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Cyanobacteria and Their Ties to Terminal Disease

Linking Corpses to Cyanobacteria

In the mid 1990s, over 70 bald eagle corpses were discovered in the southeastern United States. The public was in dismay; not only was the species endangered, but bald eagles were, and still are, the hallmark symbol for the American nation. Since the 1700s, bald eagles have been synonymous with courage, strength, dignity, and freedom; but society could do nothing but watch as the species appeared weak and lowly in the face of this unknown threat. Autopsies were performed on the bodies, and it was discovered that all of the eagles had died of a neurological disease called vacuolar myelinopathy, or VM. This disease is characterized by a buildup of white matter in the central nervous system of those infected. The white matter buildup functions as a plaque in the brains of these birds, impairing their brain function and ability to coordinate themselves correctly, ultimately leading to starvation and death. Thereafter, scientists began searching for what could have caused the unforeseen eagle deaths (Breinlinger et al. 2021).

The man-made water reservoirs scattered throughout the southeastern U.S. were quickly identified for investigation, and it was found that most were infested with the invasive plant species *Hydrilla verticillata*; the plants themselves were colonized with a species of cyanobacteria called *Aetokthonos hydrillicola* (Breinlinger et al. 2021). Cyanobacteria are a type of bacteria that can photosynthesize, like plants, to obtain energy. There are thousands of different species of cyanobacteria and they most commonly live in aquatic environments, but can be found elsewhere. Many species can produce toxins, coined as “cyanotoxins" when they come from the bacteria itself (Testai et al. 2016).

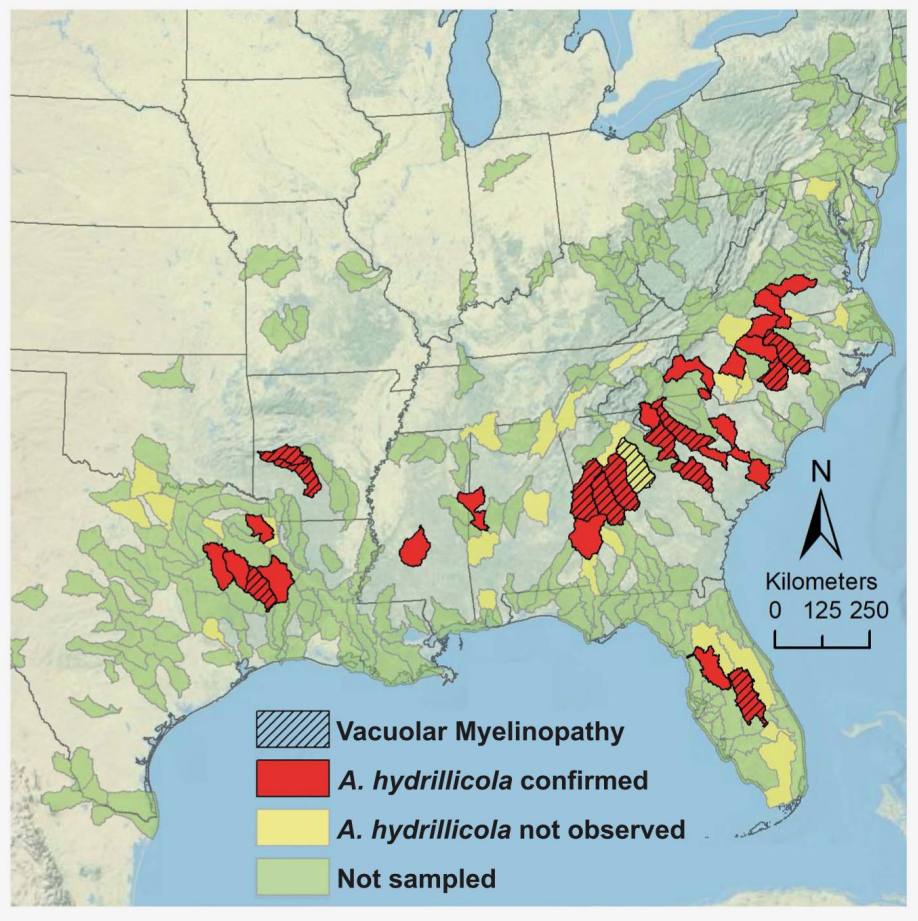


Figure 1. This map shows the correlation between the presence of cyanobacteria species *A. hydrillicola* and the diagnosis of VM in the southeastern United States, where red spaces show confirmed areas of the cyanobacteria, and the hatched line areas show areas with VM (Breinlinger et al. 2021).

In March of 2021, almost 30 years later, researchers proved that cyanotoxins produced by this specific cyanobacteria caused the terminal VM found in the eagles. Although it is reassuring to have found the cause, this realization gives rise to more concerning implications: the fact that cyanobacteria can cause terminal disease.

Confirming the Cause of VM

VM was first discovered in bald eagles, but cyanotoxins can inflict the disease in numerous avian species. When the eagle corpses were being investigated in the 1990s, researchers worked to identify what specifically caused VM in these birds, finding the true "eagle killer" in March of 2020 (Breinlinger et al. 2021).

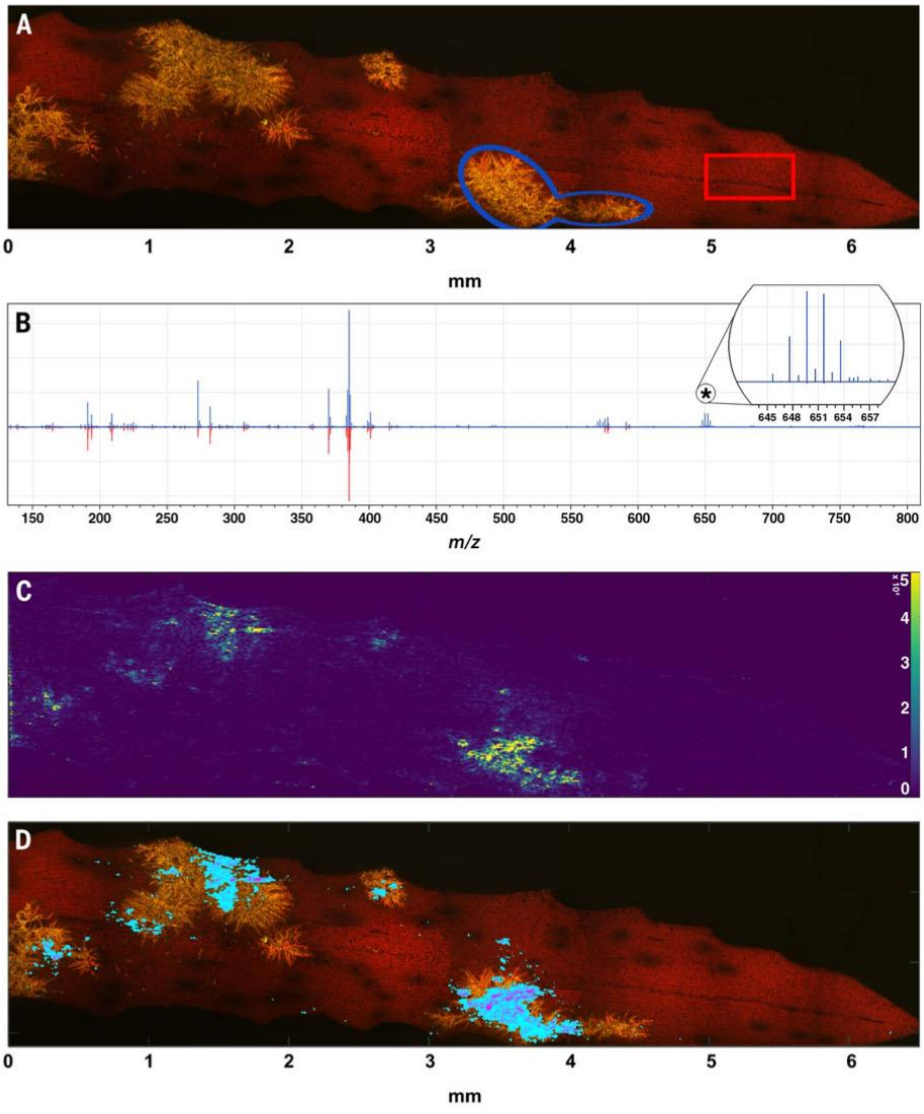


Figure 2. Shows the identification and localization of the cyanotoxin AETX in *A. hydrillicola.* Panel B is the results of mass spectrometry procedure on the marked areas in panel A. Panel C shows the results of a MALDI (matrix-assisted laser desorption/ionization) procedure used to

localize the toxin identified in panel B. Panel D is an overlay of panels A and C to emphasize the localization of the toxin in the cyanobacteria colonies (Breinlinger et al. 2021).

In figure 2, a chemistry procedure called mass spectrometry was performed on an area of a *H. verticillata* leaf, and then on an area of the leaf that was colonized with the cyanobacteria. In this procedure, a laser ionizes and splits molecules, while a detector measures the size and charge of the created ions. By doing this to both areas, the researchers could compare the molecular makeup of both, and identify a molecule exclusive to the cyanobacteria colonies (this can be seen as the enlarged starred area in panel B). After identifying the presumed cyanotoxin, the researchers performed a MALDI procedure. Here, an ionizing laser scans the entire area, and a mass spectrometry graph is produced for each spot the laser hits. Because they already knew which ions they were looking for (from panel B), they were able to measure the relative abundance of these ions in each spot, therefore localizing the presumed toxin. In panel C, the yellow spots are the areas where the most abundance of these ions occurs. Finally, panel D is a visualization that the areas of most abundance are in fact the areas of the cyanobacteria colonies, confirming that this molecule is exclusive to them (Breinlinger et al. 2021). Computer analyses of the ions were able to piece them back together, giving the chemical makeup of the cyanotoxin.

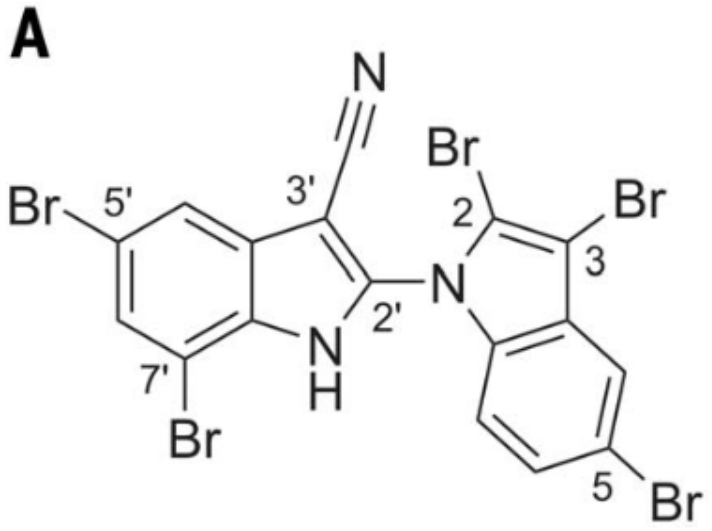


Figure 3. Shows the chemical makeup of the cyanotoxin from *A. hydrillicola*, named aetokthonotoxin, and abbreviated as AETX. The bromine atoms (shown as "Br" in the figure) contribute to the toxicity of the molecule (Breinlinger et al. 2021).

Studies on red-tailed hawks were performed to determine their susceptibility to the disease. In these studies, American coots (a species of waterfowl) infected with VM were collected and fed to rehabilitated red-tailed hawks; various tissues from these coots were fed to the hawks, not just the brain tissue where VM manifests. The control group in this experiment also consisted of rehabilitated hawks, but these hawks were fed coots who did not have VM. These hawks were only fed the tissues for 28 days, yet by the end, all five hawks that were fed the infected coots had VM themselves. The diagnosis was confirmed by examining the hawk's brain tissue, in the process confirming the non-exclusivity of the disease to bald eagles and coots (Fischer et al. 2003).

Concerned for the risk of all avian species in areas of *A. hydrillicola*, Haram et al. (2020) performed a study to determine the risk specific species faced to AETX. A team of researchers used surveillance to assess how often waterbirds used sites infested with *hydrilla*, and how likely

these waterbirds were to scavenge on deceased VM infected carcassess. The results were alarming; the researchers found that waterbird density and richness were significantly greater at areas infested with *hydrilla* (the invasive plant associated with the cyanobacteria), backed with a statistical Wilcoxon signed rank test and statistically significant p-values. They even observed uncoordinated movement and listlessness directly in American coots and ruddy ducks, signs of neurological impairment that are heavily associated with VM. They also used surveillance methods to track scavenger activity by placing dead American coots in front of motion detecting cameras.

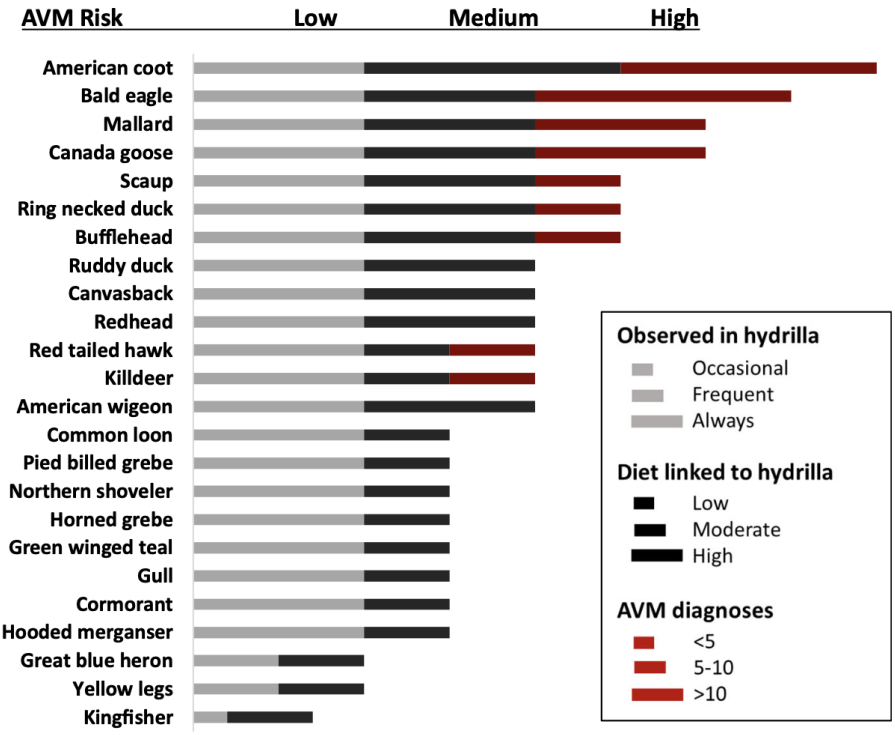


Figure 4. Shows the waterbird risk results from the Haram et al. 2020 study. The length of the bar next to the species name shows the relative risk that species face to AETX and VM, where long bars represent high risk, and short bars represent low risk (Haram et al. 2020).

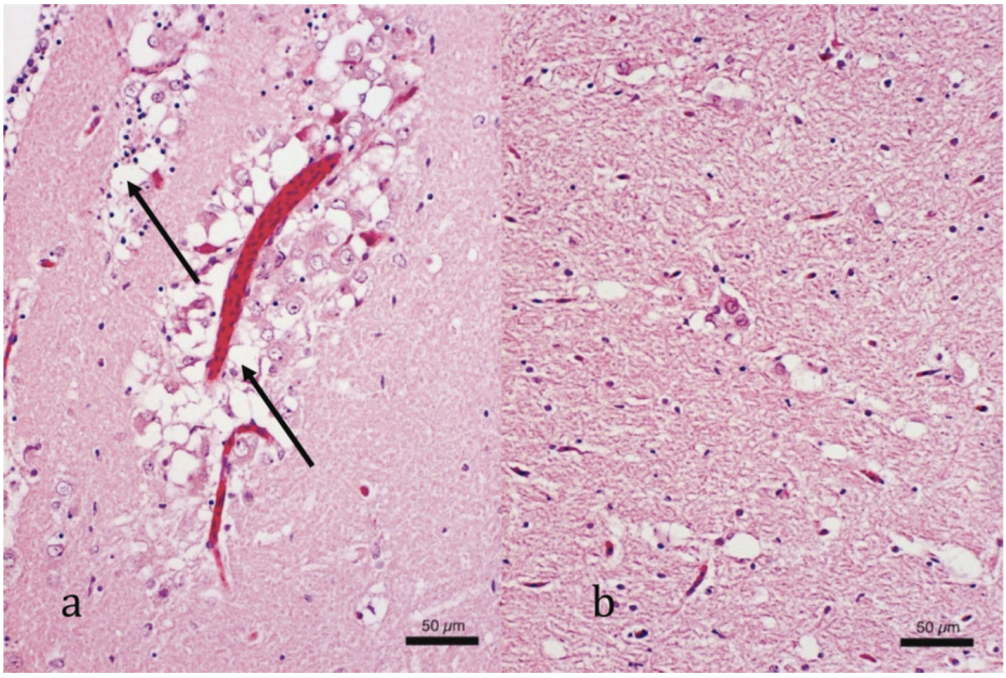
Figure 4 visualizes what factors contribute to VM risk (abbreviated AVM in the figure, for Avian Vacuolar Myelinopathy, which is clinically the same as general VM). The light grey

bars put into perspective the variety and abundance of avian species found in areas of *hydrilla;* almost all the species listed are recorded as "always" found in these areas. Because waterfowl primarily contract VM from ingesting the toxin itself or through ingesting other VM infected birds, diet is also something heavily associated with risk. It is important to note that the red bars in figure 4 only represent recorded VM cases, and do not account for every VM case to exist. It is also important to note that all waterfowl in trophic levels are at risk. Herbivorous birds are drawn to the dense foliage the invasive hydrilla provide, therefore contracting VM directly through ingesting AETX. The predatory birds are then drawn to the dense populations of prey, contracting VM indirectly through birds already infected with the disease. Not only are the populations concentrated here, but having VM impairs a bird's ability to fly, orient, and feed themselves, making them easier prey. Birds that die of the disease are easily scavenged by predators, leading to even more spread of the disease. The graph results are also alarming in that they suggest that VM risk depends on exposure and not susceptibility, meaning that no avian species is safe from AETX (Haram et al. 2020).

VM in Non-Avian Species

The trophic relationship this cyanotoxin has in relation to avian species is a concern; it means that predatory birds do not have to feed directly on AETX to contract a terminal disease. Feeding patterns in ecosystems are complex, and contribute to large, all-encompassing food webs that relate species from all corners of biodiversity. Avian species feed on, and are preyed on by non-avian species, and it is important to consider the non-avian species at risk and how they participate in the spread of VM. Studies similar to that of Fischer et al. 2003, in which red-tailed hawks were fed VM infected coots, were done on triploid grass carp and painted turtles, giving light to how non-avian species participate in the spread of VM.

Triploid Grass Carp:



Painted Turtles:

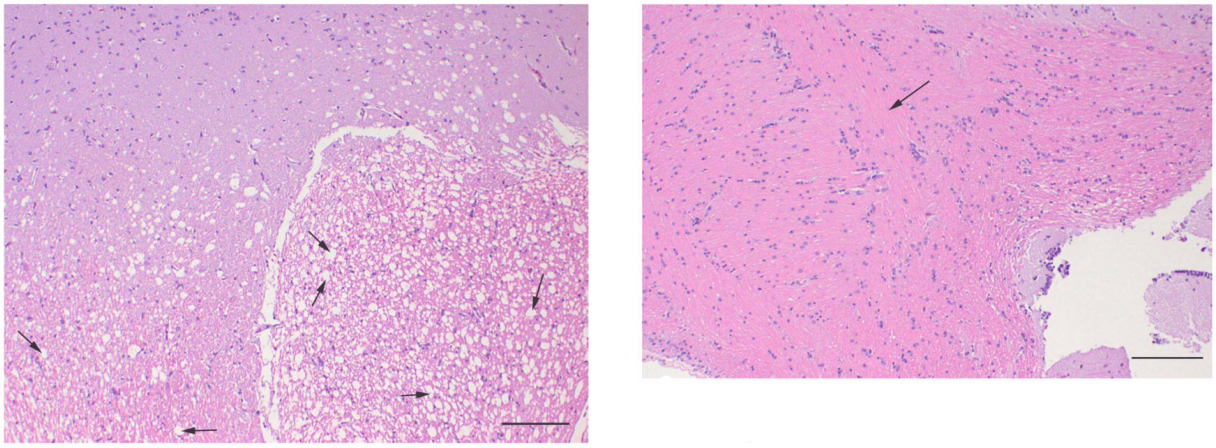


Figure 5. Shows the diagnosis of VM in both triploid grass carp (Haynie et al. 2013) and painted turtles (Mercurio et al. 2014). The right panels show healthy brain tissue, while the left panels show brain tissue from animals infected with VM.

VM is most commonly diagnosed by looking at the white matter vacuolization in the brain tissue. On a molecular level, VM causes destruction by causing a buildup of white matter

in the brain where it should not be. Here, it infiltrates the neurons of the brain and ruptures important cellular membranes, most notably the myelin sheaths of the neurons (Haynie et al. 2013). The myelin sheaths are essential for neurons to conduct electrical impulses in order to communicate with each other and control voluntary movements. This is why VM visually manifests the way it does: by impairing the organism's ability to correctly control their movements. Figure 5 makes it easy to compare brain tissues, healthy brain tissue is on the right, while the VM diagnosed brain tissue is on the left. The black arrows point to white matter, which is more abundant in the organisms with VM. The white, circular vacuoles are easy to identify, and are used to diagnose the disease. The turtles in the Mercurio et al. 2014 study were fed the AETX for almost 90 days by the time the study was over, and had numerous symptoms of VM that are documented in figure 6 (Mercurio et al. 2014). However, the grass carp in the Haynie et al. 2013 study were fed the toxin for about 35 days showed no outward symptoms of the disease (Haynie et al. 2013). It is unclear if the carp showed no symptoms because of a unique susceptibility to the disease, or because the study concluded before symptoms were able to manifest.

Table 1. Clinical signs observed in the treatment group turtles after the first observed deficits on day 82 of the experiment.

ID #	Anorexic?	Gait and movement Normal?	Able to Swim?	Mentation	Spinal and other Reflexes Normal?	Could keep head in horizontal plane when rotated and listed?
107	Yes	Would not ambulate	Floating upside down	Stupor	No attempt to right itself	Yes
104	Yes	Ataxia	No	Stupor	Unable to right itself	Reduced ability
118	No	Ataxia	Yes	Depressed	No head withdrawal, no attempt to right itself	Reduced ability
85	Yes	Ataxia	Yes	Alert	Unable to right itself and head withdrawal reflex was reduced	Yes
119	No	Ataxia	Yes	Alert	Unable to right itself	Yes

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Figure 6. Gives light to the physical manifestations and symptoms VM inflicted on the painted turtles in the Mercurio et al. 2014 experiment. Ataxia refers to general coordination, while ambulate is in reference to walking (Mercurio et al. 2014).

The fact that various aquatic species are susceptible to VM, and that they have the potential to carry it through the food web is concerning for several reasons. There is a possibility that AETX has the potential to bioaccumulate or biomagnify in the environment. Bioaccumulation refers to the buildup of a molecule over time in a single organism as it ages, while biomagnification refers to the buildup of a substance as it moves up the food web to apex predators (National Geographic Society 2021). One of the most famous cases of biomagnification in the environment was DDT, which was a toxic chemical used in pesticides in the 1940s and 50s. As predatory animals preyed upon organisms that had relatively low concentrations of DDT in their bodies, DDT would build up, and accumulate in their fatty tissues until they were the ones with the highest concentrations. Like AETX, DDT was also observed in eagles first. Here, the eagles could not produce adequate eggshells, and therefore their eaglets would die before being born (National Pesticide Information Center 1999). The likeness between two toxins are alarming; their ability to move through the food web and attack apex predators should not be taken lightly. DDT was only banned in the U.S. when Rachel Carson wrote her

infamous book, *Silent Spring*. In which she describes the dystopian future society was headed for if DDT continued to be used. The idea of a 'silent spring' devoid of life resonated with the public and brought light to the situation, ridding the environment of the toxic DDT. This is also the way these toxins can reach humans. Humans are apex predators themselves, and ingesting animals exposed to those toxins could lead us to a similar fate as them.

Cyanobacteria and Humans

The case of the cyanotoxin, AETX, produced by *Aetokthonos hydrillicola* is just a single example of a dangerous cyanotoxin; however, there are thousands of cyanobacteria species, many of which produce cyanotoxins unique to themselves. Anatoxin-a (ATX), homoanatoxin-a (HTX), Anatoxin-a(S) (ATX-s), and Saxitoxins (STXs) are all toxic cyanotoxins that function as neurotoxins found throughout the world (Testai et al. 2016).

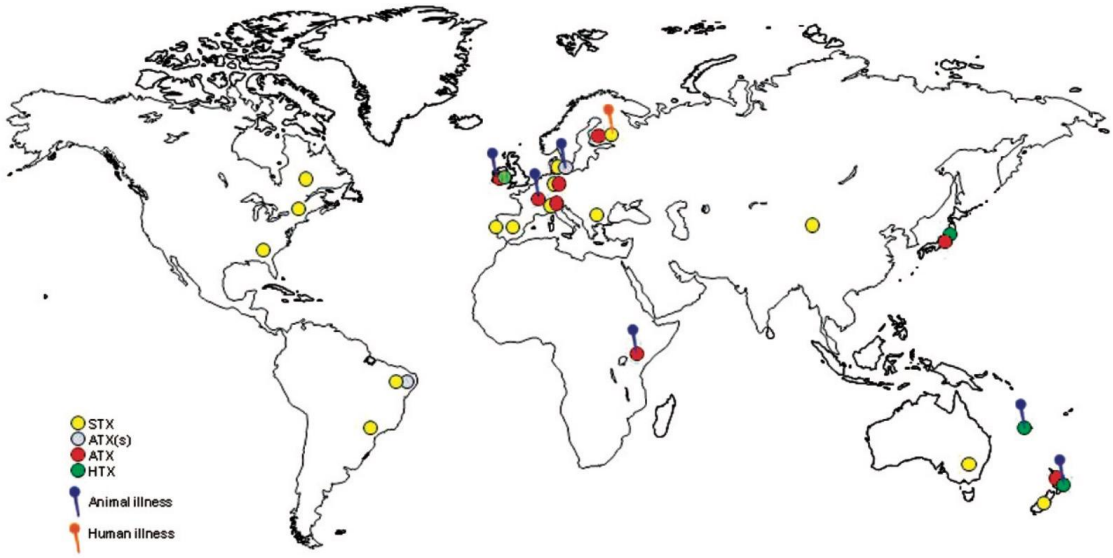

Figure 7. Demonstrates how widespread the effects of cyanotoxins are throughout the world (Testai et al. 2016).

Poisoning is common in livestock and other animals. They often function as neurotoxins when ingested, and rapidly cause symptoms in those intoxicated resulting in bodies of the deceased being discovered around infested water sources. If dogs drink or bathe in waters infested with ATX or HTX, they are diagnosed with poisoning. These cases are often reported the most due to dogs being household pets, and therefore being brought to veterinarians. However, these toxins are responsible for numerous animal mortalities, ranging from flamingos to pigs and sheep. ATX-s poisoning is often tied to respiratory failure and increased levels of acetylcholine (an important neurotransmitter that participates in muscle contraction, elevated levels are tied to convulsions and can lead to paralysis) in those intoxicated. People often assume that they are separated from animals, and cannot be affected in the same ways; however, ATX itself was responsible for the death of a 17 year old boy in 2003. He had ingested the toxin while swimming, and paid the price with his life (Testai et al. 2016). These phenomena are a rightful cause of concern, but rarely is the public informed of the true dangers of these cyanotoxins. Society should work to bring awareness to the issue, and create legislatures that work to keep people safe. Figure 8 shows these necessary laws, but few exist throughout the world.

		Country	Limits	Description	Assumptions and comments	References
ATX	Drinking water	New Zealand	6 μg/L	PMAV	–	Kouzminov (2005) and Chorus (2012)
HTX	Drinking water	New Zealand	2 μg/L	PMAV	–	
ATX	Drinking water	Ohio (USA)	20 μg/L	GV	–	Ohio Environmental Protection Agency (2015a)
ATX	Drinking water	Oregon (USA)	3.0 μg/L	Limit for drinking water	–	Chorus (2012)
ATX	Recreational water	Quebec (Canada)	3.7 μg/L	PMAC	–	Chorus (2012)
ATX	Recreational water	Oregon (USA)	20 μg/L	Provisional recreational water GV	Limit value for a boy of 20 kg body weight and an ingestion rate of 0.1 L/day	Oregon Health Authority Public Health Division (2015)
ATX	Recreational water	State of Washington (USA)	450 μg/L	Short-term recreational GV	PoD: short-term NOAEL = 2.5 mg/kg day from the systemic maternal toxicity study in mice (Fawell et al. 1999)	Hardy (2008)
ATX	Recreational water	State of Washington (USA)	75 μg/L	Subchronic recreational GV	PoD: subchronic NOAEL = 0.5 mg/kg/day, from the 7 weeks systemic toxicity in rats (Astrachan et al. 1980); UF = 1000	Hardy (2008)
ATX	Recreational water	Ohio (USA)	300 μg/L (80 μg//L)	Recreational water GV (Public Health Advisory)	–	Ohio Environmental Protection Agency (2015b)
ATX	Recreational water	California (USA)	90 μg ATX/L	action level	Based on dermal, inhalation and oral routes of exposure; 7–10-year-old swimmers considered as the most highly exposed group	Butler et al. (2012)

PMAC: provisional maximum acceptable concentration; PMAV: provisional maximum acceptable value; PoD: point of departure; GV: guidance value; UF: uncertainty factor.

Figure 8. Shows the cyanotoxin limits legally put into place around the world, notice how few even exist (Testai et al. 2016).

However dire the human health risk is to these toxins, the public knows too little to be able to protect themselves from them. Without adequate legislation in place, there will always be a risk of these toxins infiltrating drinking and recreational water sites. Too often do people observe the symptoms of poisoning in animals and neglect to realize that poisoning could transpire in their loved ones, and even in themselves. More research and awareness needs to be brought to the subject of cyanobacteria for society to be more correctly informed on how to regulate human safety.

Concluding Thoughts

Cyanotoxins have proven themselves to be harmful to ecosystems and humans, especially when they are unknowingly ingested. The fact that these molecules can potentially biomagnify through the food web is alarming, especially when organisms fall victim to terminal diseases caused by these toxins.

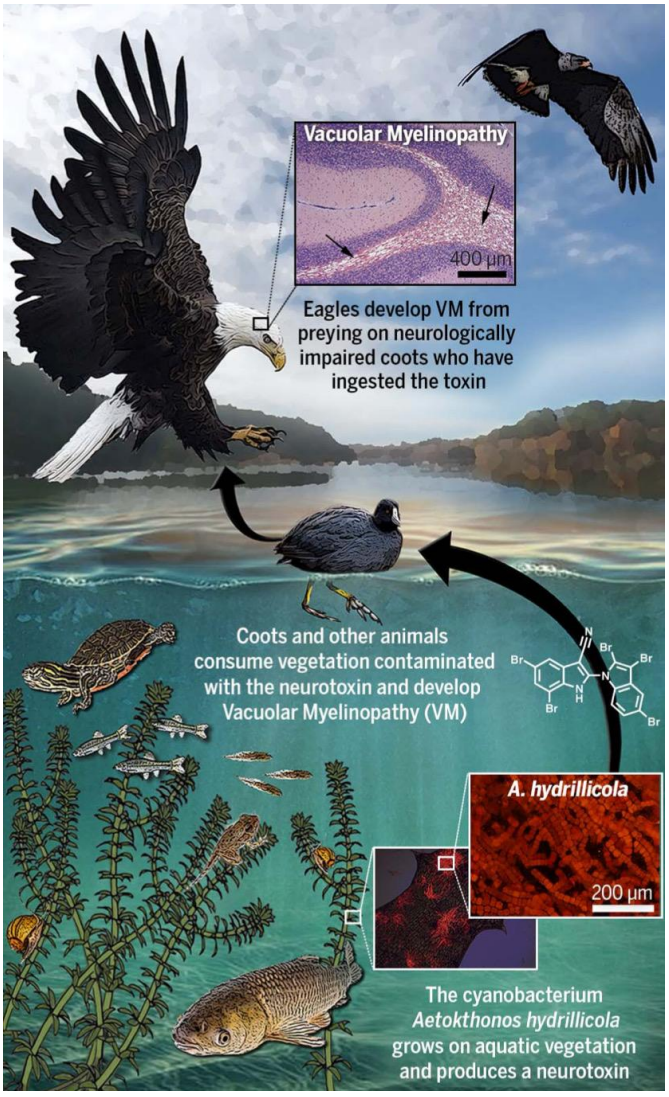

Figure 9. Shows how the cyanotoxin AETX can easily move through the food web to inflict terminal Vacuolar Myelinopathy in apex predators (Breinlinger et al. 2021).

One of the easiest and most important steps people can take to rid the environment of cyanotoxins is to help spread awareness of the subject and bring attention to research being done. For example, in the Haynie et al. 2013 study, triploid grass carp were proposed as a biological control to rid reservoirs of *H. verticillata.* They were proved to be susceptible to VM, but did not show any outward symptoms of disease, and it is still unknown if birds can contract VM from ingesting infected grass carp. More research is needed to expand the understanding of this disease and its relationship to grass carp in order to ensure its effectiveness as a biological control. In working to prevent VM, society should work to influence the stop of pollution from power plants and water treatment near man-made water reservoirs. It is from these sources that bromide is introduced into the water and incorporated into AETX to give it its toxic nature (Breinlinger et al. 2021).

It is also highly encouraged that individuals look into their local ecosystems and environments to observe cyanobacteria activity. This way, people can be educated on threats to their health and ecosystem health. For example, New York State, through the Department of Environmental Conservation, has an interactive map web page where they document harmful algal blooms, or HABs (Department of Environmental Conservation 2021).

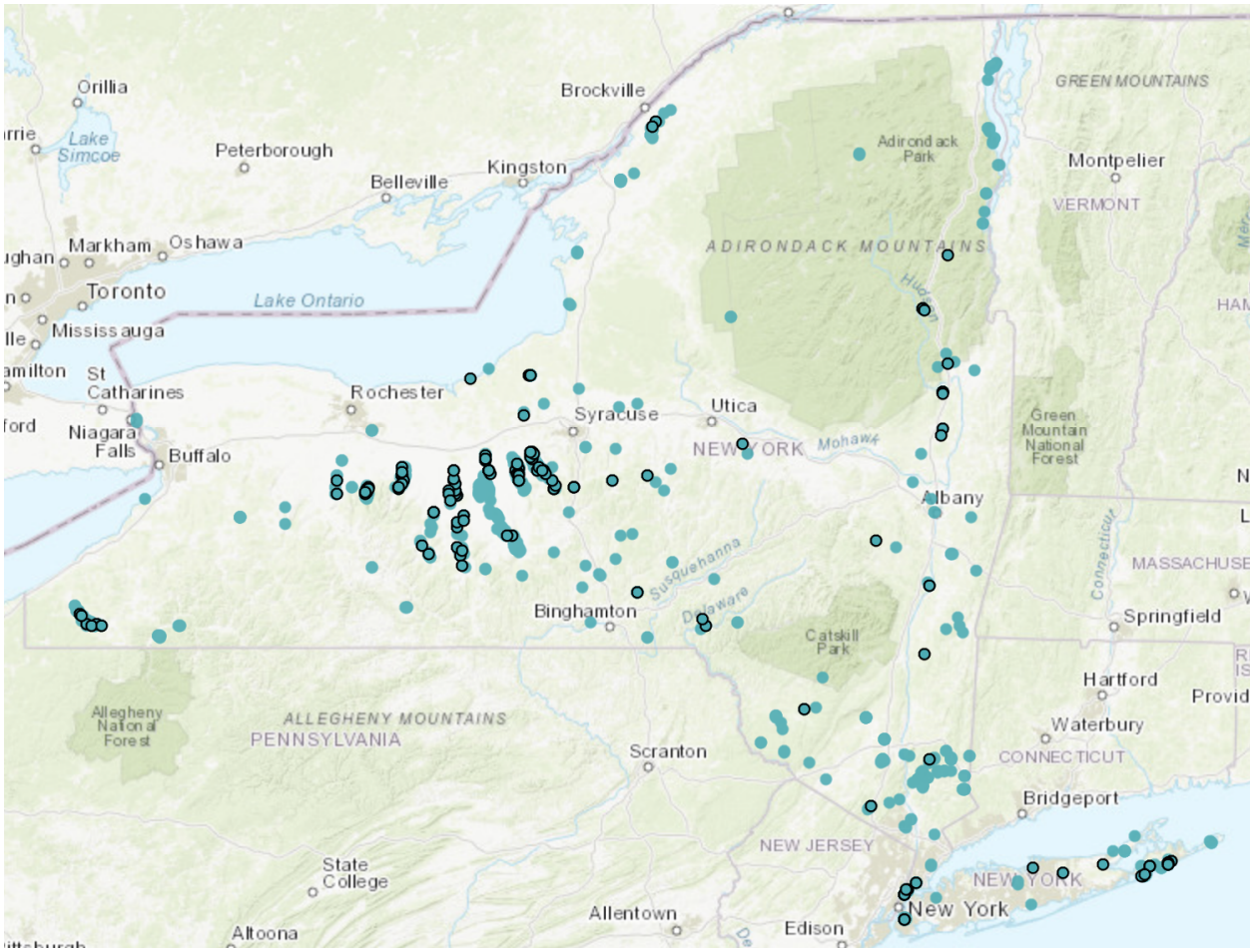

Figure 10. Shows the interactive map that documents HABs in New York State. Each sighting location has pictures and other informative details about the severity and species of the cyanobacteria found there (Department of Environmental Conservation 2021).

Just as the bald eagles represent strength in our nation, we need to instill strength in our ecosystems. Through awareness and activism, we can rid society of the cyanobacterial threat, freeing ourselves from the nasty reality of cyanotoxin poisoning. As Rachel Carson said herself, "Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts. There is something infinitely healing in the repeated refrains of nature - the assurance that dawn comes after night, and spring after winter" (Carson 1962).

Literature Cited

Breinlinger S, Phillips TJ, Haram BN, Mareš J, Martínez Yerena JA, Hrouzek P, Sobotka R, Henderson WM, Schmieder P, Williams SM, et al. 2021. Hunting the eagle killer: A cyanobacterial neurotoxin causes vacuolar myelinopathy. Science. 371(6536):eaax9050. doi:10.1126/science.aax9050.

Carson R. 1962. Silent Spring. Houghton Mifflin.

Department of Environmental Conservation. c(2012). Harmful Algal Blooms (HABs) Notifications Page [Internet]. Department of Environmental Conservation. [2021]. Available from https://www.dec.ny.gov/chemical/83310.html

Fischer JR, Lewis-Weis LA, Tate CM. 2003. Experimental Vacuolar Myelinopathy in Red-Tailed Hawks. Journal of Wildlife Diseases.:7.

Haram BN, Wilde SB, Chamberlain MJ, Boyd KH. 2020. Vacuolar myelinopathy: waterbird risk on a southeastern impoundment co-infested with Hydrilla verticillata and Aetokthonos hydrillicola. Biol Invasions. 22(9):2651–2660. doi:10.1007/s10530-020-02282-w.

Haynie RS, Bowerman WW, Williams SK, Morrison JR, Grizzle JM, Fischer JM, Wilde SB. 2013. Triploid Grass Carp Susceptibility and Potential for Disease Transfer when used to Control Aquatic Vegetation in Reservoirs with Avian Vacuolar Myelinopathy. Journal of Aquatic Animal Health. 25(4):252–259. doi:10.1080/08997659.2013.833556.

Mercurio AD, Hernandez SM, Maerz JC, Yabsley MJ, Ellis AE, Coleman AL, Shelnutt LM, Fischer JR, Wilde SB. 2014. Experimental Feeding of Hydrilla verticillata Colonized by Stigonematales Cyanobacteria Induces Vacuolar Myelinopathy in Painted Turtles (Chrysemys picta). Cunha MV, editor. PLoS ONE. 9(4):e93295. doi:10.1371/journal.pone.0093295.

National Geographic Society. c(2021). Biomagnification and Bioaccumulation [Internet]. National Geographic Society. [2021]. Available from https://www.nationalgeographic.org/activity/biomagnification-and-bioaccumulation/

National Pesticide Information Center. 1999. DDT: (General Fact Sheet). Internet. Oregon State University and the U.S. Environmental Protection Agency.

Testai E, Scardala S, Vichi S, Buratti FM, Funari E. 2016. Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. Critical Reviews in Toxicology. 46(5):385–419. doi:10.3109/10408444.2015.1137865.