

Indicators of Type E Avian Botulism Outbreaks on Lakes Erie and Ontario

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Abstract

Type E avian botulism has been a significant cause of waterfowl and shorebird deaths on the Great Lakes since the late 1990's but there is a surprising deficit in what is known about the toxin. The bacteria that produces the botulism toxin, *Clostridium botulinum*, is found naturally in the sediments of the great lakes in anoxic environments of decaying matter on the lake floor. The toxin is believed to be entering the trophic system of the lakes through the aquatic invaders the quagga mussel, *Dreissena bugensis*, that is filtering the decaying matter in the sediments of the lakes and becoming concentrated with the toxin, and the round goby, *Neogobius melanostomus*, which is preying upon the quagga mussels and it becoming concentrated with the botulism toxin. The native birds of the Great Lakes are then consuming the contaminated fish and becoming so concentrated with the botulism toxin that they die of respiratory failure from paralysis of the muscle or they drown from paralysis of their neck muscles. The invaders, however, are distributed evenly throughout the lakes but the outbreaks of type E avian botulism only occur in certain spots along the lake. This study attempts to find abiotic indicators of type E avian botulism outbreaks to help define wildlife management techniques when dealing with this epizootic.

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Advice to Future Honors Students

The most important piece of advice anyone else ever bestowed upon me before I began writing my honors thesis was to choose a topic that I was excited about. Do not choose a topic just because it is relevant to your major or just because you believe that a graduate school would appreciate your knowledge on the subject. Although the latter is a valuable concern, you are going to spend a lot of time researching, writing, and discussing this topic with others so you do not want to become disinterested in it over time. The more passionate you are about the subject, the more time and effort you will put into it and in the end, the better the thesis will be. In fact, the more you talk to other people about your topic the better. You will be surprised how much openly discussing your topic to your peers can help you in the process, especially when you come to a point in the road where you are unsure of where to go next.

For my second piece of advice on thesis writing, do as much as you can whenever you can. One of my own thesis advisors told me that everything would take longer than I expected it to and he was proven right time and time again. If you have a moment to write or read, take the opportunity to do so and even if you only write a small piece that you completely get rid of later on, that small effort may help you think of new ways to overcome problems and organize your thoughts. You will never “find time” to work on this unless you make that time happen. If you are in the Honors Program you are most likely used to pushing yourself to succeed academically so use that to your advantage. Also, keep in constant contact with your advisors and ask them to push you as well. Make appointments with them every time you meet for your next meeting and what will be expected of you once that time comes. This will focus you to always show up with progress for them to look over and you will not end up fretting over time constraints in the end. Do not choose advisors simply because they like you or you believe that

they will not expect as much work from you as someone else will. Choose advisors that will push you to do your best. You have already taken on this challenge; you might as well put your best into it and produce the best quality product that you can.

Finally, if you can, take this opportunity to study a topic and present on something that will make a difference in the academic community. Chances are, you will not cure a disease or stop violent protests in other countries, but do your best to find something that will benefit others around you. We are in the Honors Program because we have an innate desire to do the best that we know how to do and I encourage you to use your time and energy to produce something valuable to others. You will never know how the little things you do in life will affect other people, their decisions and their actions, so do your best to make a positive impact.

Acknowledgements

I would like to start out by extending my gratitude to my Honors Thesis Advisors Dr. Peter A. Rosenbaum and Dr. Christopher Blonar for their guidance and expertise. Next, I would like to thank the New York State Department of Environmental Conservation namely Kenneth Roblee and David Adams from the Buffalo and Albany divisions respectively for the data used in this study of the bird die off counts. I would also like to thank Rachel Guy and Mark Jankowski from the USGS National Wildlife Health Center, Sara Grise and Ken Hyde from the Sleeping Bear Dunes, and Helen Dombske from the NYS SeaGrant at Cornell University for their help collecting bird die off data. For the climate data, I would like to thank Dr. Skubis from the meteorology department and the North East Regional Climate Center at Cornell University for their assistance in finding and collecting the abiotic data needed for this study. I would also like to thank Dr. Scott Preston from the math department for his time and proficiency with the statistical programs used to analyze the data collected for this study. His work was very much appreciated. Finally, I would like to recognize Arlee J. Logan for his help in adapting some of the figures used in this study.

Author's Reflections

I did not choose the topic of my thesis, but rather the topic found a way of choosing me. I was out on Lake Ontario in a small boat with some friends when I noticed a large bird drowning in the water. I dove over board and brought it to shore where I discovered that it was a Double-Crested Cormorant suffering from ailment that caused neck and nictitating membrane paralysis. I called the wildlife rehabilitator Jean Soprano from Pennville and rushed the bird to her facilities, much to the bird's apparent displeasure. I called Mrs. Soprano every week to learn of the bird's progress and after his system was flushed he was eventually released back into the wild. When she told me about avian botulism and the amount of bird deaths that it caused annually I was shocked. Being an avid bird watcher with a particular affinity for aquatic birds and a zoology major, I assumed I would have known if such an epizootic was occurring but here it was going on in my proverbial backyard and I was clueless. I decided to take this topic to be my thesis because, even if I did not have the equipment or the training to find a perfect or definitive predictor of the outbreaks, at least I could raise awareness about the problem and talk to others about it. I wanted people to know what was happening to the birds and that the populations of some of our most precious species were in danger. Most importantly, I believe as humans we must take on a role of stewardship towards nature. We must understand that our actions affect wildlife and we can do things to actively manage these systems to keep them healthy.

It has been my belief from the beginning that one person's passion can ignite other people to do positive things for the environment and I have every desire to be a catalyst for that kind of change. While writing this thesis, I have discussed with many people in New York State that knew nothing about type E avian botulism but once they heard what it was doing to

the local populations of waterfowl, they were driven to actively seek out more information on the subject and were interested in finding a way to help the birds. If this paper has caused me to discover anything about myself, it is that one person can make a large difference in their community if they have the motivation and enthusiasm to do so. By talking to people and making them aware, those that are inclined to change the way humans interact with the environment can change policies and management plans just by showing their support. The Great Lakes region is an incredible freshwater system and it is invaluable as a natural resource. Living in the area and learning of its systems has been a privilege and I hope that future generations will understand this as well and keep the ecosystems as pristine as possible.

As a writer I have learned precious skills in human interactions when gathering data, time management, organization, editing, and writing. For human relations I learned that it is always important to express your appreciation for someone's time no matter how small of a favor they do for you. Not only does it make them feel good about helping you and more likely to aid you in the future, but as humans I believe our individual efforts are often taken for granted. On the other hand, I have learned to assert myself when I am in need of someone's time or attention in order to complete a task. It is often easy for someone to neglect an undergraduate student when they feel other projects or people deserve more of their attention. For time management and organization I learned that it is important to do your personal best every day. Sometimes your personal best will be to write 15 pages of well cited research while other days just having time to read a few journal articles is all one can afford. Either way, putting the energy forward to complete these tasks and being satisfied with the result was a personal achievement for me. I am often too critical of my work and when I could only afford a small amount of time to work on the thesis I would condemn myself for it. I learned, however, to

work with what time I was given and make the best of it. Organizing a project of this magnitude is daunting at first, but I learned as I went how to file papers and keep track of my progress as best as I knew how. As for the writing and editing, this process has definitely improved my skills. I was fortunate enough to be working with Dr. Rosenbaum who has been publishing scientific papers for years and is a fantastic writer so I was very pleased to have his input. I believe the writing that I produce now is much better than I have ever done before and I have learned a lot about adding graphs, figures, and tables into scientific papers and making them flow with the context.

Introduction

The Great Lakes are one of the earth's most precious resources as the water in these lakes makes up 18% of the world's surface fresh water (Danz & Regal, et al., 2005). The coastline of the Great Lakes extends along eight states of the United States of America, including Minnesota, Wisconsin, Michigan, Illinois, Indiana, Ohio, Pennsylvania, and New York, and along the provinces Ontario and Quebec of Canada containing about 23,000 km³ of fresh water and spanning over 244,000 km². Figure 1 depicts the five Great Lakes and their watershed regions (Danz & Regal, 2005).

The Great Lakes and Watershed Boundaries

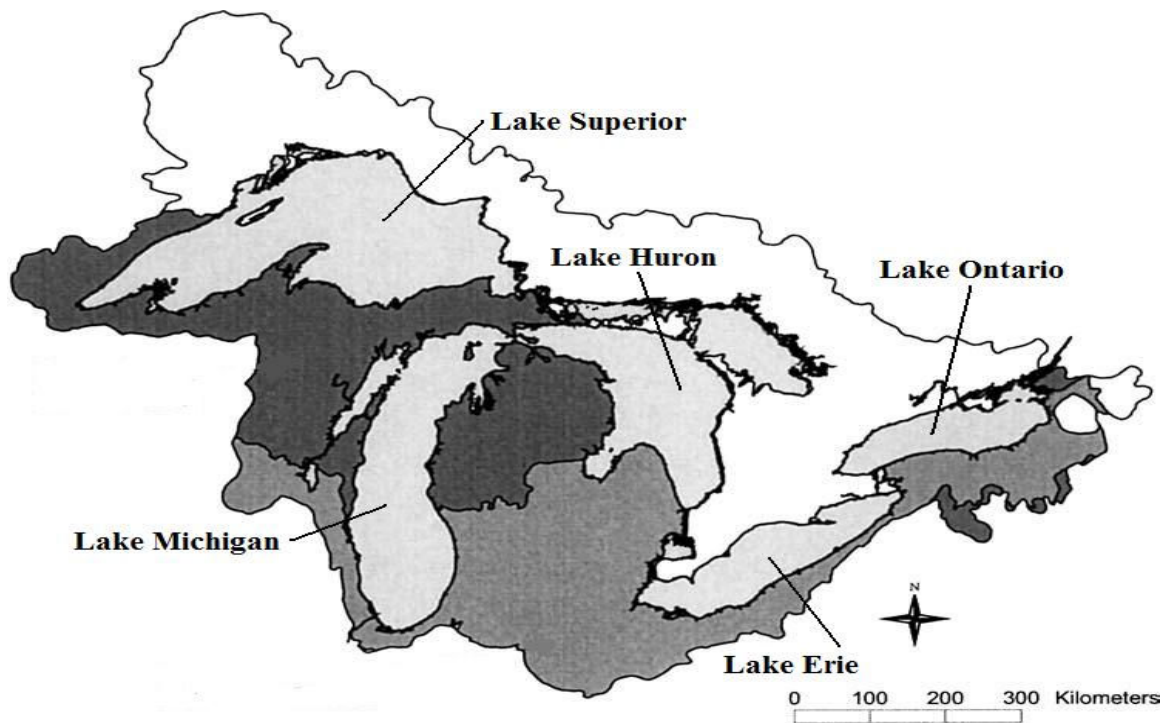


Fig 1: The Great Lakes and the surrounding watershed regions. Adapted from Danz & Regal, et al. (2005). Environmentally stratified sampling design for the development of Great Lakes environmental indicators. *Environmental Monitoring and Assessment*. 102, 41 – 65

The Great Lakes include Superior, Michigan, Huron, Erie, and Ontario of which Erie is the shallowest but Ontario is the smallest by area. Lake Superior is the largest, deepest, and

coldest of the lakes and it could fit all of the other Great Lakes within itself with room to spare (US EPA & Government of Canada, 2002). One of the Great Lake region's most important assets is its wetlands and these are of particular conservation value for their ability to support rare species, migratory birds, and to help mitigate for water run off and store and cleanse water and nutrients. Currently, although renowned for their value over two-thirds of the Great Lakes wetlands have been lost and the remaining areas are under constant threat of being drained, developed on, or polluted by run off or dumping (US EPA & Government of Canada, 2002).

The lakes themselves are major facilitators of economic growth, trade and areas of tourism and recreation while the fertile lands in the basin support a wide variety of agricultural institutions. Unfortunately, degradation and habitat loss due to the growing human population remains an increasing threat to the Great Lakes basin. Pollution, introduction of non-native species, over consumption of natural resources, and habitat loss due to residential, agricultural, or industrial development are just some of the on-going threats to the region and the impact is already being seen in the declining populations of some of the native species (US EPA & Government of Canada, 2002).

Human implications in the changing environment

According to the United States Census Bureau, as of the year 2008 the world human population was over 6,750,500,000 individuals with representatives of the human species on every continent (U.S. Census Bureau, 2008). Needless to say, the global distribution of large mammals with the ability to travel over extensive distances, transport foreign commodities in large quantities to new areas, and use natural resources to regulate its surroundings to increase its fecundity has a profound effect on the environment. The exponential growth of the human

species is an ongoing threat to the stability of a multitude of ecosystems as humans are consuming great quantities of resources relatively quickly like space, fresh water, non-renewable energy sources, and other organisms. Figure 2 depicts the global human population growth since 1950 and the projected human growth to 2050 showing a constant trend of increase in world population size (Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, 2005).

World Population from 1950 to 2050

| Year | Population |
|------|------------|
| 1950 | 2 535 093 |
| 1955 | 2 770 753 |
| 1960 | 3 031 931 |
| 1965 | 3 342 771 |
| 1970 | 3 698 676 |
| 1975 | 4 076 080 |
| 1980 | 4 451 470 |
| 1985 | 4 855 264 |
| 1990 | 5 294 879 |
| 1995 | 5 719 045 |
| 2000 | 6 124 123 |
| 2005 | 6 514 751 |
| 2010 | 6 906 558 |
| 2015 | 7 295 135 |
| 2020 | 7 667 090 |
| 2025 | 8 010 509 |
| 2030 | 8 317 707 |
| 2035 | 8 587 050 |
| 2040 | 8 823 546 |
| 2045 | 9 025 982 |
| 2050 | 9 191 287 |

Fig. 2 – Current predicted trend of global human population growth from 1950 to 2050. Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat (2006) *World Population Prospects: The 2006 Revision*

The influences of the human species on the global environment can be hard to quantify as we affect the biodiversity and integrity of habitats on such a grand scale both directly and indirectly. To begin with, every ecosystem has a carrying capacity which is the amount of individuals of a species an area can sustain without being degraded. Arguably,

without modern technology the human species would have already over populated past the carrying capacity of the earth and the global population would have declined due to lack of resources. Developments in all facets of life, however, have changed the ability to harvest and distribute goods which increases the amount of individuals that can be sustained in an area although degradation will still often occur. Improvements in agriculture, obtaining and using fuel, sanitation systems, and improving medical technologies have increased the fertility, distribution of populations, and life span of humans on all continents.

Human over use of natural resources

Humans in general consume a wide variety of organic matter from various environments in the form of vegetation and animal products that can easily be seen in any grocery store. More often than not, the every day consumer does not know whether the products they buy are made of materials or organisms that have been sustainably harvested or if they are being overexploited. Humans overexploit populations of organisms and land for commercial use, subsistence use, and even accidentally, such as in the case of by catch in fisheries (Hunter & Gibbs, 2007). Models can be made of human disturbances to calculate their scope using predictions of land use, human density and dispersal, and climate change. According to one study in 2000, land use by humans will be the greatest contributor to habitat degradation and loss by 2100 followed by climate change. In freshwater based ecosystems however, biological exchange was deemed the greatest contributor to habitat change, but all of these factors are influenced or accelerated by the human species. Biological exchange can be facilitated by humans with intent for recreation like adding non-native fish species to lakes for angling, or by

accident like transporting organisms in ballast water on ships and releasing them into freshwater systems (Sala & Chapin III, et al., 2000).

The Great Lakes basin has supported human activities for about 10,000 years but the major exploitation began with the settlements of the Europeans in the 1600 to 1700's when the area was put to immediate economic use. Originally the fur trade was one of the most lucrative practices in the region but eventually the land was developed into agricultural and logging industries that prompted the need for mass clearing of land which in turn caused soil erosion, fluctuations in lake levels, and destruction of spawning habitats for fish. The lakes themselves were used for transportation of people and goods while fisheries and canal systems also began to grow. During the industrial revolution of the late 1800's to the early 1900's, the lakes were essential to the growth of manufacturing companies including paper mills, lumber yards, the steel and coal industries, and for supporting and transporting grain and other agriculture commodities. Now along with these uses, the lakes are widely valued for sport fishing and recreation that brings in tourists from all over the nation (US EPA & Government of Canada, 2002). This extensive amount of human activity on the lakes and in the regions around them had undeniable consequences on the populations of organisms in the area.

One of the most well known instances of human overexploitation of a resource leading to extirpation, or local extinction, of a native species in the Great Lakes is of the Lake Ontario Atlantic salmon, *Salmo salar*. Figure 3 illustrates two individual Lake Ontario Atlantic salmon from a side view (Miller & Ringler, 1996)

Lake Ontario Atlantic salmon, *Salmo salar*



Fig. 3 – Two adult Lake Ontario Atlantic salmon, *Salmo salar*. Miller, D.J. & N.H. Ringler (1996). Atlantic Salmon in New York. SUNY-ESF E-Center Web site: <http://www.esf.edu/pubprog/brochure/salmon/salmon.htm>

Once plentiful in Lake Ontario, the population of Atlantic salmon plummeted under the strains of human over fishing, habitat destruction, and the creation of barriers in their spawning pathways and they were extinct in the area by 1896. Plans to reintroduce the species to the lake were futile since the environmental stresses that had caused their local extinction were not removed from the system before the reintroduction. In the place of the Atlantic salmon, other salmonids have been introduced to the lake to keep the recreational fishing industry intact such as the chinook salmon, *Oncorhynchus tshawytscha*, the coho salmon, *O. kisutch*, the rainbow trout, *O. mykiss*, and the brown trout, *Salmo trutta*. Currently, there are still plans to reintroduce the species as the quality of the Lake Ontario tributaries where the salmonids spawn have improved but the non-native species that were introduced in their absence prove to be a barrier. Studies have found that the non-natives act aggressively to the Atlantic salmon and sometimes the non-native males attempt to court the Atlantic salmon females which could lead to hybridization or lack of reproduction of the Atlantic salmon (Scott & Judge, et al., 2005). This example shows the consequences of human overexploitation of a species, the

dangers of biological exchange, and the resulting issues it causes when attempting to restore the quality of a degraded habitat.

Human induced contamination and pollution of ecosystems

Contamination of ecosystems can happen in many ways and can have damaging affects very quickly. Air quality, water quality, and even soil quality can be damaged so the media in which toxins can move through are diverse. Results of contamination can be as vast as mutation, impaired growth, sterilization, susceptibility to disease, and death. Sometimes contamination occurs by accident such as in an oil spill along a beach or continually occurs like air pollution from car exhaust or the run off from farmlands. Fresh water systems like lakes that are limited in their connection to one another are particularly susceptible to contamination effects because the flora and fauna of the area have limited mobility to escape the effects of the contamination (Hunter & Gibbs, 2007).

This problem is present on the Great Lakes like most fresh water systems that are utilized heavily by human activity. For example, agricultural fertilizers with phosphorus wash into the lakes causing mass eutrophication, which is an increase in nutrients artificially introduced to an aquatic system that allows for a significant change in the growth of aquatic plant life in the exposed region. The growth of algal blooms due to eutrophication further disrupts the natural ecosystem because the vegetation collects on the bottom of the lake and decays which in turn depletes the available oxygen. Logging industries contribute to soil erosion by clearing away large portions of integral forest habitat that would have prevented the soil from washing into lakes and streams. Once they collect in the streams and lakes, the sediments can change the habitat by reducing the amount of light that can penetrate the water, settling on important parts of the system making them unavailable to other organisms,

diverting the flow of the water, causing flooding, and reducing the water level (US EPA & Government of Canada, 2002).

Currently pollutants are one of the greatest health concerns on the Great Lakes and include DDT, dichloro-diphenyl-trichloroethane, and PCBs, polychlorinated biphenyls, which have been serious contaminants since the 1940's and 1920's respectively. Pollutants in the lakes have been known to cause tumors in fish, misshaping of bird beaks, thinning of bird eggs and potentially could cause human health hazards. Through biomagnification, when organisms have been contaminated by a toxin, each step in the food web causes an accumulation of toxins and when it reaches the top predator, often birds or large mammals, it can be concentrated at very high levels.

PCB Biomagnification in a Great Lakes Food Web

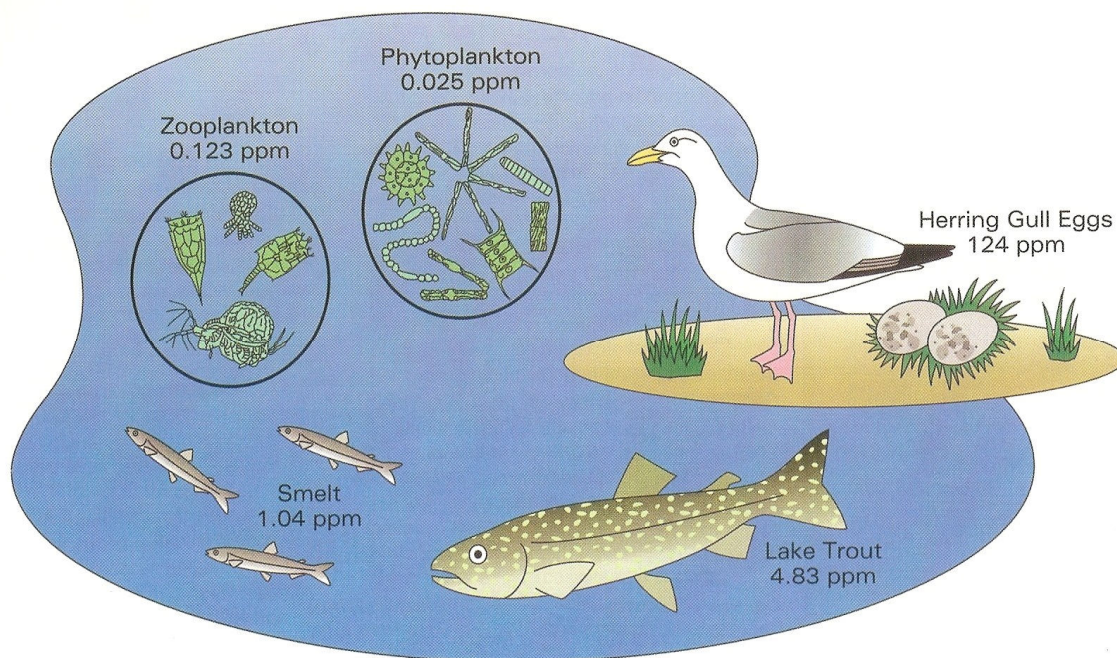


Fig. 3 – PCB accumulation (in parts per million) through biomagnification in a Great Lakes food web showing an increase in levels of the contaminate in higher taxa from feeding on contaminated food items. United States Environmental Protection Agency, Government of Canada, (2002). *The Great Lakes: An Environmental Atlas and Resource Book*. Chicago, Illinois: Great Lakes National Program Office.

Figure 3 illustrates the biomagnification of PCB's in parts per million in a trophic web from phytoplankton to a herring gull's eggs as an example of how concentrations of toxins can accumulate at very high levels in higher predators from eating contaminated prey (US EPA & Government of Canada, 2002).

Human facilitated transport of non-native species

Since the beginning of advancements in transportation, agriculture, and trade, humans have been distributing different species from one area to another including crops, herbs, domesticated animals, diseases, and pests which has had a multitude of effects on the world as we know it. When we think of detrimental effects on ecosystems, invasive species that have established in an area and changed the natural system are often one of the first issues that come to mind. Like the issues mentioned before, the Great Lakes eco-region is not an exception to degradation of this kind. It has been greatly modified by the introduction of non-native species facilitated by the movement of humans and goods throughout the region. It has been determined that at least 60% of the invasive alien species currently in the Great Lakes were introduced by commercial shipping and the Michigan Department of Environmental Quality estimated that zebra mussels alone could cause around five billion dollars in damages over the next 10 years (Horan & Lupi, 2005). Around 182 non indigenous species have been identified in the Great Lakes since 2006 and a new invader to the system is estimated to be discovered about every 28 weeks which is the highest rate ever recorded for a fresh water system (Ricciardi, 2006). In the lakes themselves some of the more commonly known invaders include the sea lamprey, *Petromyzon marinus*, the alewife, *Alosa pseudoharengus*, the zebra mussel, *Dreissena polymorpha*, the quagga mussel, *Dreissena bugensis*, and the round goby,

Neogobius melanostomus. In the terrestrial ecosystems of the Great Lakes basin purple loosestrife, *Lythrum salicaria*, and the European buckthorn, *Rhamnus cathartica*, are two of the more well known invasive species (US EPA & Government of Canada, 2002).

Zebra mussels and quagga mussels have been commonly used as examples of how invasive species can modify an ecosystem since their introduction to the Great Lakes in the late 1980's. The mussels have proliferated out of control since their introduction through the ballast water of ships that have been in the waters of the Black and Caspian sea where the mussels are native (Stone & Okoniewski, 2002). These mussels, although small, have been able to make such intense changes because of their efficiency in reproducing and feeding. A single adult dreissenid mussel can filter up to two liters of water per day, removing all zooplankton, phytoplankton and bacteria from the water that it filters (Domske & O'Neill Jr, 2004).. The mussels can attach to any solid surface so anything submerged in the water quickly becomes covered in the bivalves such as the bottom of boats, docks, buoys, pipes and rocks. Each female mussel can produce up to 1,000,000 eggs a year and the intermediate mobile life stage of the mussel, the veliger, moves along with the current and settles on any available space it can attach to. The main life style difference between the zebra mussel and the quagga mussel is that the quagga mussel can adapt to colder, deeper waters where it is better at surviving on bacteria as a main food source. Other than being a nuisance as a "biofouler" that sticks to recreational and commercial equipment the mussels have caused a reduction of nutrients available for native species by their efficient filter systems depleting the supply of plankton (Domske & O'Neill Jr, 2004). Figure 4 depicts a cluster of zebra mussels being exposed above the water (USGS, 2008).

Zebra Mussels



Fig 4 – Cluster of zebra mussels. USGS, (2008). Invasive Invertebrates: Zebra Mussel. USGS Great Lakes Science Center Web site: http://www.glsc.usgs.gov/main.php?content=research_invasive_zebramussel&title=Invasive+Invertebrates0&menu=research_invasive_invertebrates

Human conservation efforts

It is clear that humans can have a massive damaging affect on the quality of the ecosystems around them, but not all human interactions are necessarily negative. Humans have the ability to change their actions to restore degraded habitats, promote the conservation of pristine habitats, and implement management plans that can help save populations of endangered species. For example, in 2006 the United States Congress passed a revision to the Great Lakes Fish and Wildlife Restoration Act of 1990 (GLFWRA) that supports the funding of conservation efforts for fish and wildlife habitat up to 16 million dollars annually. The proposal committee that includes experts in wildlife management is overseen by the U.S. Fish and Wildlife Service (The Nature Conservancy, 2006). This shows that with conscientious effort legislation that protects wildlife and the quality of their habitats can be supported through the federal government. As long as people are aware of the conservation issues concerning the environment around them, they can make a difference by supporting certain organizations such at The Nature Conservancy or by letting government officials and

representatives know that they are concerned about these issues and want government help or support. With education, awareness, and research great advances can be made toward conservation of species, habitats, and improvement of existing management plans.

Waterfowl of the Great Lakes

The Great Lakes basin is a vital component for the continued existence of a multitude of birds that use the lakes and surrounding areas during migration or for foraging, breeding, making nests, and wintering areas. The quality of the Great Lakes ecosystems has the potential to have profound effects on the ability for these birds to reproduce and survive so the conservation of these areas is integral for these bird populations. Below are listed some of the waterfowl that utilize the Great Lakes and are under threat from degradation of the basin habitats.

- *Loons*

One of the most charismatic of these inhabitants of the Great Lakes eco-region is the Common Loon, *Gavia immer*, known for the male's long eerie call. The migratory bird breeds in the northern most parts of the United States, all of Canada and even around Greenland and Iceland. It spends the winter all along the coast of the United States, Europe, and Canada and even as far south as Mexico and the Mediterranean. They feed mainly on fish which they catch underwater after diving down extensive distances (Cornell University, 2003 (A)). Figure 5 depicts an adult Common Loon with its breeding plumage with a chick upon its back (Cornell University, 2003 (A)).

Adult Common Loon



Figure 5 – Adult Common Loon in breeding plumage with chick. Cornell University (2003)(A). All About Birds: The Common Loon. Cornell Lab of Ornithology Web site: http://www.birds.cornell.edu/AllAboutBirds/BirdGuide/Common_Loon_dtl.html#food

Red Throated Loons, *Gavia stellata*, can also be seen on the Great Lakes and have similar prey requirements and eating habits as the common loon. Smaller than the common loon, its habitat is more restricted to the northern parts of Canada and Alaska, but can be found in the extreme north of Russia and Europe. Figure 6 is a picture of an adult Red-throated Loon female in breeding plumage (Cornell University, 2003 (B)).

Adult Red-Throated Loon



Fig 6 – Adult female Red-Throated Loon in breeding plumage on the water. Cornell University, (2003)(B). All About Birds: The Red Throated Loon. Cornell Lab of Ornithology Web site: http://www.birds.cornell.edu/AllAboutBirds/BirdGuide/Red-throated_Loon_dtl.html

- *Diving Ducks*

Diving ducks are often ornate and assorted in appearance which makes them particularly interesting to bird watchers. Common Mergansers, *Mergus merganser*, are residents of the Great Lakes basin year round as well as in western states from California all the way north to the coasts of Alaska but often migrate from southern states of the U.S. and northern Mexico to Canada to breed in the summer. They also be found anywhere from northern Eurasia in the summer to northern Africa in the winter. Like most diving ducks, their diet is varied from fish, frogs, tadpoles, mollusks, crustaceans, annelids, plants, and even small mammals that they can catch far below the surface of the water. Figure 7 depicts an adult male Common Merganser (Cornell University, 2003 (C)). Red Breasted Mergansers, *Mergus serrator*, tend to have similar eating habits and prey but their territories are more extreme to the north and south when they migrate (Cornell University, 2003 (D)). Greater Scaup, *Aythya marila*, and Lesser Scaup, *Aythya affinis*, can both be found in the Great Lakes region and have the ability to dive over 50 to 60 feet deep when looking for food. Their diet consists of fish, other aquatic vertebrates and invertebrates, as well as plant matter and the lesser scaup has a summer range of Canada and the northern United States but can travel south to the southern states, Mexico, and even the north coasts of South American during the winter. Greater Scaups, however, prefer the coastlines and breed in the summer in Canada and Alaska but spend the winters on the coasts of the Atlantic and Pacific Oceans along the United States, Canada, and Eurasia, although a few winter on the Great Lakes (Cornell University, 2003 (E & F)). Once known as the Oldsquaw, the Long Tailed Duck, *Clangula hyemalis*, is an attractive diving duck that also feeds on crustaceans, fish, insects, plant matter, and fish and amphibian eggs. This duck is the greatest diver of all the diving ducks reaching depths of 200 feet and unfortunately

known to be victims of by-catch. Their summer breeding range is the polar and subpolar regions along with the northern most parts of Canada and Alaska. In the winter they migrate to the Baltic Sea, the Great Lakes, and the Atlantic and Pacific coasts of the United States (Cornell University, 2003 (G)). Finally, among the diving ducks the White-Winged Scoter, *Melanitta fusca*, has become a more common resident during the winter months on the Great Lakes which could possibly be in correlation to the invasion of zebra mussels which is utilized as a food source. Its usual prey follows the same line as the other diving ducks and the scoter breeds in Alaska and Canada during the summer time and winters on the Great Lakes, the both oceanic coasts of the United States and even on the southern coasts of Europe and China (Cornell University, 2003 (H)).

- *Gulls*

Of all the various species of birds that can be seen on the Great Lakes, the extensive populations of gulls are probably one of the most noticeable. These scavengers eat a wide variety of available foods including mollusks, echinoderms, fish, insects, bird eggs, carrion, and garbage and can drink fresh or salt water with the help of their specialized nasal glands. Year round inhabitants of the Great Lakes include the Herring Gull, *Larus argentatus*, the Ring-Billed Gull, *Larus delawarensis*, and the Great Black-backed Gull, *Larus marinus*, while the Bonaparte's Gull, *Larus Philadelphiensis*, only resides on the Great Lakes during the winter (Cornell University, 2003 (I,J,K &L)). Although seen as pests to many people, their niche in the ecosystem as scavengers helps the environment by keeping large amounts of organic matter from being left to decay and increase bacteria loads on the ecosystem.

The Growing Threat of Avian Botulism on the Great Lakes

One of the greatest threats to the populations of birds named above is avian botulism. Botulism is a neurotoxin produced by the bacteria *Clostridium botulinum* and is classified in seven serotypes that affect different organisms. Table 1 describes the different serotypes and the primary species that are affected by them, as well as the estimated risk to humans (Rocke & Friend, 1999).

Table 1: Botulinum toxins and primary species affected.

| Toxin Type | Animal Affected | Risk for Humans |
|------------|-------------------------------------|-------------------|
| A | Poultry | Occasionally High |
| B | Horses | High |
| C | Wild birds, cattle, horses, poultry | Low |
| D | Cattle | Low |
| E | Fish eating birds | High |
| F | * | Unknown |
| G | * | Unknown |

* Rarely detected in nature; too little information for species evaluations.

Table 1 – Botulism serotypes categorized by name, the most common animals that the serotype affects, and the potential risk for human exposure. Rocke, T.E. & M. Friend (1999). Chapter 38: Avian Botulism. *Field Manual of Wildlife Diseases: Birds*. 271-281.

- *Type C Avian Botulism*

Types C is well known for its ability to cause epizootics that have ended in millions of bird deaths over time. The type C strain is also known as the “western duck sickness” and caused most of the outbreaks of avian botulism before 1966 in the western states such as California and Utah (Rocke & Samuel, 1999). The proposed method of transfer for the type C strain is known as the carcass-maggot cycle which is started by the mass die off of some taxa, often fish or birds, and the carcasses that drift to the shore begin to decompose. The decomposing organic matter which is already contaminated with the *Clostridium* spores due to

its natural presence in aquatic environments provides an anaerobic substrate for the bacteria to grow in and produce the botulism toxin. Once maggots begin to develop and feed on the carcass, the toxin concentrates within the body of the maggot. Scavengers like seagulls ingest the bacterial toxin with the maggots and the dead matter and become sick or die from the toxin, continuing the production of decomposing matter (Rocke & Friend, 1999). Figure 8 illustrates the proposed method of the carcass-maggot cycle of type C avian botulism (Sea Grant, 2002)

The Carcass-Maggot Cycle

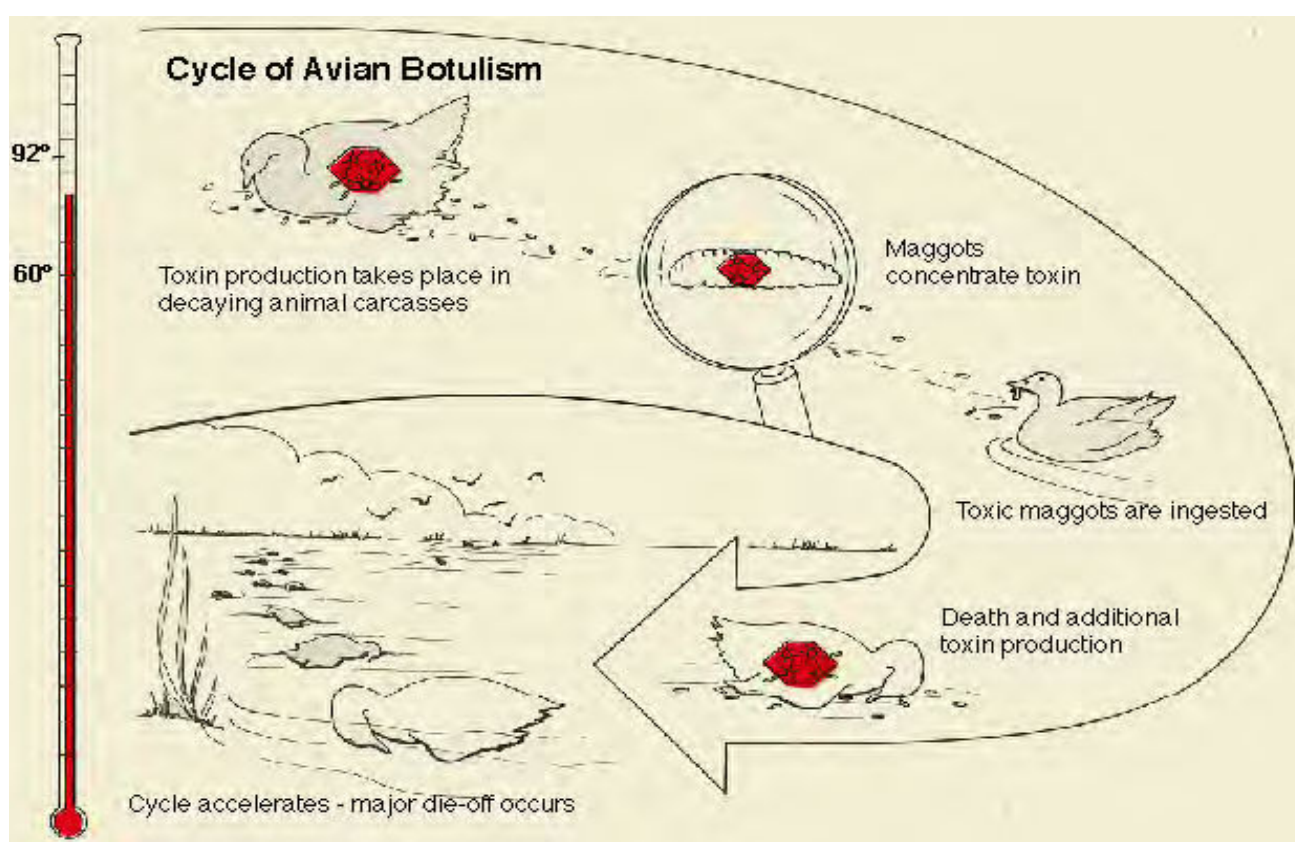


Fig 8 - The proposed method of the carcass-maggot cycle. Sea Grant (2002). *Botulism in Lake Erie Workshop Proceedings*. New York Sea Grant, Ohio Sea Grant, and Pennsylvania Sea Grant. <http://www.seagrantsunysb.edu/botulism/pdfs/Botulism-Proc02.pdf>

- *Type E Avian Botulism*

Type E avian botulism however has been contributed to other possible vector pathways but has yet to be determined. Epizootics of type E, unlike type C, have been mainly restricted to the

Great Lakes eco-region and have been recorded since the 1960's on Lakes Huron and Michigan but have only been recorded causing outbreaks on Lake Erie since 1999 (Majumdar & Huffman et al, 2005). It is assumed that the birds and fish that are involved in the epizootic are either ingesting the botulism toxin through contaminated food sources such as other infected fish or invertebrates or that the spores created by *Clostridium botulinum* have the ability to colonize the intestinal tract of vertebrates that are susceptible (Perez-Fuentetaja & Clapsadl et al, 2006). Botulism outbreaks usually occur in areas known as "hot spots" and it is assumed that in these locations the requirements for mass bacterial proliferation are met (Perez-Fuentetaja & Clapsadl et al, 2006). In 1999 alone, thousands of birds were killed in an outbreak including 700 Common Loons on Lake Huron (Campbell & Barker, 1999). An estimated 370 loons were killed by botulism type E just on the shores of New York in 2001 and the numbers of outbreaks are climbing annually (Stone & Okoniewski, 2002).

- *Symptoms*

Once birds are infected the symptoms begin with paralysis of the legs following with the nictitating membranes or eyelids and eventually the muscles around the head and neck become immobilized so the bird is no longer able to keep its head above the water. This is why many aquatic birds die from drowning before they are able to die from respiratory failure caused by the toxin. If allowed to proceed without drowning the host, the toxin will eventually cause respiratory paralysis (Rocke & Friend, 1999). The bacterial spores can be found in freshwater, marine and terrestrial habitats all over the world and they are resistant to heat and desiccation (Yule & Barker et al, 2006., Rocke & Euliss et al. 1999). Yet, since the bacteria and spores of type E are always present and the outbreaks have become annually recurrent on Lake Erie only since 1999, there is a possibility that something in the environment is

encouraging the continual growth and dispersal of the bacteria in a concentrated volume enough to cause these outbreaks (Yule & Lepage et al, 2006).

Proposed Mechanisms of Toxin Transfer by Invasive Exotics

It is widely accepted that the invasive species of zebra mussels, *Dreissena polymorpha*, the quagga mussel, *Dreissena bugensis*, and the round goby, *Neogobius melanostomus*, are key factors in the biomagnification of the toxin through the trophic web. Natives to the Black Sea, the round gobies were first spotted in the Great Lakes in 1990 north of Lake St. Clair and within just five years the fish had established populations in each of the five Great Lakes (Majumdar & Huffman et al, 2005). In annual trawl samples taken from Lake Erie, the percentage of round gobies of the total catch went from 4% in 1997 to 95% in 1999 (Murray, 2001). The dreissenid mussels have changed the soft sediment beds of the lakes they inhabit by blanketing it with the bodies of living and dead mussels and their by products, which in turn through decomposition and the shielding the sediments from the water interface creates an anaerobic environment for the bacteria to flourish in (Perez-Fuentetaja & Clapsadl et al, 2006). Significant amounts of the bacterium toxin has been found in the mussel tissues and feces leading to the conclusion that through their filtration systems, the mussels themselves are harboring higher concentrations of the bacteria compared to their surrounding, which may mean potential danger to any predators (Perez-Fuentetaja & Clapsadl et al, 2006). The appearance of the mussels in the Great Lakes also created vast limnological effects including greater water clarity from their filtration of sediments, meaning more sunlight permeates the water to the vegetation on the bottom. The problem arises when the winter comes and the increased amount of vegetation dies and decomposes at the bottom of the lake where it

inadvertantly creates a beneficial living condition for the *Clostridium* bacteria by decreasing the oxygen levels (Stone & Okoniewski, 2002). Interestingly enough, the round goby appears to be more susceptible to low doses of the botulism toxin comparatively in trials done with multiple fish species living in the Great Lakes and varying dosages of toxin (Yule & Lepage et al. 2006). Loons in general will pick out the contaminated round gobies when feeding due to the fact that they swim slower and more erratically which makes them easier prey. The round gobies are avid predators of the quagga mussels and are found at a population density of about 20 per square meter of lake bottom on a lake wide basis making them highly suspect as transmitters of this disease to fish eating birds (Campbell & Barker, 1999). The round goby also changes color once infected with the neurotoxin which may also encourage birds to prey upon them as an indicator of their decreased fitness (Yule & Barker et al, 2006) In one study from 1999 to 2000 of the stomach contents of dead birds about 38% of the stomach contents on average contained the remains of round gobies (Yule & Lepage et al, 2006). The problem remains however that a bird may not experience any symptoms in hours to days after the ingestion of enough toxin to cause death and therefore the remains of the vector will have been eliminated by the time of death (Rocke & Friend, 1999).

Clostridium botulinum and its toxin type E

Although believed to be “the most toxic substance known” there is a deficit in the amount of research that has been conducted on *Clostridium botulinum* and its toxin serotype E (Yule & Barker et al, 2006). The toxin itself is so potent that it has been known to cause death in humans with as little as .05 to .1 µg (Cherinton, 1997). The *Clostridium* bacterium thrives in anoxic substrates that are rich in organic material like the bottom of lakes, in fermenting

vegetation, or in the carcasses of dead vertebrates (Perez-Fuentetaja & Clapsadl et al, 2006). The neurotoxin created by *Clostridium botulinum* is a polypeptide that inhibits the release of acetylcholine, an important chemical messenger, from synaptic vesicles at the peripheral motor nerve terminal which blocks the chemical message from transferring across the neuromuscular junction (Sobel, 2005) Figure 9 illustrates a synaptic vesicle attaching to a pre-synaptic membrane and releasing neurotransmitters like acetylcholine into a neuromuscular junction where they bind to receptors on the post-synaptic membrane.

Vesicle Binding to a Presynaptic Membrane

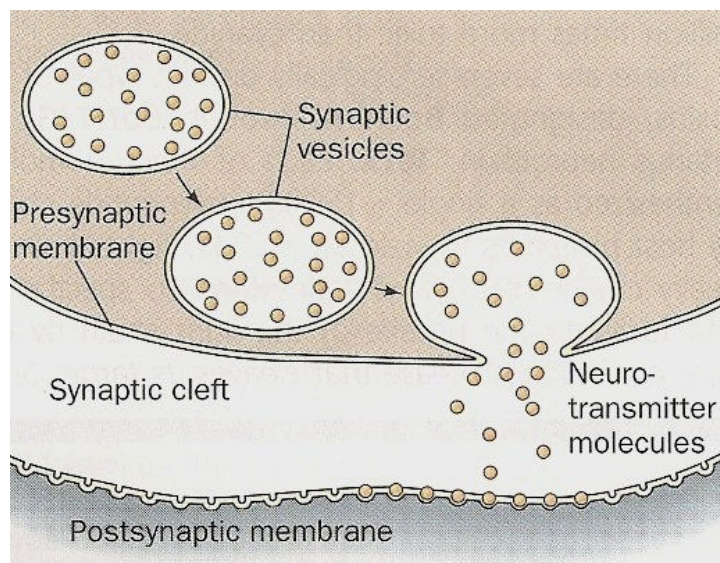


Fig 9 – The transportation of neurotransmitters via a synaptic vesicle by binding to the presynaptic membrane and being released into the neuromuscular junction. The neurotransmitters bind to the postsynaptic membrane to continue the chemical message pathway. Voet, D., J.G. Voet, & C.W. Pratt (2008). *Fundamentals of Biochemistry: Life at the Molecular Level*. Danvers, MA: John Wiley & Sons, Inc.

The single ~150 kD polypeptide chain created by the bacteria is actually cleaved within the host by the host's proteases, enzymes that cleave specific proteins, into a ~50 kD light chain and a ~100 kD heavy chain. The heavy chain binds to a motor neuron and facilitates the uptake of the light chain by endocytosis and then the light chain cleaves its target SNARE

protein at a specified site. SNARE proteins are important for chemical message transfer as they facilitate the binding of vesicles to membranes so that their chemical contents can be released outside of the membrane. Without the SNARE proteins, the phospholipids bilayers of the vesicle and the target membrane would have no mechanism that would bind them together. Figure 10 illustrates the SNARE protein complex that is created when a vesicle binds to a membrane and where the type E botulism toxin serotype cleaves the protein. The type E serotype is indicated by the BoNT/E abbreviation (Voet & Voet, et al, 2008).

SNARE complex between two membranes and Type E cleavage site

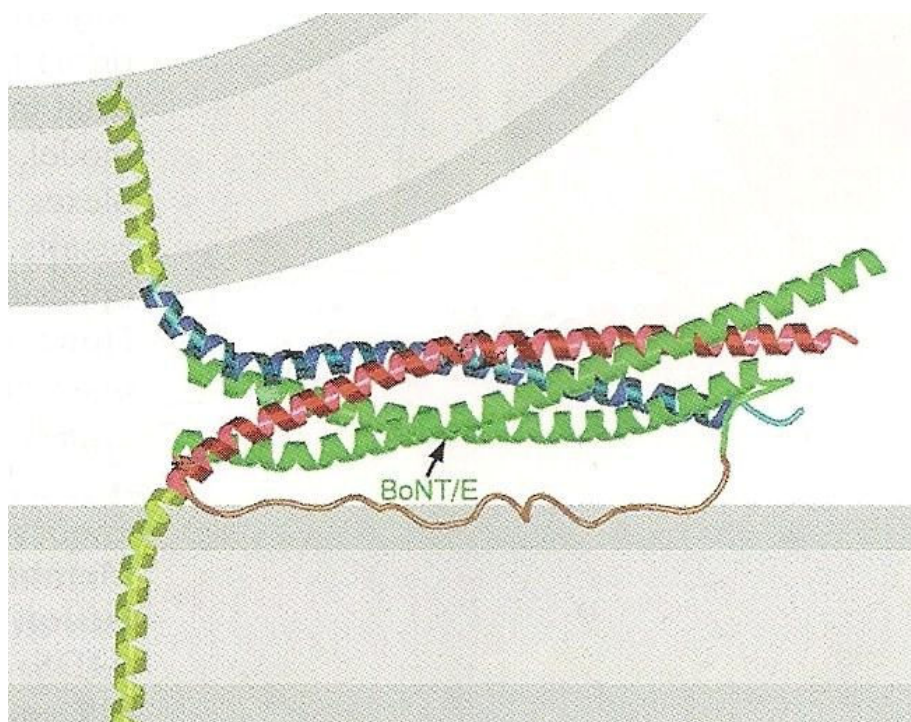


Fig 10 – Illustration of the complex of the R-SNARE protein (red, one helix) and the Q-SNARE protein (green, 2 helices) binding the membranes of a vesicle and a membrane. The cleavage site of the type E botulism toxin is indicated by a black arrow. Voet, D., J.G. Voet, & C.W. Pratt (2008). *Fundamentals of Biochemistry: Life at the Molecular Level*. Danvers, MA: John Wiley & Sons, Inc.

Type E serotype cleaves the SNAP-25 helix of the Q-SNARE which is attached to the target membrane that the vesicle would have bound to. The cleaving of the SNARE protein

discourages the formation of the complex that binds the vesicles to the membrane so that the chemical messenger is not released (Voet & Voet et al, 2008). Once the toxin protein has bound to its specific receptor the reaction can not be reversed and therefore the only means of recovery is the regeneration of axons and nerve terminals (Campbell & Barker, 1999).

Current Research on Abiotic Factors as Indicators of Outbreaks

The *Clostridium* bacteria are always present in the soil around the lakes and in the sediments of the lakes, but the outbreaks of avian botulism cause mass die offs in relatively strict areas in large amounts very quickly. Several studies have suggested possible links to abiotic factors that would contribute to the initiation of the outbreaks by creating an environment for increased bacterial growth. In a study in 1999, Roche and Samuel look samples at 32 wetlands in 9 states from 1986 to 1994 to obtain avian botulism outbreak data. They collected information on bird deaths and the temperature, pH, redox potential, conductivity and salinity of each site daily. Their results suggest that the environment for high risk of an outbreak has a pH level of >5 and a salinity of >2 ppt. (Roche & Samuel, 1999). In a study by Hyytia-Trees (1999) the researchers found that the bacterium has the ability to grow and reproduce at a temperature of 3° C but its optimal growth range is between 25 to 37° C. *Clostridium* salinity tolerance is as high as 5% and has a pH tolerance as low as 4.8 although its optimal growth is between 6.8 to 7 (cited in Majumdar & Huffman et al., 2005). This indicates that the *Clostridium* bacteria are highly efficient in regulating their life processes to thrive in different environments but there are peaks in temperature and pH that would increase their reproduction. In a study done by Murphy & Lawson et al. (2000) they proposed that while birds were molting their flight is restricted and they are therefore less able to avoid contact

with algal toxins which could provide enough additional stress to the bird's system that it would become susceptible to colonization by the bacterium in its digestive tract. At six stations, nine samples were taken of the dissolved oxygen, conductivity, redox, pH, temperature and depth of the water. Samples of sediments from the site as well as algal samples were taken and analyzed for their chemical composition and concentration. Murphy & Lawson et al. produced evidence that it is likely that the lysis of toxic algal cells during an algal bloom collapse would release the algal toxins into the water where the molting birds would be exposed to it and it could amplify the effect on their already strained systems.

Wetland flooding and draining, the use of pesticides, and other agricultural pollutants could also be a major factor in how outbreaks begin as they could cause die offs of other species and in turn create more habitats for *Clostridium* growth (Rocke & Friend, 1999). Rocke and Samuel (1999) found that the average biomass of invertebrates was higher during outbreak periods than in periods when no outbreaks occurred. The increased levels of invertebrates in correlation to outbreaks could mean that the invertebrates themselves are changing the environment during outbreaks or that they are a part of the transfer of the toxin to other taxa. The redox potential of the system, or the ability of the environment to electrochemically oxidize or reduce particle, was also found to be important as it dictates the direction of biochemical reactions and therefore the operation of enzymes and other metabolic processes that could either stimulate or inhibit bacterial growth (Rocke & Samuel, 1999). Perez-Fuentetaja et al. (2006) found a direct correlation between the bacteria's production and low redox potential and low dissolved oxygen which conflicts with Rocke and Samuel's data from their 1999 study in which they found no link to dissolved oxygen or water depth (Perez, et al. 2006., Rocke & Samuel, 1999). Perez-Fuentetaja and her associates also found that there was a

higher productivity zone of spore production of the bacteria in shallower study areas where birds would be more likely to come into contact with the spores and become contaminated. Samples of six stations in Lake Erie near Dunkirk and were tested in both “inshore” (mean depth =7.3 m) and “off shore” (mean depth = 17.6 m) locations. Water depth, dissolved oxygen, temperature, conductance, pH, redox potential, phosphorus and nitrate/nitrite levels, and sediment analyses for presence of *Clostridium botulinum* were all measured during the study period (Perez, et al. 2006)

Finally, Barras and Kadlec’s (2000) study in the Bear River Delta, Utah proposed that high precipitation and increased run off from storms may contribute to the organic matter *Clostridium* can use as an energy source. The increased amount of nutrients collected by the lakes through surface run off could possibly temporarily release *Clostridium botulinum* from competition with other microbes and therefore allow it to proliferate exponentially for a period of time after the storm or increased precipitation pattern. Although observational data has conflicted between studies and many ideas of indicators of bacterial growth have been proposed there is no evidence so far of a single biotic or abiotic indicator of an outbreak of type E avian botulism.

Premise of Study

In this study, historical abiotic factors including temperature, wind strength, precipitation, and lake levels from specific locations around the shores of Lakes Erie and Ontario have been compared against annual mortality rates of specific bird species in corresponding locations. Since these outbreaks occur annually, we predict that there should be recurring indicators of an outbreak before it happens (Soos & Wobeser, 2006). The ten station

locations for climate data that were used are located on the New York State shorelines of Lake Erie and Lake Ontario near sites where bird death data was collected. The objective of this study is to determine if there is a relationship between the bird deaths from 2002 to 2007 and the temperature, wind strength, precipitation, and lake levels of the two lakes.

- Higher temperatures are predicted to have positive correlation with outbreaks as the bacteria have been shown optimally proliferate at temperatures between 25° to 40° C (Rocke & Friend, 1999).
- Greater wind strength will presumably cause more disturbances in the water and therefore make sediments more heavily distributed. Since the *Clostridium* spores are prevalent in lake sediments, this would give them more of an opportunity to become filtered into a mussel when feeding or picked up by a fish and eventually carried on up the trophic web.
- Precipitation is predicted to have a similar effect as the wind strength since it causes disturbances to the lake sediments which would stir them up and expose more *Clostridium* spores into the open water where they could be ingested, but also could cause bird mortalities in other ways and then secondarily start a corpse-scavenger cycle of transmitting the toxin.
- Lake levels are controlled to some degree by humans, but it is possible that there is enough natural fluctuation to have an effect on the stability of the aquatic ecosystem. Lower lake levels could cause a greater concentration of *Clostridium* spores per unit of lake water making it more likely to be concentrated in the prey species of the aquatic birds. Also, lower lake levels mean that there is less of a buffer between the surface of the lake and the sediments below meaning that disturbances to the sediments would be

more dramatic. From 1963 to 1964 massive outbreaks of type E avian botulism occurred on Lake Michigan and Huron during the lowest lake levels ever recorded between 1916 and 1993 (Majumdar & Huffman et al., 2005).

The importance of defining indicators of type E avian botulism outbreaks is that it could have major implications on how wetland habitats are managed. If the vector is from the accumulation of decaying organic matter then the removal of carcasses after major die offs or large storms can be made a greater priority. If the vectors are found to be biomagnifications through invasive aliens then greater enforcement on ballast water regulations could become a management priority to help prevent more introductions from causing further damage. Research towards eliminating or controlling the populations of current invasive species should be put into action. If abiotic factors can be used as indicators then management plans that require daily recordings of the indicators can be implemented and possible controls for the factors could be made. For example, if lake levels are found to have a relationship to botulism type E outbreaks then when lakes reach the detrimental level then groups could be activated to search the beach and waters for birds in distress to remove and treat them. Bird losses annually could be cut to more manageable numbers and it would release some bird species from the threat of losing genetic diversity from loss of breeding pairs or from becoming endangered or even extinct in these areas.

Materials and Methods

Avian Guilds

The bird species used in the study were chosen for their abundance on both lakes and for their appearance in type E botulism outbreak data provided by the New York State Department of Environmental Conservation. To compare the bird die offs on Lake Erie and Lake Ontario, bird species studied were divided into guilds by their feeding preferences. The first guild was labeled as the “Loon” guild and incorporates the Common Loon, *Gavia Immer*, and the Red Throated Loon, *Gavia stellata*. The loons were placed in a separate guild because their diet consists mostly of fish with a limited portion of invertebrates, The second guild is the “diving ducks” which includes the Greater Scaup, *Aythya marila*, the Lesser Scaup, *Aythya affinis*, the White-winged Scoter, *Melanitta fusca*, the Long-tailed Duck, *Clangula hyemalis*, the Common Merganser, *Mergus merganser*, and the Red-breasted Merganser, *Mergus serrator*. Diving ducks generally eat a higher proportion of invertebrates and vegetation. Finally, the third guild was designated to “Gulls” due to their scavenging feeding behavior. The Gulls included in this guild were the Bonaparte’s Gull, *Larus philadelphia*, Sabine’s Gull, *Xema sabini*, Ring-billed Gull, *Larus delawarensis*, Herring Gull, *Larus argentatus*, Glaucous Gull, *Larus hyperboreus*, and the Great Black-backed Gull, *Larus marinus*.

Study Area

The bird die off data collected by the New York State Department of Environmental Conservation was organized by species, day of collection, and by transect the bird corpse(s) were found. From this data the birds were organized by species and guild, by the date of their collection, and by the lake on which they were found. The data was then compiled on a daily,

weekly, and monthly basis but still remained separated by bird guild and by lake they were found on. The bird die off data was obtained from the New York State DEC from both their Buffalo and Albany branches produced by Kenneth Roblee and Dave Adams respectively. Since 2001, the New York State Department of Environmental Conservation has collected bird die off data in the fall from type E avian botulism in specific transects along Lakes Erie and Ontario. The collection of bird corpses usually began between mid September to early October and ended around the beginning of January. All corpses were tested for the botulism toxin to establish cause of death before being added to the bird die off list. The list of transects studied by the NYS DEC by lake, county and latitude and longitude are included in Table 1.

Collection Site Transect Data

| Lake | Transect | Site Name | County | Start Latitude | End Latitude | Start Longitude | End Longitude |
|---------|----------|---------------------------------|------------|----------------|--------------|-----------------|---------------|
| Erie | 9ERI01 | Barcelona Beach South | Chautauqua | 42.34 | 42.3378 | -79.6038 | -79.6074 |
| | 9ERI02 | Barcelona Beach North | Chautauqua | 42.3421 | 42.3401 | -79.5987 | -79.6022 |
| | 9ERI03 | Lake Erie State Park South | Chautauqua | 42.4215 | 42.4191 | -79.4395 | -79.443 |
| | 9ERI04 | Lake Erie State Park North | Chautauqua | 42.4254 | 42.4228 | -79.4343 | -79.4378 |
| | 9ERI05 | Point Gratoit Park | Chautauqua | 42.4867 | 42.4831 | -79.3595 | -79.3603 |
| | 9ERI06 | Sunset Bay South | Chautauqua | 42.5537 | 42.5522 | -79.1486 | -79.1527 |
| | 9ERI07 | Sunset Bay North | Chautauqua | 42.5691 | 42.5656 | -79.1375 | -79.1386 |
| | 9ERI08 | Bennett Beach | Erie | 42.659 | 42.6556 | -79.063 | -79.0628 |
| | 9ERI09 | Wendt Beach Park | Erie | 42.6789 | 42.6758 | -79.0522 | -79.0537 |
| | 9ERI10 | Sturgeon Point Marina South | Erie | 42.6916 | 42.6883 | -79.0474 | -79.0492 |
| | 9ERI11 | Sturgeon Point Marina North | Erie | 42.6934 | 42.6917 | -79.0392 | -79.0433 |
| | 9ERI12 | Woodlawn Beach State Park South | Erie | 42.7904 | 42.7868 | -78.8532 | -78.8535 |
| | 9ERI13 | Woodlawn Beach State Park North | Erie | 42.8 | 42.7968 | -78.8562 | -78.8549 |
| Ontario | 6ONT36 | Lakeview Marsh WMA | Jefferson | 43.7575 | 43.7538 | -76.2139 | -76.2129 |
| | 6ONT37 | Black Pond WMA | Jefferson | 43.8039 | 43.8002 | -76.229 | -76.2278 |
| | 6ONT38 | Boomer Cove | Jefferson | 43.8409 | 43.838 | -76.2774 | -76.2746 |
| | 6ONT39 | Stony Point | Jefferson | 43.8805 | 43.8819 | -76.2543 | -76.2594 |
| | 6ONT40 | Henderson Bay | Jefferson | 43.8653 | 43.8642 | -76.2258 | -76.2208 |
| | 6ONT41 | Ben's Cove | Jefferson | 43.8889 | 43.8906 | -76.1446 | -76.1403 |
| | 6ONT42 | Gilmore Point | Jefferson | 43.9439 | 43.9409 | -76.1362 | -76.1392 |
| | 6ONT43 | Godfrey's | Jefferson | 43.9691 | 43.9691 | -76.1963 | -76.2007 |
| | 6ONT44 | J. Constance | Jefferson | 43.9854 | 43.9885 | -76.2702 | -76.2677 |
| | 6ONT45 | Bob Pitcher's | Jefferson | 44.0111 | 44.014 | -76.3016 | -76.3024 |
| | 6ONT46 | Conrad Salber's | Jefferson | 44.0585 | 44.0597 | -76.3079 | -76.3116 |
| | 6ONT47 | Cape Vincent | Jefferson | 44.0798 | 44.0824 | -76.3545 | -76.3504 |

Ontario

| | | | | | | |
|--------|---------------------|--------|---------|---------|----------|----------|
| 7ONT27 | Sitts Bluff | Cayuga | 43.3444 | 43.3458 | -76.6989 | -76.6947 |
| 7ONT28 | West Ninemile Point | Cayuga | 43.4131 | 43.4164 | -76.6225 | -76.6189 |
| 7ONT29 | SUNY Oswego | Oswego | 43.4536 | 43.4558 | -76.5478 | -76.5439 |
| 7ONT30 | Alcan | Oswego | 43.4864 | 43.4911 | -76.4764 | -76.4744 |
| 7ONT31 | Ninemile Point | Oswego | 43.525 | 43.5239 | -76.3858 | -76.3811 |
| 7ONT32 | Dempster Beach | Oswego | 43.5147 | 43.5139 | -76.3108 | -76.3056 |
| 7ONT33 | Ramona Beach | Oswego | 43.5333 | 43.5372 | -76.2281 | -76.2244 |
| 7ONT34 | Deer Creek Marsh | Oswego | 43.5964 | 43.6006 | -76.1994 | -76.1989 |
| 7ONT35 | Sandy Pond | Oswego | 43.6689 | 43.6731 | -76.1975 | -76.1983 |

Ontario

| | | | | | | |
|--------|-------------------------|---------|---------|---------|----------|----------|
| 8ONT07 | | Orleans | 43.3786 | 43.3789 | -78.4308 | -78.4292 |
| 8ONT08 | | Orleans | 43.3789 | 43.3792 | -78.4292 | -78.3494 |
| 8ONT09 | | Orleans | 43.3772 | 43.3731 | -78.2511 | -78.2536 |
| 8ONT10 | | Orleans | 43.3819 | 43.3814 | -78.1658 | -78.1569 |
| 8ONT11 | | Orleans | 43.3756 | 43.3742 | -78.0775 | -78.0675 |
| 8ONT12 | | Monroe | 43.3692 | 43.3694 | -77.9736 | -77.9544 |
| 8ONT13 | | Monroe | 43.3525 | 43.3514 | -77.8872 | -77.8772 |
| 8ONT14 | | Monroe | 43.3464 | 43.3447 | -77.7842 | -77.7858 |
| 8ONT15 | Braddock Bay | Monroe | 43.3206 | 43.3164 | -77.7083 | -77.7006 |
| 8ONT16 | | Monroe | 43.2761 | 43.2714 | -77.6325 | -77.625 |
| 8ONT17 | Durand-Eastman Park | Monroe | 43.2369 | 43.2358 | -77.5583 | -77.5506 |
| 8ONT18 | Kanatota Park | Monroe | 43.2604 | 43.2614 | -77.4521 | -77.4496 |
| 8ONT19 | 1624-1626 Lake Road | Monroe | 43.2797 | 43.2806 | -77.345 | -77.3408 |
| 8ONT20 | Bear Creek Harbor | Wayne | 43.2709 | 43.2785 | -77.2769 | -77.2861 |
| 8ONT21 | Pultneyville Yacht Club | Wayne | 43.2839 | 43.2842 | -77.1865 | -77.1923 |
| 8ONT22 | Bootlegger's Point | Wayne | 43.2872 | 43.2892 | -77.1016 | -77.1067 |
| 8ONT23 | Camp Beechwood | Wayne | 43.2705 | 43.2707 | -77.0295 | -77.118 |
| 8ONT24 | Chimney Bluffs | Wayne | 43.2707 | 43.2841 | -77.0347 | -76.9261 |
| 8ONT25 | Port Bay | Wayne | 43.3058 | 43.3052 | -76.8323 | -76.8372 |
| 8ONT26 | Blind Sodus Bay | Wayne | 43.3437 | 43.3447 | -76.7207 | -76.7147 |

Ontario

| | | | | | | |
|--------|----------------------------|---------|---------|---------|----------|----------|
| 9ONT01 | Four-Mile Creek State Park | Niagara | 43.2771 | 43.2773 | -79.0001 | -78.9949 |
| 9ONT02 | Uneeda Beach | Niagara | 43.2969 | 43.2981 | -78.9167 | -78.9116 |
| 9ONT03 | Wilson Tuscarora | Niagara | 43.3111 | 43.3129 | -78.8542 | -78.849 |
| 9ONT04 | Olcott Beach | Niagara | 43.3389 | 43.3397 | -78.7161 | -78.7106 |
| 9ONT05 | Lower Lake Road | Niagara | 43.365 | 43.3656 | -78.579 | -78.5734 |
| 9ONT06 | Lakeview Drive | Niagara | 43.3696 | 43.37 | -78.5448 | -78.5393 |

Table 1: Collection sites for botulism type E contaminated bird carcasses by transect, site name, county, latitude and longitude. Dave Adams (2008) The NYS Department of Environmental Conservation, Albany NY, personal contact

Climate Data

Daily average temperature, daily average precipitation and daily average snowfall for this study were collected by co-ops of the National Oceanic and Atmospheric Administration

(NOAA). The individual data sites were chosen for their location within 10 miles of lake Erie or lake Ontario, their general close proximity to the transects used by the NYS DEC for collecting the bird death data and for their collection of data between 2001 to 2008. The co-ops used for this climate data were Dunkirk in Chautauqua county (ID: 302198), Silver Creek in Chautauqua county (ID: SILN6), the Buffalo-Niagara International Airport in Buffalo in Erie county (ID: 301012), the Watertown International Airport in Jefferson county (ID: 309005), Wellesley Island in Jefferson county (ID: 309055), Oswego East co-op in Oswego county (ID: 306314), Albion in Orleans county (ID: 300055), Lyndonville in Orleans county (ID: 304939), the Rochester Greater International Airport in Monroe county (ID: 307167), and Sodus co-op in Wayne county (ID: 307842). Climate data was compiled by day and by station, and then purchased through the Northeast Regional Climate Center (NRCC) in Ithaca, NY for use in this study. The stations from counties on Lake Erie were separated and weekly and monthly averages were determined for each individual station. The stations from counties on Lake Ontario were separated and averages for weekly and monthly conditions were also calculated. The historical lake level data was collected by the NOAA for Lakes Erie and Ontario separately by month and compiled from their Great Lakes Environmental Research Laboratory. The historical wind direction and strength data was collected by the NOAA from their cooperative stations along the lake shores including National Data Buoy Center stations, National Ocean Service stations, National Weather Service Great Lakes Observing System stations and a National Estuarine Research Reserve System station. Figure 11 is a map depicting the locations of the stations used for climatic data in this study

Map of Weather Data Station Locations

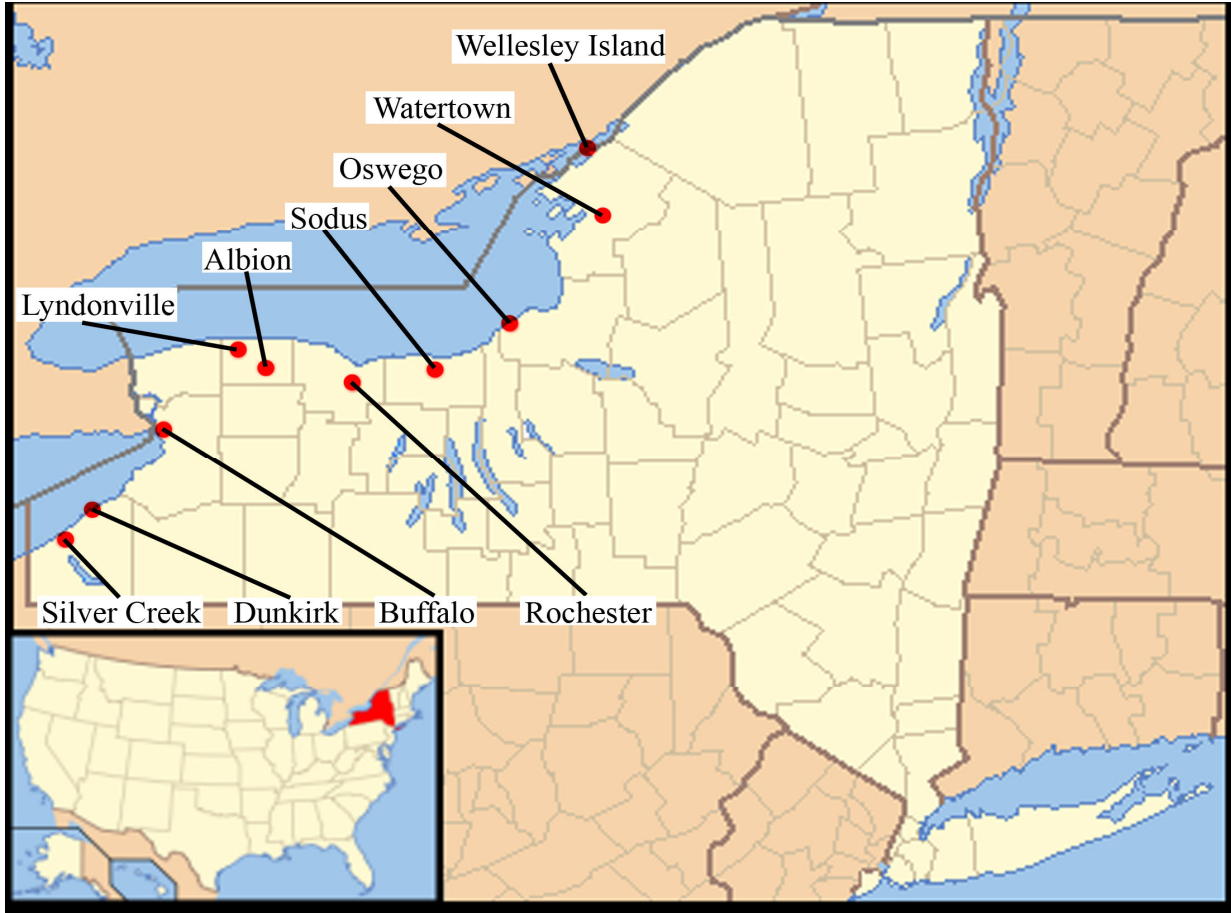


Figure 11: Map of stations where abiotic data was collected from and utilized in this study. This map was adapted from US Census website, modified by Wikimedia commons. http://en.wikipedia.org/wiki/File:New_York_Locator_Map_with_US.PNG

For the weather stations with missing data points, averages of the other stations were taken compensate.

Statistical Analysis

The data was analyzed using a multiple regression test to determine if a relationship exists between the mean bird deaths at a function of year, weekly average temperature, weekly average precipitation, weekly average snowfall, and the presence or absence of snowfall. The variable of “the day of the year” was modeled as a cyclical predictor. T tests are used for a

single comparison of two kinds of data. For example, the temperature would be one variable and the mean deaths would be a second variable and the relationship between them would be the comparison. An F test however is used when there needs to be multiple comparisons like when we tested the year by the mean deaths of the loons. The loons are a single variable, but then each year is another separate variable that needs a separate comparison to the die off data. The P value of the data is a measurement of its significance as a predictor of a bird die-off in this study. The bird deaths were expressed logarithmically to decrease the significant difference between the data points of recorded deaths during an outbreak to make them more manageable to compare. Minitab's GLM and Regression procedures were applied to fit the dependent variable of the current, previous, and second previous weeks' log transformed counts of deaths of loons and diving ducks as quantitative predictors to test if the deaths of the loons and/or diving ducks were predictors of gull deaths. For the relationship of temperature between the different study years on the gull die offs, a post hoc comparison of temperature effects for the years was conducted. *P*-values were adjusted for the multiple comparisons. The software used in this analysis included both Minitab ® and SAS/STAT ®.

Data and Results

Loons

For the study years from 2002 to 2007, the collected data suggests that the variables of weekly average temperature, weekly average snow fall, the year, and the day of the year are significant predictors of mean loon deaths. A multiple regression test was run on the abiotic factors against the mean loon deaths and Table 2 shows their fit as predictors of loon die offs including their coefficients as multiplicative factors, the standard error of the data, the ratio via a T or F test, and their P value. The year in which the bird data was collected (between 2002 and 2007) was found to be a significant predictor of the mean loon die offs ($P = 0.0011$). This means that there was a significant difference between each year the loon death data was collected and the mean loon die offs recorded. The cycle, meaning the trend of mean loon die offs and the day of the year, was also found to be a significant predictor of loon die off counts ($P = 0.0044$). The average peak in loon die offs averaged between the years occurs around November 18th as shown on Graph 1. The temperature is accepted to be an indicator of loon deaths in this study ($P = 0.0020$). For each one degree increase in the temperature, it is predicted to decrease the mean die off of loons by 6%. This is expressed from the coefficient of the temperature data found to be -0.0602 as shown in Table 2 (95% confidence interval of (2.3%, 9.3%)). The presence of snowfall is also considered to be an accepted indicator of loon deaths (P value of 0.0196). The presence of snow fall increases the die off count of loons by 2.42 times that of when snow fall is absent. This is expressed by the coefficient of the snow fall data which is 0.8829 as shown in Table 2 (95% confidence interval (1.16 to 5.05)). All together, the abiotic predictors used in the table can be used to significantly predict a loon die off during the years used in the study ($P < 0.0001$). The abiotic predictors are able to describe

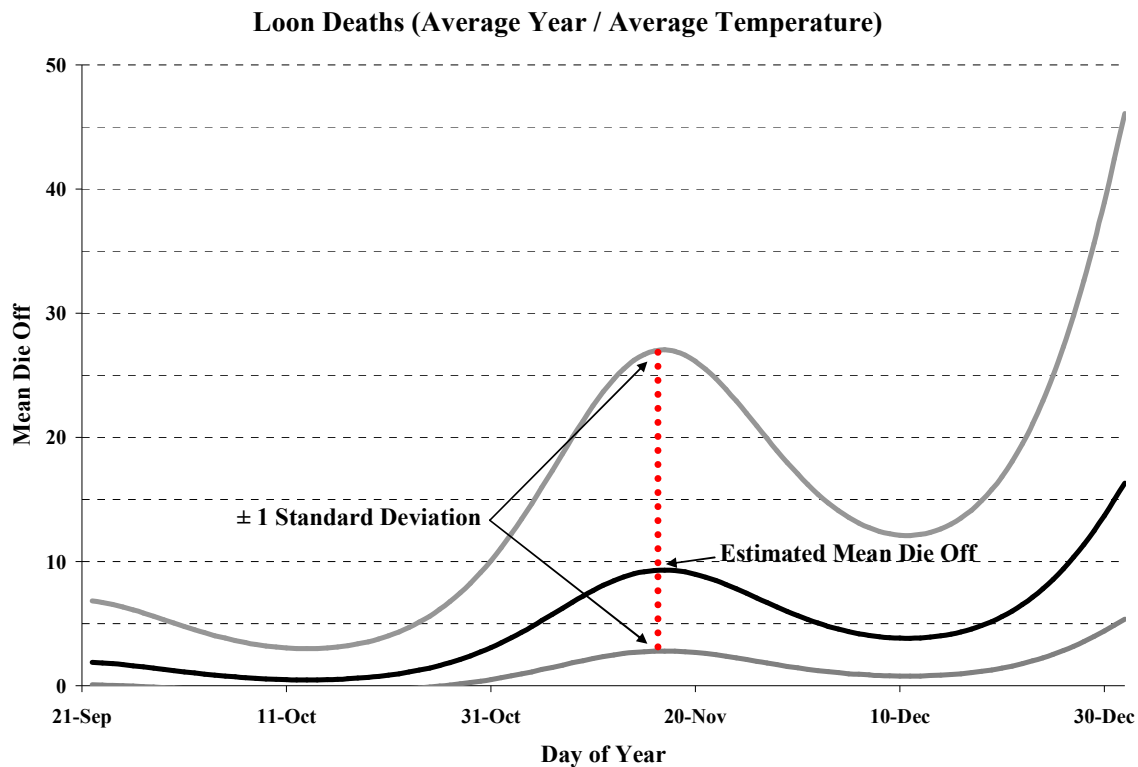
65% of the trends of mean loon deaths (R^2 value = 65%). The weekly average precipitation was found to not be a significant predictor of loon die offs (($P = 0.6113$ and $T(55) = 0.513$)) and therefore was eliminated as a possible indicator.

Loon Analysis

| PREDICTOR | COEFFICIENT | STANDARD ERROR | RATIO | P-VALUE |
|--------------|-------------|----------------|---------------------|----------|
| Temperature | -0.0602 | 0.0186 | $T(56) = 3.24$ | 0.0020 |
| Snow | 0.8829 | 0.3675 | $T(56) = 2.40$ | 0.0196 |
| Year | | | $F(5, 56) = 4.77$ | 0.0011 |
| Cycle | | | $F(3, 56) = 4.88$ | 0.0044 |
| Entire Model | | | $F(10, 56) = 10.39$ | < 0.0001 |

Table 2: Fit of abiotic predictors to loon deaths using multiple regression tests in SAS/STAT® program

Graph 1 shows the trend of mean loon die offs on collection days when no snow was present by the day of the year. The loon die off data for each year was averaged in the graph so the magnitude of the peak around this time may be different for each of the independent years but the over all trend of an increase at this time was significant for every year. The standard deviation of one above and below the estimated mean was included to give a relative range of the increase in deaths during this time for every year. Graph 1 indicates that a cyclic pattern may be occurring, but there is a definite increase in the mean loon die off count in November between the 10th and the 30th.



Graph 1: The mean loon die off versus the day of the year using averaged data from all six years in the study and averaged temperature data for the days. This plot only pertains to days when snow fall was absent.

Diving Ducks

For the diving duck guild, the abiotic variables of weekly average precipitation, snow fall, the study year, and the day of the year were found to be significant predictors of die offs. Table 3 includes the results of the multiple regression test to determine the fit of the abiotic predictors to the diving ducks die off data including their coefficients as multiplicative factors, the standard error of the data, the ratio via a T or F test, and their P value. Snow fall was found to be a significant predictor of mean diving duck die offs ($P = 0.0386$) but the relationship between snowfall and the diving ducks was not as significant as it was between the snowfall and the loons. The study year in which the diving duck deaths were recorded was found to be very significant as a predictor of die offs ($P = 0.0002$). This means that there was a high

significant difference between the magnitude of mean diving ducks that died and the year in which they were collected. The cycle, which is the day of the year during the study in which the diving ducks were collected averaged between the years of the study, was also found to be a significant predictor of diving duck die offs ($P = 0.0027$). For every weekly average increase of one inch of precipitation, the mean death count of the diving ducks in this study will decrease by 51.1%. This is shown by the coefficient of the weekly average precipitation data of -2.8910 (95% confidence interval (7%, 113%)). All together the abiotic predictors were found to be significant predictors of a diving duck die off during the study period ($P < 0.0001$). The weekly average temperature was not found to be a significant predictor of diving duck die offs for the study period ($P = 0.2204$ and $T(54) = 1.24$). The remaining predictors could be used to describe 59.5% of the estimated die offs of diving ducks during the study (R^2 value of 59.5%).

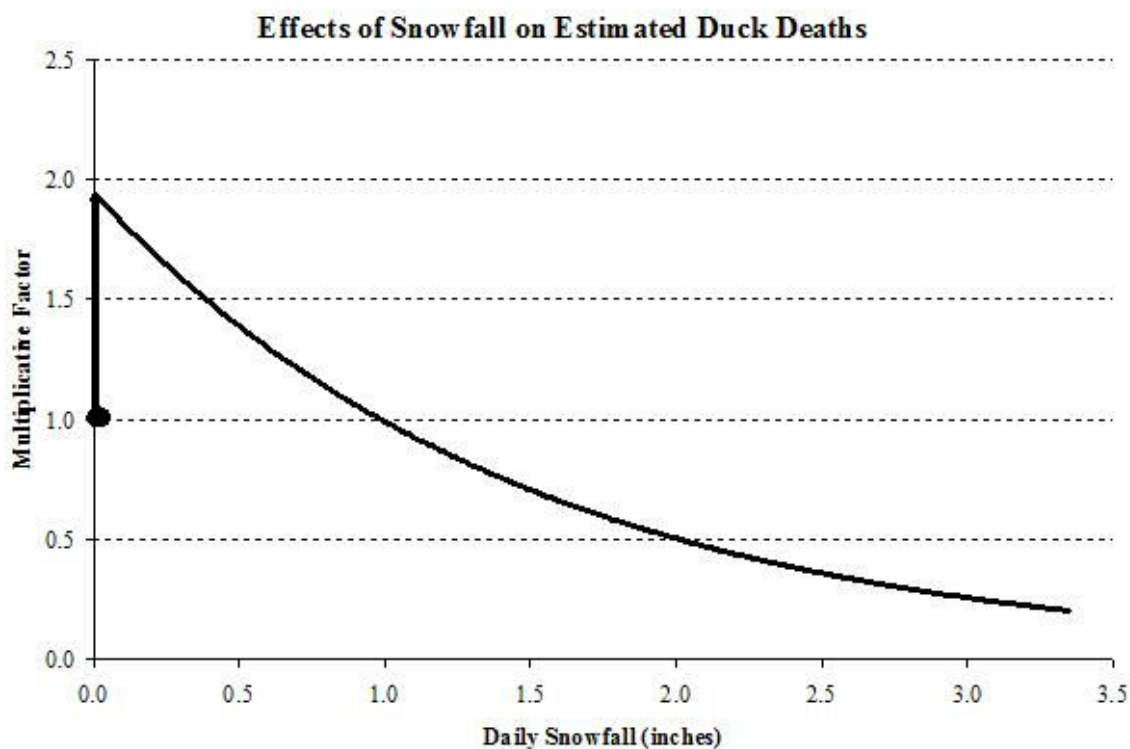
Duck Analysis

| PREDICTOR | COEFFICIENT | STANDARD ERROR | RATIO | P-VALUE |
|---------------|-------------|----------------|-------------------|------------|
| Precipitation | -2.8910 | 1.2021 | $T(54) = 2.40$ | 0.0196 |
| Snow | | | $F(2, 54) = 3.46$ | 0.0386 |
| Year | | | $F(2, 54) = 5.94$ | 0.0002 |
| Cycle | | | $F(3, 56) = 4.88$ | 0.0027 |
| Entire Model | | | $F(12,54) = 6.60$ | < 0.0001 |

Table 3: Fit of abiotic variables to diving duck die offs using multiple regression tests in SAS/STAT ® program.

Graph 2 demonstrates the linear relationship between the mean diving duck die offs as a multiplicative factor and the daily amount of snow fall in inches. The large dot on the far left of the graph indicates the estimated amount of duck deaths in the absence of snowfall and then

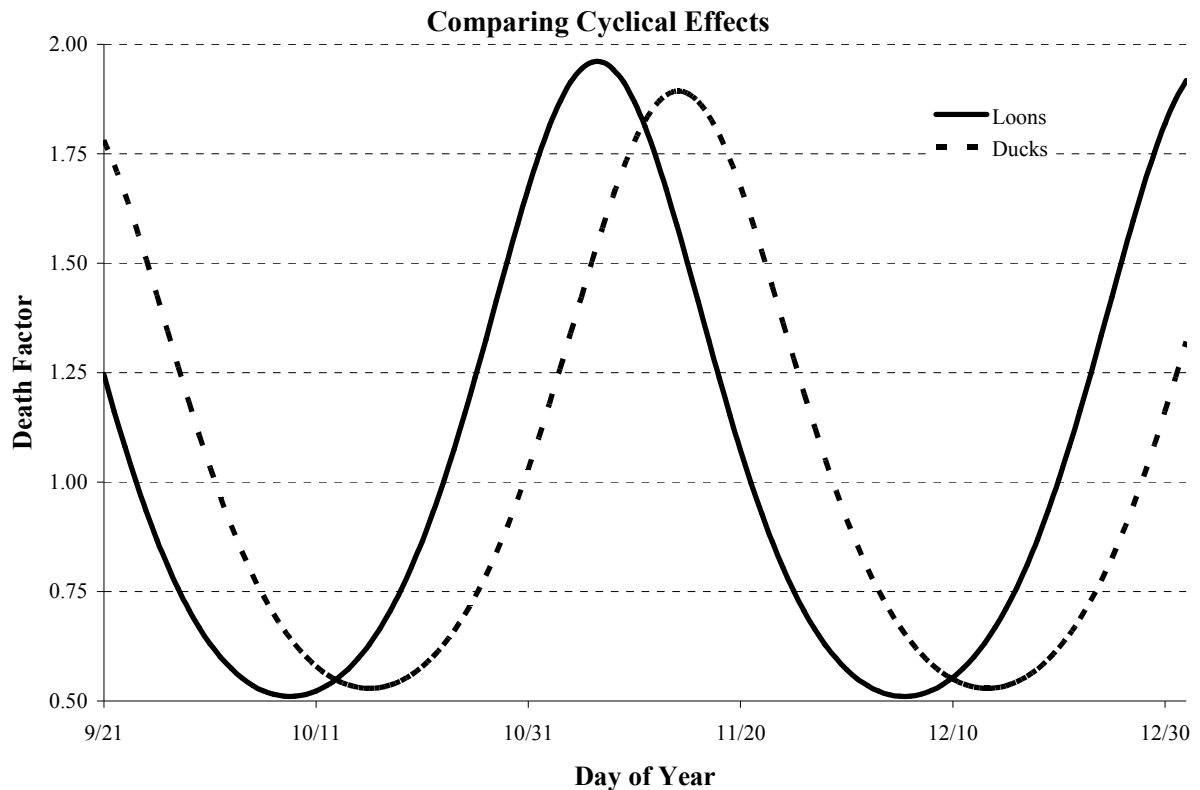
the trend line spikes at the first presence of snow fall then declines as the amount of snowfall becomes greater. This is most likely due to the correlation of snow fall at the time of the year indicated by the high significance of the cycle (or day of the year) as a predictor of diving duck deaths.



Graph 2: The mean duck deaths as a multiplicative factor by the amount of snowfall in inches during the study period

Graph 3 is a comparison of the cyclical effect of the bird die off counts for both the loons and the diving ducks by the day of the year during the study period. The study period being September 1st to December 30th averaged between all six study years using the death counts as a multiplicative factor. Although the loons experienced die offs approximately eight days before the diving ducks during the averages of the study, the loons are not a predictor of diving

duck die offs. What this graph indicates is that eight days after the loons experienced a die off, the diving ducks experienced a die off of similar magnitude and duration eight days later.



Graph 3: Comparison of diving duck and loon die offs as a multiplicative factor by the day of the year during the study period.

Gulls

The mean loon and diving duck deaths during the current week of gull deaths and the two weeks previous to the gull deaths were not found to be significant indicators of gull die offs ($P = 0.4467$ and $F(6, 35) = 0.99$). The two earliest sessions from each year were not included in assessing these effects, as two-week lags weren't computable from the given data. Table 4 shows the results of the multiple regression test to determine the fit of the abiotic predictors on the gull die off data including their coefficients as multiplicative factors, the

standard error of the data, the ratio via a T or F test, and their P values. The temperature variable was found to be a significant predictor of gull die offs ($P < 0.0001$). The abiotic variables of weekly average precipitation and presence/absence of snowfall were determined to not be significant indicators of gull die offs and were eliminated from the study variables ($P = 0.6631$, $F(2, 53)$). The predictors of the year, the temperature, and the interaction between the temperature and the study year were found to describe 48.8% of the trends of gull die offs ($R^2 = 48.8\%$) and together were significant predictors of gull die offs ($P < 0.0001$).

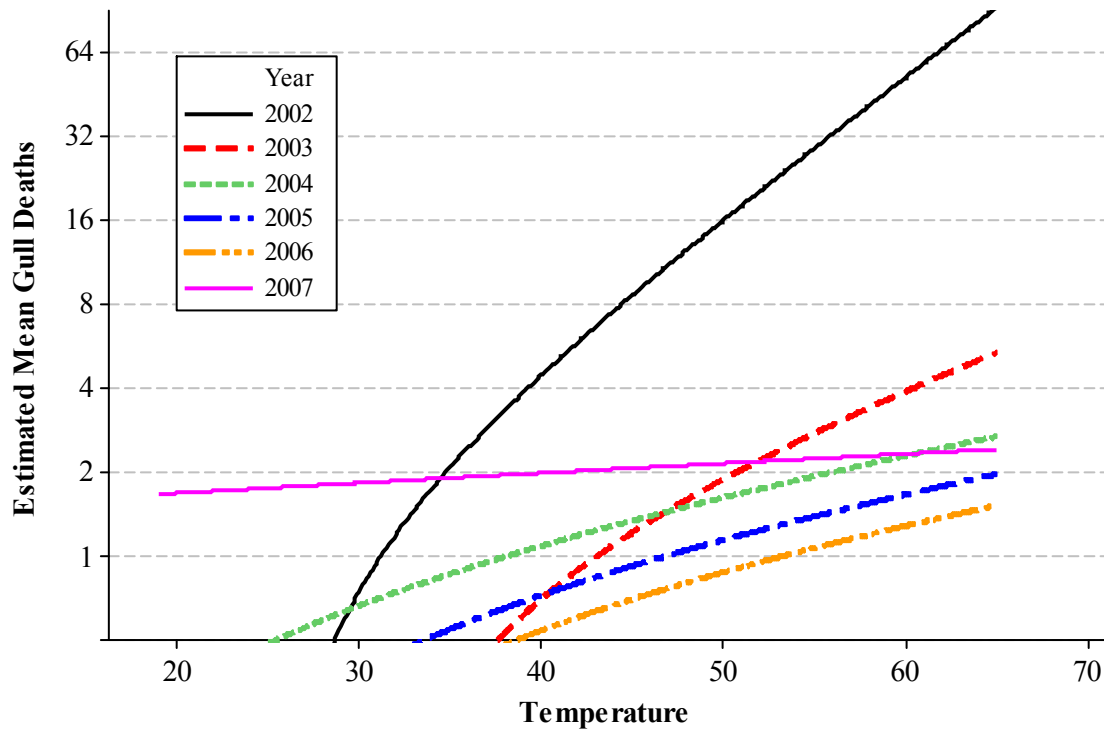
Gull Analysis

| PREDICTOR | COEFFICIENT | STANDARD ERROR | RATIO | P-VALUE |
|------------------|--|----------------|-----------------|----------|
| Temperature | 0.03928 | 0.00844 | T(55) = 4.66 | < 0.0001 |
| Year | | | F(5, 55) = 2.13 | 0.0756 |
| Year*Temperature | 2002: 0.07434 2003: 0.01346 2004: -0.01661 2005: -0.01762 2006: -0.01966 2007: -0.03391 | | F(5, 55) = 2.59 | 0.0355 |
| Entire Model | | | F(11,55) = 4.76 | < 0.0001 |

Table 4: Fit of abiotic variables to gull die offs using multiple regression tests in SAS/STAT® program.

The gull data indicated that the variable of temperature has a different effect on gull die offs among the different study years. Graph 4 shows the exponential relationship between the mean gull deaths and the temperature for each of the independent years.

The Affect of Temperature on Mean Gull Deaths by Study Year



Graph 4: Mean gull die offs by temperature for each of the study years from 2002 to 2007

As indicated by Graph 4, for the study year of 2002 temperature had the greatest exponential relationship with the gull deaths as compared to the other study years. For 2002, the mean gull deaths double for every 6 degree increase. For the other years, the relationship was not as strong. For example, for the study year of 2005 it takes a 17 degree increase in temperature to double the gull deaths. This indicates that although statistically, the average temperature was found to be a significant predictor of gull deaths, the temperature increase used to predict the gull deaths is different for every year.

Discussion

Loons

The day of the year during the study period was found to be a significant predictor of loon die offs and there was a peak in loon deaths between November 10th and November 30th. This is likely due to the fact that loons are known to gather on large fresh water lakes in preparation for their winter migration in late October to early November. During this time, they increase their food intake before their migration and this is most likely when they are becoming poisoned with botulism from the contaminated fish and invertebrates that are in the Great Lakes. The loon deaths then drop by early December since the loons that are healthy enough to migrate have left the Great Lakes region and are on the coasts of the U.S. or even as far south as Mexico (Wisconsin SeaGrant, 2007).

Temperature was a significant predictor of loon deaths from avian botulism and the analysis revealed for every one degree increase in the temperature the loon deaths would decrease by approximately 6%. This is likely to be due to the fact that the time of the year is a significant predictor of bird deaths. The weekly average temperature naturally decreases from October to December due to the change in the seasons which is the same time the loons are preparing to migrate for the winter. As the weeks continue and the temperature drops around late October and early November, the loons are collecting on the Great Lakes to feed before they migrate and they are being killed from botulism poisoning. This would explain why a decrease in temperature would have a significant effect on loon die offs as it reflects the relationship between the presence of the loons on Lakes Erie and Ontario and the time of the year that they gather there. Other studies on initiation of avian botulism outbreaks have also

found temperature to be a significant indicator of botulism outbreaks (Rocke & Euliss, et al., 1999; Rocke & Samuel, 1999).

The presence of snow fall has a similar relationship between the loon die offs as the temperature in that it is likely due to the fact that the time of the year in which the loons are present on the lakes is a significant predictor. Snow accumulation only occurs once the temperature is below freezing which usually starts to occur around mid November at the peak of the loons' presence on the lake and their peak of recorded deaths.

The study year was determined to be a significant predictor of loon deaths on Lakes Erie and Ontario but this was most likely an indicator of other biological variables not included in this study. Every independent year has different average snow fall, average temperatures, and other different biological factors such as the size of the population of loons gathering on the lakes each year, other possible pollutants or man made alterations, or even other aquatic invaders that may have altered the system. Biological systems are prone to high amounts of variation due to the extensive network of different factors that act upon them, both biotic and abiotic.

Diving Ducks

The diving ducks in this study have similar migration patterns as the loons and they collect on large bodies of fresh water until mid to late November when snow and ice accumulate. From there they migrate anywhere along the Atlantic coastline from southern Canada all the way south to North Carolina and can even be found in the Gulf of Mexico. This helps to explain why the diving ducks also had a strong relationship between their peak die offs and the day of the year used in the study. When they congregate on the Great Lakes to feed

during the fall, they are accumulating the botulism toxin in their system from the contaminated fish and invertebrates in the lakes causing die offs to rise in mid to late November. After this time period, the diving ducks that are healthy enough also leave the lakes causing the data to show a decline in the death rates of the individual birds.

This as well connects to the snow fall as being a predictor of the diving duck die offs. As indicated on Graph 2, the presence of snow fall causes a rapid increase in the mean duck die offs but as the snow begins to accumulate, the diving duck deaths also begin to decrease. This is related to the day of the year as when the temperature gets cold enough to freeze the precipitation to snow, the diving ducks are at their peak in population size on the lakes preparing to migrate. As time continues however, the snowfall accumulates but the diving ducks have most likely left on their winter migration to the coastline which explains their absence in the death counts. Like the loon guild, the diving duck guild data suggests that the year studied has a direct relationship with the mean duck die offs which is most likely due to unexplained variation in the biological system. During different years the population of diving ducks on the lakes could fluctuate, available food sources will differ, contaminants may change, and other unpredictable variables may occur.

Graph 3 indicates an interesting relationship between the mean loon die offs and the mean duck die offs over time. The graph predicts that approximately eight days after the peak die off of loons, the diving ducks will experience a die off of similar length in time and in magnitude of deaths. The loon deaths are not an indicator of diving duck die offs, but this means that the loons and the ducks are being killed by avian botulism for the same duration and by the same magnitude of mean bird deaths. One possible reason for the delay in time between the out breaks could be the feeding habits of the two guilds. The diet of a loon is more

heavily concentrated on fish while a diving duck eats a much greater percentage of invertebrates and vegetation in their diets. Since the fish are higher on the trophic web of the lakes, they are more likely to have a higher concentration of the botulism toxin in their tissues compared to an invertebrate or plant due to biomagnification. Since loons are eating higher up on the trophic web, they are eating tissues higher in the toxin and are more likely to be poisoned more quickly than a diving duck. The loons also appear to be gathering on the Great Lakes in preparation for migration slightly earlier than the diving ducks by about a week which could also be a factor in the amount of time it takes for a bird to accumulate enough toxin in its tissues to be fatal.

Gulls

It was originally predicted that due to their feeding habits as scavengers, the gulls would experience an out break of poisoning from avian botulism after out breaks of the loons and the diving ducks. The analysis, however, shows that there is no relationship between the gull die offs and the previous loon and diving duck die offs. This could be for a few reasons, the most prominent one being that the gulls are not predating as heavily on contaminated avian carcasses from botulism outbreaks. The fish die offs from contamination were not taken into account during this study and it is likely that the gulls predate on their carcasses just as much if not more often than the avian carcasses. Also, gulls have highly varied diets relying heavily on human refuse and food products when in highly populated areas like those around the Great Lakes.

The affect of temperature as a predictor of gull die offs was found to be statistically significant, but its relationship to the mean gull die offs varied greatly by study year. This

could be due to regular fluctuations in the biological system like the density of the population of gulls on the lakes each year, the deviation in weekly average temperatures each year, or the availability of contaminated food for the gulls to scavenge. Being that there is such a great deviation in the affect on temperature on gull deaths each study year, although it is a significant predictor, the use of temperature as a predictor of gull deaths in the field for conservation work may not be recommended.

Limitations of this study

Further data collection and analysis to comprehend the complexity of the issue of the type E avian botulism epizootic is needed. In this particular study, the availability of certain types of data was absent and therefore certain relationships between the outbreaks and the climate factors could not be obtained. To begin, the collection of the bird carcasses was only recorded by species, transect, and day from September to January of each year leaving a deficit of information on bird carcasses found on the shore in these regions for the rest of the year. With complete data on bird deaths all year round one could potentially determine if there is a connection between type E botulism outbreaks and lake turn over among other limnological and climatic processes. The lakes are a dynamic system that is constantly changing throughout the year and a more complete annual study would have been beneficial. Also, during the study period, the transects were not checked every single day and therefore the lack of collection of bird remains could have caused an accumulation of carcasses on the study sites if enough time had passed between collections. This also makes it difficult to correctly judge when the bird died and correlate it to climate data. A proportion of the birds almost certainly died in the water away from the shoreline and could have washed up on to the shore days to weeks later

depending on the conditions of the lake and wind direction and strength. This could also be a variable when finding a relationship between outbreaks and the abiotic factors occurring in the lakes. One could also speculate that a portion of the birds that died from type E avian botulism sank to the bottom of the lake or were scavenged by other organisms. These birds were not counted in this data and this would in turn increase the amount of individuals that were most likely killed during an outbreak. Finally, the age and sex ratio of the bird corpses found were not determined so no relationship could be established between susceptibility to botulism contamination and population demographics.

For the climate data studied, the stations that produced the historical data were in the closest proximity available to the transect sites where the bird carcasses were collected. There may have been some divergence in the data available as the conditions at the study site themselves were not directly monitored by consistent data equipment. The weather stations chosen were within ten miles of the shoreline of Lake Erie or Lake Ontario but were not directly on the shoreline where the birds would have been located and could also have been a source of minor error. Wind data from the National Buoy Data Center was considered for this study as the strength and direction of the winds could affect the dispersal of sediments in the water columns and the distribution of bird carcasses along the shoreline but the historical wind data for the desired stations was not available in a format that could not be utilized. The wind strength and direction was recorded every six minutes at each station and to average the data by day and month could have hidden outliers that would have ecological significance. As a final point, the NOAA only provided historical annual lake levels as monthly averages rather than daily averages for each lake. Despite the fact that the lake levels for Lake Ontario are

managed by humans, there may have been enough deviation in the levels daily to contribute to a stronger relationship between the outbreaks and the lake level data.

Future Studies

The intention of this study is to raise awareness of the conservation management issues surrounding the Great Lakes and the populations of waterfowl that they support. The study of this epizootic would be more complete if a relationship could be determined between other variables not mentioned within this study due to lack of availability or resources. For instance, a comparison of sewage overflows from major cities within the Great Lakes watershed during large rainstorms and the appearance of avian botulism outbreaks would be beneficial as the contaminants from the sewage could provide certain nutrients to the *Clostridium* bacteria or possible vectors. The increased nutrients could cause rapid growth and dispersal in the lakes and then be transported up the food chain until it reached the native birds that depend on the lakes. A study of historical outbreaks on all of the Great Lakes annually would also be in order to determine if the outbreaks move through the lake system in a certain order or pattern over time. This could possibly pin point an area or site that the outbreaks are originating from or help researchers narrow in on particular areas that are a problem. Since the time of the year was found to be a significant predictor for the avian botulism outbreaks on Lakes Erie and Ontario, management efforts could be concentrated from late October through November to collect the contaminated remains and possibly help find and rehabilitate contaminated individuals that are still alive. By refining wildlife management techniques like this to certain time frames, money and time can be saved while making the program more efficient by rehabilitating more individuals. Although in this study the die offs of loons and ducks were not

found to be significant predictors of gull deaths from scavenging, management plans that mandate beach clean ups of avian carcasses would still be encouraged to eliminate health risks to scavengers, pets, and humans.

Further investigation of the link between invasive species in the lakes and type E botulism outbreaks could also prove to be valuable. Previous studies on abiotic variables as predictors of type E avian botulism outbreaks have indicated that high pH, low dissolved oxygen, and lower redox potential are also all significant predictors (Rocke & Euliss, et al., 1999; Rocke & Samuel, 1999). Looking more closely into these environmental factors may be essential in managing these outbreaks. Knowing whether or not limnological or climatic data could indicate a change in growth or distribution of quagga mussels or round gobies could help in the discovery of indicators of outbreaks and change conservation management techniques. As for eliminating the source of the accumulation of the botulism toxin, the complete eradication of the quagga mussels or the round gobies may not be advisable for a few reasons. First of all removing them from the Great Lakes system would be extremely costly, a project of immense magnitude as they inhabit all of the lakes in great abundance, and the process used to eliminate them could possibly have adverse effects on the native biota. Second, the quagga mussels and the round gobies entered a system already experiencing great changes in population compositions. Now that they have established, it is quite possible that they have been integrally integrated into the system and removing them so quickly and abruptly could have adverse affects to the wildlife that now depend on them directly as a food source or indirectly through habitat modification.

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