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.

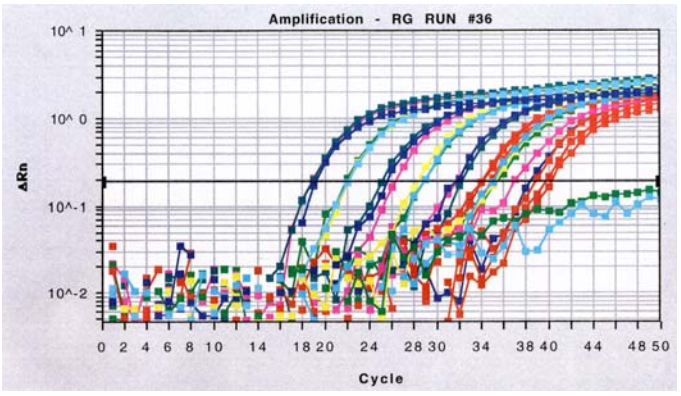
Sample processing and DNA Extraction

- 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

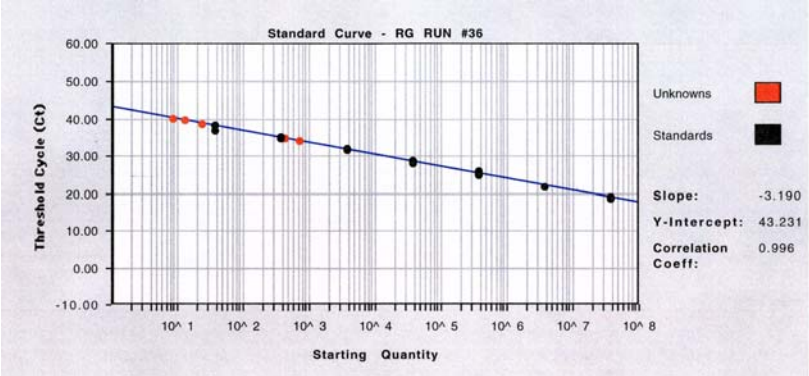
- After DNA is isolated, we can look for the toxin gene using a standard PCR amplification of a 139 base pair fragment to demonstrate the presence or absence of *C. botulinum* Type E.
- But, quantitative (real-time) PCR will provide actual numbers of *C. botulinum* Type E per gram of tissue when compared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