





# **Botulism in Lake Erie Workshop Proceedings**

Co-Sponsored by

New York Sea Grant Ohio Sea Grant Pennsylvania Sea Grant



# April 3, 2003 Buffalo, New York

Proceedings by Helen M. Domske, November 2003

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# **Other Partners:**

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#### **Publication Production:**

Ellen George, New York Sea Grant November 2003

## **Introduction – Workshop Objectives**

In response to fish and bird die-offs along the shores of Lake Erie in 2002, the extension staff of New York, Pennsylvania and Ohio Sea Grant wanted to continue their efforts to deal with botulism outbreaks that have occurred since 1999. To understand the extent of the die-offs, gather scientific information and explore the ecological impacts of these botulism outbreaks the Sea Grant programs agreed to once again organize a workshop that would get a binational group of agency representatives, researchers and stakeholders together.

Building upon the success of the first two conferences on botulism that were held in 2001 and 2002, New York, Pennsylvania and Ohio Sea Grant worked together to co-sponsor a third workshop. On April 3, 2003, the third workshop on Botulism in Lake Erie was held in Buffalo, New York, to provide updates on die-offs and an overview of current research efforts. The workshop brought together 70 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 hear presentations from researchers working on the botulism issue.

The first conference was held in January 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. 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. On February 28, 2002, a workshop on Botulism in Lake Erie was held in Buffalo, New York. Ohio Sea Grant joined the effort for the workshop that involved 100 participants. The goal of the workshop was to share information on outbreaks and to develop a research agenda. Copies of the proceedings from both conferences can be found at: <a href="https://www.nyseagrant.org/botulism/">www.nyseagrant.org/botulism/</a>

To demonstrate a research commitment to this important environmental issue, New York Sea Grant made botulism a focus area for their 2003 call for funded research proposals. As a special focus area, botulism research was assigned a high level of support and a number of research proposals were received by the New York Sea Grant Institute. We expect that the funded projects will help to answer some of the questions related to the botulism outbreaks of the past four years.

#### Lake Erie Botulism List-Serve

Realizing the importance of quick notification and advanced networking opportunities, the Sea Grant programs developed an electronic list-serve related to botulism outbreaks around Lake Erie. The e-mail listing, under the supervision of Eric Obert, sends out reports on die-offs and observations, as well as answers question ns and provides information. Members of the e-mail group include researchers, fishery and wildlife biologists, resource managers, agency representatives and concerned stakeholders. If you wish to join the list-serve, please send a message to ecol@mail.psu.edu and Eric Obert will add your name to this list.

#### **Botulism in Lake Erie - Overview**

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. Die-offs continued in 2002, with mortalities seen in benthic fishes and thousands of mudpuppies, cryptic aquatic amphibians. Bird die-offs during the 2002 fall migration were also quite large, with estimates of 17,000 dead birds in the New York waters of the lake.

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.

# 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

# Pennsylvania Update

# **Botulism in Lake Erie**

Bob Wellington – Erie County Health Department Mike Mumau – Presque Isle State Park

#### Near shore water temperature @ Erie Waterworks Intake



July 11, 2002: Fish Die-off



Blue-green algae bloom, Horseshoe Pond, Presque Isle State Park



#### Collection Procedures & Data for Dead Fish Presque Isle State Park

- Nearly 2000 fish collected on Presque Isle during summer 2002
- July 10 & 11 446 dead fish collected
- Collected regularly on Monday, Wednesday and Friday. Also early morning on weekends
- Collected by interns, placed in plastic bags, sealed and deposited in compacter
- Concentration found west of Beach 6

#### **Most Common Fish Species**

- Freshwater Drum
- Channel Catfish
- Small mouth Bass
- Common Carp
- Alewife
- Walleye
- White sucker
- Yellow Perch
- Bluegill

#### Collection Procedures & Data for Dead Birds, Presque Isle State Park

- Bird mortalities were reduced compared to 2001
- Ring-billed gulls, herring gulls in the summer
- Loons in the fall
- Cooperative agreement with Erie County Humane Society for disposal (incineration)
- Double bagged, then placed in freezer for temporary storage
- Park interns and maintenance staff work cooperatively

# Waterbird Mortality in New York Waters of Lakes Erie and Ontario Resulting from Type E Botulism

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

Botulism Observed in a Gull Colony

- The Bethlehem Steel gull colony near Buffalo contains approximately 10,000 pairs of nesting Ring-billed gulls.
- During a July 5, 2002 inspection of the colony, 946 dead gulls (adults and chicks) were counted.
- The following week approximately 3,000 dead gulls were observed.
- Type E botulism was diagnosed and mudpuppies were found in gull stomachs.

#### Lake Erie Botulism Mortality Surveys 100m Transect Survey Results by NYSDEC 10/15/02 – 1/09/03 Table 1, Section 1

Species	<b>Predicted Mortality</b>	Upper Conf.	Lower Conf.
Common Loon	2,042	3,447	637
Horned Grebe	273	482	64
Red Necked Grebe	36	84	0

#### Lake Erie Botulism Mortality Surveys 100m Transect Survey Results by NYSDEC 10/15/02 – 1/09/03 Table 1, Section 2

Species	<b>Predicted Mortality</b>	Upper Conf.	Lower Conf.
Wood Duck	18	54	0
Greater Scaup	128	213	43
Long-tailed Duck	12,616	20,808	4,424
Scoter	18	54	0
C. Merganser	18	54	0
R-b Merganser	839	1,405	273
Unidentified Merg.	383	649	117
Unidentified Duck	36	108	0

#### Lake Erie Botulism Mortality Surveys 100m Transect Survey Results by NYSDEC 10/15/02 – 1/09/03 Table 1, Section 3

Species	Predicted Mortality	Upper Conf.	Lower Conf.
Bonaparte's Gull	18	54	0
Ring-billed Gull	273	429	117
Herring Gull	474	767	181
Gr. Black-bkd. Gull	36	84	0
Unidentified Gull	91	203	0
TOTAL BIRDS*	17,301	27,566	7,036

\* Total from Table 1, sections 1, 2 & 3

# Lake Erie Waterbird Mortality New York Botulism Transect Survey Results

	2000	2001	2002
Common Loon	583	1,149	2,042
Horned Grebe	109	0	273
Long-tailed Duck	0	310	12,616
Red-breasted Merganser	2,479	91	839
Ring-billed Gull	1,714	510	273
TOTAL BIRDS	5,415	2,862	17,301

#### Lake Ontario Botulism Mortality Survey 100 M Transect Survey Results NYSDEC 2002

Species	Predicted Mortality	Upper Limit	Lower Limit
		95% Confidence	95% Confidence
Long-tailed Duck	675	1,336	0
Herring Gull	80	83	0
Ring-billed Gull	129	251	7
Greater Black-backed Gull	32	76	0
Double-crested Cormorant	32	76	0
Unknown Duck	16	48	0
Unknown Gull	64	25	3
Misc Unknown	16	48	0
TOTAL BIRDS	1,046	1,924	168



#### Niagara County Botulism Mortality Survey 100 M Transect Survey Results NYS DEC 2002

Species	Predicted Mortality	Upper Limit 95% Confidence	Lower limit 95% Confidence
Long-tailed Duck	700	1,169	231
Unknown Duck	17	50	0
Ring-billed Gull	100	201	0
Herring Gull	67	164	0
Greater Black-backed Gull	17	50	0
Unknown Gull	17	50	0
Double-crested Cormorant	17	50	0
Misc. Unknown	17	50	0
TOTAL BIRDS	950	1,565	335

#### 2002 in Summary

- Significant mortality observed at Bethlehem Steel gull colony.
- Loon, long-tailed duck and total bird mortality increased in Lake Erie.
- Botulism-caused mortality observed in gulls and long-tailed ducks in Lake Ontario.

# Long-Term Assessments of Warmwater Fish Stocks and Characteristics of Fish Kill Events in NY's Portion of Lake Erie

Don Einhouse NYS Department of Environmental Conservation Lake Erie Fisheries Unit

#### Common fish kills observed in eastern Lake Erie through the years

- March-April, Alewife & Gizzard Shad temperature stress
- May-June Smelt *Glugea* and spawning
- June-July Smallmouth Bass spawning & upwellings?
- June-August Warmwater Fish upwellings





































![](_page_20_Picture_0.jpeg)

![](_page_20_Figure_1.jpeg)

![](_page_21_Figure_0.jpeg)

# Smallmouth Bass Diets from Annual September Sampling

		Percent of stomachs with selected prey			
Year	Sample (n)	<u>crayfish</u>	<u>clupeids</u>	<u>smelt</u>	<u>goby</u>
1985 - 87	173	23.7 %	9.8 %	4.6 %	0.0 % Prior to Goby presence
1999	80	11.3 %	15.0 %	10.0 %	42.5 %
2000	170	4.1 %	1.2 %	2.4 %	89.4 %
2001	58	1.7 %	1.7 %	6.9 %	58.6 %
2002	22	13.6 %	4.5 %	0.0 %	81.8 %

![](_page_21_Picture_3.jpeg)

![](_page_22_Figure_0.jpeg)

#### NYS DEC - Lake Erie Unit Research Partnerships

- NYS DEC Wildlife Pathology Unit
- Cornell University Aquatic Animal Health Program
- SUNY Fredonia Biology Department

#### **Questions?**

- Where is the anaerobic environment in eastern Lake Erie?
- Some benthic fish species are conspicuous by their absence in fish kills, ex. suckers, yellow perch?
- We have experienced abundant mussel densities for over a decade, but botulism only recently?

# Botulism in Fish and Wildlife in NYS Waters of Lake Erie

Ward Stone – Pathologist, NYS Department of Environmental Conservation (Slide presentation – not available)

This presentation focused on the work being done by NYSDEC pathologist Ward Stone and his lab colleagues. He indicated that there were more than 7,000 submissions for botulism testing during the year. Type E botulism was diagnosed for the first time in 2002 and the toxin was found in sheepshead and smallmouth bass.

Regarding the birds that were examined, Stone indicated that the birds were not thin, and it appeared that these fish-eating birds looked like they drowned. He noted that round gobies and mudpuppies were found inside many of these birds. Some of the bird carcasses were scavenged by other mammals, which would seem to explain the opossum and 2 raccoons that were recovered and tested positive for botulism. He did mention that 1,100 loon carcasses were found over a small area, indicating the intensity of the die-off.

He also mentioned that shorebirds ate fly maggots and the larvae were in the gizzards which concentrated the toxin. Stone surmised that long-tailed ducks were eating quagga mussels and were probably picking up the type E toxin directly from the mussels.

Ward Stone discussed experiments that his lab worked on, including feeding pieces of loon livers that contained the type E toxin to round gobies. He stated that round gobies carry the toxin quite readily and are highly susceptible. Within 12 hours the gobies are usually dead, but can survive up to 24 hours.

Concerning the outbreak in Lake Ontario, Stone gave the following information on species impacted by type E: A ring-billed gull was collected at Fair Haven on July 16, 2002. A long-tail duck was found at Four Mile Creek, and a common goldeneye was collected at Fort Niagara on October 29<sup>th</sup>. A herring gull was collected at West Brighton, Pt. Breeze on November 4, 2002.

Stone also noted that his lab found 10 different types of botulism type E in specimens collected and also a variety of botulism types were found in the Lake Erie sediments.

2000	2001	2002	
106	303	434	Common Loon
11	5	128	Horned Grebe
2	29	19	Double-crested Cormorant
1	44	4,877	Long-tailed Duck
424	16	627	Red-breasted Merganser
70	58	151	Herring Gull
427	160	856	Ring-billed Gull
33	22	22	Great Black-backed Gull
0	23	2	Sanderling
13	3	22	Bonaparte's Gull
1,100	706	7,202	TOTAL

#### Found and Examined Type E Botulism Outbreak

# Type E Botulism in Fish-eating Birds

# **Ontario 2002**

## **Jeff Robinson**

**Canadian Wildlife Service** 

**Ontario Region, London** 

# G. Douglas Campbell and Ian K. Barker

**Canadian Cooperative Wildlife Health Centre** 

**Ontario Veterinary College** 

**University of Guelph** 

# What are we studying in Canada?

- Stomach examination of fish eating birds to determine food habits (ongoing, OMNR, Canadian Co-operative Wildlife Health Centre)
- Distribution of fish and bird mortality events (OMNR, CWS)
- Loon population analysis for Ontario breeding lakes (CWS)
- Experimental dosing of fish with Type E toxin to:
  - evaluate toxic dose
  - fish behavior relevant to consumption by fish-eating birds
  - tissue distribution

(R.D. Moccia - University of Guelph & graduate student)

![](_page_25_Figure_0.jpeg)

Case #	Date	Location	Species	Result	Stomach contents
W321.02	28 June	Mohawk Is.	Herring Gull	+	
W322.02	44	55	RB Gull	-	
W338.02	9 July	Pt. Pelee NP	RB Gull	-	
W360.02	14 July	66	RB Gull	+	
W386.02	2 August	Mohawk Is.	Caspian Tern	-	
W387.02	66	22	DC Cormorant	-	
W388.02	66	39	RB Gull	NT	
W415.02	4 Sept.	Pt. Dover	RB Gull	-	
W449.02	31 August	Long Pt.	Sanderling	-	
W450.02	"		DC Cormorant	-	unidentified fish bones
W451.02	66	99	Golden Ployer	+	
W503.02	15 October	Kincardine	Common Loon	-	unidentified fish bones
W504.02	66	55	Red Necked Grebe	+	round goby
W515.02	**	**	Common Loon	-	unidentified fish bones
W516.02	**	55	Red Breasted Merganser	+	unidentified fish bones
W517.02	**	93	Red Necked Grebe	-	empty
W518.02	66	99	Pied Grebe	-	empty
W519.02	66	55	RB Gull	-	unidentified fish bones. Physells spp., zn
W520.02	30 October	Lincoln	Ruddy Duck	-	
W521.02	31 October	Burlington	Long Tailed Duck	-	empty
W550.02	9 November	Hoover Pt	Common Loon	NT	unidentified fish bones, zm
W551.02	"	»	Red Breasted Merganser	NT	undentified fibil cones, and
W552.02	**	33	Horned Grebe	NT	
W553.02	**	33	Long Tailed Duck	NT	
W554.02	9 November	Quarrie Rd	Common Loon	NT	
W555.02	66		Long Tailed Duck	NT	
W556.02	0 November	Long Beach	Common Loon	+	
W557.02	"	""	Long Tailed Duck		
W561.02	9 November	Nickle Beach	Common Loon	NT	empty
W562.02	"	33	Horned Grahe	NT	empty
W562.02	66	**	Long Tailed Duck	NT	
W566.02	0 November	Long Pt	Common Loon	+	
W579.02	> 140 verificer	John Pearce PP	RB Gull	NT	
W580.02		Dt Bruce	PB Gull	NT	
W581.02		Dt Burwall DD	RB Gull	NT	
W582.02		Pt. Stanlay	PD Gull	NT	

		November 14, 20	002	
Long Point NWA			<u>Port Bruce</u> Bonaparte's Gull Harring or Ping billed Gull	26
Common Loon	720	30 per km of beach surveyed	Great Black-backed Gull	1
Gull spp.	254	10.6	Common Loon	7
Red-breasted merganser	43	1.8	Horned Grebe	3
Scaup spp.	7	0.3	Double-crested Cormorant	2
Long-tailed duck	4	0.2	Red-breasted Merganser	6
White-winged scoter	1		scaup species	1
Horned Grebe	1		Total	81(160/km
carp	100		100	01(100)101
Total	830(3	4/km)	Port Stanley	
			Bonaparte's Gull	16
Points west of Long Point NW	Ą		Ring-billed Gull	95
Port Burwell			Herring Gull	15
Bonaparte's Gull	60		Common Loon	3
Ring-billed Gull	76		Red-breasted Merganser	4
Herring Gull	11		Total	133(50/km
Herring or Ring-billed	31		10.00	100(00)100
Great Black-backed Gull	1		Rondeau Provinical Park(Octob	per 25, 2002)
Common Loon	11		Common Loon	82
Horned Grebe	1		Red-breasted Merganser	40
White-winged Scoter	1		Gull spp	4
Red-breasted Merg	8		Total	126(13/km
Greater Scaup	1			. 20(10/km
Total	201(5	0/km)		

![](_page_26_Figure_1.jpeg)

	Gizzard content (co	s analysis from 20 nfirmed and susp	ected)	ents
Empty	Common Loon Erie Nov 9 17	Common Loon Huron Oct 15 -	LT Duck Erie Oct 30 1	LT Duck Nov 9 1
Fish(no ID)	25	6	5	-
Perch(white and yellow)	5	-	-	-
Goby	1	-	-	-
Shad	1	-	-	-
Shiner	2	-	-	-
Zebra Mussels	2	1	18	3
Other items	5	-	1	-

![](_page_27_Figure_1.jpeg)

Comn	non Loor	1								
Year	Strat	um 1	Strat	um 2	Strat	um 3	Strat	um 4	Tota	l
1990	4856	1166	2346	636	4424	978				
1991	3458	929	4278	1030	5407	1476	18979	3890	32122	4386
1992	3192	813	2304	512	5407	1745	23511	4124	34414	4580
1993	3746	962	5520	1303	7374	1964	25846	5746	42485	6285
1994	2882	763	5013	1156	10815	2788	28239	5568	46948	6379
1995	3458	1544	6323	1712	7692	1998	24786	7002	42258	7637
1996	1773	657	2484	697	5838	1590	29929	6229	40024	6499
1997	3192	1100	5493	1003	14010	2890	39038	9200	61733	9758
1998	2660	786	5332	1203	8173	1423	28194	5585	44358	5940
1999	1419	452	7097	1394	9807	1857	28194	5654	46517	6129
2000	2305	731	5962	1510	11208	3062	24724	4970	44199	6074
2001	5047	1618	7593	1688	8406	1822	37303	6688	58349	7316
2002	1820	499	8517	1547	7005	1483	40339	7401	57680	7722

![](_page_28_Figure_1.jpeg)

# Environmental Parameters Associated with Outbreaks of Botulism in Eastern Lake Erie

Alicia Perez-Fuentetaja, Theodore Lee, Mark Clapsadl Biology Department, SUNY Fredonia

#### **Outline:**

- Overall view of two-year project
- Goals
- First field season
- Methods
- Field results
- Future work

#### **Project:** "Botulism Type E in Lake Erie: Ecology and Lower Food Web Transfer"

- Funding:
  - 2002-2003 US Fish and Wildlife Service
  - 2003-2004 US EPA/GLNPO
- Research team:
  - SUNY-Fredonia / Biology Department
  - NYDEC / Dunkirk Office (B. Culligan, D. Einhouse)

#### **Project Goals:**

- (1) To identify environmental conditions in Lake Erie associated with the presence of *Clostridium botulinum* type E.
- (2) To determine whether benthic food items (mussels, and other benthic organisms) contain the botulism bacterium in their tissues, becoming a food web link between sediment and fish.

#### Hypotheses to be tested:

<u>Assumption</u>: There are pockets of anaerobic conditions in the benthic ecosystem of Lake Erie's Eastern basin.

<u>Hypothesis</u>: Anaerobic sites provide habitat for *C. botulinum* type E, which multiply and infect (or are carried by) organisms that inhabit the lake sediment.

Assumption: Outbreaks of botulism are discrete events.

<u>Hypothesis</u>: There must be a set of environmental conditions necessary for the lysis of the *C*. *botulinum* type E bacteria and the release of the toxin.

![](_page_30_Picture_0.jpeg)

![](_page_30_Picture_1.jpeg)

### **Physico-Chemical Parameters:**

- 0.5 m above sediment.
- Multiparameter Meter YSI 556:
  - Temperature
  - Dissolved Oxygen
  - Conductivity
  - Salinity
  - Total Dissolved Solids
  - рН
  - Redox Potential

### Water Samples:

- 0.5 m above sediment
- Acrylic Alpha Bottle
- Parameters:
  - Nitrates
  - Phosphate
  - Chloride
  - Chlorophyll a

![](_page_31_Figure_18.jpeg)

![](_page_32_Figure_0.jpeg)

![](_page_32_Figure_1.jpeg)

![](_page_33_Figure_0.jpeg)

![](_page_33_Figure_1.jpeg)

![](_page_34_Figure_0.jpeg)

#### In Summary:

- One large algal bloom in June was correlated with decreased visibility and fish mortalities.
- Temperature increased and Dissolved Oxygen and pH decreased during the two outbreak events (June/July, August).
- Redox levels experienced a marked decrease from mid to late August, associated with an increase in Total Dissolved Solids and nitrates, and an outbreak of fish and bird mortalities.

#### **Data Being Processed:**

• Weather events during the season 2002 that may explain mixing of the water column and changes in Lake Erie conditions.

![](_page_35_Figure_0.jpeg)

# **Data Being Processed:**

• Analysis of tissue from benthic organisms, including quagga mussels, dipteran larvae, nematoda, amphipoda and mayfly larvae, as well as pseudofeces, to detect the toxin from *C. botulinum* type E using PCR.

#### Season 2003:

- Additional sampling sites along the coast.
- Monitoring of physico-chemical parameters following depth profiles.
- Li-cor light penetration measurements, including photosynthetically active radiation (PAR).

#### **Acknowledgements:**

- Captain and crew of the R/V Argo (DEC).
- Dean NSSPS, SUNY-Fredonia.
- William Culligan and Donald Einhouse, NYSDEC.
- Dr. Pamela Marshall, SUNY-Fredonia.
- Students Jessica Wuerstle and Daniel Sek.

![](_page_36_Picture_0.jpeg)

![](_page_36_Picture_1.jpeg)

![](_page_37_Picture_0.jpeg)

![](_page_37_Picture_1.jpeg)

• Fish intestinal contents, liver, and blood are processed to concentrate their DNA.

Sea Grant

• This multi-step procedure provides purified DNA that can be assayed for the presence of the *C. botulinum* Type E toxin gene.

![](_page_37_Picture_4.jpeg)

ORNELL

![](_page_38_Picture_0.jpeg)

![](_page_38_Picture_1.jpeg)

See Crant 2002 Fi	ish Collect	ion Totals
Lake E	rie Lake Ontario	
Spring 265	8	
Summer 176	71	
Fall 186	30	
Smallmouth Bass	286	
Freshwater Drum	a 208	
Round Goby	148	
Yellow Perch	24	
Alewife	20	
Brown Bullhead	11	
Other species	41	and the second

![](_page_39_Picture_1.jpeg)

![](_page_40_Figure_0.jpeg)

![](_page_40_Picture_1.jpeg)

# **Comparative Toxicity of Type E Botulinum Toxin in Several Lake Erie Fish Species**

R.D. Moccia, A.Yule, I.K. Barker and J. Austin University of Guelph and Health Canada Sponsored by Environment Canada

### THE PARADOX:

- How to explain the 'Fish' pathway of toxin ingestion in species like loons and mergansers which feed nearly exclusively on 'live' fish.
- Lack of apparent correlation between fish botulism epizootics and avian mortality patterns.

# THE QUESTIONS:

- Are 'living' fish a reasonable transport mechanism by which BoNT (*Clostridium botulinum neurotoxin*) moves from point of origin to the bird?
- Is it plausible that live, but moribund, fish are selective prey species?
- Can 'live' fish harbour bird-lethal doses of BoNT?
- Do fish display a varying 'tolerance' to BoNT related to phylogeny, natural life history or primary feeding habitat? eg. benthic versus pelagic species
- Is there a potential human health hazard to people who consume BoNT laden fish?

# Setting Out to Answer One Very Small Piece of the Botulism Ecology Puzzle

#### **RESEARCH OBJECTIVES:**

- 1. Develop fish botulism exposure model ("FBEM")
- 2. Test comparative sensitivity and temporal aspects of clinical intoxication for several candidate fish species
- 3. Develop Dose-Response Model
- 4. Determine Toxin titres in BoNT Mortalities

"FBEM"

![](_page_41_Picture_18.jpeg)

Trout, goby, mudpuppy, perch ...

#### **GENERAL APPROACH:**

- Produce and encapsulate BoNT-E at known Dosages (eg. 400 MLD)
- Intubate test fish
- Map the temporal aspects and clinical progression of intoxication
- Assay mortalities for BoNT titres
- Calculate expected toxin transport to bird

#### **TOXIN PRODUCTION METHODS:**

#### Method used to prepare the BoNT

- *C. botulinum* type E strain
- grown in tryptone-peptone-glucose yeast extract broth at 25°C
- atmosphere of 10% H2, 80% N2, 10% CO2 for 4 days
- harvested by centrifuge
- concentration of type E neurotoxin assayed by mouse bioassay
- toxicity neutralized by monospecific type E antiserum: therefore, toxic effect was only due to type E neurotoxin (no other toxins in preparation)

#### **CAPSULE PREPARATION:**

- starch filler (60mg) was added to each gelatin capsule
- 100 ul of culture supernatant (containing 400MLD) was quickly added to gelatin capsule
- filled capsules immediately frozen

#### **CONTROLS:**

- possible effects of starch filler on toxicity was checked
- gelatin phosphate and buffer and starch filler mix had no effect

#### PRELIMINARY MORBIDITY SIGNS IN RAINBOW TROUT (400MLD)

TIME	COMMENT
0	- fish intubated
Х	- time of capsule degeneration and toxin release
XX	- normal behavior
у	- restless, agitated, increased swimming behavior
уу	<ul> <li>first equilibrium loss (loss gradually increases), postural changes, righting ability gradually lost, progressive immobility</li> </ul>
ууу	- sporadic swimming (fish upside down and on side), no fin coordination
or	righting ability, irregular 'breaching' behavior noted
Z	- complete loss of any motor function
ZZ	- loss of respiratory reflex/death

Once Temporal Response Pattern Has Been Establish for the Various Species, Their Sensitivities and Onset of Clinical Signs Can Be Compared.

![](_page_43_Figure_1.jpeg)

# PRELIMINARY RESULTS-IMPLICATIONS TO BIRD PREDATORS:

1. LOSS OF EQUILIBRIUM: In a natural setting, fish showing equilibrium loss could represent "easy" prey for live-fish eating birds. Thus, such birds could be targeting intoxicated fish due to their abnormal behavior.

![](_page_43_Picture_4.jpeg)

2. BREACHING BEHAVIOR: In a natural setting, fish showing breaching behavior would present an "easy" target for predators and maximize BoNT ingestion.

![](_page_44_Picture_1.jpeg)

#### **Alternate Species**

Once the "FBEM" is established in the rainbow trout the following species will be tested:

- Round Goby
- Mudpuppy
- Yellow Perch
- Walleye
- others

#### **EARLY THOUGHTS:**

- Prolonged moribund state with high BoNT exposure
- Progressive clinical stages with respiratory reflex lost late in sequence
- Behavioural response may 'target' predators
- Live fish may be significant vector for toxin
- No idea yet of inter-species sensitivity

# Type E Botulism in the Great Lakes Conference Overview

Grace McLaughlin USGS National Wildlife Health Center

#### *Clostridium botulinum* type E

- Spores found primarily in cold water environments (Great Lakes, Baltic Sea)
- Toxin production NOT dependent on a bacteriophage
- Primarily afflicts fish and fish-eating birds
- Causes disease in humans

#### Type E botulism outbreaks in the Great Lakes

Year	Lake_	<u>Number</u>	<u>Species</u>
1963-1964	Michigan	>12,000	Gulls, Loons
1976-1983	Michigan, Huron	>1800	Gulls, Loons
1998-2002	Huron, Michigan	~2500	Mergansers, Gulls, Loons
1999-2001	Erie	>25,000	Mergansers, Gulls, Loons
2002	Erie	>25,000	Long-tail ducks, Gulls,
			Loons, Mergansers,
			Cormorants

![](_page_45_Figure_9.jpeg)

#### Locations of other type E outbreaks in birds

- Canche Estuary, France 1996 5-10,000 gulls
- Salton Sea (California) isolated cases pelicans, cormorants
- Alaska isolated cases

#### **Epizootiology of Avian Botulism**

![](_page_46_Picture_1.jpeg)

#### Factors involved in avian botulism outbreaks

- Toxigenic bacteria
- Appropriate environmental conditions
- Proper substrate
- Availability of toxin to birds

![](_page_46_Figure_7.jpeg)

#### **Research Needs**

- Spatiotemporal distribution of type E spores and cells in Great Lake sediments and fish
- Sources of cells and toxin for fish and birds
- Environmental correlates
- Population effects in birds
- Non-avian mortality

#### Pennsylvania Update 2002

Bob Wellington and Mike Mumau

- March, May dead alewives, turtles
- June dead gobies & mudpuppies less algae than in past years
- July rapid temperature drop  $75^{\circ} \longrightarrow 50^{\circ}$ F in 2 days
- Monitored water temperature  $-70^{\circ}$ -80°F in July-September, 50°F November
- Invertebrate mortality
- Several invasive species
  - Alewives, gobies
  - Algae (*Cladophora*? If so, long ago.)
  - Invertebrates (mussels, amphipods)
- Blue-green algae blooms
- $\sim 2000$  fish collected  $\sim 10$  species
  - July 10-11 = 446 (22.3% of total)
- Fewer dead birds than 2001
- Gulls in Summer
- Loons in Fall
- Invertebrates could be substrate for significant toxin production.

#### New York Update 2002

Don Einhouse

- Fish mortalities effects on trends, abundance
  - March-April: alewives, gizzard shad temperature stress
  - May-June: smelt spawning, *Glugea*
  - June-July: smallmouth bass spawning? upwelling?
  - June-August: warmwater species., upwelling
- June 50% gobies
- July 44% sheepshead
  - Also thousands of mudpuppies
- September mortality: 81% sheepshead
  - Only 4% net (live) samples, yellow perch 40%
  - Why don't yellow perch and gizzard shad die?
- Smallmouth bass survival has not changed with Botulism E
- Forage fish composition
- Gobies
  - 1994 Cleveland
  - 1995 inconclusive numbers
  - 1996 moving East and West in Central basin, into West
  - 1997 into eastern basin
  - 1999 well into NY waters, but low numbers
  - 2000 declining in western basin, including East
  - 2001 huge number in eastern basin
  - 2002 declining in abundance

#### **Sturgeon Mortality**

- Rare to find on beach pre-2000
  - 5 in 1996 upwelling
- >25 in 2001
  - Corresponded with high goby numbers
- Fewer in 2002
  - also fewer gobies in trawls

#### **Changes in Food Web**

- Shift to gobies post 1998
- Sheepshead eat mussels

#### **Questions:**

- Where is anaerobic environment?
  - In anything that dies. Pockets in substrate.
- Benthic fish species absent from kills why?
  - Different susceptibility?
- Role of mussels?
- How to determine botulism E mortality?

#### NY Avian Mortality

#### Ken Roblee

- >3000 Gulls in June & July
  - Concurrent with mudpuppy mortality
- October-December estimated >17,000 birds
  - >12,600 long-tailed ducks
  - >2000 loons
  - >1000 mergansers

# NY Pathological Investigations

Ward Stone

- 7000+ submissions for botulism in 2003
- First diagnosis of type E 2000
- Food habits: gobies, mudpuppies
- Sheepshead, bass
  - Moribund fish
- Feeding experiments
  - Gobies gull liver
- Toxin identified in gizzard contents
  - Gulls in summer mudpuppies
  - Mergansers mudpuppies, gobies
  - Shorebirds fly larvae from carcasses
  - Long-tailed ducks mussels, gobies
- Few puddle ducks
- Scaup undetermined cause of death

#### **New York Mortality – bird pickup numbers**

	2000	2001	2002	Totals
Totals	1100	706	7202	9008
Long-tailed ducks	1	44	4877	4913
Gulls	543	228	>1030	>1800
Red-breasted Merganser	424	16	627	1067
Loons	106	303	434	843

Birds still being collected from 2002-2003 Estimates of dead birds much higher

#### **Food Habits**

- Gobies:
  - Loons: 56-61%
  - Horned grebe: 54%
  - Long-tailed Ducks: 60% of 169
- Mudpupppies
  - Gulls 17-82%
  - Mergansers: 20-40%

#### **Feeding Experiments – New York**

- Feeding livers assuming problem is botulism toxin, some negative samples used also
- Gobies, Centrarchids, fatheads susceptible
- Yellow Perch and Painted Turtles show some resistance
  - Yellow Perch impaired up to 1 week
  - Altered swimming ability
  - Change in activity patterns

#### **Botulism Type E Genetics**

- 10 different type E strains
  - Fish, sediments
- How do these compare to?
  - Other years
  - Huron
  - Michigan
  - Superior

- Mediterranean, Baltic
- France
- Alaska

#### Canadian Update

Jeff Robinson

- Mortality distribution
- Food habits
- Loon population data
- Experimental dosing
- Loons
  - Believe Stratum 4 breeders
  - Population 19,000 to 40,000 breeding pairs
- Several Lake Erie events
  - June, July, August: gulls, terns, cormorants
  - September: gulls, cormorants
  - October: gulls
  - Late October & November: Common Loons, Long-tailed Ducks, Red-breasted Mergansers
- Lake Huron October
  - Grebes, mergansers, Common Loons
  - Goderich and Port Elgin
- Ontario (not confirmed)

#### Environmental Parameters Associated with Outbreaks of Botulism in Lake Erie

Alicia Perez-Fuentetaja, Ted Lee, Mark Clapsadl

Identify environmental conditions in Lake Erie associated with presence of *Clostridium botulinum* type E.

Are there areas of low oxygen where the bacteria are found?

10m inshore, 20m offshore depths.

Triplicate samples

Physico-chemical parameters 0.5 m above sediment.

- Algal bloom at time of June-July outbreak
- Oxygen, Redox, pH drop in August
  - Rocke, Samuel JWM
- Weather event in August
  - strong winds, wave action mixing
- Sample processing in progress

#### **Botulism: Atypical Pathogenesis in Other Species**

Dr. Robert H. Whitlock, New Bolton Center, University of Pennsylvania

- Cattle
  - Grains oat and rye silage
  - 1 Cat carcass killed 431 of 441 cows
  - Feeding in avian botulism site

- Horses
  - Ravens as vectors from carcasses
  - Wounds castration,
    - drainage contaminated feed
  - 1979 racetrack 30 horses died
- Diagnosis, vaccine, treatment

#### <u>Botulism in Fish</u>

Getchell, Bowser, et al.

- Rule outs bacteria, viruses, weather (750)
- For Botulism E quantitative PCR
  - Process intestinal contents, liver, etc.
  - Looking for light chain E toxin gene (not toxin)
- Found in sheepshead from July 2001 dieoff
  - Kd, Lv, Sp pool; 3K genome equivalents
- 15-23K genome equivalents GIT contents
  - Only found in very few fish
- 200 148K in bird samples
- What about in healthy fish? No vegetative cells

#### Fish Susceptibility to Botulism E

Moccia et al.

- Fish Botulism Exposure Model
  - Standardize methods
- Temporal aspects, Sensitivity
  - Trout, goby, perch, walleye, mudpuppy
- Dose-response model
- Toxin titers
  - Tissue distribution
- Methods
  - Dosages to intubate fish
  - Temporal pattern, tissue distribution
  - Calculate up-web transport
- Temporal Observations
  - Restless, agitated, inc. swimming
  - Disequilibrium, altered posture, righting response lost
  - Lethargy interspersed with swimming, lack of coordination
  - Head up/tail down posture, breaching
  - Loss of motor function except respiration
  - Respiratory failure
- Prolonged course
- Altered behavior increased predation risk?
- Tissue distribution?
- Persistence of toxin?

# Agenda – Lake Erie Botulism Conference Thursday, April 3, 2003 Holiday Inn, Amherst, New York

#### 9:00 - Welcoming Remarks:

NYS Assemblyman, Richard Smith

#### **Botulism Overview/Introduction:**

Helen Domske, NY Sea Grant, Great Lakes Program - University at Buffalo

#### Pennsylvania Update:

Eric Obert, PA Sea Grant; Mike Mumau, Assistant Park Manager, Presque Isle State Park, Department of Conservation and Natural Resources, Bureau of State Parks; Bob Wellington, Erie County Department of Health

#### New York Update:

Waterfowl Mortalities - Ken Roblee, Senior Wildlife Biologist, NYSDEC Fish Mortalities Relative to Recent Fish Population Trends - Don Einhouse, Senior Fisheries Biologist, NYSDEC

#### Botulism in Fish and Wildlife in NYS Waters of Lake Erie

Ward Stone, NYSDEC Pathologist

#### **Ohio Update:**

Frank Lichtkoppler, Ohio Sea Grant

#### 12:00 - Lunch

#### **Canadian Update:**

Jeff Robinson, Canadian Wildlife Service, Dr. Ian Barker, CCWHC - Univ. at Guelph

#### **Research Presentations:**

Environmental Parameters Associated with Outbreaks of Botulism in Lake Erie Dr. Alicia Pérez-Fuentetaja, Biology Department, SUNY-Fredonia

Botulism: Atypical Pathogenesis in Other Species Dr. Robert H. Whitlock, New Bolton Center, University of Pennsylvania

#### Afternoon Break

Prevalence of Botulism in Fish in the Lower Great Lakes Dr. Paul Bowser, Aquatic Animal Health Program, Cornell University

Comparative Toxicity of Type E Botulinum to Several Lake Erie Fish Species Dr. Rich Moccia, University of Guelph

#### Wrap-Up:

Next Steps – Conclusions, Dr. Grace McLaughlin, National Wildlife Health Center, USGS

#### 4:30 - Adjourn

![](_page_53_Picture_0.jpeg)

![](_page_53_Picture_1.jpeg)

# Botulism In Lake Erie Workshop - Evaluation Thursday, April 3, 2003 Buffalo, New York

Please help us to evaluate the educational program by responding to the following statements. We ask that you complete this evaluation in its entirety.

69 participants 43 responses 62% return rate	KEY 1 = Strongly Disagree 2 = Disagree 3 = Neither Disagree nor Agree 4 = Agree 5 = Strongly Agree	e Stro	ongly		Str	ongly			
Please circle	e your response.	Disa	igree		Ag	ree	_N N	/IEA	N +/_
1) The workshop achie	ved its goal of sharing information.	1	2	3	4	5	43	4.58	.58
2) The workshop achie opportunities.	ved its goal of providing networking	1	2	3	4	5	43	4.30	.67
3) The botulism overvi	ew was worthwhile.	1	2	3	4	5	42	4.29	.77
4) The state & Canadia	n updates were worthwhile.	1	2	3	4	5	43	4.51	.59
5) The research present	ations were worthwhile.	1	2	3	4	5	43	4.47	.70
6) The wrap-up was wo	orthwhile.	1	2	3	4	5	32	3.97	1.18
The educational mate	rials and content of the workshop:								
7) Helped me better un botulism in Lak	derstand the issues surrounding e Erie.	1	2	3	4	5	43	4.30	.60
8) Provided information	n relevant to my work.	1	2	3	4	5	43	4.19	.70
9) Were well organized	L	1	2	3	4	5	43	4.33	.57
10) Were easy to under	stand.	1	2	3	4	5	43	4.33	.64
11) Presented informati	ion that will help me.	1	2	3	4	5	43	4.35	.57

Please circle your response. The educational materials and content of the workshop:		Strongl Disagre		y S xe A		ongly ree	_N MEAN +/_	
12) Will be of great use to me.			1	2	3	4	5	43 3.95 .75
13) I gained new knowledge from this wo	orkshop.		1	2	3	4	5	43 4.49 .59
14) I plan to share the information learne with others.	d at this works	shop	1	2	3	4	5	43 4.23 .81
15) I plan to take some action as a result I learned at this workshop.	of the informa	tion	1	2	3	4	5	42 3.74 1.06
<ul><li>16) I attended a previous botulism works</li><li>2001 or Buffalo, NY in 2002.</li></ul>	Please cir hop in Erie, PA N=43	cle or fill A in YES	in y 56	our 5%	respor NO 4	1se.  4%		
17) I shared the information I learned at p workshop(s) with others.	previous botuli N=29	ism YES	86	%	NO 1	4%	14 no	ot applicable
Who did you share the information with?	23 re	espondents	8					
18) I took action as a result of what I lear botulism workshop(s).	ned at previou N=30	s YES	60	%	NO	40%	13 no	ot applicable
What action did you take?	16	responde	nts_					
19) Would you be interested in attending topic next year?	a workshop or N=43	n this YES	10	0%	NO	0%		
20) What could we do to improve this wo	orkshop?							
20 respondents								

Thank you for completing this questionnaire. Please return your completed evaluation to Frank Lichtkoppler before you leave or mail it to: Ohio Sea Grant 99 East Erie Street, Painesville, Ohio 44077

#### Evaluation Comments - Botulism in Lake Erie Workshop - April 3, 2003 - Buffalo, NY

#### Question 17. I shared the information I learned at previous botulism workshop(s) with others.

#### Who did you share the information with? 23 participants responded

- Other extension staff, researchers, stakeholders
- Sport fishermen, media, agency staff
- Co-workers
- Co-workers, public, sportsmen
- Other researchers, grad students
- Staff members at DEC
- Co-workers
- Students, natural resources administrators and biologists, research collaborators
- Northeast loon work group
- Colleagues, community members
- Intra-agency, anglers
- Co-workers
- Co-workers
- Did get proceedings from website have shared with students
- General public, sport fishermen
- Mortality Info/species impacts
- Co-workers and sportsman
- Media, public, agency staff, researchers
- Public Mostly
- Other biologists, researchers and public
- Other colleagues
- Various sport angling groups and legislators
- Anglers and others contacting DEC for information

#### Question 18. I took action as a result of what I learned at previous botulism workshop(s).

#### What action did you take? 16 participants responded

- Initiated research program
- Informed staff of the potential of wound botulism
- Initiated a research project
- Developed research project revised surveillance
- Started water bird mortality surveys on Lake Ontario
- Research project
- Response to public inquiries, presentations to clients groups, media interviews, articles for agency newsletter
- Have given out posters from Sea Grant and or talked with public
- Started genotyping loons may not have had not connected to this group
- Several magazines articles, newspaper articles written
- Media, public, agency staff, researchers

- Collection of specimens, support of research
- Assisting in ongoing Sea Grant Research
- Various sport angling groups and legislators
- Collaboration
- Use information to guide my own actions regarding sportfishing activities in Lake Erie

### Question 20. What could we do to improve this workshop? 20 participants responded

- Not sure. Format worked well, speakers were effective and well-regarded. I'd suggest: action items, format for next year, identify research needs.
- As the proceedings are prepared, it may be a good idea to put Grace McLaughlin's wrap up presentation up on the web first
- Great graphics Don Einhouse Great. Some positive news, not gloom and doom! Good food, fish next year? Mudpuppy pie for desert!! Next year should have more results, relevant studies all appear to be just underway with no real results, Cornell, Ward Stone, University of Guleph. All appear to be on right tract. Look forward to their results.
- (Cold room) Otherwise great day!!!
- More time for informal interactions. I didn't know a lot of the "players" (only by name) and would have wanted more time to meet and talk with them.
- Perhaps less time on summaries with bird counts, and more presentations on research.
- Perhaps a short session where the group can pose suggestions on what to do next. Make a list of priorities. Dr. McLaughlin's recap at the end was unnecessary repetitive of what we just saw in the previous presenters.
- A little more time to network. Heat.
- A little more systematic observations required in "status reports". More info correlating limnology with biological events. We have too few "lake" people.
- Today's workshop was excellent improved over last year due to research presentations. Thanks for a great job.
- Obviously a warm room! I appreciate this opportunity wish I could have come for pizza on Wednesday night more networking time would have been nice perhaps also more info about funding opportunities and who is funded to do what.
- Standardize presentation display machinery.
- Just some heat...
- Hold workshop in September/October to develop/refine research questions. Circulate status reports before workshop (or post them) and spend more time on research findings and development of joint proposals. We didn't need to hear about cows and horses in such detail.
- Review what possible links this issue may impact/have on the food chain. Health concerns. Would like copies of power-point slides for each presentation.
- Continue to vary the content. Economic impacts?
- Presenters and podium block the view of the attendees.
- Handouts prior to meeting
- Encourage other states/provinces to invest as much effort as New York has in studying botulism. The amount of federal participation in Great Lakes botulism efforts appears to be very low, which is true, is a real shame. Please continue to engage Canadians in workshop participation.

# Botulism in Lake Erie Attendees/Contact List April 3, 2003

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