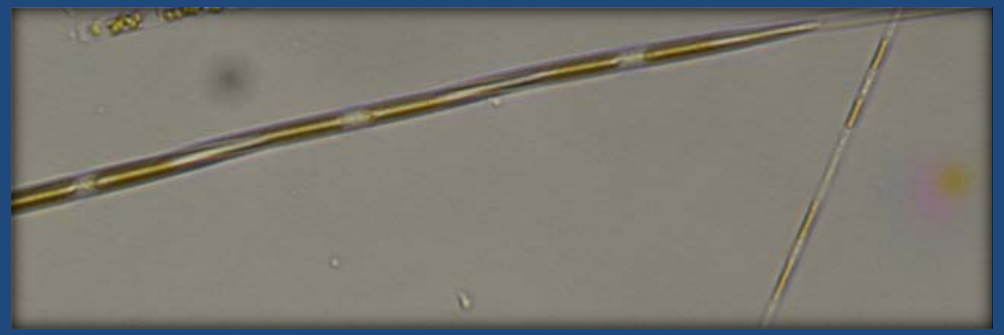


RIDEM

RI DOH

Harmful Algal Bloom Monitoring and Response Plan:

General structure of the plan

September 12, 2017

Coastal Institute Building, URI

National Shellfish Sanitation Program (NSSP)

Guide for the Control of Molluscan Shellfish 2015 Revision

B. Marine Biotoxin Monitoring.
In those areas where toxin-forming organisms are known to occur periodically and the toxins are prone to accumulate in shellfish, and when appropriate at those times when marine Biotoxins can be reasonably predicted to occur, representative samples of the water and/or shellfish shall be collected during harvest periods. The samples shall be collected from indicator stations at intervals determined by the Authority. Water samples will be assayed for the presence of toxin-forming organisms and shellfish meat samples shall be assayed for the presence of toxins.

RI HAB Plan approach:

- 1. Presence of HAB species In plankton (cell counts)
- 2. Analysis of biotoxin in plankton
- 3. Analysis of biotoxin in shellfish meats

RI DEM Shellfish Program HAB monitoring

- Collect phytoplankton samples in conjunction with bacteria sampling
- Phytoplankton collected year-round:
  - Net tows, cell counts: *Alexandrium spp, Dinophysis spp, Pseudo-nitzschia spp.*
- Cell count threshold for plankton biotoxin testing

Photo: Katie Rodrigue

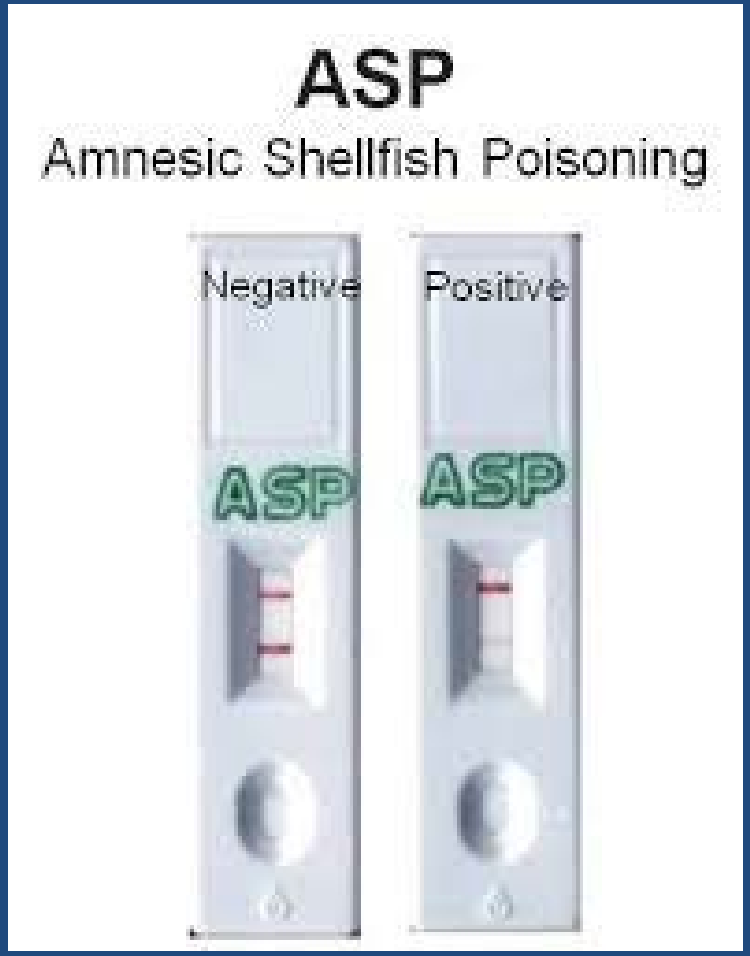
HAB plan: general structure

- 1. Presence of HAB species In plankton (cell counts)
- 2. Analysis of biotoxin in plankton
- 3. Analysis of biotoxin in shellfish meats

- RI DOH Screening kits for biotoxin in plankton

- If NEGATIVE
  - Continue HAB phytoplankton monitoring

- If POSITIVE
  - Initiate shellfish biotoxin monitoring



RI HAB Plan approach:

- 1. Presence of HAB species In plankton (cell counts)
- 2. Analysis of biotoxin in plankton
- 3. Analysis of biotoxin in shellfish meats

RI HAB Monitoring: Shellfish collection

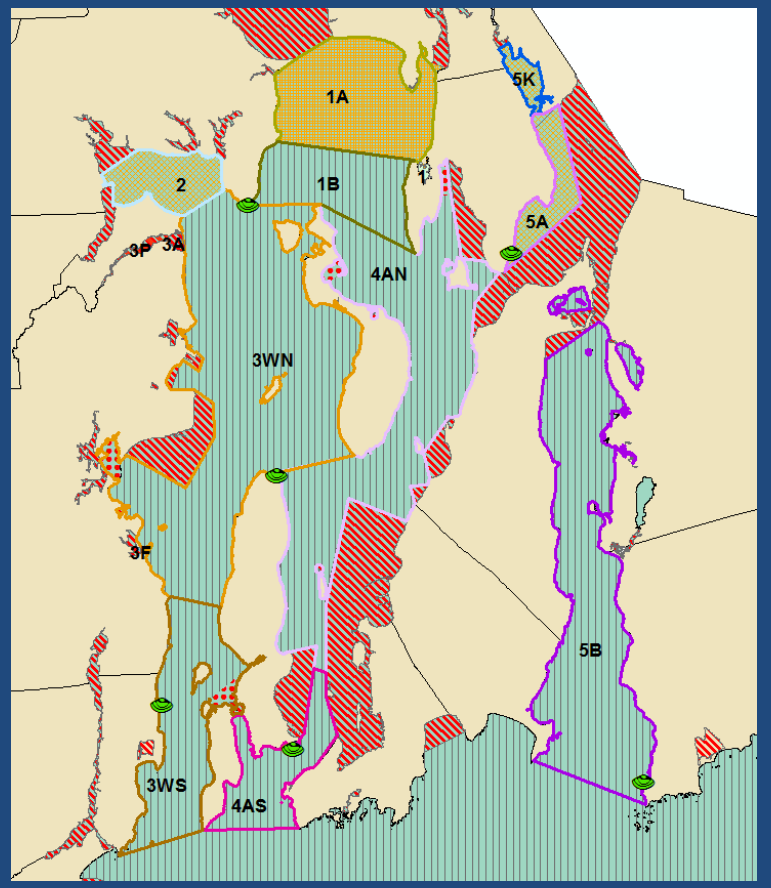
- Not always straight forward!

- DEM DMF Sentry Shellfish Program
  - 6 stations established representing Narragansett Bay harvest areas.

- DEM OWR
  - Interactive shellfish resource map

- RIDOH Food Safety
  - Collect meat samples from dealers

Current mussel sentry sites



Shellfishing Area Closure:

- DOH Food Protection contacts dealers; dealer stock from affected area held and tested
- DEM DMF helps communicate closure to shellfish community (listserve, etc)
- DEM Enforcement patrol enforces closure
- US FDA Regional Specialist alerted
- Shellfish programs in nearby states alerted
- DEM OWR and DOH continue monitoring and sampling

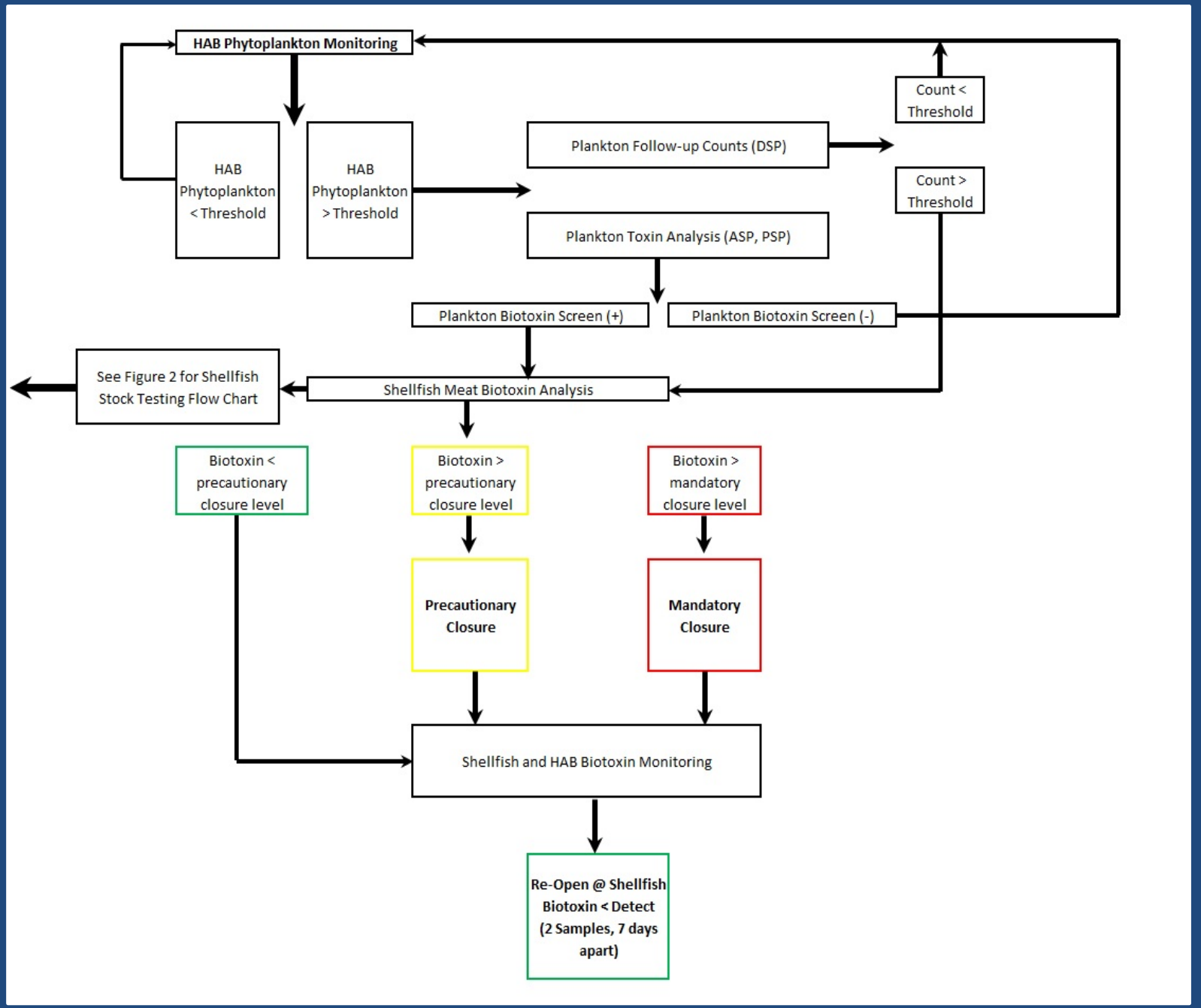
Shellfish Area Reopening:

Precautionary Closure:

- Declining HAB cell counts
- Absence of biotoxin in shellfish via quantitative methods

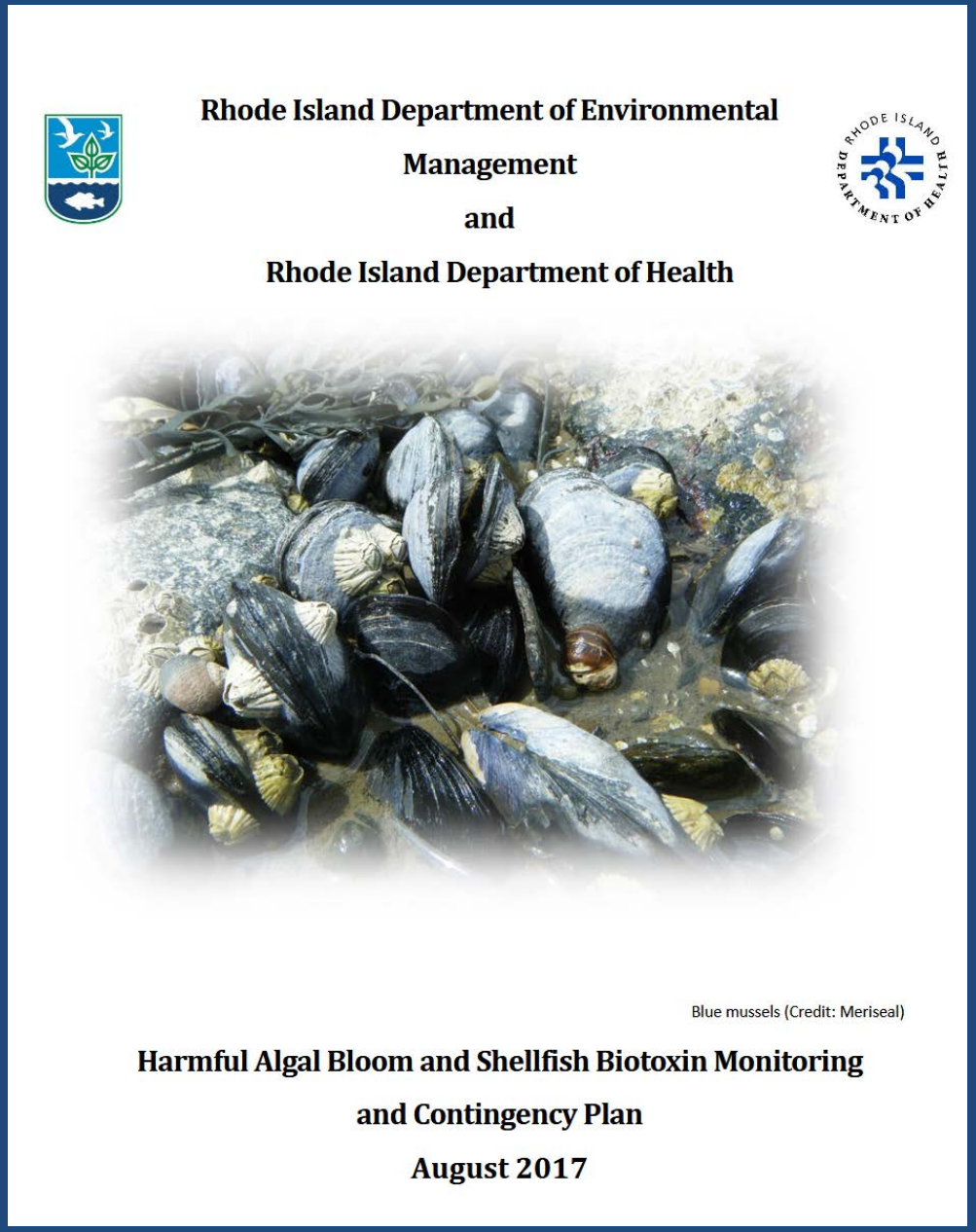
Mandatory Closure:

- Shellfish from closed area with biotoxin less than precautionary closure levels in shellfish via FDA-approved quantitative methods
- Two shellfish samples seven days apart


HAB Phytoplankton Monitoring
HAB Phytoplankton < Threshold
HAB Phytoplankton > Threshold
Plankton Follow-up Counts (DSP)
Count < Threshold
Count > Threshold
Plankton Toxin Analysis (ASP, PSP)
Plankton Biotoxin Screen (+)
Plankton Biotoxin Screen (-)
See Figure 2 for Shellfish Stock Testing Flow Chart
Shellfish Meat Biotoxin Analysis
Biotoxin < precautionary closure level
Biotoxin > precautionary closure level
Biotoxin > mandatory closure level
Precautionary Closure
Mandatory Closure
Shellfish and HAB Biotoxin Monitoring
Re-Open @ Shellfish Biotoxin < Detect (2 Samples, 7 days apart)

RI HAB Monitoring plan revisions Summary of changes:

- Increased frequency and spatial coverage of HAB monitoring (DEM-OWR, DEM-DMF)
- Mussel sentry program (DEM-DMF)
- Improved biotoxin quantification capability (DOH)
- Formalized sampling protocols, closure and re-opening procedures consistent with FDA and NSSP guidance
- Improved coordination between State and Federal agencies



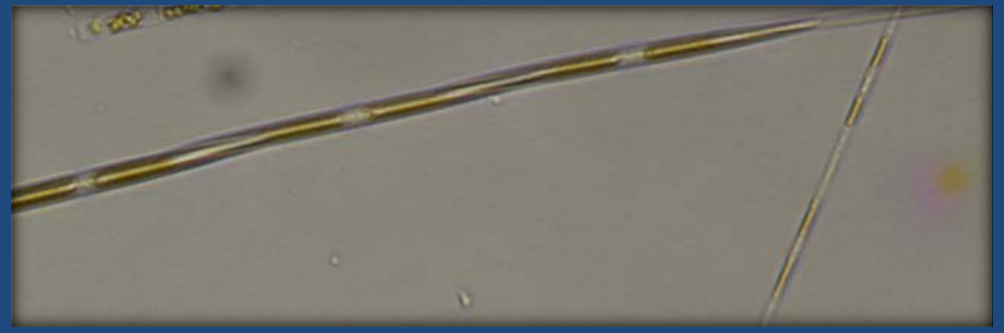
Thank you!

Shellfish Program

401.222.4700

DEM.shellfish@dem.ri.gov

The revised RI HAB Plan is available at:
http://www.dem.ri.gov/programs/benviron/water/shellfsh/pdf/habplan.pdf



RIDEM

RI DOH

Harmful Algal Bloom Monitoring and Response Plan:

Phytoplankton monitoring

September 12, 2017

Coastal Institute Building, URI

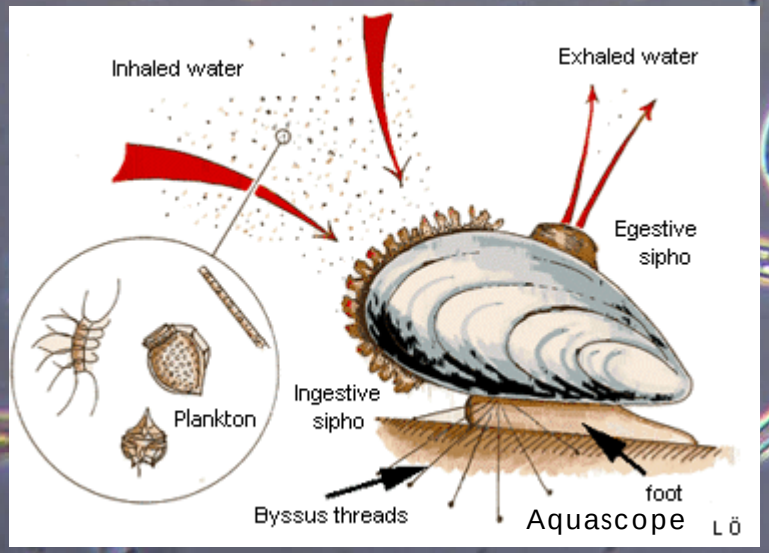
RI HAB plan: general structure

- Presence of HAB species In plankton (cell counts)
- Analysis of biotoxin in plankton
- Analysis of biotoxin in shellfish meats

RI HAB phytoplankton monitoring

Thousands of phytoplankton species

- **99%+ benign, base of marine food webs**



The problem:

- **NE US: ~45 *potentially* harmful species**
- **some *may* produce shellfish vectored biotoxins**

RI HAB monitoring: history

- Mouse assays (1970s – 1990s)
- 2000- on: Phytoplankton ‘early warning’
- Prior to 2016:
  - Collect phytoplankton samples ~2x week at stations in areas open for harvesting
  - May to October HAB samples
  - ~ 50 to 100 HAB samples per year

Photo: Katie Rodrigue

RI HAB Phytoplankton monitoring

HAB phytoplankton genera of concern that may produce shellfish vectored toxins in RI:

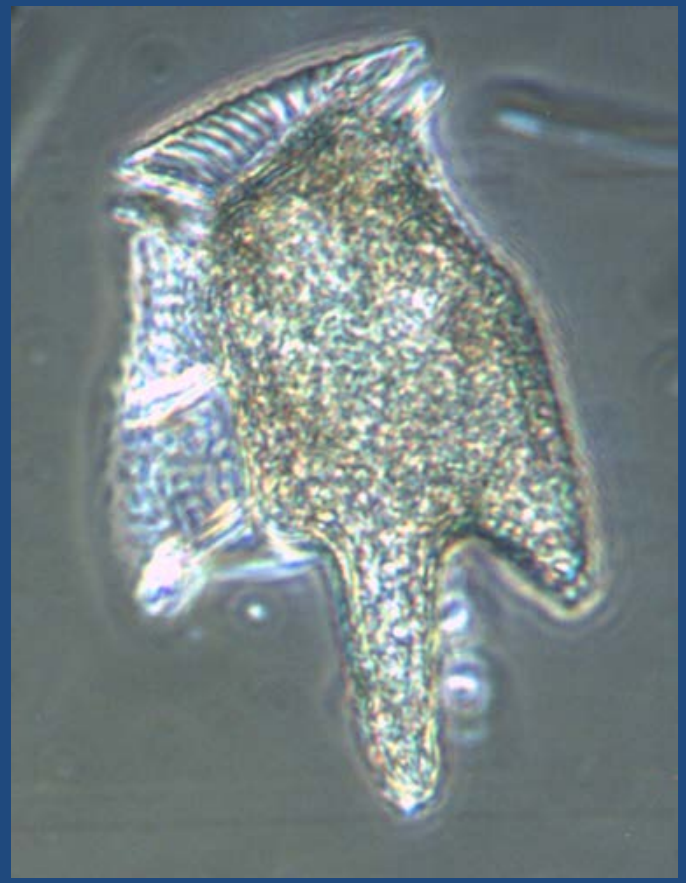
- ***Alexandrium* spp.**
- ***Dinophysis* spp.**
- ***Pseudo-nitzschia* spp.**

Alexandrium spp., PSP (Saxitoxin)

- *Alexandrium* PSP saxitoxin in RI?
  - 1970s to 1990s: 1,000s of mouse assays, only 2 (+) for PSP
  - 2000s to now: Cell counts & screening kits
    - Cell count warning never reached
    - 2005 Gulf of Maine bloom = no toxin detect in RI shellfish
  - **Extremely rare in RI shellfish (2 occurrences during1970s; none since 1979)**

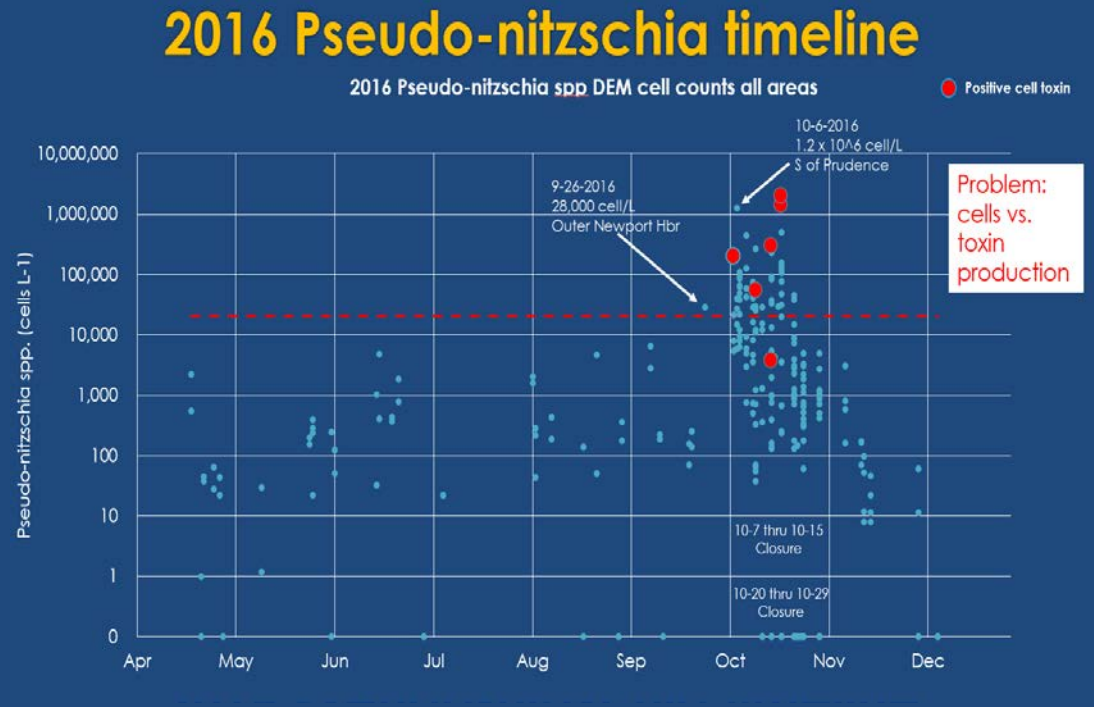
Dinophysis spp., DSP (Okadaic Acid)

- Dinophysis and Okadaic Acid in RI?
  - Cell count threshold never exceeded 2000s to present
  - No testing for biotoxin required 2000s to present



Pseudo-nitzschia spp., ASP (Domoic Acid)

- ASP biotoxin in RI?
  - Cell count warning exceeded 29 separate events (2000-2015)
  - Follow-up screening for Domoic Acid presence in shellfish ALL NEGATIVE (2000-2015)
  - That changed in 2016…..
  - Oct 2016: Precautionary Closure
  - March 2017: Mandatory Closure



RI HAB Phytoplankton monitoring Concerns raised by 2016 & 2017 bloom:

- Standard methods for quantitative phytoplankton counts?
  - Needed for standardized results
- Need for year-round monitoring?
  - 2016 & 2017 indicated that *Pseudo-nitzschia* blooms occur Maine-RI during previously non-sampled 'winter season'
- Sample Frequency adequate to detect blooms?
  - Prior to revisions, gaps of greater than 100 days between HAB samples common in some shellfish growing areas
-

RI HAB Monitoring plan revisions

- Goals:
  - 1. Standardize quantitative methods
  - 2. Year-round sampling
  - 3. Increase HAB plankton sample frequency in all shellfish growing areas
- Changes:
  - 1. Standardize protocols to yield quantitative (cells/L) HAB results
  - 2. Begin year-round HAB sampling 2016 - 2017
  - 3a. Increase # sites plankton collection during bacteria monitoring
  - 3b. Add synoptic plankton sampling cruises during peak bloom season (May – October)

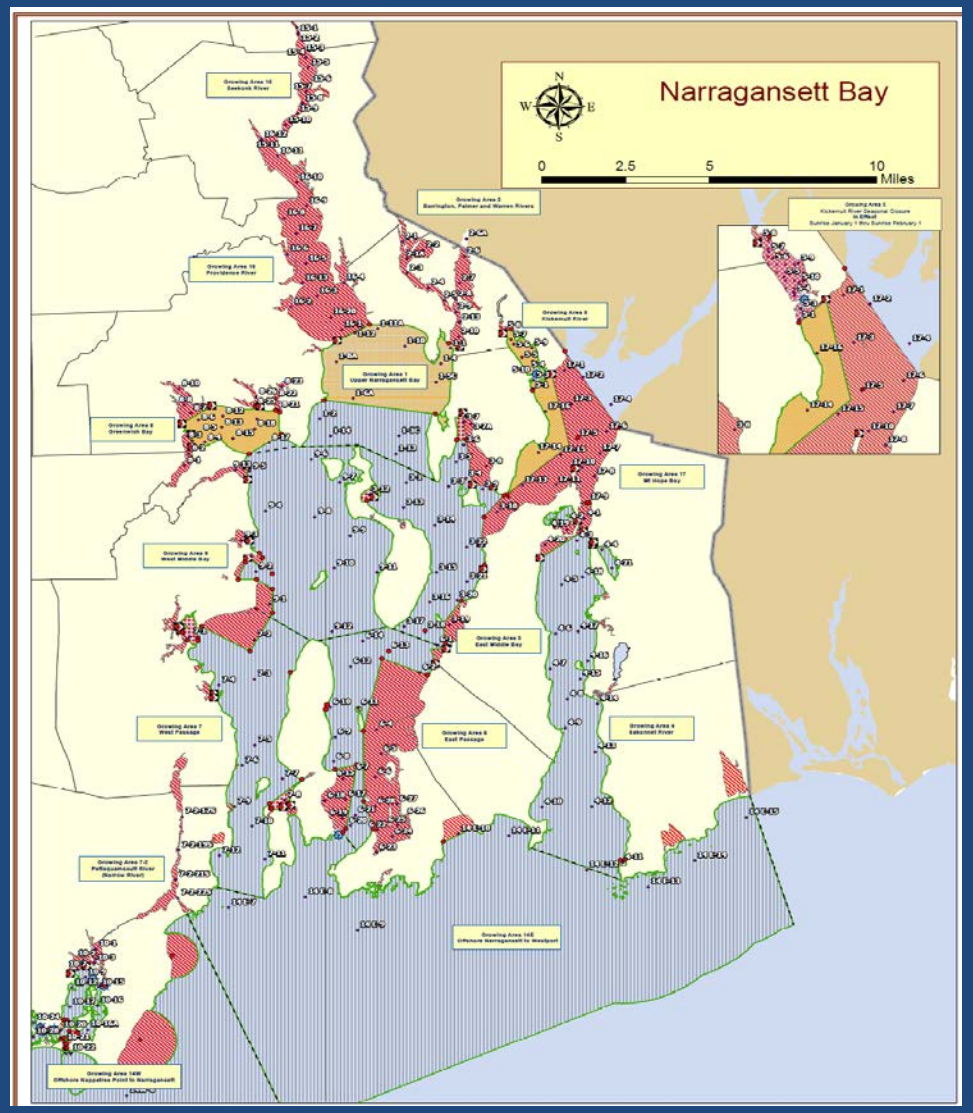
RI HAB monitoring: Standardize methods

- 2016 Revisions:
  - Year-round HAB monitoring
  - Phytoplankton methods:
    - 20 μm mesh, net tows, with either flow meters or known volume (20L by bucket) into net
    - cell counts *Alexandrium spp, Dinophysis spp, Pseudo-nitzschia spp.*
    - Live counts, 250X-500X microscopy
    - Abundance estimates in cells/L
- Cell count threshold to trigger:
  - Additional sampling
  - Shellfish biotoxin testing

Photo: Katie Rodrigue

Monitoring plan revisions: Added Sampling

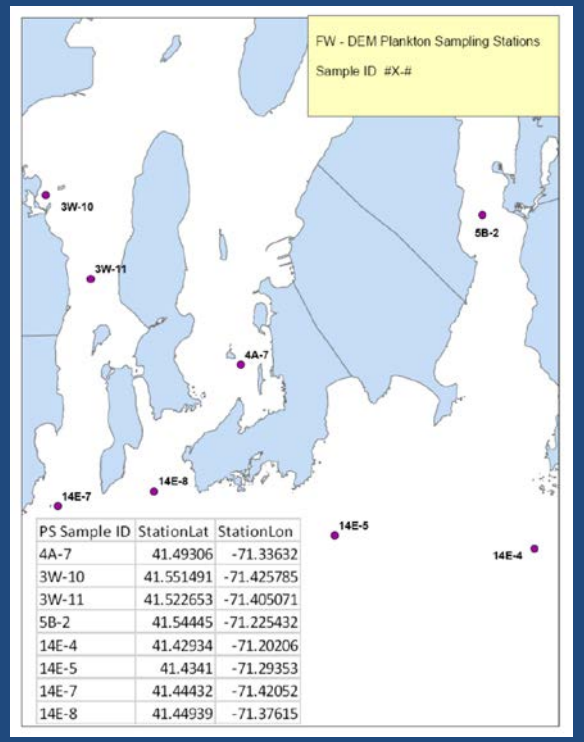
- Extend routine HAB sampling to year-round
- Increase # stations sampled per growing area



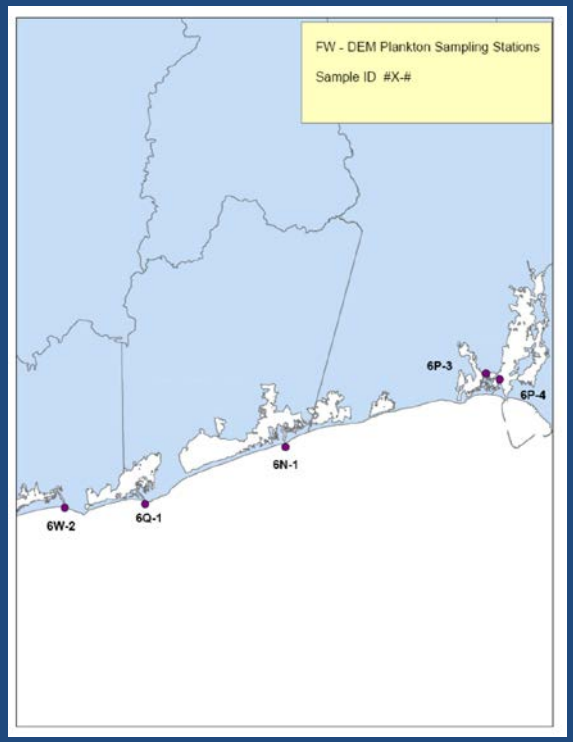
Grow Area	Name	# FIXED Phyto stations	# Times sampled per year (routine)	# Routine Phyto samples /year
1	Upper Narragansett Bay	3	12	36
3	East Middle Bay	2	6	12
4	Sakonnet River	2	6	12
5	Kickemuit River	1	12	12
6	East Passage	2	6	12
7	West Passage	2	6	12
8	Greenwich Bay	2	12	24
9	West Middle Bay	2	6	12
10	Pt. Judith & Potter Ponds	2	6	12
11NG	Ninigret & Green Hill Ponds	2	6	12
11QW	Quonochontaug & Winnapaug Ponds	2	6	12
12	Little Narragansett Bay	1	6	6
13	Block Island	1	12	12
14BI	Offshore - Block Island	2	2	4
14E	Offshore - East	3	2	6
14W	Offshore - West	2	2	4
17	Mt Hope Bay	2	12	24
			TOTAL	224

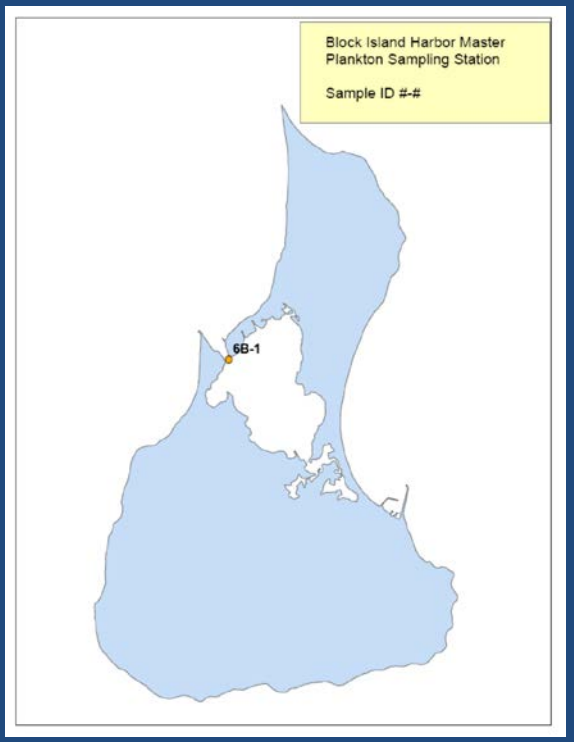
RI HAB Monitoring plan revisions: Synoptic Sampling

- Increase sample frequency and spatial coverage
- Synoptic (6X year; May-Oct) plankton monitoring stations
- (n = 23 stations)



PS Sample ID	StationLat	StationLon
4A-7	41.49306	-71.33632
3W-10	41.551491	-71.425785
3W-11	41.522653	-71.405071
5B-2	41.54445	-71.225432
14E-4	41.42934	-71.20206
14E-5	41.4341	-71.29353
14E-7	41.44432	-71.42052
14E-8	41.44939	-71.37615





RI HAB Monitoring plan revisions

- Increase sample frequency; 6 synoptic sampling trips (May-Oct)

Schedule of 6X per year synoptic HAB phytoplankton monitoring sample collection.

- ADDED SYNOPTIC CRUISES DURING PEAK SEASON
- 6X per year (May to October)
- Reduce sampling gaps during peak season

Grow Area	Name	# Stations	# TIMES sampled /year	TOTAL # Phyto Samples /year
1	Upper Narragansett Bay	3	6	18
3	East Middle Bay	2	6	12
4	Sakonnet River	2	6	12
6	East Passage	2	6	12
7	West Passage	2	6	12
8	Greenwich Bay	1	6	6
9	West Middle Bay	2	6	12
10	Pt. Judith & Potter Ponds	2	6	12
11NG	Ninigret and Green Hill Ponds	1	6	6
11QW	Quonochontaug and Winnapaug Ponds	2	6	12
14E	Offshore - East	3	6	18
17	Mt Hope Bay	1	6	6
		23		138

RI HAB Monitoring plan revisions

- Increase sample frequency; 6 synoptic sampling trips (May-Oct)

Combined schedule of HAB phytoplankton monitoring sample collection.

-APPROXIMATE TRIPLING OF PAST EFFORTS

Grow Area	Name	# TIMES sampled /year	TOTAL # Phyto Samples /year
1	Upper Narragansett Bay	18	54
3	East Middle Bay	12	24
4	Sakonnet River	12	24
5	Kickemuit River	12	12
6	East Passage	12	24
7	West Passage	12	24
8	Greenwich Bay	18	30
9	West Middle Bay	12	24
10	Pt. Judith & Potter Ponds	12	24
11NG	Ninigret and Green Hill Ponds	12	12
11QW	Quonochontaug and Winnapaug Ponds	12	24
12	Little Narragansett Bay	6	6
13	Block Island	12	12
14BI	Offshore - Block Island	2	4
14E	Offshore - East	8	24
14W	Offshore - West	2	4
17	Mt Hope Bay	18	30
		TOTAL	362

RI HAB Monitoring plan revisions:

Sample date	Conditional Areas Sample interval (days)	Open Areas Sample interval (days)
15-Jan	1st sample	
15-Feb	31	
15-Mar	29	
15-Apr	31	
1-May	16	1st sample
15-May	14	14
1-Jun	17	17
15-Jun	14	14
1-Jul	16	16
15-Jul	14	14
1-Aug	17	17
15-Aug	14	14
1-Sep	17	17
15-Sep	14	14
1-Oct	16	16
15-Oct	14	14
15-Nov	31	
15-Dec	30	
AVG (annual)	19.7	
AVG (May - Oct)	15.3	15.2

Additional HAB Sampling:

- Better geographic coverage
- Reduced sample interval to ~2 weeks May to October
- 2017 actual sampling
  - Conditional areas: every 17.9 days
  - Open areas: every 18.6 days

HAB Monitoring plan revisions

- HAB cell count contingency levels
- Literature search, FDA, National HAB Plan guidance
- Early warning
- Triggers:
  - expanded sampling
  - biotoxin screening in plankton

HAB Taxa	Biotoxin	Shellfish Syndrome	Abundance threshold
Alexandrium spp.	Saxitoxins	Paralytic Shellfish Poisoning (PSP)	1,000 cells L^{-1}
Dinophysis spp.	Okadaic Acid	Diarrhetic Shellfish Poisoning (DSP)	30,000 cells L^{-1}
Pseudo-nitzschia spp.	Domoic Acid	Amnesic Shellfish Poisoning (ASP)	20,000 cells L^{-1}

RI HAB Plan approach:

- 1. Presence of HAB species In plankton (cell counts)
- 2. Analysis of biotoxin in plankton
- 3. Analysis of biotoxin in shellfish meats

HAB Phytoplankton Monitoring revisions Summary:

- Standardized quantitative HAB phytoplankton monitoring consistent with national HAB program guidance

- Year-round HAB monitoring

- Increased frequency and spatial coverage of HAB monitoring
    - Goal of sample in each grow area every ~14 days

HAB Phytoplankton Monitoring 2017 update:

- Working under plan revisions since January 2017

- Through 9/8/2017:
  - 315 HAB phytoplankton samples collected and analyzed
    - *Alexandrium* spp. = 0 threshold exceedances
    - *Dinophysis* spp. = 0 threshold exceedances

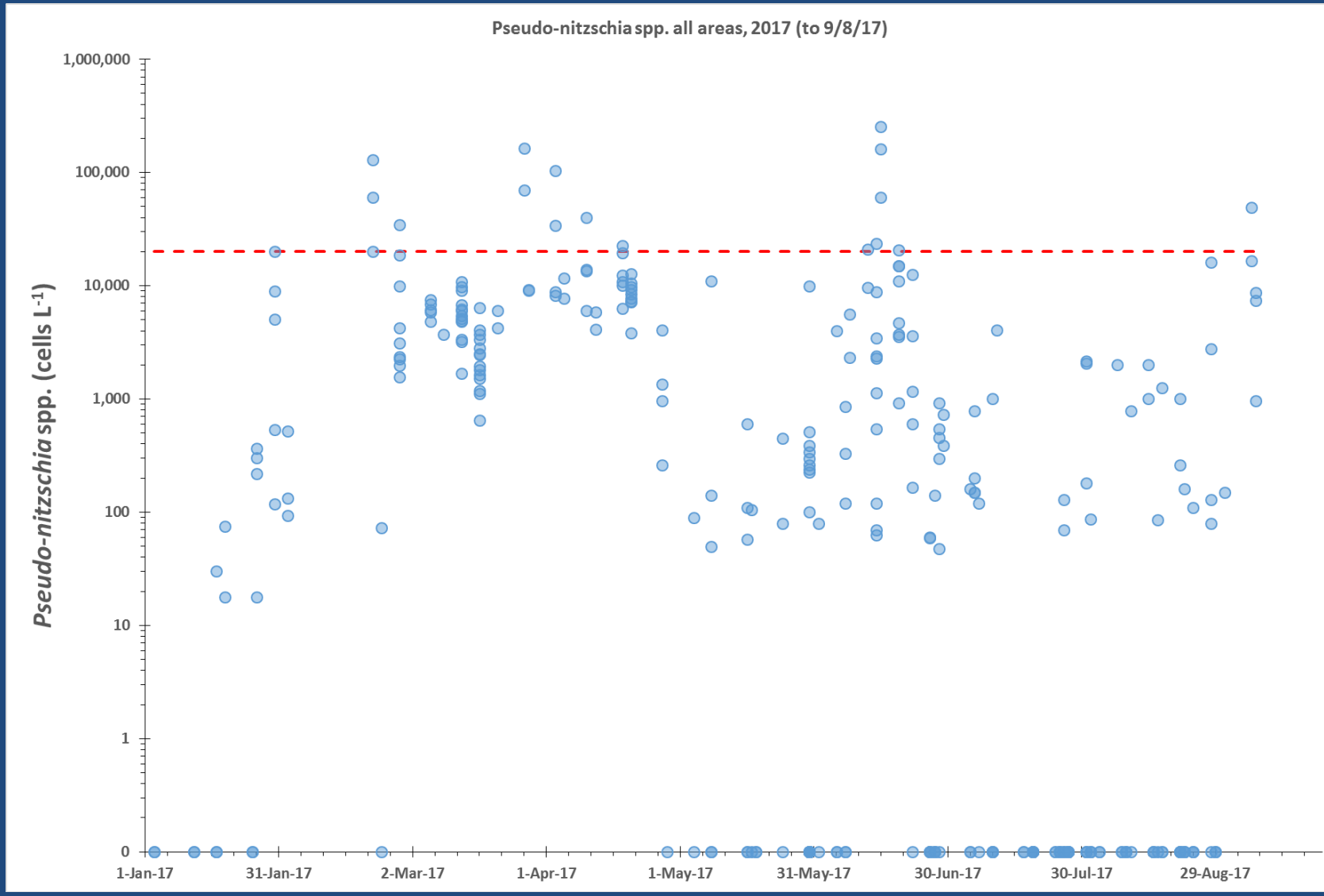
HAB Phytoplankton 2017 update:

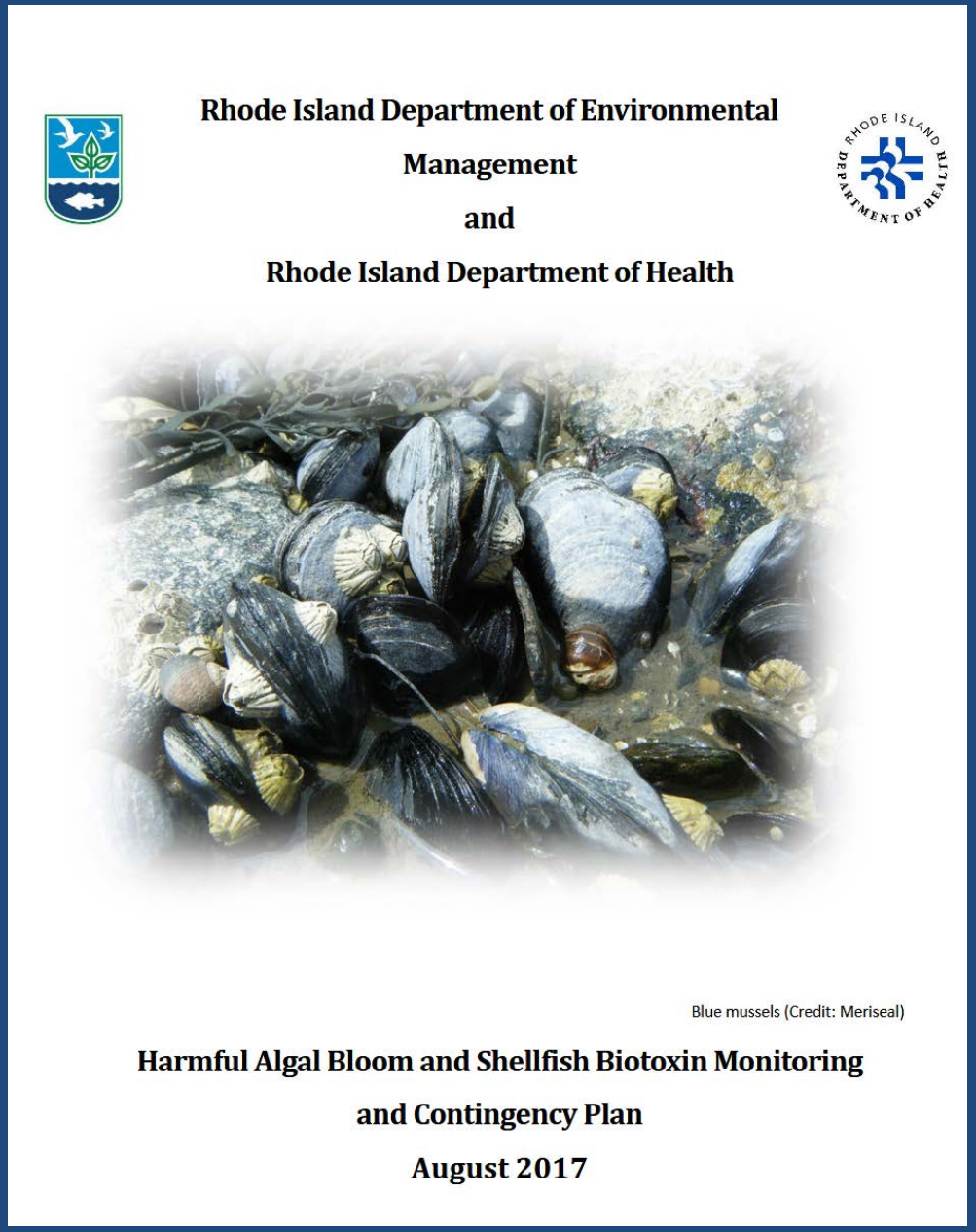
Pseudo-nitzschia spp. 2017

- Abundance peaks in:
  - March (lower Bay)
  - June (BI)
  - increasing Sept.?

- 18 threshold exceedances

- 3 of 18 (+) for DA in plankton





Thank you!

Shellfish Program

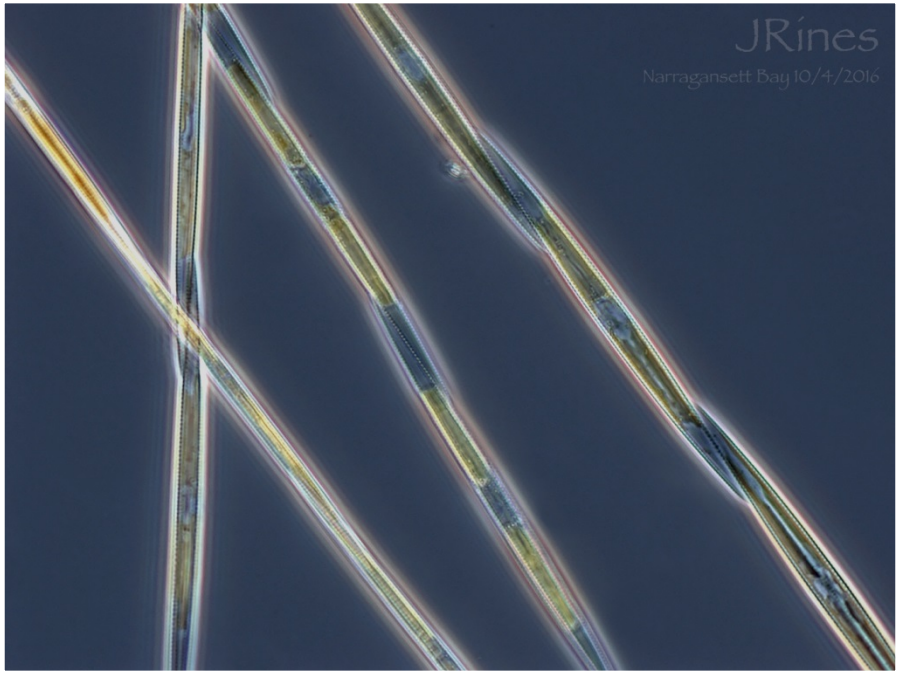
401.222.4700

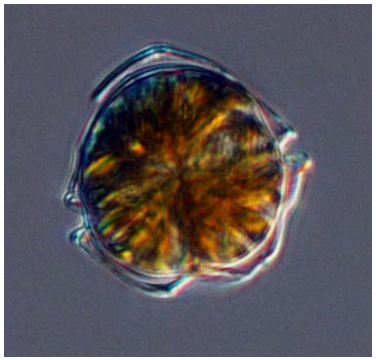
DEM.shellfish@dem.ri.gov

The revised RI HAB Plan is available at:
http://www.dem.ri.gov/programs/benviron/water/shellfsh/pdf/habplan.pdf

RI Sound as a potential source of HAB toxin for Narragansett Bay

Lucie Maranda, David Ullman, and Marta Gomez-Chiarri
University of Rhode Island
Conor McManus
RI DEM





https://www.eoas.ubc.ca/

THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxin for Narragansett Bay

Our proposal:

1. **seek the most appropriate sentinel station in RIS**

2. **establish the occurrence, abundance, and distribution of the *Pseudo-nitzschia* spp., *Alexandrium* spp., and *Dinophysis* spp.**

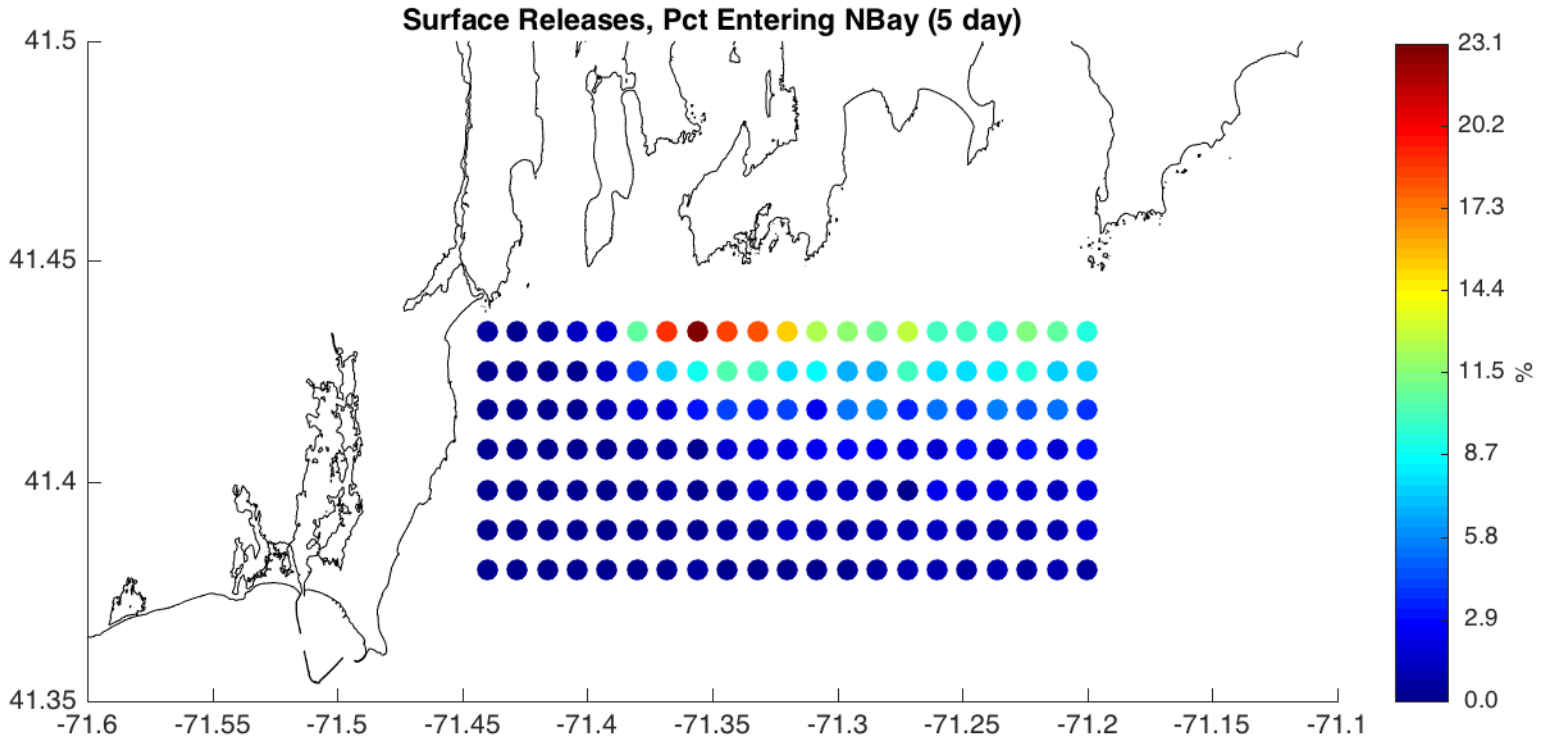
3. **relate domoic acid uptake in blue mussels (*Mytilus edulis*) with *Pseudo-nitzschia* abundance and toxin content**

THINK BIG WE DO

RI Sound as a potential source of HAB toxin for Narragansett Bay

1. Sentinel station:

Passive particle-tracking methods with realistic model circulation fields

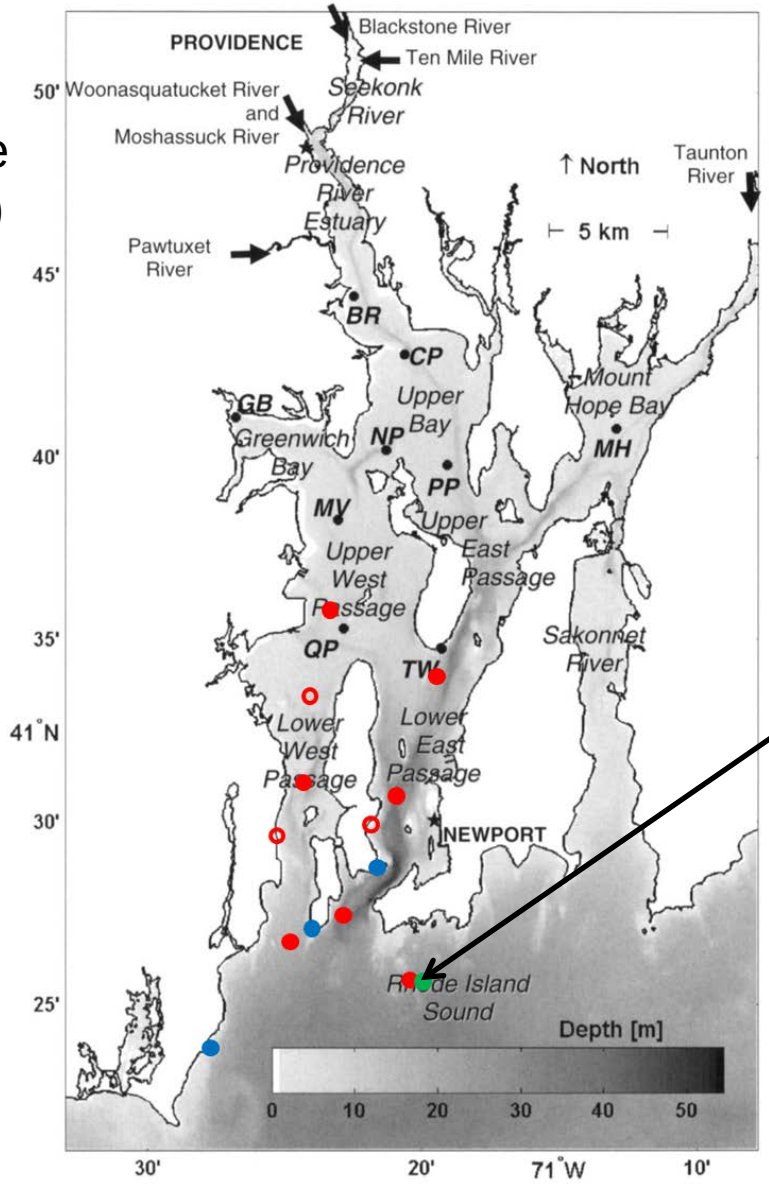


THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxin for Narragansett Bay

- Physical parameters
  Phytoplankton abundance
  (3 depths)
- Phytoplankton net tows
  (toxin assay)
- Wild mussel station
  (toxin assay)

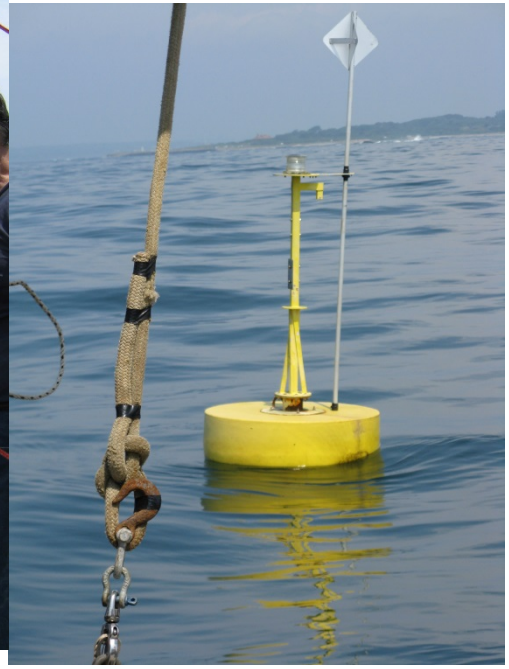


Sentinel station

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

Mussels, CTDs, and ADCP at sentinel station on July 13th
Sampling at stations started August 3rd, every other week



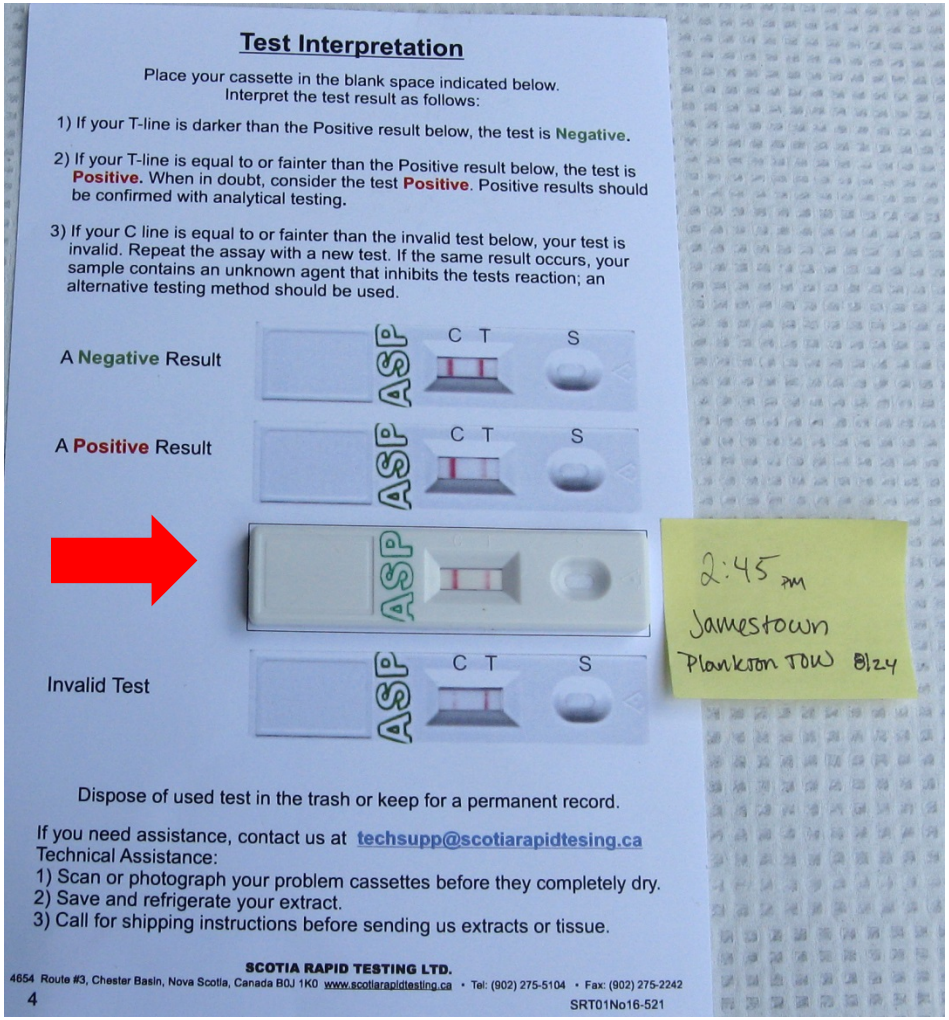
THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

August 22 2017
Phytoplankton tow
Jamestown town dock

ASP test positive



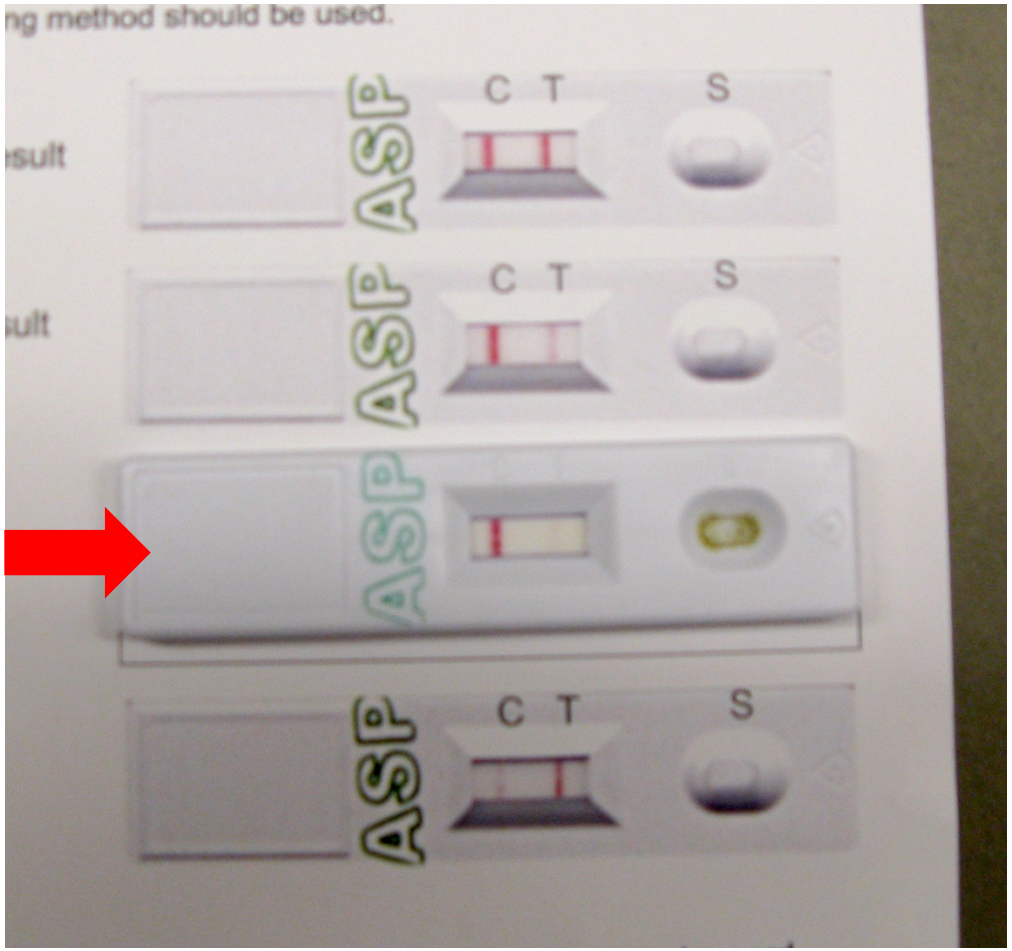
THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

September 6 2017
Phytoplankton tow
Jamestown town dock
350 cells extracted

ASP test positive

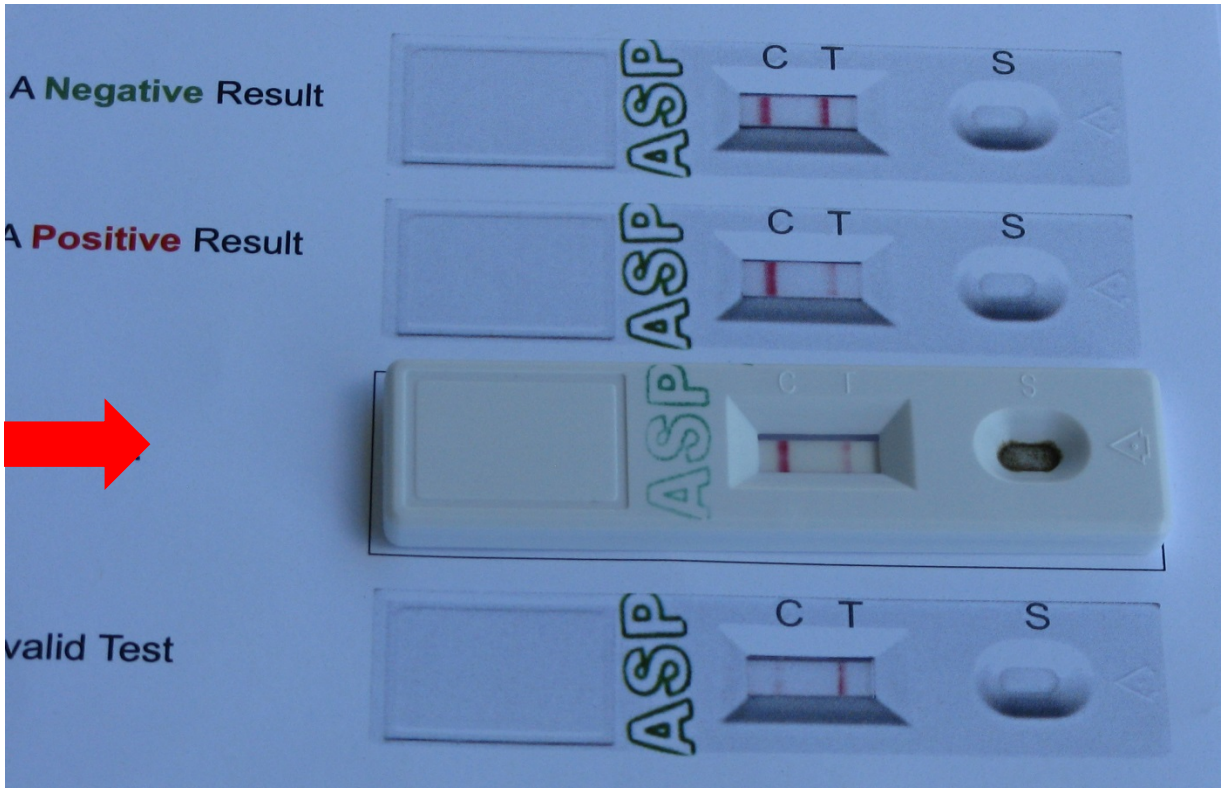


RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

September 8 2017
Phytoplankton tow
GSO dock

ASP test positive



THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

September 4 2017
Mussels sentinel station

ASP tests negative

Surface

Bottom

THINK BIG WE DO

RIDEM
September 12 2017

RI Sound as a potential source of HAB toxins for Narragansett Bay

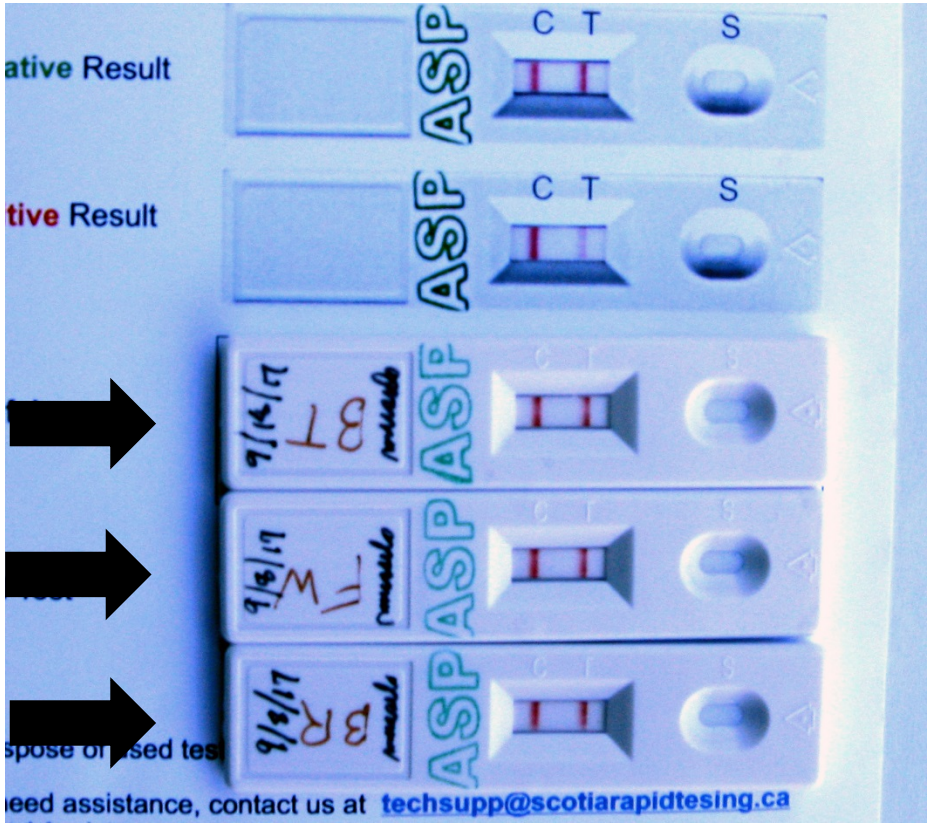
September 8 2017
Mussels sentinel station

ASP tests negative

Beavertail

Fort Wetherhill

Bass Rock



RI Sound as a potential source of HAB toxins for Narragansett Bay

Cell enumeration ongoing

So far, sentinel and stations at the mouth of the Bay have higher densities of *Pseudo-nitzschia* up to 40,000 L^{-1}

So far, very few *Alexandrium*

Three species of *Dinophysis*

THINK BIG WE DO

RIDEM
September 12 2017

RHODE ISLAND
DEPARTMENT OF HEALTH

RIDOH Laboratories' Role

HAB and Shellfish Contingency Plan
and
Rhode Island Shellfish Initiative

At the RIDOH Laboratories…

With our partners in the Rhode Island Shellfish Initiative we are determined to help make Ocean State shellfish the healthiest and safest for all people to enjoy.

Since 1888 we have protected Rhode Islanders from health threats large and small through early detection.

As the state's FDA approved shellfish laboratory our job is to be prepared for every worst-case-scenario.

RIDOH Division of Laboratories

Center for Environmental Sciences

Environmental Microbiology – drinking, beach, recreational, shellfishing waters, dairy, shellfish, plankton, cyanobacteria

Food and Water Chemistry - water, food, shellfish

Organic Chemistry - water, food, shellfish, agriculture

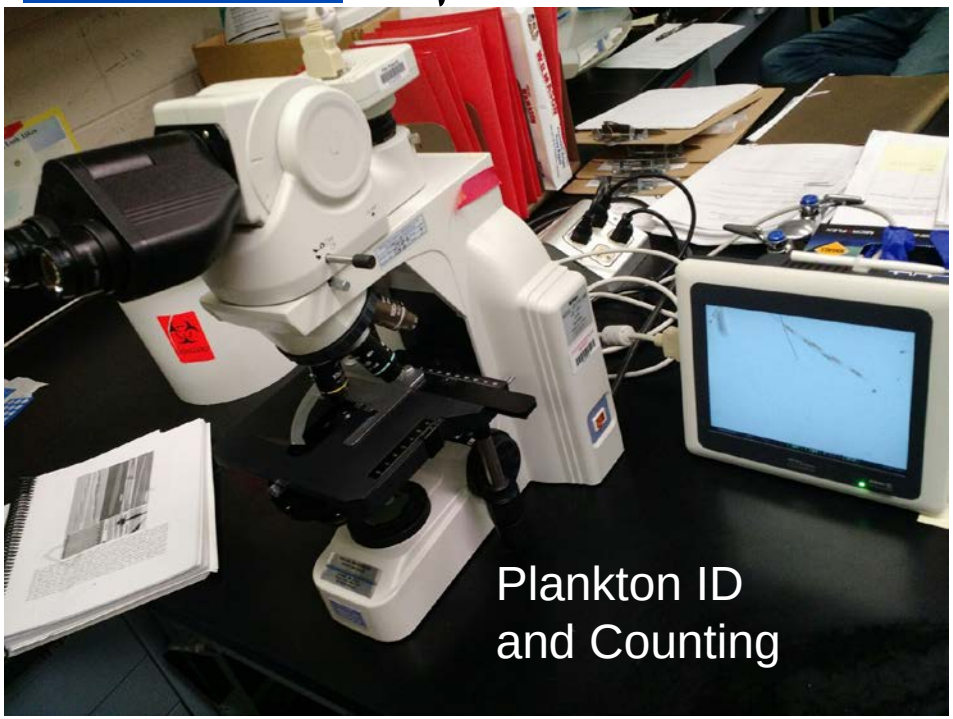
Air Pollution Monitoring – ambient air (7 field stations)

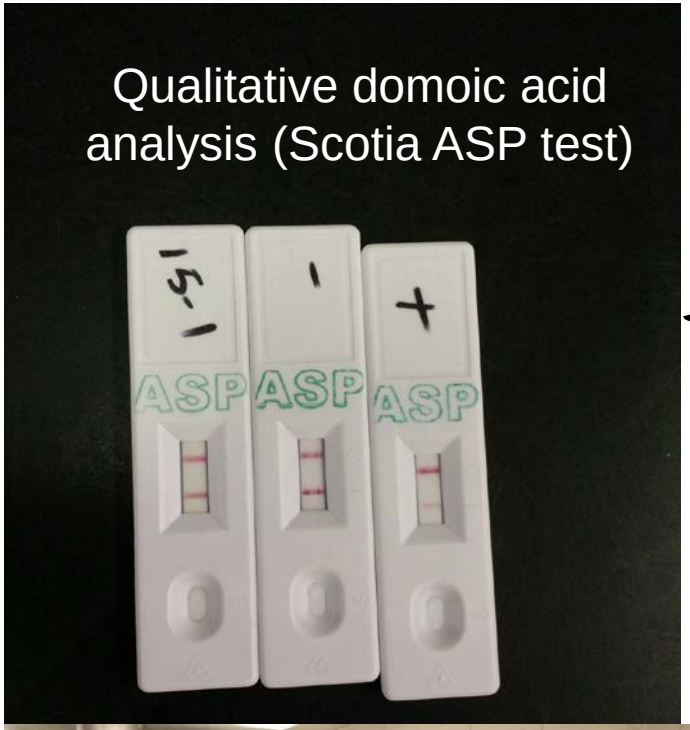
Center for Biological Sciences (Clinical)

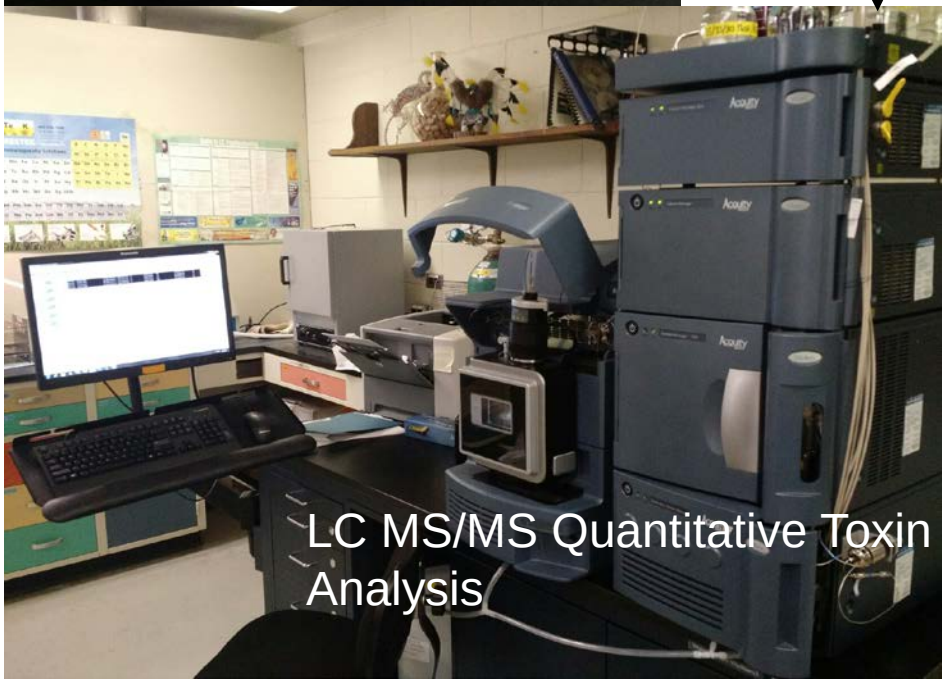
Molecular biology, special pathogens, serology, biomonitoring

Center for Forensic Sciences

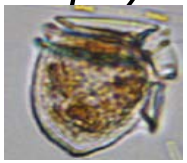
Drug chemistry, Toxicology, DNA, Breath Analysis


Plankton ID
and Counting


Qualitative domoic acid
analysis (Scotia ASP test)
-
-
+
ASP
ASP
ASP


LC MS/MS Quantitative Toxin
Analysis

HAB phytoplankton contingency threshold abundances

HAB Taxa	Biotoxin	Shellfish Syndrome	Abundance threshold
Alexandrium spp.	Saxitoxins	Paralytic Shellfish Poisoning (PSP)	1,000 cells L^{-1}
Dinophysis spp. 	Okadaic Acid	Diarrhetic Shellfish Poisoning (DSP)	30,000 cells L^{-1}
Pseudo-nitzschia spp.	Domoic Acid	Amnesic Shellfish Poisoning (ASP)	20,000 cells L^{-1}

Role of Water Microbiology Laboratory

HAB Phytoplankton Counting

< Threshold

continue HAB monitoring

> Threshold

Plankton Toxin Screen
(ASP and PSP only)

Plankton Toxin (-)

Plankton Toxin (+)

Request shellfish for toxin testing

Extraction and LC MS/MS Quantitative
(Organic Chemistry Laboratory)

ASP - Domoic Acid

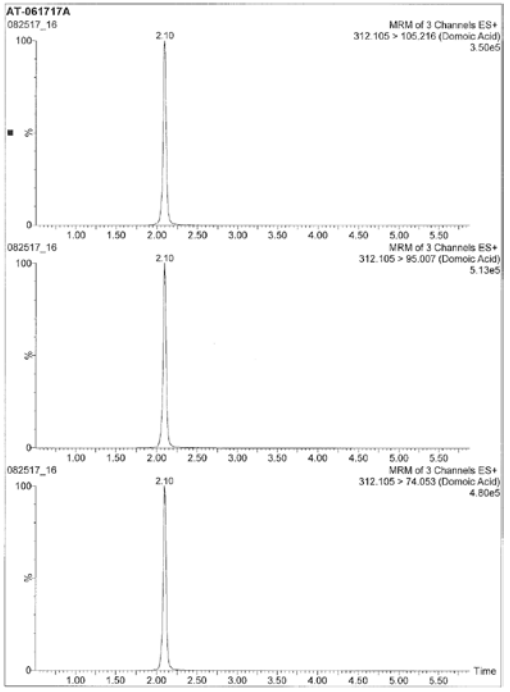
Extraction and Immunoassay
(qualitative screen)

ASP – domoic acid
PSP - saxitoxin
DSP – okadoic acid

Quantitative Analysis of Shellfish Toxin

FDA laboratory approval is not required to make precautionary and mandatory closure decisions.

RIDOH Laboratories uses LC MS/MS quantitative testing to support closure decisions based on the concentration of toxin in shellfish.



Decisions to reopen closed areas must be based on FDA approved laboratories using quantitative analysis.

Reopening closed areas currently is based on an FDA approved shellfish laboratory in Maine using quantitative analysis.

Shellfish meat biotoxin concentration contingency levels for PSP, DSP and ASP (after NSSP, 2015).

Toxin	Concentration	Action
Saxitoxin (PSP)	0.40 – 0.79 ppm	Precautionary Closure
Saxitoxin (PSP)	>/= 0.80 ppm	Mandatory Closure
Okadaic Acid (DSP)	> 0.08 – 0.15 ppm	Precautionary Closure
Okadaic Acid (DSP)	>/= 0.16 ppm	Mandatory Closure
Domoic Acid (ASP)	10 – < 20 ppm	Precautionary Closure
Domoic Acid (ASP)	>/= 20 ppm	Mandatory Closure

RIDOH Laboratories looking for resources necessary to expand its scope of FDA approved shellfish testing

Current scope of FDA approved testing:

m-TEC (fecal coliform), APC and MPN on shellfish, MPN for wet storage of shellfish on UV treated process water

Scope of FDA approved testing necessary to reopen closed harvesting areas and to address other emerging threats to the safety shellfish:

HAB biotoxins, coliphage, vibrio

RIDOH Laboratories is Committed to the Rhode Island Shellfish Initiative

While we are looking for the additional resources necessary to expand our support as the state's FDA approved shellfish testing laboratory, our focus is always to protect the reputation and strength of the shellfish industry and public health.

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