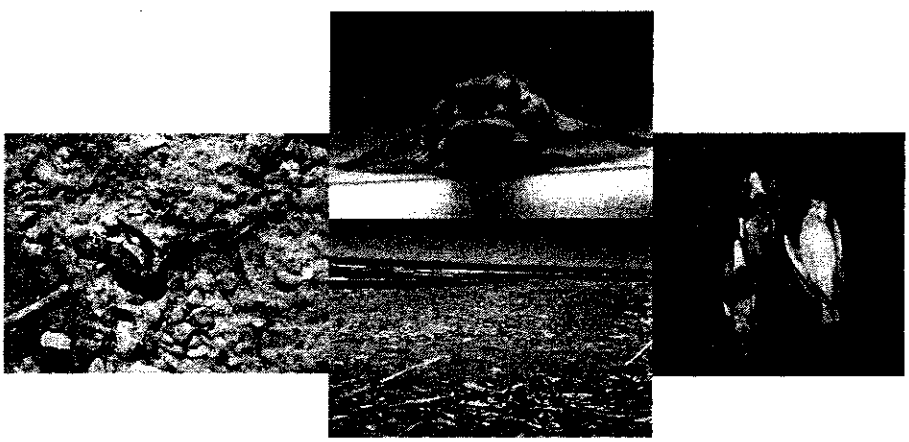
NYSGI-W-04-001 C2

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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Bill Culligan, NYS Department of Environmental Conservation
Helen Domske, New York Sea Grant Extension

Publication Production:
Ellen George, New York Sea Grant
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 Moccia, Dr. Ian 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
Erie 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
Erie 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
Erie 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 mudpuppy 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:

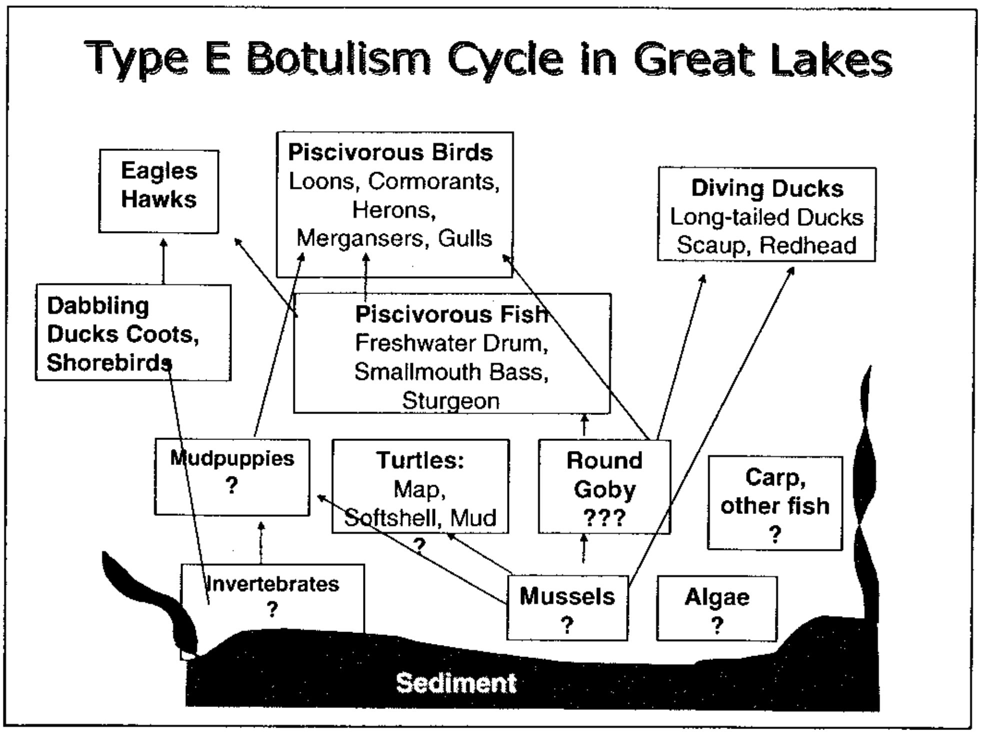
- **Dr. Campbell** – Gull mortality summer to fall. Loon and merganser die-off in late fall. Die-offs seem to occur following changes in weather.
- **Dr. Baker** – Suggested to look for perturbations on the ecosystem, like significant change in wind or weather patterns.
- **Dr. Murphy** – Suggested that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



Presenters 2002

- Ward Stone (NYDEC) -- Presented overview of pathological work conducted in his lab in 2001.
- 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 *Cladaphora* 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, *Glugea*
  - 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

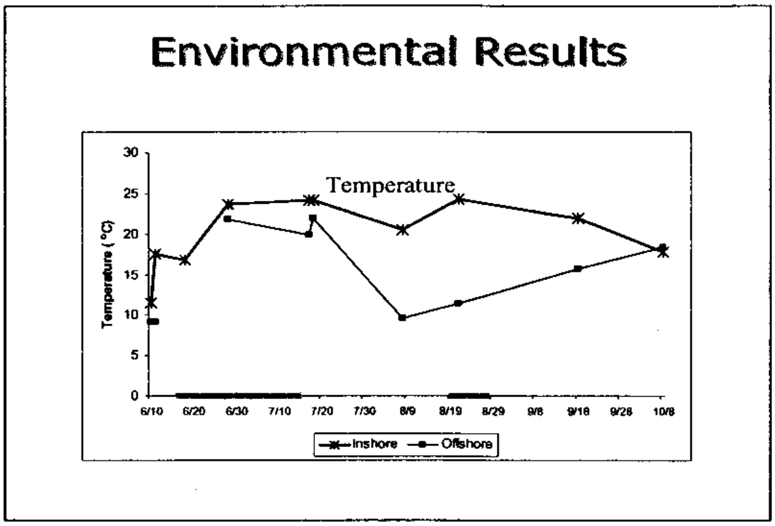
Grace McLaughlin (USGS) Type E botulism Outbreaks in the Great Lakes

Year	Lake	Number	1° Species
1963-4	Michigan	>12,000	Gulls, Loons
1976-83	Michigan, Huron	>1800	Gulls, Loons
1998-2002	Huron, Michigan	~2500	Mergansers, Gulls, Loons
1999-2001	Erie	>25,000	Mergs, Gulls, Loons
2002	Erie	>25,000	Long-tailed ducks, Gulls Loons, Mergansers Cormorants

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.



Perez *et al.*

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.*

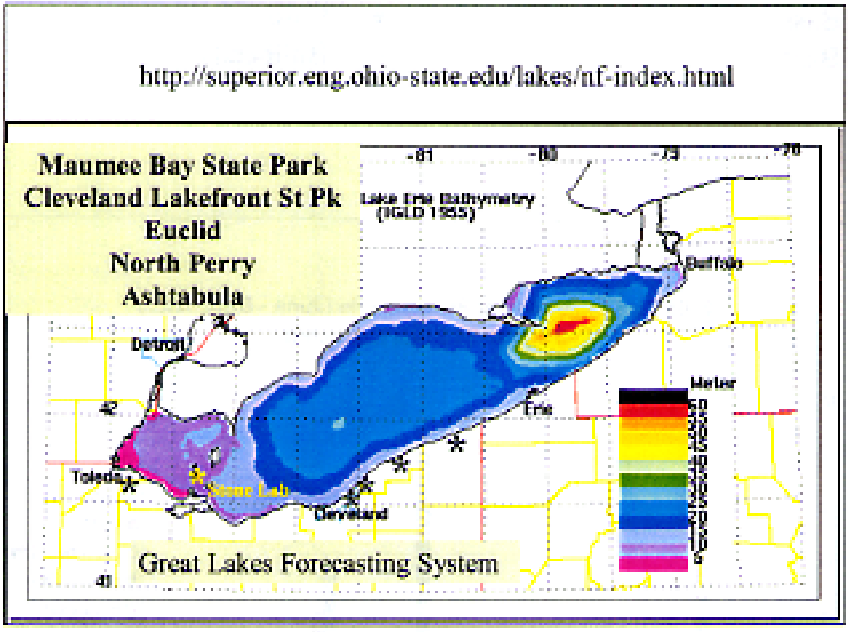
- LOSS OF EQUILIBRIUM: In a natural setting, fish showing equilibrium loss could represent "easy" prey for live-fish eating birds. Thus, such birds could be targeting intoxicated fish due to their abnormal behavior.
- BREACHING BEHAVIOR: In a natural setting, fish showing breaching behavior would present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



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:

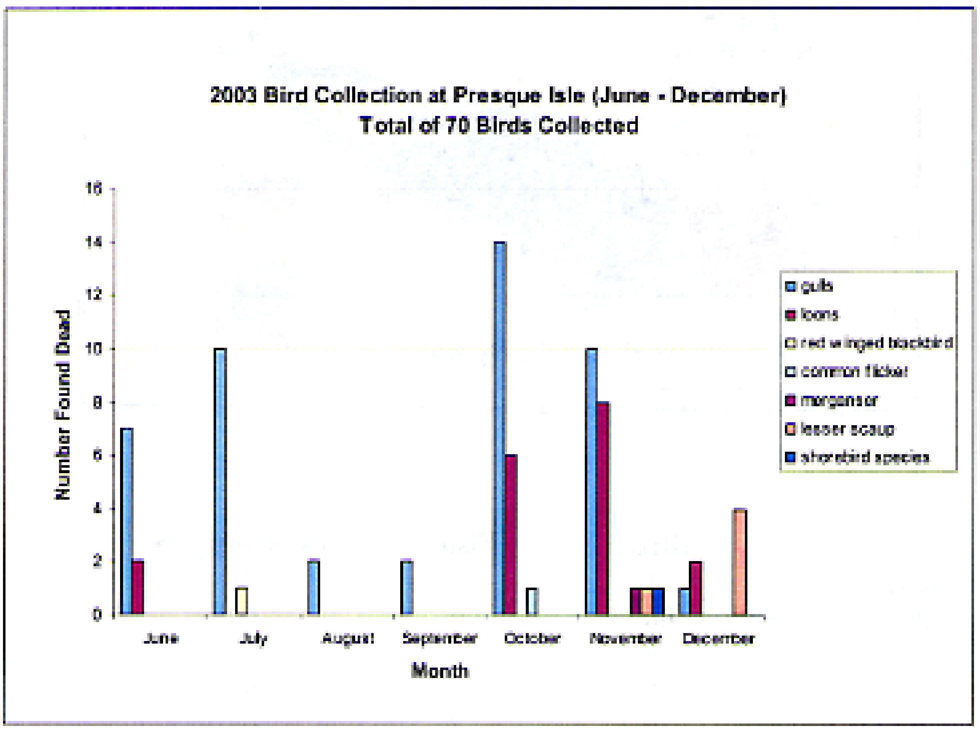
- No significant fish or bird kills in 2003 were reported by the manager of Stone Laboratory, which is located on Gibraltar Island.
- Only a few, isolated (in space and time) dead birds or fish were observed by five volunteers who lived on or regularly worked on the Ohio Lake Erie shoreline.
- "No big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

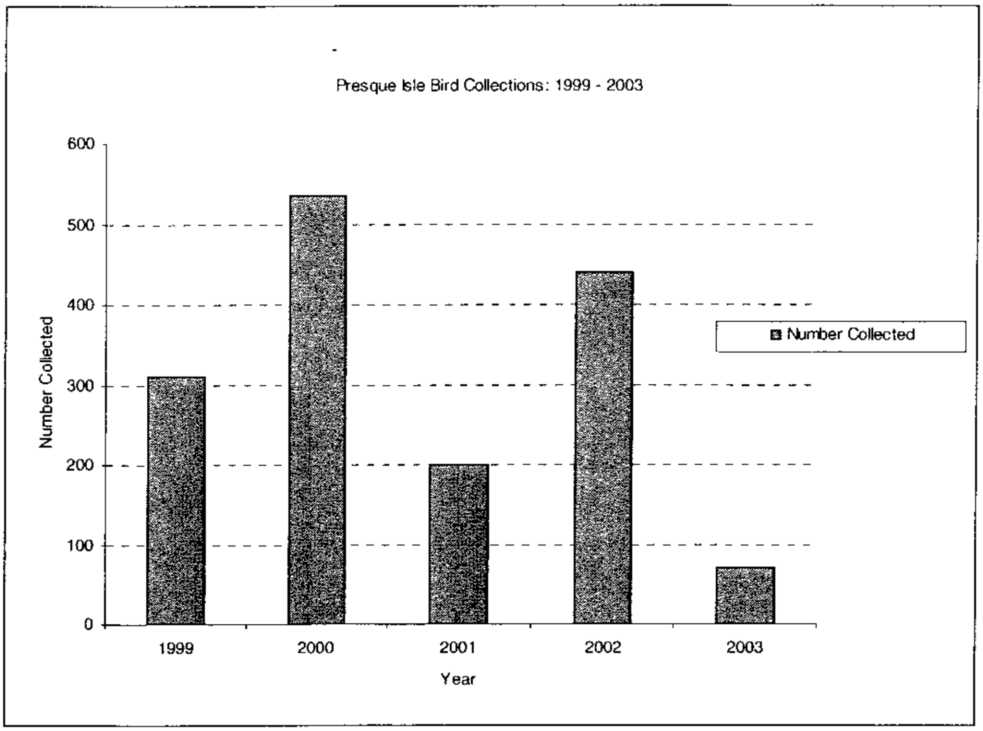
Pennsylvania Lake Erie 2003 Botulism Update

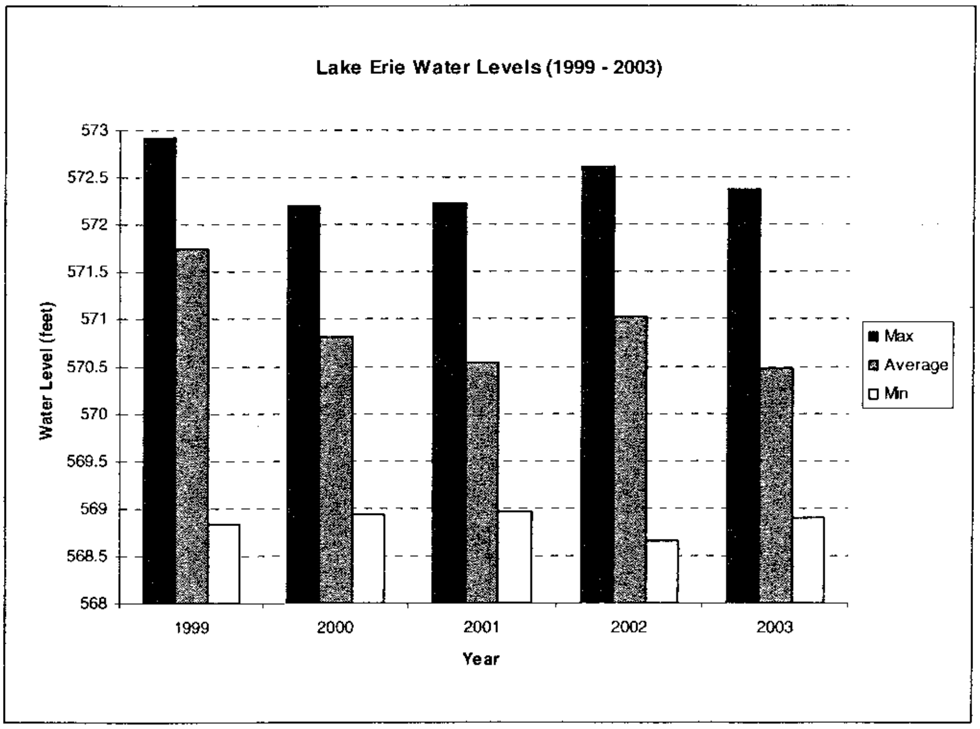
Bob Wellington
Erie County Health Department

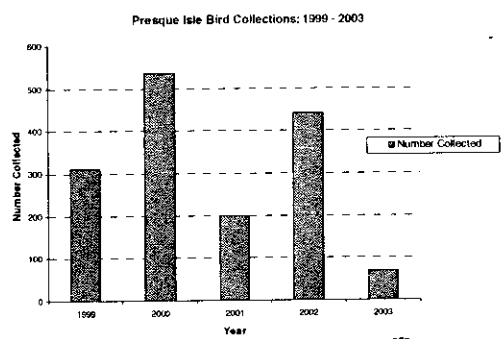
Large Fish Die-Off in Presque Isle Marina noted June 11, 2003

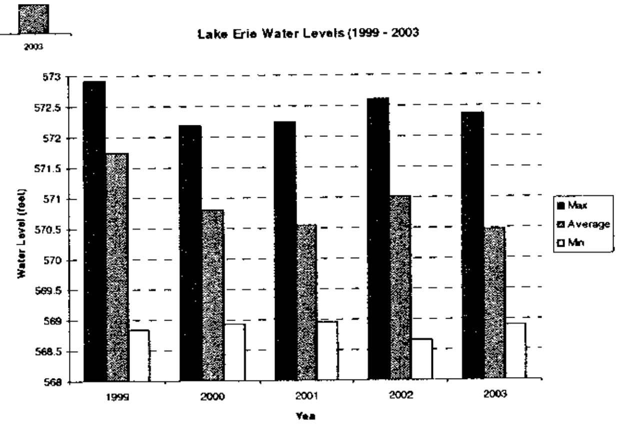
- Bluegill
- Sunfish
- Largemouth Bass
- Smallmouth Bass
- Freshwater Drum
- Yellow perch
- Carp
- Bullheads

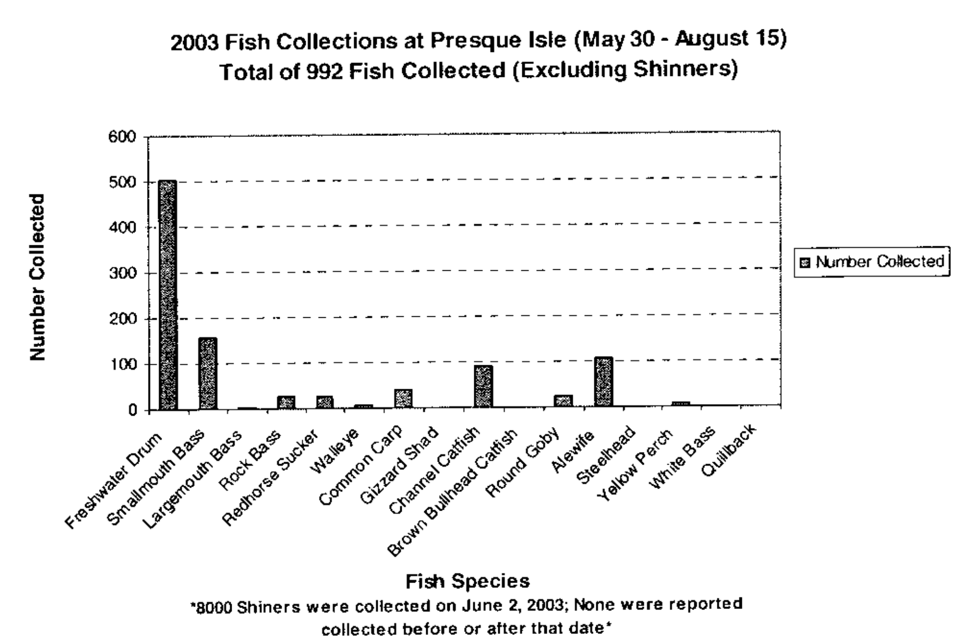



Presque Isle Bird Collections: 1999 - 2003
600
500
400
300
200
100
0
Number Collected
1999
2000
2001
2002
2003
Year
Number Collected

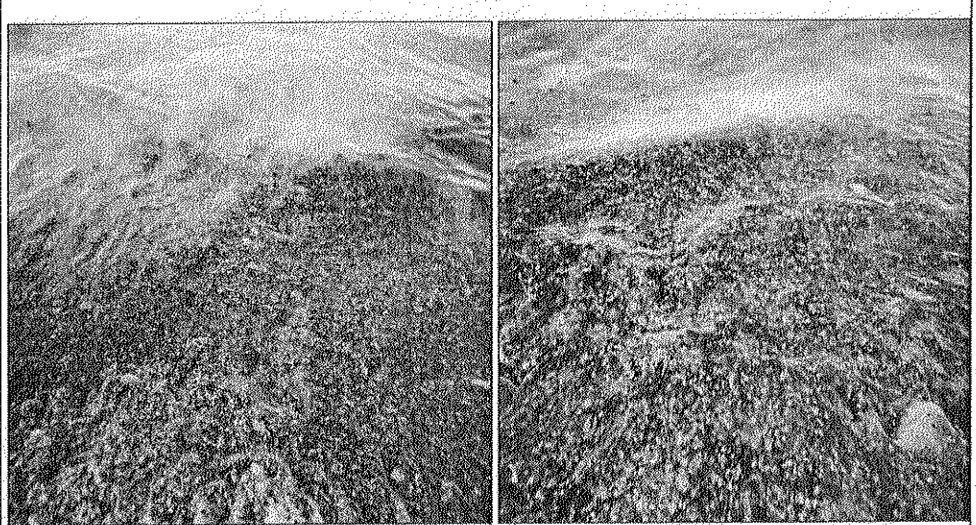

Lake Erie Water Levels (1999 - 2003)
573
572.5
572
571.5
571
570.5
570
569.5
569
568.5
568
Water Level (feet)
1999
2000
2001
2002
2003
Year
Max
Average
Min


Presque Isle Bird Collections: 1999 - 2003
Number Collected
Year
1999
2000
2001
2002
2003
Number Collected

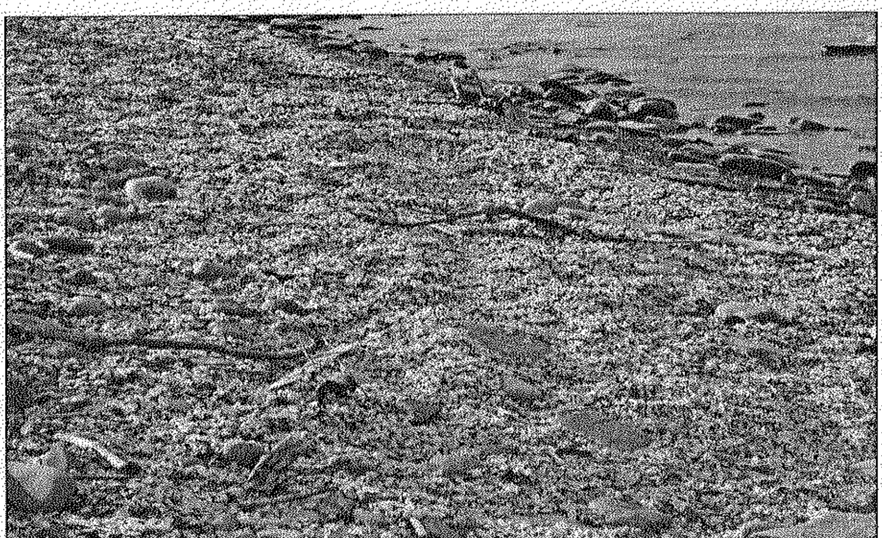

Lake Erie Water Levels (1999 - 2003
Water Level (feet)
573
572.5
572
571.5
571
570.5
570
569.5
569
568.5
568
1999
2000
2001
2002
2003
Max
Average
Min


2003 Fish Collections at Presque Isle (May 30 - August 15)
Total of 992 Fish Collected (Excluding Shinners)
Number Collected
600
500
400
300
200
100
0
Number Collected
Freshwater Drum
Smallmouth Bass
Largemouth Bass
Rock Bass
Redhorse Sucker
Walleye
Common Carp
Gizzard Shad
Channel Catfish
Brown Bullhead Catfish
Round Goby
Alewife
Steelhead
Yellow Perch
White Bass
Quillback
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

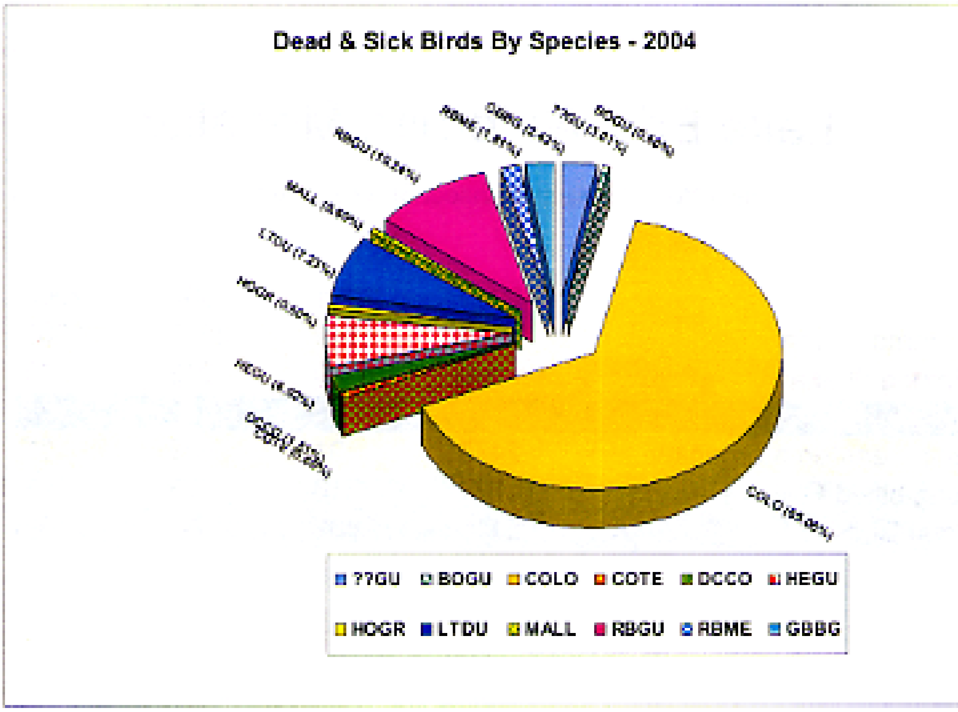
May 5, 2003 – DEC inspection of the Bethlehem Steel ring-billed gull colony shows no evidence of botulism mortality in gulls.

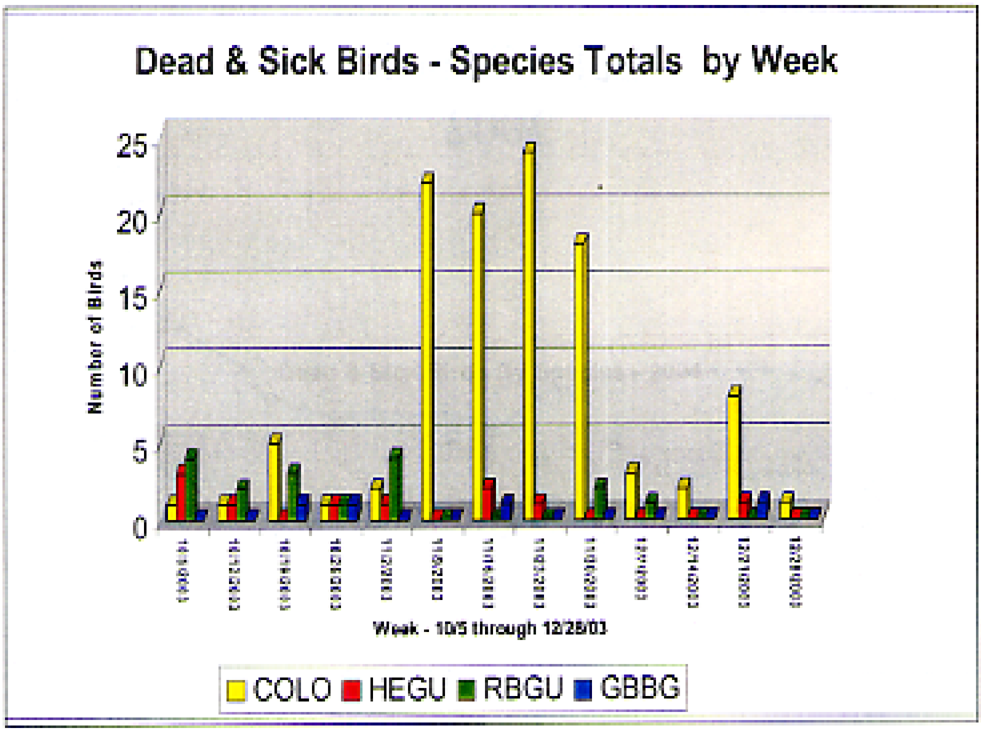
November 4, 2003 – Type E botulism was confirmed in a gull from Dunkirk (Lake Erie) by George Hannett of the NYSDOH.

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

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

Species	Predicted Mortality	Upper Limit 95% Confidence	Lower Limit 95% Confidence
Common Loon	1969	2759	1179
Horned Grebe	18	52	0
Double-crested Cormorant	55	129	0
Mallard	18	52	0
Long-tailed Duck	219	502	0
Red-breasted Merganser	55	129	0
Bonaparte's Gull	18	52	0
Ring-billed Gull	292	436	147
Herring Gull	182	285	79
Great Black-backed Gull	73	151	0
Unidentified Gull	91	172	10
Common Tern	18	52	0
Total Birds	3008	4000	2016

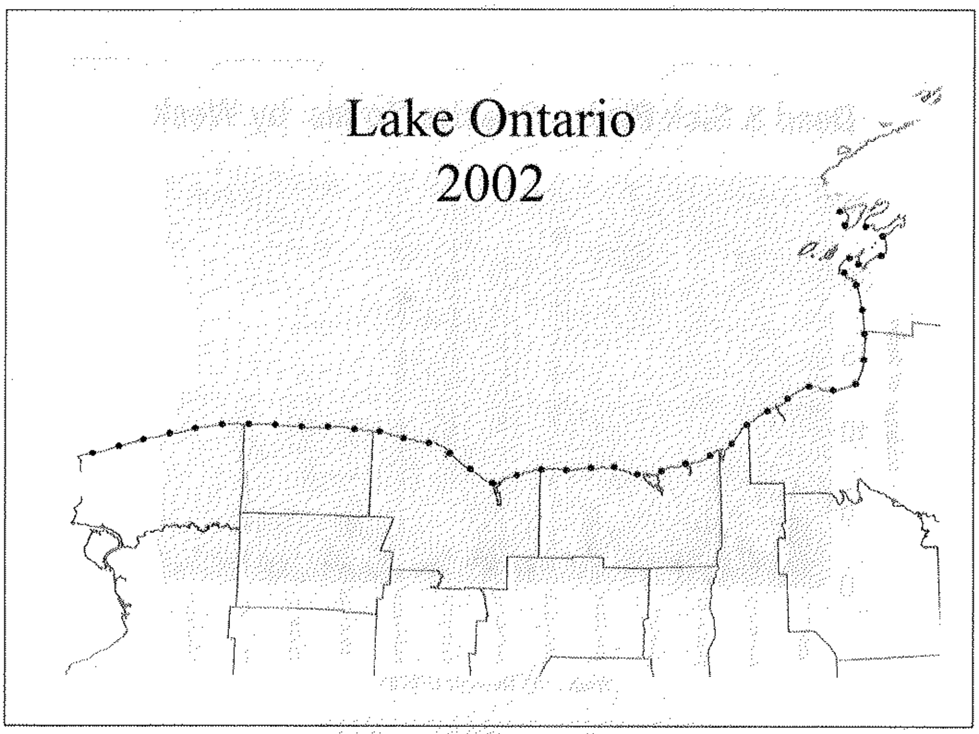

Dead & Sick Birds By Species - 2004
??GU
BOGU
COLO
COTE
DCCO
HEGU
HOGR
LTDU
MALL
RBGU
RBME
GBBG


Dead & Sick Birds - Species Totals by Week
25
20
15
10
5
0
Number of Birds
Week - 10/5 through 12/28/03
COLO
HEGU
RBGU
GBBG

Lake Erie Waterbird Mortality

New York Botulism Transect Survey Results

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



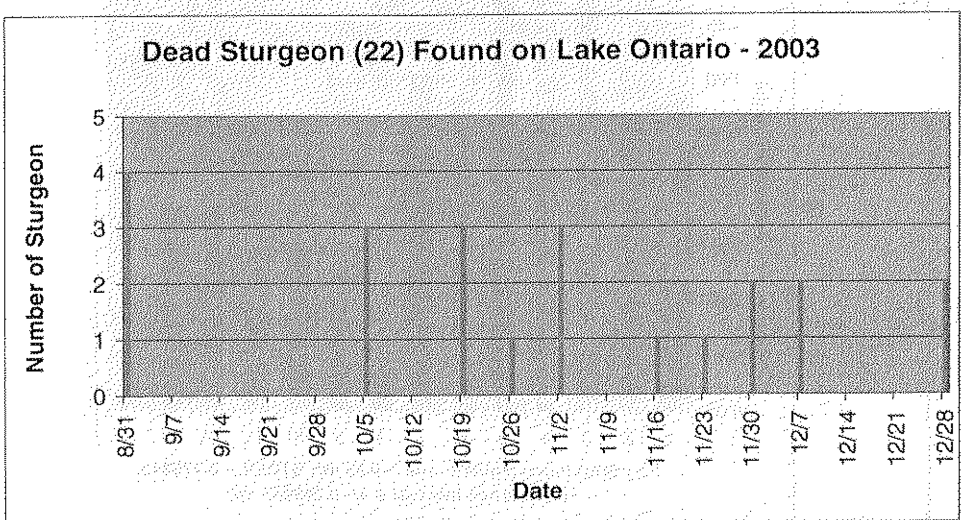
March 23, 2004 – Type E botulism was confirmed by the University of Pennsylvania in a ring-billed gull collected near Youngstown, Lake Ontario, in 2003.

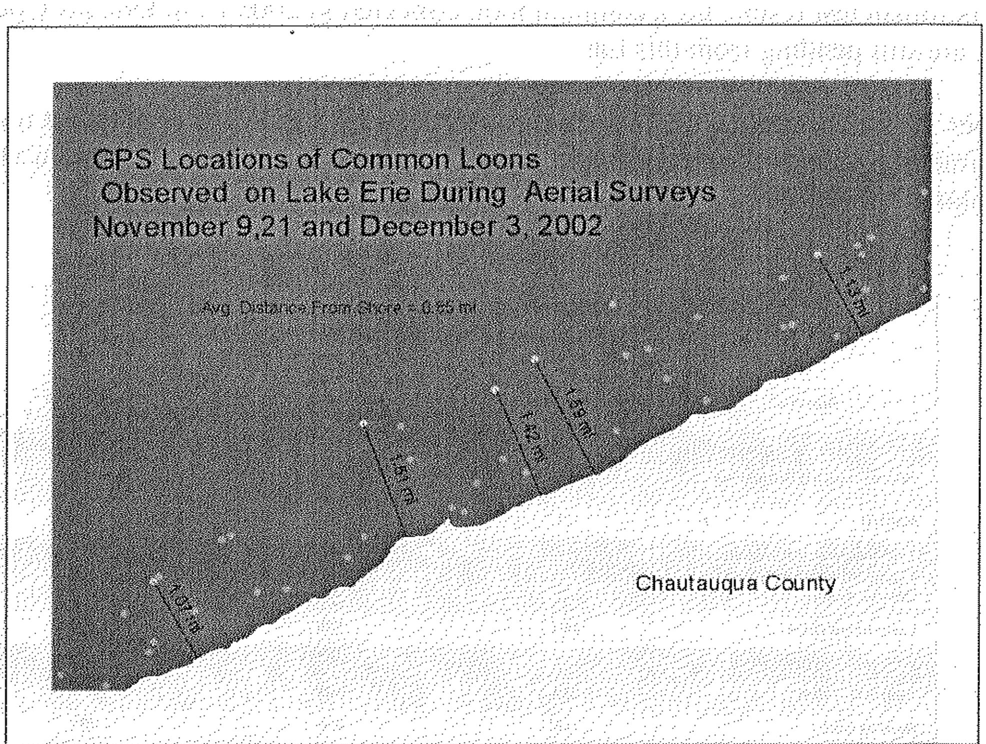
Type E botulism test results for a common loon collected in 2003 from Orleans County, Lake Ontario, are still pending from this Lab.

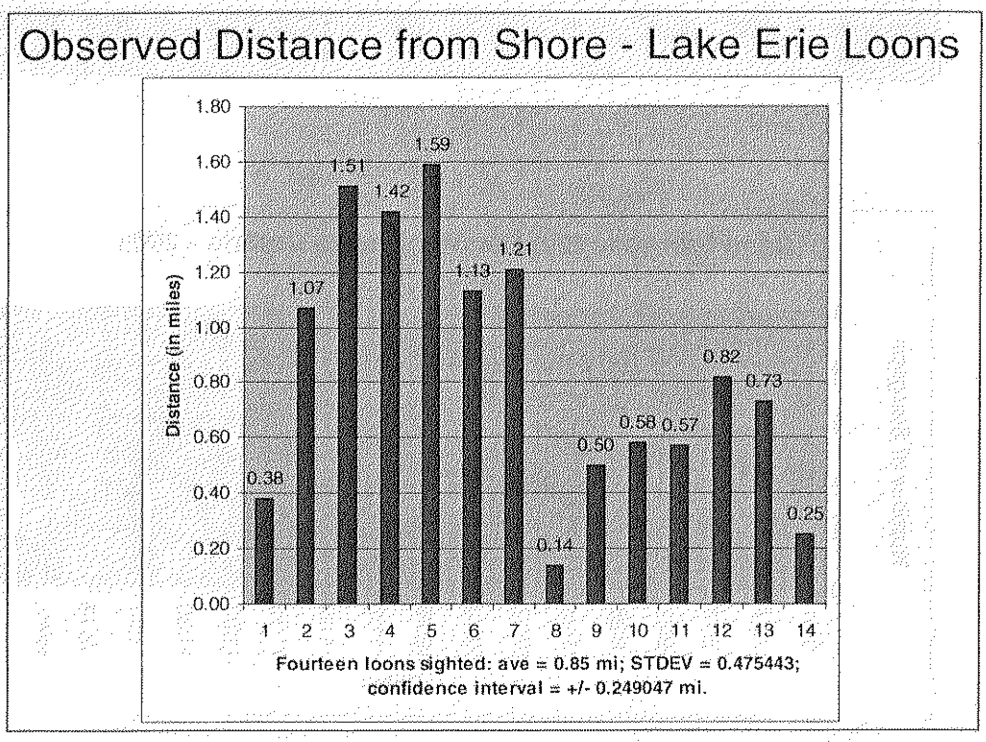
December 12, 2003 – Type E botulism toxin was detected by George Hannett of the NYSDOH in the alimentary canal contents of a lake sturgeon collected at Wilson-Tuscarora State Park, Lake Ontario, on October 27, 2003.

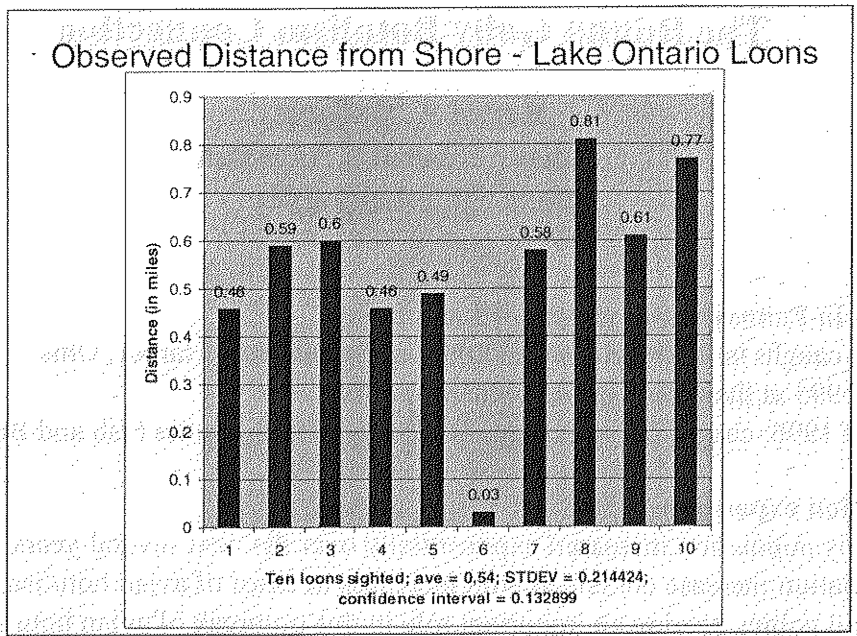
Table 2: New York Shoreline Lake Ontario Botulism Mortality Surveys
100m Transect Survey Results
NYSDEC 10/09/03-12/29/03

Species	Predicted Mortality	Upper Limit 95% Confidence	Lower Limit 95% Confidence
Common Loon	132	238	25
Double-crested Cormorant	66	128	4
Mallard	16	49	0
Red-breasted Merganser	16	49	0
Greater Scaup	33	78	0
Long-tailed Duck	263	525	2
White-winged Scoter	280	487	72
Ring-billed Gull	263	552	0
Herring Gull	164	294	35
Great Black-backed Gull	66	128	4
Unidentified Gull	148	291	5
Common Goldeneye	49	121	0
Red-throated Loon	16	49	0
Total Birds	1529	2438	621




GPS Locations of Common Loons
Observed on Lake Erie During Aerial Surveys
November 9,21 and December 3, 2002
1.59 mi
1.42 mi
Chautauqua County


Observed Distance from Shore - Lake Erie Loons
Distance (in miles)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.38
1.07
1.51
1.42
1.59
1.13
1.21
0.14
0.50
0.58
0.57
0.82
0.73
0.25
1 2 3 4 5 6 7 8 9 10 11 12 13 14
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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Noelle Rayman
Steve Robb
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Jason Telecky
Mike Todd
Ben Weber
Tony Zerkle

The Round Goby Botulism Connection

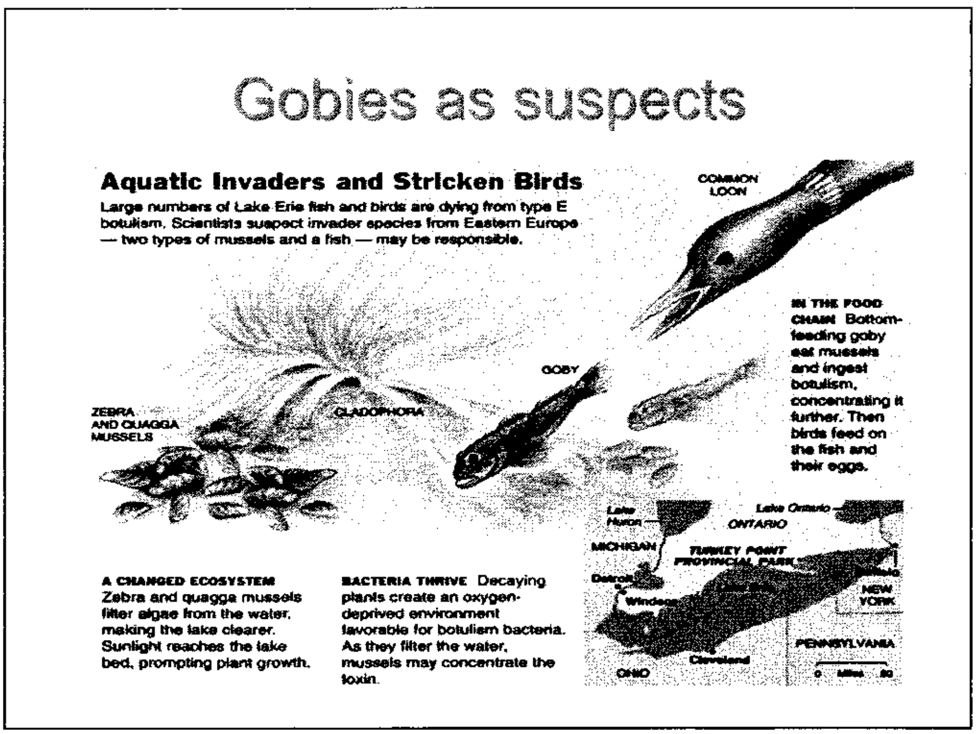
Renea A. Ruffing
Graduate Research Assistant
Penn State University

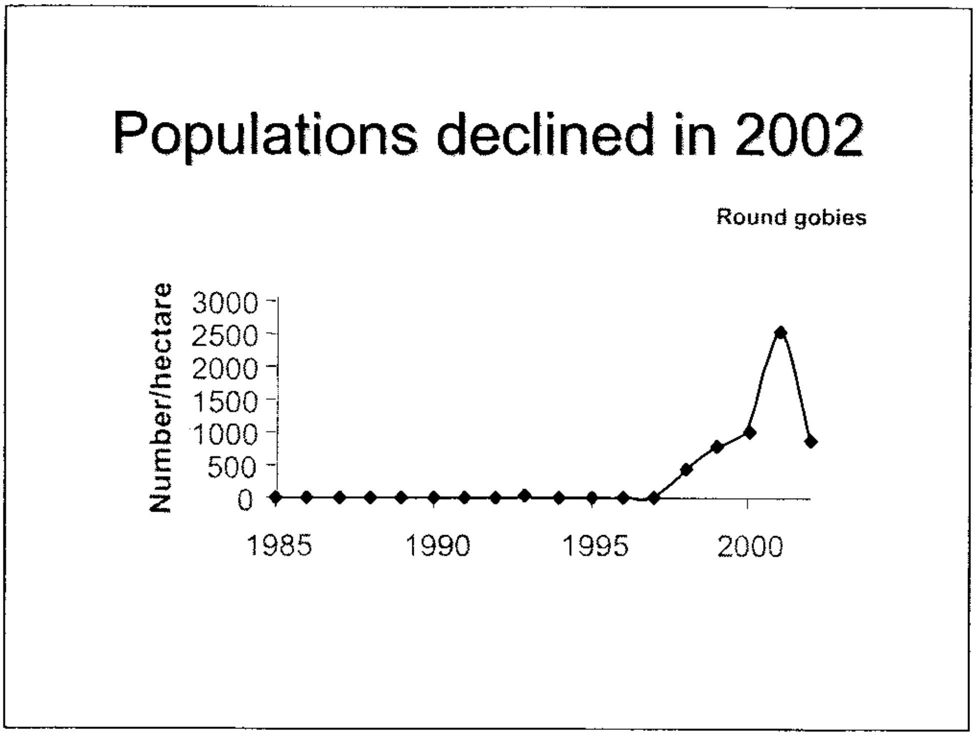
Round gobies in Pennsylvania waters of Lake Erie

- 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?





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.

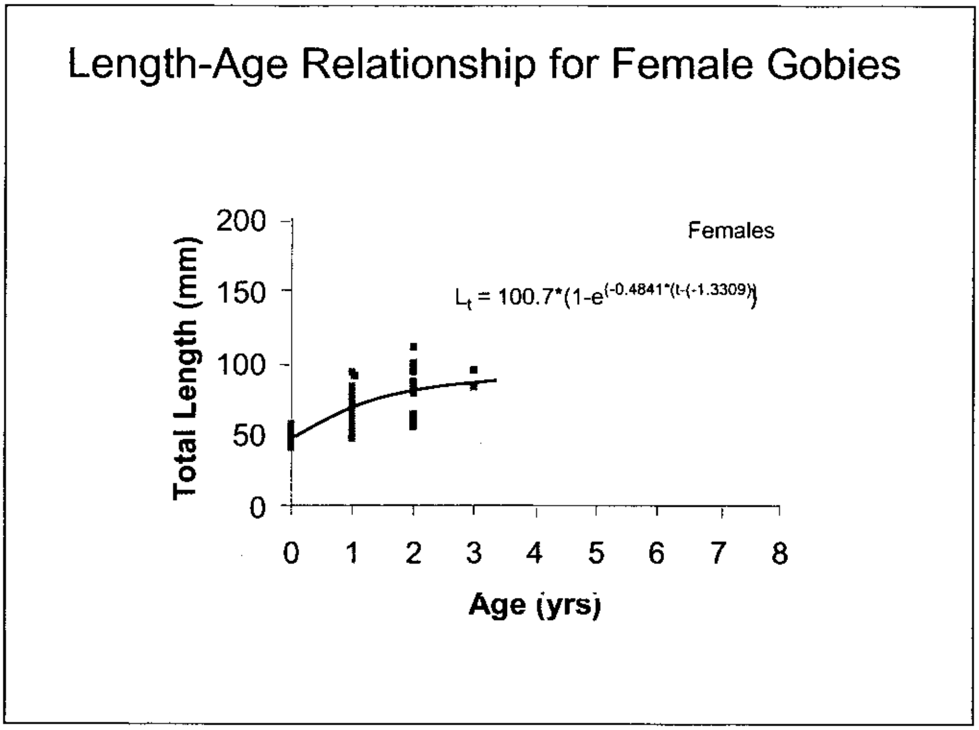
Sick Phase

- 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.

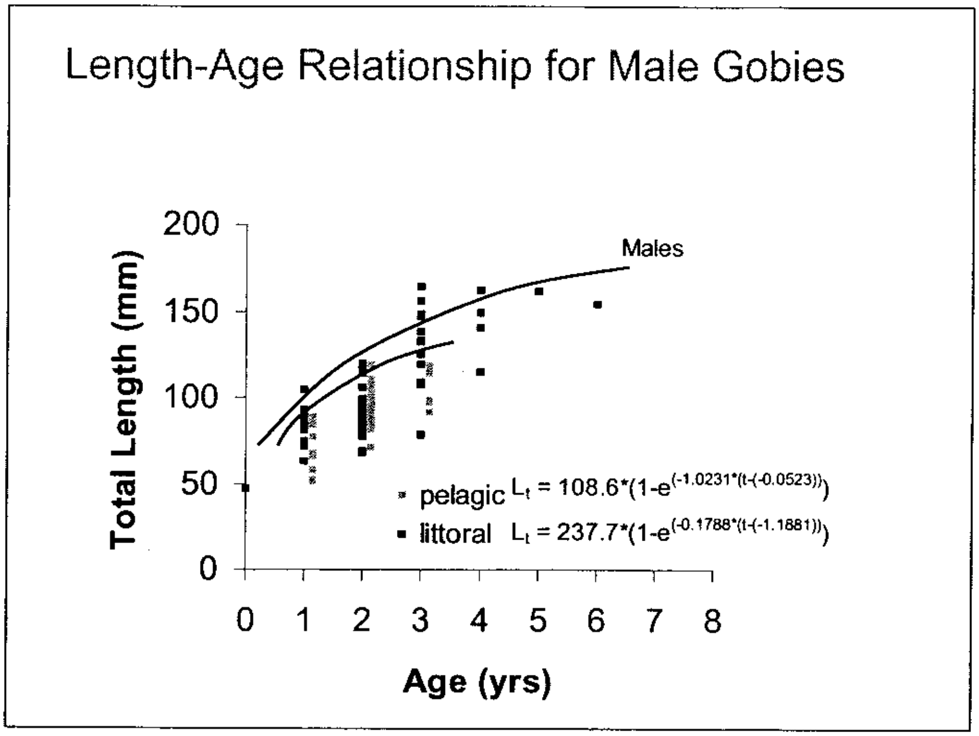
Goby Studies

- 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 Relationships for females

- The Von Bertalanffy equation for female gobies.
- $L_l=100.7*(1-e^{(-0.4841*(t-(-1.3309))})$
- 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.

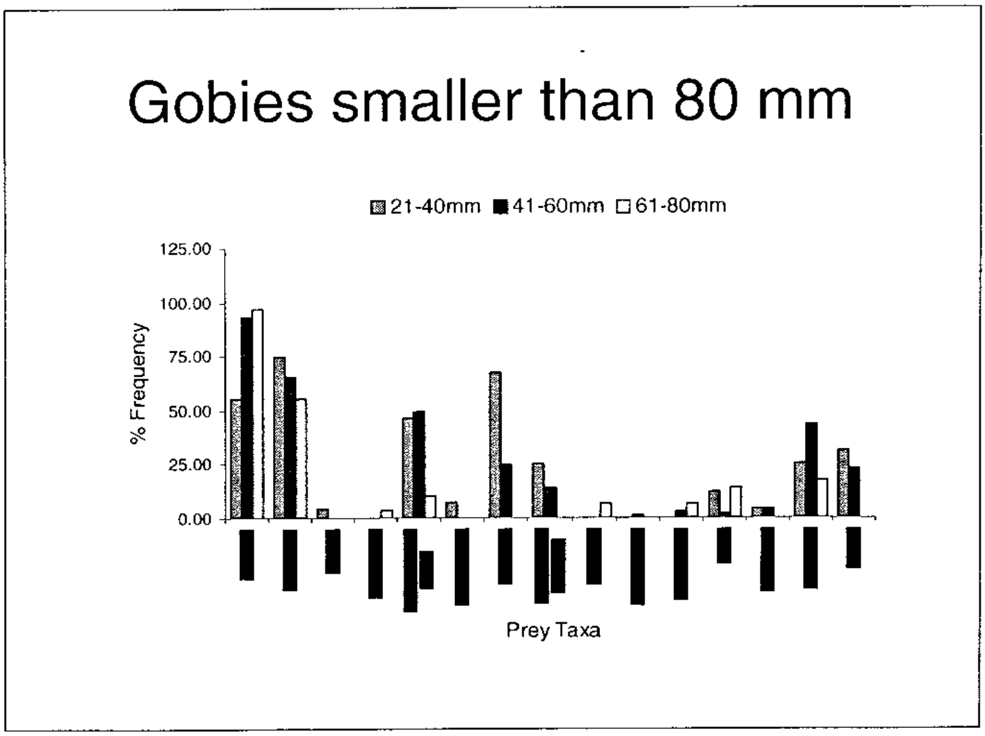


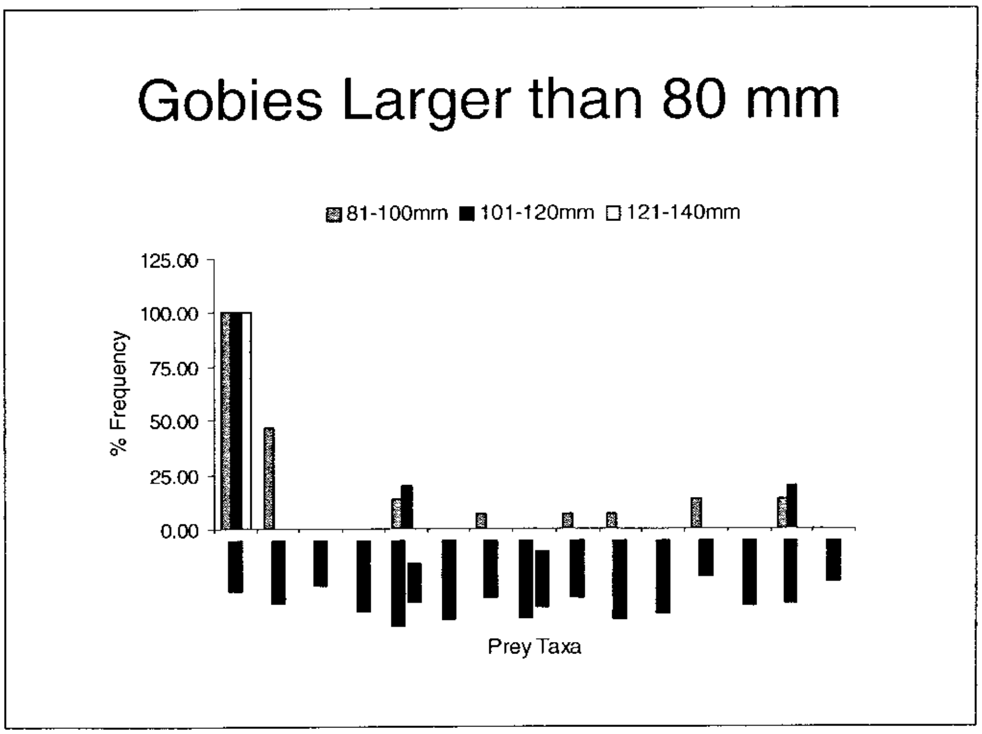
Length Age Relationships for Males

- 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

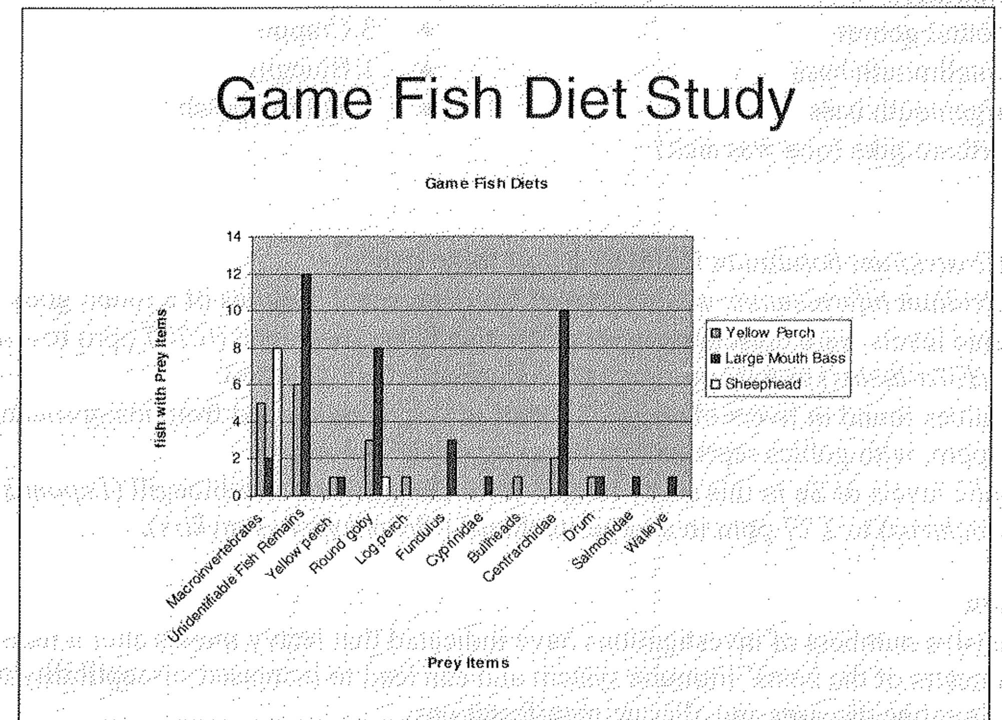
- Two different studies in which several different comparisons were made.
- Differences between stream vs. lake gobies.
- Diets of males vs. females.
- Comparisons of diets among different size classes.


Gobies smaller than 80 mm
21-40mm 41-60mm 61-80mm
125.00
100.00
75.00
50.00
25.00
0.00
% Frequency
Prey Taxa


Gobies Larger than 80 mm
81-100mm 101-120mm 121-140mm
125.00
100.00
75.00
50.00
25.00
0.00
% Frequency
Prey Taxa

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

- June 2001 - Oct 2003 PAFBC collected 927 yellow perch.
- 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 Sultan 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 Erie 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.

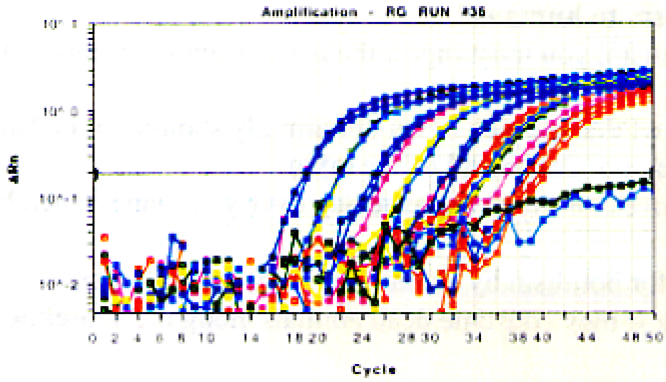
Sample processing and DNA Extraction

- 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

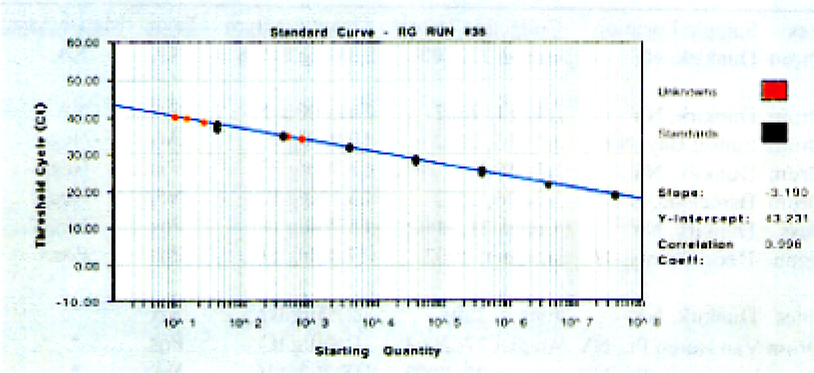
- After DNA is isolated, we can look for the toxin gene using a standard PCR amplification of a 139 base pair fragment to demonstrate the presence or absence of *C. botulinum* Type E.
- But, quantitative (real-time) PCR will provide actual numbers of *C. botulinum* Type E per gram of tissue when compared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

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

	2002	2003
Lake Erie		
Spring	265	175
Summer	176	71
Fall	186	282
Lake Ontario		
Spring	8	21
Summer	71	75
Fall	30	54

	2002	2003
Smallmouth bass	286	295
Freshwater drum	208	166
Round goby	148	185
Yellow perch	24	30
Alewife	20	0
Brown bullhead	11	0
Other species	41	2

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

Species	Sample Location	Collection Date/s	Quantity/Gram	Toxin	Mouse Assay
FWDrum	Dunkirk, NY	August 17, 2001	3,000/g K,L,S	NA	NA
FWDrum	Dunkirk, NY	July 11, 2002	208,000/g IC	NA	NA
FWDrum	Sunset Bay, NY	July 18, 2002	10,900/g IC	Pos.	Neg.
FWDrum	Dunkirk, NY	July 18&30, 2002	21,700/g IC	Pos.	Neg.
FWDrum	Barcelona, NY	July 26, 2002	23,100/g IC	Neg.	Neg.
SMBass	Dunkirk, NY	August 21, 2002	15,200/g IC	Pos.	Neg.
Sturgeon	Door County, WI	Summer, 2002	17,400/g SC	Pos.	Pos.
RGobies	Dunkirk, NY	June 4, 2003	2,700/g IC	NA	*
FWDrum	Van Buren Pt., NY	August 27, 2003	1,100/g IC	Pos.	*
FWDrum	Van Buren Pt., NY	August 27, 2003	15,200/g IC	Neg.	*
FWDrum	Van Buren Pt., NY	August 27, 2003	42,300/g IC	Pos.	*

IC – Intestinal contents; SC – Stomach contents included two goby-like fish; K,L,S – Combined kidney, liver, and spleen. POS or Neg – Positive or negative assay with botulism toxin rapid detection kit (Osborn Scientific Group, Lakeside, AZ). NA – Not assayed, no tissue available; *Mouse bioassay results pending.

WI Sturgeon QPCR Results

Species	Sample Location	Collection Date/s	Quantity/Gram	Toxin	Mouse Assay
Sturgeon	Green Bay, WI	July 25, 2003	250,000,000/g Li	Neg.	*
			3,000,000/g SC	NA	
Sturgeon	Green Bay, WI	July 28, 2003	15,900,000/g Li	NA	*
			4,000,000/g IC	NA	
			10,700,000g/ SC	NA	
Sturgeon	Green Bay, WI	August 6, 2003	2,400,000/g IC	NA	*
Sturgeon	Green Bay, WI	August 13, 2003	6,900,00/g Li	NA	
			2,300,000/g IC	NA	
			2,700,000/g SC	NA	
Sturgeon	Green Bay, WI	August 14, 2003	780,000/g IC	NA	
			39,900,000/g SC	Pos.	*
Sturgeon	Green Bay, WI	Sept. 5, 2003	570,000/g IC	Pos.	*
Sturgeon	Green Bay, WI	Sept. 17, 2003	4,170,000/g Li	NA	*
			977,000/g IC	NA	
			42,000/g SC	NA	

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 (Osborn Scientific Group, Lakeside, AZ); NA – Not assayed, no tissue available; *Mouse bioassay results pending.

2001 Avian *C. botulinum* Type E QPCR Results

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

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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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

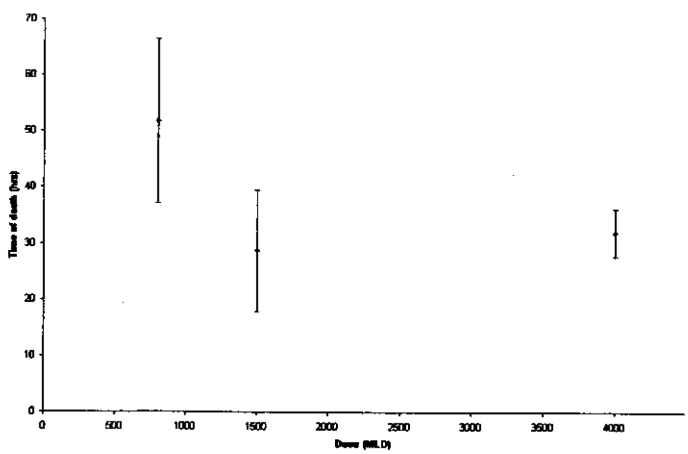
Lake Erie Bird Mortalities
- Type E botulism is considered the primary cause of avian deaths.

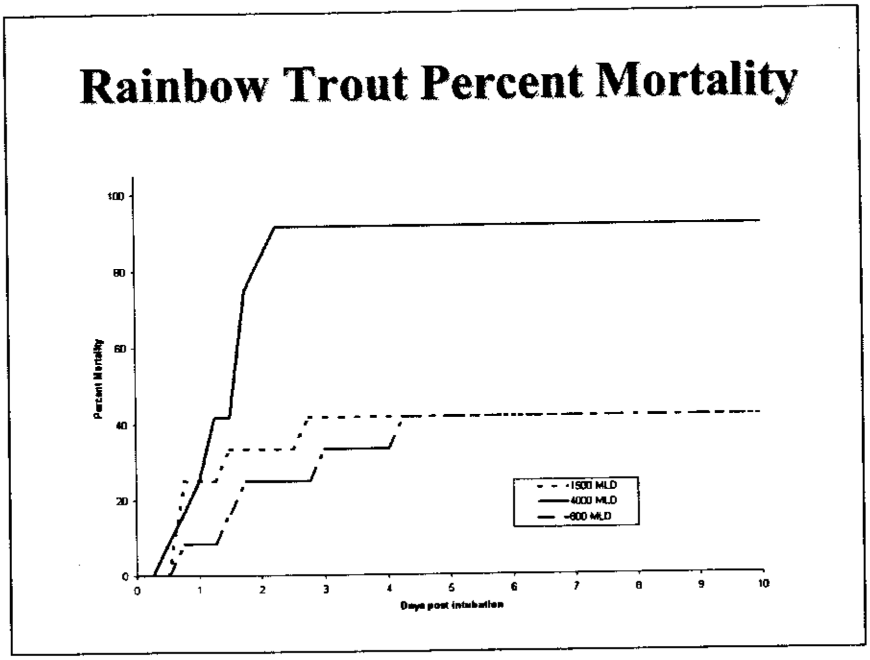
The Paradox?
- How to explain the 'fish' pathway of toxin ingestion in birds which feed exclusively on 'live' fish (e.g. loons, common and red-breasted mergansers, grebes)?
- Lack of apparent correlation between fish botulism epizootics and avian mortality patterns.

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



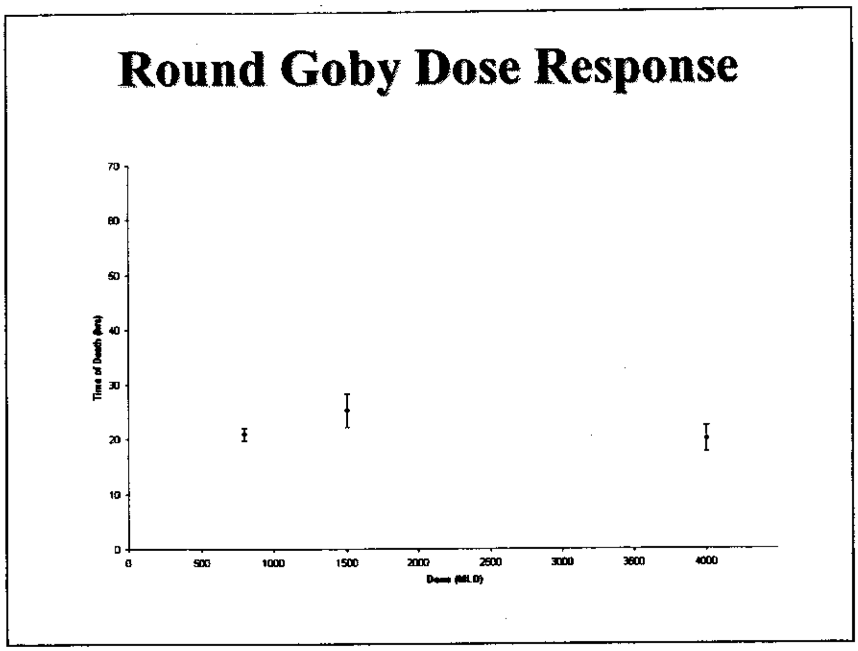


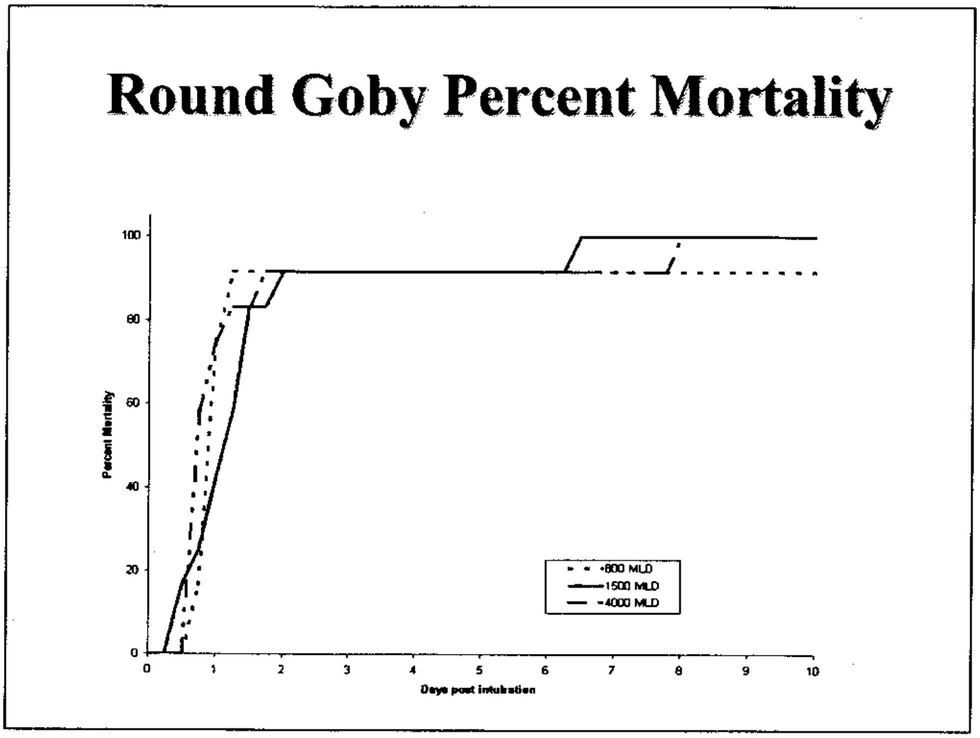
Goby - Onset of Pigment Change

- Normal, pale coloration.
- Very early onset of darkening pattern.

Progressive Discoloration

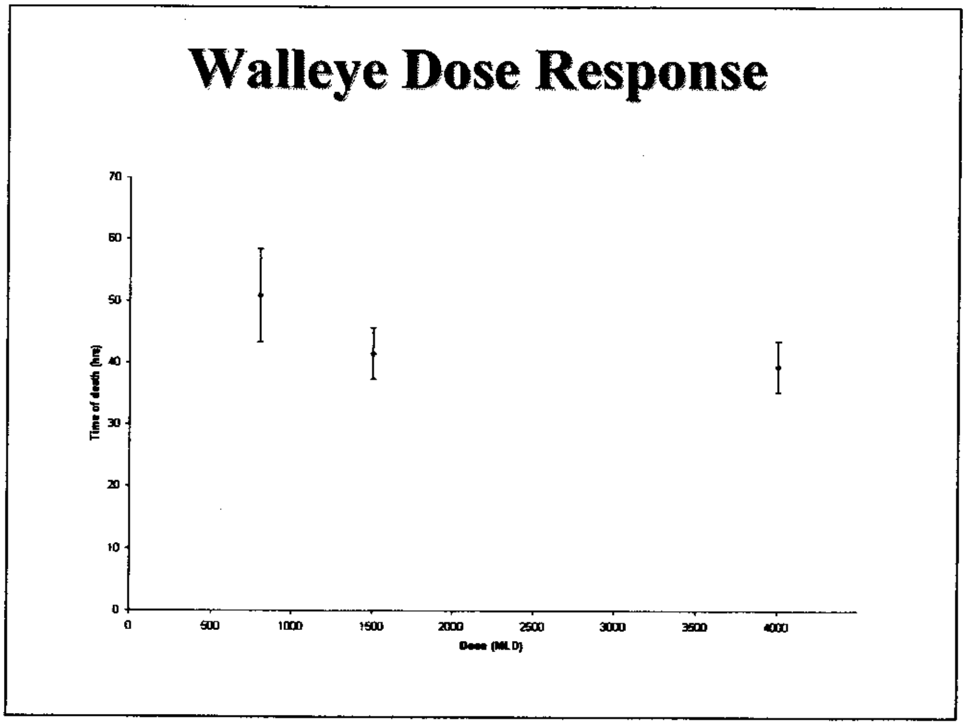
- Formation of the progressive, pigment 'band.'
- Complete pigment change, followed closely by death.



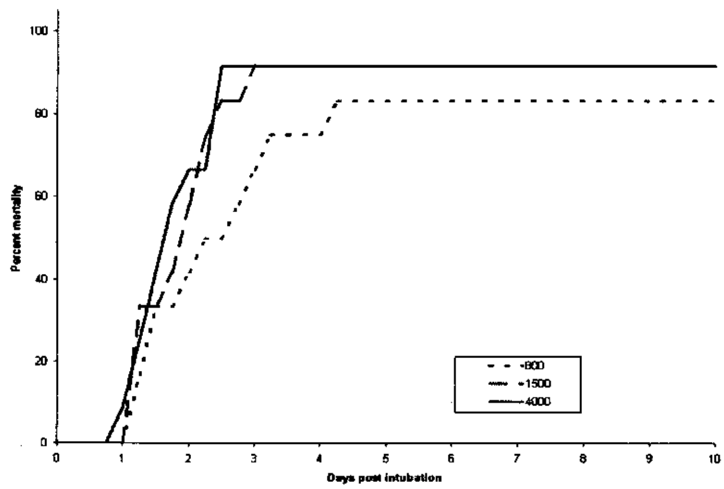


Walleye: Onset of Respiratory Distress

- Severe distention of operculum: erratic swimming behavior (often breaching surface).
- Fish exhibiting the "head up-tail down" orientation.



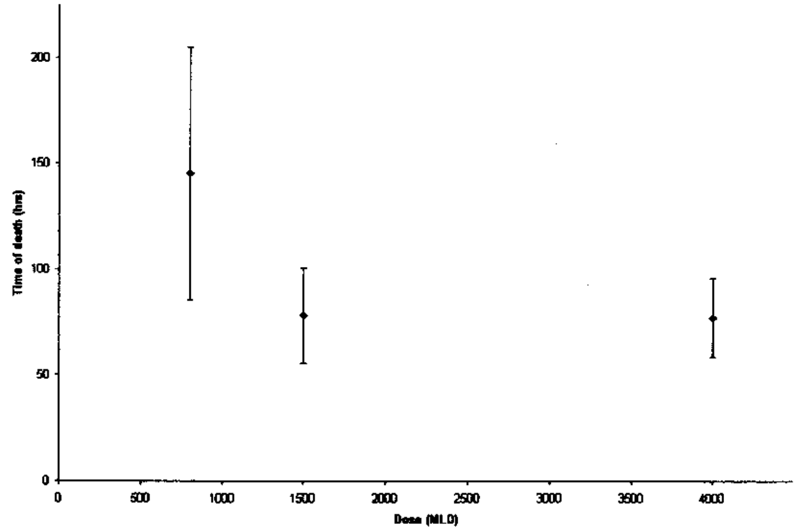
Walleye Percent Mortality



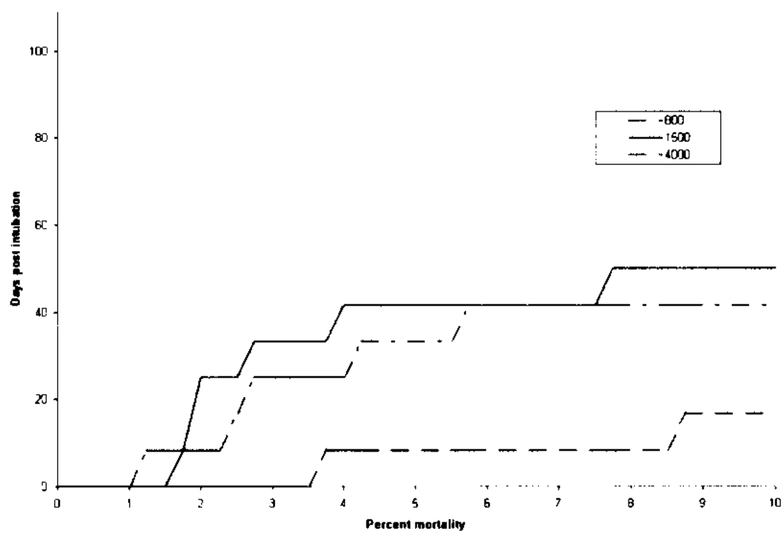
Perch: Onset of Pigment Change

- Slight pigment change (darker).
- Drastic pigment change.
- Equilibrium loss, fish often breaching the surface.

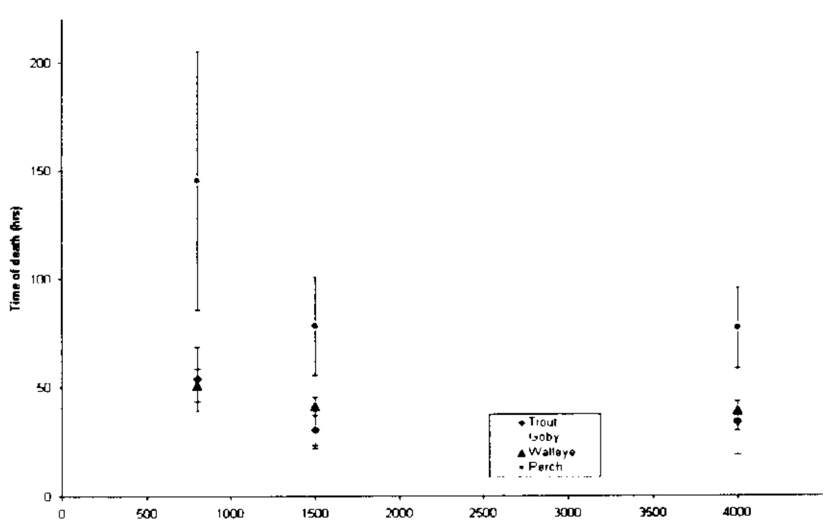
Perch Dose Response



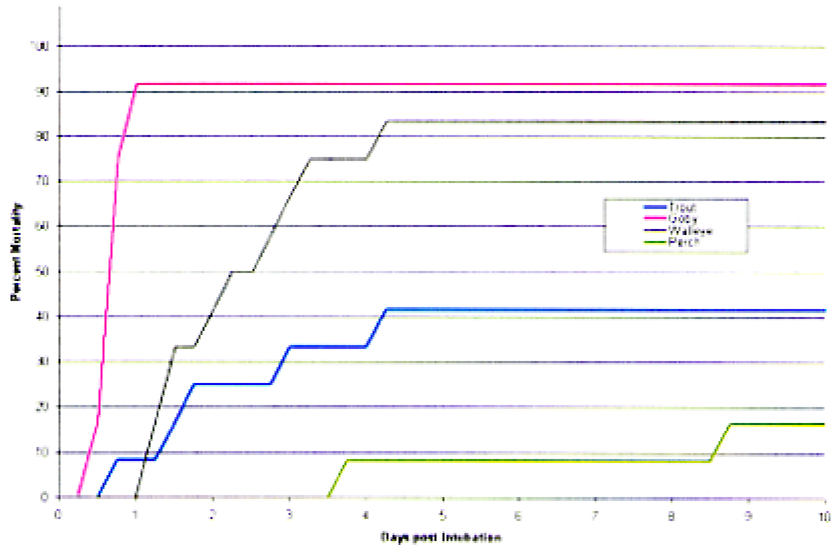
Perch Percent Mortality



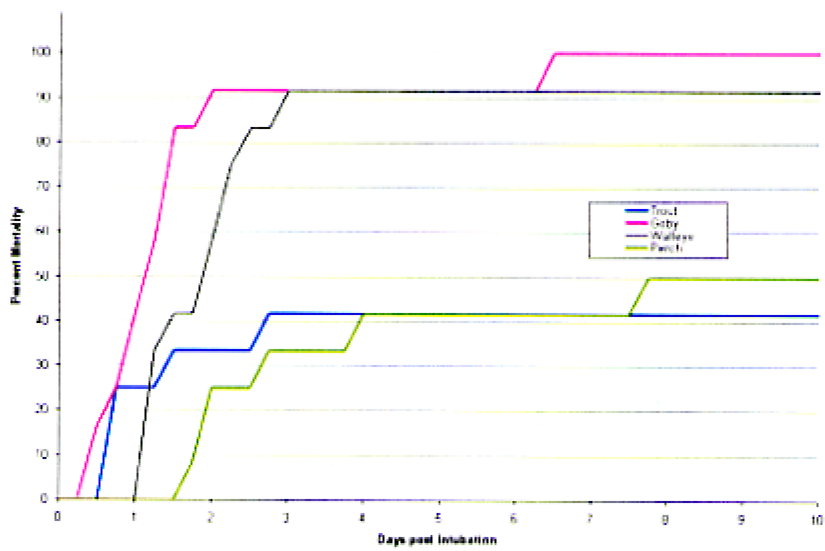
Interspecies Dose Response

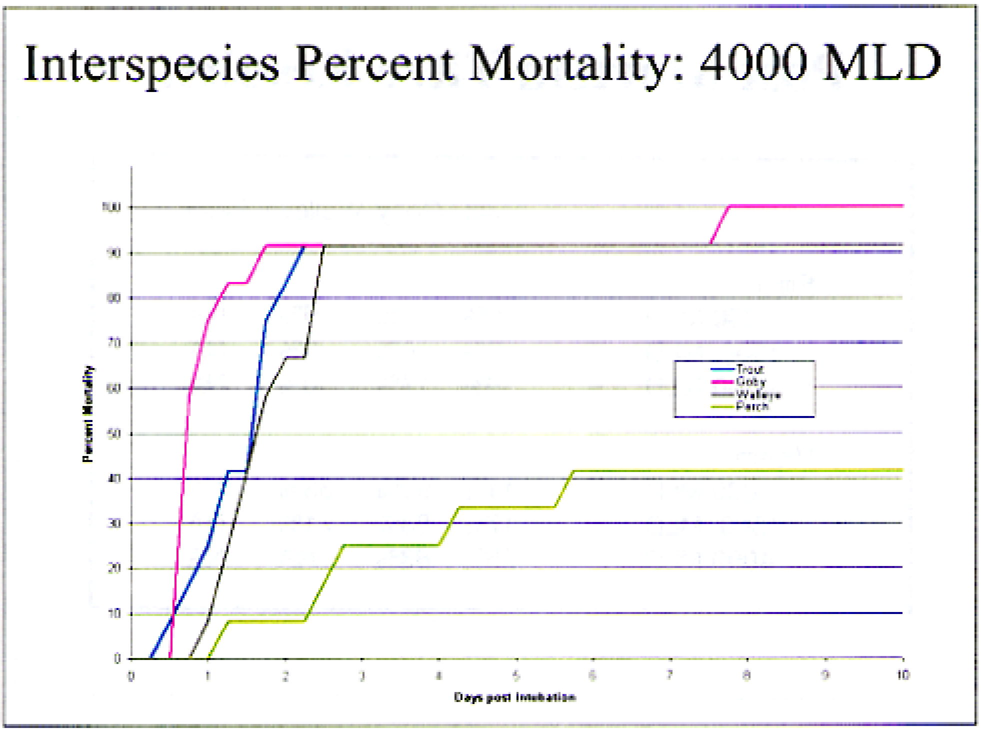


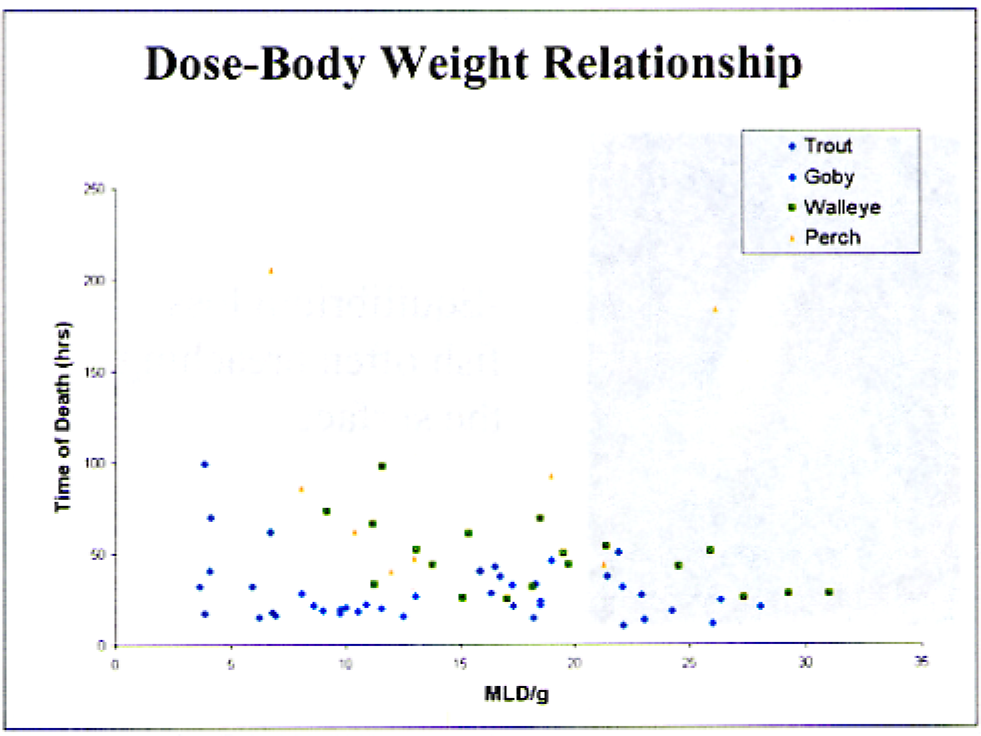
Interspecies Percent Mortality: 800 MLD



Interspecies Percent Mortality: 1500 MLD



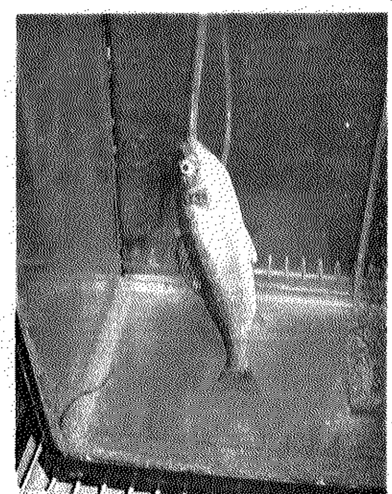




Tissue Titres of Botulinum Neurotoxin
In other words, what's left in a dead fish?

Post Mortem Titre Results:

	Trout	Goby	Walleye	Perch
Fillet				
800 MLD	0%	0%	0%	NA
1500 MLD	0%	0%	0%	NA
4000 MLD	0%	17%	0%	NA
Vicera				
800 MLD	11%	92%	33%	NA
1500 MLD	17%	83%	71%	NA
4000 MLD	25%	83%	NA	NA



-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

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

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

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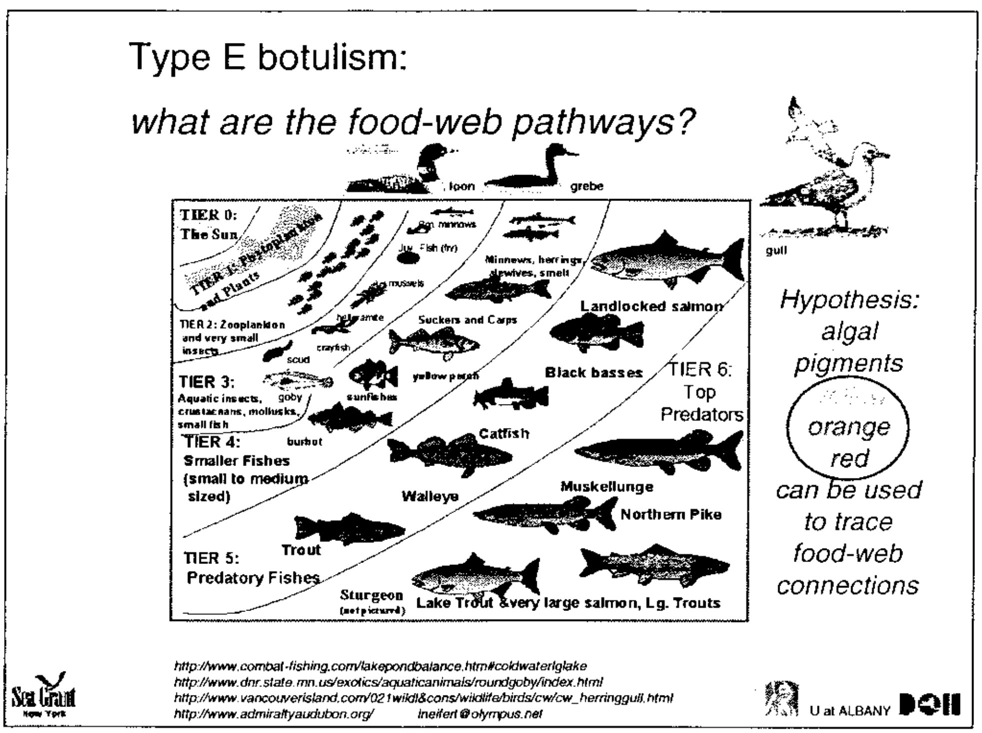
Bill Culligan, NY Department of Environmental Conservation, Region 9
Kofi Fynn-Aikins, Ph.D.; Betsy Trometer; Mike Goehle, USFWS LGLFR

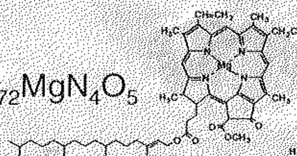


Common to all photosynthetic algae

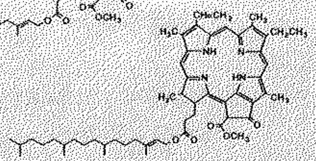
β-carotene $C_{40}H_{56}$

+ retained from diet

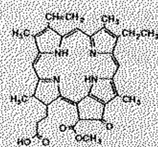
chlorophyll a $C_{55}H_{72}MgN_4O_5$



pheophytin a $C_{55}H_{74}N_4O_5$



pheophorbide a $C_{35}H_{36}N_4O_5$
(chl a degradation)



- degraded, excreted;
fecal pellets

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Y. Tsukii June 2001 http://protist.i.hosei.ac.jp/

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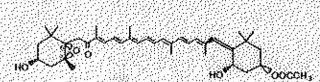
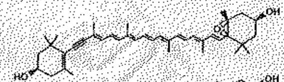
Diatoms

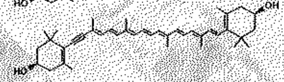
fucoxanthin $C_{42}H_{60}O_6$



diadinoxanthin $C_{40}H_{54}O_3$



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



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	93 %	*Aulacoseira islandica* (centric)
spring Lake Ontario	78 %	*Aulacoseira islandica* (centric)
summer Lake Erie	32 %	*Fragilaria crotonensis* (pennate)
summer Lake Ontario	7 %	*Fragilaria crotonensis* (pennate)

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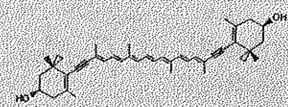
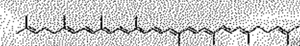
Cryptophytes

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 minutae*
spring Lake Ontario	13.%	*Rhodomonas minutae*
summer Lake Erie	17.%	*Rhodomonas minutae*
summer Lake Ontario	23.%	*Cryptomonas erosa*

http://huey.colorado.edu/LTER/images/pictures/lakes/plankton/rhodomonas/Rhodomonas_891204_T2D.html

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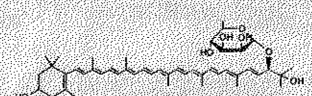
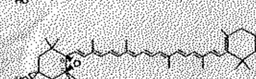
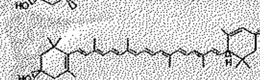
Chlorophytes

violaxanthin $C_{40}H_{56}O_4$ 

antheraxanthin $C_{40}H_{56}O_3$ 

lutein $C_{40}H_{56}O_2$ 

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant	
spring Lake Ontario	not abundant	
summer Lake Erie	36%	*Pediastrum simplex, Pediastrum spp.*
summer Lake Ontario	19%	*Staurastrum gracile, Oocystis borgei, tetraedron min.*
Epilithic chlorophytes	___??%	*Cladophora glomerata, C. vagabonda*

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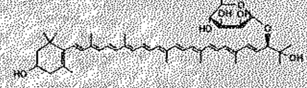
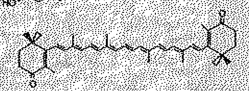
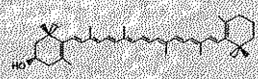
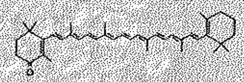
Cyanobacteria

myxoxanthophyll $C_{46}H_{66}O_7$ 

zeaxanthin $C_{40}H_{56}O_2$ 

canthaxanthin $C_{40}H_{52}O_2$ 

β-cryptoxanthin $C_{40}H_{56}O$ 

echinenone $C_{40}H_{54}O$ 

Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	1%	*Anacystis montana*
summer Lake Erie	10%	*Microcystis spp*

Yuuji Tsukii Zenko-ji Nagano 2003 http://protist.i.hosei.ac.jp/

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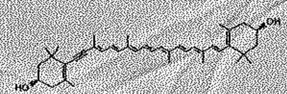
Chrysophytes

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

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	5.5% *Dinobryon divergens*

© Michael R. Martin 2002 http://www.cedareden.com/micro/diatom.html

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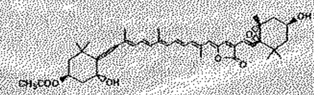
Dinoflagellates

peridinin $C_{39}H_{52}O_7$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant	
spring Lake Ontario	8.9%	*Gymnodinium helveticum*
summer Lake Erie	4.9%	*Ceratium hirundinella*
summer Lake Ontario	46.%	*Ceratium hirundinella*

Yuuji Tsukii: Daidarabotchi-ike, Iizuna-kogen, Nagano 2003
http://protist.i.hosei.ac.jp/

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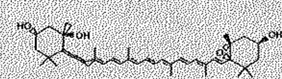
Euglenophytes

neoxanthin $C_{40}H_{56}O_4$



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

Yuuji Tsukii: Sugadaira, Nagano, 2001 http://protist.i.hosei.ac.jp/

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Seasonal changes: Lake Erie

Phytoplankton	spring*	summer*	pigments expected in diet	
diatoms	93%	32%	fucoxanthin	$C_{42}H_{60}O_6$
			diadinoxanthin	$C_{40}H_{54}O_3$
			diatoxanthin	$C_{40}H_{54}O_2$
cryptophytes	5%	17%	alloxanthin	$C_{40}H_{52}O_2$
			lycopene	$C_{40}H_{56}$
chlorophytes	-	36%	violaxanthin	$C_{40}H_{56}O_4$
			antheraxanthin	$C_{40}H_{56}O_3$
			lutein	$C_{40}H_{56}O_2$
cyanophytes	1%	10%	myxoxanthophyll	$C_{46}H_{66}O_7$
			zeaxanthin	$C_{40}H_{56}O_2$
			canthaxanthin	$C_{40}H_{52}O_2$
			β-cryptoxanthin	$C_{40}H_{56}O$
			echinenone	$C_{40}H_{54}O$

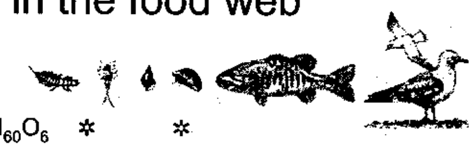
* Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

Seasonal changes: Lake Ontario

Phytoplankton	spring*	summer*	pigments expected in diet	
diatoms	78%	7%	fucoxanthin	$C_{42}H_{60}O_6$
			diadinoxanthin	$C_{40}H_{54}O_3$
			diatoxanthin	$C_{40}H_{54}O_2$
cryptophytes	13%	23%	alloxanthin	$C_{40}H_{52}O_2$
			lycopene	$C_{40}H_{56}$
chlorophytes	-	19%	violaxanthin	$C_{40}H_{56}O_4$
			antheraxanthin	$C_{40}H_{56}O_3$
			lutein	$C_{40}H_{56}O_2$
chrysophytes	-	6%	diatoxanthin	$C_{40}H_{54}O_2$
dinoflagellates	-	46%	peridinin	$C_{39}H_{52}O_7$

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

Carotenoids found in the food web



diatoms &	fucoxanthin	$C_{42}H_{60}O_6$	*	*		
chrysophytes	diatoxanthin	$C_{40}H_{54}O_2$		*	*	
cryptophytes	alloxanthin	$C_{40}H_{52}O_2$		*	*	
chlorophytes	lutein	$C_{40}H_{56}O_2$	(*)	*	*	*
cyanobacteria	zeaxanthin	$C_{40}H_{56}O_2$	*	*	*	*
	cantha-	$C_{40}H_{52}O_2$			*	*
	β-crypto-	$C_{40}H_{56}O$			*	*
	echinenone	$C_{40}H_{54}O$	*		*	*
euglenophytes	neoxanthin	$C_{40}H_{56}O_4$			*	
dinoflagellates	peridinin	$C_{39}H_{52}O_7$	NF	NF	NF	NF
crustacean metabolism	astaxanthin	$C_{40}H_{52}O_4$	*	*	*	*

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Biomarkers and time: prey vs predator profiles

	food resources: present biomarkers of prey	past and future biomarkers of predator *and* past ingestion
tissue analyzed:	recent ingestion	long-term accumulation
	stomach	liver
	intestines	muscle
	(spleen)	integument (skin)
		eye
		brain
		gonads, eggs

http://www.dnr.state.wi.us/org/water/fhp/fish/species/index.shtml
http://www.dnr.state.mn.us/exotics/aquaticanimals/roundgoby/index.html

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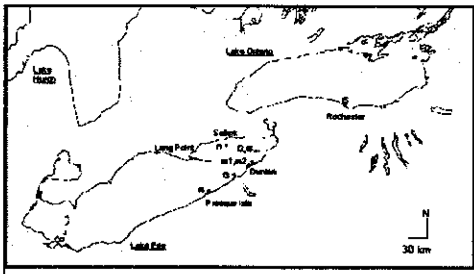
Sample collection: Lake Erie (Lake Ontario)



A. Perez Fuentetaja (SUNY-Fredonia)

	Macroinvertebrates
	Sept - Oct
diptera	weekly (Aug)
oligochaeta	weekly (Aug)
dreissenids	June, Aug (6)

EPA GLNPO: late 2003

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

Fish	2003
smelt	Oct
round gobies	Sept - Oct
smallmouth bass	Sept
rock bass	Sept - Oct
black crappie	Sept
2003	sheepshead
white perch	Sept - Oct
yellow perch	Sept
spottail shiner	Sept

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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

i dissect specimen
ii homogenize tissue
iii freeze dry and determine percent dry weight
(mussels ~ 5% dw; fish ~ 20% dw)
iv weigh specimen to be extracted: 75 - 300 mg dw (1500 mg ww)
v solvent extract in acetone, sonnicate in dark, cold
vi saponify KOH, methanol; solvent exchange, cleanup, dry
vii concentrate extract: dry N_2
viii filter extract, 0.2 μm, into screw-cap vial

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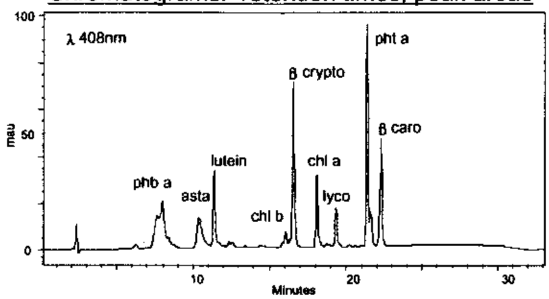
HPLC-photodiode array analysis

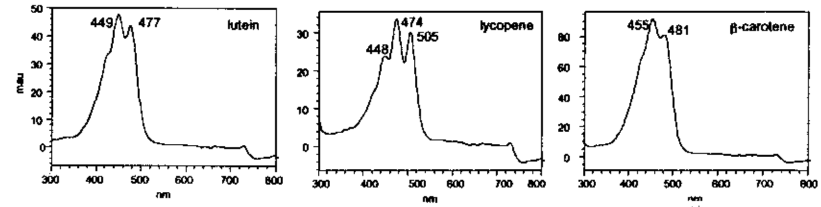
Chromatograms: retention times, peak areas



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)



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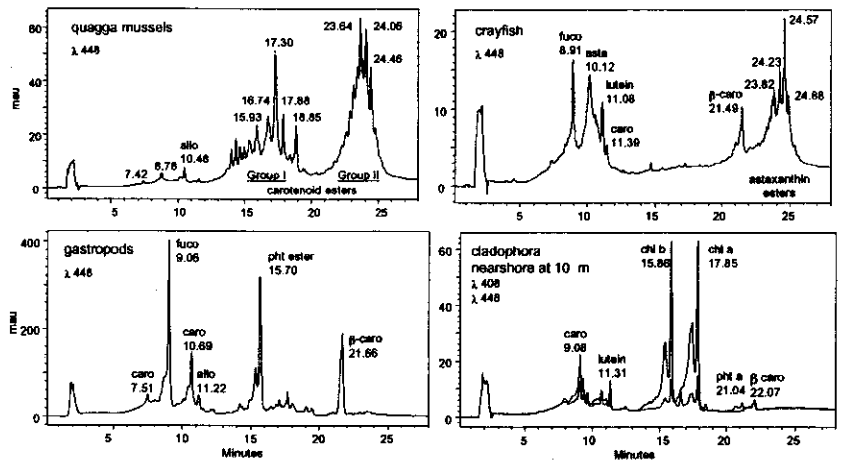
Lake Ontario samples, Aug 2002*



NY GLPF small grant: Alben, Makarewicz 2002-3

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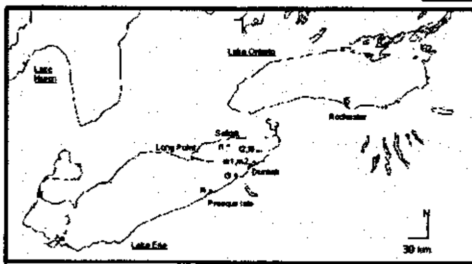
NY Sea Grant 2004-6: Lake Erie (Lake Ontario)



Culligan (NYDEC)
Getchell, Bowser (Cornell)
Fynn-Aikins, 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
trout	????

A. Perez Fuentetaja (SUNY-Fredonia)

Macroinvertebrates	collection
diptera	weekly
oligochaetes	weekly
quagga mussels	weekly

Others of interest	Birds
crayfish	gulls
mudpuppies	long-tailed ducks
	loons
	grebes

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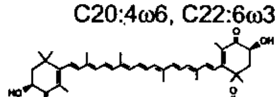
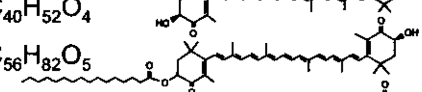
Crustaceans: astaxanthin; fatty acid (FA) esters

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

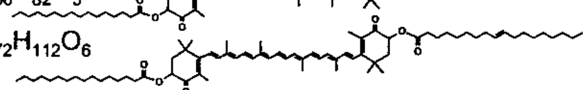
'free' astaxanthin $C_{40}H_{52}O_4$



C16:0 monoester $C_{56}H_{82}O_5$

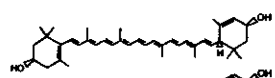


C16:0-C18:1 diester $C_{72}H_{112}O_6$

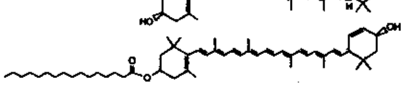


mollusks, fish, birds - also possible

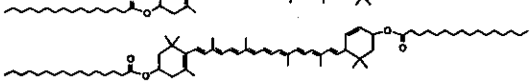
'free' lutein $C_{40}H_{56}O_2$



C16:0 mono- $C_{56}H_{86}O_3$



C16:0 diester $C_{72}H_{116}O_4$
(helenien)



Whitney Stocker: http://webby.cc.denison.edu/~stocker/limosus.html

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Acknowledgements

Students - insights
- Jamie Woodall*xanthin*
- Justin George*xanthin*
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- Abdulabasit Luke*xanthin*
- Kaitlin Harrington*xanthin*
- Christine Simmons*anthin*
- Jamie Iannacone*none*
- Yuliana De los Santos*anthin*
- Ikenna Anaka*xanthin*
- Jessica Reiner*xanthin*

Collaborators - field sites, samples
- Joe Makarewicz, SUNY Brockport
- Ted Lewis, SUNY Brockport
- Alicia Perez Fuentetaja, SUNY Fredonia
- Chris Mayer, University of Toledo
- Bill Culligan, NY DEC Region 9
- Paul Bowser, Cornell
- Rod Getchell, Cornell
- Kofi Fynn-Aikins, USFWS
- Betsy Trometer, USFWS
- Mike Goehle, USFWS

Sponsors - exploratory research projects and foodweb ventures
- Hudson River Foundation - Polgar Fellowship Program
- McNair Scholars Progam and CSTEP, SUNY Albany
- Great Lakes Protection Fund Small Grant Program
- US EPA Great Lakes National Program Office
- New York Sea Grant

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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

Sea Grant Great Lakes Network

Sea Grant Great Lakes Network

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

Please circle your response.	Strongly Disagree				Strongly Agree	N	Mean	+/-
1) The workshop achieved its goal of sharing information.	1	2	3	4	5	**28**	**4.56**	**.83**
2) The workshop achieved its goal of providing networking opportunities.	1	2	3	4	5	**28**	**4.54**	**.74**
3) The botulism overview was worthwhile.	1	2	3	4	5	**28**	**4.32**	**.95**
4) The state & Canadian updates were worthwhile.	1	2	3	4	5	**26**	**4.42**	**.63**
5) The research presentations were worthwhile.	1	2	3	4	5	**28**	**4.68**	**.67**
6) The discussion session was worthwhile.	1	2	3	4	5	**15**	**4.60**	**.83**
The educational materials and content of the workshop:								
7) Helped me better understand the issues surrounding botulism in Lake Erie.	1	2	3	4	5	**27**	**4.59**	**.69**
8) Provided information relevant to my work.	1	2	3	4	5	**26**	**3.88**	**.91**
9) Were well organized.	1	2	3	4	5	**27**	**4.37**	**.74**
10) Were easy to understand.	1	2	3	4	5	**27**	**4.33**	**.68**
11) Presented information that will help me.	1	2	3	4	5	**27**	**4.22**	**.80**

Please circle your response. The educational materials and content of the workshop:	Strongly Disagree				Strongly Agree	N	Mean	+/-
12) Will be of great use to me.	1	2	3	4	5	**27**	**3.89**	**.70**
13) I gained new knowledge from this workshop.	1	2	3	4	5	**28**	**4.64**	**.68**
14) I plan to share the information learned at this workshop with others.	1	2	3	4	5	**28**	**4.36**	**.99**
15) I plan to take some action as a result of the information I learned at this workshop.	1	2	3	4	5	**28**	**3.71**	**.98**

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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