin is accessible to the zooplankton that feed on these small phytoplankton, making transfer up the food chain possible. Higher levels of BMAA in zooplankton than in the phytoplankton may also indicate that BMAA can be transferred laterally via the microbial food web when zooplankton selectively feed on phagotrophic protists, such as ciliates, that have accumulated the toxin (Zguna et al. 2019). In Province Lake, biomagnification was observed only from phytoplankton to zooplankton. The large BMF of 44.8 may be due to a combination of both a low phytoplankton toxicity and a relatively high zooplankton toxicity. In 2013, BMAA concentrations were five or six times higher in the lake's phytoplankton but approximately half as high in zooplankton compared to the present values, yielding a BMF about 1/10 that found in the current study (Cocchiola and Kott 2013). However, the small values reported here are particularly susceptible to errors (the current concentration in phytoplankton, 0.12, 0.02–0.22 µg g^{-1} DW gives a percentage error of 83%) and are a source of variability in results. Additionally, the comparison of all phytoplankton and zooplankton is simplistic and may overestimate the biomagnification factor; since the phytoplankton fraction collected from the pocket ZAPPR is mostly too large to be grazed by the zooplankton, and a comparison with the <50 µm edible fraction might be more realistic in future studies. Dry weights of the edible fraction were not determined in this study.

Biomagnification also depends on the tissues assayed. As can be seen from the differences in BMAA concentration in different tissues for the Pumpkinseed Sunfish and Common Loon, the tissues that are studied affect the BMF values obtained. It is therefore important to study either a consistent tissue throughout the trophic levels or a much wider range of tissues for better comparison. As in all studies, performing analysis on more samples from each trophic level would strengthen the results and allow for more robust statistical analyses.

Biodilution seemed to occur at higher trophic levels, with the fish having lower BMAA concentrations than zooplankton. This might be explained by the fact that fish were collected near the lake shore while plankton were collected in the middle of the lake; thus the fish may have been feeding on other more littoral food sources such as insects or benthic crustaceans, which are known components of some freshwater sunfish diets. Similarly, although Common Loons feed primarily on perch, sunfish and other fish, they will seek alternative food sources from crustaceans, snails, leeches and insect larvae if water turbidity is unconducive to fishing (Cornell Lab of Ornithology 2019, accessed 20 June 2020). The shallow waters of Province Lake are also inhabited by molluscs which, as filter feeders, may be particularly susceptible to accumulating cyanotoxins from the water and it is possible that these were fed to the young loon, contributing to its exposure to BMAA. Conclusions drawn about biomagnification are constrained by the assumptions made about dietary linkages and the simplified food chain that is not representative of the complicated interactions occurring in the lake food web. Future studies should investigate a broader range of food sources for organisms in Province Lake and use stable isotope analysis to establish quantitatively the trophic connections.

The animals' varying life histories and time lags between data collection must also be taken into account when studying the trophic transfer of cyanotoxins. Plankton have a lifespan ranging from a few days to weeks and the loon in this study lived for 33 days before its death. Fish can live, and thus potentially accumulate toxins, for many years. Organisms will consume prey that may not have reached full size and the young loon was probably not eating large mature sunfish like the Pumpkinseed Sunfish examined but may have been feeding directly on the smaller fish. The young age of the bird may explain why more BMAA had not accumulated in its tissues; thus, it would be helpful to examine the blood or tissues of adult loons in Province Lake to see if they are higher. Moreover, the plankton and fish samples collected in September may not be reflective of the environmental conditions that the loon was exposed to in July and August. Studies have pointed out the need for temporal and spatial resolution in bioaccumulation research to overcome these limitations (Zguna et al. 2019).

There is limited information in the literature about BMAA in birds. It has been detected in small amounts in feathers from Lesser Flamingos collected from the freshwater Lake Bogoria and Lake Nakuru ecosystems (77 and 59 ng g^{-1} DW, respectively) during a mass mortality event (Metcalf et al. 2013). Lesser Flamingos feed primarily on cyanobacteria and deposition into feathers could be a way of excreting cyanotoxins from their bodies. No other studies were found in which BMAA has been detected and quantified in the tissues of naturally exposed birds. BMAA from epiphytic Stigonematales cyanobacteria growing on aquatic *Hydrilla verticillata* plants has been linked with the neurological disease avian vacuolar myelinopathy (AVM) in wetland birds in the southeastern United States, and the presence of these cyanobacteria has been confirmed in Florida freshwater (Bidigare et al. 2009, Williams Jr et al. 2009). However, BMAA has not been detected in wild birds diagnosed with AVM from one of these wetland reservoirs (Haynie et al. 2015). Quails dosed with BMAA were shown to deposit the toxin in their eggs (Andersson 2018). If the adult loons of Province Lake were exposed to BMAA and deposited it in their eggs, the chick may already have been exposed to the toxin before hatching, with unknown consequences.

Toxic Effects of BMAA – The immediate cause of death for the 33-day-old female loon chick was determined from the necropsy to be trauma, likely resulting from an attack by an intruding loon (Samuelson 2020). However, the possible indirect contribution of BMAA to its survivability cannot be ruled out. Since BMAA has been linked to neurological damage, it is possible that the chick was functioning at subpar capacity and was less able to avoid or defend itself against the attack. In nature and under strong selective pressures, it is likely that the major impact of BMAA is at sublethal rather than acute concentrations. Sublethal effects of BMAA administered in the water have been demonstrated in larvae of the mangrove rivulus fish (*Kryptolebias marmoratus*), significantly affecting their movement and predatory behavior, leading to an increased failure rate of prey capture and possibly higher energy expenditure to successfully capture the same amount of prey (Carion et al. 2018). Dietary exposure to BMAA has also been shown to lead to neurotoxicity, decreased olfactory learning and increased mortality in honeybees (Okle et al. 2013). Quantitative data on the chronic effects of BMAA in humans and other animals are severely lacking. The presumptive median lethal dose (LD_{50}) for female and male mice injected with various acute concentrations of BMAA was 3 mg g^{-1} body weight, at which dosage BMAA was detected in brains and livers at similar concentrations (by UPLC-UV) or concentrations a few times higher (by LC/MS/MS) compared to the levels in the loon in this study (Al-Sammak et al. 2015). Half of the mice treated with this dose of BMAA became moribund and developed adverse clinical symptoms including muscle twitches, convulsions, and uncontrolled urination and defecation (2015). While data for mice and birds are not directly comparable, considering the paucity of data, acute toxicity figures for mice may be helpful in understanding quantitatively the possible effects of BMAA in birds. It has not

been ascertained whether toxicity would be caused by the acute or chronic effects of BMAA.

BMAA may also have interacted with other toxins to impact the loon's health. For example, it has synergistic effects with mercury by depleting the antioxidant glutathione (Rush et al. 2012). Province Lake has a history of high levels of mercury resulting in fish consumption advisories from the state. Common Loon eggs in New Hampshire have moderate levels of mercury, although the concentration in Province Lake loon eggs is not known (Loon Preservation Committee 1999, NHDES 2018). Additionally, the co-occurrence of BMAA with other cyanobacterial toxins such as microcystins has been demonstrated (Metcalf et al. 2013). Therefore, it would be valuable to analyze the current study's tissue samples for microcystins as well to determine in what concentrations they are present (if at all) and compare their transfer in the food chain to that of BMAA.

Alternative Sources of BMAA – It is remarkable that a loon that had spent only five weeks on the lake had accumulated relatively high levels of BMAA in its tissues, although it was already mentioned that exposure to the toxin may have occurred even before hatching. The relatively high level of BMAA in lung tissue, compared with liver tissue, raises questions about the source of BMAA. During this research, it was assumed that the loon chick was exposed to BMAA via its diet. Although possible, it seems unlikely that the BMAA in the lungs came from the digestive system. These results suggest an alternative route of exposure to BMAA from aerosols that the loon breathes in and accumulates first in the lungs. It is possible that the BMAA is then passed on to the liver via the bloodstream. This alternative route of BMAA transfer is illustrated in Fig. 4. Both (pico)cyanobacterial cells and cyanotoxic microcystins have been identified in aerosols from New England lakes, including Province Lake (Murby and Haney 2016, Langley 2019). Furthermore, BMAA and microcystins have been detected in aerosols from Lake Mascoma, NH, near which a cluster of ALS cases were found (Banack et al. 2015).

Cyanotoxins in water are known to be aerosolized by continuous evaporative processes that occur both during the day and at night (Langley 2019). Lake aerosols can also occur through disturbances by recreational activities, wave action and bubble bursting while cyanobacteria living in cooling towers may be another potential source of airborne BMAA that can then be breathed in by humans (Stommel et al. 2013). The presence of cyanobacterial cells within the upper respiratory tract and central airway of humans suggested an aerosol route of exposure to cyanotoxins (Facciponte et al. 2018). Cyanobacteria cells were detected in 84.6% of postmortem lung tissue patients previously living within ¼ mile from a water body (the high risk group); half of these patients also exhibited neurodegeneration (compared with only 7.7% of low risk patients harbouring cyanobacteria in their lungs and none with observed neurodegeneration) (Henegan et al. 2017).

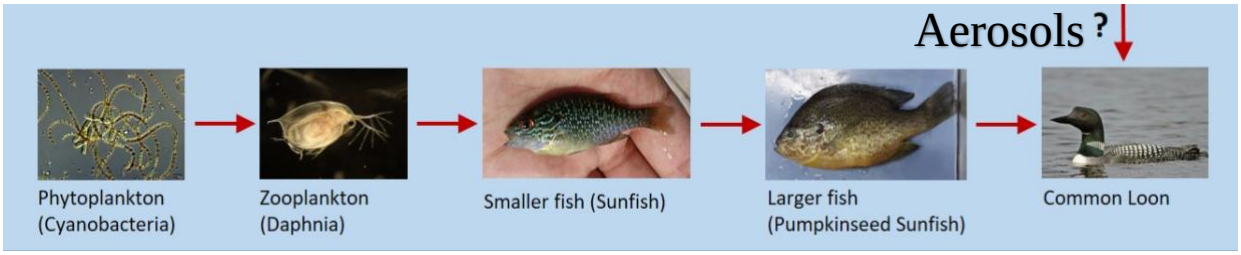

Fig. 4: Model of BMAA transfer in Province Lake, illustrated using the organisms studied. BMAA may be transferred up the food chain; aerosols represent another potential source of cyanobacteria and their toxins. Image credits: "Juvenile Northern Sunfish", by BenitoJuarez98, licensed under (CC BY-SA 4.0); "Common-loon", by Wikisrsshsd

It has been hypothesized that cyanobacteria present in human organs could chronically secrete neurotoxins such as BMAA over extended periods of time to induce neurological

disease in people (Stommel et al. 2013). Aerosols should be considered as a potentially significant source of BMAA exposure and have implications for the health of wildlife and people using or living near water bodies or other areas where cyanobacteria grow.

Summary

- Free BMAA was present in compartments of the Province Lake freshwater ecosystem and entered at the base of the food chain, with 44.8X biomagnification from phytoplankton to zooplankton but with apparent biodilution (<1) at higher trophic levels.
- Differences in BMAA concentration were observed between lung, liver, and muscle tissues. Its presence in common loon lung tissue suggests intake via aerosols (besides dietary exposure) with possible subsequent transport through the bloodstream.
- The neurotoxin may have had sublethal effects on the young loon, contributing indirectly to its death, and could possibly have interacted synergistically with other cyanotoxins and/or mercury.
- Future biomagnification studies using stable isotope analysis could better define trophic connections and should take into account seasonal and spatial variation in cyanotoxin concentrations and compare toxin concentrations in zooplankton specifically with concentrations in the edible (<50 µm) fraction of phytoplankton.

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