Botulism in Lake Erie Workshop Proceedings

Co-Sponsored by

New York Sea Grant
Ohio Sea Grant
Pennsylvania Sea Grant

March 25, 2004
Erie, Pennsylvania

Proceedings by Helen M. Domske, September 2004
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September 2004
2004 Workshop Overview

The workshop brought positive and negative news regarding botulism die-offs in the lower Great Lakes. The positive note was that there were fewer die-offs of fish and waterfowl in the summer and fall of 2003 in Lake Erie. On the negative side, there has been an increase of die-offs in Lake Ontario. This leaves scientists wondering if Lake Ontario will see the same ecological impacts of botulism that has been witnessed in Lake Erie over the past five years.

Botulism is a disease caused by the bacterium *Clostridium botulinum*. Although type C has caused the die-off of thousands of waterfowl (especially ducks) across the western United States, type E has been somewhat restricted to fish-eating birds in the Great Lakes. Type E toxin has also been known to affect fish and the toxin is suspected in recent fish die-offs in the lower Great Lakes.

In order to bring researchers, agency staff members and concerned stakeholders together to exchange data and ideas, New York Sea Grant again joined forces with Pennsylvania and Ohio Sea Grant to hold a binational workshop on the botulism issue. The fourth annual workshop on botulism was held on Thursday, March 25, 2004, at the Stull Nature Center in Erie, Pennsylvania.

Approximately 40 participants gathered to hear reports from New York, Ohio, Pennsylvania, and Ontario. The reports from the various state and provincial agencies provided information on the fish and waterfowl die-offs from 2003. Ken Roblee of the New York Department of Environmental Conservation reported that waterfowl die-offs decreased from over 17,000 in 2002 to approximately 3000 in 2002. Roblee, a wildlife biologist, also reported that 22 dead lake sturgeon were collected along the Niagara County shoreline of Lake Ontario, a significant mortality for these threatened fish. These mortalities concern biologists since the Niagara River is an established spawning area for lake sturgeon and these slow-reproducing fish could be impacted by the loss of reproductive-age fish.

After the reports on bird and fish die-offs were finished, participants heard from researchers representing Cornell University, Penn State University, Wadsworth Center- New York State Department of Health and the University of Guelph – Health Canada. The research at Cornell and the Wadsworth Center are efforts that were funded through New York Sea Grant.

The NYSG funded research at Cornell is being carried out by Dr. Paul Bowser and Dr. Rod Getchell. Through this work, the Cornell team has developed a faster, safer and more affordable way to detect botulism using a molecular assay to screen samples. Their research will also focus on testing sediments, quagga mussels and other invertebrates in the future. This will help to validate the hypothesis that botulism is being moved from the sediments up into the food chain by filter-feeding quagga mussels.

As a true binational effort, participants heard from researchers in Ontario. Dr. Rich Mocia, Dr. lan Barker and graduate student, Adam Yule, from the University at Guelph, presented their findings on interspecies toxicity of Type E botulism in fish. This research demonstrated that fish such as round gobies, walleye, yellow perch and rainbow trout show different sensitivities to the
botulism toxin. Not only was there a difference in mortality for different species of fish, some species like the round goby seemed to show pigment changes and others like trout showed marked behavioral changes. These changes in behaviors, such as erratic swimming or “breaching” (where the fish swims head-first upwards in water), may actually help to “lure” bird predators to the affected fish. This may increase the likelihood for fish-eating birds to prey on fish that contain the toxin, resulting in illness or death for birds like loons and mergansers.

Evaluation results from the workshop indicated that 98% of the participants will share the information and data from this workshop with others. Participants have shared information from previous workshops with colleagues, students, administrators, general public, media, sportfishing groups and the Commissioners from the International Joint Commission.

When asked if they plan to take some action as a result of the information learned at this year’s workshop, nearly all of the participants indicated that they would. These actions ranged from initiating research projects, working on bird or fish surveys, making observations, collecting samples, and writing articles, to making oral and written briefings for agency colleagues.

Conference organizers were pleased by the positive responses concerning interest in the continuation of annual workshops. Participants overwhelmingly indicated that there is a need to keep the flow of information and data ongoing.
Botulism Workshop Highlights
2001-2003
Eric Obert, Pennsylvania Sea Grant

The Beginning
1998 Pennsylvania and Canadian coastline
- Warmest February and least amount of snowfall on record.
- July - Many dead channel catfish washed up on Presque Isle Beaches.
- August - Complaints of sick and dying gulls by local rehabilitator, Wendy Campbell.
- Dying birds reported along Canadian shoreline.

April to Early October 1999: The Mystery Begins
Eric Morning News Article by Jack Grazier, October 7, 1999
- April – Reports of thousands of dead alewives and gizzard shad along Pennsylvania eastern shores.
- June-July – Heavy beds of Cladophora algae washing up onshore.
- Wildlife Rehabilitator, Wendy Campbell, is brought more than a dozen gulls with symptoms of muscular weakness/paralysis.
- Pennsylvania Game Commission Officer, Larry Smith, reports dead gulls appearing all along Lake Erie Shoreline.
- Over 150 dead gulls picked up at Presque Isle State Park.

The Mystery Continues
Eric Morning News Article by Jack Grazier, October 22, 1999
- Pennsylvania Game Commission suspects poisoning as possible cause of gull deaths.
- Canadian Wildlife Service reports shorebirds, gulls and carp are washing ashore at Pelee National Park, Rondeau Provincial Park, and Long Point.
- Pennsylvania sends gulls to the National Wildlife Health Center in Madison, Wisconsin, for testing.

The Mystery Begins to be Unraveled
Eric Morning News Article by John Bartlett, November 2, 1999
- NWHC lab in Madison, confirms Type E Botulism as cause of death of gulls collected from Presque Isle.
- A major die-off of over 6,000 birds is reported (90% mergansers) between Rondeau Provincial Park and Point Pelee on Canadian shores.
  - Type E toxin is confirmed.

Botulism Moves East – 2000
- New York DEC Reports fish and muddypuppy die-offs from Pennsylvania state line to Dunkirk, New York.
- Alewives in March, smallmouth bass in April through June, and 8 sturgeon in August.
- Tests done in late November on carp, zebra mussel, and goby from the Dunkirk area, all were negative for Type E botulism.
- **Nov. 16, 2000** – First calls of dead water birds, Type E botulism toxin was cause of mortality.
- **Nov. 27-28, 2000** – Estimate of 5,400-6,500 dead birds on shoreline.
- **Dec. 4, 2000** – 1,100 birds collected, scattered along the shoreline.

**Avian Botulism in Lake Erie Workshop**
**January 24-25, 2001 – Erie, Pennsylvania**
- Co-Sponsored by: New York and Pennsylvania Sea Grant
- Goal was to share information bi-nationally.
- Create a functioning network of government agency and university experts.
- Collaborate on research issues and develop a response plan for future outbreaks.

**State and Provincial Updates for 2001**
- Reports of extensive fish and mudpuppy kills (20 species affected). Freshwater drum, smallmouth, rock bass, sturgeon, carp, catfish and other benthic species.
- Soft-shell and map turtles dying in Presque Isle Bay. Botulism?
- Extensive *Cladophora* algal blooms wash ashore.
- Pennsylvania Fish and Boat Commission near-shore trawl data, gobies 70% of biomass.
- Freshwater drum 81% of dead fish on New York shoreline, 27 dead lake sturgeon collected.
- Lake Erie mortality 100 meter transect surveys 2,862 waterfowl of 18 species.
- Increased occurrences of round gobies in the die-off events of fish.
- Summary of Type E Botulism tests conducted from 1998-2001, and stomach contents from birds dying in botulism events. Round gobies and other fish were the main food identified in the stomach.
- Dead birds reported in Lake Ontario (unconfirmed).

**Summary of First Workshop**
- More than 60 researchers, fishery and wildlife biologists attend.
- Current knowledge and history of avian botulism outbreaks.
- Research and outreach priorities were discussed and developed. Botulism Taskforce was formed to improve communication.

**Research:**
- **Dr. Campbell** – Gull mortality summer to fall. Loon and merganser die-off in late fall. Die-offs seem to occur following changes in weather.
- **Dr. Baker** – Suggested to look for perturbations on the ecosystem, like significant change in wind or weather patterns.
- **Dr. Murphy** – Suggested that research is needed on the impacts of microcystines on fish and ducks.

**February 28, 2002 – Buffalo, New York**
- Co-Sponsored by: New York, Ohio and Pennsylvania Sea Grant
- Over 100 in attendance.
Presenters 2002

- **Dr. J. Michael Campbell** (Mercyhurst College) – Presented history of botulism Type E outbreaks in the Great Lakes and showed historical correlations with declining lake levels, invasive species (alewives) invasion, and association of kills with major storm events and internal seiche events.

- **John C. Lyons** (MD, FACS, MSME) – Presented facts about botulism in humans, particularly the bacterial genus *Clostridium*, and the interrelationship with avian botulism. The pathogenesis of *Clostridium*, clinical syndrome and treatments, and survival rates were discussed.

- **Dr. Rod Getchell** (Cornell University) – Discussed a project that 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.

Four questions to be addressed in this study:

A. Is *Clostridium 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 *Clostridium botulinum* than another?

C. Does *Clostridium botulinum* toxin form in these fish ante- or post-mortem?

D. Are fish carrying *Clostridium botulinum* associated with waterfowl mortality events?

- **Dr. Grace McLaughlin** (USGS) – Discussed the National Wildlife Health Center’s role and summarized the findings of Type E botulism in the Great Lakes.

  Non-avian mortality
  Algae
  Environmental correlates
  Population effects

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**Type E Botulism Cycle in Great Lakes**

- **Eagles Hawks**
- **Piscivorous Birds**
  - Loons, Comorants, Herons, Mergansers, Gulls
- **Diving Ducks**
  - Long-tailed Ducks, Scaup, Redhead
- **Dabbling Ducks Coots, Shorebirds**
- **Piscivorous Fish**
  - Freshwater Drum, Smallmouth Bass, Sturgeon
- **Mudpuppies**
- **Turtles:**
  - Map, Softshell, Mud
- **Round Goby**
- **Carp, other fish**
- **Invertebrates**
- **Mussels**
- **Algae**

---

Sediment
Presenters 2002
- Type E botulism was first found in Lake Erie in 1999, NYSDEC first identified Type E in the fall of 2000.
- Long-tailed ducks (2001) tested positive for Type E and were feeding on quagga mussels.
- Type E was found in fish alimentary canals (gut content).
- Type E was found in freshwater drum gut and tissue samples.
- Type E was found in mudpuppies.
- Maggots (fly larvae) had Type E toxin.
- Mudpuppies and round gobies were found in the guts of gulls, mergansers and loons.

Update 2002 Pennsylvania
- March, May – dead alewives, turtles observed.
- June – dead gobies and mudpuppies.
- Less Cladophora algae than in past years.
- July – Dying smallmouth bass sent for analyses (-negative)
- Mortality probably due to rapid temperature drop 75°F to 50°F in 2 days.
- Large die off of common loons in October.
- Ohio – Report of dead gulls showing up on eastern beaches.

Update 2002 New York
- Fish kills observed similar to Pennsylvania.
  - March-April: alewives, gizzard shad – temperature stress
  - May-June: smelt – spawning, Clupea
  - June-July: smallmouth bass – spawning? upwelling?
  - June-August: warmwater species, upwelling
- Gobies forage fish composition
  - 2000 - declining in Western basin, increasing in the east.
  - 2001 - huge numbers in Eastern basin.
  - 2002 - decline in abundance.
- Ward Stone – 7,000 submissions for botulism testing.
- Round gobies and mudpuppies found in many birds.
- 1 opossum and 2 raccoons positive for Type E.
- Long-tailed ducks eating quagga mussels - source of Type E.
- Fed loon livers to gobies and induced mortality.
- Waterfowl positive for Type E in Lake Ontario.

Canada Update
- Several Erie events
  - June, July, August - gulls, terns, cormorants
  - September - gulls, cormorants
  - October - gulls
  - Late October and November - common loon, long-tailed duck, red-breasted merganser
- Huron - October
  - Grebes, mergansers, loons
  - Goderich and Port Elgin

April 3, 2003 – Buffalo, New York
Co-Sponsored by: New York, Ohio and Pennsylvania Sea Grant

Grace McLaughlin (USGS) Type E botulism Outbreaks in the Great Lakes

<table>
<thead>
<tr>
<th>Year</th>
<th>Lake</th>
<th>Number</th>
<th>1° Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963-4</td>
<td>Michigan</td>
<td>&gt;12,000</td>
<td>Gulls, Loons</td>
</tr>
<tr>
<td>1976-83</td>
<td>Michigan, Huron</td>
<td>&gt;1800</td>
<td>Gulls, Loons</td>
</tr>
<tr>
<td>1998-2002</td>
<td>Huron, Michigan</td>
<td>~2500</td>
<td>Mergansers, Gulls, Loons</td>
</tr>
<tr>
<td>1999-2001</td>
<td>Erie</td>
<td>&gt;25,000</td>
<td>Mergs, Gulls, Loons</td>
</tr>
<tr>
<td>2002</td>
<td>Erie</td>
<td>&gt;25,000</td>
<td>Long-tailed ducks, Gulls</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Loons, Mergansers, Cormorants</td>
</tr>
</tbody>
</table>

Research Update from the Lake Erie Botulism Conference of April 3, 2003
Research findings from:
Perez et al. – SUNY Fredonia
Bowser et al. – Cornell Univ.
Robinson et al. – Ontario/Guelph

Physical-Chemical Parameters being studied by SUNY Fredonia researchers
- 0.5 m above sediment.
- Multiparameter Meter YSI 556:
  - Temperature
  - Dissolved Oxygen
  - Conductivity
  - Salinity
  - Total Dissolved Solids
  - pH
  - Redox Potential

Summary of Work by Perez et al.
- 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).
- Weather events during the season 2002 that may explain mixing of the water column and changes in lake conditions.
Research Plans for Cornell - Bowser et al.
- Make a greater effort to collect fish during botulism outbreaks, particularly round gobies and freshwater drum.
- Collect sediment and quagga mussels from outbreak areas to further analyze the food chain path that Type E Botulism is following.

Results from Cornell
- Measured significant numbers of *Clostridium botulinum* Type E in dead and dying freshwater drum during three die-offs in July of 2001 near Dunkirk and Barcelona Harbor on Lake Erie.
- Measured detectable levels of *Clostridium botulinum* Type E in one apparently healthy five fish pool of smallmouth bass from Dunkirk, New York.

Research in Canada - Robinson et al.
- Distribution of fish and bird mortality events (OMNR, CWS).
- Stomach examination of fish eating birds to determine food habits (OMNR, Canadian Cooperative Wildlife Health Centre).
- 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).

Results from Moccia et al.
- 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.
- BREACHING BEHAVIOR: In a natural setting, fish showing breaching behavior would present an “easy” target for predators and maximize botulism toxin ingestion.
Ohio Update 2003
Frank Lichtkoppler, District Specialist, Ohio Sea Grant

**Beach Watch Volunteers were asked to:**
1) Make weekly observations (from week of June 14 to November 15)
2) Report large kills to ODNR
3) Compile a report (check week and record counts)

http://superior.eng.ohio-state.edu/lakes/nf-index.html

![Map of Lake Erie](image)

**Location** | **Birds** | **Fish** | **Mudpuppies**
---|---|---|---
Maumee Bay State Park | 1 | 0 | 0
Cleveland Lakefront State Park | 80 | 0 | 0
Euclid | 0 | 0 | 0
North Perry | 9 | 35 | 0
Ashtabula | 10 | 2 | 0

**Mallards, Gulls, Canada Geese, 2 Loons**

**Good News:**
- No significant fish or bird kills in 2003 were reported by the manager of Stone Laboratory, which is located on Gibraltar Island.
- Only a few, isolated (in space and time) dead birds or fish were observed by five volunteers who lived on or regularly worked on the Ohio Lake Erie shoreline.
- “No big kills this past year... just a few minor episodes of fish kills from minor upwelling events,” according to K. Kayle, ODNR.
Pennsylvania Lake Erie 2003 Botulism Update
Bob Wellington
Erie County Health Department

Large Fish Die-Off in Presque Isle Marina noted June 11, 2003
- Bluegill
- Sunfish
- Largemouth Bass
- Smallmouth Bass
- Freshwater Drum
- Yellow perch
- Carp
- Bullheads

2003 Bird Collection at Presque Isle (June - December)
Total of 70 Birds Collected
2003 Fish Collections at Presque Isle (May 30 - August 15)
Total of 992 Fish Collected (Excluding Shinners)

Fish Species
*8000 Shiners were collected on June 2, 2003; None were reported collected before or after that date*
Quagga Mussels Along Shoreline (November 19, 2003)

Quagga Mussel Shells Covering the Shoreline (November 26, 2003)
Botulism Caused Fish & Waterbird Mortality
*In New York Waters of Lakes Erie & Ontario - 2003*

Prepared by
David Adams & Kenneth Roblee & Ward Stone – NYS DEC
Presented by
Kenneth Roblee - NYS DEC Region 9

May 5, 2003 – DEC inspection of the Bethlehem Steel ring-billed gull colony shows no evidence of botulism mortality in gulls.

November 4, 2003 – Type E botulism was confirmed in a gull from Dunkirk (Lake Erie) by George Hannett of the NYSDOH.

November 10, 2003 – Type E botulism was confirmed in a common loon from Dunkirk, Lake Erie by George Hannett of the NYSDOH.

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Table 1: Lake Erie Botulism Mortality Surveys
100m Transect Survey Results
NYSDEC 10/06/03-1/02/04

<table>
<thead>
<tr>
<th>Species</th>
<th>Predicted Mortality</th>
<th>Upper Limit 95% Confidence</th>
<th>Lower Limit 95% Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Loon</td>
<td>1569</td>
<td>2799</td>
<td>117</td>
</tr>
<tr>
<td>Horned Grebe</td>
<td>18</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>Double-crested Cormorant</td>
<td>55</td>
<td>129</td>
<td>0</td>
</tr>
<tr>
<td>Mallard</td>
<td>16</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>Long-tailed Duck</td>
<td>239</td>
<td>502</td>
<td>0</td>
</tr>
<tr>
<td>Red-breasted Merganser</td>
<td>35</td>
<td>129</td>
<td>0</td>
</tr>
<tr>
<td>Bonaparte’s Gull</td>
<td>16</td>
<td>52</td>
<td>0</td>
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<tr>
<td>Ring-billed Gull</td>
<td>282</td>
<td>439</td>
<td>147</td>
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<td>Horned Gull</td>
<td>282</td>
<td>285</td>
<td>79</td>
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<td>Great Black-backed Gull</td>
<td>73</td>
<td>151</td>
<td>0</td>
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<tr>
<td>Unidentified Gull</td>
<td>91</td>
<td>172</td>
<td>10</td>
</tr>
<tr>
<td>Common Tern</td>
<td>18</td>
<td>52</td>
<td>9</td>
</tr>
<tr>
<td>Total Birds</td>
<td>906</td>
<td>4000</td>
<td>2015</td>
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Lake Erie Waterbird Mortality

New York Botulism Transect Survey Results

<table>
<thead>
<tr>
<th></th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
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<tr>
<td>Common Loon</td>
<td>583</td>
<td>1149</td>
<td>2042</td>
<td>1969</td>
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<tr>
<td>Horned Grebe</td>
<td>109</td>
<td>0</td>
<td>273</td>
<td>18</td>
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<tr>
<td>Long-tailed Duck</td>
<td>0</td>
<td>310</td>
<td>12616</td>
<td>219</td>
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<tr>
<td>Red-breasted Merganser</td>
<td>2479</td>
<td>91</td>
<td>839</td>
<td>55</td>
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<tr>
<td>Ring-billed Gull</td>
<td>1714</td>
<td>510</td>
<td>273</td>
<td>292</td>
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<tr>
<td>Total Birds</td>
<td>5415</td>
<td>2862</td>
<td>17301</td>
<td>3008</td>
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</tbody>
</table>

Lake Ontario

2002

March 23, 2004 – Type E botulism was confirmed by the University of Pennsylvania in a ring-billed gull collected near Youngstown, Lake Ontario, in 2003.
Type E botulism test results for a common loon collected in 2003 from Orleans County, Lake Ontario, are still pending from this Lab.

December 12, 2003 – Type E botulism toxin was detected by George Hannett of the NYSDOH in the alimentary canal contents of a lake sturgeon collected at Wilson-Tuscarora State Park, Lake Ontario, on October 27, 2003.

### Table 2: New York Shoreline Lake Ontario Botulism Mortality Surveys

<table>
<thead>
<tr>
<th>Species</th>
<th>Predicted Mortality</th>
<th>Upper Limit 95% Confidence</th>
<th>Lower Limit 95% Confidence</th>
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<tbody>
<tr>
<td>Common Loon</td>
<td>134</td>
<td>238</td>
<td>25</td>
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<tr>
<td>Double-crested Cormorant</td>
<td>66</td>
<td>128</td>
<td>8</td>
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<tr>
<td>Mallard</td>
<td>16</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Red-breasted Merganser</td>
<td>18</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Greater Scapen</td>
<td>33</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>Long-tailed Duck</td>
<td>263</td>
<td>525</td>
<td>2</td>
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<tr>
<td>White-winged Scoter</td>
<td>200</td>
<td>487</td>
<td>72</td>
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<tr>
<td>Ring-billed Gull</td>
<td>263</td>
<td>525</td>
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<tr>
<td>Herring Gull</td>
<td>164</td>
<td>294</td>
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<tr>
<td>Great Black-backed Gull</td>
<td>66</td>
<td>128</td>
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<tr>
<td>Unidentified Gull</td>
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<td>Common Goldeneye</td>
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<tr>
<td>Red-throated Loon</td>
<td>18</td>
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<tr>
<td>Total birds</td>
<td>1420</td>
<td>2838</td>
<td>621</td>
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</tbody>
</table>

### Dead Sturgeon (22) Found on Lake Ontario - 2003

![Graph showing the number of sturgeon found on Lake Ontario from 8/31 to 12/28, 2003.](image)
GPS Locations of Common Loons
Observed on Lake Erie During Aerial Surveys
November 9, 21 and December 3, 2002

Chautauqua County

Observed Distance from Shore - Lake Erie Loons

Fourteen loons sighted: ave = 0.85 mi; STDEV = 0.475443;
confidence interval = +/- 0.249047 mi.
In Summary:
Type E botulism was confirmed in Lake Erie for the fourth consecutive year and in Lake Ontario for the second consecutive year.

In Lake Erie, mortality declined in most waterbird species except for ring-billed gull and common loon.

In Lake Ontario, common and red-throated loon mortality, presumable due to Type E botulism (pending diagnoses), was observed for the first year.

Botulism toxin was available to fish and wildlife during cooler lake temperatures, with many specimens collected during mid to late November and December.

Type E toxin botulism was diagnosed in a Lake Sturgeon from Lake Ontario. Twenty-two dead sturgeon were collected and significant mortality to other fish species was observed along the Niagara County, Lake Ontario shoreline.

Acknowledgements:
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Dennis Falknham  Irene Mazzocchi  Mike Todd
Rich Farmer  Melissa Neely  Ben Weber
Jim Farquhar  Patti Nelson  Tony Zerkle
Bill Gordon  Joe Okoniewski
Floyd Knowlton  Josh Patchen
The Round Goby Botulism Connection

Renea A. Ruffing
Graduate Research Assistant
Penn State University

Round gobies in Pennsylvania waters of Lake Erie
- First goby caught in Lake Erie was in 1993, in Grand River Harbor, Ohio.
- Found in 1995 at the mouth of the Ashtabula River.
- October of 1996, caught off of Presque Isle by the Pennsylvania Fish and Boat Commission.

Goby population expansion
- Round goby population increased exponentially over the next several years.
- This population increase coincided with increases in cases of avian botulism.
- Were round gobies playing an important role in this outbreak of avian botulism?

Gobies as suspects

Aquatic Invaders and Stricken Birds
Large numbers of Lake Erie fish and birds are dying from type E botulism. Scientists suspect invader species from Eastern Europe — two types of mussels and a fish — may be responsible.

A CHANGED ECOSYSTEM
Zebra and quagga mussels filter algae from the water, making the lake clearer. Sunlight reaches the lake bed, prompting plant growth.

BACTERIA THRIVE
Decaying plants create an oxygen-deprived environment favorable for botulism bacteria. As they filter the water, mussels may concentrate the toxin.

IN THE FRESHWATER CHAIN
Bottom-feeding goby eat mussels and ingest botulism, concentrating it further. Then birds feed on the fish and their eggs.
Are round goby populations being effected by avian botulism?
No real evidence of this.
- Inshore populations have increased rather than decreased.
- No large amounts of gobies found when there have been large fish kills, with the exception of the summer of 2002 and this appeared to occur with a cold-water upwelling.
- Large males are known to die after spawning events.
- Do not see evidence for this while diving.

Do round gobies contribute to the avian botulism problem by carrying botulism?
- Diet studies have shown that gobies in the lakes do consume large numbers of zebra mussels.
- Gobies could perhaps acquire botulism from the ingestion of mussels.
- Transfer botulism to fishes and birds that prey on them.

Ward Stone Lab Results
- Gobies are susceptible to the botulism toxin.
- Majority died within 24-hours of ingesting botulism infected loon livers.
- Botulism infected gobies tend to move slowly and erratically.
- Remained on the bottom even after death.

Sick Phase
- Make them more susceptible to predatory fishes.
- Transfer method for botulism from mussels to larger game fish.
One problem with this is that you would expect to see more bottom scavenging fishes, like catfish and carp, affected as well.

**Goby Studies**
- Age vs. Length and Sex.
- Diet studies examining frequency of goby prey by size class between the lake and bay.
- Examining the total amounts of prey between stream, bay-dwelling gobies by season.
- Diets of game fishes.

**Length-Age Relationship for Female Gobies**

\[ L_t = 100.7 \times (1-e^{-0.4841(t-1.3309)}) \]

**Length Age Relationships for females**
- The Von Bertalanffy equation for female gobies.
- \( L_t = 100.7 \times (1-e^{-0.4841(t-1.3309)}) \)
- The oldest female was approximately 3-years of age at a length of 110.3 mm.
- Substantial variation within age classes.
- Probably caused by multiple spawning events throughout the season.
Length-Age Relationship for Male Gobies

\[ \text{Total Length (mm)} \]

- Pelagic: \( L_t = 108.6 \times (1 - e^{(-1.0231)(t-0.0523)}) \)
- Littoral: \( L_t = 237.7 \times (1 - e^{(-0.1788)(t-1.1681)}) \)

**Length Age Relationships for Males**
- Oldest male was approximately 6-years old at 164.5 mm.
- Again, there is substantial variation within age classes.
- There was no difference between theoretical maximum length of pelagic and littoral males.
- Differences in K indicate that pelagic and littoral males are on different growth trajectories.
- Difference may be due to different habitat or sampling differences.

**Goby Diet Studies**
- Two different studies in which several different comparisons were made.
- Differences between stream vs. lake gobies.
- Diets of males vs. females.
- Comparisons of diets among different size classes.
Goby Diet Results
- Female gobies (which tend to be smaller) have a more diverse diet.
- Stream gobies, regardless of size, tend to eat many fewer (almost no) mussels, as compared to lake gobies.
- Gobies larger than 80 mm (Age 1+) feed almost exclusively on mussels.

Diet Study
- 22% of largemouth bass, 16% of yellow perch, and 10% of sheepshead fed on gobies.
- Sheepshead also fed on large amounts of chironomid larvae.
- Other fishes, such as blue gills, occasionally fed on gobies as well.

PAFBC Yellow Perch Diet
- 19% of fish with prey in stomachs were eating round gobies.
- Only 5% of these fish were eating gobies of age 2+.

Discussion
- Results of our studies and others indicate that almost all predatory fishes are feeding on round gobies.
- Larger gobies (greater than 80 mm) are feeding almost entirely on mussels.
- If there is a connection between avian botulism found in mussels and gobies it is probably affecting fishes that can feed on larger gobies.
Goby Toxicity
• Fishes were collected randomly from May 2002 through May 2003, from the Pennsylvania water of Lake Erie (mostly from Presque Isle Bay) by hook and line and boat electro-fishing.
• Pennsylvania Animal Diagnostic Laboratory System at New Bolton Center received gobies.
• Conducted heavy metal analysis and assayed for botulinum toxin.

Fishes examined
• 50 Round gobies
• 25 Smallmouth bass
• 2 Largemouth bass
• 5 Northern pike (one was sick)
• 3 Crappie
• 1 Bluegill
• 1 Yellow perch

Results
• No Clostridium botulinum found in any of these fishes.
• Clostridium bifermentans was recovered from intestinal samples of a round goby.
• Arsenic levels were detectable in all of the fish livers examined (0.207 ppm in a northern pike (Esox lucius) to 6.07 ppm in a pooled group of goby livers).
• Hg values found in livers of sampled fishes in this study ranged from insignificant (<0.05) to 9.42 ppm, with gobies representing the extremes of the range.
• Hepatic levels of Se in this study ranged from insignificant in a bluegill (Lepomis macrochirus) to 2.27 ppm in a large steelhead (Oncorhynchus mykiss).

Discussion
• Extensive numbers of investigations have indicated that heavy metals alter a number of parameters of the hosts’ immune system and can lead to increased susceptibility to infection auto immune diseases and allergic manifestations.
• High levels of mercury, arsenic or selenium could be transferred up the food chain causing immunosuppression in fish-eating birds.
• For example, high levels of Se were found in a pelican that died from Type C avian botulism in the Sulton Sea in California.

What does all this tell us about the goby avian botulism connection?
• Gobies below 80 mm in length are not consuming large numbers of mussels.
• Gobies are not immune to botulism toxins.
• Larger gobies’ behavior does not make them easy prey items for birds.
• Game fish are consuming large numbers of gobies; however, yellow perch appear to consume smaller sized gobies.
• We may need to examine multiple stressors in order to better understand this problem.

Acknowledgements
• Pennsylvania Sea Grant
• Erie County Conservation District
• Chuck Murray and the PAFBC
• PA Coastal Zone Management
• PA DEP
Diagnosing Botulism in Fish in the Lower Great Lakes

Investigators: Paul Bowser, Cornell University, Rod Getchell, Cornell University

Collaborators: Bill Culligan, NYSDEC Dunkirk, Don Einhouse, NYSDEC Dunkirk
Steve LaPan, NYSDEC Cape Vincent, Web Pearsall, NYSDEC Avon
Ward Stone, NYSDEC Delmar, James & Fina Casey, Cornell University
Claudia Sutton, Cornell University and Robert Whitlock, U Penn

Introduction to Clostridium botulinum Type E
- A common aquatic bacteria, Clostridium botulinum Type E produces a potent toxin under the high nutrient and anaerobic (oxygen-free) conditions that occur in dead organisms.
- The Type E strain of Clostridium botulinum is the most frequently found strain in the aquatic environment.
- Animals, especially fish-eating birds, ingest the bacteria in their diet, become paralyzed by the botulinum toxin, and often die. Their carcasses then become culture vessels for more Clostridia.

Signs of Type E Botulism in waterfowl and fish
- Signs of Type E Botulism occur when the botulinum toxin binds to nerve receptors that then leads to a descending paralysis.
- Birds usually cannot hold their heads up and so waterfowl often drown.
- Birds like gulls can sometimes walk, but not fly. You often see them dragging one or both of their wings.
- Fish may flounder or swim erratically near the surface of the water. The location of affected fish on the water’s surface is often identified by the presence of feeding gulls.
- Fish usually die quickly and are most likely seen washed up on shore.

Risks of Type E Botulism to humans?
- To get Type E Botulism, you must ingest the toxin, usually by eating an infected fish, bird, or marine mammal.
- Any fish or waterfowl that are sick or act abnormally should not be harvested or eaten because cooking may not destroy all of the toxin.
- Wear disposable gloves, or invert a plastic bag over your hand, when handling sick, dead, or dying animals.
- You are not at risk for botulism by swimming in the Great Lakes.
- Your pets are at risk if they consume dead animals along the shoreline.

Background on Type E Botulism in Lake Erie
- Type E Botulism outbreaks have killed thousands of waterfowl on Lake Erie in each of the last 5 years.
- Fish kills have been associated with some of these events.
- Are live or moribund fish a vector for Type E Botulism in loons and mergansers?
- The public hazard from these outbreaks needs to be clarified. Are apparently healthy fish safe to eat, while sick fish are not safe to consume?
Fish collections and sample necropsy

- NYSDEC fisheries personnel are collecting healthy, sick, and fresh dead fish from Lakes Eric and Ontario.
- At Cornell, fish are necropsied and tissues are tested for various pathogens, including *Clostridium botulinum* Type E.
- Tissues are frozen for later molecular analysis.

Sample processing and DNA Extraction

- The traditional method for botulism diagnoses is either by anaerobic culture or the mouse bioassay.
- We have developed a molecular assay to screen samples because it is faster, safer, and more affordable.
- Fish intestinal contents and liver are processed to concentrate their DNA.
- This multi-step procedure provides purified DNA that can be assayed for the presence of the *C. botulinum* Type E toxin gene.

Quantitative (real-time) PCR

- After DNA is isolated, we can look for the toxin gene using a standard PCR amplification of a 139 base pair fragment to demonstrate the presence or absence of *C. botulinum* Type E.
- But, quantitative (real-time) PCR will provide actual numbers of *C. botulinum* Type E per gram of tissue when compared to a series of standards.

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**Quantitative PCR data**

- Quantitative (real-time) PCR data output from ABI 7700.
- Samples tested from a freshwater drum’s intestine and liver that was caught on July 11, 2002 near Dunkirk, NY appear in this plot as red squares.
**QPCR standard curve**

- QPCR standard curve showing sample data (●) and standards (○) from plasmid DNA containing the 139 bp fragment of the *C. botulinum* Type E toxin gene.

### 2002-2003 Fish Collection Totals

<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Erie</td>
<td></td>
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</tr>
<tr>
<td>Spring</td>
<td>265</td>
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<td>Summer</td>
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<td>Fall</td>
<td>186</td>
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<td>Lake Ontario</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>8</td>
<td>21</td>
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<tr>
<td>Summer</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Fall</td>
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<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2003</th>
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</thead>
<tbody>
<tr>
<td>Smallmouth bass</td>
<td>286</td>
<td>295</td>
</tr>
<tr>
<td>Freshwater drum</td>
<td>208</td>
<td>166</td>
</tr>
<tr>
<td>Round goby</td>
<td>148</td>
<td>185</td>
</tr>
<tr>
<td>Yellow perch</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Alewife</td>
<td>20</td>
<td>0</td>
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<tr>
<td>Brown bullhead</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Other species</td>
<td>41</td>
<td>2</td>
</tr>
</tbody>
</table>

**Botulinum toxin rapid detection kit**

- Several QPCR-positive samples have been tested with a botulinum toxin rapid detection kit.
- The sample tested here was from a lake sturgeon’s stomach contents that contained two goby-like fish that were partially decomposed.
- The sturgeon had washed up on a Lake Michigan beach in Door County, Wisconsin, during the summer of 2002.
### 2001-2003 C. botulinum Type E QPCR Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample Location</th>
<th>Collection Dates</th>
<th>Quantity/gram</th>
<th>Toxin</th>
<th>Mouse Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWDrum</td>
<td>Dunkirk, NY</td>
<td>August 17, 2001</td>
<td>3,000/g KLS</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>FWDrum</td>
<td>Dunkirk, NY</td>
<td>July 11, 2002</td>
<td>208,000/g IC</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>FWDrum</td>
<td>Sunset Bay, NY</td>
<td>July 18, 2002</td>
<td>10,900/g IC</td>
<td>Pos.</td>
<td>Neg.</td>
</tr>
<tr>
<td>FWDrum</td>
<td>Dunkirk, NY</td>
<td>July 18, 2002</td>
<td>21,700/g IC</td>
<td>Pos.</td>
<td>Neg.</td>
</tr>
<tr>
<td>FWDrum</td>
<td>Barcelona, NY</td>
<td>July 26, 2002</td>
<td>23,100/g IC</td>
<td>Nec.</td>
<td>Nec.</td>
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<tr>
<td>SMBass</td>
<td>Dunkirk, NY</td>
<td>August 21, 2002</td>
<td>15,200/g IC</td>
<td>Pos.</td>
<td>Neg.</td>
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<tr>
<td>Sturgeon</td>
<td>Door County, WI</td>
<td>Summer, 2002</td>
<td>17,400/g SC</td>
<td>Pos.</td>
<td>Pos.</td>
</tr>
<tr>
<td>RGobies</td>
<td>Dunkirk, NY</td>
<td>June 4, 2003</td>
<td>2,700/g IC</td>
<td>NA</td>
<td>*</td>
</tr>
<tr>
<td>FWDrum</td>
<td>Van Buren Pt., NY</td>
<td>August 27, 2003</td>
<td>1,100/g IC</td>
<td>Pos.</td>
<td>*</td>
</tr>
<tr>
<td>FWDrum</td>
<td>Van Buren Pt., NY</td>
<td>August 27, 2003</td>
<td>15,200/g IC</td>
<td>Neg.</td>
<td>*</td>
</tr>
<tr>
<td>FWDrum</td>
<td>Van Buren Pt., NY</td>
<td>August 27, 2003</td>
<td>42,300/g IC</td>
<td>Pos.</td>
<td>*</td>
</tr>
</tbody>
</table>

IC = Intestinal contents. SC = Stomach contents included two goby-like fish. KLS = Kidney, liver, and spleen. POS or Neg = Positive or negative assay with botulism toxin rapid detection kit (Coburn Scientific Group, Lakeviile, AY). NA = Not assayed, no tissue available. *Mouse bioassay results pending.

### WI Sturgeon QPCR Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample Location</th>
<th>Collection Dates</th>
<th>Quantity/gram</th>
<th>Toxin</th>
<th>Mouse Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>July 25, 2003</td>
<td>250,000,000/g Li</td>
<td>Neg.</td>
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<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>July 28, 2003</td>
<td>1,000,000/g SC</td>
<td>NA</td>
<td>*</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>August 6, 2003</td>
<td>15,900,000/g Li</td>
<td>NA</td>
<td>*</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>August 13, 2003</td>
<td>4,000,000/g IC</td>
<td>NA</td>
<td>*</td>
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<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>August 14, 2003</td>
<td>10,700,000/g SC</td>
<td>NA</td>
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<td>Sturgeon</td>
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<td>August 15, 2003</td>
<td>2,400,000/g IC</td>
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<tr>
<td>Sturgeon</td>
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<td>6,900,000/g Li</td>
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<td>Sturgeon</td>
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<td>August 17, 2003</td>
<td>2,300,000/g IC</td>
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<td>*</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>August 18, 2003</td>
<td>2,700,000/g SC</td>
<td>NA</td>
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<tr>
<td>Sturgeon</td>
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<td>780,000/g IC</td>
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<td>Sturgeon</td>
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<td>39,900,000/g SC</td>
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<td>September 6, 2003</td>
<td>9,700,000/g Li</td>
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<tr>
<td>Sturgeon</td>
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<td>September 7, 2003</td>
<td>4,170,000/g Li</td>
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<td>Sturgeon</td>
<td>Green Bay, WI</td>
<td>September 8, 2003</td>
<td>42,000/g SC</td>
<td>NA</td>
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</tbody>
</table>

IC = Intestinal contents. SC = Stomach contents included two goby-like fish. Li = Liver. POS or Neg = Positive or negative assay with botulism toxin rapid detection kit (Coburn Scientific Group, Lakeviile, AY). NA = Not assayed, no tissue available. *Mouse bioassay results pending.
2001 Avian *C. botulinum* Type E QPCR Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Case Number</th>
<th>Sample Location</th>
<th>Quantity/Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Loon</td>
<td>01-45-19B</td>
<td>Lake Erie</td>
<td>148,000/g ACC</td>
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<tr>
<td>Common Loon</td>
<td>01-45-23</td>
<td>Lake Erie</td>
<td>40,700/g ACC</td>
</tr>
<tr>
<td>Common Loon</td>
<td>01-45-29</td>
<td>Lake Erie</td>
<td>36,200/g SC</td>
</tr>
<tr>
<td>Coot</td>
<td>01-45-22</td>
<td>Lake Erie</td>
<td>340/g ACC</td>
</tr>
<tr>
<td>Long Tail Duck</td>
<td>01-45-04F</td>
<td>Lake Erie</td>
<td>40,800/g GC</td>
</tr>
</tbody>
</table>

ACC = Alimentary canal contents; SC = Stomach contents; GC = Gizzard contents.

Results from 2002-2003

- In 2002, 736 fish were examined.
- Significant numbers of *C. botulinum* Type E were measured in dead and dying freshwater drum during die-offs in July of 2002 near Dunkirk, Sunset Bay, and Barcelona Harbor on Lake Erie.
- Detectable levels of *C. botulinum* Type E measured in an apparently healthy five-fish pools of smallmouth bass and freshwater drum.
- In 2003, 678 fish were examined.
- In late August of 2003, significant numbers of *C. botulinum* Type E were again measured in dead and dying freshwater drum collected off Van Buren Point, also in Lake Erie.
- Detectable levels of *C. botulinum* Type E measured in an apparently healthy five-fish pool of round gobies.

Future research plans

- Confirm that *C. botulinum* Type E levels in moribund fish are high enough to kill waterfowl, as well as other fish.
- Continue to collect fish during botulism outbreaks.
- Redouble our collection efforts on Lake Ontario.
- Collect and test sediment, quagga mussels, and other invertebrates from outbreak areas, as well as designated sites in both lakes.
- Collaborate with a regional diagnostic lab to validate our molecular assay methods, i.e. sensitivity and specificity analyses.

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**Cornell Fish Pathology Lab**: Greg Wooster, Susan Bartlett, Steffanie Grimmett, Natalija Topic-Popovic, Cheryl Sangster, Megan Kirchgessner, Connie Lee, Chun-Yao Chen

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Lake Ontario Ecosystem Project: Mark Bain, Kristi Arend and Gail Steinhart

USFWS: Emily Zollweg and Rob Elliot

New York Sea Grant: Helen Domske

Captain Doug Stein

Janice M. Plante
Interspecies Toxicity of Type-E Botulinum in Fish: 
A Bird’s-Eye View

A.M. Yule, R.D. Moccia, J. Austin and I.K. Barker

Lake Erie Bird Mortalities
• Type E botulism is considered the primary cause of avian deaths.

The Paradox?
• How to explain the ‘fish’ pathway of toxin ingestion in birds which feed exclusively on ‘live’ fish (e.g. loons, common and red-breasted mergansers, grebes)?
• Lack of apparent correlation between fish botulism epizootics and avian mortality patterns.

What Are The Questions?
• Are ‘living’ fish a reasonable transport mechanism moving Botulinum Neurotoxin from point of origin to the bird?
• Is it plausible that live, but moribund, fish are selective prey species?
• Does phylogeny, natural life history or primary feeding habitat influence sensitivity?
• Is there a potential human health hazard to people who consume Botulinum Neurotoxin laden fish?

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. Determine toxin titres in Botulinum Neurotoxin mortalities.

Rainbow Trout Dose Response
Rainbow Trout Percent Mortality

Goby - Onset of Pigment Change
- Normal, pale coloration.
- Very early onset of darkening pattern.

Progressive Discoloration
- Formation of the progressive, pigment ‘band.’
- Complete pigment change, followed closely by death.
Walleye: Onset of Respiratory Distress
- Severe distention of operculum: erratic swimming behavior (often breaching surface).
- Fish exhibiting the “head up-tail down” orientation.
Walleye Percent Mortality

Perch: Onset of Pigment Change
- Slight pigment change (darker).
- Drastic pigment change.
- Equilibrium loss, fish often breaching the surface.

Perch Dose Response
Perch Percent Mortality

Interspecies Dose Response
Interspecies Percent Mortality: 4000 MLD

Dose-Body Weight Relationship

*Tissue Titres of Botulinum Neurotoxin*
In other words, what's left in a dead fish?
### Post Mortem Titre Results:

<table>
<thead>
<tr>
<th></th>
<th>Trout</th>
<th>Goby</th>
<th>Walleye</th>
<th>Perch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fillet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 MLD</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>1500 MLD</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>4000 MLD</td>
<td>0%</td>
<td>17%</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>Vicera</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 MLD</td>
<td>11%</td>
<td>92%</td>
<td>33%</td>
<td>NA</td>
</tr>
<tr>
<td>1500 MLD</td>
<td>17%</td>
<td>83%</td>
<td>71%</td>
<td>NA</td>
</tr>
<tr>
<td>4000 MLD</td>
<td>25%</td>
<td>83%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

-Equilibrium loss, fish often breaching the surface
Implications of Results
- Each species shows a unique set of clinical signs.
- Behavioral (e.g. breaching, erratic swimming) and pigmentation changes in all species could "lure" bird predators to affected fish.
- Prolonged moribund state in fish, even with high Botulinum Neurotoxin levels.
- Live fish can be significant vector for toxin transfer through trophic levels.
- The round goby is not highly tolerant as was previously hypothesized.
- Perch are very resistant.
- The high percentage of positive titre results in the goby could implicate them in the bird mortalities.
- Unlikely human health significance to live fish carrying toxin (perch?).

Rainbow Trout: Onset of Clinical Signs

<table>
<thead>
<tr>
<th>TIME</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>fish intubated</td>
</tr>
<tr>
<td>T1</td>
<td>time of capsule degeneration and toxin release</td>
</tr>
<tr>
<td>T2</td>
<td>normal behavior</td>
</tr>
<tr>
<td>T3</td>
<td>restless, agitated, increased swimming behavior</td>
</tr>
<tr>
<td>T4</td>
<td>first equilibrium loss (loss gradually increases)</td>
</tr>
<tr>
<td>T5</td>
<td>no fin co-ordination or righting ability, irregular ‘breaching’ behavior noted</td>
</tr>
<tr>
<td>T6</td>
<td>loss of any voluntary motor function</td>
</tr>
<tr>
<td>T7</td>
<td>loss of respiratory reflex/death</td>
</tr>
</tbody>
</table>

Acknowledgements

Special thanks to Environment Canada for providing research funding to support this work, and the Ministry of Natural Resources for aiding in fish acquisitions.

Thanks also to Ms. Sandra George and Jeff Robinson for their enthusiastic contributions to this project.
Algal Pigments as Biomarkers Linking Fish and Benthic Organisms With Type E Botulism

Katherine T. Alben, Ph.D. (PI)
Wadsworth Center, New York State Department of Health
Department of Environmental Health Toxicology, SUNY-Albany

Collaborators:
Alicia Perez-Fuentetaja, Ph.D. (co-PI), Department of Biology, SUNY Fredonia
Paul Bowser, Ph.D.; Dr. Rod Getchell, Ph.D., Veterinary Medicine, Cornell University
Bill Culligan, NY Department of Environmental Conservation, Region 9
Kofi Fynn-Aikins, Ph.D.; Betsy Tromer; Mike Goehle, USFWS LGLFR

Type E botulism:

what are the food-web pathways?

Hypothesis:
algal pigments
orange can be used red to trace food-web connections

http://www.combat-fishing.com/basketballbalance.jpg#basketba.png
http://www.csr.state.ny.us/mwater/healthywater/healthy/swimming/index.html
http://www.almityeaquarium.org/"mikef@dolphins.net"
Common to all photosynthetic algae

\[ \beta\text{-}\text{carotene} \quad C_{40}H_{56} \]
\[ \text{retained from diet} \]

\[ \text{chlorophyll a} \quad C_{55}H_{72}\text{MgN}_{4}O_{5} \]

\[ \text{pheophytin a} \quad C_{55}H_{74}N_{4}O_{5} \]
\[ (\text{chla degradation}) \]

\[ \text{pheophorbide a} \quad C_{39}H_{38}N_{4}O_{5} \]
\[ \text{degraded, excreted; fecal pellets} \]

Diatoms

\[ \text{fucoxanthin} \quad C_{42}H_{60}O_{6} \]
\[ \text{diadinoxanthin} \quad C_{40}H_{54}O_{3} \]
\[ \text{diatoxanthin} \quad C_{40}H_{44}O_{2} \]

Lake Guardian phytoplankton survey 1998 (Barbier, Tuchman 2000)

- spring Lake Erie: 93% \( \text{Aulacoseira islandica (centric)} \)
- spring Lake Ontario: 78% \( \text{Aulacoseira islandica (centric)} \)
- summer Lake Erie: 92% \( \text{Fragilaria crotonensis (pennate)} \)
- summer Lake Ontario: 7% \( \text{Fragilaria crotonensis (pennate)} \)
Cryptophytes

- alloxanthin \( C_{40}H_{52}O_2 \)
- lycopene \( C_{40}H_{56} \)

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)
- spring Lake Erie: 5.2% Rhodomonas minuta
- spring Lake Ontario: 13% Rhodomonas minuta
- summer Lake Erie: 17% Rhodomonas minuta
- summer Lake Ontario: 23% Cryptomonas erosa

Chlorophytes

- violaxanthin \( C_{40}H_{58}O_4 \)
- antheraxanthin \( C_{40}H_{56}O_3 \)
- lutein \( C_{40}H_{58}O_2 \)

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)
- spring Lake Erie: not abundant
- spring Lake Ontario: not abundant
- summer Lake Erie: 36% Pediasstrum simplex, Pediasstrum spp.
- summer Lake Ontario: 19% Staurastrum gracile, Oocystis bergeyi, tetraedron min.

Epithitic chlorophytes: ??% Cladophora glomerata, C. vagabonda
Cyanobacteria
myxoxanthophyll $C_{46}H_{66}O_7$
zeaxanthin $C_{40}H_{56}O_2$
canthaxanthin $C_{40}H_{56}O_2$
$\beta$-cryptoxanthin $C_{40}H_{56}O$
echinonone $C_{40}H_{54}O$

Lake Guardian phytoplankton survey 1998 (Barbiero, Tochman 2000)
spring Lake Erie 1% Anacystis montana
summer Lake Erie 10% Microcystis spp.

Chrysophytes
diatoxanthin $C_{40}H_{54}O_2$

Lake Guardian phytoplankton survey 1998 (Barbiero, Tochman 2000)
spring Lake Erie not abundant
spring Lake Ontario not abundant
summer Lake Erie not abundant
summer Lake Ontario 5.5% Dinobryon divergens
Dinoflagellates

peridinin \( \text{C}_{30}\text{H}_{52}\text{O}_{7} \)

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)
- spring Lake Erie: not abundant
- spring Lake Ontario: 9.9% *Gymnodinium helveticum*
- summer Lake Erie: 4.9% *Ceratium hirundinella*
- summer Lake Ontario: 48.2% *Ceratium hirundinella*

Euglenophytes

neoxanthin \( \text{C}_{40}\text{H}_{56}\text{O}_{4} \)

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)
- spring Lake Erie: not abundant
- spring Lake Ontario: not abundant
- summer Lake Erie: not abundant
- summer Lake Ontario: not abundant
### Seasonal changes: Lake Erie

<table>
<thead>
<tr>
<th>Phytoplankton</th>
<th>spring*</th>
<th>summer*</th>
<th>pigments expected in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>diatoms</td>
<td>93%</td>
<td>32%</td>
<td>fucoxanthin C_{42}H_{60}O_{6}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diadinoxanthin C_{40}H_{54}O_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diatoxanthin C_{40}H_{54}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>alloanthin C_{40}H_{52}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lycopene C_{40}H_{56}</td>
</tr>
<tr>
<td>cryptophytes</td>
<td>5%</td>
<td>17%</td>
<td>violaxanthin C_{40}H_{58}O_{4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>antheraxanthin C_{40}H_{56}O_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lutein C_{40}H_{56}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>myxoxanthophyll C_{40}H_{58}O_{7}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>zeaxanthin C_{40}H_{56}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>canthaxanthin C_{40}H_{52}O_{2}</td>
</tr>
<tr>
<td>cyanophytes</td>
<td>1%</td>
<td>10%</td>
<td>β-cryptoxanthin C_{40}H_{56}O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>echinenone C_{40}H_{54}O</td>
</tr>
</tbody>
</table>

* Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

### Seasonal changes: Lake Ontario

<table>
<thead>
<tr>
<th>Phytoplankton</th>
<th>spring*</th>
<th>summer*</th>
<th>pigments expected in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>diatoms</td>
<td>78%</td>
<td>7%</td>
<td>fucoxanthin C_{42}H_{60}O_{6}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diadinoxanthin C_{40}H_{54}O_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diatoxanthin C_{40}H_{54}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>alloanthin C_{40}H_{52}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lycopene C_{40}H_{56}</td>
</tr>
<tr>
<td>cryptophytes</td>
<td>13%</td>
<td>23%</td>
<td>violaxanthin C_{40}H_{58}O_{4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>antheraxanthin C_{40}H_{56}O_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lutein C_{40}H_{56}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>myxoxanthophyll C_{40}H_{58}O_{7}</td>
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<td></td>
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<td></td>
<td>zeaxanthin C_{40}H_{56}O_{2}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>canthaxanthin C_{40}H_{52}O_{2}</td>
</tr>
<tr>
<td>chlorophytes</td>
<td>-</td>
<td>19%</td>
<td>β-cryptoxanthin C_{40}H_{56}O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>echinenone C_{40}H_{54}O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>peridinin C_{36}H_{52}O_{7}</td>
</tr>
</tbody>
</table>

* Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)
### Carotenoids found in the food web

<table>
<thead>
<tr>
<th>Group</th>
<th>Carotenoid</th>
<th>Formula</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms &amp; Fucoxanthin</td>
<td>C₄₂H₆₀O₆</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chrysophytes</td>
<td>Diatoxanthin</td>
<td>C₄₀H₅₄O₂</td>
<td>*</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>Alloxanthin</td>
<td>C₄₀H₅₂O₂</td>
<td>*</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td>Lutein</td>
<td>C₄₀H₅₈O₂</td>
<td>(*)</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Zeaxanthin</td>
<td>C₄₀H₅₆O₂</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>C₄₀H₅₂O₂</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>β-Cryptoxanthin</td>
<td>C₄₀H₅₆O</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Echinenone</td>
<td>C₄₀H₅₄O</td>
<td>*</td>
</tr>
<tr>
<td>Euglenophytes</td>
<td>Neoxanthin</td>
<td>C₄₀H₅₆O₄</td>
<td></td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>Peridinin</td>
<td>C₃₉H₄₂O₇</td>
<td>NF</td>
</tr>
<tr>
<td>Crustacean</td>
<td>Astaxanthin</td>
<td>C₄₀H₅₂O₄</td>
<td>*</td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Biomarkers and time: prey vs predator profiles

<table>
<thead>
<tr>
<th>Tissue Analyzed</th>
<th>Recent Ingestion</th>
<th>Long-Term Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Intestines</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>(Spleen)</td>
<td>Integument (Skin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eye</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gonads, Eggs</td>
<td></td>
</tr>
</tbody>
</table>

[Links:](http://www.dnr.state.mn.us/exotic/exoticanimals/houndgoby/index.html)
### Sample collection: Lake Erie (Lake Ontario)

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smelt</td>
<td>Oct</td>
</tr>
<tr>
<td>Round gobies</td>
<td>Sept - Oct</td>
</tr>
<tr>
<td>Smallmouth bass</td>
<td>Sept</td>
</tr>
<tr>
<td>Rock bass</td>
<td>Sept - Oct</td>
</tr>
<tr>
<td>Black crappie</td>
<td>Sept</td>
</tr>
<tr>
<td>White perch</td>
<td>Sept - Oct</td>
</tr>
<tr>
<td>Yellow perch</td>
<td>Sept</td>
</tr>
<tr>
<td>Spottail shiner</td>
<td>Sept</td>
</tr>
<tr>
<td>Sheephead</td>
<td>2003</td>
</tr>
</tbody>
</table>

**Culligan (NYDEC)**
**Getchell, Bowser (Cornell)**
**Fynn-Aikins, Trometer, Goehle (FWS)**

<table>
<thead>
<tr>
<th>Macroinvertebrates</th>
<th>Collection Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diptera</td>
<td>Sept-Oct</td>
</tr>
<tr>
<td>Oligochaeta</td>
<td>Weekly (Aug)</td>
</tr>
<tr>
<td>Dreissenids</td>
<td>June-Aug (6)</td>
</tr>
</tbody>
</table>

**A. Perez Fuenteaja (SUNY-Fredonia)**

**EPA GLNPO:** late 2003

### Sample preparation: pigments in tissue specimens

**Precautions**

* Specimen storage: -80°C, dark (use dry ice in field)
** Biological safety cabinet (level II) including chemical fume hood

* *protect pigments*  **protect analyst - C. b. type E toxin, pathogens

**Procedures**

1. Dissect specimen
2. Homogenize tissue
3. Freeze dry and determine percent dry weight
   (mussels ~ 5% dw; fish ~ 20% dw)
4. Weigh specimen to be extracted: 75 - 300 mg dw (1500 mg ww)
5. Solvent extract in acetone, sonicate in dark, cold
6. Saponify KOH, methanol; solvent exchange, cleanup, dry
7. Concentrate extract: dry N₂
8. Filter extract, 0.2 μm, into screw-cap vial
HPLC-photodiode array analysis

Chromatograms: retention times, peak areas

Mixed standards
21 carotenoids
6 chlorophylls

Concentration ranges
0.05 - 1.0 μg/mL
1.2 - 20 μg/g dw mussels
0.5 - 5 μg/g dw fish

Spectra (characteristic wavelengths for pigment identification)

Lake Ontario samples, Aug 2002*

Quagga mussels
Crayfish
Gastropods
Cladophora

NY GLPF small grant: Alben, Makarewicz 2002-3
NY Sea Grant 2004-6: Lake Erie (Lake Ontario)

Culligan (NYDEC)
Getchell, Bowser (Cornell)
Fynn-Akins, Trometer, Goehle (FWS)

Fish collection
smelt summer, fall
round gobies summer, fall
smallmouth bass summer, fall
sheepshead summer, fall
yellow perch fall
walleye fall
white perch fall
trot  ????

Others of interest Birds

Diptera

Oligochaetes weekly
quagga mussels weekly

Macroinvertebrates collection

Crustaceans: astaxanthin; fatty acid (FA) esters

FAs saturated monounsaturated PUFAs
C14:0, C16:0, C18:1n9 C18:3ω3, C18:2ω6, C20:5ω3
C18:0, C20:0 C20:4ω6, C22:6ω3

'free' astaxanthin C₄₀H₅₂O₄
C₁₆:0 monoester C₉₆H₁₅₅O₅
C₁₆:0-C₁₈:1 diester C₇₂H₁₁₂O₆

Mollusks, fish, birds - also possible

'free' lutein C₄₀H₅₆O₂
C₁₆:0 mono-C₉₆H₁₈₆O₃
C₁₆:0 diester C₇₂H₁₁₆O₄
(helenien)

Whitney Stocker: http://webby.coe.unc.edu/~stocker/filosaus.html
# Acknowledgements

<table>
<thead>
<tr>
<th>Students - insights</th>
<th>Collaborators - field sites, samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jamie Woodall<strong>xanthin</strong></td>
<td>Joe Makarewicz, SUNY Brockport</td>
</tr>
<tr>
<td>Justin Georgexanthin</td>
<td>Ted Lewis, SUNY Brockport</td>
</tr>
<tr>
<td>Wellington Guzmantein</td>
<td>Alicia Perez Fuentetaja, SUNY Fredonia</td>
</tr>
<tr>
<td>Abdulabasit Lukexanthin</td>
<td>Chris Mayer, University of Toledo</td>
</tr>
<tr>
<td>Kaitlin Harringtonxanthin</td>
<td>Bill Culligan, NY DEC Region 9</td>
</tr>
<tr>
<td>Christine Simmonsanthin</td>
<td>Paul Bowser, Cornell</td>
</tr>
<tr>
<td>Jamie lanaconenone</td>
<td>Rod Getchell, Cornell</td>
</tr>
<tr>
<td>Yuliana De los Santosanthin</td>
<td>Kofi Fynn-Aikins, USFWS</td>
</tr>
<tr>
<td>Ikenna Anakaxanthin</td>
<td>Betsy Trometer, USFWS</td>
</tr>
<tr>
<td>Jessica Reinerxanthin</td>
<td>Mike Goehle, USFWS</td>
</tr>
</tbody>
</table>

**Sponsors - exploratory research projects and foodweb ventures**

- Hudson River Foundation - Polgar Fellowship Program
- McNair Scholars Program and CSTEP, SUNY Albany
- Great Lakes Protection Fund Small Grant Program
- US EPA Great Lakes National Program Office
- New York Sea Grant
Great Lakes Botulism Conference  
Thursday, March 25, 2004  
9 am - 4 pm  
Stull Nature Center  
Presque Isle State Park, Erie, PA

9:00 Welcoming Remarks:  
Helen Domske, New York Sea Grant, Great Lakes Program – University at Buffalo

Botulism Overview/Lake Erie and Ontario 1999–2003:  
Eric Obert, Pennsylvania Sea Grant, Penn State Erie

Ohio Update 2003:  
Frank Lichtkoppler, Ohio Sea Grant, Ohio State University

Pennsylvania Update 2003:  
Bob Wellington, Erie County Health Department, Pennsylvania

New York Update 2003:  
Ken Roblee, Senior Wildlife Biologist, NYSDEC

Canadian Update:  
Jeff Robinson, Canadian Wildlife Service, Ontario

12:00 Lunch - Provided by Pennsylvania Sea Grant

1:00 Research Presentations:

Botulism Caused Fish and Waterbird Mortality in New York Waters of Lakes Erie and Ontario 2003  
Ken Roblee, Senior Wildlife Biologist, NYSDEC

Round Goby Interactions and Relationship to the Botulism Outbreak  
Renea Ruffing, Penn State University

Diagnosing Botulism in Fish in the Lower Great Lakes  
Rodney G. Getchell, College of Veterinary Medicine, Cornell University

Interspecies Toxicity of Type-E Botulinum in Fish: A Bird’s Eye View!  
Adam Yule and Rich Moccia, University of Guelph, Ontario

Carotenoid Tracers of Food Web Pathways for Type E Botulism  
Katherine Alben, NYS Department of Health

3:00 Discussion Session:  
Monitoring and Research Plans for 2004?  
Approaches for a possible Outbreak Decline in coming years?

4:00 Adjourn
## Evaluation Results

**Botulism In Lake Erie Workshop**  
Pennsylvania Sea Grant - New York Sea Grant - Ohio Sea Grant  
Thursday March 25, 2004 > Stull Nature Center – Erie, PA.

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

**50 participants**  
28 surveys returned  
56% return rate

### KEY
1 = Strongly Disagree  
2 = Disagree  
3 = Neither Disagree nor Agree  
4 = Agree  
5 = Strongly Agree

<table>
<thead>
<tr>
<th>Please circle your response</th>
<th>Strongly Disagree</th>
<th>Strongly Agree</th>
<th>N</th>
<th>Mean</th>
<th>+/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) The workshop achieved its goal of sharing information.</td>
<td>1 2 3 4 5</td>
<td>28 4.56 .83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) The workshop achieved its goal of providing networking opportunities.</td>
<td>1 2 3 4 5</td>
<td>28 4.54 .74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) The botulism overview was worthwhile.</td>
<td>1 2 3 4 5</td>
<td>28 4.32 .95</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4) The state &amp; Canadian updates were worthwhile.</td>
<td>1 2 3 4 5</td>
<td>26 4.42 .63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) The research presentations were worthwhile.</td>
<td>1 2 3 4 5</td>
<td>28 4.68 .67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) The discussion session was worthwhile.</td>
<td>1 2 3 4 5</td>
<td>15 4.60 .83</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### The educational materials and content of the workshop:

7) Helped me better understand the issues surrounding botulism in Lake Erie.  
8) Provided information relevant to my work.  
9) Were well organized.  
10) Were easy to understand.  
11) Presented information that will help me.
Please circle your response.

The educational materials and content of the workshop:

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>12) Will be of great use to me.</td>
<td>1 2 3 4 5</td>
<td>27 3.89 .70</td>
</tr>
<tr>
<td>13) I gained new knowledge from this workshop.</td>
<td>1 2 3 4 5</td>
<td>28 4.64 .68</td>
</tr>
<tr>
<td>14) I plan to share the information learned at this workshop with others.</td>
<td>1 2 3 4 5</td>
<td>28 4.36 .99</td>
</tr>
<tr>
<td>15) I plan to take some action as a result of the information I learned at this workshop.</td>
<td>1 2 3 4 5</td>
<td>28 3.71 .98</td>
</tr>
</tbody>
</table>

Please circle or fill in your response.

16) I attended a previous botulism workshop in Erie, PA in 2001, Buffalo, NY in 2002 or Buffalo, NY in 2003. N=28 YES 68% NO 32%

17) I shared the information I learned at previous botulism workshop(s) with others. N=26 YES 73% NO 27%

Who did you share the information with? ______ 19 Respondents ______

18) I took action as a result of what I learned at previous botulism workshop(s). N=25 YES 56% NO 44%

What action did you take? ______ 14 Respondents ______

19) Would you be interested in attending a workshop on this topic next year? N=25 YES 100% NO 0%

20) What could we do to improve this workshop?

11 Respondents

Thank you for completing this questionnaire.
Please return your completed evaluation before you leave or mail it to:
Frank Lichtkoppler, Ohio Sea Grant
99 East Erie Street, Painesville, Ohio 44077
Botulism in Lake Erie Workshop – Evaluation
Thursday, March 25, 2004

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

Who did you share the information with? Open Ended Responses:

1. Colleagues, students
2. Regional staff; administrators
3. General Public
4. Fellow office workers/general public
5. Staff and fish & wildlife users
6. Toxicology students at Cornell; Fish Health researchers at meetings
7. Friends in Ohio – NYSOC employees
8. Public, Administrators, Media
9. Public, colleagues
10. Briefings to IJC Commissioners and IJC Water Quality Board/also OSU Stone Lab fish ecology students
11. Peers and other Great Lakes stakeholders
12. Various sportfishing groups
13. Monroe County Health Department, Irondequoit Bay Coordinating Committee, MC Water Quality coordinating committee
14. Co-workers
15. Colleagues
16. Co-workers, students
17. Limited discussion with Health Department Staff
18. Supervisor, others in my agency, Public
19. Colleagues, sportsman’s groups, Media

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

What action did you take? Open Ended Responses:

1. Planned and executed research project
2. Avian surveys Lake Erie/Ontario
3. Wrote several informational articles on the topic
4. Made more careful observation on Lake Erie
5. Conducted botulism research
6. Collected samples for investigators
7. Research initiation
8. Included in oral and written briefings on the changing Lake Erie ecosystem
9. Increased literature research
10. Benefit in evaluations NYSG proposal for funding
11. Coordinated observation at Ontario Beach submitted moribund gulls to NYSDEC
12. Applied for grant money to do research
13. Small amount of preparation in case of bird or fish die off
14. Share information with co-workers and public

Item 20. What could we do to improve this workshop?

Open Ended Responses:

1. Provide detailed handout prior to event
2. Like the informality!
3. To be in the acknowledgements
4. Spend more time on discussion of research direction/opportunities – have updates posted or handed out beforehand and ask for any questions rather than spend half day on updates.
5. Appropriate division of program between die-off updates and research updates. Good one-day format. My only suggestions having “noon-to-noon” meeting with overnight in the middle for more networking and informal interactions.
6. Provide print outs of the power point presentations, the authors are okay with it
7. Very nicely done
8. Don’t use Styrofoam plates for lunch!
9. The greatest value of these workshops is the coordination of research effort and the exchange of information. It may be helpful to select research topics that compliment each other to the extent possible, or at least clearly relate to the topic of the meeting.
10. This was a well-organized conference. Nice job on achieving a good mix of issues relevant to Botulism (fish, birds, zooplankton, mussels, etc.)
11. Very well organized and excellent presentations. Thank you!
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March 25, 2004

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