



Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

Canadian DSP Symposium

Vancouver, BC

November 27, 2012

## Canadian Food Inspection Agency



## *Testing for Marine Toxins and DSP*

### **Our vision:**

To excel as a science-based regulator, trusted and respected by Canadians and the international community.

### **Our mission:**

Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

*Wade Rourke*

*Canadian Food Inspection Agency Dartmouth Laboratory*

*Dartmouth, NS*

Canada

# Outline

- Information about toxins and toxin groups
- Information about different methods
- Canadian context and perspective
- Observations from BC samples

# What are we talking about?

Okadaic acid and analogues (DSP toxins)

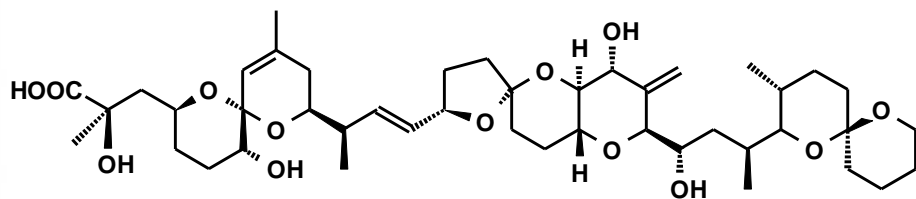
Pectenotoxins

Azaspiracids

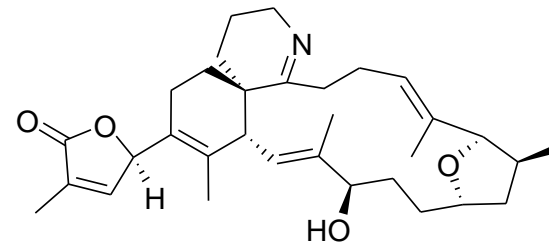
Yessotoxins

Cyclic Imines

# Chemical Structures

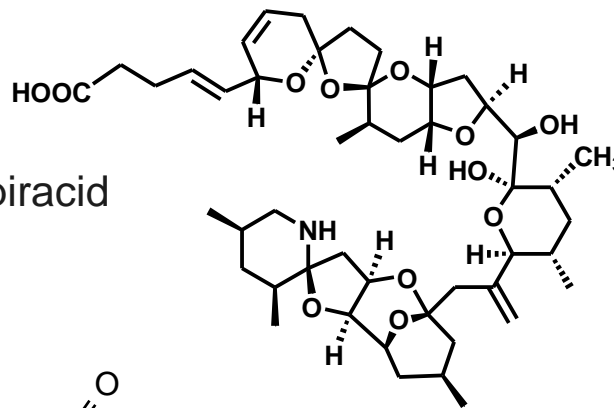


Okadaic Acid

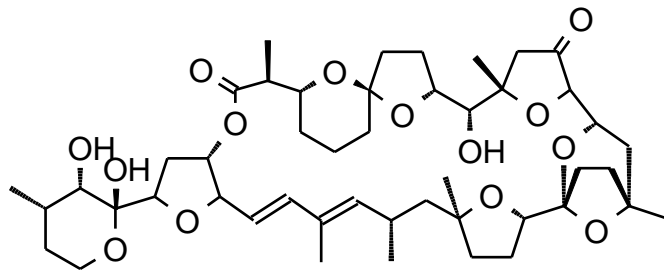
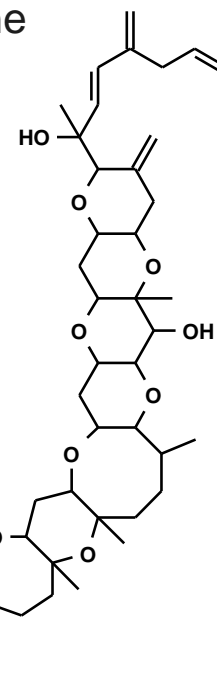


Gymnodimine

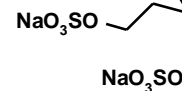
Azaspiracid



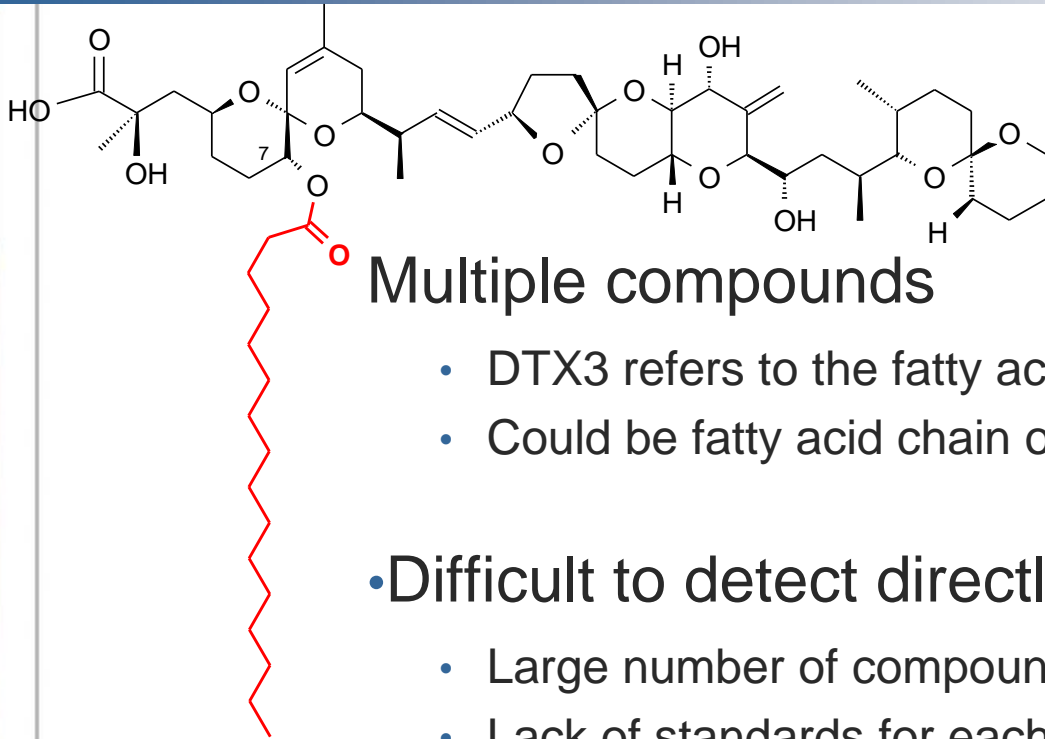
Yessotoxin



Pectenotoxin



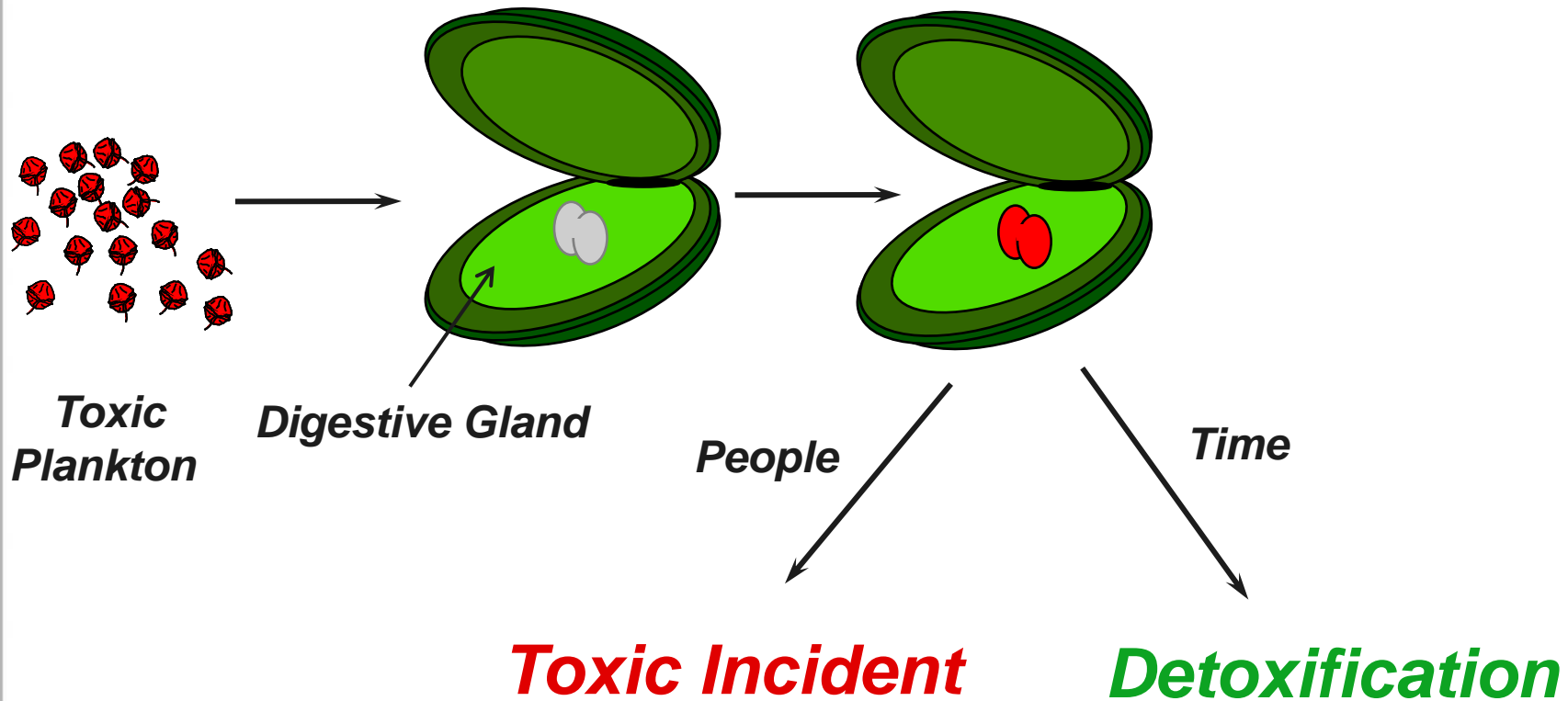
# “DTX3”



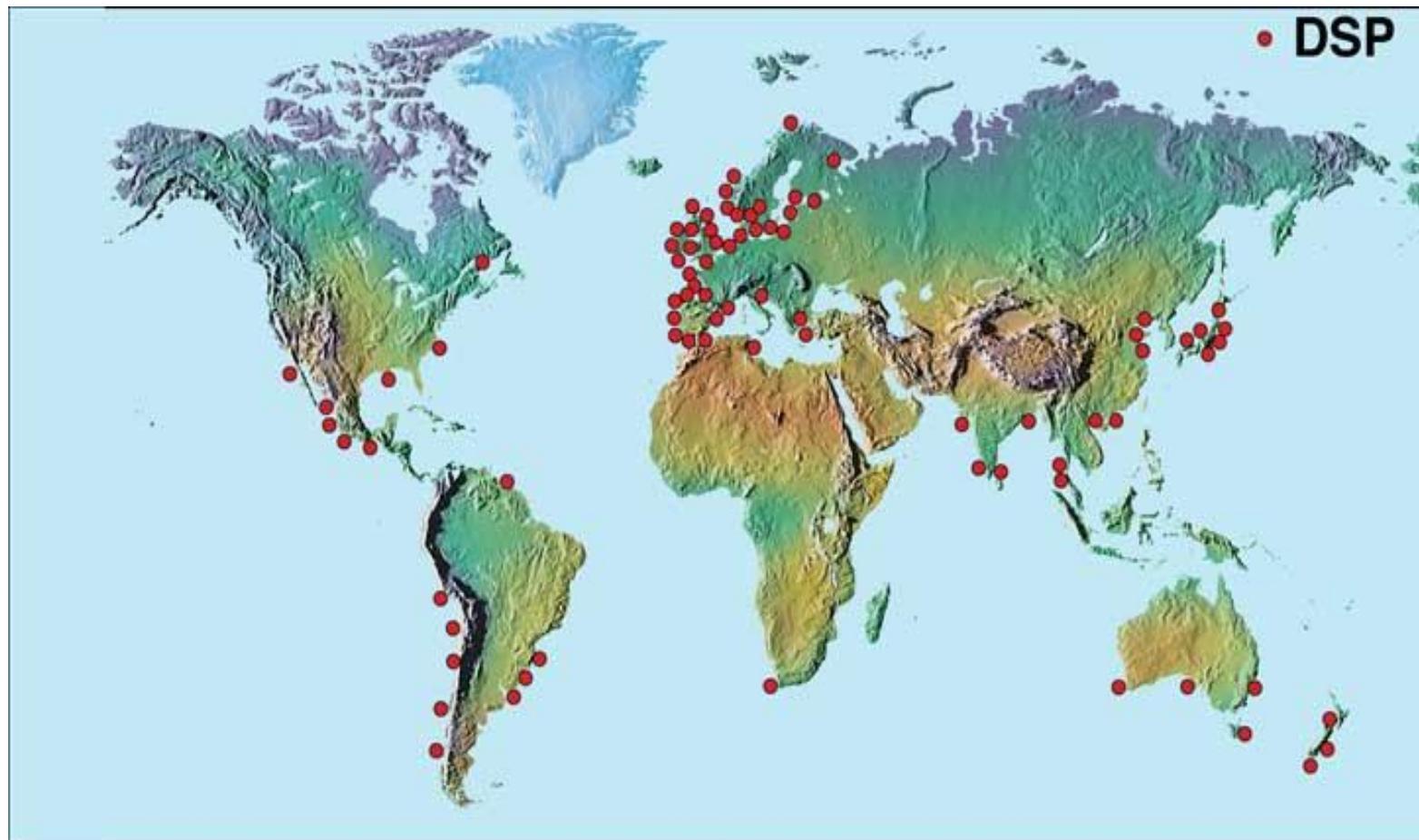
## Multiple compounds

- DTX3 refers to the fatty acid forms of OA, DTX1 and DTX2
- Could be fatty acid chain of 14-22 carbons
- Difficult to detect directly
  - Large number of compounds
  - Lack of standards for each compound
- Use enzymatic or alkaline hydrolysis procedure

# The problem starts...with hunger



# World Distribution



<http://www.who.edu/redtide/page.do?pid=18103&tid=542&cid=65844&c=3>



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# Mouse Bioassay

- Samples are extracted and then injected into 2-3 mice
  - May need multiple extractions depending on toxin profile
- Mice are monitored for 24 hours
  - Positive result:  $\geq 2$  of 3 mice die
  - Negative result:  $\geq 2$  of 3 mice live



# Methods of Analysis: Biological Methods I

Method	Advantage	Disadvantage
Biological Methods – General	<ul style="list-style-type: none"><li>• Produce a single result</li><li>• Complex instruments not required</li></ul>	<ul style="list-style-type: none"><li>• Need several assays to monitor all toxin groups</li><li>• No toxin profile data</li></ul>
Live Animal Bioassays	<ul style="list-style-type: none"><li>• Potential to detect unknown toxins</li></ul>	<ul style="list-style-type: none"><li>• Poor reproducibility and sensitivity</li><li>• Non-specific</li><li>• Use animals</li><li>• False positives and negatives</li></ul>

# Methods of Analysis: Biological Methods II

Method	Advantage	Disadvantage
Immuno Assays	<ul style="list-style-type: none"><li>•Sensitive</li><li>•Fast</li><li>•Very specific</li></ul>	<ul style="list-style-type: none"><li>•Good only as screen</li><li>•React to antibody not receptor</li><li>•Different reactivity of analogues to antibody</li></ul>
Functional Assays	<ul style="list-style-type: none"><li>•Very sensitive</li><li>•Fast</li><li>•Test at receptor level</li><li>•Independent of toxin profile</li></ul>	<ul style="list-style-type: none"><li>•Requires reliable enzymes</li><li>•Must know toxin mode of action</li></ul>

# Methods of Analysis Chemical

Method	Advantage	Disadvantage
Chemical Methods - General	<ul style="list-style-type: none"> <li>•Toxin profile data</li> </ul>	<ul style="list-style-type: none"> <li>•Complex instruments required</li> <li>•Require individual standards and TEFs</li> </ul>
LC-FLD	<ul style="list-style-type: none"> <li>•Good within a group, not between groups</li> </ul>	<ul style="list-style-type: none"> <li>•Difficult to add new toxins</li> <li>•Derivatization required</li> </ul>
LC-MS/MS	<ul style="list-style-type: none"> <li>•Specific and sensitive</li> <li>•Analyse toxins directly</li> <li>•Multi-toxin analysis</li> <li>•Easiest to add new toxins</li> </ul>	<ul style="list-style-type: none"> <li>•Prone to matrix effects</li> <li>•Most expensive instruments</li> </ul>

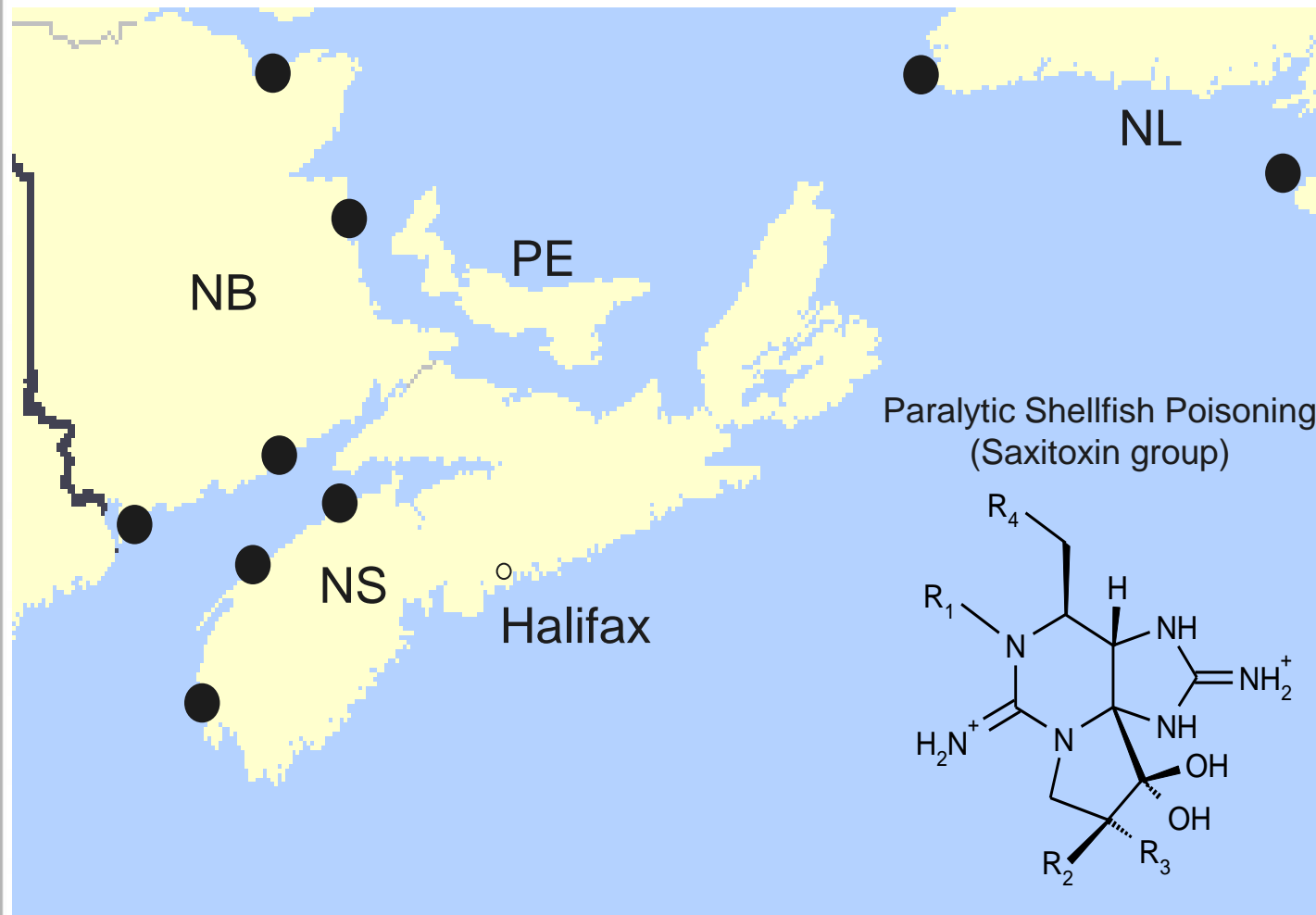


# EFSA Recommendations - 2008

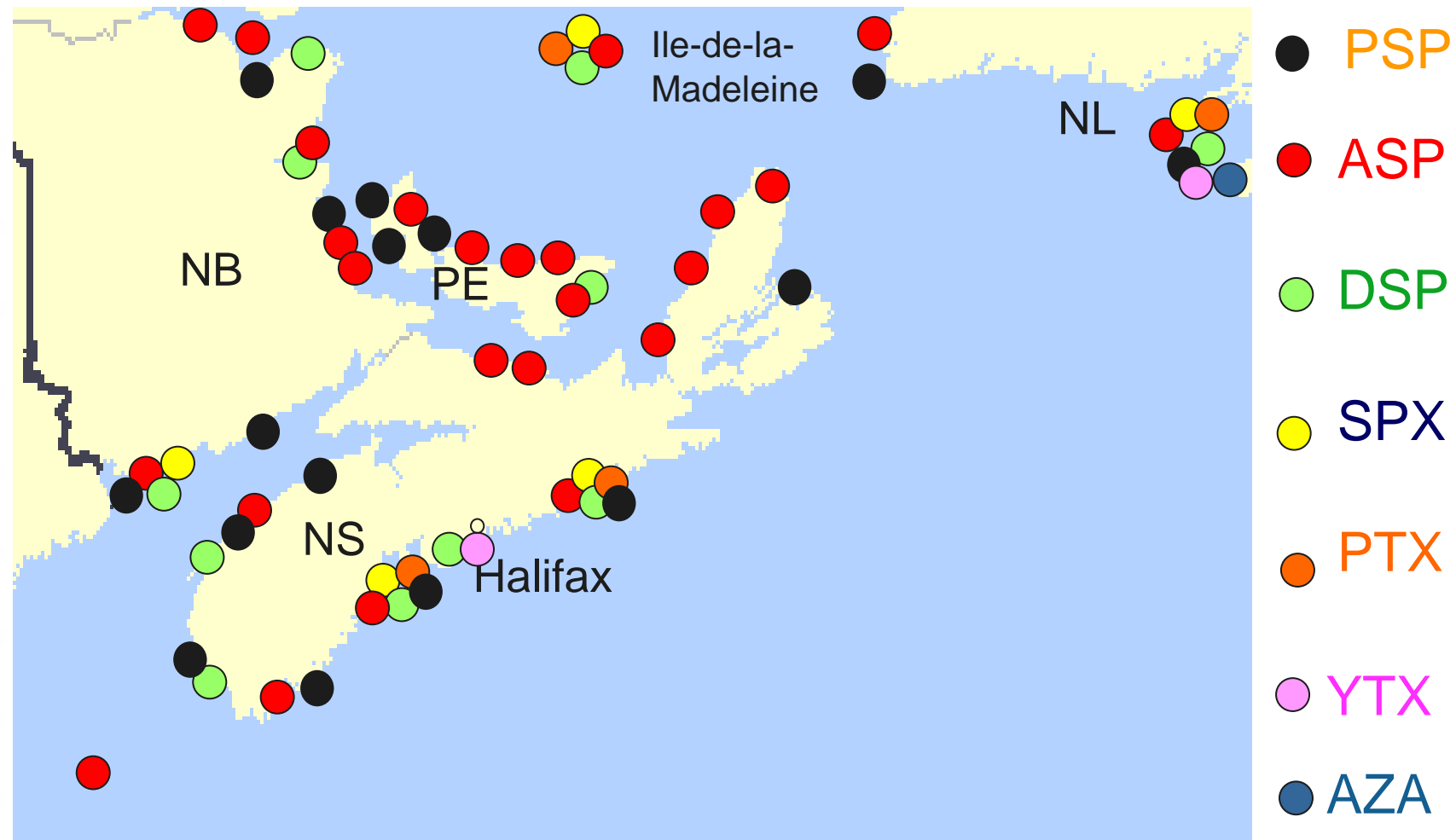
- Change action limits
  - Decrease limits for OAs, PTXs, AZAs
  - Increase limit for YTXs
- Group toxins by structure/mode of action not symptoms
- Change reference method
- Use TEFs to reflect toxicity of individual compounds
- **Resulted in reference method changing from MBA to LC-MS/MS in 2011**



# Toxins in Atlantic Canada before 1987



# Toxins in Atlantic Canada, 2005



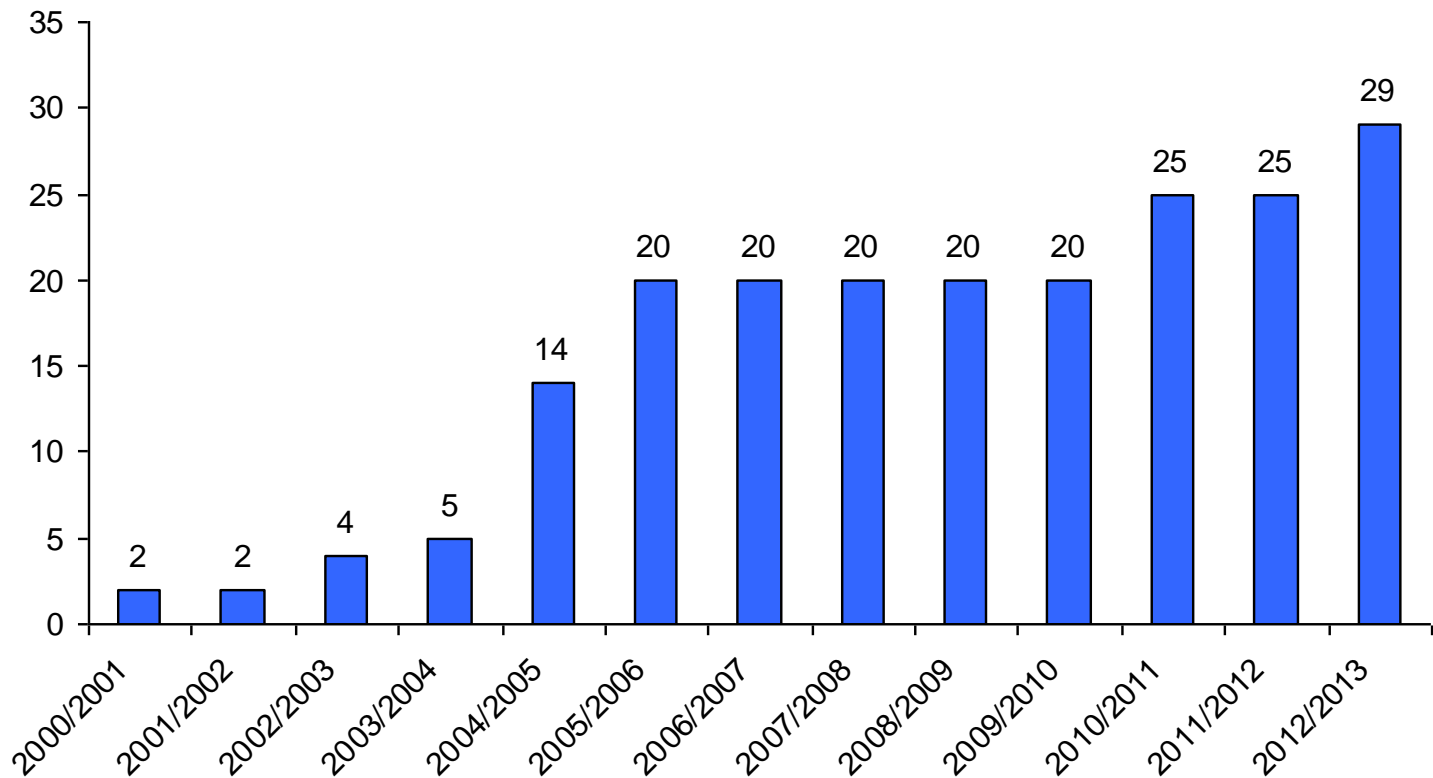
# Changing Times

- Adopting new technology
  - HPLC-FLD with derivatization
  - HPLC-MS
  - UPLC-MS/MS
  - Simplified, efficient extraction
- Ability to monitor toxins directly
- Ability to monitor multiple toxin classes simultaneously
- Proactive monitoring vs Reactive testing



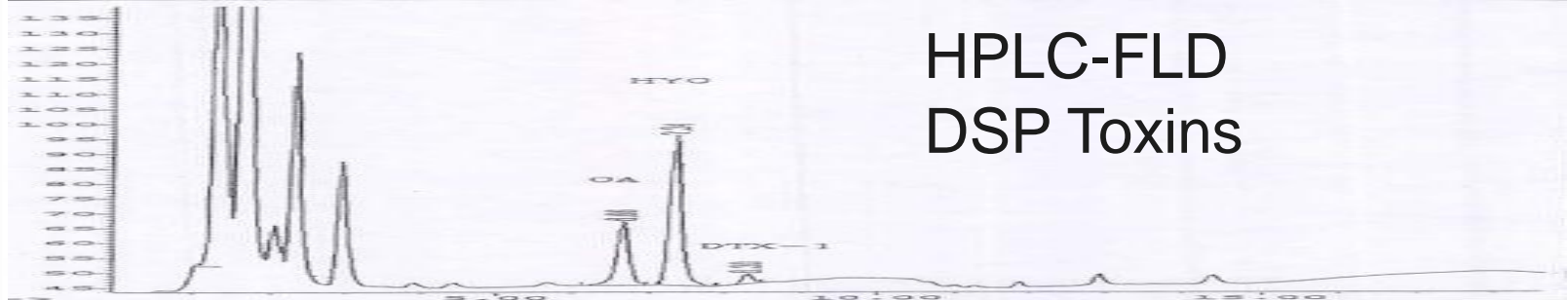
# Dartmouth Laboratory – LSTs

# of Toxins Monitored





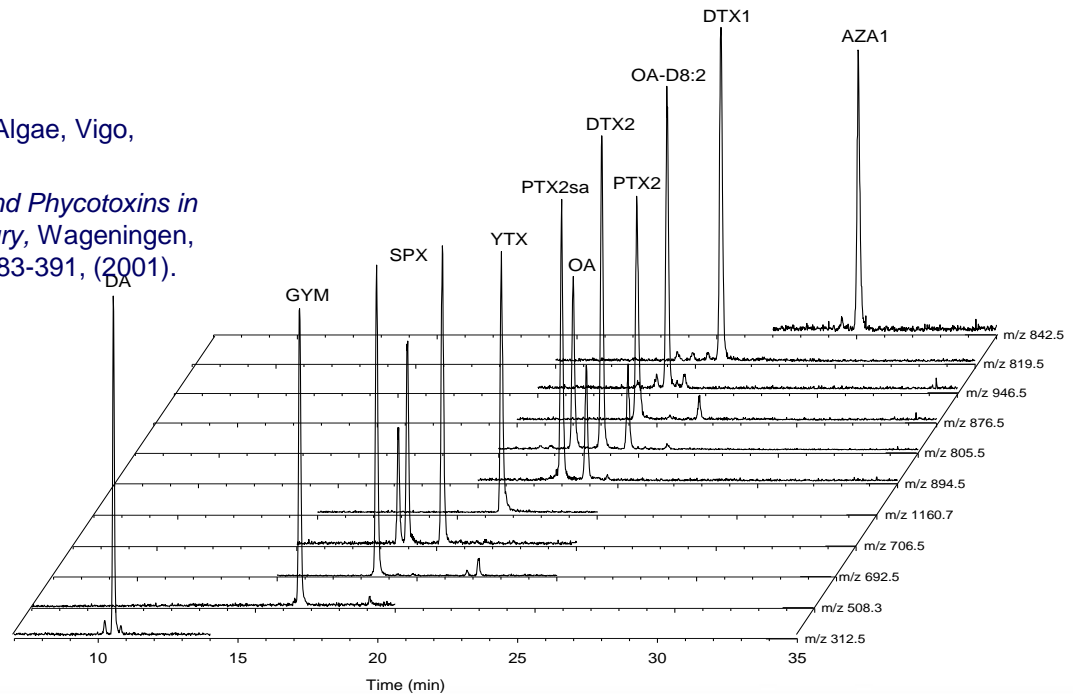
# Changing Times



MA Quilliam. 8th Int. Conf. Harmful Algae, Vigo, Spain, June 1997.

MA Quilliam et al., In: *Mycotoxins and Phycotoxins in Perspective at the Turn of the Century*, Wageningen, The Netherlands, W.J. deKoe, pp. 383-391, (2001).

## LC-MS Multi-toxin



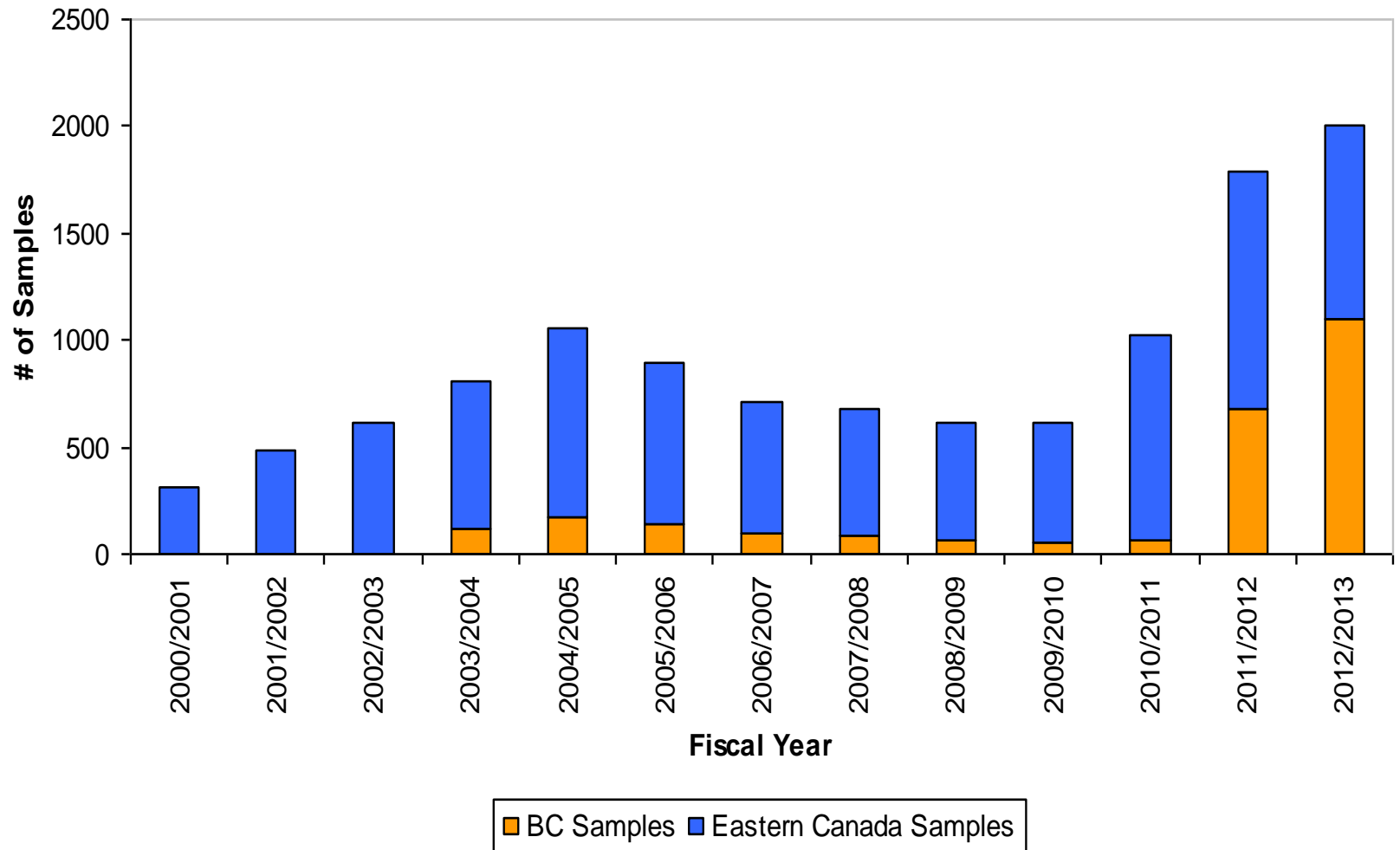
# Standards and CRMs

- Needed for instrumental methods
- Difficult to get
- NRC CRMP only supplier of CRMs
- Need to rely on in-house standards and gather material during toxic episodes
- Collaboration within community
- Difficult to validate methods without reliable standards, especially for toxin regulations of different countries with different toxic profiles

# CRM Availability

- National Research Council Canada
  - Certified Reference Material Program
- Available LST CRMs
  - OA, DTX1, DTX2, PTX2, AZA1, AZA2, AZA3, YTX, homoYTX, GYM, SPX1, DSP Mus, AZA Mus
- New materials in planning or in progress
  - Zero Mus, FDMT, PnTX-G

# East vs West Sample Distribution



# Method Transition

- Comparison and validation of new EU reference method began in April, 2011
- Saw performance and efficiency gains
- Implemented new method in May, 2012
- Average turn-around-time
  - November, 2009 – April, 2012: 4.1 days (2900 samples)
  - May, 2012 – November, 2012: 3.4 days (1300 samples)



# Turn-Around-Time: Western Samples

Monday	Tuesday	Wednesday	Thursday	Friday
		<ul style="list-style-type: none"> <li>•Samples arrive in afternoon</li> <li>•Day 1</li> </ul>	<ul style="list-style-type: none"> <li>•Samples are extracted and analysed</li> <li>•Day 2</li> </ul>	<ul style="list-style-type: none"> <li>•Data are analysed and results reported</li> <li>•Day 3</li> </ul>
<ul style="list-style-type: none"> <li>•Samples are extracted and analysed</li> <li>•Day 2</li> </ul>	<ul style="list-style-type: none"> <li>•Data are analysed and results reported</li> <li>•Day 3</li> </ul>			<ul style="list-style-type: none"> <li>•Samples arrive in afternoon</li> <li>•Day 1</li> </ul>

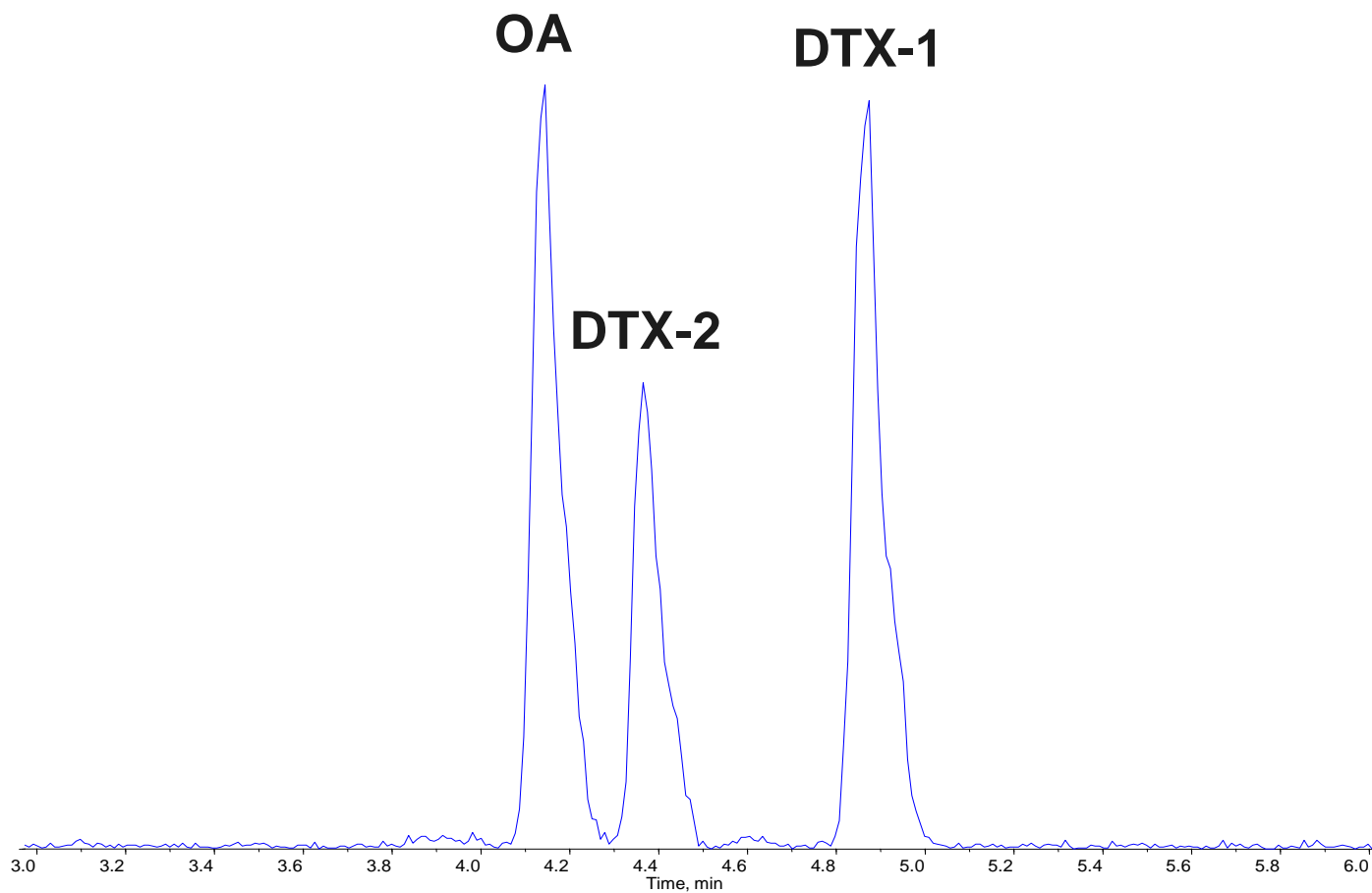
# Turn-Around-Time

- Laboratory service standard is 5 business days
- Average turn-around-time is 3.4 days
  - No difference between Eastern and Western samples
- Applies to regular samples
- Priority samples are analysed as soon as possible



# LC-MS/MS Chromatogram

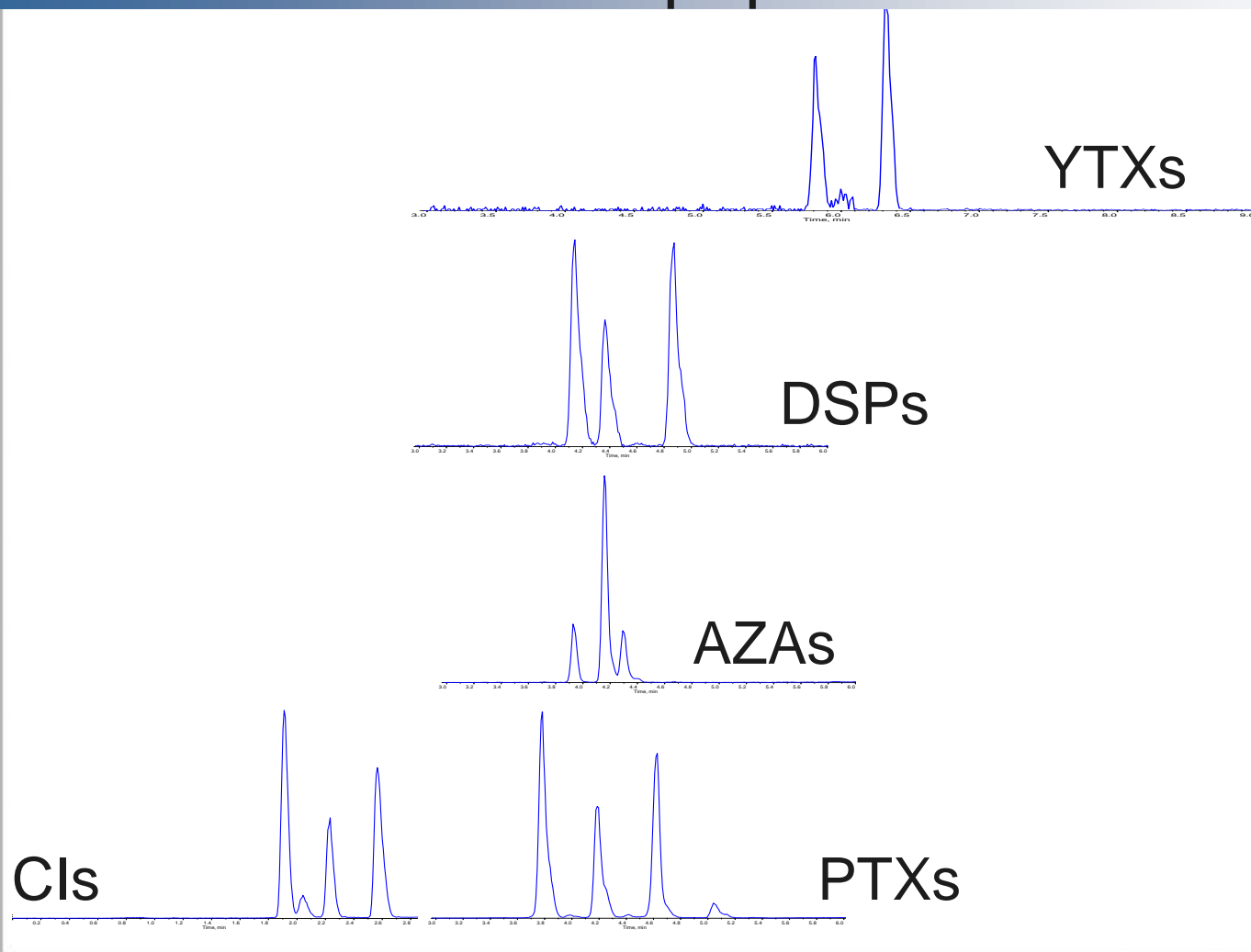
## DSP Toxins





# LC-MS/MS Chromatogram

## Lipophilic Shellfish Toxins



# Yessotoxins - EU

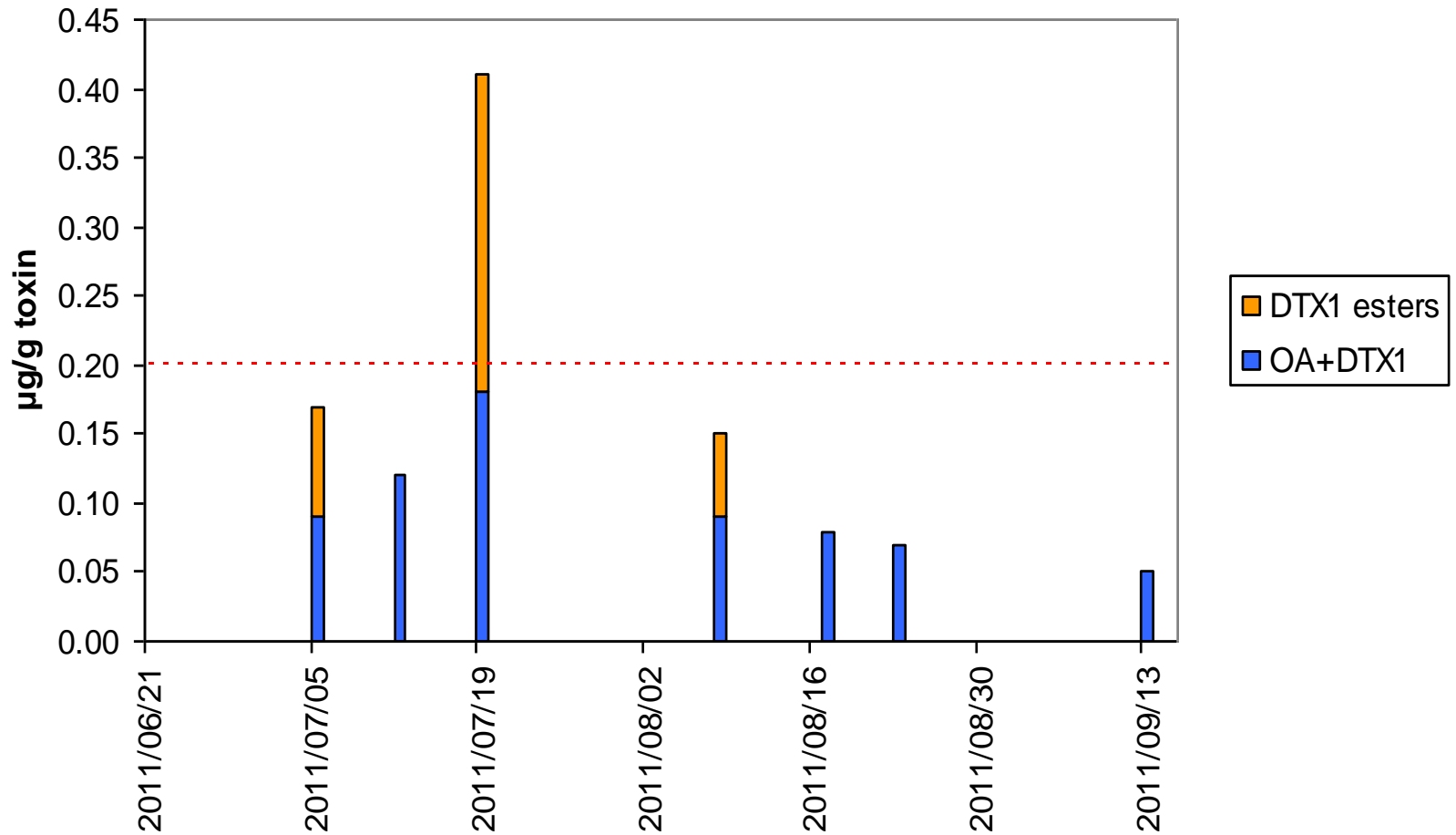
- No human illnesses have been linked to YTXs
- Regulated in EU but not in Canada
  - YTX, homoYTX, 45-OH YTX, 45-OH homoYTX
- EU maximum limit: 1.0 µg/g

# Yessotoxins - Canada

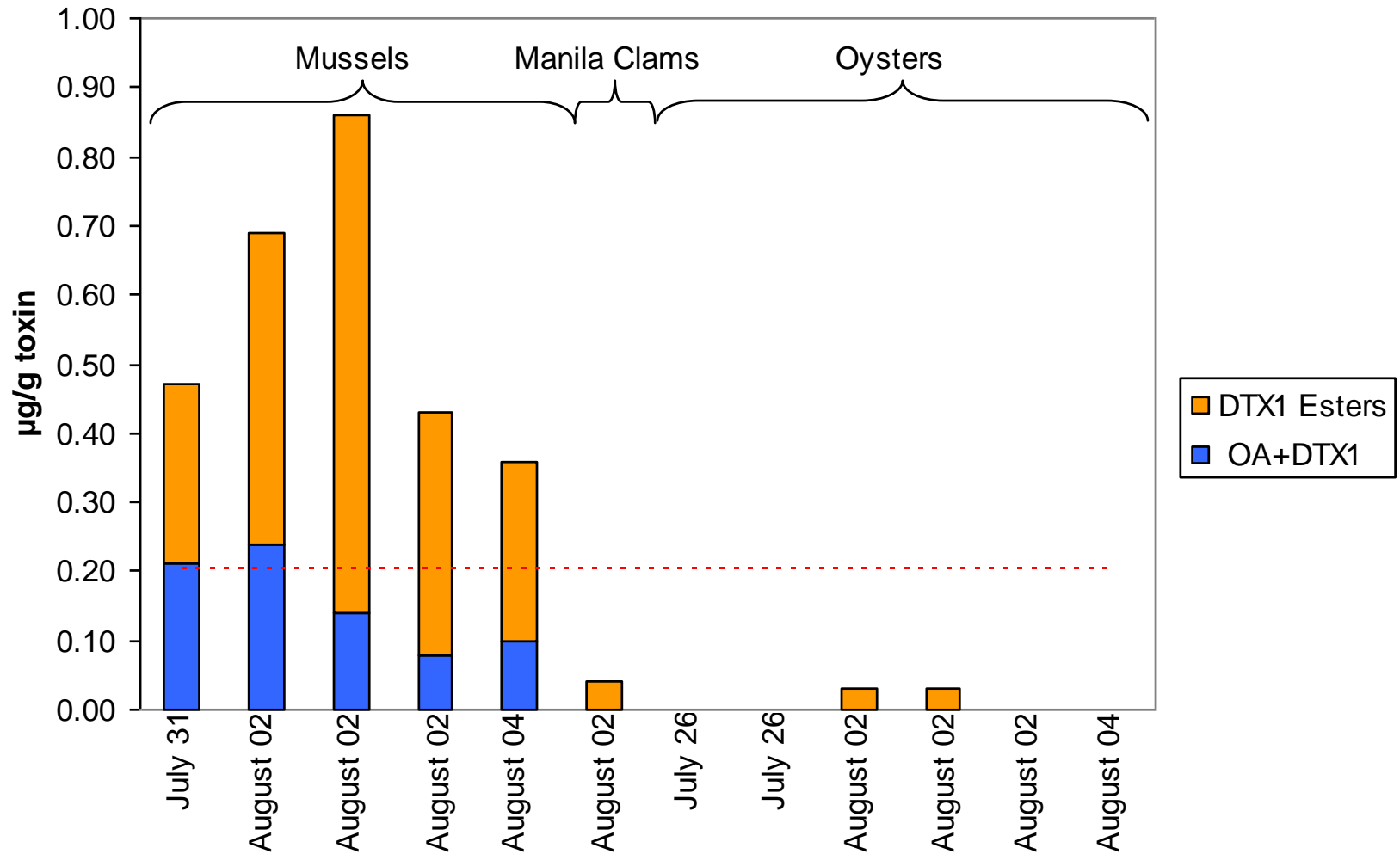
- Maximum YTX levels found in Canada: 12 µg/g
  - Combination of YTX and 45-OH YTX
- Results forwarded to HC for risk assessment
- These levels do not pose human health risk



# Gorge Harbour - Monitoring



# Gorge Harbour - Investigation



# Summary

- Lipophilic Shellfish Toxins (LSTs) include many different toxins, including the DSP toxins
- Many different methods, but LC-MS/MS offers most potential for multi-toxin analysis
- Taking advantage of newer technology provides potential to decrease turn-around-time
- CFIA looks for regulated and “emerging” toxins
- Monitoring program in place to prevent outbreaks



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