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Development and application of LC/MS based analysis for marine algal toxins in Hood Canal

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Development and application of LC/MS based analysis for marine algal toxins in Hood Canal

Sang Seon Yun, Seth Book, Ron Figlar-Barnes, Lisa Bellevueau, Aaron Bentson-Royal, and Kenneth Collins

Department of Natural Resources, Skokomish Indian Tribe
Overview

• Background
• Methods
• Results
• Discussion
• Future studies
• Acknowledgements
Background: Marine algal toxins

- **Hydrophilic toxins**
  - Domoic acid (ASP+)
  - Saxitoxin (PSP+)
  - Gonyautoxin (PSP+)
  - Neosaxitoxins (PSP+)

- **Lipophilic toxins**
  - Okadaic acid (DSP)-
  - Dinophysistoxins (DSP)-
  - Pectenotoxins (DSP)+
  - Yessotoxin-
Background: Marine algal toxin analysis

• Climate change and harmful algal blooms in Hood Canal?
  • Increasing surface water temperature
  • Increasing weather events
  • Increasing nutrient input

• Skokomish Tribe’s initiative to establish an early warning system for marine algal toxins in Hood Canal
  • Focusing on water and phytoplankton samples

• BIA funded algal toxin monitoring program launched

• Analytical methods
  • Mouse bioassay (MBA)
  • ELISA
  • Receptor binding assay
  • Chemical analysis
    • HPLC
    • LC/MS
Background: LC/MS based analysis

- Combines LC separation with mass spectrometric detection
- Individual compounds can be identified and quantified
- Offers very sensitive detection
- Enable detection and quantitation of multiple compounds in one run
- Requires costly equipment
Methods: Solid Phase extraction

Lipophilic Toxins

- Oasis HLB
- Methanol
- 250 ml seawater
- Oasis HLB
- Methanol

Hydrophilic Toxins

- Envi Carb-graphitized carbon
- 20% acetonitrile
- Up to 12 ml
- DI water
- 40% acetonitrile
Methods: LC/MS analysis

LC parameters

- Column
  - Luna C18 (50 x 2.1 mm)

- Solvents
  - A: DI water with 2 mM ammonium formate + 50 mM formic acid
  - B: 95% Acetonitrile with 2 mM ammonium formate + 50 mM formic acid

- Gradient
  - Negative mode: 10 min gradient
  - Positive mode: 9 min gradient

Mass spectrometric parameters

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Analytical toxin standards were obtained from NRC’ CRM (Canada) and used for method development and quantitation.

References: McCarron et al., 2014; Silver et al., 2010; Wang et al., 2007
Methods: Hood Canal monitoring sites

- Sampling sites
  - Sequim Bay (SB)-a reference point
  - Hood Canal Bridge (HC)
  - Quilcene (QC)
  - Pointe Whitney (PW)
  - Triton State Park (TS)
  - Ayock (AY)
  - Glen Ayr (GA)
  - Lilliwaup (LW)
  - Dewatto Beach (DB)
  - Rensland (RS)
  - Union Dock (UD)
  - Hood Sport (HS)
  - Port of Allyn (PA)

- Sampling and monitoring period
  - June 1 – September 30, 2017
Results: Separation and Detection of toxins

Negative mode detection
OA eluting at 4.29 min Detection limits: 4 ng/L
DTX-2 eluting at 4.50 min Detection limits: 4 ng/L
DTX-1 eluting at 5.04 min Detection limits: 4 ng/L

Positive mode detection
DA eluting at 3.41 min Detection limits: 40 ng/L
PTX-2 eluting at 5.19 min Detection limits: 4 ng/L
Results: Toxin monitoring in Hood Canal

Domoic acid

• DA is present throughout the monitoring period in HC
• DA surged at the 13\textsuperscript{th} week (Aug 25th) peaking at the 14\textsuperscript{th} week (Sept 1)
• Some sites observed over 2 µg/L DA concentrations-NOAA’s recommended risk limits
• DA levels subsided after the 15th week
• No phytoplankton and toxicity data available
Results: Toxin monitoring in Hood Canal
Dinophysistoxin-1

• Low levels of DTX-1 was observed in Hood Canal, while higher concentrations in Sequim Bay
• Slight variation in DTX-1 concentrations over sampling sites and sampling period in Hood Canal
• Sequim Bay maintained higher concentrations over time—although toxicity is not known
Results: Toxin monitoring in Hood Canal

Pectenotoxin-2

- Low levels of PTX-2 were present during the monitoring period (below 100 ng/L) in Hood Canal
- Sequim Bay exhibited fluctuating concentrations over the sampling period
- In Sequim Bay, PTX-2 reached a peak over 2.5 µg/L
- No toxicity information arising from this toxin is known
Results: Method development for saxitoxin

- Porous graphitized carbon (PGC) cartridges tested
- Sigma’s Envi carb cartridge works best
- zHILIC column found most reliable
- A 10 min gradient program developed
- Due to the limitation of volume that can be extracted, the detection limits of this method will be over 1 µg/L
- Further refinement may need

References: Armstrong, 2017; Brag et al., 2015; Halme et al., 2012
Conclusions

• Sensitive analytical methods for 5 toxins using LC/MS were developed
• Pilot monitoring study conducted on 13 sampling sites demonstrated that the LC/MS analysis can provide reliable measurements of 5 toxins in water and phytoplankton samples
• SPE and LC/MS methods for hydrophilic toxins have been worked out and will be deployed to monitor in 2018
• Current chemistry data need to be combined with phytoplankton abundance and shellfish toxicity data
• Further collaborations with DOH and Sound Toxins
• Further refinement of analytical protocols
Further Studies

- Deployment of LC/MS analysis for hydrophilic toxins (STX, GTX)
- Phytoplankton analysis: identification and abundance
- Environmental fate studies for algal toxins
- Dynamics of toxins in foodwebs
- Preparation for future accreditation
- Open to collaborations
Acknowledgements

• Skokomish Indian Tribe Council for their general support
• EPA for their support for the Skokomish Tribe’s water quality lab.
• BIA for funding this project

Thanks for listening