

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



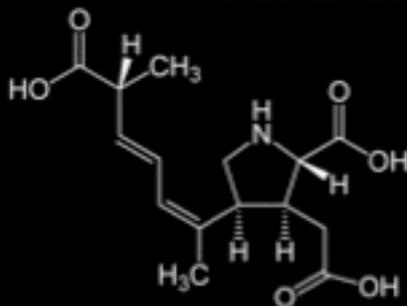
The toxic diatom *Pseudo-nitzschia*

Broadly distributed

- In all major oceans
- From the equator to the poles

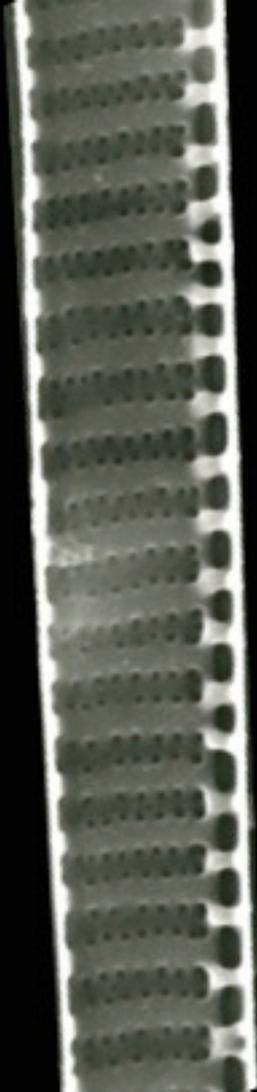
Currently ~50 described species in genus (many look identical via light microscopy)

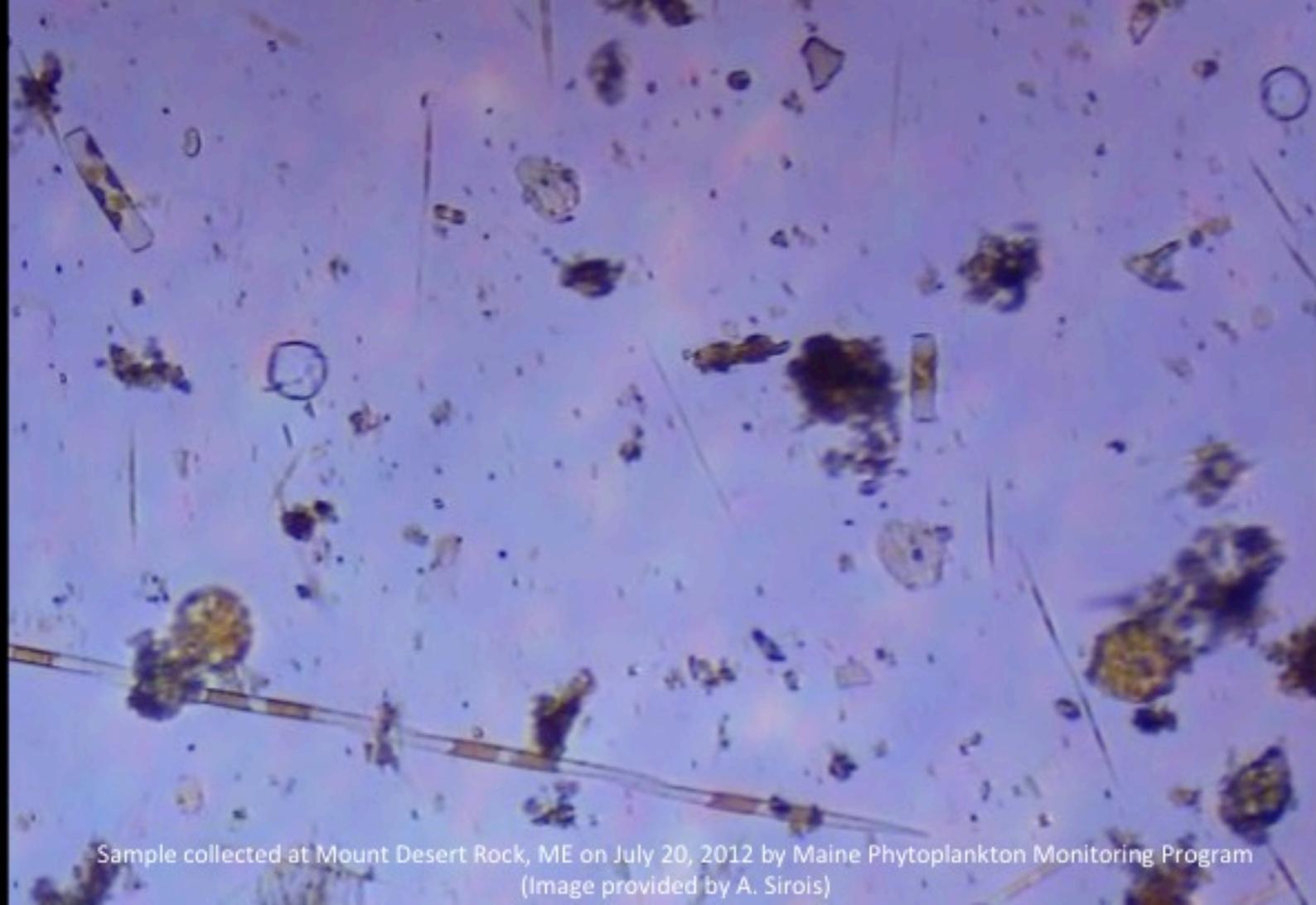
Some species produce neurotoxin domoic acid (DA)...



Domoic acid causes Amnesic Shellfish Poisoning in humans and Domoic Acid Poisoning in marine birds and mammals

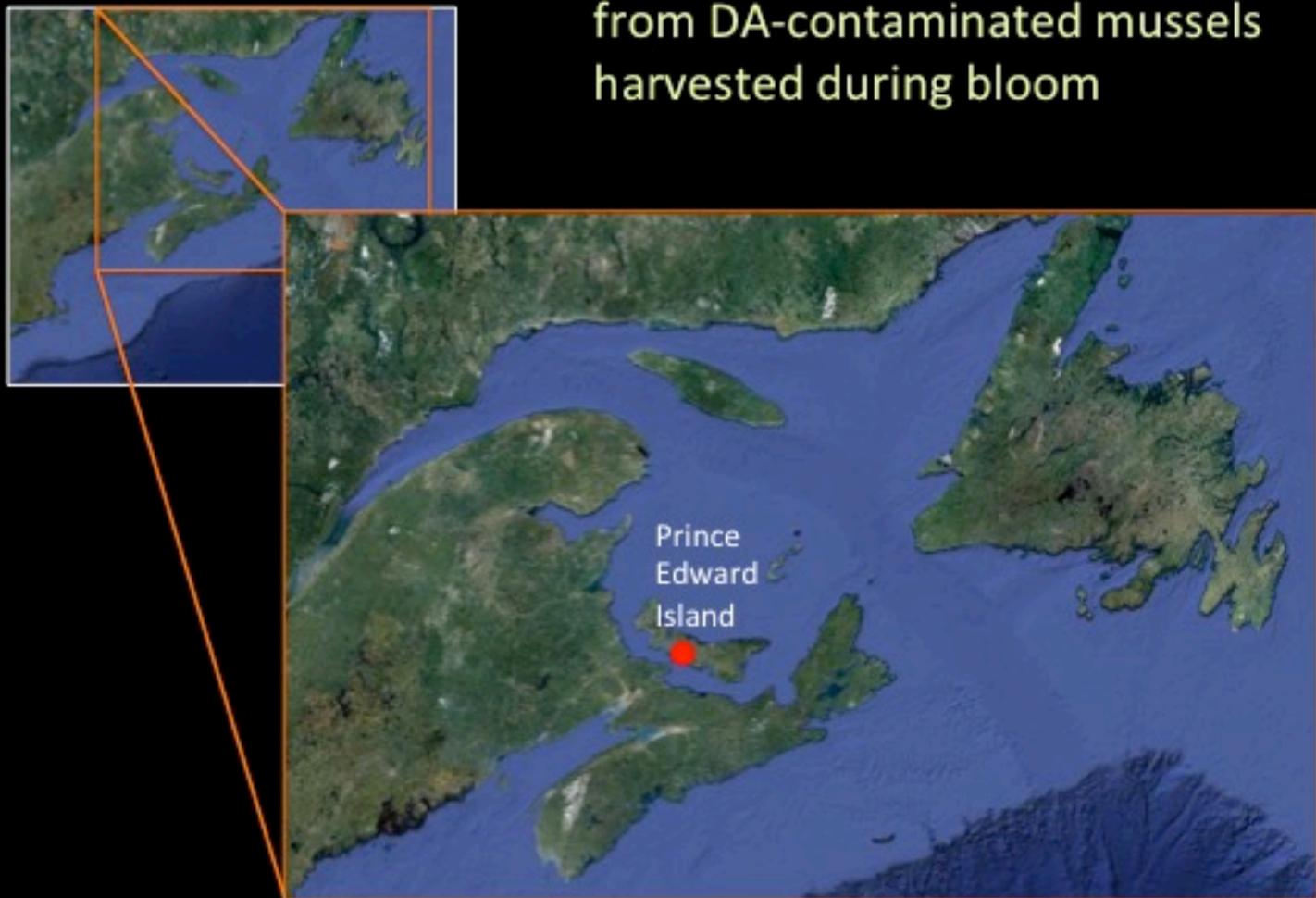
2 μ m





Sample collected at Mount Desert Rock, ME on July 20, 2012 by Maine Phytoplankton Monitoring Program
(Image provided by A. Sirosis)

First ASP event in 1987: >100 humans sick and 3 mortalities
from DA-contaminated mussels
harvested during bloom

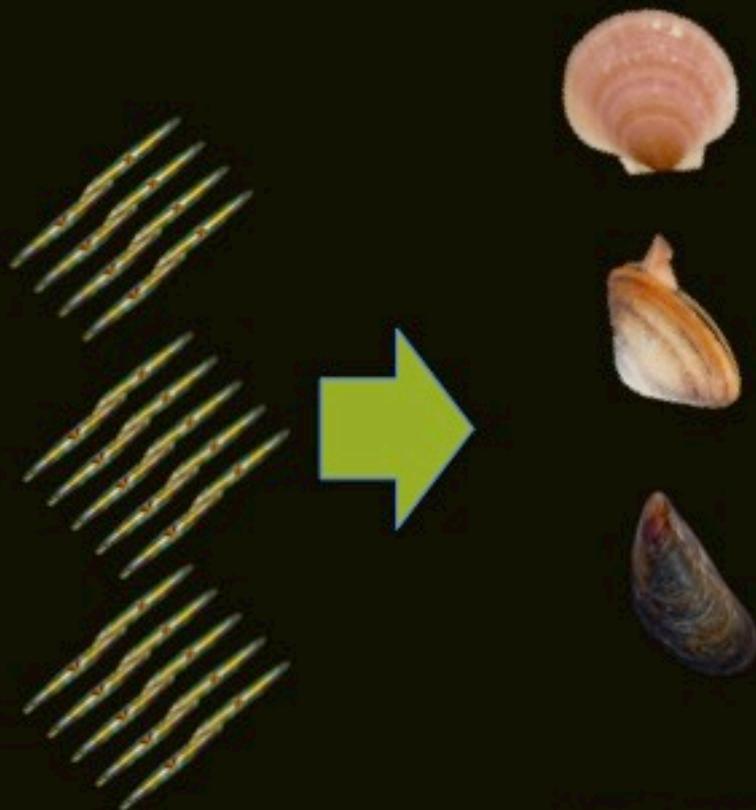


• ASP



Modified from www.whoi.edu

Transfer of domoic acid up the food web- bivalves



Scallops:

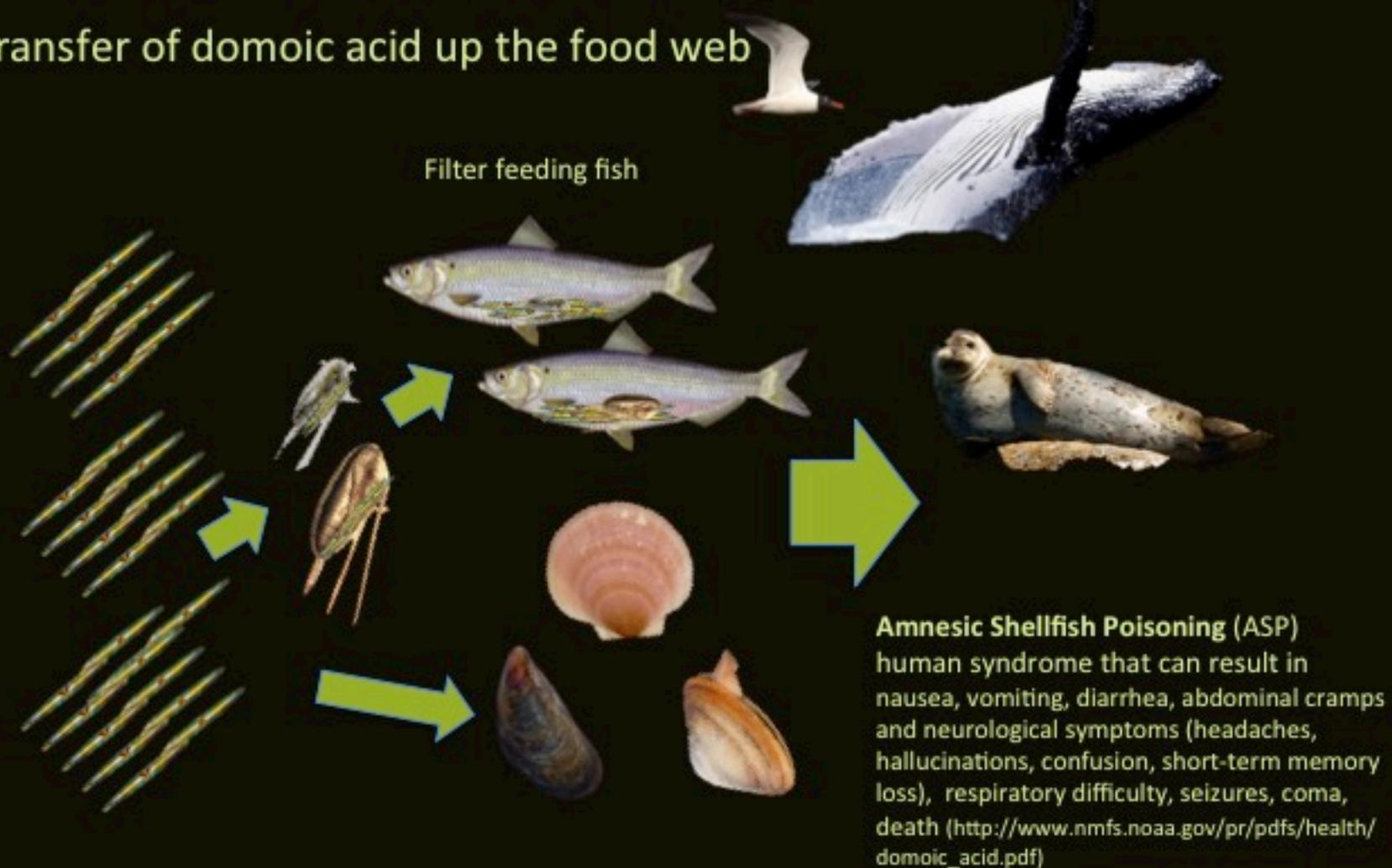
- DA accumulates and depurates slowly
- primarily in non-edible tissue (Stewart et al. 1997)
- EU guidelines designed to allow harvest (separate testing and threshold of 4.6 ppm for edible tissue)

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



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

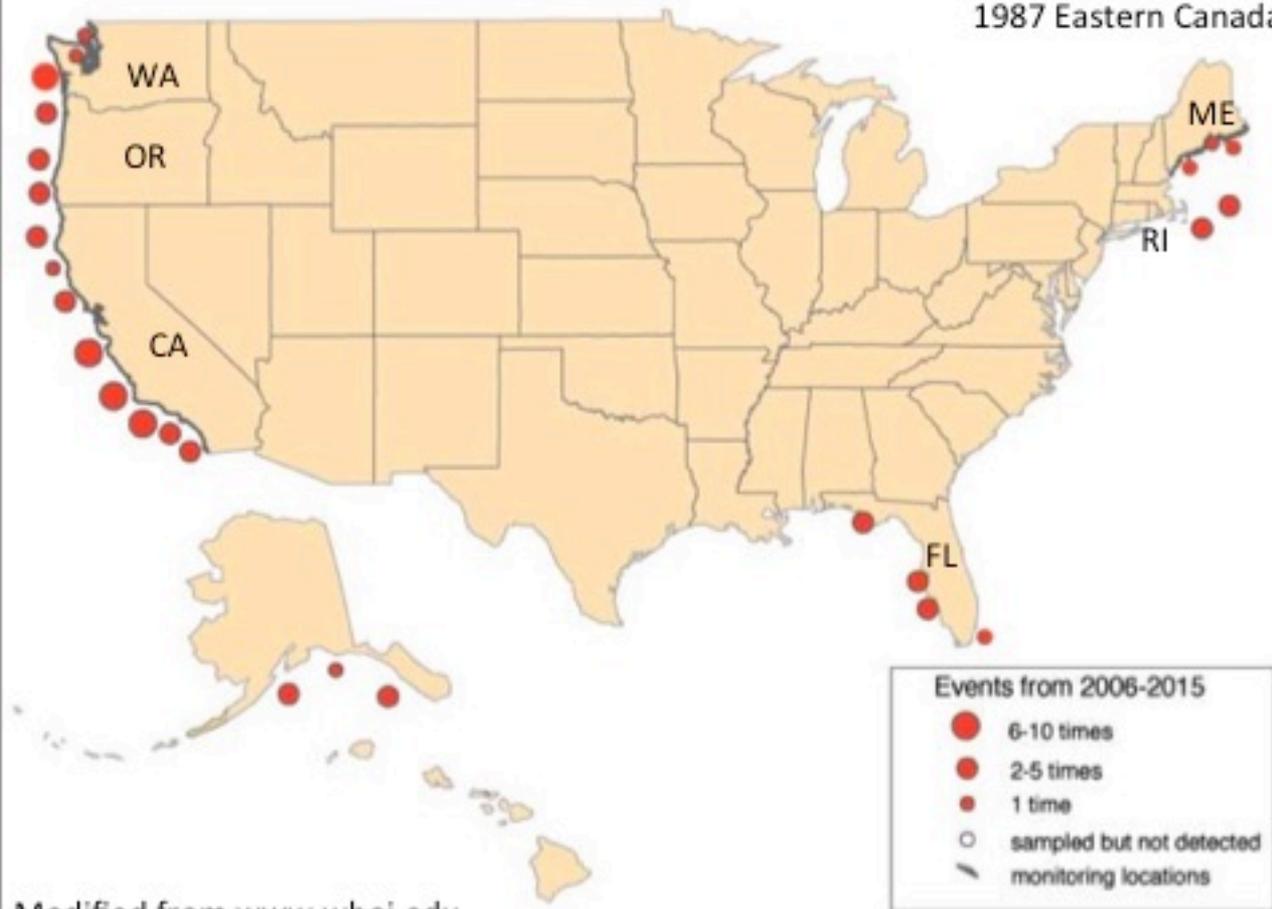


Presence of ASP Toxins in Seafood in the U.S.

1987 Eastern Canada

West Coast
closures
from early
'90's

East Coast
closures
more
recent
~2013



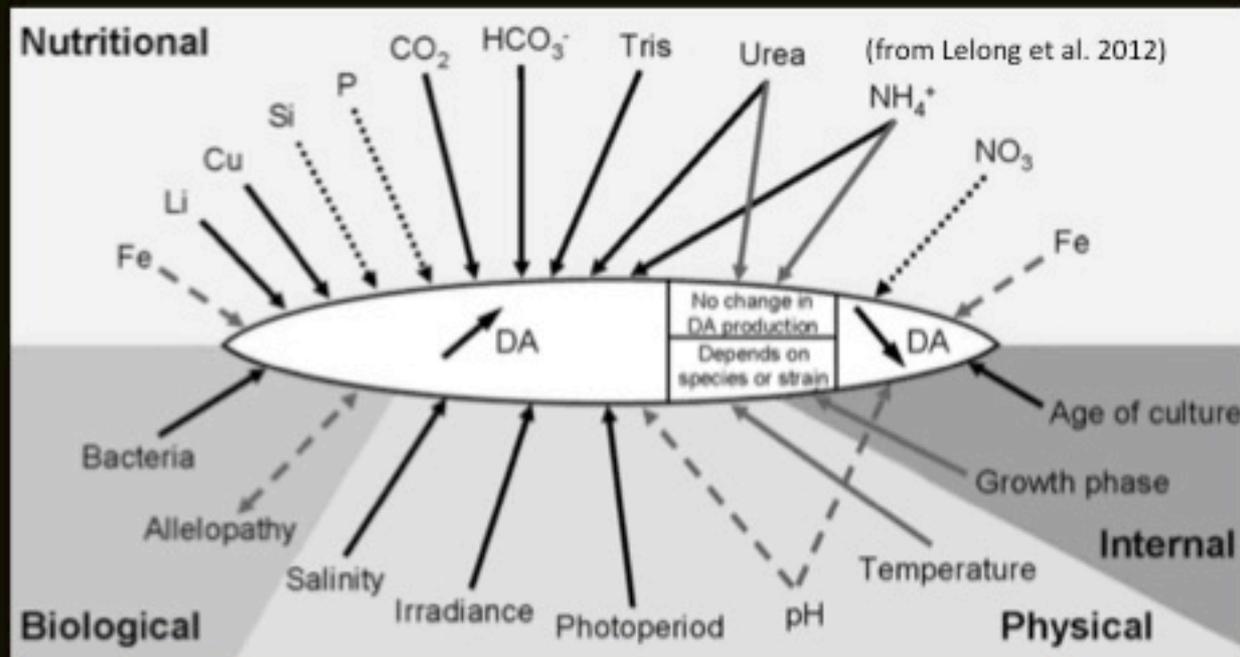
Modified from www.whoi.edu

Many factors have been shown to affect domoic acid production

→ Effect of increased parameter

⋯ → Effect of decreased parameter

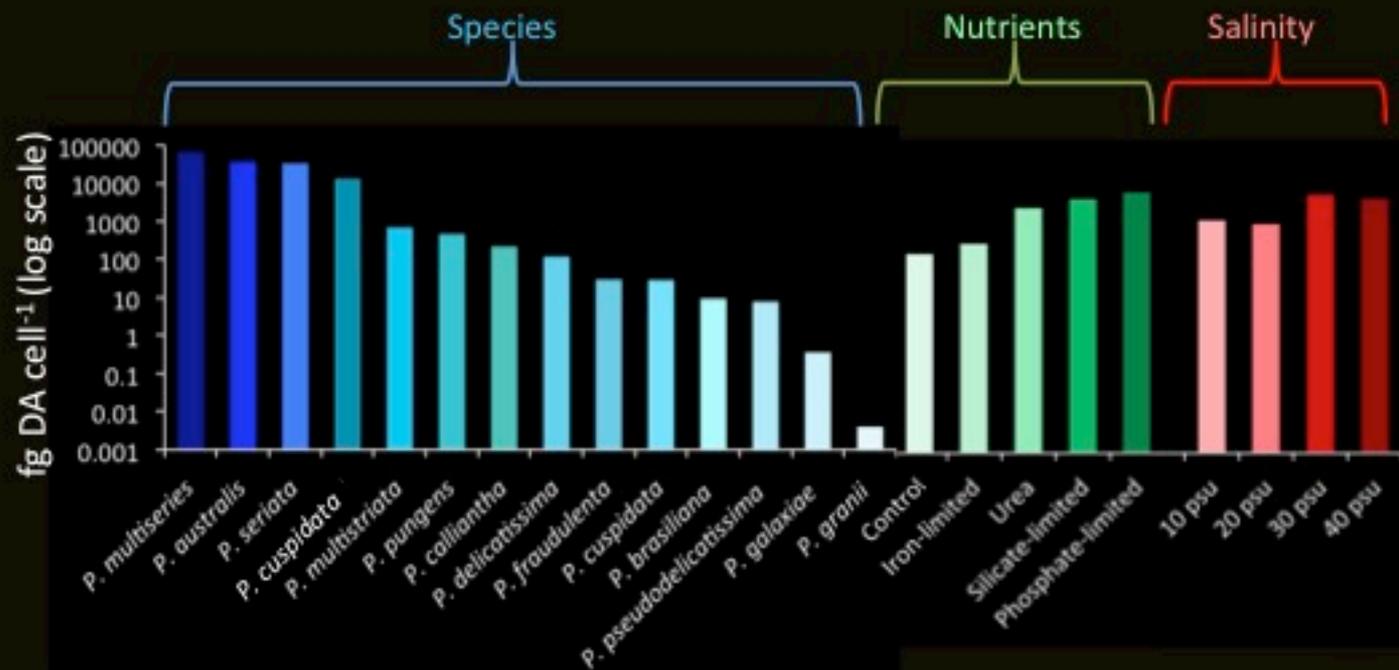
--- → Variable results



Reproduction
Grazing
Life cycle

These can also impact growth – so how do you get a toxic bloom??

Variability in DA production



Zooplankton grazing



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

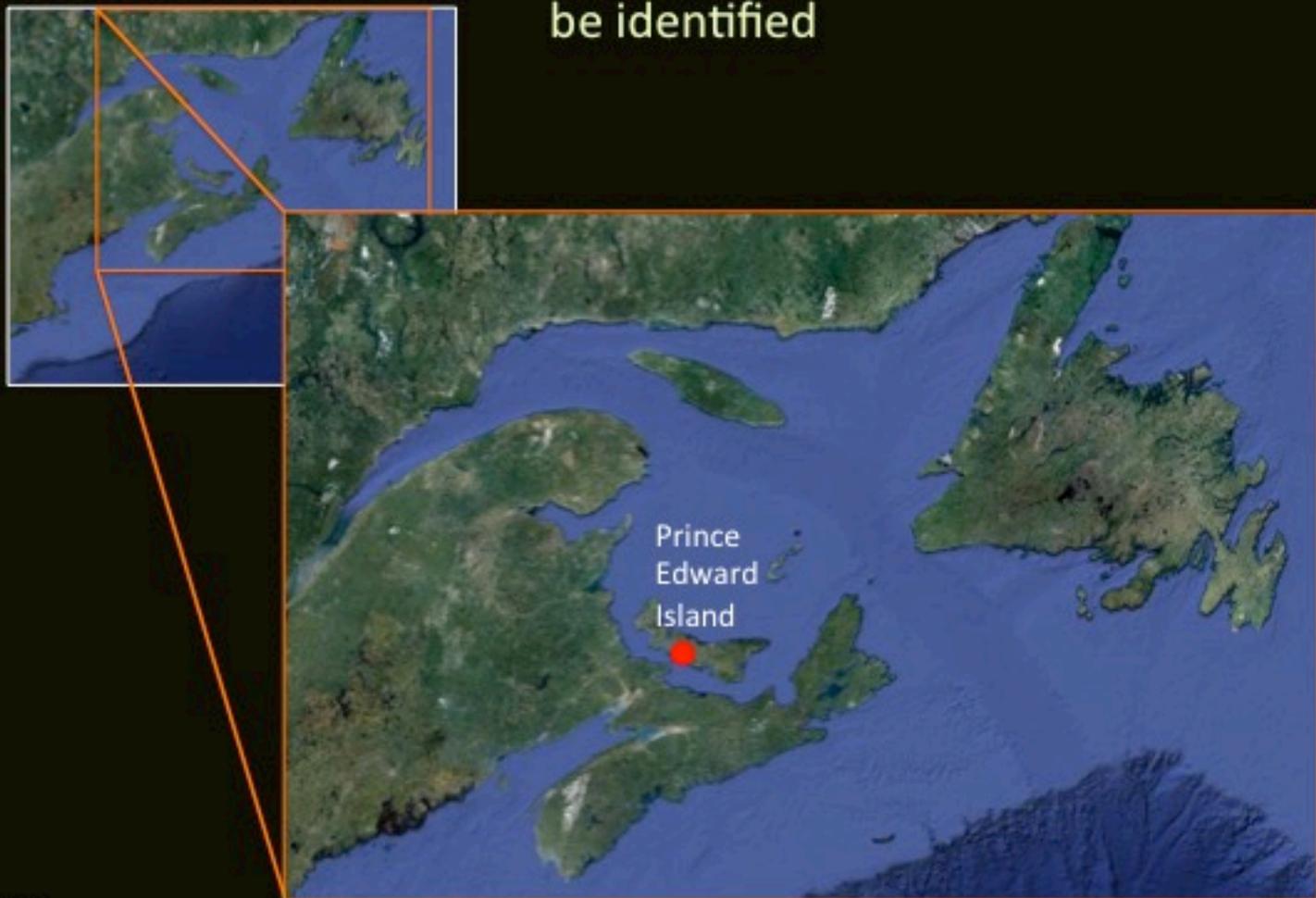
(M. Parker et al., unpub. data)

(Doucette et al., 2008 *Nova Hedwig*.)

(Harðardóttir et al., 2015; Tammilehto et al., 2015)

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

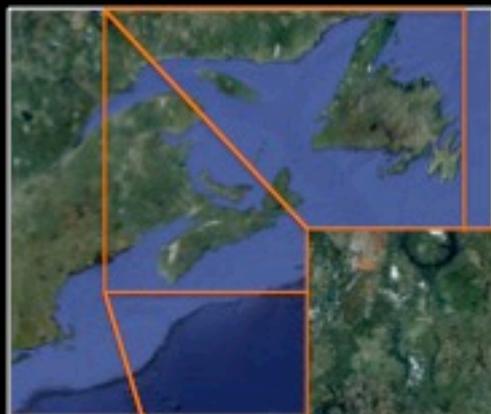
- Federal, state, academic, citizen sampling
- Focuses on screening for known and novel HABs

Biotoxin testing

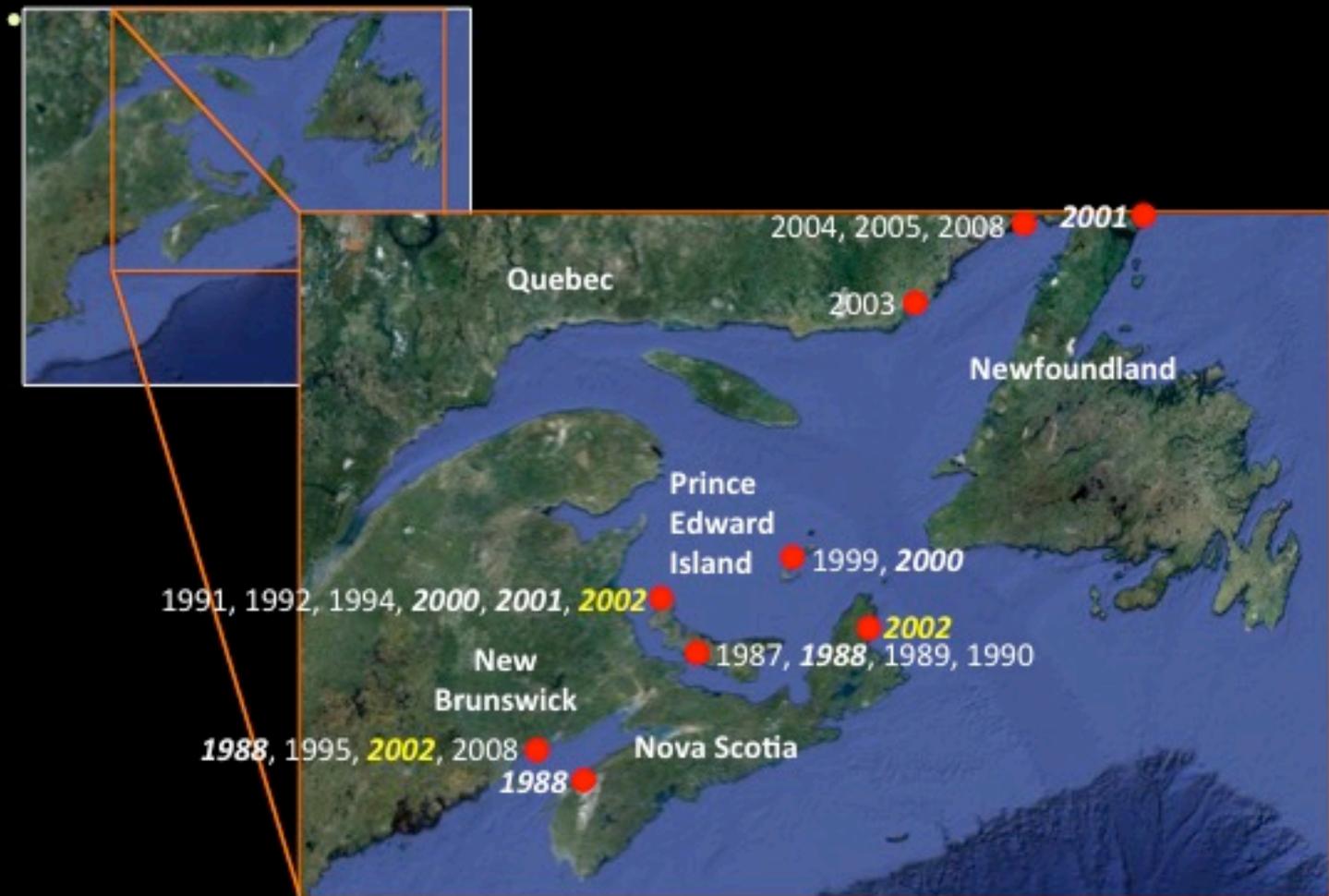
- FDA-approved laboratories that follow national regulations
- Different methods approved for primary HAB toxins that impact the US → rapid vs. regulatory screening for ASP toxins

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

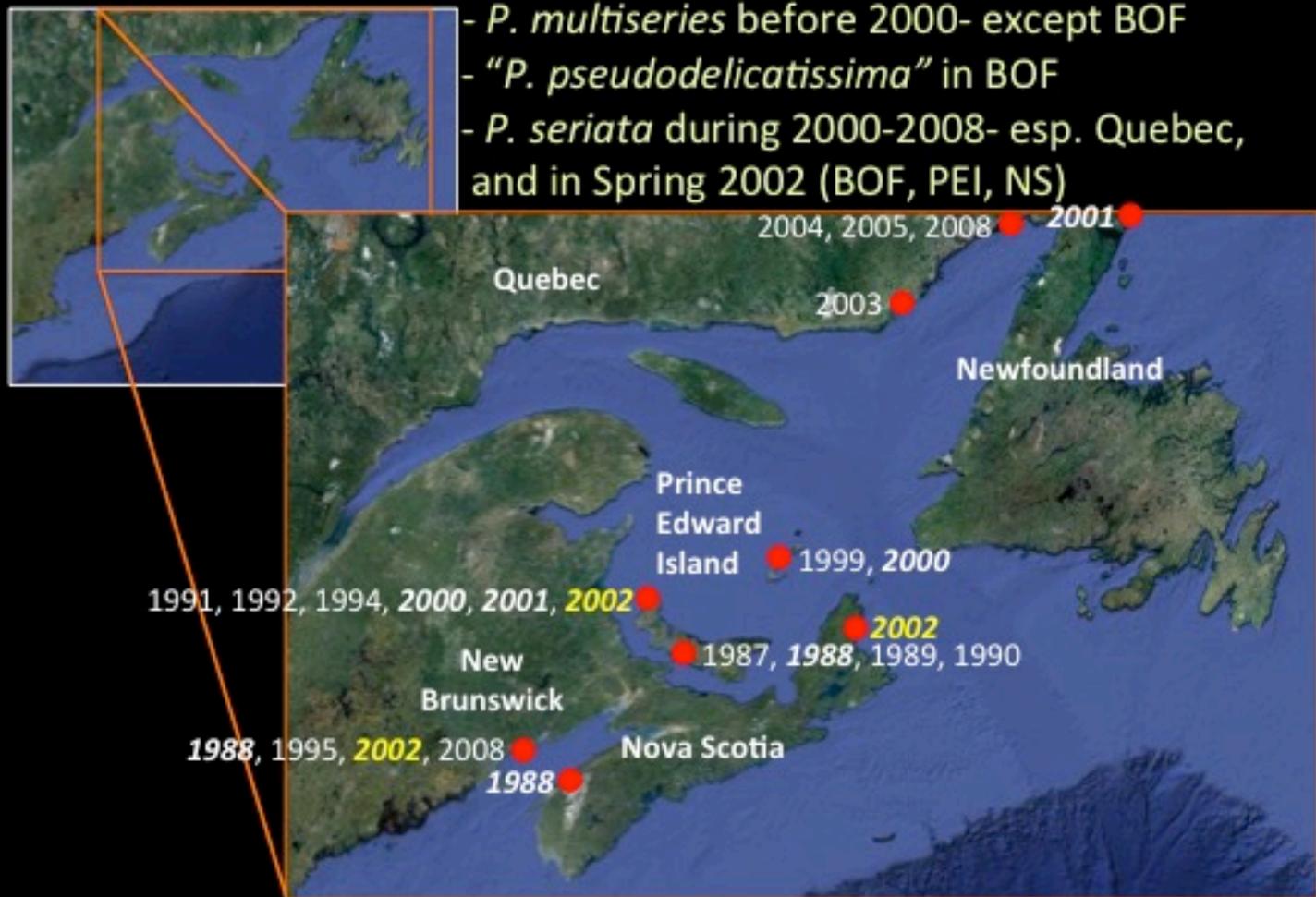


Closures occur July-October... except during Spring 2002



Different *Pseudo-nitzschia* species during toxic events

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



A History of Domoic Acid in Eastern North America

ASP Closures in eastern Canadian provinces



A History of Domoic Acid in Eastern North America

ASP Closures in eastern Canadian provinces



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?

P. australis

Monitoring Approaches for Early Warning of Domoic Acid Events in Washington State

BY VERA L. TRAINER AND MARC SUDDESON

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 toxin, via filter feeding of *Pseudo-nitzschia* or ingestion of filter feeding organisms, to shellfish, crustaceans, seabirds, finfish, 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 (Wickell 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.

Neah Bay

Makah Bay

Kalaloch

Copalis

Twin Harbors

Willapa Bay

Long Beach

Olympic Peninsula

Commercial aquaculture

<https://wdfw.wa.gov/fishing/shellfish/oysters/>

Puget Sound

● Seattle

8 week commercial season with recreational harvests

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



SOUND TOXINS

An Early Warning Program for Harmful Algal
Blooms in Puget Sound

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

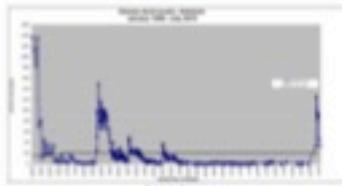
[https://www.nwfsc.noaa.gov/publications/documents/
FINAL%20SoundToxins%20050317.pdf](https://www.nwfsc.noaa.gov/publications/documents/FINAL%20SoundToxins%20050317.pdf)



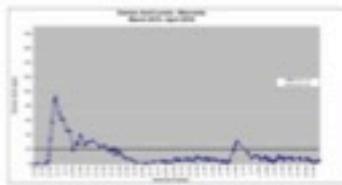
Kalaloch



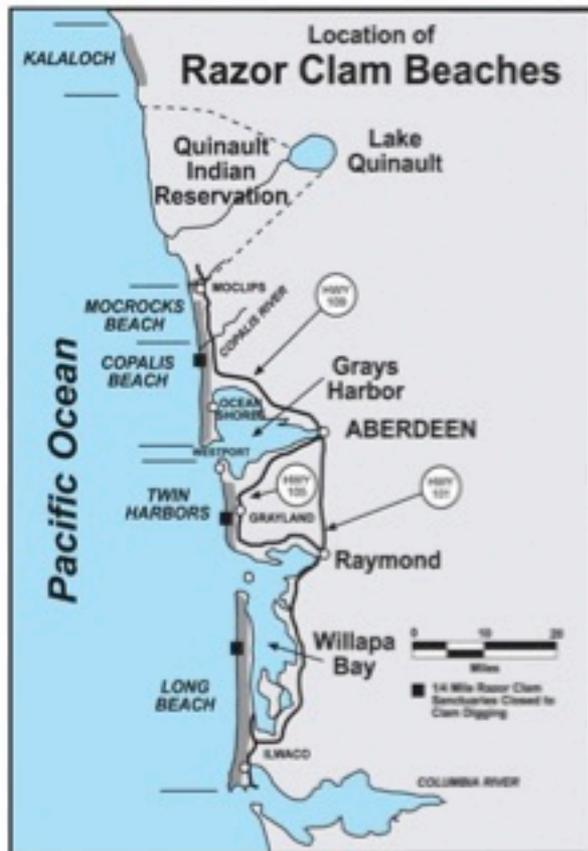
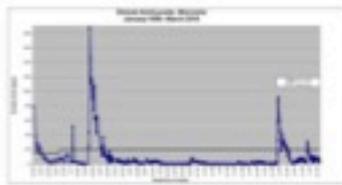
2018	
Sample Date	Level (ppm)
03/20/18	21.0



Mocrocks



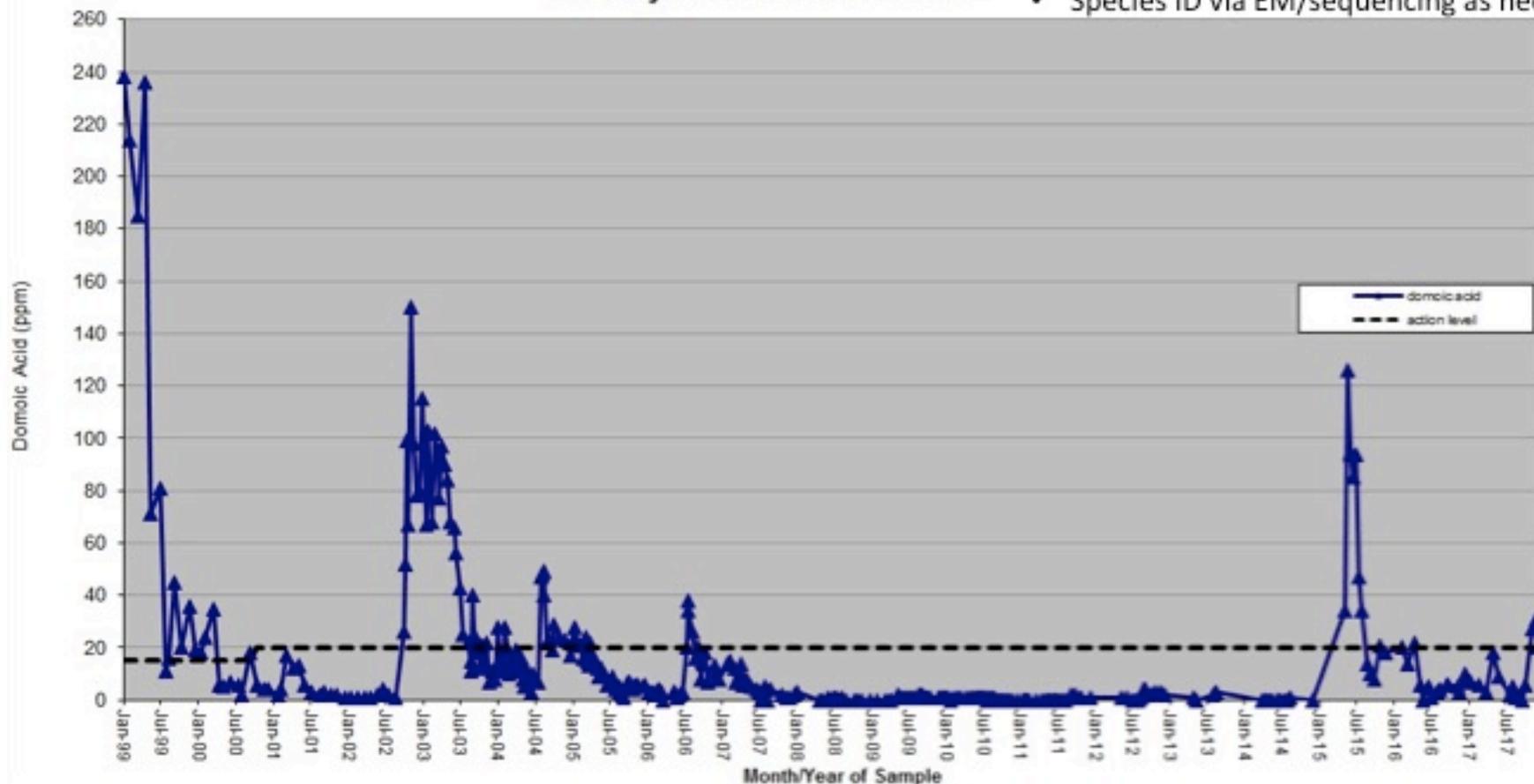
2018	
Sample Date	Level (ppm)
01/14/18	8.0
01/20/18	6.0
02/12/18	6.0
02/14/18	8.0
02/26/18	5.0
03/06/18	3.0
03/21/18	3.0
04/21/18	5.0



https://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html

Domoic Acid Levels - Kalaloch January 1999 - December 2017

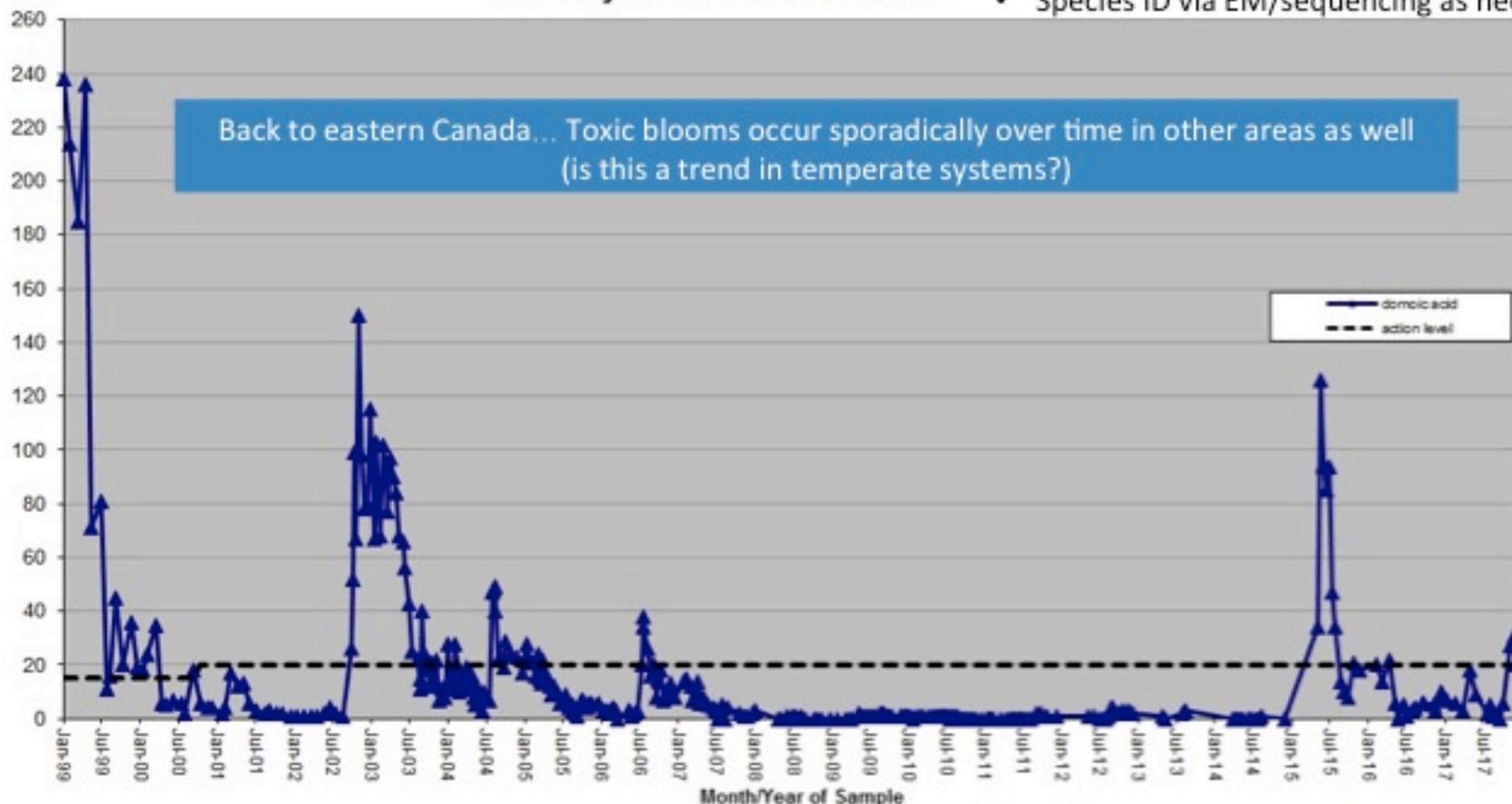
- Routine screening via light microscopy and toxin testing
- Species ID via EM/sequencing as needed



https://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html

Domoic Acid Levels - Kalaloch January 1999 - December 2017

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https://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html

West Coast 2015 *Pseudo-nitzschia* bloom: the worst ASP event to date??

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



Estimated \$100 million decrease in value from Dungeness crab harvest closures alone



Image: <https://wdfw.wa.gov/fishing/washington/Species/6747/>

“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

Advancing *Pseudo-nitzschia* monitoring efforts in the Gulf of Maine

State phytoplankton/toxin monitoring



CELLULAR ABUNDANCE BINS	
# grids to get 500	Cell abundance bins
>100	<7500 cells/L
100 to 50	7500-15,000 cells/L
49 to 16	15,306 to 46,875
15	50,000
7	107,142
<7***	***wholewater count

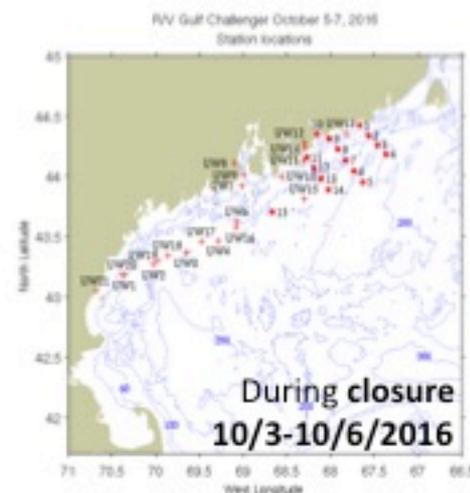
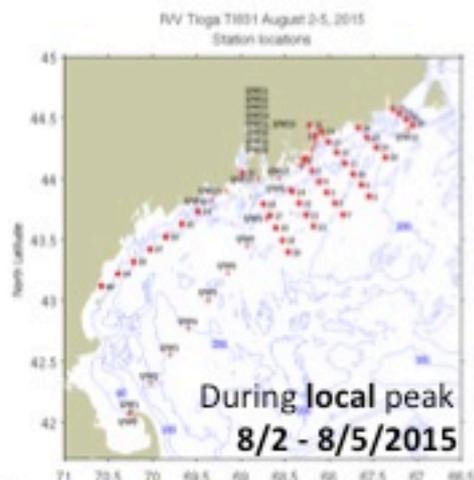
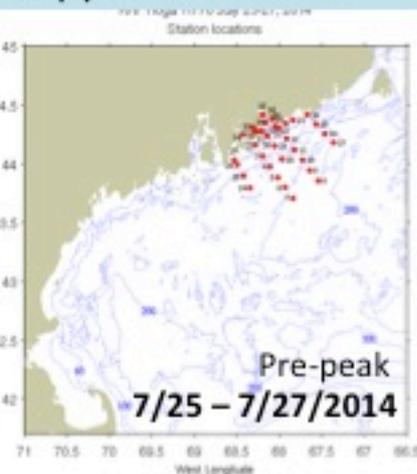
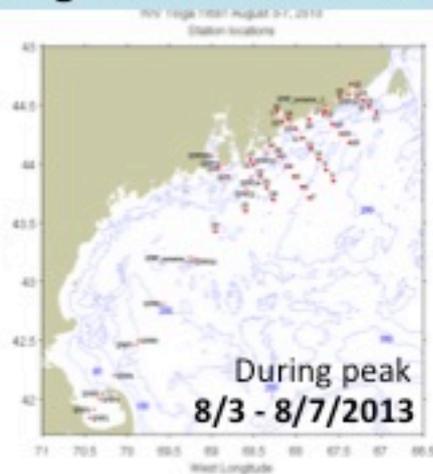
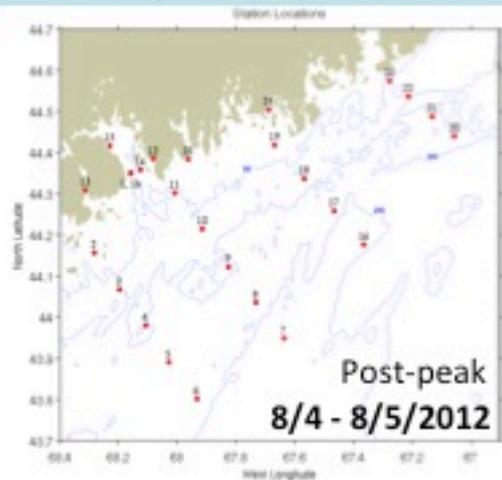
Toxin testing

“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

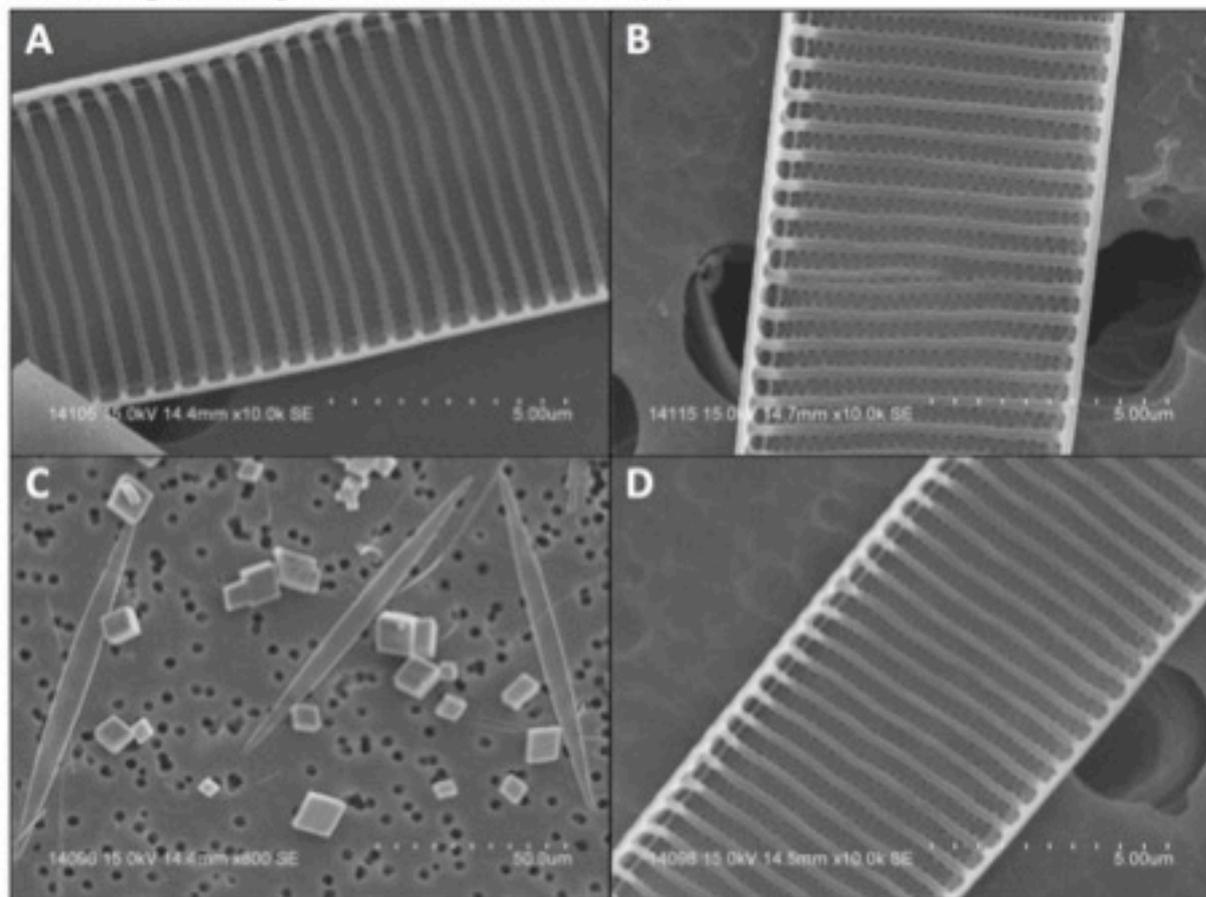
Rapid Response sampling for *Pseudo-nitzschia* spp. 2012-2016



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

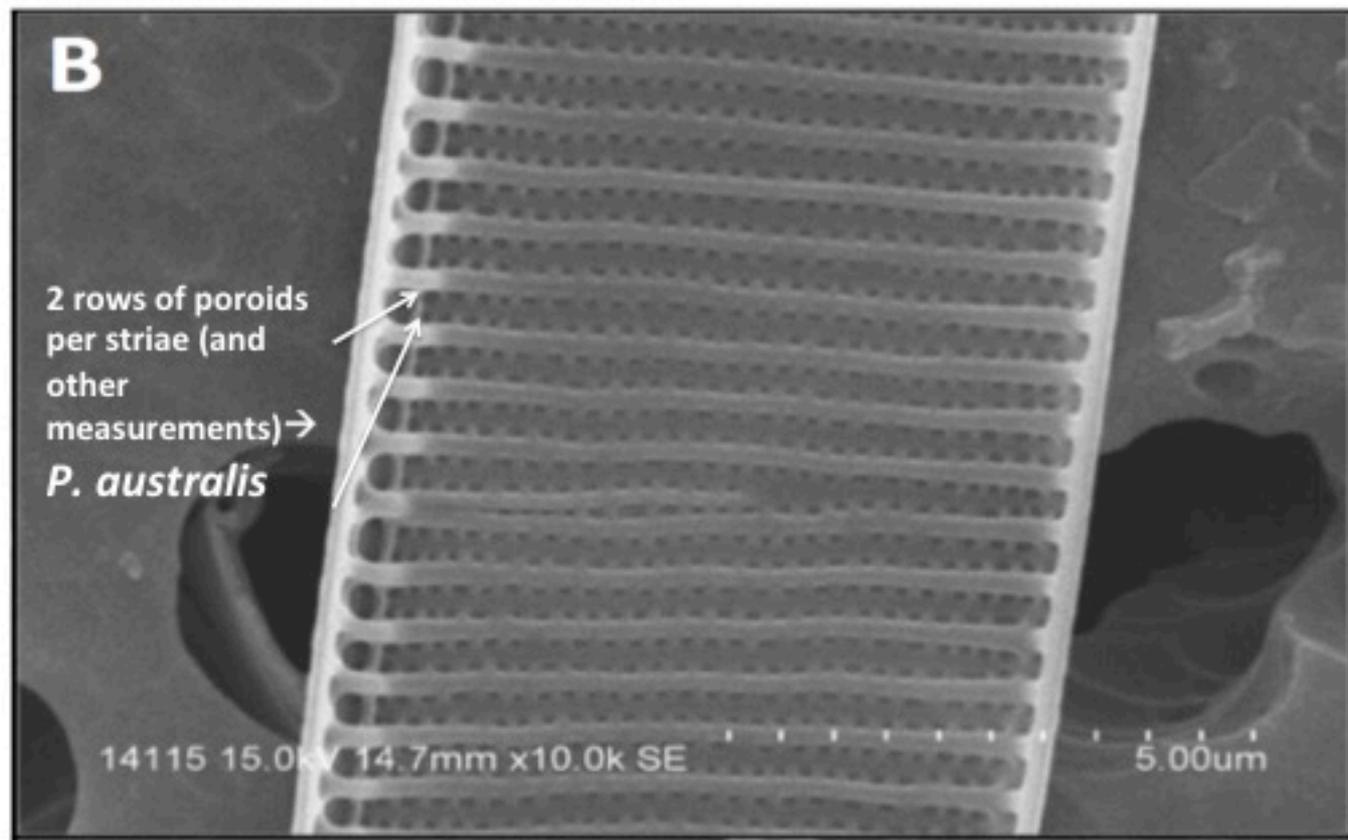
I. Scanning (and light) electron microscopy



Hubbard et al. *in prep*

First observation of *Pseudo-nitzschia australis* in the Gulf of Maine

I. Light and scanning electron microscopy

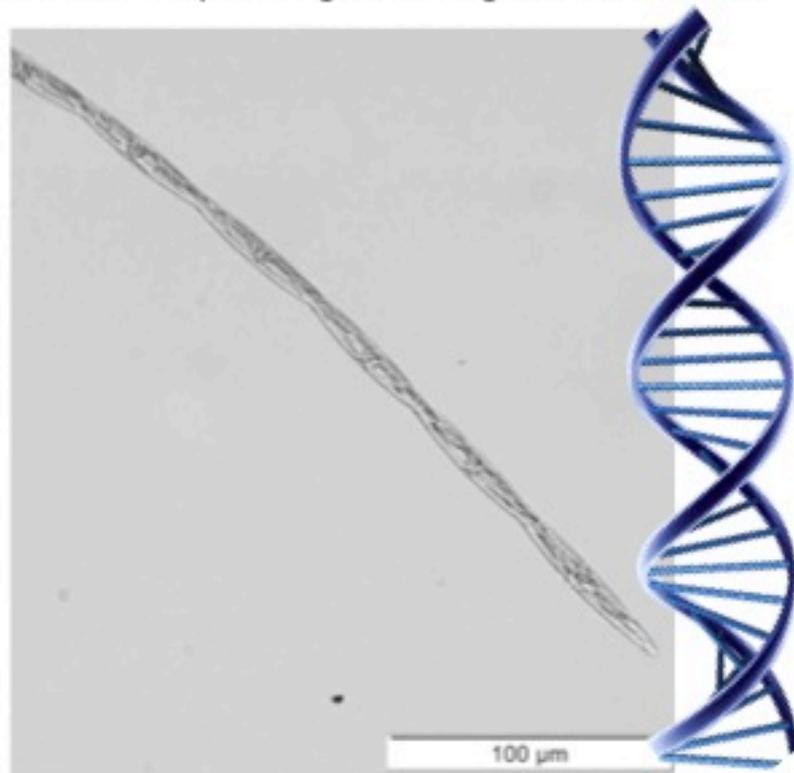


Hubbard et al. *in prep*

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

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



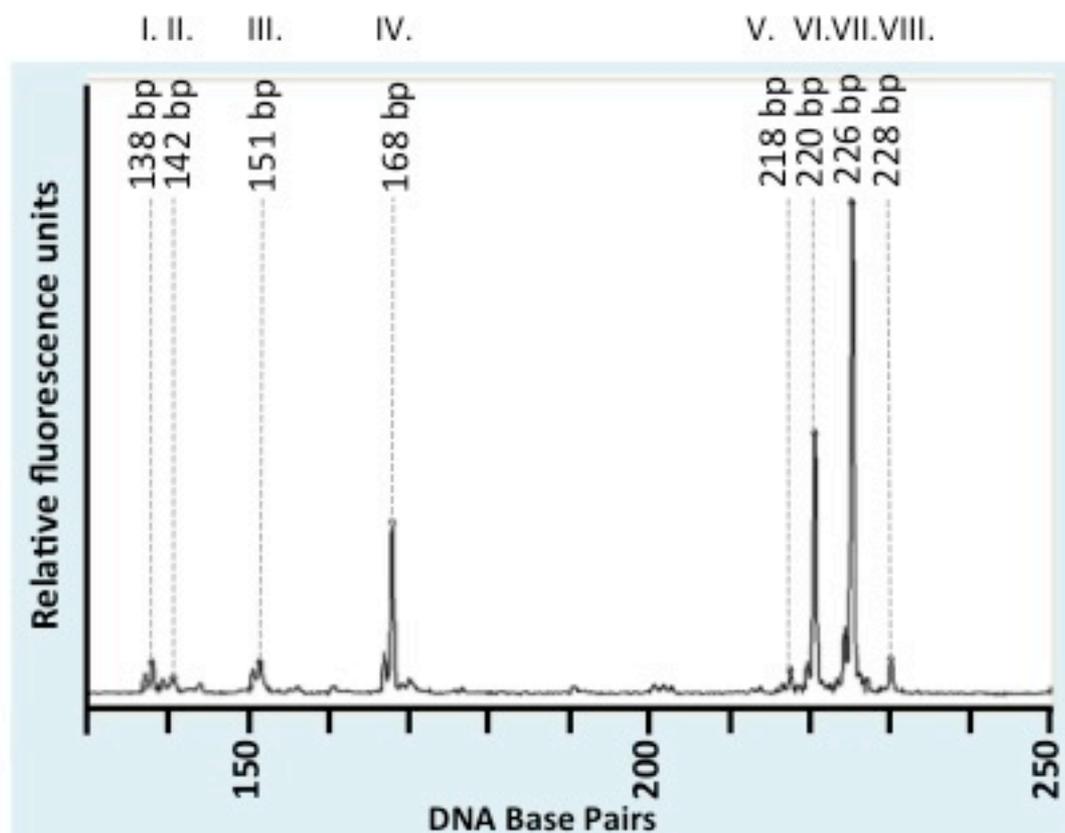
Pseudo-nitzschia species DNA fingerprinting

Hubbard et al. 2008 *J. Phycol.*

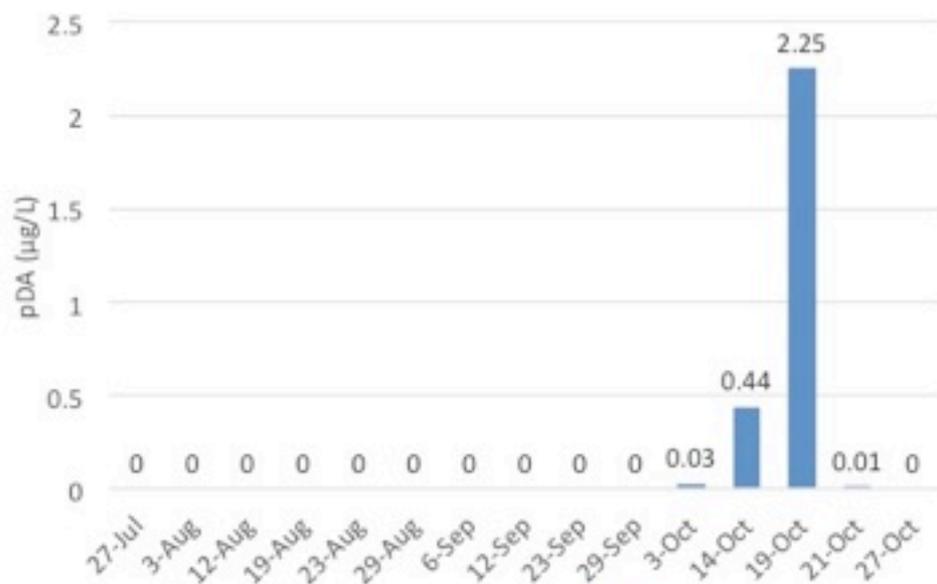
Hubbard et al. 2014, *MEPS*

- I. *P. sabit*
- II. *P. pungens*
- III. *P. seriata*
- IV. *P. delicatissima*
- V. Unknown
- VI. Unknown
- VII. *P. plurisecta*
- VIII. *P. caciantha*

NEW: P. australis

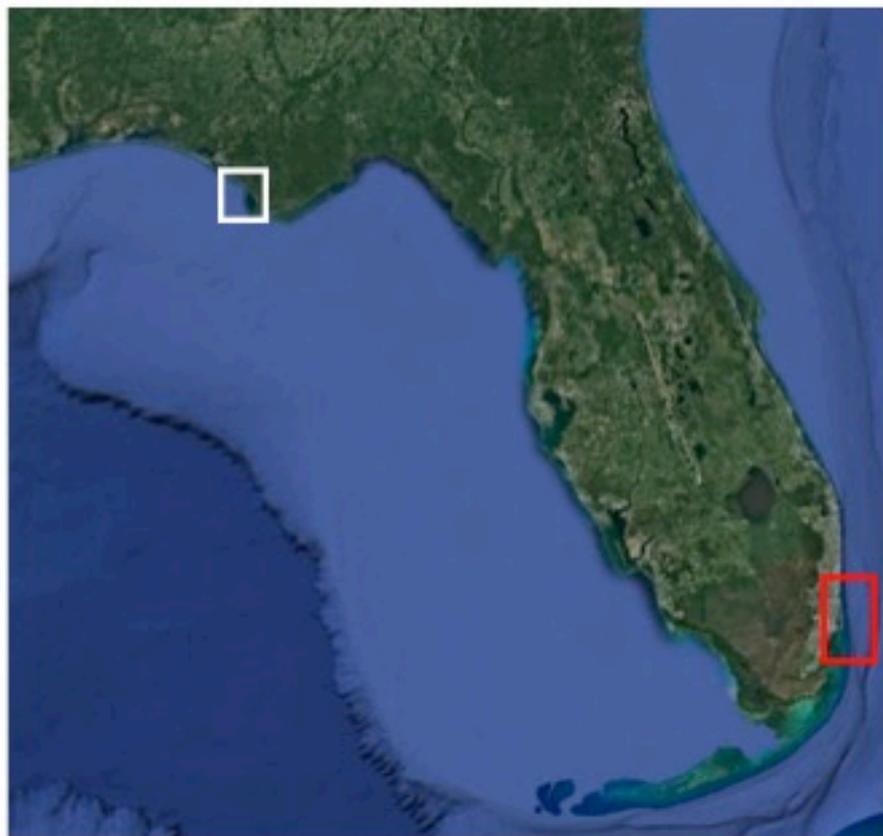


Pseudo-nitzschia species as early warning of toxicity?



P. australis first detected using fingerprinting in 9/29 samples from Salisbury Cove

ASP closures vary for shellfish and commercial species: Florida 2017



Biotoxins sometimes coincident in time and/or space

FWC Red Tide Status Update

PSP
ASP
NSP
Brown tide
w/ fish kills



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