



# Botulism in Lake Erie Workshop Proceedings

*Co-Sponsored by*

New York Sea Grant  
Ohio Sea Grant  
Pennsylvania Sea Grant



*Bob Wellington with sturgeon (Erie, PA)*

February 28, 2002  
Buffalo, New York

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# **Botulism in Lake Erie Workshop Proceedings:**

## *Introduction*

## Introduction – Workshop Objectives

Responding to fish and bird die-offs along the shores of Lake Erie from 1999-2001, the staffs of New York, Pennsylvania and Ohio Sea Grant wanted to understand the extent of the die-offs, gather scientific information and explore the ecological impacts of these botulism outbreaks. In order to achieve these goals, the Sea Grant programs realized a need to create a functioning network of involved agencies and individuals and organize a workshop that would get this diverse binational group working together.

Working from the success of the first conference on avian botulism that was held in 2001, New York, Pennsylvania and Ohio Sea Grant worked together to co-sponsor a workshop that was designed to develop a research agenda to deal with this ecological problem. On February 28, 2002, a workshop on Botulism in Lake Erie was held in Buffalo, New York. That workshop brought together 100 researchers, fishery and wildlife biologists, resource managers, and agency representatives. The goal of the workshop was to share information from the American and Canadian shores and to develop a research agenda for future efforts.

The original conference, held on January 24-25, 2001, was co-sponsored by New York and Pennsylvania Sea Grant and was held in Erie, Pennsylvania. That workshop focused on avian botulism, since at that time most mortalities were occurring in fish-eating birds like loons and mergansers. Organizers wanted to determine the extent of the avian botulism problem based on geography and environmental conditions that existed during the outbreaks. Although organizers realized that the first conference was premature from a data standpoint, they wanted to create a functioning network of scientists who would collaborate on research issues and respond to future outbreaks.

### Botulism in Lake Erie

Botulism, a disease caused by *Clostridium botulinum*, has been recognized as a major cause of mortality in migratory birds since the early 1900s. Although type C botulism has caused the die-off of thousands of waterfowl (especially ducks) across the western United States, type E has been mainly restricted to fish-eating birds in the Great Lakes. Other outbreaks of type E have sporadically occurred in Alaska, Florida, and California, with periodic outbreaks occurring in Lake Michigan and Lake Huron over a twenty-year period beginning in 1964. During 1999 and 2000, a large die-off of waterfowl occurred in Lake Erie and type E botulism was isolated in these outbreaks. In 2001, a large die-off of benthic fishes like sheepshead occurred along the shores, followed in the fall by another die-off of fish-eating birds.

The bacterium is classified into seven types (A-G) by using characteristics of the neurotoxins that are produced. The toxins produced by *C. botulinum* are among the most potent biological poisons, warranting human health and safety concerns. These neurotoxins bind to the receptors on nerve endings, impacting neuromuscular function, which results in the paralytic effect on birds. Impacted waterfowl typically show signs of weakness, dizziness, inability to fly, muscular paralysis, and respiratory impairment. Often, the inner eyelid or nictitating membrane becomes paralyzed, impairing the bird's normal vision.

Although type C and type E avian botulism outbreaks occurred in the Great Lakes in the past, there are some significant differences between the two types. Type C botulism primarily impacts dabbling ducks and bottom-feeding waterfowl, although shorebirds may also fall victim to this type of botulism. In type C botulism, the bacterium, *C. botulinum*, does not produce toxin unless it is infected by a specific "phage" or virus. This relationship with a phage is not known to exist with type E. Type E botulism typically impacts fish-eating birds like loons and grebes. Several species of gulls that are common in the Great Lakes region have been impacted by type C and type E botulism. While live fish can carry spores of type E botulism, it is not known whether they can carry the toxin itself or become sick and die from the toxin. Type E toxin has been found in carcasses of several species of Great Lakes fish, including round gobies, and researchers are studying the role this invader may play, if any, in recent outbreaks of the disease in Lake Erie.

Spores of both type C and type E botulism are naturally found in anaerobic habitats such as soils and aquatic sediments, and can also be found in the intestinal tracts of live, healthy animals. The spores can remain in the ecosystem for extended periods of time, even years, and are quite resistant to temperature extremes and drying. In the absence of oxygen, with a suitable nutrient source, and under favorable temperatures and pH, spores can germinate and vegetative growth of bacterial cells can occur (Brand, *et. al* 1988).

Botulism toxin is only produced during vegetative growth, not when the bacterium is in its spore stage. Decaying animal and insect carcasses provide favorable conditions for botulism toxin production since the decay process uses up oxygen and creates anaerobic conditions (Friend, *et al.* 1996).

It has long been known that type C botulism is perpetuated through a carcass-maggot cycle. Researchers have now determined that type E botulism can also be spread through this cycle. Birds and fish that have died from botulism decay and become hosts for maggots. The maggots may contain the botulism toxin and if fed upon by birds, the cycle is continued.

The following illustration shows the cycle for Type C Botulism:

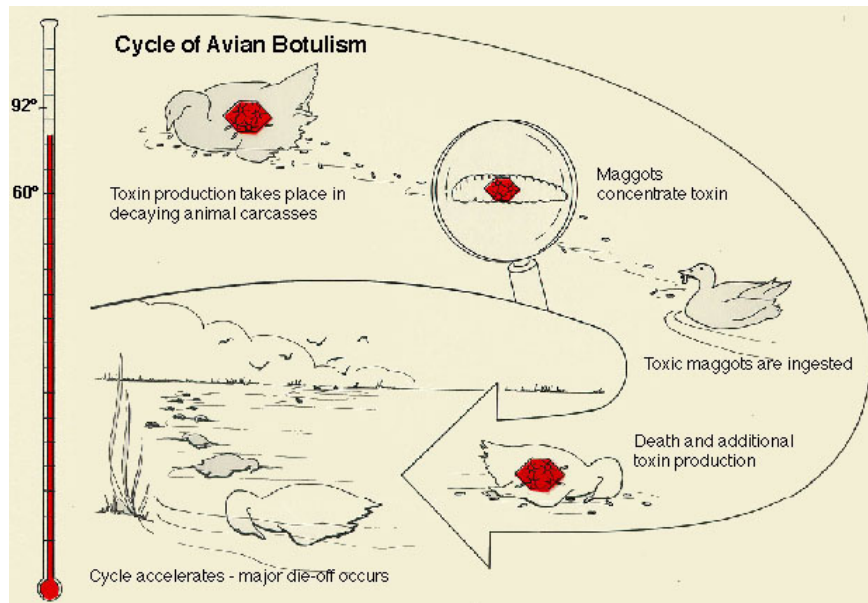


Illustration from: U.S. Geological Survey, Avian Botulism Factsheet

## **Introduction – Human Health Considerations**

Human botulism is typically caused by eating improperly canned or stored foods and normally involves type A or type B botulism toxin. There have been several fatalities during the 1960s in the Great Lakes basin attributed to type E toxin, but these were caused by eating improperly smoked or cooked fish that contained the toxin. Humans, dogs, and cats are generally considered resistant to type C avian botulism (Friend, *et al.* 1996).

The toxin found in food items will be killed by proper cooking of fish and waterfowl. When canning or smoking fish or waterfowl, methods should be used that incorporate sufficient heat to insure that any toxins will be killed off. Anglers and hunters should avoid harvesting any sick or dying fish or waterfowl, or those demonstrating unusual behavior, in areas where avian botulism has occurred. People should not handle dead birds or fish with bare hands. The use of gloves or an inverted plastic bag is recommended in order to avoid risks. If a diseased or dead bird is handled without gloves, hands should be thoroughly washed with hot soapy water or an anti-bacterial cleaner.

In case of a die-off, individuals are urged to contact local agencies responsible for fish and wildlife management to notify them of fish and bird mortalities. It is important to record the location, type of birds or fishes, and number of carcasses found. Stakeholders should follow agency recommendations in handling dead fish and wildlife. In certain areas, burying of the carcasses is allowed, in other areas incineration may be recommended. If birds are to be collected, they should be placed in heavy plastic bags to avoid the spread of botulism-containing maggots.

### **References:**

Brand, Christopher J., Stephen M. Schmitt, Ruth M. Duncan and Thomas M. Cooley, *An Outbreak of Type E Botulism Among Common Loons (Gavia immer) in Michigan's Upper Peninsula*, Journal of Wildlife Diseases, 24(3), 1988, pp. 471-476.

Friend, Milton, Louis N. Locke and James J. Kennelly, National Wildlife Health Laboratory, Madison, Wisconsin. 1996.

Avian Botulism Factsheet: <http://www.nwhc.usgs.gov/facts/avian.html>

## **Introduction – Synopsis of Research Questions/Issues from 2001 Botulism Workshop**

### **Research Questions:**

- What role do round gobies (*Neogobius melanostomus*) play in botulism outbreaks?
- What role do *Dreissenid* mussels play in botulism outbreaks?
- Do lower lake levels have something to do with outbreaks? Any connections?
- Do weather or limnological conditions play a role in botulism outbreaks?
- What unique climatic and hydrological conditions are present before/or during outbreaks?
- What role does water clarity and resulting fish movements/feeding areas play in outbreaks?
- What role does *Microcystis* or other toxic algae play in botulism outbreaks?
- What other environmental stressors may be involved in botulism outbreaks?

### **Other Pertinent Questions:**

- During outbreaks - are fish/waterfowl safe to eat?
- During non-outbreak periods, are there concerns about eating fish/waterfowl?
- What is the human health threat of handling, eating contaminated fish/waterfowl?

### **Desired Outcomes/Needs:**

- Test for spores/toxin in *Dreissenid* mussels and other possible vectors.
- Test healthy fish/birds for botulism toxin during outbreaks.
- Test for toxins in area around dead fish/birds for botulism spores/toxin.
- Test mudpuppies and other benthic organisms for botulism spores/toxin.
  
- Improve/expand fish pathology capabilities and develop standardized analysis protocol.
- Develop a model to predict potential for outbreaks using various environmental factors.
- Develop a cohesive database: fish, bird die-offs, limnological data.
- Standardize methods of reporting mortality/episodes.
- Standardized method for fish/waterfowl collection, disposal.
- Standardized public outreach information (education).
- Establish “fast response” team to collect data during outbreak periods.
  
- Need agency (Health Canada, US Federal Agencies, State Health Departments) assistance in developing coordinated advisories/information for other agencies to distribute to the public.

### **Public needs information on:**

- Unified message on the botulism issue.
- Proper cooking techniques for fish.
- Does freezing impact toxins?
- Proper handling of birds found during outbreaks.
- Proper carcass disposal.

## **Introduction – Contacts**

For additional information on the botulism outbreaks in Lake Erie, the NY/OH/PA Sea Grant co-sponsored workshop, or on follow-up activities, please contact:

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### **Acknowledgements:**

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### **Other Partners:**

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### ***Special Thanks:***

Ellen George, New York Sea Grant



**Botulism in Lake Erie  
Workshop Proceedings:**

*Conference Presentations*

## **Environmental Correlates with Outbreaks of Type E Avian Botulism in the Great Lakes**

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### **Brief Introduction to Avian Botulism**

#### **What it is**

- A paralytic disease of birds caused by a potent neurotoxin produced by the bacterium *Clostridium botulinum*
- Of the seven known serotypes of *C. botulinum* (A-F), two (types C and E) cause paralysis and death of waterfowl (*Rocke and Friend 1999*)

#### **Types C and E compared**

##### **Major similarity: Progressive symptoms in birds for both types**

- Inability to fly
- Paralysis of the legs
- Paralysis of the inner eyelid and neck muscles “limberneck” condition - may lead to drowning
- Respiratory failure and death (*Rocke and Friend 1999*)

##### **Major differences between Types C and E**

- Outbreaks of avian botulism **type C** most often affect ducks
- Type C outbreaks have occurred on every continent except Antarctica
- Have killed millions of waterfowl
- Most outbreaks reported in North America in the past 35 years have occurred west of the Mississippi River (*Rocke and Friend 1999*)

##### **Type E avian botulism**

- Outbreaks affect fish-eating birds: gulls (*Larus* sp.) and loons (*Gavia* sp.)
- Type E outbreaks mostly confined to the North American Great Lakes
- Isolated cases documented in Alaska, Florida, and the the Salton Sea of southern California (*Brand 2001*)

##### **How Type C *Clostridium botulinum* kills ducks**

- Spores of *C. botulinum*, type C, are widely distributed in wetland sediments and can be found in the tissues of most wetland inhabitants
- Type C spores germinate in tissues that become anaerobic after an animal dies
- Neurotoxin production is dependent on association with bacteriophages that carry the toxin gene
- Wetland birds become poisoned when they ingest food items (usually invertebrates) containing the toxin (*Rocke and Friend, 1999 and Williamson et al., 1999*)

### **Wide distribution of Type E spores in environment**

- Spores of *C. botulinum*, type E, are found in marine coastal sediments of Canada, Alaska, Greenland, and Russia, as well as Great Lakes sediments
- Spores are carried primarily by bottom-feeding fishes in marine habitats (*Hyytia-Trees 1999*)
- In the Great Lakes, spores of *C. botulinum*, type E, are carried by a wide variety of fish, which are considered active agents for disseminating the bacterium (*Bott et al. 1966, 1968*)

### **How Type E *Clostridium botulinum* kills fish-eating birds**

- Fish and aquatic invertebrates ingest the spores during feeding and become a substrate for bacterial growth when they die (*Bott et al. 1966, 1968*)
- Bottom-feeding fish may ingest foodstuffs that include dead material already containing botulism toxin
- Birds may become sick when they feed on:
  - o Dead contaminated fish
  - o Moribund fish with type E neurotoxin in their gut (*Brand et al. 1983, 1988*)
  - o Animals that feed on contaminated carcasses

### **A common feature of both types of Avian Botulism: The “carcass-maggot cycle”**

- Toxin produced in a decaying animal carcass is ingested by maggots
- The maggots are lethal to birds that eat the maggots (*Rocke and Friend 1999*)
- May account for some of the gull mortality in outbreaks of both types of botulism

## **Diagnostic Procedures for type E Avian Botulism**

### **Phenotypic characteristics of Type E**

- Can grow and produce toxin at temperatures as low as 3° C
- Optimum growth occurs in the range of 25-37° C
- Tolerates salt conditions up to 5% and a pH as low as 4.8 (optimum 6.8-7) (*Hyytia-Trees 1999*)

### **Why diagnosis of type E botulism as the primary cause of a bird die-off in the Great Lakes is problematic**

- Neurotoxins of C and E types of *C. botulinum* produce the same symptoms in waterfowl
- Both pathogen types may be involved in large die-offs (*Fay 1966*)
- Both are part of the natural bacterial flora of the system (*Bott et al. 1966, Williamson et al. 1999*)
- There are other causes of botulism-like symptoms other than botulism, including algal or castor bean poisoning (*Rocke and Friend 1999*)

### **What is ideally required to make a definitive diagnosis?**

- Eliminating other possible causes of botulism-like poisoning in birds
- Positive results from each of three different procedures, including:
  - 1) Traditional bacteriological tests to demonstrate that a population of *C. botulinum* capable of producing toxin is present in the environment
  - 2) Tests to identify the specific type(s) of *C. botulinum* isolated in the environmental samples (sediment, water, or fish carcasses)
  - 3) Tests to confirm that moribund or recently dead birds contain the same type of botulism toxin that has been demonstrated in the environment.

### **Environmental sample processing**

- First need to do tests for identification of *C. botulinum* Group II (types B-F) as suggested in Buchanan (1975) and CDCP (1996)
- *C. botulinum* can be grown in a variety of enrichment media that include a protein source (cooked meat or meat extract), yeast extract, and a carbohydrate source (usually glucose), or meat-egg yolk media can be used
- Samples placed in enrichment media are incubated in anaerobic containers at varying temperatures (25°-37° C) in either broth or agar cultures
- Subsequent tests of an isolate from these cultures which conclude gram positive bacilli with subterminal (to central) endospores with appendages that are non-proteolytic and ferment glucose, but not mannose to acid lead to the conclusion that the organism is a Group II *Clostridium botulinum*

### **Conclusive tests for determining *C. botulinum* type E utilize**

- Serological methods: Mouse bioassay, ELISA
- Or genomic methods: PCR-based methods using RAPD or nested PCR
- Each technique has its advantages and disadvantages (Recent reviews in *Williamson et al. 1999* and *Hyytia-Trees 1999*)

### **Mouse bioassay with seroneutralization**

- The traditional serological approach (e.g. *Bott et al. 1966*) requires antitoxins for each of the *C. botulinum* toxin types possibly involved
- Uses ICR strain mice, and therefore requires an animal facility and protocols approved by an institutional animal use committee

### **Enzyme-linked immunosorbent assay (ELISA)**

- The serological alternative to the mouse bioassay
- Developed for detecting specific botulinum toxins (*Rocke and Friend 1999*)
- ELISA's are not commercially available
- *Hyytia-Trees (1999)* believes that they have not been adequately evaluated

### **Genomic methods based upon the polymerase chain reaction (PCR)**

- Have been developed to replace the mouse bioassays for identifying the various types of *C. botulinum*
- Most of the PCR-based methods involve extraction of the genomic DNA from a culture-enriched bacteria population
- *Hyytia-Trees (1999)* used a PCR-based method called RAPD (randomly amplified polymorphic DNA assay) and pulsed-field gel electrophoresis to characterize the genetic variability of type E isolates of *C. botulinum* found in fish samples

### **Disadvantage of techniques using enriched media to grow bacteria prior to extracting DNA for PCR**

- May not provide an accurate representation of the abundance of toxin-producing *C. botulinum* cells in the natural environment
- Before concluding that any type of *C. botulinum* identified in environmental samples is a potential cause of a bird die-off, it should be known whether there were sufficient numbers of toxin-producing, vegetative cells versus endospores, which are non-toxic and possibly ubiquitous in the environment (*Williamson et al. 1999*)

### **Nested PCR assay**

- Has been developed for detecting the botulinum neurotoxin gene (BoNT) for type C
- Does not require prior processing of sediment samples in enrichment culture (*Williamson et al. 1999*)
- NWHC is working on a method of extracting DNA from vegetative cells (while leaving spores intact) that would enable PCR to be used to assess the prevalence of toxin-producing type C cells in environmental samples (*Williamson et al. 1999*)
- Hopefully, once these techniques are available, they can be modified for use with type E

### **Final tests required to indicate whether a type of *C. botulinum* is the cause of a bird die-off**

- Requires obtaining blood sample from moribund or recently dead birds
- Serum fraction of blood obtained from a bird is used in a mouse bioassay (with seroneutralization) or ELISA tests
- Problem with using the mouse bioassay to detect neurotoxin type E in both dead birds and environmental samples: It may produce false-negative results, because the type E neurotoxin is unstable in the environment (NWHC unpubl. report)
- Advantage of the ELISA test: has the advantage of being able to detect inactive as well as biologically active toxin (*Rocke and Friend 1999*)

## **Comparative Analysis of Past and Current Type E Avian Botulism Outbreaks**

### **Past (historical) outbreaks: 1963-1983**

### **Current outbreaks: 1999-2001**

#### **Factors considered**

- Locations and Timing of Outbreaks
- Outbreaks in relation to lake levels
- Dietary factors
- Outbreaks in relation to changes in fisheries
- The zebra mussel connection

#### **Location of avian botulism outbreaks: past and present**

- Over two-thirds of the confirmed type E die-offs from 1963 to 1983 occurred in Lake Michigan, and the rest occurred in adjoining Lake Huron (*Fay et al. 1965, Fay 1966, Fay 1969, Brand et al. 1983, Brand et al. 1988*)
- All but one of the current type E outbreaks have occurred in Lake Erie
- An east-to-west progression evident in the locations of major outbreaks in Lake Erie over the current three-year record of bird die-offs especially apparent along the Canadian side of the lake

### **Seasonal timing of die-offs**

In both past and current outbreaks:

- Gull die-offs peaked sometime during the summer
- Major loon, merganser (*Mergus* spp.), and grebe (*Podiceps* spp.) mortalities occurred in the fall, corresponding to the timing of their migratory movements through the lakes

### **Timing of bird die-offs within seasons**

- Noted that loon die-offs in the past outbreaks often occurred in discrete episodes or “waves” (Fay *et al.* 1965; Fay 1969)
- Many of the bird deaths reported in the current series of outbreaks occurred in episodes that appeared to follow major storm events accompanying cold fronts
- Coincidence of bird die-offs following storms was noted on both sides of Lake Erie in 2000 and 2001
- Indicates possible connection to lake thermal phenomena

### **Outbreaks in Relation to Lake Levels**

- Analysis of historical hydrological data for Lake Michigan-Huron (Fuller and Shear 1995) indicates that the larger bird die-offs occurred during periods of very low or rapidly declining lake levels
- Current outbreaks are occurring during a significant period of low Lake Erie levels

### **Past outbreaks in relation to Lake Michigan water levels**

- 1963 and 1964, years of the worst episodes of type E avian botulism in the past, were years of lowest levels for the period of record from 1916-1993
- Smaller events in 1965 through 1968 corresponded to a period of gradually increasing lake levels from the 1963-64 minimum (Fuller and Shear 1995)
- Among the outbreaks reported from 1976-1983, the larger events (1976, 1983) were in years when seasonal lake level declines from late summer-fall were unusually steep in Lake Michigan (Fuller and Shear 1995)
- Minor outbreaks in the intervening years occurred during years of more typical lake level fluctuations

### **Current outbreaks in relation to Lake Erie levels**

- 1999-2001 type E die-offs commenced as Lake Erie water levels fell to their lowest point in several decades
- Question raised: What do water level changes have to do with outbreaks of avian botulism?

### **Correlations with water level changes and Type C avian botulism**

- Water level fluctuations (especially summer draw-downs in wetlands) have been identified as one of the environmental factors correlating with type C avian botulism outbreaks (Rocke and Friend 1999)
- Draw-down events apparently increase the amount of substrate available for growth of *C. botulinum*, type C, by increasing the mortality of wetland invertebrates

### **How could this phenomenon apply to Type E outbreaks in the Great Lakes?**

- Perhaps sharply decreasing levels in Lake Michigan, Huron and Erie contribute to the production of carcasses supporting the growth of the type E bacterium

- Is there a link between low lake levels and more frequent fish kills?
- Summer anoxic conditions at bottom develop sooner in central basin of Lake Erie when water levels are low (Carey Knight, Fairport Fish Station, Fairport, Ohio)
- Perhaps low lake levels have a similar effect on development of isolated pockets of anoxia in the eastern basin

### **How pockets of anoxia may contribute to fish kills**

- Internal seiche by itself causes stress to bottom fish populations via rapid temperature changes
- Internal seiche may also disperse anoxic waters from “isolated pockets” and compound the stresses
- Adult fish already weakened by spawning activity may be especially susceptible (including round goby males on nests – Cary Knight)
- Off-shore water temperatures were at an all-time high level in the central basin of Lake Erie in the summer of 1999 (*Wellington 2001*)
- Perhaps warmer temperatures in the top half of the water column additionally predisposed near-shore fish populations to thermal shock from cold-water incursions during storm events and internal seiche

### **Dietary Factors - birds’ diets in past studies of type E avian botulism**

- Alewives (*Alosa pseudoharengus*) were the major food-item in gizzards of dead gulls and loons examined in 1963 and 1964 die-offs (*Fay 1966*)
- Experiments by Fay demonstrated toxicity of dead alewives (collected from Lake Michigan beaches) fed to captive ring-billed (*Larus delawarensis*) and herring gulls (*L. argentatus*)
- Dead alewives collected from the beaches and bottom (at a depth of 25 feet) of Lake Michigan found to contain sufficient levels of type E toxin to sicken kill gulls (*Monheimer 1968*)

### **Fish species other than alewife implicated in past bird die-offs**

- Sculpins (*Cottus bairdi*), smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*) found in the gizzards of many birds killed in the type E outbreaks in 1963 and 1964 (*Fay 1966*)
- Type E toxin found in high levels in decaying carcasses of smelt and sculpins from lake Michigan (*Fay 1969*)
- 1980 type E outbreak among gulls in southern Green Bay (*Brand et al. 1983*) corresponded to die-off of alewife and suckers (*Catostomus* sp.) on the eastern shore of Green Bay
- Type E toxin detected in carcasses of burbot (*Lota lota*), alewife, and smelt found on a beach at the site of the 1983 loon die-off (*Brand et al. 1988*), although numbers of dead fish on beach were not “unusually large”

### **Birds’ diets in current outbreaks**

- Bones of small fish found in the gizzards of mergansers collected during 1999 outbreak on the Canadian shore of Lake Erie (*Campbell and Barker 1999*)
- Gulls and loons examined in Pennsylvania in 2000 also contained fish remains (*Campbell and Gauriloff 2001*)
- One Pennsylvania loon contained a partially digested goby; one merganser contained a mudpuppy (*Necturus maculosus*)

- Round goby reported to be main food item in gizzards of loons and mergansers examined during the 2000 type E die-off on the Canadian side of Lake Erie (CWS unpubl. report)

### **Type E Avian Botulism Outbreaks in Relation to Changes in Fisheries**

- Fay's (1966) hunch: suggested possibility of "some connection between the accumulation of dead alewives and the bird mortalities"
- Link between type E avian botulism outbreaks and major changes in lake fisheries suspected with the first major bird die-offs in Lake Michigan (*Fay et al. 1965; Fay 1965*)
- Fay (1965) reported that during the 1963-1965 period, the invasive alewife became the dominant fish (reportedly 95% by weight) in lake Michigan
- Fay also noted that each year the alewife die "en masse," (*Fay 1966*)

### **How published records of changes in the fisheries of Lakes Michigan now confirm Fay's (1966) suggestion**

- Alewives had entered the upper Great Lakes before the 1950s, but their population did not begin to increase significantly in Lakes Michigan and Huron until the 1960s (*Smith 1970*)
- The first period of rapid alewife population growth occurred from 1957 to 1963, immediately preceding the first major bird die-offs due to type E
- Second period of significant population increase by alewives occurred from 1964-1967, concurrent with the second wave of type E outbreaks

### **Why massive die-offs of alewife occurred**

- Alewife is not well adapted to the thermal structure of the Great Lakes, which explains why massive die-offs of the fish are common (*Christie 1974*)
- Adult alewife apparently spend the winter in deeper water and then move inshore to spawn in the late spring
- As the fish move from deeper water to nearshore areas, they may traverse temperature gradients faster than they can acclimate, resulting in death due to temperature shock (*Christie 1974*)

### **Fisheries reports further substantiated**

- Annual, late spring die-offs of alewife, producing many dead fish on beaches and nearshore lake bottom, were common in the 1960s (*Smith 1970*)
- Alewife die-offs were apparently worse in Lake Michigan than in Lake Huron (*Christie 1974*), consistent with the initial type E botulism outbreaks being concentrated in Lake Michigan.

### **Avian botulism type E outbreaks and fisheries changes in 1970s and 1980s**

- 1976 loon die-off (*Brand et al. 1983*) occurred during same year as unusual sharp decline in the bottom-dwelling slimy sculpin (*Cottus cognatus*) in Lake Michigan (*Eck and Wells 1987*)
- Alewife populations in Lake Michigan also declined sharply from 1975 to 1977 (especially during the first year)
- Alewife recovered from 1977 to 1979, then another sharp decline occurred in 1980, corresponding to the 1980 type E die-off of gulls in Green Bay (*Brand et al. 1983*)



- The year alewife populations fell to their ten-year minimum (1983) was same year as last major loon die-off in Lake Michigan (*Brand et al. 1988*)

### **Fisheries changes/die-offs and current type E avian botulism outbreaks**

- Unusual fish and/or mudpuppy die-offs reported prior to or during many (*but not all*) of the current type E avian botulism outbreaks
- Most frequently mentioned species in the current die-offs are nearshore bottom-dwellers, including sheepshead (*Aplodinotus grunniens*), mudpuppy (*Necturus maculosus* - amphibian), rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), round goby (*Neogobius melanostomus*), and sturgeon (*Acipenser fulvescens*)
- Temperature intolerance related to storm and internal seiche phenomena has been a frequently cited explanation for die-offs (*Murray 2001; Culligan 2001; CWS unpubl. report*)

### **Possible key role of round goby in current fish and bird die-offs**

- Link to moribund sheepshead found by NYSDEC in August, 2001
- Similarities between round goby invasion of Lake Erie and alewife population boom in Lake Michigan/Huron in the 1960s
- Goby abundance in annual trawl samples from Lake Erie (offshore from PA) increased from 4% of the total catch in 1997 to 95% in 1999 (*Murray 2001*)
- Phil Ryan's observation (at Jan. 2001 avian botulism conference) that expanding spatial distribution of goby appears correlated with west-to-east advance of type E avian botulism outbreaks (that continued in Fall 2001)

### **Other goby facts suggesting a possible link**

- Initial introduction to Great Lakes via ballast water discharged from a tanker from the Black Sea (*Jude et al. 1992*)
- Goby first sighted in 1990 north of Lake St. Clair; now is in all five of the Great Lakes (*Jude, 1997*)
- Goby population apparently grew fastest in Lake Erie (the shallowest and warmest lake), where principle food (*Dreissenid* mussels), were abundant: same location of current series of type E avian botulism outbreaks

### **Goby connection may best explain why current avian botulism outbreak is mostly confined to Lake Erie.**

#### **Round goby ecology (similar to alewife)**

- Apparent warm-water preference
- Nearshore spawning habit
- Predilection for overwintering in deep water both in Black Sea (*Jude 1997*) and Lake Erie (Carey Knight – Ohio)
- Possible susceptibility to thermal shock during internal seiche events, particularly when males are on nests (Carey Knight)

### **If gobies are a key element in the current series of avian botulism outbreaks, then why hasn't the fish been more prominent in the recent fish die-offs reported around the lake?**

- The possibility that the death of large numbers of small gobies could go unnoticed (*Stone 2000*)
- Dead gobies apparently sink (Carey Knight) or remain on bottom
- Dead males occur regularly in trawl samples in Ohio (Carey Knight) although not in very large numbers
- *Live* gobies may transmit botulism toxin (from food in their gut) to other predators, including fish-eating birds or other fish (NYSDEC)
- Suggestion that live, moribund fish transmit type E toxin to birds was made in previous studies of bird die-offs in Lake Michigan (*Brand et al. 1983,1988*)
- Can account for puzzling type E mortality of loons and mergansers, which do not normally feed on dead fish, and occurrence of bird die-offs in absence of obvious fish-kills

#### **Possible role(s) of zebra and quagga mussels in avian botulism outbreaks**

- Ward Stone's suggestion that botulism toxin in zebra mussels could be picked-up by feeding gobies and transmitted to mergansers and loons that feed on live gobies
- Large die-offs of zebra or quagga mussels are apparently occurring, suggested by common observations of shell accumulation on Lake Erie beaches following storm events
- Mussels may provide a major source of decaying animal matter to fuel the growth of *C. botulinum* on the nearshore lake bottom
- Dense mussel beds may degrade water quality (*Sullivan and Endris 1998*), which may add to stresses experienced by bottom-dwelling fishes
- Live zebra mussels may play a role in fish die-offs by concentrating potential fish toxins produced by the alga *Microcystis* in Lake Erie (*Murphy 2001*)

#### **Dietary factors supporting the zebra/quagga mussel link**

- Mollusks are important food items in the diets of round gobies (*Jude 1997*)
- Mollusks also important in diets of sheepshead and sturgeon in Lake Erie (*Bolsenga and Herdendorf, 1993*)
- Mudpuppies are known to feed on mollusks and carrion (*Harding 1997*)
- Ingestion of dead zebra mussels contaminated with *C. botulinum*, type E, may have contributed to some of the recent unusual fish and amphibian die-offs

#### **Role of carcass-maggot cycle?**

- Summer-to-early-fall gull die-offs likely to be partly attributable to this cycle
- Overall role may be minor, if Lake Erie gulls feed more on live fish than carrion

### **General Summary**

#### **Common elements of past and current type E avian botulism outbreaks**

- The seasonal timing of gull versus loon/merganser die-offs and "episodic" character of outbreaks within seasons
- Correlation of type E avian botulism outbreaks with unusually low lake levels
- Correlation between fish and bird die-offs, with some exceptional major bird die-offs in the apparent absence of fish-kills
- Probable role of an invasive fish species, apparently adapted to warmer temperatures, during a period of initial rapid population growth

### Unconfirmed difference between the past and current outbreaks

- The potential role of invasive mussels in the current Lake Erie outbreaks

### Some questions for investigation suggested by these analyses

- Do low lake levels affect off-shore or nearshore ecology in ways that increase the likelihood bird- and fish-kills?
- Are round goby susceptible to thermal shock from cold water incursions, and are goby die-offs occurring that are not detectable at the surface?
- Are zebra or quagga mussel die-offs supporting the growth of *C. botulinum*, type E, and/or are live mussels concentrating the bacteria or toxins?
- Are moribund fish and/or mudpuppies that contain type E toxin responsible for outbreaks involving birds that normally do not eat dead fish?

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## Observations of fish-kills, turtles, and birds

Robert Wellington, Erie County Department of Health  
 Mike Campbell, Mercyhurst College  
 Eric Obert, PA Sea Grant



## Summary of avian botulism-related findings for Pennsylvania in 2001

- Robert Wellington, Erie County Health Department
- Chuck Murray, PA Fish & Boat Commission
- Larry Smith, PA Game Commission and Randy Neyer, DCNR Presque Isle State Park
- Harry Leslie, DCNR Presque Isle State Park

## Lake Erie Fish Die-offs in Pennsylvania – 2001

- > 20 species affected
- Die-offs noted from April to October
- Nearshore, bottom-dwelling species most affected

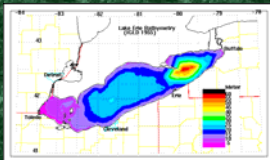
## Patterns and Trends in Fish Kills

- Fish species most frequently reported, in order of decreasing frequency:
  - Sheepshead (drum)
  - Bass (largemouth and smallmouth)
  - Rock bass (occasionally large numbers)
  - Sturgeon (several incidents with individual fish)
  - Carp (occasionally large numbers)
  - Mudpuppies
  - Catfish, bullhead, stonecat



## Timing and Locations of Fish Kills

- Early season kills (April – June) involved a wide variety of species and were concentrated in bay-connected habitats of Presque Isle
- Most of the late season kills (July – October) were at locations east of Presque Isle, at the southwestern edge of the eastern basin of Lake Erie (sturgeon, drum, mudpuppy)



## Unusual Turtle Die-offs: (mostly observed on the bay side of Presque Isle from April to July)

- Softshell turtles (several); one sick one diagnosed with TB by the NWHC
- Map turtles (several)
- Snapping and musk turtles (1 each)
- Good News: lots of live turtles (apparently Map turtles) observed at Presque Isle's Misery Bay in the fall



Softshell Turtle

## Dead Bird Reports East of Presque Isle in PA

- Great blue heron (1)
- Cormorant (1)
- Occasional “long-dead” gulls



## Algae Problems Noted by Wellington

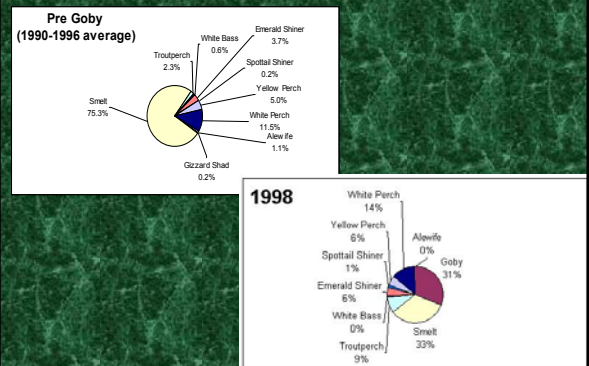
- Most reported as incidents separate from fish and turtle die-offs
- Large accumulations of dead, rotting *Cladophora* in mid-late July
- Late July bluegreen algae bloom at Presque Isle’s Horseshoe Pond: *Anabaena* and *Microcystis*

## Zebra Mussels

- Plate sampler pulled from Presque Isle Marina in mid-October found to be less populated than in past
- Numbers approximately one-third the densities observed 10 years ago
- Secchi disk readings less than 2-3 years ago, apparently more algae due to less zebra mussels

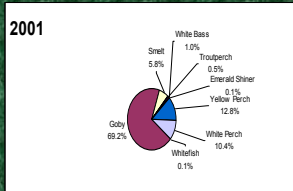


## Trawl Data by PA Fish & Boat Commission Provided by: Chuck Murray



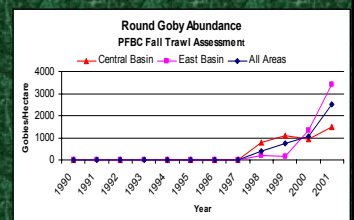
## Trawl Data by PA Fish & Boat Commission Provided by: Chuck Murray

- Round gobies still dominant (as in prior two years)
- Many more YOY Yellow and White perch caught in 2001 than in previous years at eastern basin sites
- Rainbow smelt, emerald shiner, and trout perch numbers still low compared to catches during years prior to round goby boom (pre-1999)
- YOY lake whitefish in trawls from eastern basin sites: first in 10 years of trawling



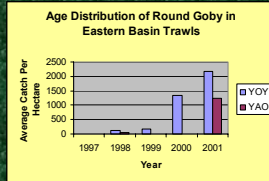
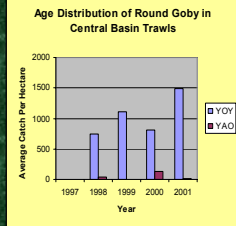
## Round Goby Population Trends at Central and Eastern Basin Sites Compared

- Population boom appears to have leveled-off in central basin
- Population in eastern basin still increasing exponentially



## Age Structure of Round Goby in Trawl Catches

- Relatively higher numbers of YAO (yearlings and older) goby in trawl catches from eastern compared to central basin sites



• Is adult goby survivorship better in eastern basin?

## Bird Mortality Records at Presque Isle – 2001

Larry Smith

– Pennsylvania Game Commission

Randy Neyer

– DCNR Presque Isle State Park

- Less than half as many dead birds found in 2001 compared to 2000
- Few specimens sent to NWHC for determination of cause of death
- Gulls were the most commonly affected species, followed by loons; no mergansers this year
- Timing of gull and loon die-offs similar to prior years when Type E botulism was demonstrated to be the cause of bird deaths: gull die-off mainly in summer, loon die-off in fall

## Tally of dead birds collected through November 2001

	Prior to September 16	After September 16	Total
Ring-billed gull and unidentified	85	30	115
Herring gull	18	22	40
Gr. black-backed gull	8	5	13
Bonaparte's gull	0	3	3
Common Loon	0	17	17
Unid. grebe	0	1	1
Cormorant	0	1	1
Shorebirds	0	3	3
Goose	0	1	1
Bufflehead	0	2	2
L. Scap duck	0	2	2
Ruddy duck	0	1	1
Flicker	1	0	1
<b>Totals</b>	<b>112</b>	<b>88</b>	<b>200</b>

## Notes on Improvements in Procedure for Disposing of Dead Birds Collected at Presque Isle State Park

By Harry Leslie – DCNR Presque Isle State Park, Park Operations Manager

- **Main problems encountered in 2000:**
- Union concerns with safety issue of maintenance crew burning dead birds in a homemade incinerator unit
- Concerns about handling dead birds, fall-out of ash material, and foul odors of decaying specimens

## Improvements made in 2001:

- Cooperative arrangement made with Erie County Humane Society (no cost)
- Birds collected from beaches were double plastic-bagged, and placed in a large chest freezer in park maintenance area for temporary storage
- Dead (frozen) bird carcasses transported once per week by dump truck from park to Humane Society incineration unit (five miles from park), and included with their incineration work
- Park maintenance crew was pleased with the arrangements

Kenneth Roblee  
NYS Department of Environmental Conservation  
Bureau of Wildlife, Region 9

### **Botulism Caused Waterbird Mortality in New York Waters of Lake Erie - 2001**

Department of Environmental Conservation (DEC) staff in Region 9 are familiar with repeated outbreaks of type C botulism which cause heavy mortality to mallards frequenting sewage contaminated watercourses in the Buffalo metro area. Type E botulism and its devastating effect on migrating waterbirds is a new phenomenon to the western New York region. The first outbreak of this disease during November 2000, killed an estimated 5,400 waterbirds on Lake Erie and was reported at the Avian Botulism in Lake Erie Workshop held at Erie, Pennsylvania in January 2001.

In 2001, waterbird mortality from type E botulism continued in Lake Erie with the first reports of dead ring-billed gulls being received on June 27. The summer of 2001 was notable for heavy die-offs of fish including, among others, sheepshead, smallmouth bass and rock bass. The shoreline at many locations was littered with fish carcasses, mats of algae and spent mussel shells, either zebra or quagga. Ring-billed, herring and great black-backed gulls were observed foraging in this mix of debris and specifically on fish carcasses. Other dead birds species collected along the Lake Erie shore during this period included double-crested cormorants and a bald eagle. DEC's Wildlife Pathology Unit subsequently confirmed type E botulism in ring-billed gulls, cormorants and a bald eagle. In September 2001, shorebirds were observed dying and 23 sanderlings, 1 dunlin and 1 semi-palmated sandpiper were collected. Type E toxin was identified in the ingesta of the sanderlings and the sandpiper. No attempt was made to quantify the mortality to these species during summer, but it is expected that several thousand gulls were affected.

In anticipation of repeated common loon mortality during the fall migration, shoreline transects established in 2000 were relocated and identified. Sixty-five 100m transects were relocated using a hand-held GPS unit and marked with flagging. The transects were surveyed once each week for 10 weeks beginning in early October. This required the tremendous task of inspecting, recording observations for and collecting dead birds and fish from a total of 65,000 m of Lake Erie shore. Table 1 provides a summary of the predicted waterbird mortality from the 2001 surveys. Predicted mortality values for all of New York's shoreline were calculated by multiplying the mean number of birds killed per 100 m by 1,185, the number of 100 m transects possible along the shoreline. Some major differences between the 2000 and the 2001 mortality are apparent in comparison of the data. The common loon mortality of 1,149 predicted from transects in 2001 was nearly double the mortality of 583 predicted in 2000. Although this increase could be attributed to a longer survey period during the later year, the author believes that loon mortality in 2001 was in fact higher than the previous year. This is because the 2000 transects were surveyed on the November 28 and 29, late enough to cover the majority of the mortality observed during the extended survey period in 2001. It is assumed that birds dying previous to the November 28-29 survey dates would have accumulated on the shoreline and would have been observed and counted. Double-crested cormorants, greater scaup and long-tailed ducks were found dead on transects in 2001, but not in 2000. Greater scaup and long-



tailed duck were also confirmed as positive for type E botulism in 2001. The predicted herring gull mortality of 401 was nearly double the 237 predicted in 2000, while the predicted ring-billed gull mortality of 510 was less than one third of the 1,714 predicted for 2000. Again, this represents only a portion of the gull mortality as gull mortality was not measured prior to October. Horned grebes were not found on transects in 2001, although they were observed in 2000. Especially noticeable was the decline in the predicted red-breasted merganser mortality from 2,479 in 2000 to 91 in 2001.

**Table 1: Lake Erie Botulism Mortality Surveys  
100 m Transect Survey Results  
NYSDEC 10/04/01-12/21/01**

Species	Predicted Mortality	Upper Limit 95% Confidence	Lower Limit 95% Confidence
Common Loon	1,149	1,509	789
Horned Grebe	0	0	0
Double-crested Cormorant	91	184	0
American Black Duck	0	0	0
Mallard	0	0	0
Greater Scaup	18	54	0
Long-tailed Duck	310	473	147
Bufflehead	0	0	0
Red-breasted Merganser	91	184	0
American Coot	18	54	0
Sabine's Gull	18	54	0
Bonaparte's Gull	18	54	0
Ring-billed Gull	510	693	327
Herring Gull	401	593	209
Great Black-backed Gull	128	231	25
Unidentified Gull	73	143	3
Common Tern	18	54	0
Belted Kingfisher	18	54	0
American Crow	0	0	0
<b>Total Birds</b>	<b>2,862</b>	<b>3,447</b>	<b>2,277</b>

Figure 1 depicts waterbird mortality observed in transects chronologically. It is important to note the decline in gull mortality observed in October to the relatively low levels observed in November and December. Healthy herring gulls and ring-billed gulls were observed within and near transects during these later months and did not seem to be as susceptible to type E botulism. A decline in fish mortality and the resulting availability of fish carcasses was also observed during this period and may be related to the improved gull survival. The highest number of dead loons was observed on transects during the week of November 12. This is believed to represent high mortality to a large flight of loons into Lake Erie, immediately prior to

this date. Dead loons observed on transect surveys, during succeeding weeks, appeared mostly to have died during this early event, as the carcasses observed exhibited a progressively decomposed state and few if any fresh carcasses were observed.

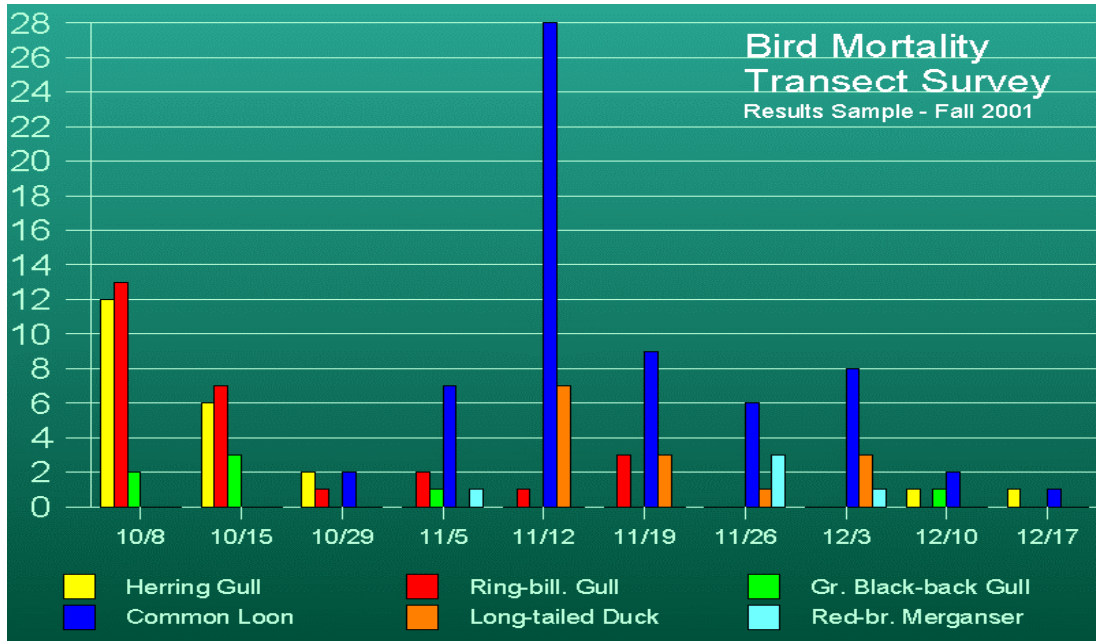


Figure 1

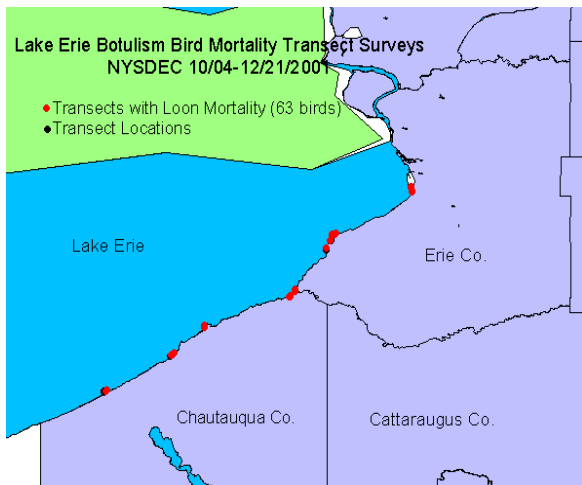


Figure 2

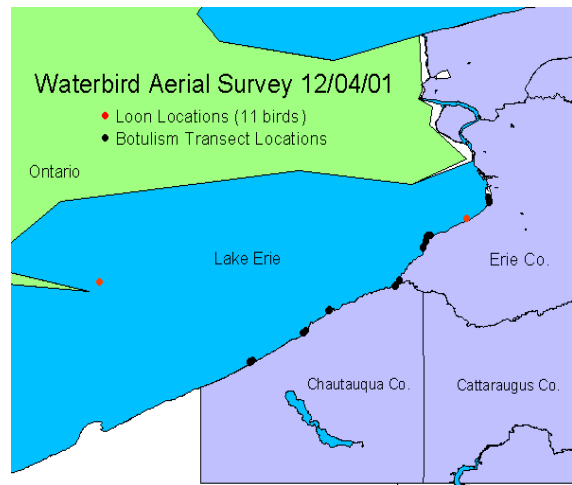
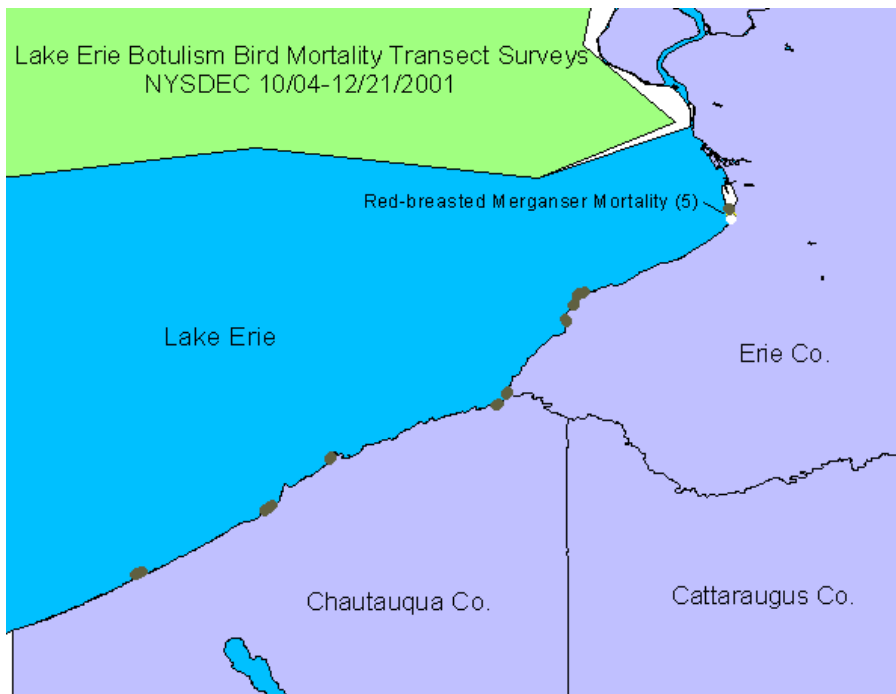


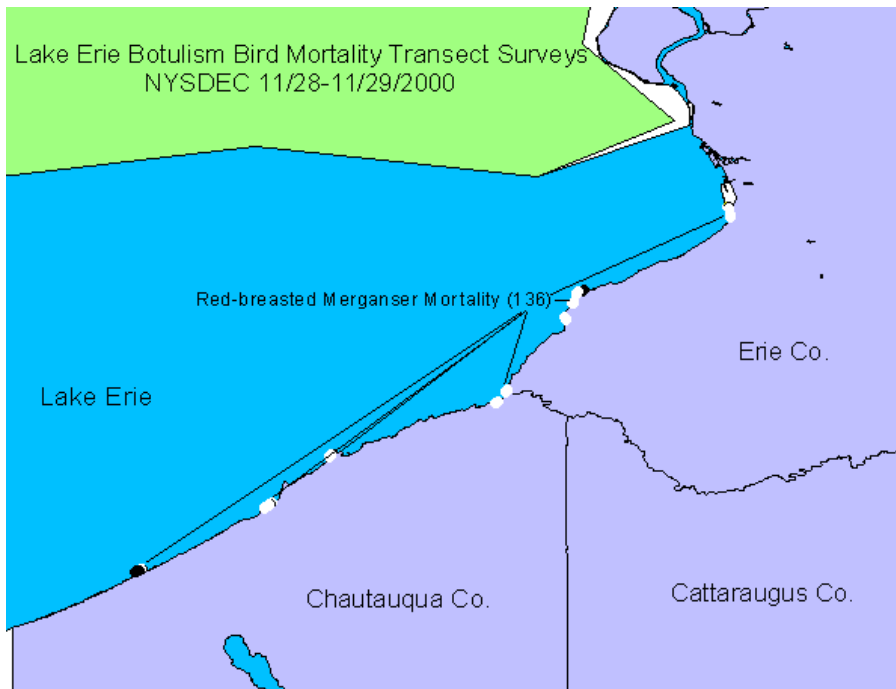
Figure 3

Transect survey results indicated that loon mortality in 2001 was distributed throughout the New York shoreline. Figure 2 depicts transects with loon mortality. In order to determine if loons were present on Lake Erie after the heavy mortality observed during the week of November 12, aerial surveys of Lake Erie were conducted for waterbirds on November 20, December 4, 2001, and January 2, 2002. Surveys were limited to New York waters, except on December 4, when a search of the waters near Long Point, Canada, was included (it was thought that Long Point could be a source of loon carcasses drifting into New York waters). Common loons were detected only during the December 4 flight (Figure 3), when 4 living loons were observed offshore of Eighteen-mile Creek in New York, and 7 living loons were observed near the east tip of Long Point in Canada. It should be noted that all loons were observed within about 1 mile of the shore. The number of loons observed is small compared to the 1,149 predicted mortality to loons 3-weeks prior to this date. Especially disturbing is the lack of loons observed during the November 20, 2001 flight. Loons are reported to rest on the Great Lakes for approximately 1 week, during migration. More loons should have been observable on the lake only 8 days after the high mortality event recorded beginning November 12. Currently, the mortality rate to loons which migrate through Lake Erie is unknown, but expected to be high. The aerial surveys indicate that significant numbers of loons were not lingering on Lake Erie on and after November 20. Any surviving loons must have left Lake Erie prior to or immediately after the November 12 event.

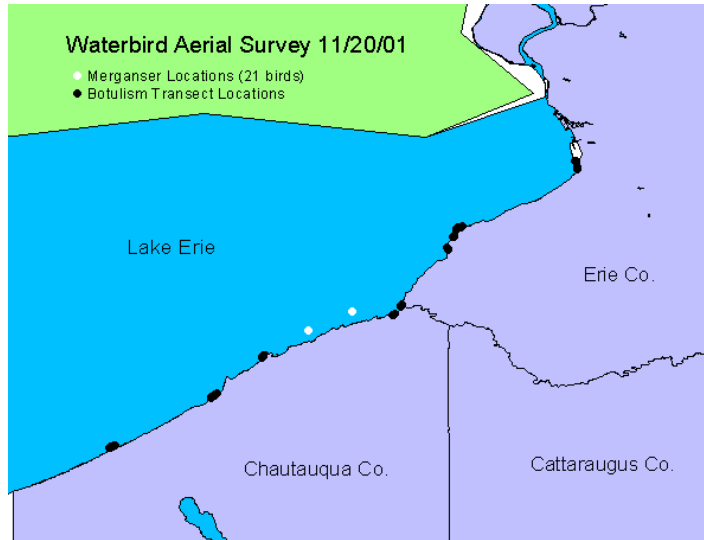
As mentioned previously, transect surveys indicated that red-breasted merganser mortality in 2001 was reduced substantially from that observed in 2000. In addition, the location of mortality was restricted in 2001 to transects at Woodlawn Beach State Park at the extreme eastern end of New York's Lake Erie shoreline (Figure 4). Transect surveys completed in 2000 showed red-breasted merganser mortality was much more widespread, occurring on 48 of 65 transects, and distributed throughout the New York shoreline (Figure 5). Aerial survey indicated that mergansers were present in small numbers (21) along the New York shoreline on November 20 (Figure 6). Common and red-breasted mergansers were observed during aerial surveys and could not be reliably differentiated during counts. Succeeding aerial surveys on December 4, 2001, (Figure 7) and January 2, 2002 (Figure 8) showed that mergansers were widespread along the shoreline by these dates in substantial numbers (690 and 669), although much less than the 2,479 red-breasted mergansers predicted killed in 2000. The aerial survey data indicate that in 2001 flights of mergansers entered Lake Erie after the date of high mortality of loons (November 12, 2001) for that year and for the previous year (November 16). Upon their arrival very little mortality was observed and seems to indicate that type E toxin was not readily available to these birds at this time.



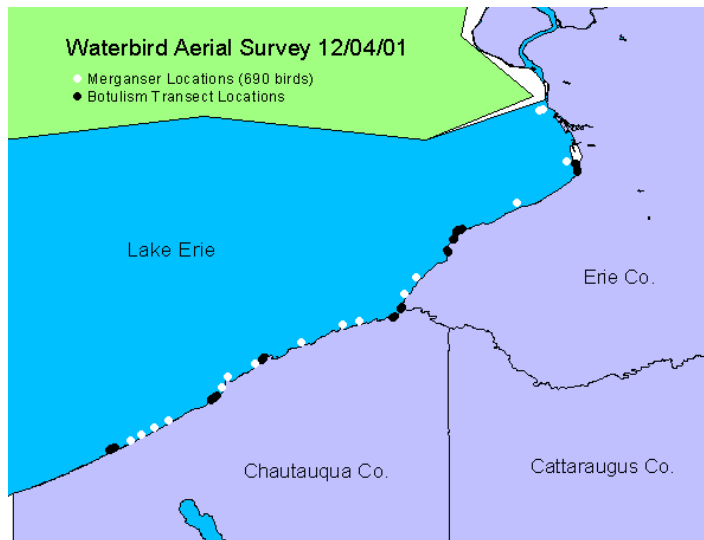
**Figure 4**



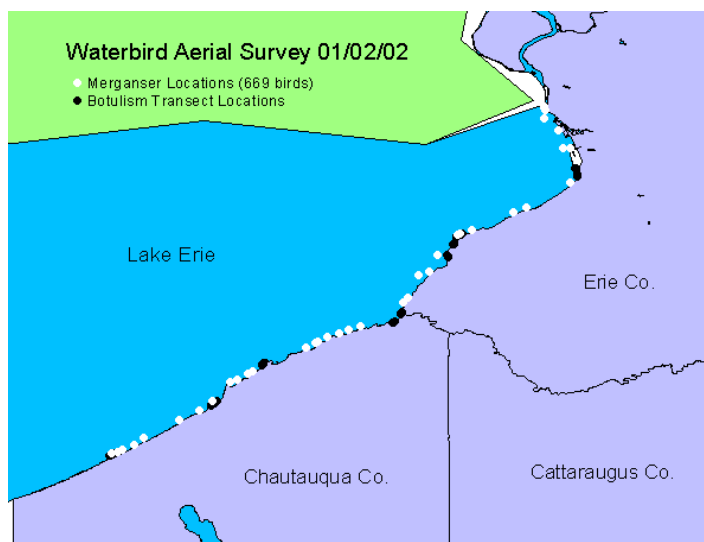
**Figure 5**



**Figure 6**



**Figure 7**



**Figure 8**

## Fish Related Mortalities Along the NY Portion of Lake Erie



by Bill Culligan  
New York Dept. of  
Environmental Conservation  
Lake Erie Unit- Dunkirk

## Primary Fish Species Affected

*Sheepshead (Freshwater Drum)*  
*Rock Bass*  
*Smallmouth Bass*  
*Channel Catfish*  
*Stone Cats*  
*Round Goby*  
*Lake Sturgeon*

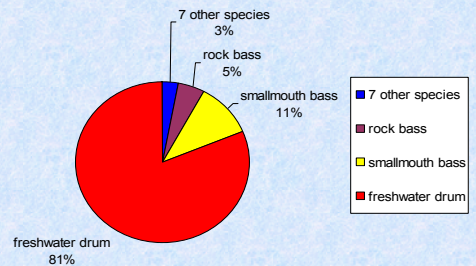
**Nearshore Benthic Species**

## Timing of NY Mortalities

- Late July thru Early October
- Peak Mortality- August
- Total Kill - Tens of Thousands of Fish

## NYSDEC - September Survey

Frequency of dead fish species observed  
along Lake Erie beaches, September, 2001



Information from NYSDEC

## Significance



### Lake Sturgeon

- \* Threatened species in NY
- \* Usually 1-2 sightings/year

### 2000

- \* 8 dead fish collected

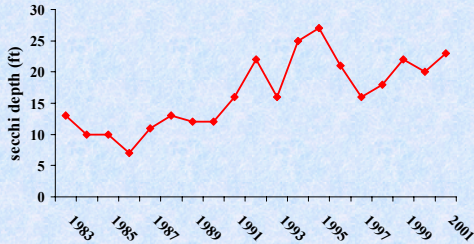
### 2001

- \* 27 dead fish collected
- \* Size: up to 80 inches

## Recent Ecological Changes in the Eastern Basin

- Water Clarity Changes
- Introduction of Quagga Mussels (*D. Bugensis*)
- Introduction of Round Goby (*N. melanostomus*)

## Average Water Clarity NY Waters of Lake Erie



## Quagga Mussels

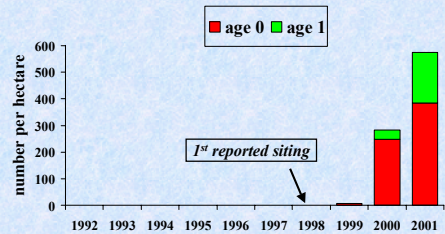
- First Appeared- 1991, East Basin
- More Abundant than *D. polymorpha* by 1993
- 2001- Almost 100% of Dreissenids
- Characteristics:
  - Deeper, Colder, Offshore Waters
  - Different Food Habits
  - Faster Growth Rates

## Round Goby



- First Collected - 1998
- Abundant - 2000
- Feed Heavily on Dreissenid Mussels
- Fed upon Heavily by Fish Predators

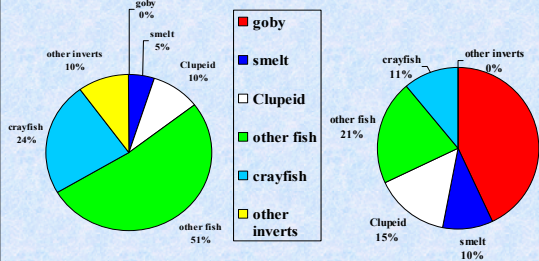
## Round Goby Abundance NY Waters of Lake Erie



## Smallmouth Bass Diet Comparisons

September 1985 - 87

September 1999



## Summary

- Major Fish Mortalities (nearshore benthic species)
- Sturgeon Mortalities may have population level significance
- Recent Ecological Changes may be playing a major role (Quagga Mussels and Goby)

### **Fish and Bird die-offs on Canadian side of Lake Erie – 2001 – Jeff Robinson, Canadian Wildlife Service**

The July to September period fish and bird die-offs were reported by various citizen and government agents along the Canadian shoreline of Lake Erie starting in late June. Fish die-off events of small to moderate scale are a relatively normal occurrence along certain shore reaches of the north shore. Citizens living in these areas have observed these in the past and are not generally alarmed by such events when they occur. In recent years, the numbers of dead fish has increased dramatically with the increased occurrence of round gobies in these die-off events. Following is a chronology of die-off events reported in 2001.

July 31: Reports of dead birds and fish east of Port Dover associated with a rapid drop in water temperature in the near shore area. Similar event occurred in the area of Dunkirk, New York, during the same week. Birds involved were primarily gulls. One report included 30 dead gulls on the beach east of Port Dover, with some numbers of dead gulls observed as far east as the mouth of the Grand River. Gulls and shorebirds were reported from Point Pelee National Park, as well, but no reports of large numbers of dead birds other than at Port Dover. Gulls collected from Port Colborne at the mouth of the Welland Canal tested positive for botulism type E. Twenty dead ring-billed gulls encountered on south beach of Long Point National Wildlife Area and 2 sick birds exhibiting botulism type E symptoms, but none were collected for testing. Several hundred apparently healthy cormorants and gulls foraging and roosting along the north shore of Long Point. No dead fish observed on north or south beaches.

August 16: Fish die-off at Wheatley did not result in any unusual bird mortalities, but had large numbers of gulls foraging on thousands of dead or dying gobies for several days. A similar fish die-off/lake thermal event occurred along the shoreline from Port Dover to possibly Port Colborne on the same day. Higher numbers of bird mortalities were reported early in the week and were investigated on August 23 and 24th at beaches between Hoover Point and Featherstone Point where 38 dead birds, 1 mudpuppy and 3 shorebirds were counted by a citizen. Canadian Wildlife Service collected 4 birds for blood samples and several dead ring-billed gulls, black-backed gulls, 2 Caspian terns, 13 cormorants. Earlier in the week, 2 great blue herons were picked up by local animal control agency as sick animals (Jeff Robinson observed another apparently sick heron on an offshore island, but was not able to get out to the island). In the area there were numerous apparently healthy ring-billed gulls, cormorants, Caspian terns, mallard ducks, Canada geese and great blue herons. The only dead fish evident on the shore were freshwater drum (sheepshead) and the citizen reporting indicated he had not seen any remarkable numbers of dead gobies or other fish on the shoreline. He noted there had been some higher number of dead birds evident last fall when similar die-offs had occurred elsewhere, but it was not reported at the time. During 2002 there were more dead birds than last year. Jeff Robinson visited the citizen again on August 24, and picked up 2 more dead cormorants and 1 ring-billed gull and 1 black-backed gull which were all the new dead birds from the day before.

Staff of the Long Beach Conservation Area east of Lowbanks had been encountering numerous dead gulls on their beaches for several days, but had not noticed unusual numbers of dead fish or mudpuppies. Picked up 3 ring-billed gulls and 1 black-backed gull from staff, as well as 1 ring-billed gull from the beach area where 14 had been reported earlier in the day.

West of Lowbanks: Surveyed a beach where a dead bald eagle had been reported on August 23. The dead eagle was not found (the citizen was quite confident it was an eagle, as he correctly identified black backed gulls also on the beach there). Picked up several dead ring-billed gulls, black-backed gulls and cormorants on this beach (1 gull and 1 cormorant were banded). This beach is within sight of Mohawk Island National Wildlife Area which has nesting colonial water birds (gulls, cormorants and terns) on it which are monitored for contaminants and productivity by Environment Canada/Canadian Wildlife Service personnel on a regular basis. On August 23, the island was visited by researchers who noted the following: approximately 30 dead double-crested cormorants (hundreds of live ones) and several sick ones; 50 dead Caspian terns including a few chicks; 10 dead great black-backed gulls; and a few dead ring-billed and herring gulls. There were also 2 sick ring-billed gulls; lying on their backs; also noted about 5 dead gulls on the east pier at Port Maitland (but none were retrieved). The citizen reporting the bald eagle indicated that other dead birds had been gathered up earlier in the week and burned by another individual.



Rock Point Provincial Park reported dead gulls, cormorants and shorebirds were being collected and buried by maintenance staff. A citizen just west of Port Maitland reported dead cormorants, gulls and fish as well on August 25. They reported dead 8" catfish and drum, but no gobies in any numbers. This die-off seems related to a lake thermal event triggered by a storm with moderate to strong north winds on August 17.

Canadian Wildlife Service staff on Long Point National Wildlife Area just west of this reported die-off did not encounter any remarkable gull mortalities on the beaches during this week.

September 6: Site visit to Mohawk Island National Wildlife Area east of Port Maitland in Lake Erie, which is a 2 hectare rocky island, 2 kilometers off shore.

Several hundred cormorants, several hundred common terns and Bonapartes gulls and less than 100 Caspian terns were evident on the island and flew off as we approached the island. Also, there were ring-billed gulls, greater black-backed gulls, mallard ducks and several shorebirds evident on the island.

We walked the island systematically and counted and identified (as much as possible) all dead birds encountered. As well, live, sick birds were collected live for testing at the CCWHC at the University of Guelph. Few dead fish were observed on shore, finding 1 dead mudpuppy, 1 rock bass, numerous sheepshead, and a sucker species floating nearby. No gobies in evidence on shore here.

Counts of dead birds:

Double-crested Cormorants:	128
Ring-billed gulls	94
Herring gulls	54
Greater Black-backed gull	30
Caspian tern	50
Common tern	2
Bonaparte's gull	3
Shorebirds	22
Blue-winged teal	2
Mallard	<u>2</u>
Total	387

Many dead chicks (especially terns) were not included in the count, as they were not related to the botulism outbreak that was being monitored. The dead birds were quite dried out and were in various stages of decomposition. Many of the Caspian terns had been dead for some time, being observed in the apparent nesting area (an area where mussel shells have piled as a result of wave and ice action). There were some relatively fresh Caspian terns, but none were recent enough to enable testing for botulism. Cormorants and black-backed gulls were in 3 different apparent episodes; some carcasses had pupating flies, some had active maggot infestation and a smaller number were relatively fresh or sick, but still alive. We retrieved 1 cormorant, 1 black-backed gull, 1 ring-billed gull and 1 immature herring gull alive for analysis at CCWHC. Test results were negative for serum, but birds exhibited classic symptoms. Ring-billed gulls observed included a high proportion of birds which died at the end of the nesting period likely from other natural causes found in colonial waterbird colonies. Six live, but sick shorebirds were also captured for assessment at the CCWHC (these included a lesser yellow-legs, a semi-palmated plover, a white-rumped sandpiper). Also, approximately 6 more dead shore-birds were collected for possible analysis at the CCWHC, with the live shorebirds. Most shorebird remains were relatively fresh. The blue-winged teal had been dead for some time, one skeleton had the wings detached as it had been obviously scavenged and the other was an articulated skeleton. The 2 mallards were more recent deaths, but were not usable specimens. We counted 63 cormorant nests on the island.

September 8: Phil Ryan from the Lake Erie Fisheries Assessment Office in Port Dover investigated a reported fish and bird die-off between Port Dover and Selkirk.

- Large amount of decaying Cladophora inshore
- Counted 29 sheepshead, 2 rock bass, 1 white perch, 1 smallmouth bass, 2 burbot, 1 cormorant and 1 seagull dead along .3 km of shoreline

Selkirk Provincial Park access to beach

- Counted 19 sheepshead, 2 rock bass, 2 gobies and 2 seagulls

East of Peacock Point – sandy bay with Cladophora on beach

- Counted 15 sheepshead, 1 rock bass, 1 bullhead, 1 stone-cat, 3 cormorant, 2 seagull and 1 great blue heron (collected)

General Comments:

- Strong winds on the weekend brought fish into beach on weekend, but offshore wind may have carried some away (Sunday night)
- Fish had been dead for a few days to a week
- Recent mortalities of birds had occurred – 1 seagull, 3 cormorants, 1 heron

Burbot were not full sized, and may be immature fish. Observation is significant as burbot unlikely to bloat and wash up on beach.

September 18: Report of 20 dead gulls on beach on west side of Rondeau Provincial Park. Park staff investigate, but no specimens retrieved for testing. No large scale mortalities encountered subsequent to this. Some dead gobies noted in same beach area. Continued reports of sick or dead birds from beaches east of Port Dover to Lowbanks.

October 5: Sixteen black-backed gulls reported from Pelee Island in western Lake Erie with specimens submitted to Guelph (no positive test for botulism type E).

October 19: Bird watchers report seeing loons flying overhead in the London area; co-operators on both sides of lake alerted to watch for sick loons in the eastern basin east of Port Dover. Public reports dead cormorants on beach at Burlington, on Lake Ontario, which Canadian Wildlife Service staff investigate and determine mortality was due to wire strikes. Some reports of dead birds continue.

October 29: Storms over the previous 2 days on Lake Erie. Check beach areas from Port Bruce to Lowbanks for dead birds. Low numbers of birds encountered, one dead common loon at Hoover-Featherstone Point beach area where high numbers of gulls had been encountered in August and September.

November 3: Telephone calls in morning of numerous loons coming ashore dead and alive east of Port Dover. No live loons are encountered on the site visits, but many are observed and collected for botulism testing. Counts are not done on this day as the extent of the shoreline effected and attempts to gather fresh specimens are priority.

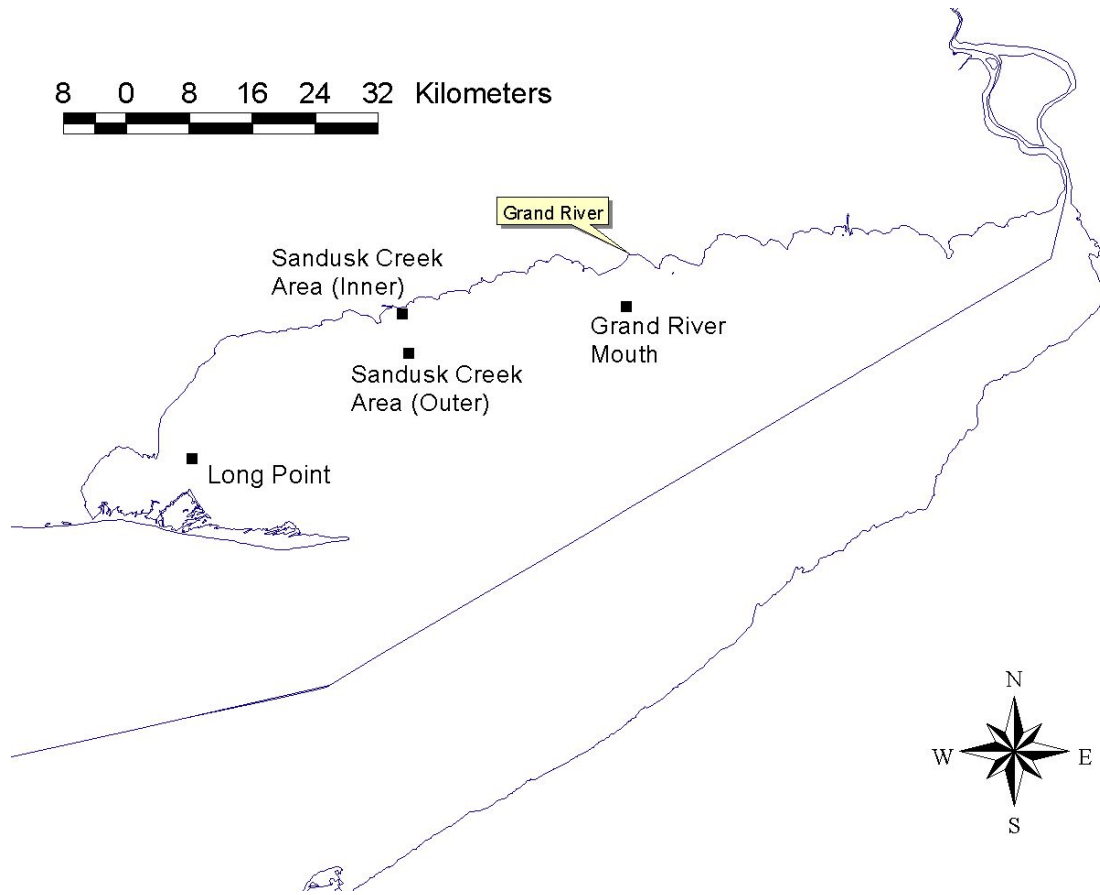
November 15: Counts of dead birds on known beach areas are conducted for 1 km at each area. Port Bruce and Port Burwell each have 1 loon per kilometer. Long Point has 35 loons, which is approximately 1 loon per kilometer of shoreline surveyed. Port Dover beach has no loons on the beach surveyed, but is the only beach with high numbers of diving ducks (12 scaup, 4 red-breasted mergansers, 1 grebe and 1 white winged scoter). Featherstone to Hoover Point; 60 red-breasted mergansers, 35 common loons, 1 grebe, 2 long-tailed ducks, 2 ring-billed gulls, 3 scaup. Knights beach area, 1 cormorant, 33 loons, 2 ring-billed gulls. Shoreline opposite Mohawk Island, 7 common loons, 1 black-backed gull, 1 long-tailed duck. Lowbanks, 5 common loons, 1 black-backed gull, 1 long-tailed duck. Other locations reported some dead birds, but no large concentrations. Virtually no dead loons were reported from other basins of the lake, with 1 report of several dead loons from the Eriean shoreline in November, but specimens were not submitted for testing.

Below is a summary of dead bird surveys conducted by Long Point Bird Observatory volunteers on 3 km of beach at the tip of Long Point in the fall of 2001. The tip is a well used roost for gulls and several thousand double-crested cormorants regularly roosted on a 5 km long sandbar west of the tip of Long Point throughout the late summer and early fall of 2001. Note that no loons were encountered at this location in this period.

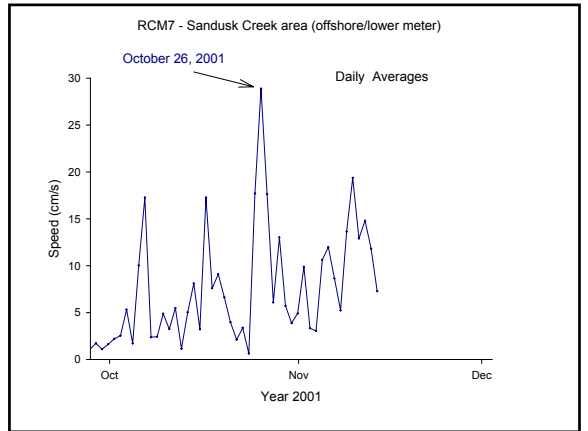
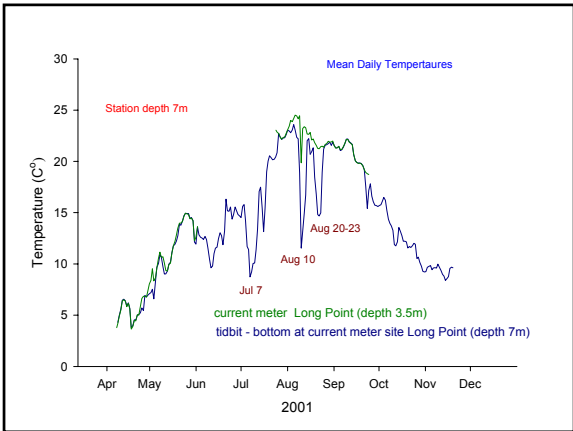
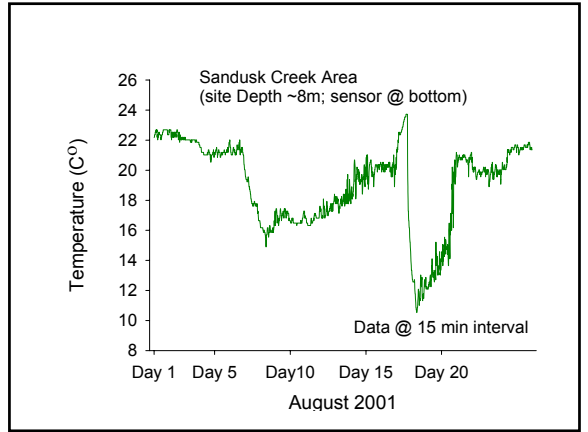
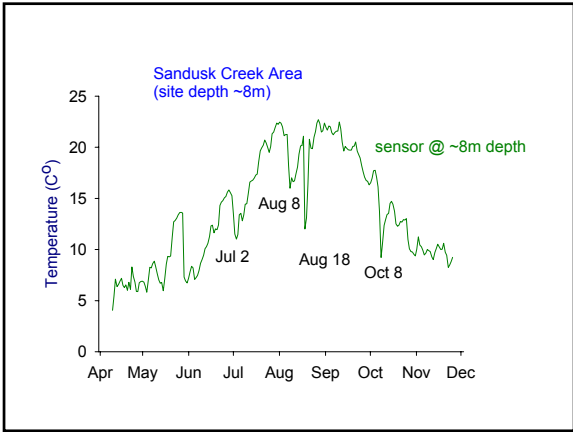
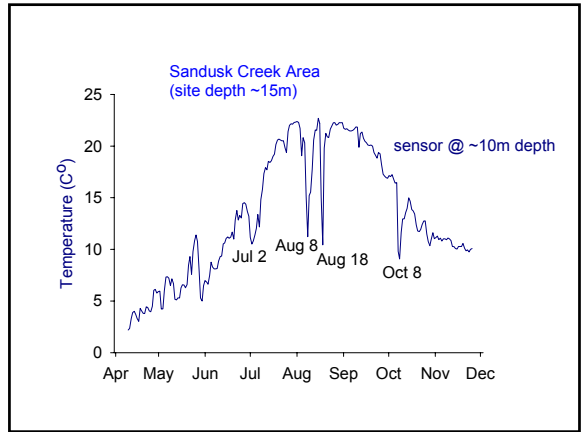
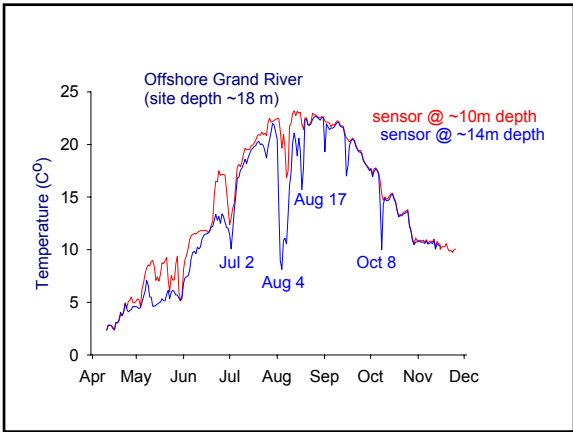
Beached Bird Counts Tip of Long Point (Fall 2001)										
	Aug 10- Sep 6	13 Sep	20 Sep	27 Sep	4 Oct	11 Oct	19 Oct	25 Oct	1 Nov	8 Nov
Double-crested Cormorant	2	1	0	4	0	1	0	0	0	0
Ring-billed Gull	19	1	4	6	8	15	3	2	0	1
Herring Gull	8	1	0	1	2	5	1	0	0	0
Great Black-backed Gull	8	1	0	1	0	0	2	0	0	0
Bonaparte's Gull	1	0	1	0	0	1	0	0	0	0
Common Tern	0	0	0	0	0	0	1	0	0	0
Lesser Scaup	0	0	0	0	0	1	1	2	0	0
Greater Scaup	0	0	0	0	0	0	0	0	0	1
Green Heron	0	0	0	1	0	0	0	0	0	0
Red-breasted Merganser	0	0	0	0	0	0	0	0	0	2
Totals	38	4	5	13	10	23	8	4	0	4
Overall Total for fall	109									
Total Species	10									

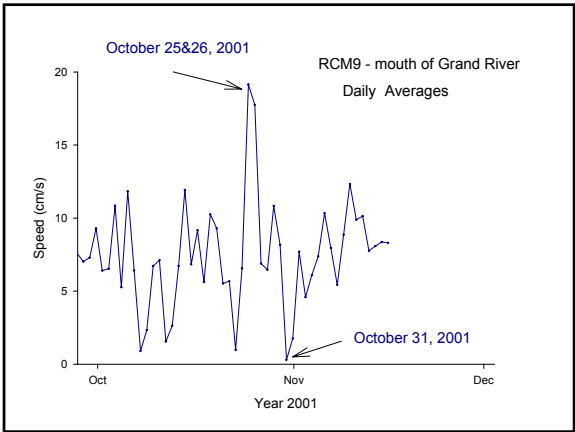
First week of December: Beach surveys conducted following another storm event in early December found no new bird mortalities in the eastern basin of Lake Erie.

Temperature profiles were recorded for sites from Port Dover to the mouth of the Grand River and results are presented as powerpoint slides.



Location of temperature probes in 2001.





## Type E Botulism Summary of Tests Conducted

	1998	1999	2000	2001
# Submissions	6	25	18	44
# Submissions tested	3	19	12	22
# Positive tests	2	9	11	8

## Type E Botulism Bird Species Affected

	1998	1999	2000	2001
Common Loon	C	C	C	P
RB Merganser	NT	C	C	C
RB Gull	NT	P	P	C
Herring Gull	P	P	P	C
GBB Gull	NT	P	P	C
Bonaparte's Gull	NT	P	NT	C
DC Cormorant	NT	NT	P	C
Diving Ducks	NT	P	P	P
Shorebirds	NT	P	P	P
Grebes	NT	NT	C	C

C - Confirmed      P - Presumptive      NT - Not Tested

## Stomach Contents Birds from Type E Botulism Events Lake Erie and Lake Huron 1999-2001

Species	N	Gobies	Other Fish +	Zebra/Quagga	Other *	Unidentifiable	No food
Common Loon	43	13	13	1	0	14	4
Red Throated Loon	1	1	-	-	-	-	-
RB Merganser	10	6	4	1	-	1	-
Eared Grebe	4	4	3	-	2	-	-
Red Necked Grebe	2	1	1	1	1	-	-
Oldsquaw	2	1	-	-	-	-	1
RB Gull	12	5	2	-	3	3	-
Herring Gull	4	1	2	1	2	-	-
GBB Gull	5	-	2	-	-	3	-
Bonaparte's gull	1	-	-	-	-	-	1
DC Cormorant	1	-	-	-	-	1	-
<b>Total</b>	<b>85</b>	<b>32</b>	<b>27</b>	<b>4</b>	<b>8</b>	<b>22</b>	<b>7</b>

\* Includes fingernail clams, mudpuppies, frogs, hemipterans  
+ includes alewife, smelt, cyprinids, sheepshead, gizzard, shad, salmonids

## Type E Botulism Identification of Stomach contents Birds Dying in Botulism Events

Food items identified	Frequency of item in stomach
Gobies	32/85
Other Fish	27/85
Zebra/Quagga Mussels	4/85
Other (frogs, mudpuppies, invertebrates)	8/85
Unidentifiable remains	22/85
No Food Present	7/85

## Type E Botulism Canadian Detection and Diagnosis

### *Detection, collection and reporting of dead birds and outbreaks*

- Canadian Wildlife Service
- Ontario Ministry of Natural Resources
- Parks Canada – Point Pelee
- Ontario Parks – Rondeau, Pinery
- Public Health Units
- Members of the public

### *Post Mortem Examination of Dead Birds*

- Canadian Cooperative Wildlife Health Centre (Guelph)

### *Laboratory testing of tissue samples*

- Animal Health Laboratory (University of Guelph)

### *Analysis of birds' stomach contents*

- Ontario MNR Fisheries Research Station (Wheatley)

## Type E Botulism Post-Mortem Examination of Dead Birds

- 1) Selection of specimens for post-mortem examination
  - species
  - geographic area
  - carcass condition
- 2) Gross necropsy of carcasses
  - evaluation of body condition
  - description of gross lesions
  - collection of samples for further testing
- 3) Diagnostic testing of samples
  - botulism testing by mouse inoculation
  - histology
  - toxicology (metals, cyanide)
  - virology (virus isolation)

**Type E Botulism**  
**Testing of Gobies**  
**Dr. Philip J. Byrne**

- MNR, Port Dover, collected gobies by trawl, and they were transported to Guelph
- Fish were held for observation in Guelph
- Sick fish were killed and examined
- Protocol:
  - Gross examination
  - Routine bacteriology
  - Histology
  - Mouse inoculation
  - Electron microscopy

**Type E Botulism**  
**Testing of Gobies**  
**Dr. Philip J. Byrne**

**Results:**

- Gross observations - many gobies lost skin in the caudal half of their bodies
  - apparent paralysis of tail
- Botulism testing - all negative

**Conclusion:**

- Initially healthy fish
- Botulism was not the cause of death
- Death likely due to stress and/or opportunistic infections



## **Botulism in Fish and Wildlife in New York State Waters of Lake Erie**

### **Ward Stone, NYS Department of Environmental Conservation's Pathologist**

Ward Stone provided an overview of NYSDEC's work with the botulism outbreak in New York's waters of Lake Erie, including the following information:

- The Delmar, New York, Laboratory took in 1,000 lbs. of loon in one shipment alone during the height of the avian die-offs in 2001.
- Old Squaw (Long tailed ducks) that tested positive for Type E botulism were feeding on quagga mussels (*Dreissena bugensis*).
- Type E botulism diagnosed was first found in Lake Erie in 1999. The NYSDEC lab first diagnosed type E botulism in the fall of 2000.
- 2001 - Type E botulism was found in fish alimentary canals (in gut content).
- 2001 - Type E botulism was found in sheepshead, both in gut and tissue samples.
- 2001 - Type E toxin was found in mudpuppies (salamander-like, aquatic amphibians).
- Mats of *Cladophora*, a filamentous algae, were found during some of the outbreaks.
- Shorebirds were impacted. Sanderlings were found with type E botulism. The birds were feeding on maggots from dead sheepshead.
- Maggots (fly larvae) – had type E toxin.
- Bald Eagle – Hatched in 2001 Delaware River (Peter Nye) was found dead in Chautauqua County near Lake Erie. The bird tested positive for Type E botulism. The eagle had large fish in gullet, including smallmouth bass.
- Type E botulism was found in the following species of birds: common loon, double-crested cormorant, long-tailed duck, red-breasted merganser, ring-billed gull, herring gull and great black-backed gull.
- Mudpuppies and round gobies were found inside some of the gulls.
- Mudpuppies were found in some of the mergansers and loons.

Despite three years of monitoring the Lake Erie outbreaks, the NYSDEC has been unable to determine where many of the animals were exposed to the toxin, particularly the loons and diving ducks that died in late fall. To date, there have been practically no observations of sick loons or ducks; they almost invariably have washed ashore dead. Because winds may transport

floating carcasses great distances, and because there are few people normally out on the lake at that time of year, the mystery remains.

Since type E's appearance in New York in 1999, staff of the Department of Environmental Conservation's (DEC) Division of Fish, Wildlife and Marine Resources have documented the extent of mortality, tried to understand toxin pathways in the ecosystem, and made recommendations about human health concerns and potential remedies. Lake Erie Fisheries Unit staff have observed fish morbidity and mortality, collected dead fish and birds, and collected sediment, mussels and healthy fish for studies on diet and *Clostridium* growth. Staff from DEC's Buffalo office and the Wildlife Pathology Unit (WPU) in Delmar have systematically monitored many beaches, bays and harbors for dead fish and birds. All of the birds and some of the fish are transported to the WPU's laboratory where post-mortem examinations are completed. Such examinations include selection of samples for botulism testing, observations of ingested food items, and the identification of other causes of death. This work has confirmed the suspected importance of the round goby in the toxin pathway and identified mudpuppies as a probable toxin victim and vector.

# Botulism in Humans

John C. Lyons, MD, FACS, MSME

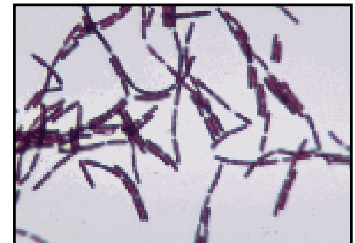


## Definition:

- Botulism is a syndrome induced by poisoning through proteins produced by the bacterium *Clostridium botulinum*.
- These proteins produce a paralysis of the muscles and death by respiratory insufficiency.
- *Clostridium botulinum* is but one strain of the bacterial genus *Clostridium*. According to *Bergey's Manual of Systemic Bacteriology* the genus includes 83 strains of which 30 are pathologic in humans.

## *Clostridium* (From: Gorbach Bartlett Blacklow):

- Some common pathologic strains of the *Clostridium* species and their associated condition include:
  - *Clostridium difficile* – diarrhea
  - *Clostridium perfringes* – gas gangrene
  - *Clostridium tetani* – tetanus
  - *Clostridium botulinum* – botulism
- *Clostridium* species are ubiquitous in nature, in both soil and water, and are generally innocuous to humans.
- They are resilient, forming spores when growth conditions are marginal and remaining viable in the environment for extended periods of time.
- *Clostridium botulinum* spores are ubiquitous in soils and aquatic sediments.
- They are activated under conditions of low oxygen tension and pH >4.6
- Under these and further conditions, the bacteria may produce clinical disease in animals or humans.
- Typically gram-positive (in late tissue recovery, may appear gram-negative).
- The microscopic appearance is that of fat, boxcar-shaped rods.
- The bacteria may be subsetting on the basis of other characteristics such as biochemistry, metabolism or other features.
- *Clostridium* species are primarily anaerobic.
- Some *Clostridium* species are quite “aerotolerant,” such as:
  - *Clostridium perfringes*
  - *Clostridium septicum*
  - *Clostridium histolyticum*
  - *Clostridium tertium*
- Some *Clostridium* species lack certain enzymes.
- Spore formation and aquatic toxin production may vary.
- Similarly, the strains themselves may be subsetting.



From: Gorbach Bartlett Blacklow

## **Groupings & Epidemiology** (From: Gorbach Bartlett Blacklow):

### ***Clostridia botulinum:***

- Has seven types: A, B, C, D, E, F, G
- And two subtypes: C<sub>1</sub> & C<sub>2</sub>
- Based on differences in the serology of the toxins.
- Historically, as outbreaks of botulism would occur, the investigations led to the discovery of different types of toxins associated with the *botulinum* bacteria.
- Outbreaks in cattle, chickens and other animals revealed variances in the toxic proteins, and subsetting was based on these differences for source identification.
- The common characteristic of these strains is the ability to produce a potent neurotoxin.
- The likely purpose of the toxin is not necessarily the death of the host, but instead, resistance against the host defense mechanisms.
- The toxin is so effective against the host and its defenses that:
  - the lethal human dose is 100 billionths of a gram (*Science News*)
  - without intervention, the host dies 70 percent of the time.
- Its specific antitoxin neutralizes the biologic activity of each of the seven types of botulinum bacteria toxin.
- There is no cross-neutralization to rely upon (limited reciprocal E&F).
- Type C is unique:
  - Antitoxin from the Bengtson botulism strain (isolated from a chicken outbreak) neutralized toxin from the Seddon strain (cattle outbreak), but not the reverse. C<sub>1</sub> and C<sub>2</sub> subtypes were established to distinguish the subsets.
  - Subtype C<sub>1</sub> produces two toxins, with C<sub>2</sub> producing only one of the two.
- Further blurring the subtyping: type C organisms may also produce some type D toxin, and type D may produce some C<sub>2</sub> toxin.
- Type C and D neurotoxins are phage mediated; G is likely plasmid; and A, B, E, F are assumed chromosomal.
- Avian botulism and botulism in domesticated/wild mammals are frequently types C and D.
- *Clostridia botulinum* may also be classified physiologically into four groups based on metabolism:
  - Group 1 - proteolytic organisms – all type A toxin producers, some type B, E or F
  - Group 2 - nonproteolytic organisms – all type C and D toxin producers
  - Group 3 - mixed proteolysis characteristics
  - Group 4 - unique argentine type G toxin
- These groupings may be of some importance.
- Each of the four groups is clearly DNA distinguishable.
- Some non-neurotoxic organisms are genetically related to the groups:
  - *Clostridium sporogenes* matches Group 1
  - *Clostridium novyi* matches Group 3
- Thus, there are toxigenic and nontoxigenic members of the same “species.” See Table 233-1.

**TABLE 233–1 ■ Groups of *Clostridium botulinum* and Other Species Capable of Producing Botulism Neurotoxin: Nontoxicogenic Species**

GROUP OR SPECIES	TYPE OF TOXIN	GLUCOSE FERMENTATION	CASEIN DIGESTION	GELATIN LIQUEFACTION	Reactions on EYA	
					Lip	Lec
I	A,B,F	+	+	+	+	–
II	B,E,F	+	–	+	+	–
III	C,D	+	±	+	+	±
<i>C. argentinense</i> †	G	–	+	+	–	–
<i>C. baratii</i>	F	+	–	–	–	+
<i>C. butyricum</i>	E	+	–	–	–	–

Key: EYA, egg yolk agar; Lip, lipase; Lec, lecithinase; A, acetic; iB, isobutyric; B, butyric; iV, isovaleric.

\*Volatile metabolic acids produced in peptone-yeast extract-glucose, analyzed by gas-liquid chromatography.

†Also commonly known as *Clostridium botulinum* type G.

From: Gorbach Bartlett Blacklow

### **Human Botulism** (From: Gorbach Bartlett Blacklow):

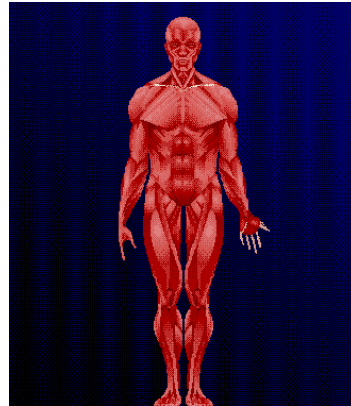
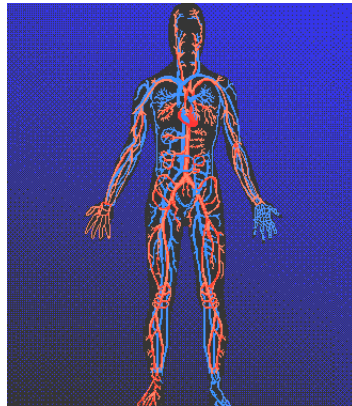
- Restricted to toxin types A, B, and E.
- There is evidence for type F, in five instances (*Center for Disease Control*)
- One food outbreak in France was type C, but only on the basis of recovery of the organism from the food:
  - No toxin was found in the food
  - No clinical samples were taken
- One outbreak in Chad was type D based on recovered type D toxin in salted ham.
- Hauschild's worldwide review of food-borne botulism (1950 – 1988, 1989 publication date) revealed 1015 cases:
  - Type A – 391
  - Type B – 170
  - Type E – 150
  - Type F – 4
  - Unknown – 300
- Type E food-borne botulism is common in Alaska, associated with fish and traditional food preparations.
- In the lower 48 states, type A predominates in the west, type B in the east.

### **Summary of *Clostridium Botulinum*:**

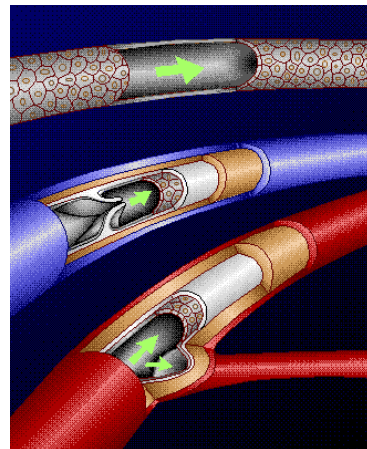
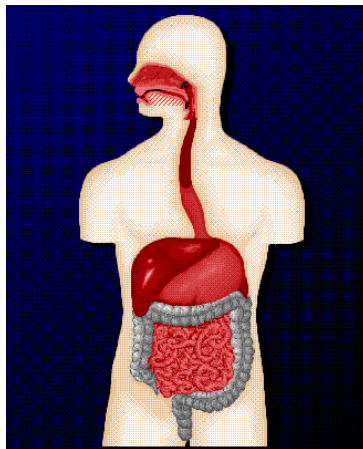
- Type A, B, and E are of primary clinical concern.
- Type C, D, and F are of questionable clinical significance.
- Avian botulism outbreaks are more associated with types C or D.
- Clinically important type E botulism is related to fish consumption.

## ***Pathogenesis of Clostridium*** (From: Gorbach Bartlett Blacklow):

- *Clostridium* species produce a binary A-B toxin
  - The A and B subunits are released separately from the bacteria and combine to specific receptors on nerve cell surfaces.
  - Synergistically the subunits penetrate the cell and poison the acetylcholine transmitter release system.
  - Muscles receive no neural stimulation and go flaccid.
- The toxins are dichain peptide molecules, molecular weight approximately 150 kd.
- A recent 3-D study revealed a belt of amino acids that protect the lethal elements of the toxin, changing the structural perceptions of the toxins and their precise mechanism of action. (*Science News*)
- Botulism primarily attacks the nerves to the skeletal muscles, although the neurotoxin may interfere with some autonomic and even cardiac function.



- Food-borne botulism toxin is absorbed through the intestine and transferred to neurogenic receptor sites via the circulation system.



- At the nerve ending, a portion of the toxin binds to the receptor and a portion is inserted into the cell to poison acetylcholine release, resulting in flaccid paralysis.

**Clinical Syndrome and Treatment** (From: Gorbach Bartlett Blacklow):

- Signs and symptoms may vary as a function of :
  - toxin type
  - dose
  - host parameters
  
- Onset of symptom presentation may range from 18 to 36 hours, with outliers from six hours to ten days (Center for Disease Control)
  
- Typical symptoms include (see Table 233-3):
  - blurred vision
  - dysphagia
  - generalized weakness
  - nausea/vomiting
  - vertigo
  - abdominal cramps
  - paresthesias (sensation of prickling, tingling or creeping in the skin) in only 1 percent

**TABLE 233-3 ■ Symptoms and Signs of Illness Observed in Food-Borne Botulism Outbreaks 1953-1973; Number of Outbreaks in Which One or More Patients Experienced Symptom or Exhibited Sign**

	07	10	00	0	00	272	Sign or Symptom
<b>Symptoms</b>							
Blurred vision, diplopia, photophobia	31	13	9	1	40	94	90.4
Dysphagia	27	14	3		35	79	76.0
Generalized weakness	22	12	4		22	60	57.7
Nausea and/or vomiting	15	13	10	1	19	58	55.8
Dysphonia	25	8	5		19	57	54.8
Dizziness or vertigo	8	4	5		15	32	30.8
Abdominal pain, cramps, fullness	5	6	3		7	21	20.2
Diarrhea	5	6			5	16	15.4
Urinary retention or incontinence	2	2	1		2	7	6.7
Sore throat	4	2	1			7	6.7
Constipation	2	2		1	3	6	5.8
Paresthesias	1					1	1.0
<b>Signs</b>							
Respiratory impairment	32	7	7		30	76	73.1
Specific muscle weakness or paralysis	23	9	3		13	48	46.2
Eye muscle involvement, including ptosis	16	9	3	1	17	46	44.2
Dry mouth, throat, or tongue	7	6	2		7	22	21.2
Dilated, fixed pupils	3	4	2		8	16	15.4
Ataxia	3	1		1	4	9	8.7
Postural hypotension			1		2	3	2.9
Nystagmus	1		1		1	3	2.9
Somnolence			1			1	1.0

\*Toxin type undetermined or unspecified.

(Data from CDC: Botulism in the United States, 1899-1977; Handbook for Epidemiologists, Clinicians, and Laboratory Workers. Atlanta, USPHS, 1979).

- Typical signs include:
  - respiratory impairment
  - specific muscle weakness or paralysis
  - eye movement impairment
  - dry mouth, throat, or tongue
  - dilated, fixed pupils
  - ataxia
  - postural hypotension
- Diagnosis is made on the basis of signs and symptoms, with a degree of clinical suspicion.
- The differential may be difficult and similar presentations may be seen in:
  - Guillain-Barre
  - myasthenia gravis
  - stroke
  - chemical poisoning
- Confirmation requires detection of the toxin, whether by serum or stool via mouse bioassay (48-hour results vs. five-seven day incubation results).
- State Health Departments or the Center for Disease Control will arrange for antitoxin.
- Antitoxin simply binds the circulating toxin, it does not reverse the paralysis.
- The destruction of the neurotransmitter is irreversible.
- Trivalent antitoxin (for types A, B, E) is available for non-infants (infantile human antitoxin is different).
- Human trials have shown efficacy, although animal studies reveal that unless the antitoxin is given early it is ineffective.
- Pentavalent botulism toxoid (A, B, C, D, E) is available from the Center for Disease Control for researchers.
- Antibiotics are ill advised, as the death of the bacterium may release more toxin.

***Survival Rates*** (From: *Gorbach Bartlett Blacklow*):

- Survival has improved, due to the supportive care now available, i.e. mechanical ventilation.
- Fatality rate (*Gale Encyclopedia of Medicine*):
  - 1910 – 70 percent
  - 1980 – 12 percent
  - 1993 – 2 percent
- There are forms of botulism poisoning other than food-borne infections:
  - Wound
  - Infantile
- WRT wound botulism is exceedingly rare (*Gale Encyclopedia of Medicine*), including 47 cases in the United States from 1943 to 1990.
- Wound botulism requires special conditions, e.g. unattended tissue necrosis, etc. – and tetanus is far more of a clinical concern.
- If staff is concerned with bird carcass handling, vaccination could be used.



## ***Botulism in Humans - Interrelationship with Avian Botulism:***

### ***Possible Health Concerns:***

- Avian botulism may impact human health in two ways:
  - The infected specimens may pose a direct health hazard, depending on issues such as botulism type.
  - The occurrence may serve as a marker for an environmental factor of concern, e.g. associated fish contamination and fish consumption.
- Little concern for direct consumption of infected gulls:
  - Not a sought after dish
  - Subtypes C and D typical to avian botulism not generally linked to human disease, but there are questions...where did the gulls encounter their exposure and would other consumables be affected?
- What is the botulism type?
- Perhaps concern for food chain contamination is involved when a predator or scavenger fish consumes the gull carcass.
- Little concern for direct inoculation from carcass handling:
  - Wound botulism exceedingly rare
  - Avian type C and D botulism are of questionable clinical concern
- Still, collection and handling precautions are prudent, particularly when the botulism type is unknown.
- May be prudent for researchers to consider the pentavalent vaccine.
- Certainly seems prudent from a public health perspective to research the phenomena to:
  - eliminate the direct source contact potential
  - ensure safety of the food chain - particularly fish

### ***The Fish Problem:***

- Lake Erie is an angler's paradise and fish consumption is widespread and essentially year-round.
- Fish preparation varies widely and lends itself to potential botulism issues.
- Lake Erie fish are smoked, dried, salted – small fish are flash cooked.

### ***Botulism Outbreaks Occur With Fish Consumption:***

- Has been demonstrated in:
  - tuna fish, lobster, whitefish and other varieties
  - canned, smoked, salted and dried preparations
  - Alaskan outbreaks most frequent
  - New York City, November 1987, eight cases due to uneviscerated, dry-salted, air-dried, whole, whitefish - *botulinum* type E (*Food and Drug Administration*)
- Anglers may have unrecognized exposure if the phenomenon is not studied.

### ***Clostridia botulinum* is a Resistant Organism:**

- Spores survive for hours in boiling water – pressure processing required (*E L Andress*).
- Toxin may be destroyed by boiling for 10 minutes at sea level (+ 1 min/1000 ft above) (*S. S. Sumner*).
- The vegetative cells are heat sensitive.
- Bacteria are inactive in acidic medium (pH < 4.6), but fish are not acidic:
  - As a result, usual fish preparation may kill vegetative *botulinum* bacteria, but will not address existing neurotoxins or spores.
  - If processed for storage (smoked, dried, salted) risk is increased.
- Therefore, avian botulism in Lake Erie appears an important matter for study in terms of public health and safety.
- First, the bacterial type is important to determine:
  - A, B, E are bad ones
  - C, D and F are of some question
  - G is not known to pose a risk
- Second, does the type vary – should track for level of certainty?
- Third, is there public exposure?
  - Direct – probably not of too much concern – the bacterium is ubiquitous
  - Indirect
- Either way, the knowledge gained by the investigations will have an important impact on the public:
  - Either to dissuade fears
  - Or to guide for safer enjoyment of Lake Erie

### **Botulism in Humans – Conclusions:**

- Botulism poisoning is rare.
- Botulism poisoning is deadly, but if discovered in time is no longer highly fatal.
- Only certain types of botulism seem to effect humans, but there remain questions in this regard.
- Avian Botulism in Lake Erie may have a human health impact, and warrants future study of:
  - types
  - fish
  - food chain issues, other than fish
- Avian botulism in Lake Erie may be studied in relative safety given the:
  - likely bacterial types
  - availability of pentavalent vaccine
  - medical treatments for the disease
- Avian botulism in Lake Erie should not be ignored because of the potential health risks it may entail:
  - Particularly, if any, for fisherman
  - Possible but low likelihood for the public
  - The certain health risk for the birds
  - Possible association with fish kills

## ***References:***

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E. L. Andress - [http://edis.ifas.ufl.edu/BODY\\_FS001](http://edis.ifas.ufl.edu/BODY_FS001)



## Conceptual Model of Type E Botulism in Lake Erie

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USGS National Wildlife Health Center

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## NWHC Role

- Diagnostic services
  - Federal, State, local agencies
- Education, Outreach
- Research – Salton Sea, ND
  - Persistence of spores in ecosystems
  - Environmental correlations of outbreaks
  - Presence of bacteria and toxin in fish
  - Risk evaluation
  - Control methods
  - Diagnostic tools



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## Summary of Type E Botulism in the Great Lakes

Year	Lake	#	1° Species
• 1963-4	Michigan	12,000	Gulls, Loons
• 1976	Michigan	1000	Com Loons
• 1980-1	Michigan	160	RB Gulls
• 1980*	Huron	130	Gulls
• 1983	Michigan	580	Com Loons
• 1999-2001	Erie	25,000?	Merg, Gulls, Loons, Ducks
• 2000	Huron	1000	Loons
• 2001*	Michigan	50-500	RB Gull

\* Other diseases present also: Type C bot, Salmonellosis, undetermined.  
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## Findings – Type E in Great Lakes

- 1960s: *C. botulinum* E in all Great Lakes
  - 1980-3: Pre-formed type E toxin in sick and dead birds
- Type E toxin in dead fish  
Both types C & E toxin in dead birds  
Carcass-maggot cycle not necessary



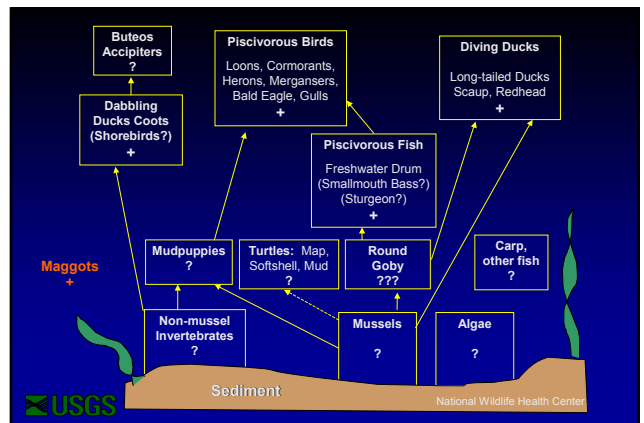
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## Findings cont.

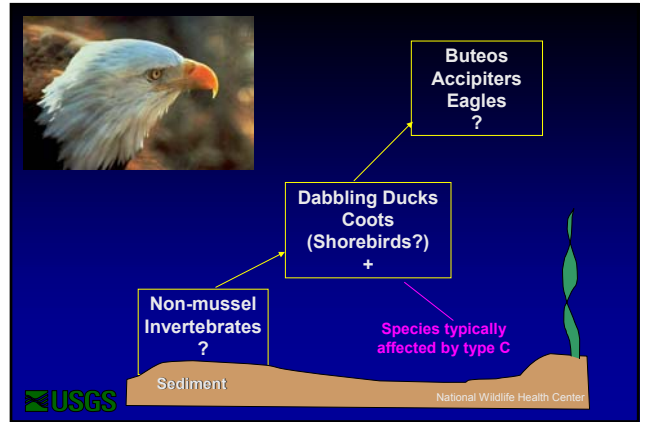
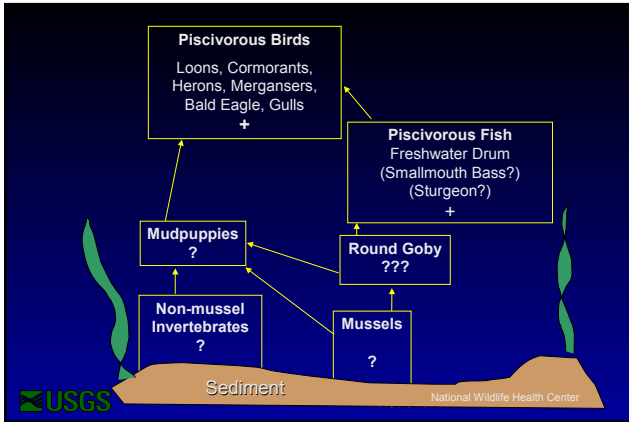
- 1999-2002
  - Documentation of type E toxin in many birds
  - Demonstration of toxin in flesh of sick fish
  - Demonstration of toxin in mussels from dead bird GI tract



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## Research Areas

- Spatiotemporal Distribution of Type E
- Non-Avian Mortality
- Algae
- Environmental Correlates
- Population Effects

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## Spatiotemporal Distribution of Outbreaks of Type E

- Different scales
  - Global, regional, local
  - Decades, annual, intra-annual
- Analyze data to look for cycles, trends
  - Need large amounts of data
- Environmental Correlates
  - More on this later

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## Spatiotemporal Distribution of *C. botulinum* Type E

- **Vegetative cells**
  - Where found, when active?
- **Spores**
  - What conditions lead to formation, activation?
- **Toxin**
  - Where and when produced?

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## Where is the toxin being produced?

- Under what conditions?
- Where in the system?
  - Substrate?
    - Under mats of dead algae?
    - Dead animals?
  - Live mussels or other invertebrates?
  - GI tract of mussel/invertebrate eaters?
  - GI tract of fish eaters, scavengers?

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## Non-Avian Mortality

Why are turtles, mudpuppies, and fish dying?

Sudden temperature changes

Low dissolved O<sub>2</sub> from algal die-offs? Disease?

Nutritional stress? Algal toxins?

Toxins? Spawning stress?



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## Algae

- Algal toxin production?

- When, where, why?

- Fish kills? Bird kills?

- Synergistic effects with botulism?

- Low dissolved O<sub>2</sub> from blooms, die-offs?

- Fish, invertebrate kills?

- Activation of *C. botulinum* spores?



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## Environmental Correlates

Temperature  
Rainfall  
Turnovers



pH  
Salinity  
Nitrates  
Phosphates

Changes in  
plant distribution?

sewage treatment facilities  
industrial effluent  
other anthropogenic factors?

Sediment changes?



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## Population Effects

- Red-Breasted Mergansers

- 8000?

- Common Loons

- 2500?

- Gulls

- Long-Tail Ducks

- Scaup

- Shorebirds



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## Control Options

- Carcass pick up

- Rehabilitation

- Vaccination?

- ???



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## Where Do We Go From Here?



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## A Project to Study the Prevalence of Botulism in Fish in the Lower Great Lakes

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**Co-Principal Investigator:** Dr. Rod Getchell

**Affiliation:** Dept. of Microbiology and Immunology,  
College of Veterinary Medicine,  
Cornell University, Ithaca, New York

### OBJECTIVE:

This project will focus on the role of fish in the recent documented outbreaks of botulism in waterfowl and the suspect botulism in fish in the Lower Great Lakes. The specific objectives of the project will be to determine the prevalence of *Clostridium botulinum* in apparently healthy, moribund, and dead fish in areas of confirmed outbreaks of avian botulism and unaffected areas within the Lower Great Lakes. We will also quantify the amount of *C. botulinum* and toxin in carrier fish.

Four questions will be addressed in this work:

- (A) Is *C. botulinum* more likely to be present in the intestinal tract or tissues of healthy, moribund, or dead fish?
- (B) Is one species of fish more likely to carry *C. botulinum* than another?
- (C) Does *C. botulinum* toxin form in these fish ante- or post-mortem?
- (D) Are fish carrying *C. botulinum* associated with waterfowl mortality events?

### RESEARCH PLAN:

**Prevalence studies:** In a cooperative effort with the NYSDEC, we will collect fish from both Lake Erie and Lake Ontario. The focus will be on the collection of carp and round gobies, as they have been the most commonly effected fish species. Other species of fish will be examined on a case-by-case basis. Standard necropsies will be performed on all fish sampled. This will be done to assess the cause of any mortalities as well as assess what other pathogens are present in these populations. In addition, fish will be tested with a PCR test for *C. botulinum*. This is a sensitive test that is less expensive and time-consuming than the mouse bioassay.

The research effort will include scheduled collections of fish as well as sampling during active outbreaks of botulism in waterfowl. Apparently healthy, moribund, and recently dead fish will be collected. Collection sites will include those locations where documented or suspect outbreaks of botulism have occurred on Lake Erie and Lake Ontario.

**PCR diagnostics:** A PCR technique to identify specific *C. botulinum* genes has been developed by several research teams. The presence of the gene is an indication of the presence of the *C. botulinum* bacterium. Our focus will be on the gene for toxin type E, which is the most common botulism toxin found in fish and the toxin identified in the waterfowl mortalities in 2000. The presence of *C. botulinum*, as indicated by the PCR test for type E toxin gene will be determined from fish intestine samples as well as selected organs such as liver. In those samples

that test positive, we will perform quantitative real-time PCR to enumerate the number of bacteria present.

***C. botulinum* toxin analysis:** For those fish samples that are PCR-positive, we will ship frozen sub-samples of intestinal contents to the Veterinary Diagnostic Laboratory at the University of Pennsylvania's New Bolton Center to test for pre-formed *botulinum* toxin type E by the mouse bioassay method.

## **BACKGROUND:**

Botulism was documented in waterfowl during several mass mortality events on Lake Erie in 1999, 2000, and 2001. Concurrent or preceding these outbreaks were mortalities in fish species such as carp, alewives, gizzard shad, drum, stonecat, and round gobies. One unconfirmed theory as to why the fish were dying was that storm-induced turnover of the lake may have brought up cold, less-oxygenated water that precipitated the fish kills, but no definitive diagnoses have been reached. Temperature intolerance is known to kill gizzard shad. Historically low water levels and algal blooms of *Microcystis* and *Cladophora* were also suggested as possible causes of anoxia and subsequent fish kills. Type E *C. botulinum* toxin developing in dead, rotting fish is well documented, but species of waterfowl that were dying in these outbreaks, such as common loons and mergansers, are not known to feed on dead fish. They feed mainly on live fish raising the question about whether live fish may carry the deadly toxin.

The Canadian Cooperative Wildlife Health Centre has an excellent discussion of this issue on their website: <<http://wildlife.usask.ca/bookhtml/botulism/botulisme.htm>>. The following three paragraphs are from this site entitled, "Ecology of Type E Botulism in Canadian Wild Birds."

Very little is known about the ecology of Type E botulism. It is known that the spores of *Clostridium botulinum* Type E are abundant in the water of many Canadian lakes and that the spores also can readily be found in the gills and digestive tracts of fish from such lakes. These spores, themselves, are harmless. Type E botulism occurs only under conditions when these spores grow and produce toxin. The ecological role of the bacterium appears to be that of a decomposer - a bacterium of putrefaction. The bacterium will grow only in a rich nutrient substrate that is free of oxygen. Fish that die for any reason and that contain the bacterial spores in their tissues are suitable substrate for growth and toxin production by the bacterium.

It seems clear that the fish-eating wild birds that have died of Type E botulism have become poisoned from eating fish that contain the toxin. It is not clear exactly how this happens. Birds such as loons and mergansers normally capture and eat only live fish. Yet, *C. botulinum* Type E should not grow and produce toxin in living fish. It may be that there are circumstances under which toxin is produced in the tissues of live, possibly dying, fish, possibly within their digestive tracts. Alternatively, it may be that the fish captured alive and eaten by the birds had themselves fed on some source of Type E toxin. Thus, it would be the toxin in the digestive tracts of the live fish that was the source of toxin for the birds in these outbreaks. It is even possible that the live fish captured by the birds were partially paralysed by the Type E toxin they had recently eaten and thus were



particularly easy prey for the birds. This might account for preferential feeding on toxin-containing fish by the affected birds.

It seems likely that there are outbreaks of Type E Botulism only when a variety of particular ecological factors occur simultaneously. There must be toxin produced in food material eaten by fish, and those fish must then be eaten by birds. The two outbreaks in Canada and the United States occurred in the fall when fish-eating birds congregate in favourable feeding habitat during fall migration.

Obviously, there are a number of questions about the ecology of type E botulism and what the causal factors are that lead to outbreaks of type E botulism. More specifically, until we understand the circumstances under which fish-eating birds can become intoxicated from eating live or moribund fish we will not be able to mitigate or prevent outbreaks of type E botulism in wild birds.

**Botulism in Lake Erie  
Workshop Proceedings:**

*Appendices*

# Botulism in Lake Erie Workshop

February 28, 2002

## Final Agenda:

8:30 - Registration (*Coffee & Refreshments*)

9:00 - Welcome/Introductions

## Morning Presentations:

- Dr. Mike Campbell, Mercyhurst College – Re-emergence of Avian Botulism Type E in the North American Great Lakes
- Pennsylvania – Fish and Wildlife Update: Dr. Mike Campbell, Mercyhurst College, Eric Obert, PA Sea Grant
- New York – Waterfowl and Fish Mortalities: Ken Roblee, Senior Wildlife Biologist, Bill Culligan, Supervising Aquatic Biologist - NYSDEC

## *Coffee Break*

- Canadian Update – Jeff Robinson, Canadian Wildlife Service, Dr. Doug Campbell, CCWHC, University at Guelph
- Ward Stone, NYSDEC Pathologist – Botulism in Fish and Wildlife in NYS Waters of Lake Erie

12:00-1:00 – Lunch (*Provided*)

## Afternoon Presentations:

- Botulism in Humans – Dr. John Lyons, M.D., Erie, Pennsylvania
- Conceptual Model of Type E Botulism in Lake Erie – Dr. Grace McLaughlin, National Wildlife Health Center, USGS
- Cornell Botulism Research Project – Dr. Rod Getchell, Aquatic Animal Health Program, Cornell University

Overview of Research Agenda

## *Coffee Break*

2:45 - Break-Outs to Formulate Research Priorities

3:30 - Group Discussion

4:30 - Wrap-Up & Next Steps

5:00 - Adjourn

## **Botulism in Lake Erie 2002 – Work Group Breakouts**

### **Group 1 – Fish Group**

#### **Top Priorities:**

- Framework – Hypothesis to test – experiment design?  
    Lab and Field – cause and effect
- Water Level effects
- West/Central/East Basin differences
- Location of anaerobic conditions
- Dose Response
- Fish Workshop and Mussels
- GIS Database (whole lake)
- Oxygen Monitoring
- Long Term Continuous Monitoring
- Zebra/Quagga Feeding Habits  
    Bacteria feeding/sediment oxygen
- European Literature Review
- Special Relationships with Gobies and Fish
- Microbial community – Other bacteria, etc.  
    and influence of low water

### **Group 2 – Education and Outreach Group**

In regards to Dr. Lyons' presentation:

- More information is needed on proper way to cook fish.
- Risk levels – range of toxin?
- Smoking fish by individuals - proper way, especially if found in more popular fish species.
- Consistent guidelines on fish consumption.
- Health warnings – how long before wear off after botulism outbreak?
- Need to evaluate risk vs. cost to industry.

Standard information – is it possible?

- Preparing fish – so many different ways used.
- Experimentation is needed to develop dosage.
- Risk with not properly handled fish has always been a problem.
- Level needed for fish botulism – experiment study needed.
  
- Important to know – can fish be made sick by botulism, or are they the carrier?
- Are symptoms of all sick fish botulism?
- More testing needs to be done, especially on sheepshead.
- Need to get word out not to cook sick looking fish and birds.
- 1/100 billion of dose – does it have an effect on public water systems or swimming?
- Pooling of bacterial levels – needs to be defined if this is possible.
- Need for focus – study what are the risks?

- Sample *Cladophora* for botulism.
- Great Lakes Fishery Commission looking for projects on botulism.
- Agencies need to know what others are doing – more networking needed.
- How do we create a network?
  - What are other states doing?
- What is going on in the lake – is something promoting it?
- Lake Levels – predictive?
- Lady bugs – any impact on system changing?

### **Important issues:**

- What dosage produces human risk?
- Standardize message to public:
  - Level of dosage in fish?
  - What are the risks in improper cooking?
- Symptoms of botulism in humans needs to be defined:
  - Will education make the public look for it?
  - Send information to doctors making them aware of botulism.
  - What type of botulism are we dealing with?
  - Type E in Lake Erie - Does the public need to be concerned about type?

### **Group 3 - Wildlife-Bird Group**

#### **Research Needs – High Priority**

- Mussels and other potential sources of toxin
- Better diagnostics
- Population effects
- Aerial surveys
- Standardized protocol for necropsy samples
- Standardized data collection and reporting from outbreaks
- Distribution of spores, vegetative cells, toxin
- Synergisms with algal and other toxins, infectious agents, stress
- Environmental profiles of outbreak sites
- Lake Ontario?
- Fast response teams
- Coordinator/Coordination

#### **Research Needs – Medium Priority**

- Do loons eat dead and sick fish?
- Feeding trials with gulls – does fasting and then ad libitum feeding set up conditions allowing toxin to be produced in the bird guts?
- Model – risk analysis
- Baltic Sea

#### **Research Needs – Lower Priority**

- Testing healthy birds for toxin
- Species susceptibilities

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**February 28, 2002**

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## Botulism in Lake Erie Workshop – Evaluation Results

### 1.) Do you think the workshop achieved its overall goal of sharing information, developing a research agenda and providing networking opportunities?

Yes 87 No 0 Uncertain 1

#### Comments:

- Sharing information – good. Research agenda – need better focus, more time. Networking – good.
- Excellent for all of the above.
- Information sharing, networking was excellent; research agenda? – time will tell.
- Good overview of issues, but should have also addressed some primary meeting objectives.
- The networking is really a very positive opportunity.
- Very beneficial.

### 2.) Were the presentations, breakout sessions and summary session effective/worthwhile?

Yes 86 No 0 Uncertain 1

#### Comments:

- Presentations – good. Breakout (fish) – not very effective.
- Excellent talks, all interesting, excellent mix of information. Couldn't have been better in terms of the choice of speakers.
- Presentations excellent; breakouts supportive; summary, okay.
- Generated good discussion – maybe reassemble sub-groups periodically throughout the year to further discuss topic and research.
- Fishing concerns.
- Although some were of limited use. Hard to comprehend many of the data slides. Couldn't see them.
- Need to keep people on time and have them stick to their talk topics rather than summarize day's talks.
- Breakout sections could be longer.
- Very nice overviews – good diversity in presentations.
- Very informative.
- Keep speakers on schedule, all presentations are good, but suffered from running on.

### 3.) Which portion(s) of the workshop did you find the most informative and interesting?

#### Comments:

- Dr. Mike Campbell, Dr. John Lyons.
- Dr. Mike Campbell, Dr. John Lyons – excellent overview and very informative.
- Descriptions of human impacts, concerns.
- Need more time to focus on questions to be addressed.
- Breakout workshop.
- Botulism in Human Health presentation.

- Most of the workshop was very interesting.
- Dr. Campbell; Dr. Lyons.
- Most of them.
- Mike Campbell’s summary, Dr. Lyons excellent. Dr. McLauhlin’s flow chart very helpful.
- Wildlife update NY and PA.
- Human health and risk assessment talk – *i.e.* put problem into real perspective as regards to human health. Conceptual model talk.
- Talks, group discussion.
- Botulism in Humans.
- Presentations and data.
- Mike Campbell’s background. Health talk (Dr. Lyons).
- Everything!
- Ward Stone; Dr. Lyons; Bill Culligan.
- Fish and human health.
- Presentations.
- Dr. Mike Campbell and Dr. John Lyons.
- The overall organization of this workshop was excellent.
- Talks by Culligan and Stone.
- Presentations.
- Dr. Campbell - history and background. Dr. Lyons - Botulism in Humans.
- Botulism in Humans.
- Dr. Stone and Dr. Campbell.
- Dr. Lyons.
- Human Health Concerns and Dr. Mike Campbell.
- Information specific to botulism, species affected and “links” in transmission.
- The NYS research, results and data presentations.

**4.) Was the conference well organized?**

Yes **88** No **0** Uncertain **0**

Comments:

- Very well organized, no wasted time.
- Great job!
- Excellent.
- Helen did a great job!
- Excellent job – Helen *et.al.* Thank you very much.

**5.) Were the facilities/food suitable?**

Yes **87** No **0** Uncertain **0**

Comments:

- Outstanding – nice to have elbow room to write!
- Very good food!
- But no fish!!!

- Great lunch!
- Great lunch!
- Very good.
- Excellent.

**6.) Would you be interested in attending a follow-up workshop on this topic in the future?**

Yes    **84**

No    **0**

Uncertain    **4**

Comments:

- Perhaps next year.
- But focus on accomplishments regarding research issues.
- Absolutely.
- Needs to be at least periodically re-visited unless “fixed.”
- Would like to hear more about health and public policy needs.
- How can citizens help?
- Absolutely!

**7.) Please provide any additional suggestions or topic areas that may prove beneficial to organizers and researchers involved with this project?**

- Perhaps fewer presentations and more time allocated to developing approaches to address specific questions. We know there is a problem (dead birds, fish). What should we do now? Focus on experimental design to get to the cause. Thanks so much for organizing such an informative workshop!
- Issues related to recreational opportunities and critter disposal. Is it safe to swim/wade etc. in areas with large quantities of fish carcasses? What are impacts to recreational opportunities like swimming, fishing, etc.?
- Bob Beltran, USEPA Region 5 GLNPO, has working hypothesis on whole picture, check with him.
- Not enough breakout time.
- With resources of course. I am able to include information to coastal and river residents and shoreline anglers.
- Maybe more information on analytical aspects. Modeling efforts?
- This workshop has obviously been valuable and productive in focusing research and awareness on Type E botulism. Sea Grant truly did an outstanding job with this!
- Thank you.
- Perhaps make the proceedings available on a CD.