Challenges and successes in monitoring the toxic diatom *Pseudo-nitzschia*:
Linking research, management, and observation

ASP Workshop 4/10/19
K. Hubbard

Woods Hole Center for Oceans and Human Health
Woods Hole Oceanographic Institution
The toxic diatom *Pseudo-nitzschia*

**Broadly distributed**
- In all major oceans
- From the equator to the poles

Currently \( \sim 50 \) described species in genus (many look identical via light microscopy)

Some species produce neurotoxin domoic acid (DA)...

Domoic acid causes Amnesic Shellfish Poisoning in humans and Domoic Acid Poisoning in marine birds and mammals
Sample collected at Mount Desert Rock, ME on July 20, 2012 by Maine Phytoplankton Monitoring Program
(Image provided by A. Sirois)
First ASP event in 1987: >100 humans sick and 3 mortalities from DA-contaminated mussels harvested during bloom
Modified from www.whoi.edu
Transfer of domoic acid up the food web - bivalves

Scallop:
- **DA** accumulates and depurates slowly
- primarily in non-edible tissue (Stewart et al. 1997)
- EU guidelines designed to allow harvest (separate testing and threshold of 4.6 ppm for edible tissue)

Blue mussels:
- DA accumulates and depurates rapidly after exposure (72 hours or more) - good sentinel species

Regulatory closure limit = 20 ppm
Transfer of domoic acid up the food web

Filter feeding fish

Amnesic Shellfish Poisoning (ASP) human syndrome that can result in nausea, vomiting, diarrhea, abdominal cramps and neurological symptoms (headaches, hallucinations, confusion, short-term memory loss), respiratory difficulty, seizures, coma, death (http://www.nmfs.noaa.gov/pr/pdfs/health/domoic_acid.pdf)
Domoic Acid Poisoning in marine birds and mammals – sometimes presents as “unusual” behavior

This is a California sea lion on Long Beach, Washington, apparently experiencing seizures from domoic acid poisoning in May 2015.

DAN AYRES/WASHINGTON DEPARTMENT OF FISH AND WILDLIFE
Presence of ASP Toxins in Seafood in the U.S.

West Coast closures from early ‘90's

1987 Eastern Canada

East Coast closures more recent ~2013

Modified from www.whoi.edu
Many factors have been shown to affect domoic acid production

These can also impact growth – so how do you get a toxic bloom??
Variability in DA production

species  nutrients  salinity  zooplankton grazing

"3300% increase in DA production when P. seriata exposed to grazing; induced toxin production in "non-toxic" species"

15 species have been confirmed in the Gulf of Maine using traditional approaches (electron microscopy and DNA sequencing); by rapid genetic methods, predict >25 species likely; at least 8 are toxic
First ASP event in 1987 → 30 years ago; toxin had to be identified
Phytoplankton monitoring

- Federal, state, academic, citizen sampling
- Focuses on screening for known and novel HABs

Biotoxin testing

- FDA-approved laboratories that follow national regulations
- Different methods approved for primary HAB toxins that impact the US → rapid vs. regulatory screening for ASP toxins

Biotoxin testing only program (e.g. Canada)
Domoic Acid Closures: After 1987 → Canadian shellfish routinely tested by Canadian Food Inspection Agency; phytoplankton monitoring declined over last decade.
Closures occur July-October... except during Spring 2002
Different *Pseudo-nitzschia* species during toxic events

- *P. multiseries* before 2000 - except BOF
- "*P. pseudodelicatissima*" in BOF
- *P. seriata* during 2000-2008 - esp. Quebec, and in Spring 2002 (BOF, PEI, NS)

Map showing the occurrence of different species in various regions.

Quebec

Prince Edward Island

New Brunswick

Nova Scotia

Map markers:
- 2001

Source: ASP Workshop 4/10/19 K. Hubbard
A History of Domoic Acid in Eastern North America

ASP Closures in eastern Canadian provinces

87 88 89 90 91 92 93 94 95 96 97 98 99 00 01 02 03 04 05 06 07 08 09 10 11 12 13

- Closures in one province
- Closures in more than one province
A History of Domoic Acid in Eastern North America

ASP Closures in eastern Canadian provinces

87 88 89 90 91 92 93 94 95 96 97 98 99 00 01 02 03 04 05 06 07 08 09 10 11 12 13

- Closures in one province
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Domoic acid and closures in eastern US waters

- 1991 Low levels, shellfish, Nantucket Shoals (Nassif and Timperi 1993)
- 1995 Very high levels, scallops, Browns and Georges Banks (Stewart et al. 1997)
- 2003 N/A; Whale mortalities (NOAA NEFSC 2003)
- 2004-2006 Low levels, scallop study, Georges Bank (Day et al. 2008)
- 2005-2006 Low levels, whale feces, krill (Leandro et al. 2010)
- 2012, 2013 Eastern ME Precautionary DA closures; 2013 first FL closure (Gulf of Mexico)
- 2014 2nd FL Closure
- 2015 Major West Coast bloom (Not east coast!!)
- 2016 First Gulf of Maine ASP closure → high levels in eastern Maine bivalves
- 2017 First Rhode Island ASP closure, 2nd Maine ASP closure, 3rd FL ASP closure
- 2018 4th FL ASP closure?
HARMFUL ALGAL BLOOMS

Monitoring Approaches for

Early Warning of Domoic Acid Events in Washington State

By Vera L. Trainer and Marc Sudoleson

On the U.S. west coast, blooms of the potentially toxic diatom Pseudo-nitzschia can cause amnesic shellfish poisoning (ASP), resulting in economic impacts to coastal economies and public health concerns. The transfer of toxins, via filter feeding of Pseudo-nitzschia or ingestion of filter feeding organisms, to shellfish, crustaceans, seabirds, fish, and marine mammals has led to mortalities of brown pelicans, Brandt's cormorants, and sea lions.

A unique problem on the outer coast of the Olympic Peninsula in Washington State, is that the Pacific razor clam, Siliqua patula (Figure 1), can retain high concentrations of the algal toxin, domoic acid (DA), for over one year (Widdel et al., 1994; Adams et al., 2000). During toxic events, recreational, commercial, and tribal subsistence harvest of clams, valued at over $20 million annually (Anderson, 1995), is suspended and public health is threatened.

8 week commercial season with recreational harvests

https://wdfw.wa.gov/fishing/shellfish/oysters/Puget Sound

Olympic Peninsula

Commercial aquaculture

Figure 1. Olympic Region Harmful Algal Bloom (HAB) sampling locations include major areas of razor clam harvest (shown in red). Razor clams (Patula) are harvested year round for recreational, commercial, and tribal subsistence purposes. Throughout the text, Kalaloch and Copalis are the western beaches, whereas Twin Harbors and Long Beach are the southern beaches.

Sean Nicole, 2019
Samples collected and analyzed by trained volunteers

https://www.soundtoxins.org/index.html: 31 sites sampled weekly
Support provided by Sea Grant, NOAA, and >20 partners

Domoic Acid Levels - Kalaloch
January 1999 - December 2017

- Routine screening via light microscopy and toxin testing
- Species ID via EM/sequencing as needed

https://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html
Domoic Acid Levels - Kalaloch
January 1999 - December 2017

- Routine screening via light microscopy and toxin testing
- Species ID via EM/sequencing as needed

Back to eastern Canada... Toxic blooms occur sporadically over time in other areas as well (is this a trend in temperate systems?)

https://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html
West Coast 2015 *Pseudo-nitzschia* bloom: the worst ASP event to date??

<table>
<thead>
<tr>
<th>2015</th>
<th>Shellfish Harvest and Fishery Closures with Maximum Domoic Acid Values</th>
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<tbody>
<tr>
<td>7-May</td>
<td>Quinault tribe razor clam harvest closure (WA)</td>
</tr>
<tr>
<td>8-May</td>
<td>Commercial, tribal &amp; recreational razor clam harvest closure (WA)</td>
</tr>
<tr>
<td>9-May</td>
<td>Razor clam harvest closure (northern OR)</td>
</tr>
<tr>
<td>14-May</td>
<td>State wide razor clam harvest closure (OR)</td>
</tr>
<tr>
<td>15-May</td>
<td>Shellfish harvest closure (BC Canada)</td>
</tr>
<tr>
<td>29-May</td>
<td>Anchovy viscera maximum 1671 ppm (CA)</td>
</tr>
<tr>
<td>1-Jun</td>
<td>Anchovy, sardine fishery closure (CA)</td>
</tr>
<tr>
<td>3-Jun</td>
<td>Dungeness crab maximum 65 ppm (WA)</td>
</tr>
<tr>
<td>5-Jun</td>
<td>Dungeness crab fishery closure (WA)</td>
</tr>
<tr>
<td>3-Jul</td>
<td>Anchovy, sardine, mussel, &amp; clam closures expanded to southern CA</td>
</tr>
<tr>
<td>11-Sep</td>
<td>Dungeness crab maximum 140 ppm (northern CA)</td>
</tr>
<tr>
<td>27-Oct</td>
<td>Razor clam maximum 170 ppm (southern OR)</td>
</tr>
<tr>
<td>3-Nov</td>
<td>Dungeness crab &amp; rock crab warning for recreational harvest (CA)</td>
</tr>
<tr>
<td>6-Nov</td>
<td>Commercial rock crab fishery closed (CA)</td>
</tr>
<tr>
<td>8-Nov</td>
<td>Dungeness crab maximum 70 ppm (southern OR)</td>
</tr>
<tr>
<td>11-Nov</td>
<td>Dungeness crab &amp; rock crab recreational &amp; commercial fishery closure (CA)</td>
</tr>
<tr>
<td>22-Nov</td>
<td>Dungeness crab maximum 270 ppm (northern CA)</td>
</tr>
<tr>
<td>23-Nov</td>
<td>Rock crab maximum 1000 ppm (southern CA)</td>
</tr>
<tr>
<td>23-Nov</td>
<td>Delayed opening of commercial Dungeness crab fishery (WA, OR, CA)</td>
</tr>
<tr>
<td>9-Feb-2016</td>
<td>CA seeks federal disaster declaration for commercial crab fishery</td>
</tr>
</tbody>
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Estimated $100 million decrease in value from Dungeness crab harvest closures alone

“the most valuable fishery on the U.S. West Coast”

- State of California requested a federal fishery disaster declaration from the U.S. Department of Commerce
- Related large whale mortality event in British Columbia/Alaska

McCabe et al. 2016
Advancing *Pseudo-nitzschia* monitoring efforts in the Gulf of Maine

State phytoplankton/toxin monitoring

**CELLULAR ABUNDANCE BINS**

<table>
<thead>
<tr>
<th>grids to get 500</th>
<th>Cell abundance bins</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;100</td>
<td>&lt;7500 cells/L</td>
</tr>
<tr>
<td>100 to 50</td>
<td>7500-15,000 cells/L</td>
</tr>
<tr>
<td>49 to 16</td>
<td>15,306 to 46,875</td>
</tr>
<tr>
<td>13</td>
<td>50,000</td>
</tr>
<tr>
<td>7</td>
<td>107,142</td>
</tr>
<tr>
<td>&lt;7***</td>
<td>wholewater count</td>
</tr>
</tbody>
</table>

“Pilot” study initiated in 2013 → integrated simple genetic and DA filtering into DMR Volunteer routine sampling @ 2 sites in Maine, Bar Harbor Town Pier and Salisbury Cove

Samples collected ~weekly during summer since 2013

NH trained in 2017 and provided event response samples
Rapid Response sampling for Pseudo-nitzschia spp. 2012-2016

- Post-peak: 8/4 - 8/5/2012
- During peak: 8/3 - 8/7/2013
- Pre-peak: 7/25 - 7/27/2014
- During local peak: 8/2 - 8/5/2015
- During closure: 10/3-10/6/2016

Funding through Woods Hole Center for Oceans and Human Health (2013-2016), NOAA (2012, 2016), Bigelow (2016)
First observation of toxic species, *P. australis*, in Gulf of Maine

I. Scanning (and light) electron microscopy

Hubbard et al. *in prep*
First observation of *Pseudo-nitzschia australis* in the Gulf of Maine

I. Light and scanning electron microscopy

![Image of *P. australis* with 2 rows of poroids per striae](image)

*P. australis*

Hubbard et al. *in prep*
First observation of *Pseudo-nitzschia australis* in the Gulf of Maine

I. Light and scanning electron microscopy

II. Direct DNA sequencing from single cells or chains

~400 bp of taxonomically informative rRNA operon showed 100% identity to *P. australis*

Hubbard et al. *in prep*
First observation of *Pseudo-nitzschia australis* in the Gulf of Maine

I. Light and scanning electron microscopy

II. Direct DNA sequencing from single cells or chains

III. Genetic fingerprinting from DNA extracts

Hubbard et al. *in prep*
**Pseudo-nitzschia** species DNA fingerprinting

Hubbard et al. 2008 *J. Phycol.*
Hubbard et al. 2014, *MEPS*

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1. *P. sabit*
2. *P. pungens*
3. *P. seriata*
4. *P. delicatissima*
5. Unknown
6. Unknown
7. *P. plurisecta*
8. *P. caciantha*

**NEW:** *P. australis*
Pseudo-nitzschia species as early warning of toxicity?

P. australis first detected using fingerprinting in 9/29 samples from Salisbury Cove.
ASP closures vary for shellfish and commercial species: Florida 2017

Closures
2013
2014
2017
2018?
Biotoxins sometimes coincident in time and/or space

Statewide *Karenia brevis* concentrations
03/29/2018 - 04/05/2018

*Karenia brevis* (cells/liter)
- not present/background (0-1,000)
- very low (>1,000-10,000)
- low (>10,000-100,000)
- medium (>100,000-1,000,000)
- high (>1,000,000)
Phytoplankton monitoring
- Federal, state, academic, citizen sampling

Biotoxin testing
- FDA-approved laboratories that follow national regulations
- Different methods approved for primary HAB toxins that impact the US → facilitate rapid screening for ASP toxins (methods used by DMR, FWC, other states)

Each state has different concerns, resources, and approaches to ASP management in response to recent DA events