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

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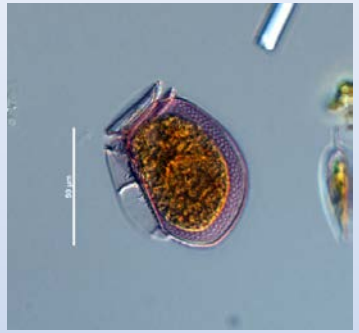
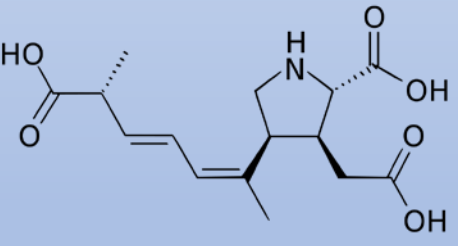
Overview

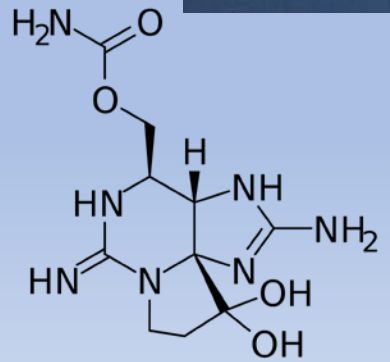
- Background
- Methods
- Results
- Discussion
- Future studies
- Acknowledgements

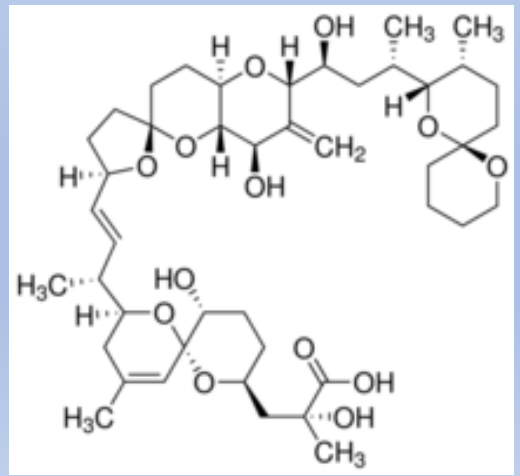
Background: Marine algal toxins

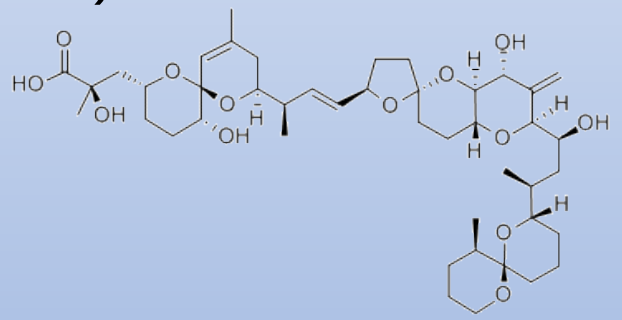


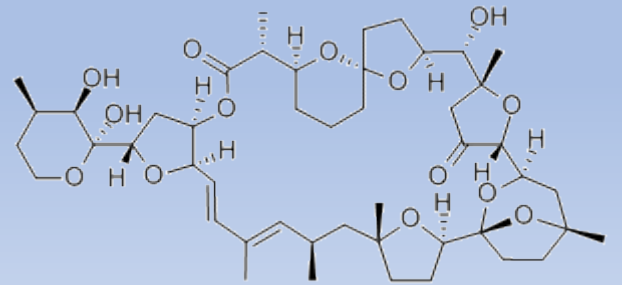
- Hydrophilic toxins
  - Domoic acid (ASP)+
  - Saxitoxin (PSP)+
  - Gonyautoxin (PSP)+
  - Neosaxitoxins (PSP)+
- Lipophilic toxins
  - Okadaic acid (DSP)-
  - Dinophysistoxins (DSP)-
  - Pectenotoxins (DSP)+
  - Yessotoxin-











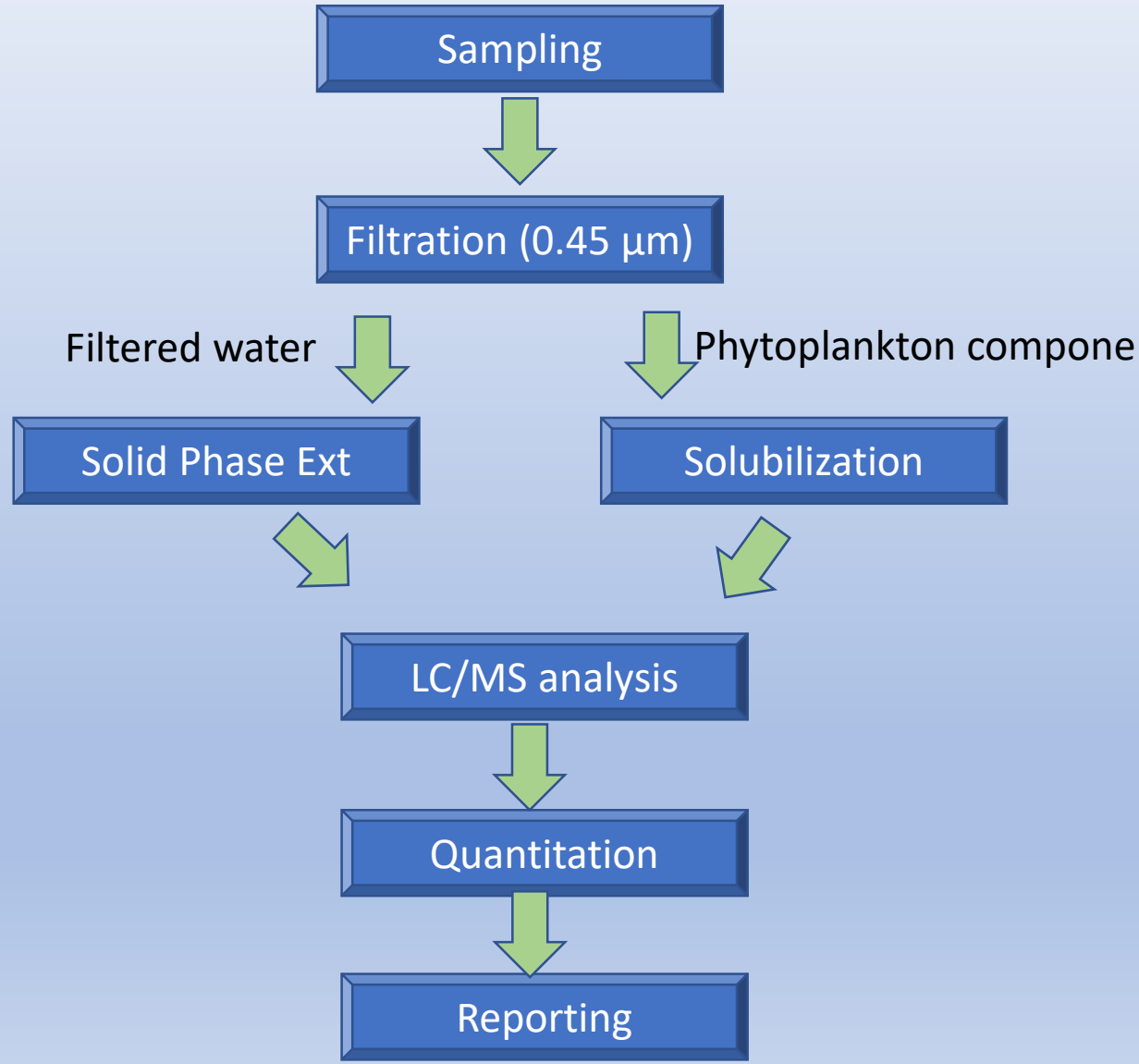
Background: Marine algal toxin analysis

- 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		Hydrophilic Toxins
Oasis HLB	SPE media	Envi Carb-graphitized carbon
Methanol	Priming	20% acetonitrile
250 ml seawater	Loading	Up to 12 ml
Oasis HLB	Cleaning	DI water
Methanol	Elution	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 formaic acid
- Gradient
  - Negative mode: 10 min gradient
  - Positive mode: 9 min gradient

Mass spectrometric parameters

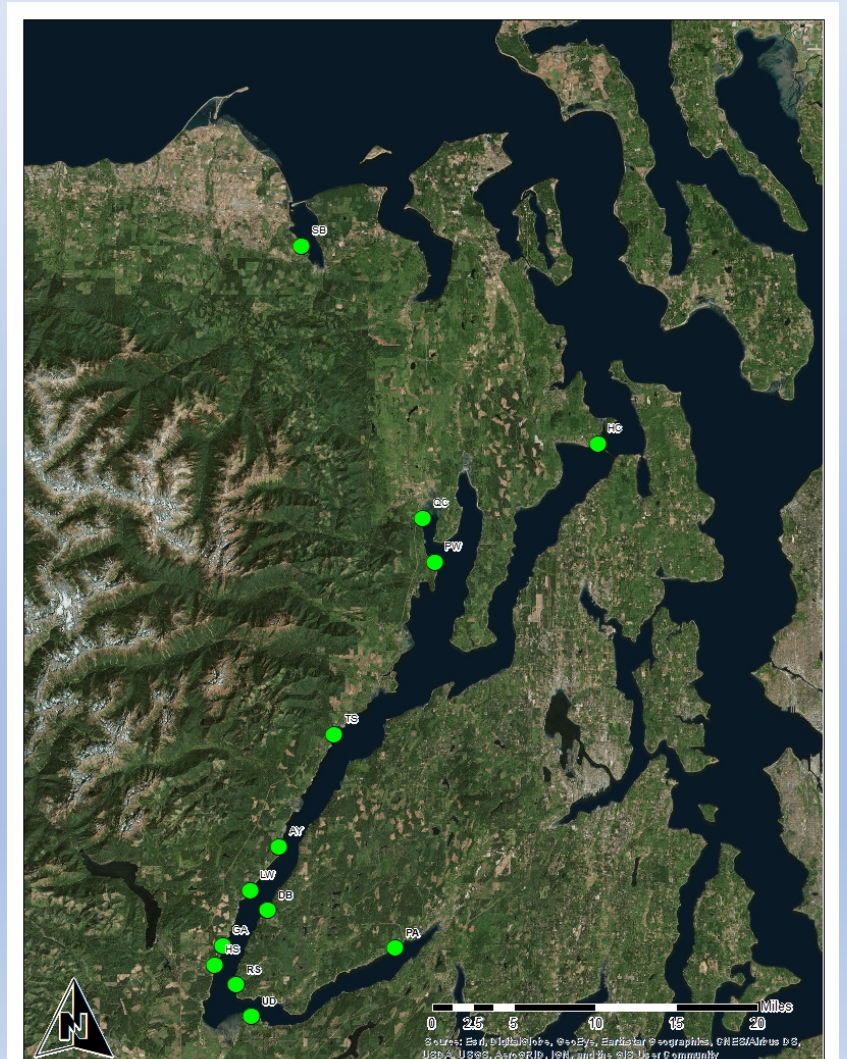
	Q1	Q3	Dwell (msec)	CE	CXP
OA	803.607	255.20	150	-64	-23
	803.607	113.20	150	-82	-13
DTX-2	803.598	255.100	150	-60	-15
	803.598	113.200	150	-82	-11
DTX-1	817.603	255.200	150	-64	-23
	817.603	113.100	150	-82	-13

	Q1	Q3	Dwell (msec)	CE	CXP
DA	312.292	266.300	150	21	28
	312.292	161.100	150	33	18
PTX-2	876.414	85.200	150	109	18
	876.414	95.200	150	105	14

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

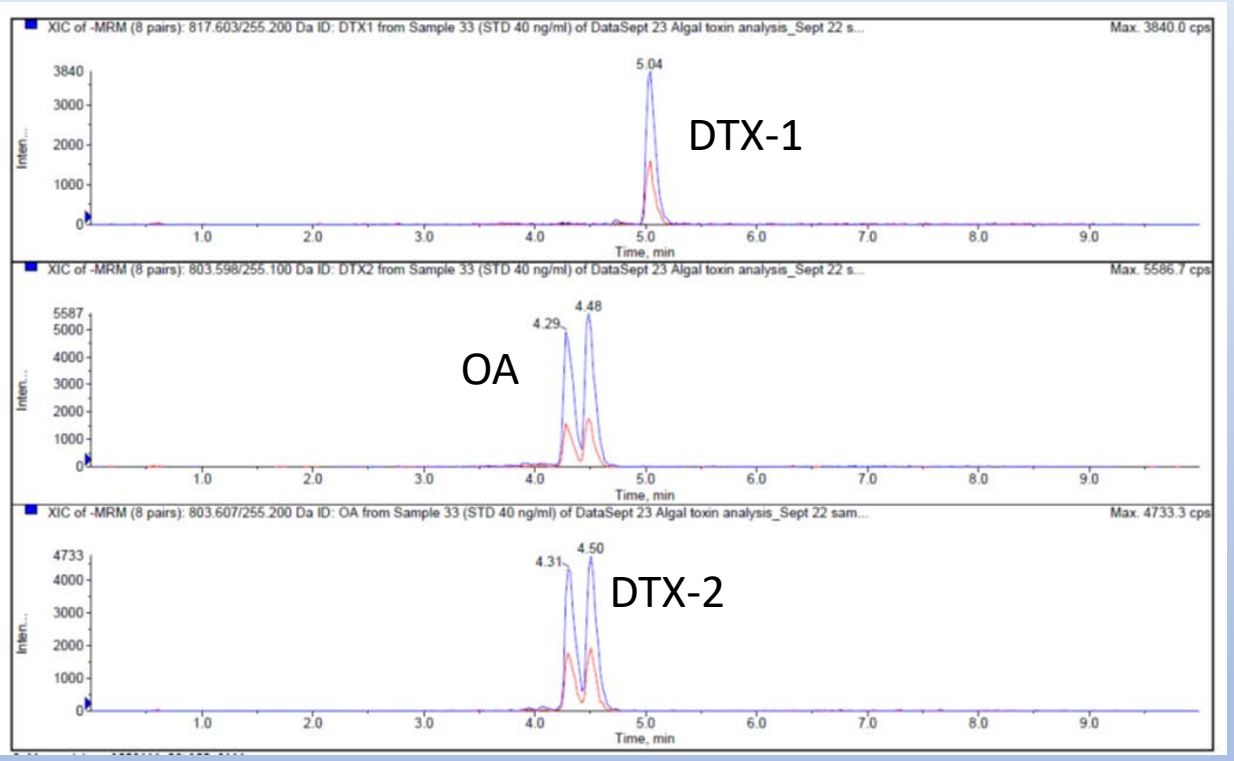
References: McCarron et al., 2014; Silver et al., 2010; Wang et al., 2007

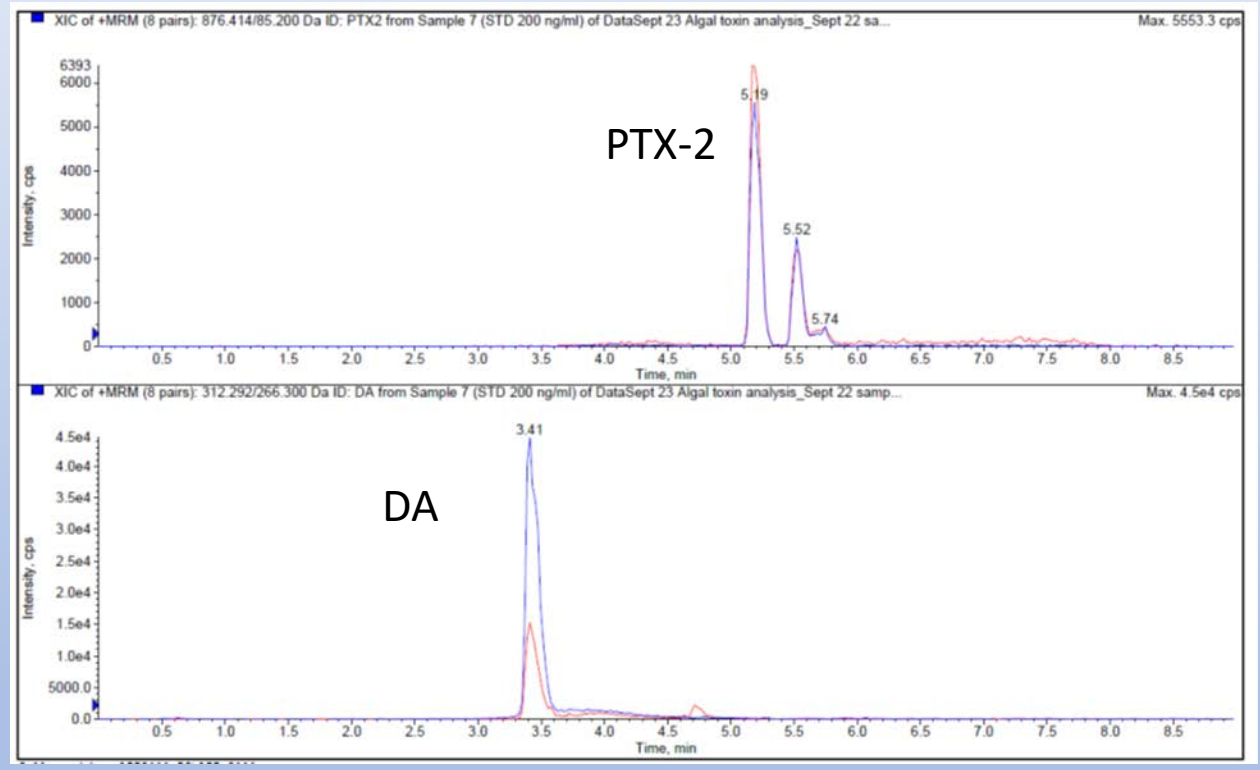
Methods: Hood Canal monitoring sites



- 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

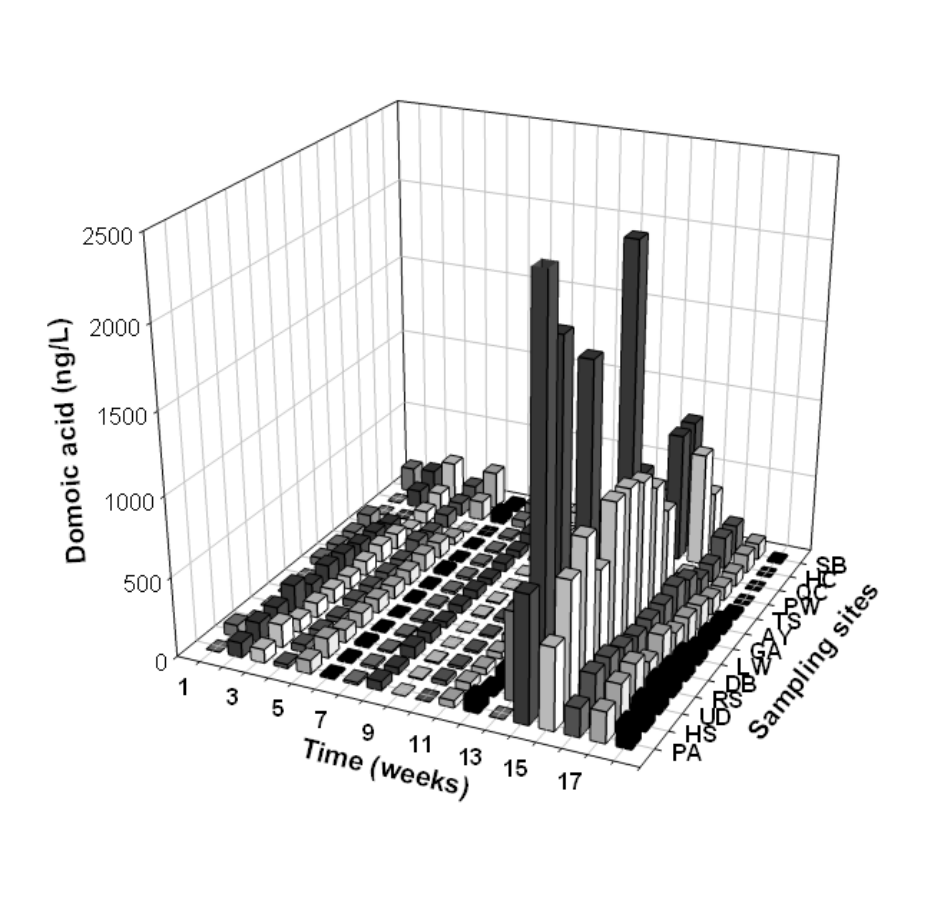




Negative mode detection
OA eluting at 4.29 min Detection limits: 4 ng/L
DTX-2 eluting at 4.50 min Detection limits: 4 ng/L
DTX-1 eluting at 5.04 min Detection limits: 4ng/L

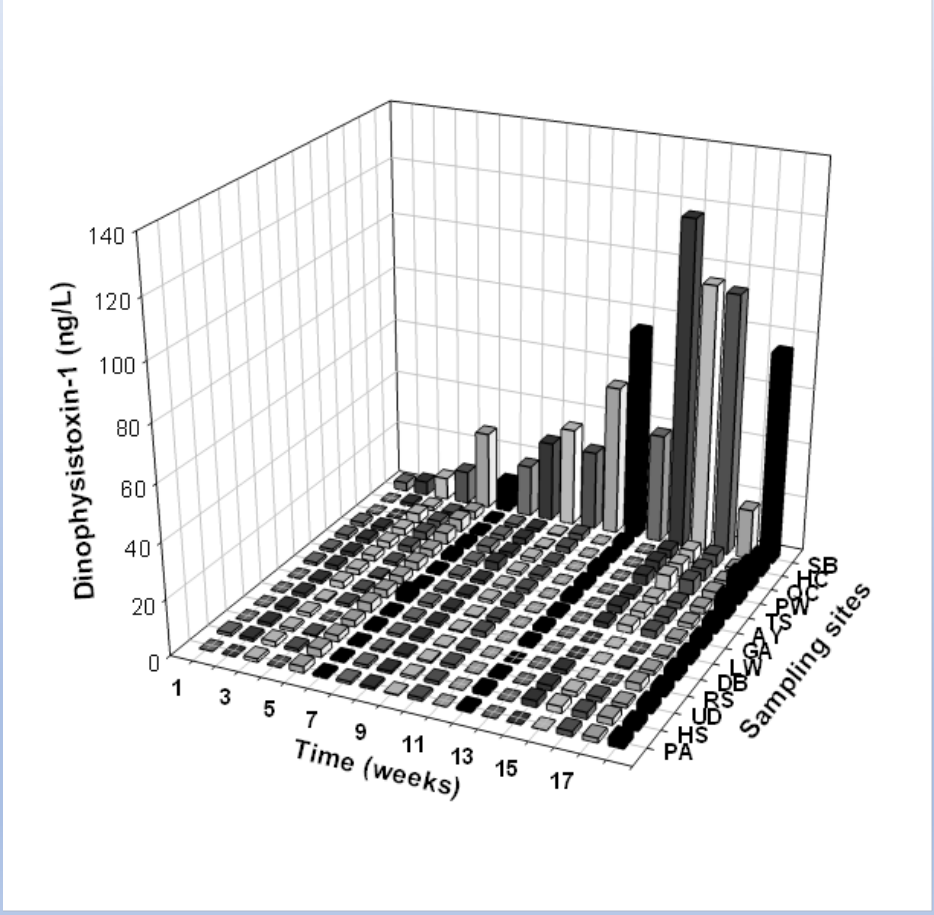
Positive mode detection
DA eluting at 3.41 min Detection limits: 40 ng/L
PTX-2 eluting at 5.19 min Detection limits: 4 ng/L

Results: Toxin monitoring in Hood Canal Domoic acid



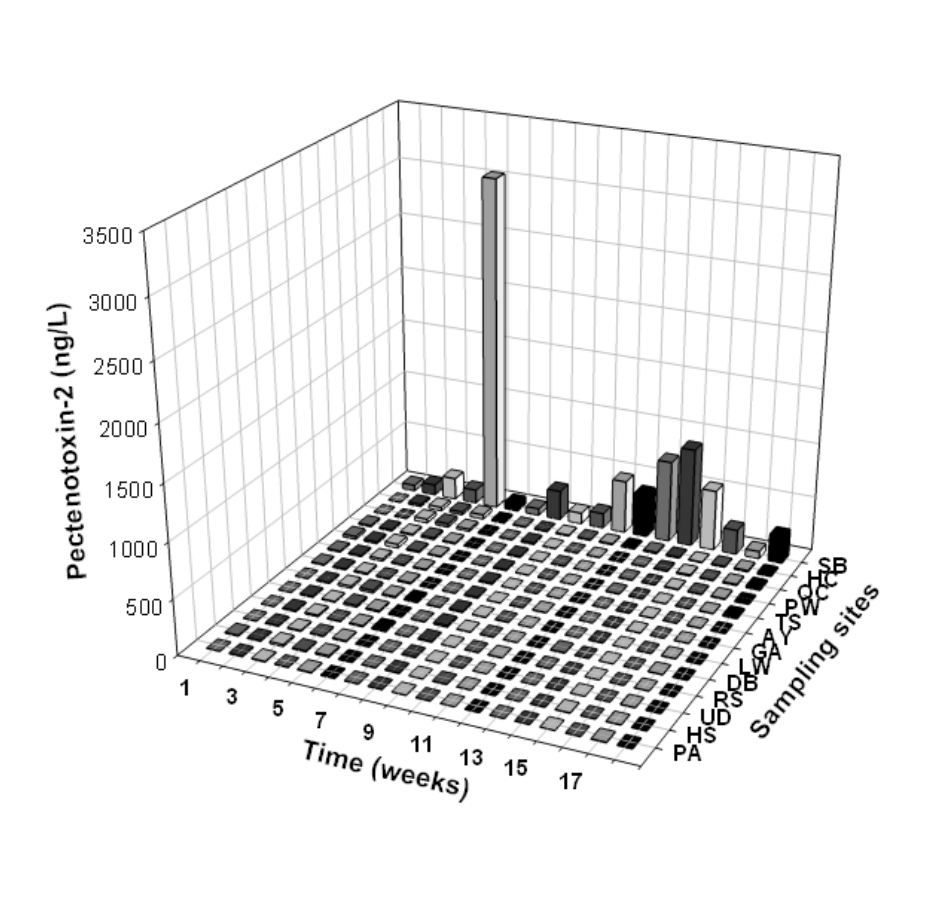
- DA is present throughout the monitoring period in HC
- DA surged at the 13th week(Aug 25th) peaking at the 14th week (Sept 1)
- Some sites observed over 2 µg/L DA concentrations-NOAA's recommended risk limits
- DA levels subsided after the 15th week
- No phytoplankton and toxicity data available

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

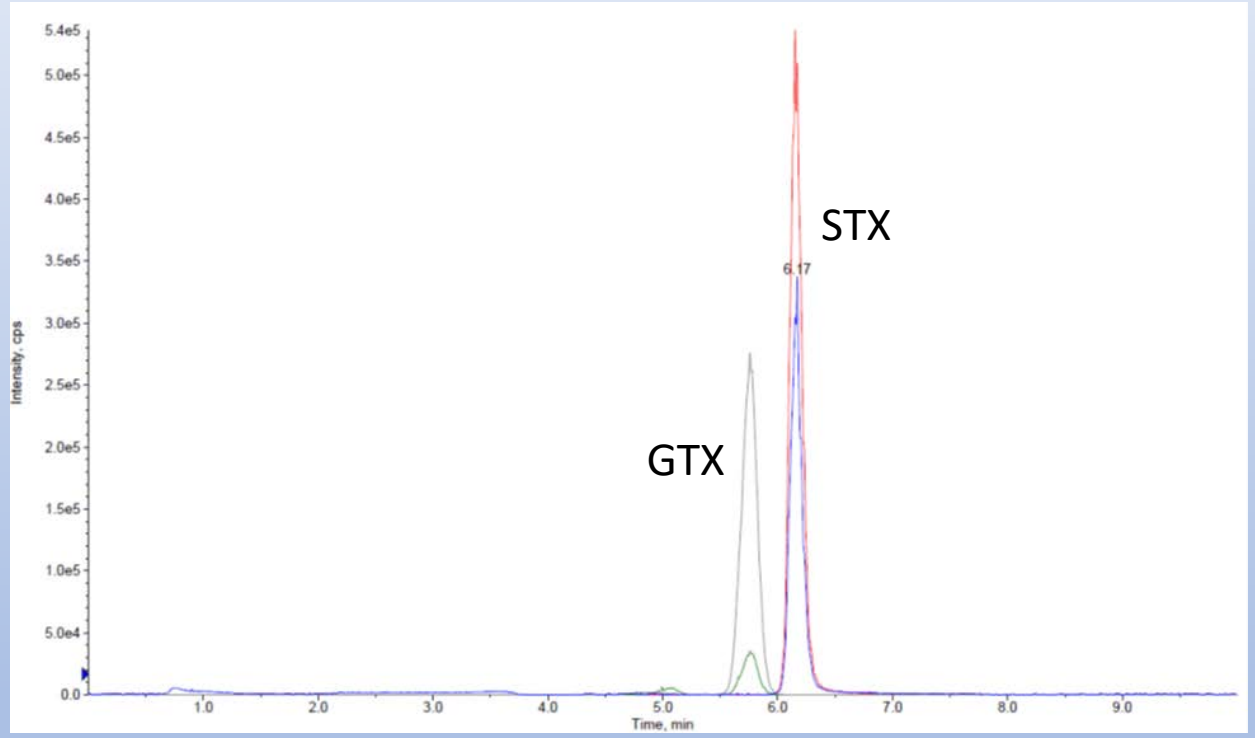
Results: Toxin monitoring in Hood Canal Pectenotoxin-2



- 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 cab be extracted, the detection limits of this method will be over 1 µg/L
- Further refinement may need



References: Amstrong, 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