





# **Role of State Health Laboratories in Ensuring Safe Shellfish Supplies**

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State Health Laboratories (SHL)

Rhode Island Department of Health

Do you trust  
your shellfish?



"GO AHEAD... MAKE MY DAY."

# State of Rhode Island and Providence Plantations Facts

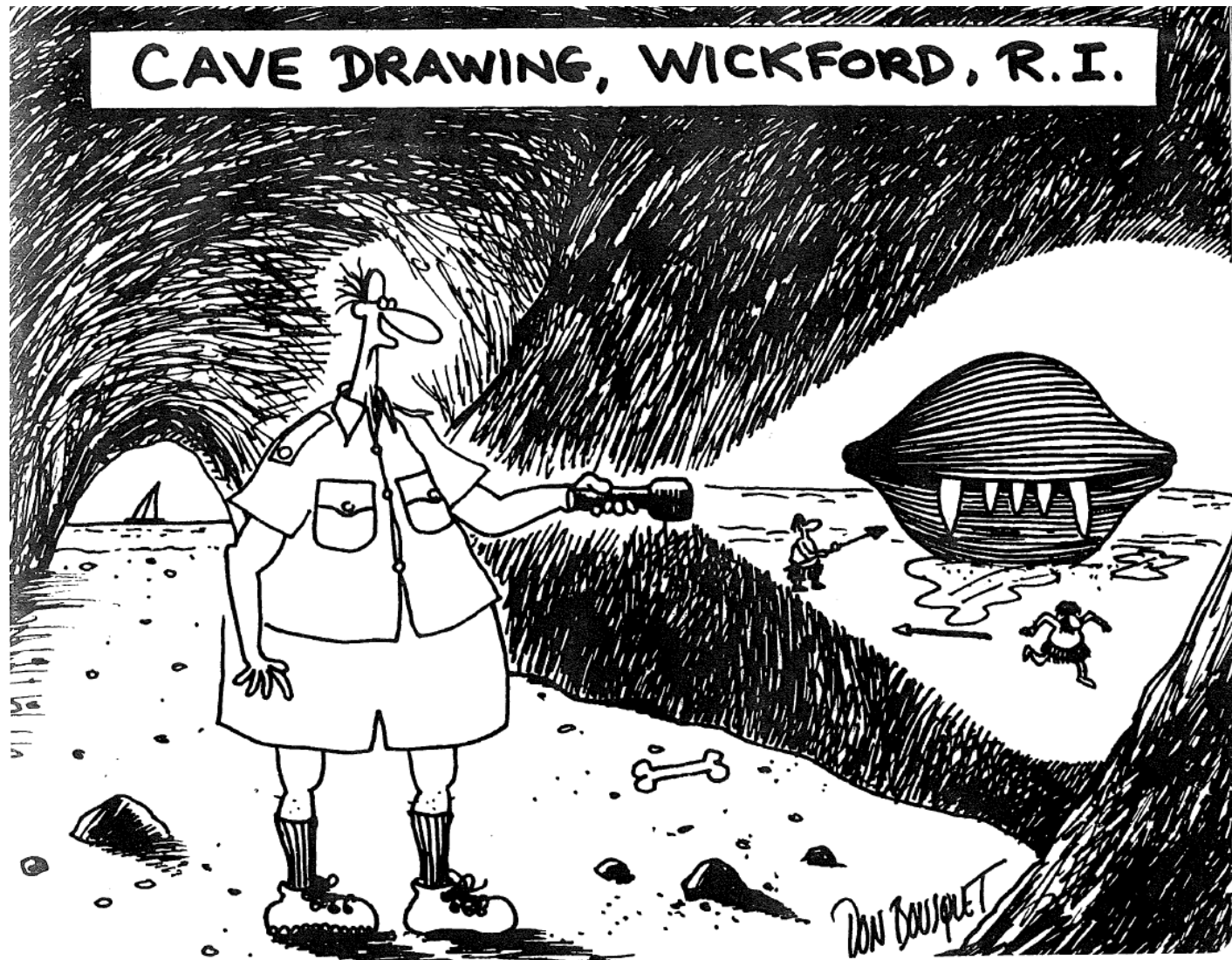


- longest name
- smallest in US (Alaska > 400x larger)
- area 1,214 mile<sup>2</sup> (1045 land + 169 water)
- 48 miles N-S, 37 miles E-W
- coastline 384 miles/618 km
- population 1.05 M
- density 1,018 per square mile

# State of Rhode Island Location



# Early shellfish harvesting



# FDA - National Shellfish Sanitation Program (NSSSP)



## Guide for the Control of Molluscan Shellfish 2011 Revision

From the U.S. Food and Drug Administration website

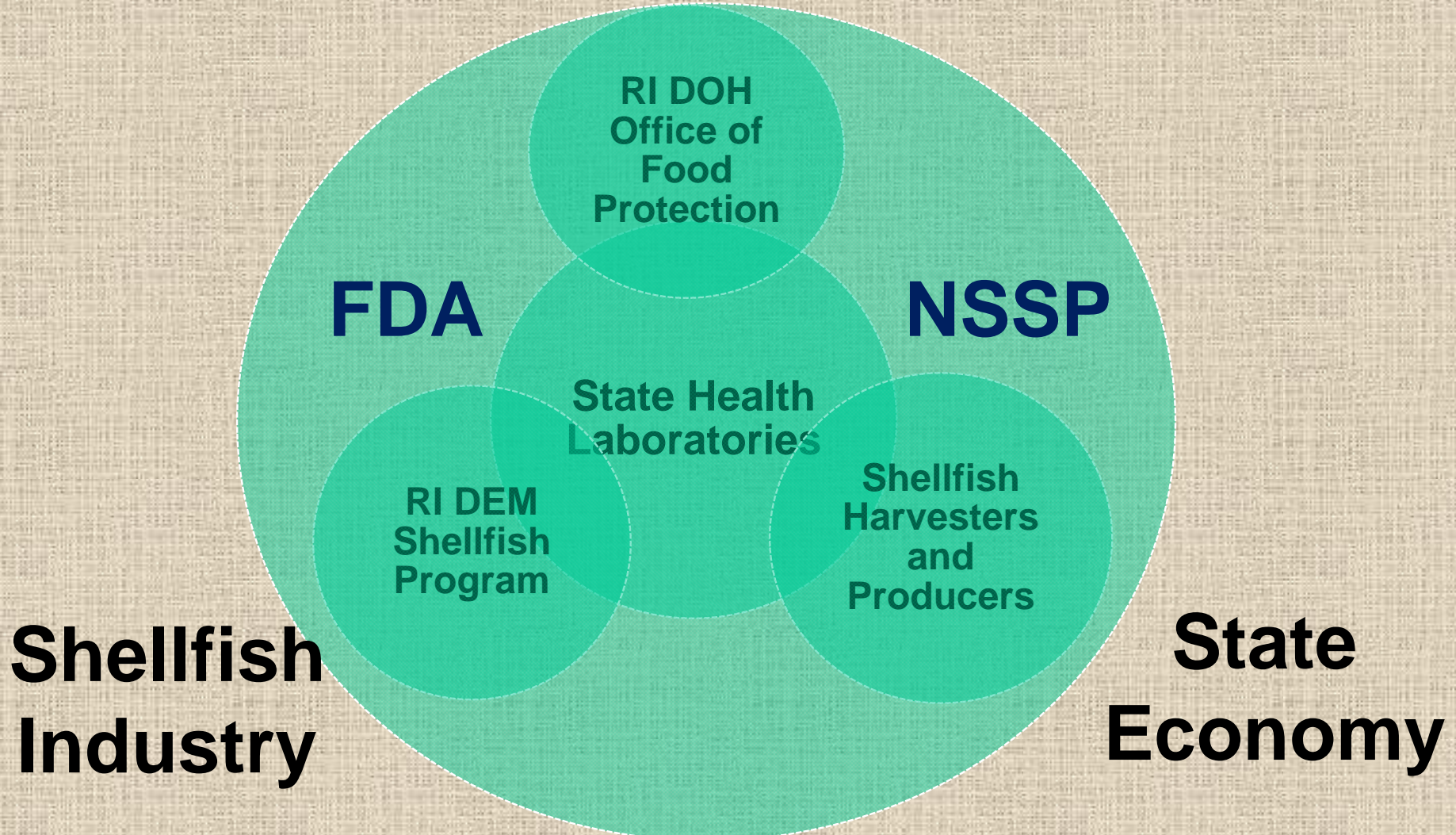
<http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm>



# Pivotal Role of SHL



## Public Health Protection



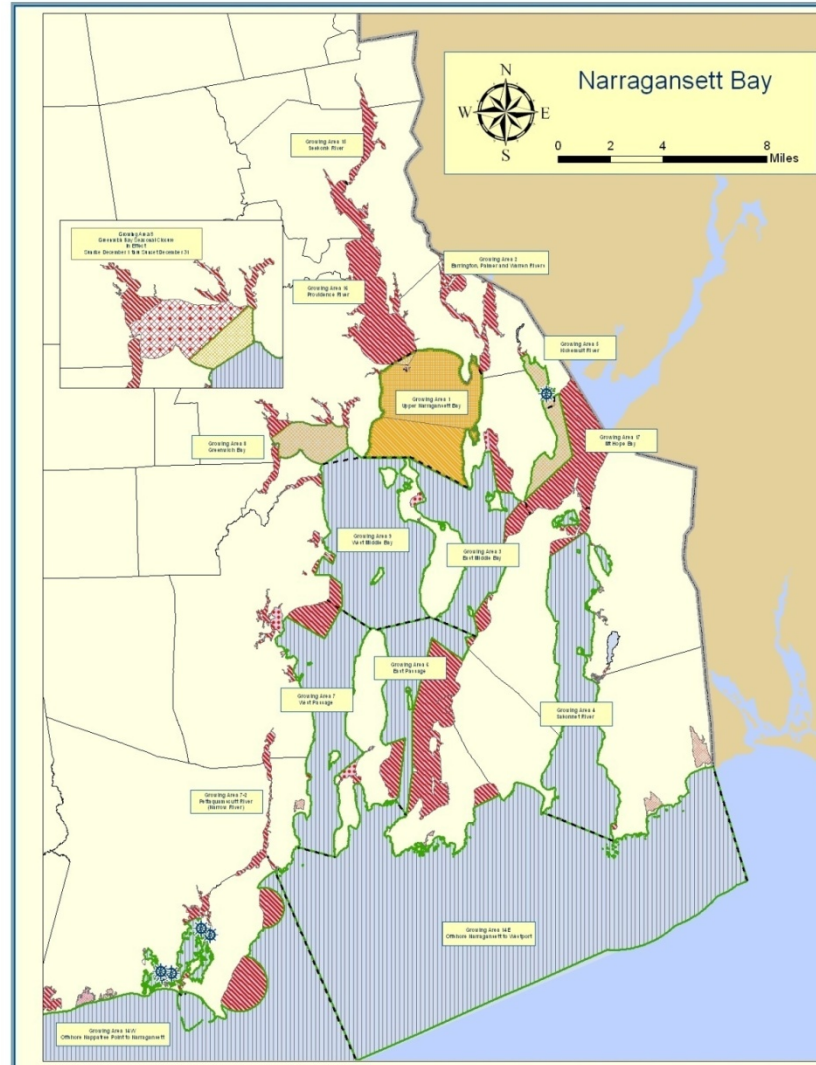


# State Health Laboratories (SHL)

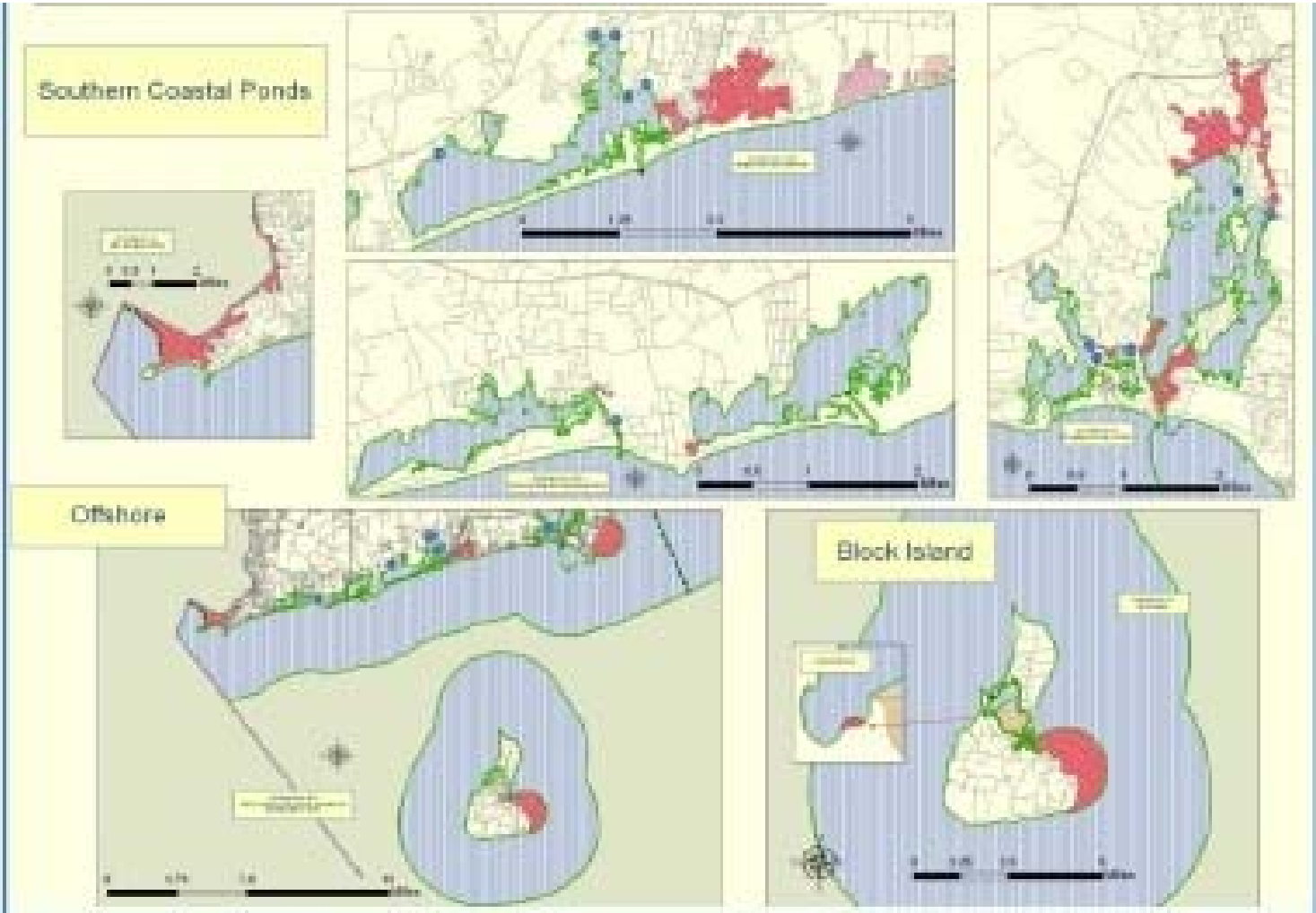


- Public health protection (state and national)
- Safe shellfish production
- \$30 million annually to state economy
  - shellfish harvesters
  - shellfish farms and processors
- Interstate shellfish industry

# Narragansett Bay, Rhode Island



# Southern RI Coastal Ponds and Block Island



# Public Health Protection and Shellfish Program Support



## SHL Responsibilities:

- Sample receiving and Chain of Custody
- Capacity for rapid testing
- Immediate notification of test results to shellfish program and industry clients
- Maintain Quality System according to FDA NSSP
- Analyze about 3000 shellfish related samples/year

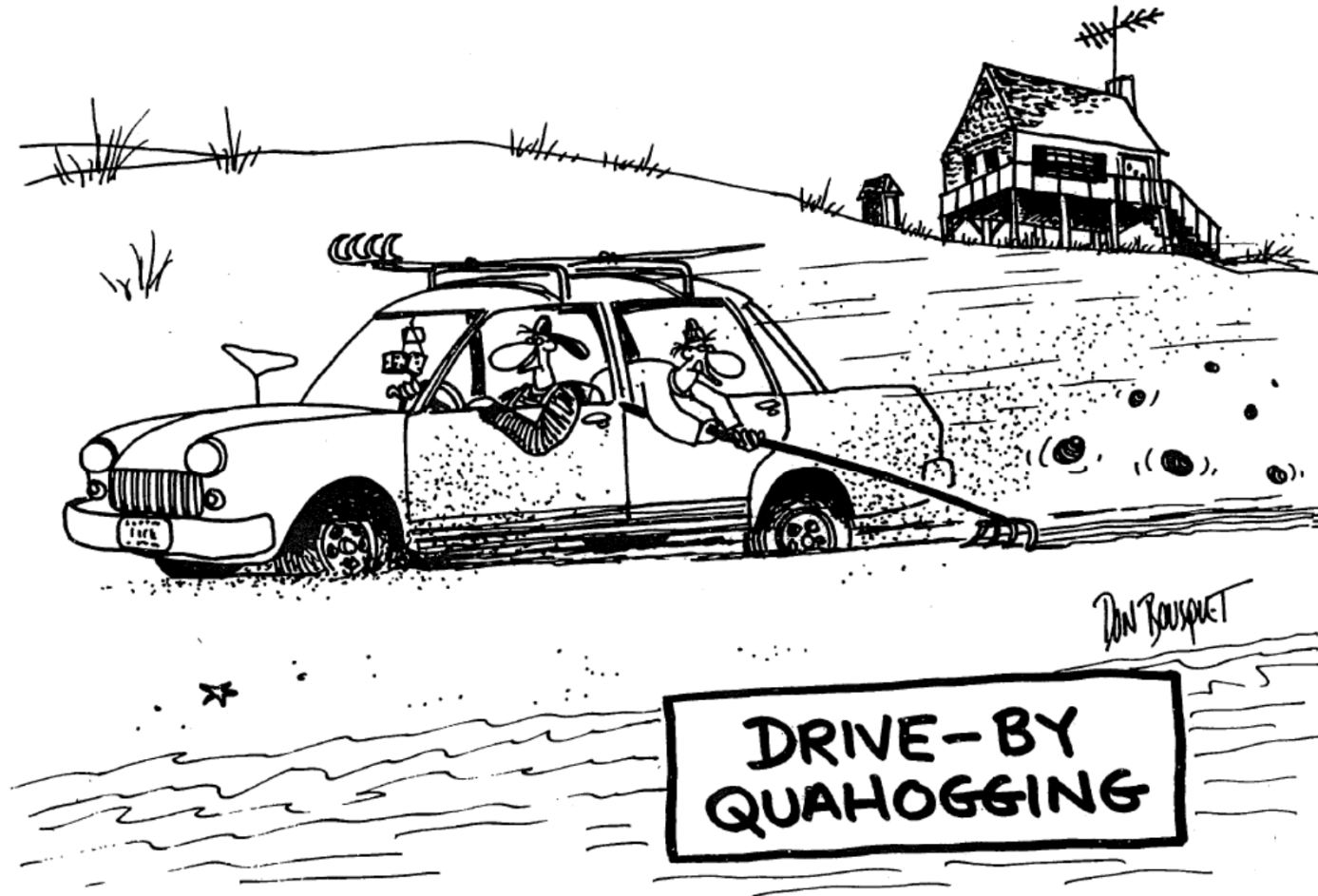


# Sample Receiving and LIMS

Sample submission - Chain of Custody

Sample management – Laboratory Information Management System (LIMS) tracks samples from receipt through analysis and upon review reports results electronically or hardcopy to clients.

# Sample Collection



# Samples Analyzed by SHL



- Shellfish harvesting waters
- Shellfish farming waters
- Shellfish holding facilities
- Harvested shellfish



# Bay Runs



Water samples collected from the harvesting and growing areas of the bay.

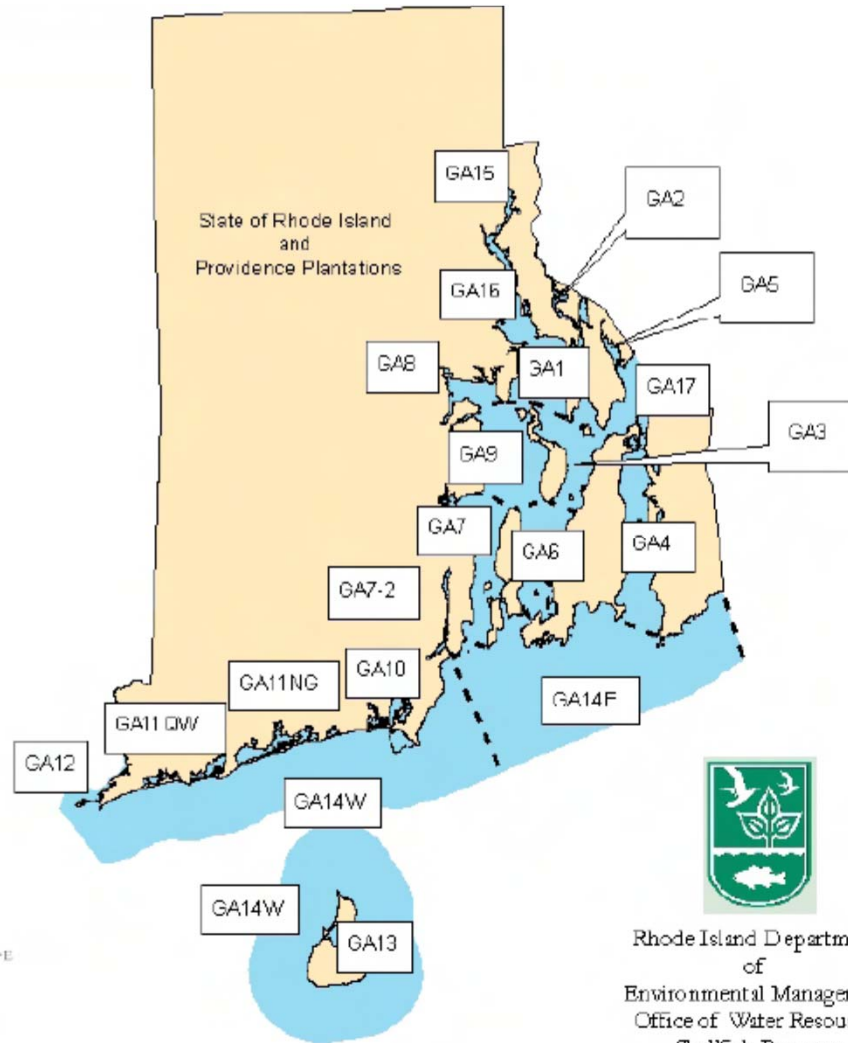




# Shellfishing Classification Maps

## Guide for Individual Growing Area (GA) Map

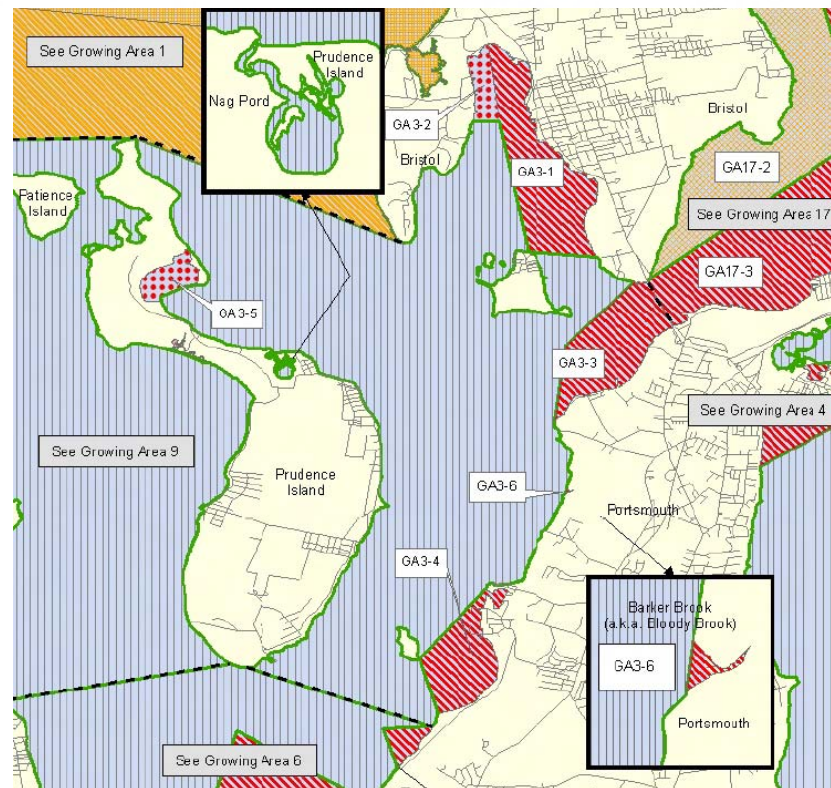
May 2013



# Shellfish Growing Area Classification Codes



-  Approved
-  Seasonal Closure
-  Shellfishing Prohibited
-  Growing Area Boundary
-  Conditional Closure
-  Upper Narragansett Bay Conditional Area - A
-  Upper Narragansett Bay Conditional Area - B
-  Unassessed - Shellfishing Prohibited



# Shoreline Survey

Water samples collected where run-off is apparent.



# Shellfish Transplantation – A Coordinated Effort Between Government Regulators and Commercial Harvesters



## **EAST BAY SHELLFISHERMEN TO CONDUCT A VOLUNTARY QUAHOG TRANSPLANT IN BRISTOL HARBOR ON TUESDAY, MAY 21**

**Shellfishermen Interested in Taking Part in this Voluntary Transplant Should Report  
to DEM Vessel to Sign In on Morning of Event**

PROVIDENCE -The Department of Environmental Management's Marine Fisheries section announces that East Bay shellfishermen will conduct a voluntary "dig and dump" quahog transplant from Bristol Harbor to the Bristol Transplant Bed on Tuesday, May 21 from 8:00 a.m. until noon. Shellfishermen will harvest quahogs from the high-density beds within the closed waters of Bristol Harbor and distribute the clams throughout the Bristol Transplant Bed from their own vessels.

# Shellfish Relocation (Transplanting)



Controlled harvests in closed areas of the bay and relocation to protected clean areas allow shellfish to depurate over time. Lab results determine when transplanted shellfish are safe to harvest.



# Conservation



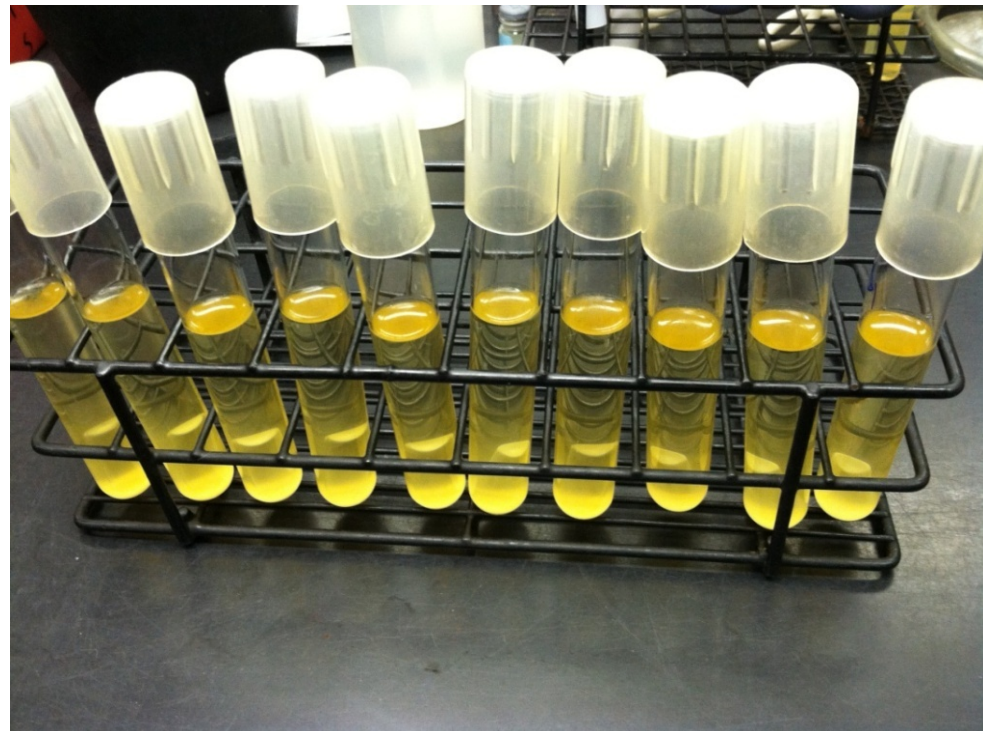
Transplanted shellfish spawn, helping to maintain the commercial fishery in the bay.



# Multiple Tube Fermentation (MTF)



Most Probable Number (MPN) of fecal coliform colonies / 100 mL estimated according to # of positive tubes in sets containing multiple sample dilutions.



# Multiple Tube Fermentation (MTF)



Presumptive phase – Total Coliform	Confirmation phase – Fecal Coliform
Lauryl Tryptose Broth (LTB)	EC Broth
24 hrs incubation at $35 \pm 0.5^{\circ}\text{C}$ 48 hrs if negative	24 hrs incubation at $44.5 \pm 0.2^{\circ}\text{C}$
Gas production indicates positive	Gas production indicates positive

MTF Fecal coliform tests have  
48 – 72 hour turn around times



# Fecal Coliform by Membrane Filtration (MF) with mTEC Agar



RI DEM Shellfish Program switched from MTF to MF membrane thermotolerant E. coli (mTEC) in 2012 due to increased rapidity and accuracy of mTEC test.

# Membrane Filtration mTEC, a single step method, direct count of fecal coliform in 24 hrs, confirmation not required



Sample is vacuum filtered on sterile membrane, retains bacteria

Membrane placed on mTEC agar, selective and differential medium

Incubated at  $35 \pm 0.5^\circ \text{C}$  for 2 hours to resuscitate injured or stressed bacteria

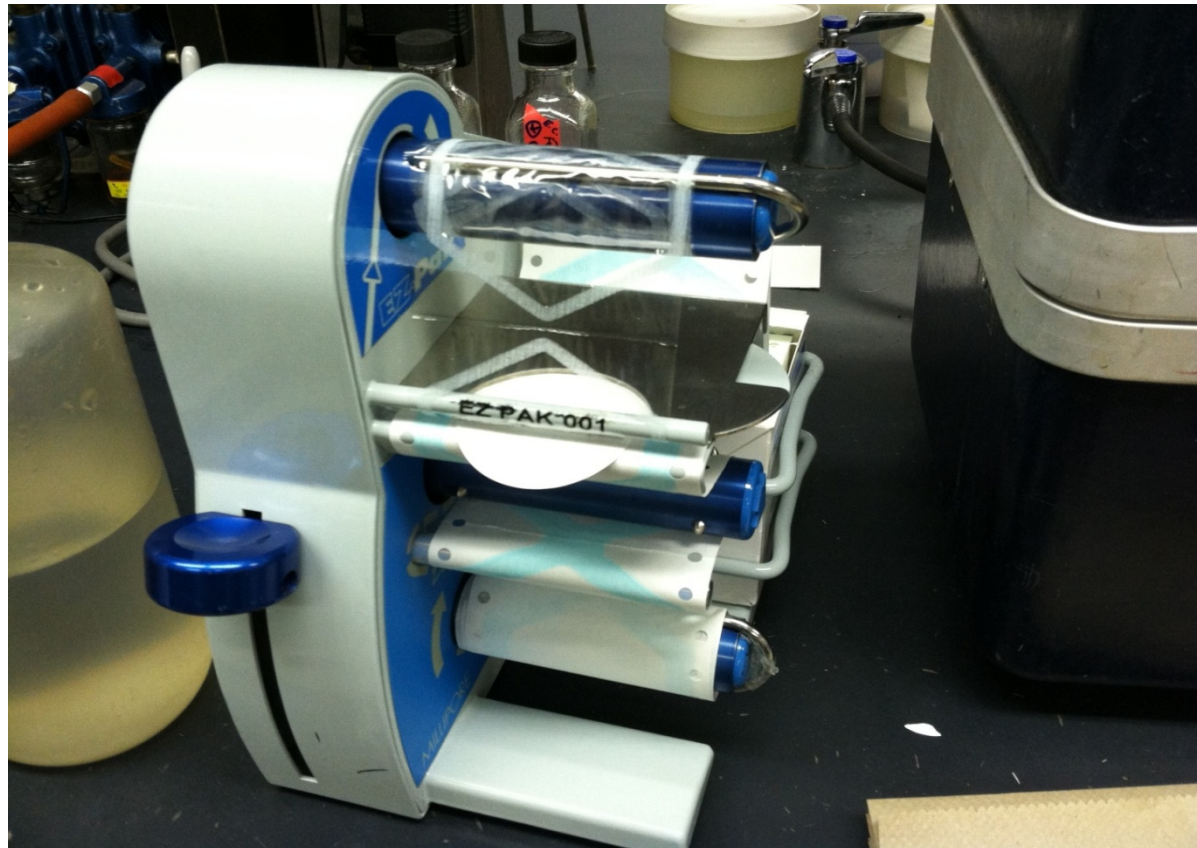
Transferred to  $44.5 \pm 0.2^\circ \text{C}$  water bath incubator for  $22 \pm 2$  hrs

Reporting range of  $< 2$  to  $>1600$  CFU/100ml

# Membrane Filtration Apparatus



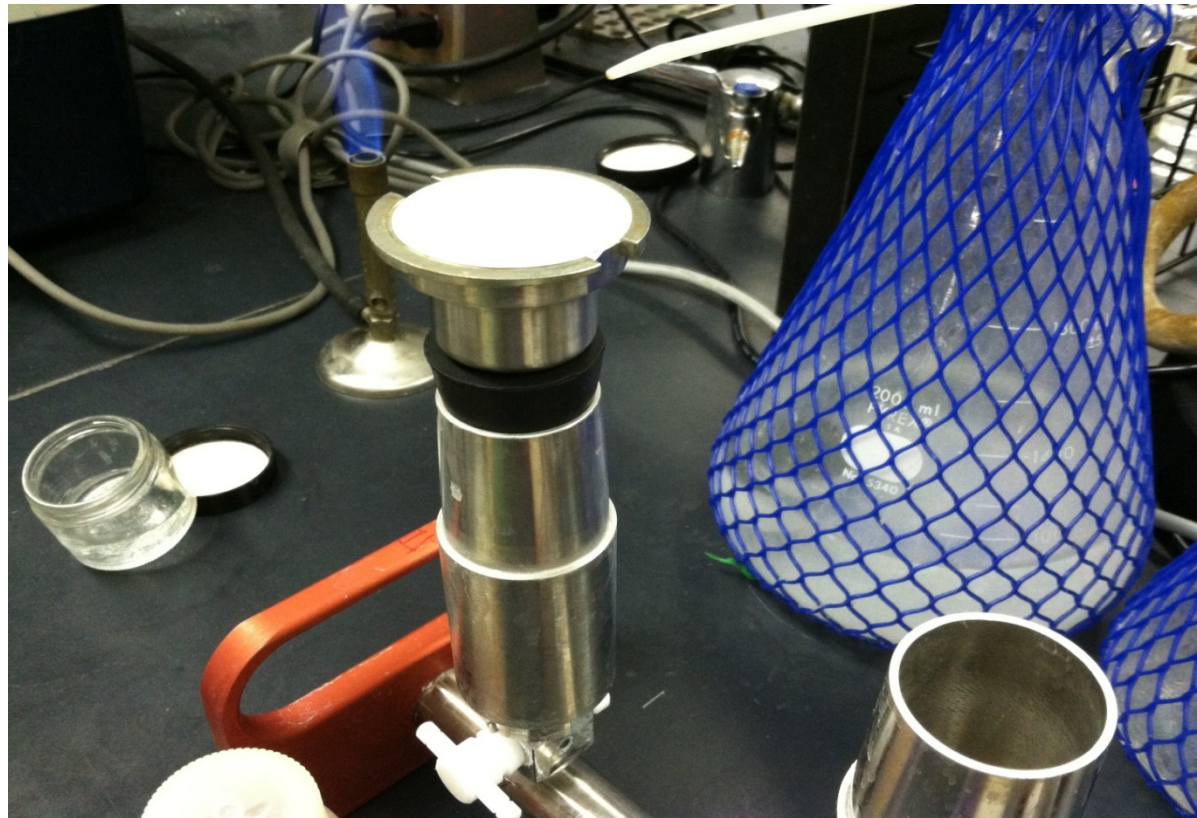
# Sterile Membrane Filters



# UV Sterilization Chamber for Filtration Equipment



# Membrane Filter Support



# Measuring Sample Volume

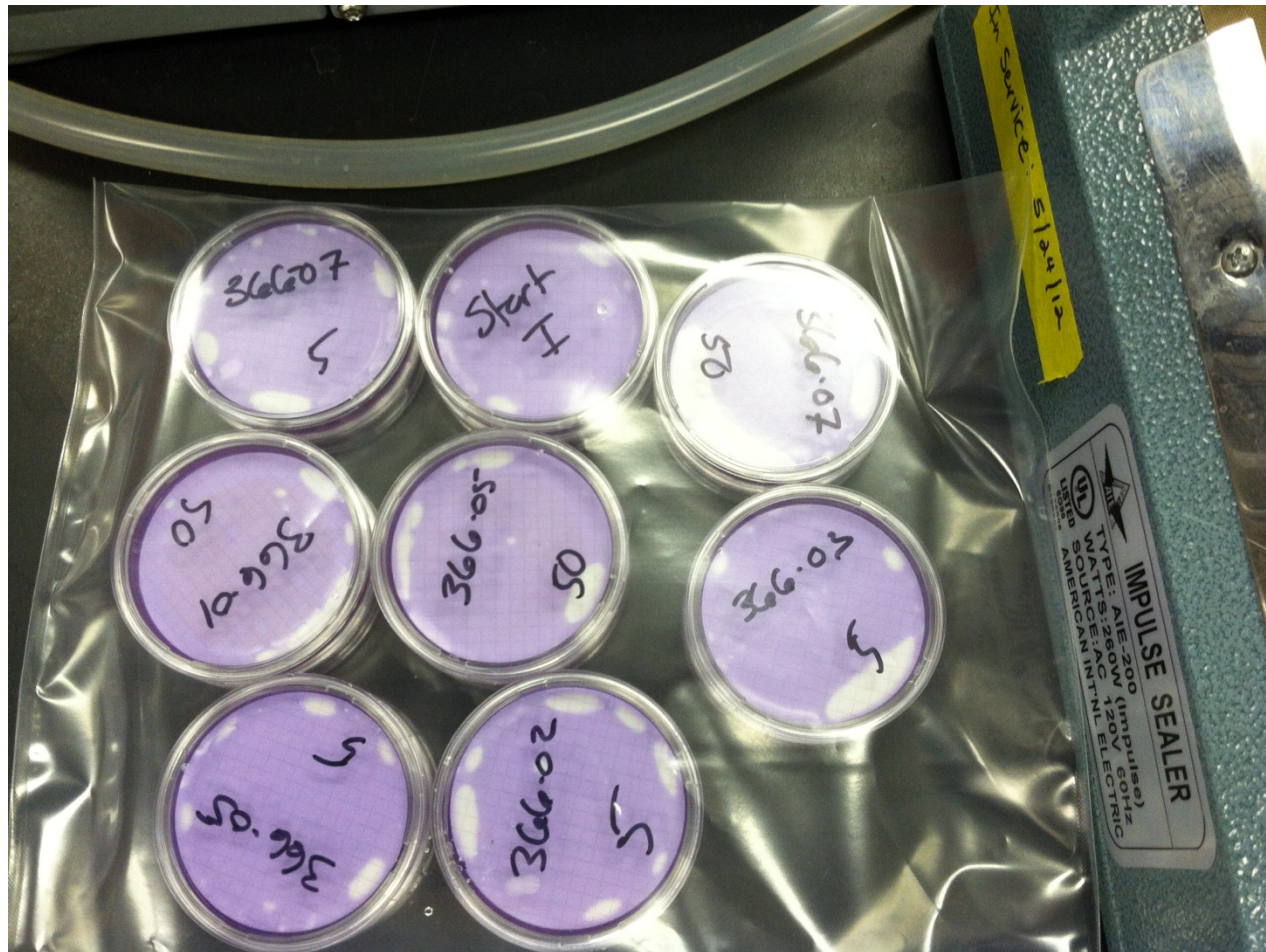


# Sample Filtration





# Membrane on mTEC agar, sealed in bags for 22 hour submersed incubation



# mTEC Membranes After Incubation



# Counting Fecal Coliform Colonies on mTEC Membrane



# Fecal Coliform Criteria



- Multiple Tube < 10% of samples cannot exceed GEO mean of 49 counts,
- mTEC < 10% of samples cannot exceed GEO mean 31 counts,
- RI DEM will collect mTEC data for several more years and reduce the criteria from 49 -31 colonies as sample size increases.

# Transplanted Shellfish



- Heavy Metals in shellfish:  
Cu, Pb, Cr, Fe, Ni, Cd, Zn  
Microwave digestion / ICP-MS analysis
- Fecal coliform by MTF (5 tube)
- Heterotrophic Plate Count

# Male-Specific Coliphage



Coliphage are viruses (bacteriophages) that infect coliform bacteria and are indicators of fecal contamination.

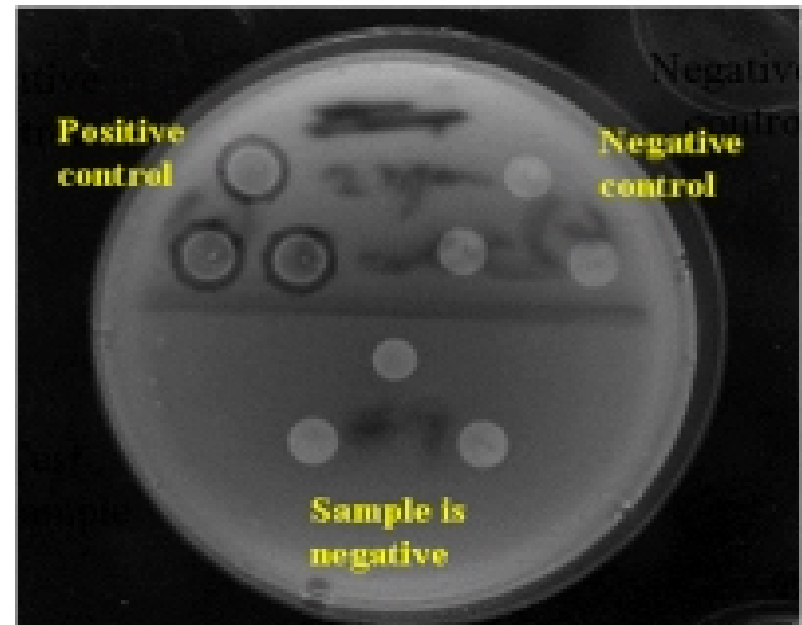
Antibiotic-resistant host-culture strain *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for male-specific coliphage.

Coliphage infect and lyse *E. coli* enriched media and produce plaques on plates within  $18 \pm 2$  hours at  $35-37 \pm 0.5$  °C.

# Male-Specific Coliphage



Samples are spotted onto a lawn of host *E. coli* and incubated. Positive results for coliphage appear as a clear halo around the spot. Bacteria from the sample grow on the spot, while phage radiate from the spot to lyse the surrounding *E. coli* lawn. Zones of lysis within the spot are also considered indicative of a positive result.



Positive results for coliphage appear as a clear halo around the spot in the surrounding *E. coli* lawn.

**Photo from USGS**

# When is Male-Specific Coliphage Testing Required?



Emergency closures of harvest areas after raw untreated sewage discharge from large community sewage collection system or wastewater treatment plant.

Collected no sooner than seven (7) days after contamination has ceased and from representative locations in each growing area potentially impacted.

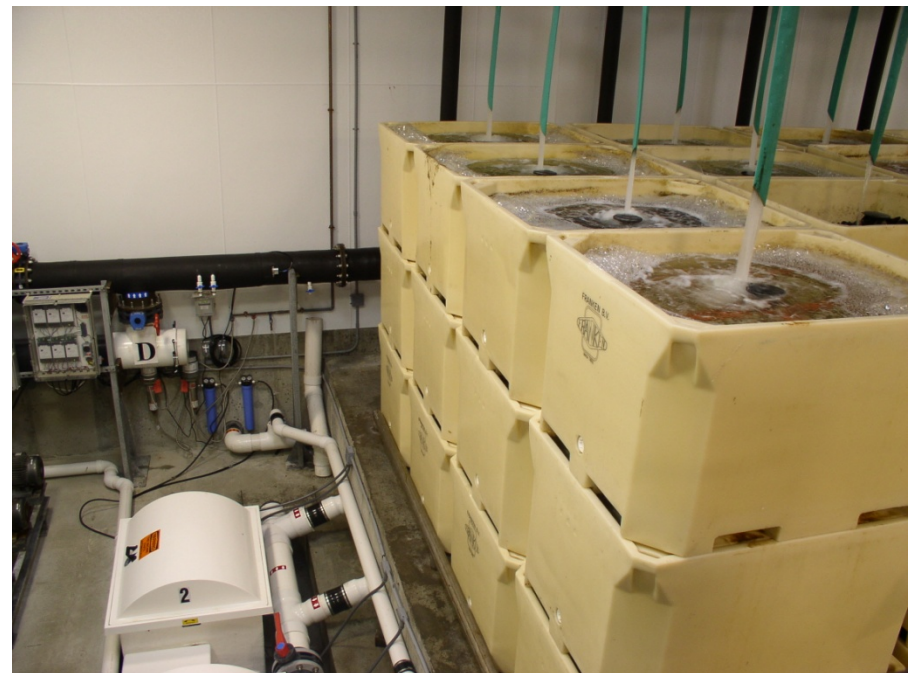
Laboratory results shall not exceed background levels or a level of fifty (50) male-specific coliphage per 100 grams from shellfish samples.



# Shellfish Suppliers and Processors



Water from shellfish farm holding and depuration facilities are tested for total / fecal coliform bacteria and turbidity and must meet DW standards.



# Farmed Shellfish



- Placed into facility seawater system on day of harvest.
- Seawater sterilized to minimize the bacteria and refrigerated to slow the metabolic rates.
- Salinity, pH, oxygen levels and the current speed of the seawater are regulated to allow the animals to clear themselves of grit and bacteria (depuration.)
- Shellfish are harvested to order, packed, and shipped for delivery.

# National Shellfish Sanitation Program (NSSP) Laboratory Evaluation Checklist – (36 pages)



<b>Check the applicable analytical methods:</b>		
<input type="checkbox"/>		Multiple Tube Fermentation Technique for Seawater (APHA)[PART II]
<input type="checkbox"/>		Multiple Tube Fermentation Technique for Seawater using MA-1 [PART II]
<input type="checkbox"/>		Membrane Filtration Technique for Seawater using mTEC [PART II]
<input type="checkbox"/>		Multiple Tube Fermentation Technique for Shellfish Meats (APHA)[PART III]
<input type="checkbox"/>		Standard Plate Count for Shellfish Meats [Part III]
<input type="checkbox"/>		Elevated Temperature Coliform Plate Method for Shellfish Meats [PART III ]
<input type="checkbox"/>		Male Specific Coliphage for Soft-shelled Clams and American Oysters
<b>PART 1 - QUALITY ASSURANCE</b>		
CODE	REF.	ITEM
K	8, 11	<b>Quality Assurance (QA) Plan</b>

# Quality Assurance



QC control tests on each batch of microbiological media prepared:

- Sterility test,
- Positive control,
- Negative control
- Processivity control (incubator and waterbath checks using specific media)

# Quality Assurance



HDPE 125 ml sample bottles are reused.

Before they are sent out for sample collection:

- Washed,
- Sterilized,
- Tested for pH and growth inhibition,
- Tested for sterility

# What does the future hold?



Concerns microbiological threats may increase with changes in climate.

- Toxic plankton blooms – shellfish poisoning
- *Vibrio* contamination and infection

# Toxin Producing Plankton Blooms



SHL currently examine plankton tow samples to identify and count toxin producing species.

When toxin producing plankton bloom in growing waters shellfish concentrate the toxins to unsafe levels in their tissue.

# Biotoxin Contingency Plan



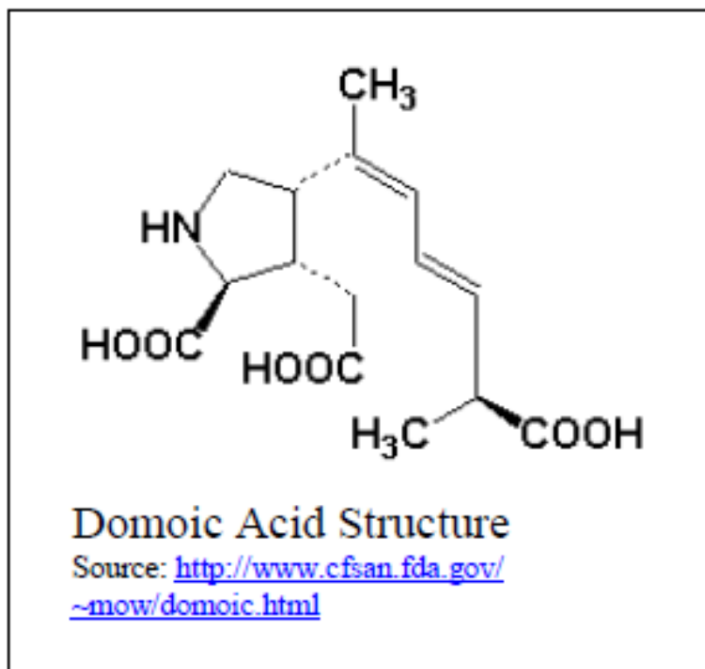
1. When SHL identifies 15 or more biotoxin producing organisms per slide of seawater samples,
2. DEM Shellfish Program will collect shellfish samples from the suspect stations, properly identified with location, date and time of collection,
3. SHL will analyze a minimum of 12 animals, providing at least 150 grams of shellfish meat to determine the concentration of toxin in the tissue.



# Amnesic Shellfish Poisoning (ASP)



Caused by consuming shellfish with concentrated levels of neurotoxin Domoic Acid (DA) produced by Pseudo-nitzschia, a diatom found worldwide



Chains of *Pseudo-nitzschia multiseriata* Source: [http://www.nwfsc.noaa.gov/hab/HABs\\_Toxins/HAB\\_Species/PseudoN/index.htm](http://www.nwfsc.noaa.gov/hab/HABs_Toxins/HAB_Species/PseudoN/index.htm)

# Amnesic Shellfish Poisoning (ASP)

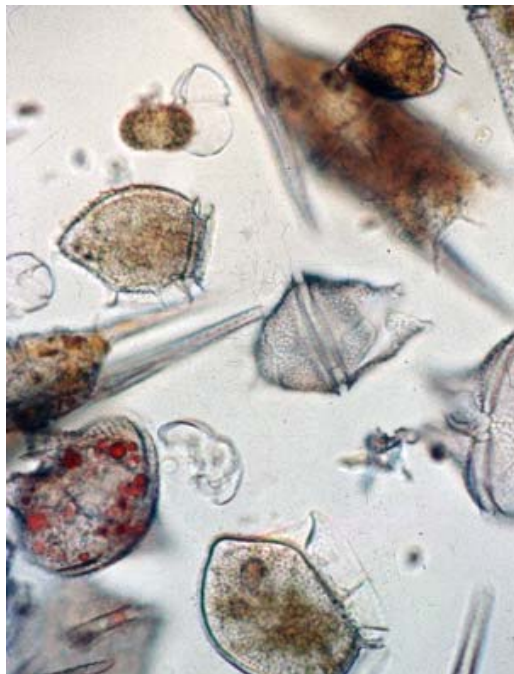


- Several species produce DA found in US on both coasts and the Gulf,
- Mild symptoms nausea, vomiting, diarrhea, abdominal cramps in 24 hrs of consuming contaminated shellfish,
- Severe cases headaches, hallucinations, confusion, short-term memory loss, respiratory difficulty, seizures, coma, and death in extreme cases.

# Paralytic Shellfish Poisoning (PSP)



Caused by consuming shellfish concentrated with saxitoxin produced by dinoflagellate species found worldwide



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## Moderately Large Potential for Red Tide Outbreak

ARTICLE | NEWS | APRIL 22, 2009 - 7:11AM

### Researchers Report 'Moderately Large' Potential for Red Tide Outbreak

*Toxic bloom expected to be smaller than last year, but still significant*

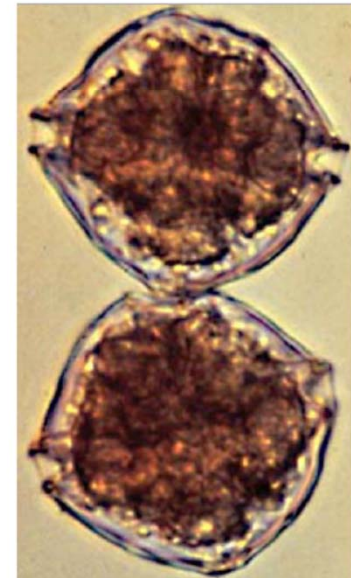
The Woods Hole Oceanographic Institute (WHOI) reports that the potential for an outbreak of the phenomenon commonly called "red tide" is expected to be "moderately large" this spring and summer, according to researchers at Woods Hole and North Carolina State University (NCSU).

Their advisory is based in part on a regional seafloor survey of quantities of *Alexandrium fundyense* - the algae notorious for producing a toxin that accumulates in clams, mussels, and other shellfish and can cause paralytic shellfish poisoning (PSP) in humans who consume them. The survey maps are used with computer models that simulate different scenarios of weather and oceanographic conditions to indicate where and in what abundance the toxic cells might be expected in 2009.

The researchers found concentrations of *Alexandrium* cysts - the dormant seed-like stage of the algae's life cycle - in the Gulf of Maine to be 40 percent lower than the historically high levels observed prior to last year's bloom, but still higher than the level preceding a major regional bloom in spring 2006 that closed shellfish beds from Canada to Massachusetts Bay.

The *Alexandrium* survey has been conducted each fall since 2004 as part of several research and event response projects funded by the National Oceanic and Atmospheric Administration (NOAA) Center for Sponsored Coastal Ocean Research (CSCOR). Fall concentrations of *Alexandrium* cysts are one of the indicators of the magnitude of a potential bloom in spring.

In October 2008, a survey team led by Don Anderson, a senior scientist in the WHOI Biology Department and the lead investigator of the Gulf of Maine Toxicity (GOMTOX) study, spent 10 days collecting



Cells of *Alexandrium*. (Don Anderson, Woods Hole Oceanographic Institution)

# Paralytic Shellfish Poisoning (PSP)



- Mild exposure - tingling sensations or numbness, headaches, fever, rash, dizziness, and gastrointestinal illness.
- Severe case - muscular paralysis, pronounced respiratory difficulty, choking sensation, and death may occur through paralysis and respiratory failure.
- Despite the severity victims begin to recover within 12-24 hours of intoxication.

# ASP and PSP Action Levels and Tests



FDA action levels for shellfish GA closure:

ASP - Domoic Acid 2 mg/100 g tissue (20 ppm)

PSP - Saxitoxin 80  $\mu$ g/100 g tissue (0.8 ppm)

ELISA quantitative testing sufficient to close GA and embargo shellfish.

ASP - HPLC to confirm that levels are safe to reopen harvesting areas

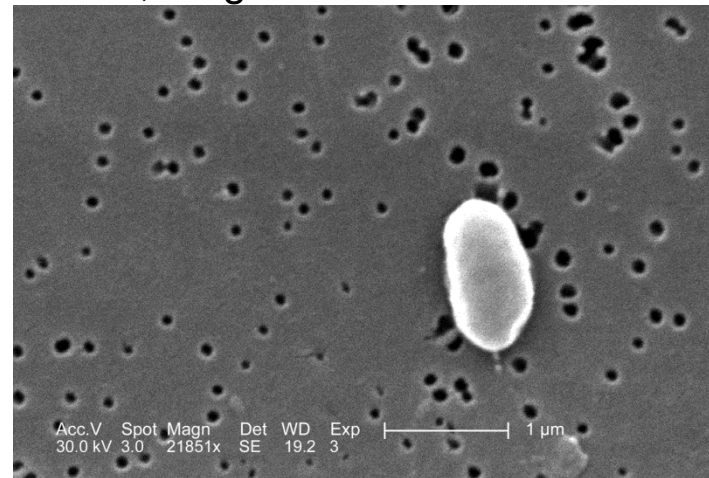
PSP - Mouse Bioassay to confirm levels are safe to reopen growing areas

# Vibrio Species



- Over 80 species known
- Most common causes of human illness:
  - *V. cholerae* (O1, O139, non-O1, non-O139)
  - *V. parahaemolyticus*
  - *V. vulnificus*
  - *V. fluvialis*
  - *V. alginolyticus*
  - *Grimontia hollisae*

SEM depicts a *Vibrio parahaemolyticus* bacterium; Mag. 21851x



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# Vibrio Transmission



- Ingestion of raw or inadequately cooked seafood (especially raw oysters)
- Ingestion of food contaminated by handling raw seafood or rinsing with contaminated water
- Open wound exposed to warm seawater

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# Vibriosis cases, New England, 2011-2012



State	2011	2012
Maine	4	10
New Hampshire	1	3
Vermont	0	0
Massachusetts	52	71
Rhode Island	2	11
Connecticut	25	24

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# Vibrio Infection



- Symptoms:
  - Watery diarrhea
  - Abdominal cramps
  - Septicemia
  - Wound infections
- Isolated from:
  - Blood
  - Stool
  - Wounds

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# Vibriosis Reporting



- State and National notifiable condition
- Specific national form used
  - Laboratory testing results
  - Clinical illness
  - Epidemiologic risk factors
    - Travel
    - Seafood exposure
    - Water exposure
  - Seafood investigation section
    - Combination inspection results and seafood consumption

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# Vibrio Analysis



BAM method for Vibrio is labor intensive.

FDA is working on developing an alternative method using PCR.

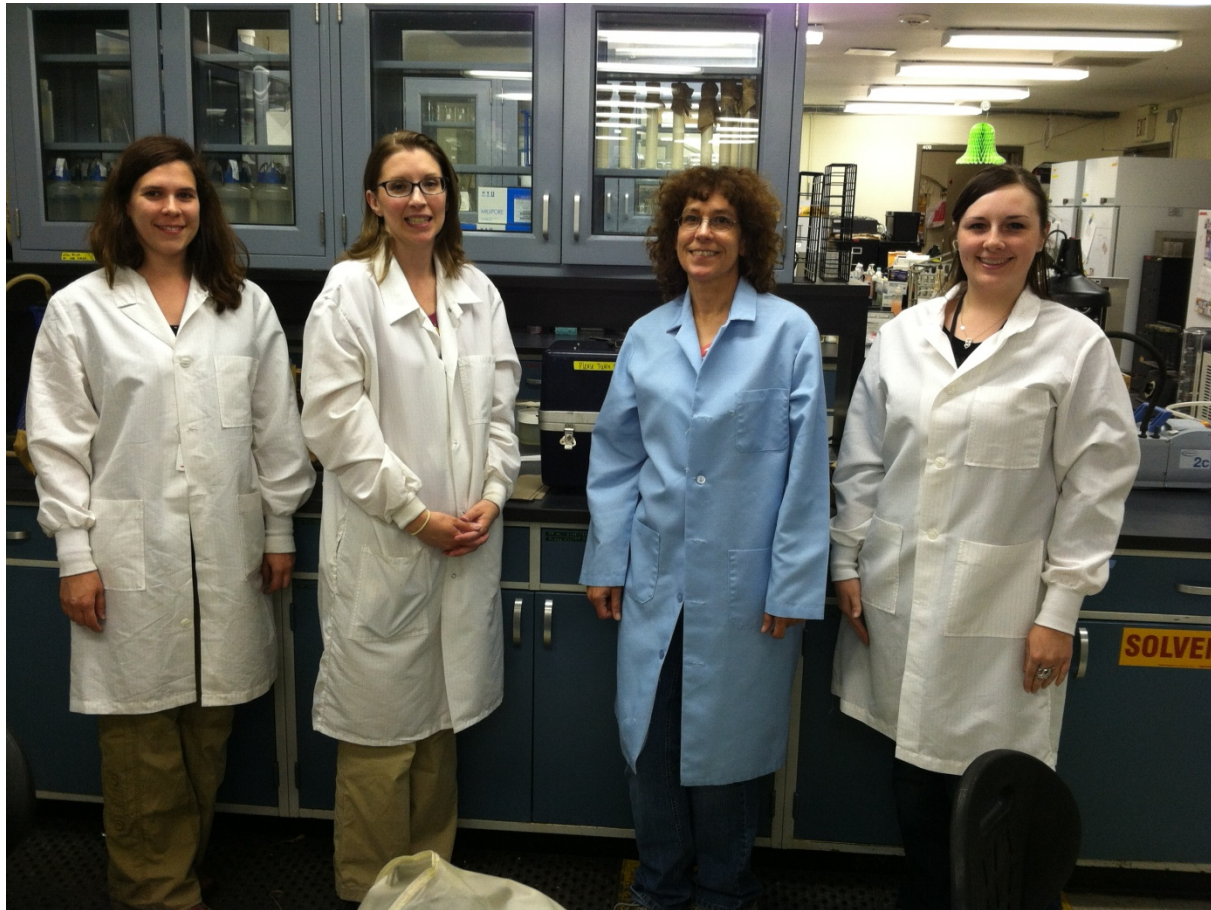
# Environmental Investigation



- Violations included:
  - No separation of raw vs. cooked foods
  - Food temperatures not checked
  - Improper cold storage of foods
  - Lack of proper consumer advisory notification on menu
  - Lack of cleanliness/fly infestations
  - Potential for cross-contamination

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# Microbiologists Responsible for NSSP and Other Environmental Microbiological Testing at the SHL



Jennifer Kulik, Kirsten Skelly,  
Kerry Patterson (supervisor), Samantha Jalette (L to R)



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