Prevalence of Botulism in Fish in the Lower Great Lakes

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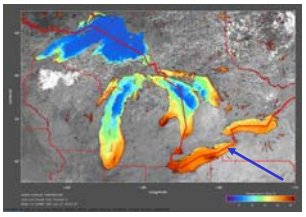
Type E Botulism: Why are we studying it?

- Type E Botulism outbreaks have killed thousands of waterfowl on Lake Erie in each of the last 4 years.
- Fish kills have been associated with many of these events.
- 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?



How are we conducting the research?

- 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.

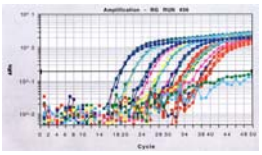
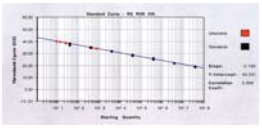
How are we conducting the research?

- Fish intestinal contents, liver, and blood 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.

How are we conducting the research?

- Standard PCR amplification of a 139 base pair fragment of the light chain of the botulism toxin gene demonstrates the presence or absence of *C. botulinum* Type E.
- But, quantitative (real-time) PCR will provide actual numbers of *C. botulinum* Type E when compared to a series of standards.
- The sequences of the primers and probe used in the QPCR assay are as follows:
  AATATTGTTTCTGTAAAAGGCATAAG
  GAAATCAATATGTATCGAAATAAATA
  ATGGTGAGTTATTTTTTGTGGCTTCCG
  AGAATAGTTATAATGATGATAATATA
  AATACTCCTAAAGAAATTGACGATAC
  AGTAACTT



What are the results we have found so far?

- We have measured significant numbers of *C. botulinum* Type E in dead and dying fresh water drum during three die-offs in July of 2001 near Dunkirk and Barcelona Harbor on Lake Erie.
- We also measured detectable levels of *C. botulinum* Type E in one apparently healthy five fish pool of smallmouth bass from Dunkirk, NY.





2002 Fish Collection Totals

	Lake Erie	Lake Ontario
Spring	265	8
Summer	176	71
Fall	186	30

Smallmouth Bass	286
Freshwater Drum	208
Round Goby	148
Yellow Perch	24
Alewife	20
Brown Bullhead	11
Other species	41

Preliminary 2001-2002 *C. botulinum* Type E QPCR Results

Species	Sample Location	Collection Date/s	Quantity/Gram
FWDrum	Dunkirk, NY	July 11, 2002	19,800/g IC
FWDrum	Dunkirk, NY	July 18&30, 2002	21,700/g IC
FWDrum	Barcelona, NY	July 26, 2002	23,100/g IC
SMBass	Dunkirk, NY	August 21, 2002	15,200/g IC
Sturgeon	Door County, WI	Summer, 2002	17,400/g SC

Species	Sample Location	Collection Date/s	Quantity/Gram
FWDrum	Dunkirk, NY	August 17, 2001	3,000/g K,L,S

IC = Intestinal contents; SC = Stomach contents included two goby-like fish; K,L,S = Combined kidney, liver, and spleen

Preliminary 2001 Avian *C. botulinum* Type E QPCR Results

Species	Sample Location	Quantity/Gram
Common Loon	Lake Erie	148,000/g ACC
Common Loon	Lake Erie	40,700/g ACC
Common Loon	Lake Erie	36,200/g SC
Coot	Lake Erie	340/g ACC
Long Tail Duck	Lake Erie	40,800/g GC

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

Where do we go from here?

- We will make a greater effort to collect fish during botulism outbreaks, particularly round gobies and freshwater drum.
- We will also collect sediment and quagga mussels from outbreak areas to further analyze the food chain path that Type E Botulism is following.