FINAL REPORT

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Scallop Research Set Aside Project 04-SCA-008

Preliminary Investigation of Marine Biotoxins on the Northwest Atlantic Continental Shelf

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Glossary of acronyms used in the text and tables throughout this report

ASP – Amnesiac Shellfish Poison – federal standard for closure is 20µg/g domoic acid.

AOAC – Association of Official Analytical Chemists – standardizing body for chemical testing.

CCEHBR – Coastal Center for Environmental Health and Biomolecular Research

CFSAN / FDA – Center for Food Safety and Applied Nutrition

DMF – Massachusetts Division of Marine Fisheries

FDA - U.S. Food and Drug Administration

GSI - Gonadalsomatic Index

HPLC – High Performance Liquid Chromatography

ISSC – Interstate Shellfish Sanitation Conference

JRT – Jellett Rapid Test

JRTA_G and JRTA_V - Jellett Rapid Test for ASP on the gonad or viscera

JRTP_G and JRTP_V – Jellett Rapid Test for PSP on the gonad or viscera

LC- MS - Liquid Chromatography – Mass Spectrometer

R / M – Roe to Meat ratio

MBA – Mouse Bioassay

NEFMC – New England Fishery Management Council

NOAA – National Oceanic and Atmospheric Administration

NOS – National Ocean Service, Charleston, S.C.

NMFS – National Marine Fisheries Service

PSP – Paralytic Shellfish Poison – federal regulatory standard is 80µg/100g STX equivalents

RBA – Receptor Binding Assay

RSA – Research Set Aside program and source of funding

STX equiv. – Saxitoxin equivalents

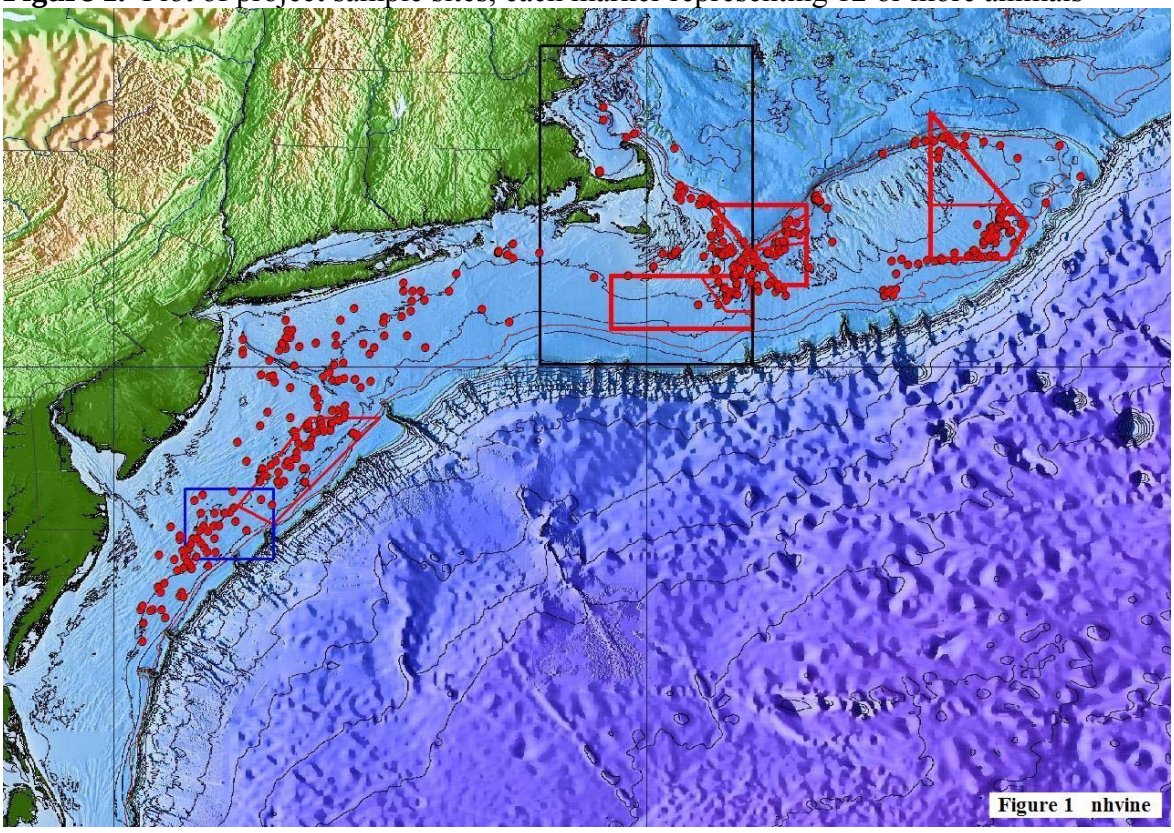
1. Project Summary

Project partners conducted testing of Atlantic sea scallop tissues for marine biotoxins responsible for Paralytic and Amnesic Shellfish Poisoning (PSP and ASP) to provide toxin distribution data critical to the development of a roe-on scallop fishery. Over 6000 animals were collected from 500 sample locations across the U.S. range of the species, using the commercial scallop industry, and the NOAA R/V Albatross IV as vessels of opportunity. Testing for biotoxins was accomplished using two newly developed methods, Jellett Rapid Test (JRT) and Receptor Binding Assay (RBA), with a subset cross-checked with High Performance Liquid Chromatography (HPLC), and the ISSC approved Mouse Bioassay (MBA).

In roe samples obtained in 2004 we found toxicity to be localized to the Nantucket Lightship Closed Area. In early 2005 an unusually large bloom of the toxic dinoflagellate *Alexandrium fundyense* caused sharp increases in PSP toxins in scallop viscera in the Great South Channel and Nantucket Shoals area, yet only 4 out of the 57 roes tested by RBA quantitative methods, were considered toxic by federal standards. Viscera toxicity for those 4 samples was 40 times the public safety standard yet the mean for that area was only 2.5 times higher. In both years, 100% of PSP results from the Mid-Atlantic and Georges Bank roes were well below federal toxicity standards. All ASP results were less than half of the 20 µg/g federal regulatory action levels in all areas at all times.

Here we propose consideration of a flexible regulatory structure for the sea scallop resource in which we identify areas where a roe-on scallop and whole-animal fishery is likely to be completely safe, and other areas where the current conditions determine the status of the area for harvest. Continued open communication and collaboration between industry and regulators is seen as a necessity.

Figure 1. Plot of project sample sites, each marker representing 12 or more animals



2. The opportunity, the constraints, and the project approach

2.1 Background on market opportunity for roe-on scallop product in the European Union

There has been a thriving demand for roe-on scallops within the European Union for many years yet the U.S. Atlantic sea scallop (*Placopecten magellanicus*) fishery currently discards that portion the animal, partially due to the potential for retention of biotoxins in the roe and viscera, and partially due to the complexity and lack of availability of testing methods. Toxin retention and measurement is of critical concern because while the scallop meats do not retain toxins, roe and viscera can become highly toxic when the infrequent *Alexandrium* blooms occur. France is the largest consumer of roe-on scallops, but its market is larger than can be met by their domestic fishery. Additionally there are sporadic supply problems with imported sources due to periodic blooms of toxic algae and closures of external production areas.

Roe-on scallops, as preferred by European consumers, consist of the adductor muscle with gonad attached. Preliminary market assessment indicates that France consumed in excess of 3,000 metric tons of roe-on scallops (*Pecten maximus*) in 2005 (MC Montfort 2006, personal comm). Shumway (2006) reports Canadian exports beginning in 1987 range between 10 and 100 metric tons of shell-off / roe-on scallops, proving a solid E.U. acceptance of *Placopecten magellanicus*.

As the size and thus market acceptability of scallop roe varies seasonally, we estimate that a roe-on product could immediately increase the saleable poundage of the US sea scallop industry by up to 15%. The marketing of scallop eye-rings, which are not similarly seasonal, could add another 10%. Sale of whole scallops, of interest to the primarily Asian markets, would increase utilization up to 100%, however the total current volume is small, estimated at less than 0.1 %. Significantly, these additional landings can be realized without increasing mortality on the scallop stock.

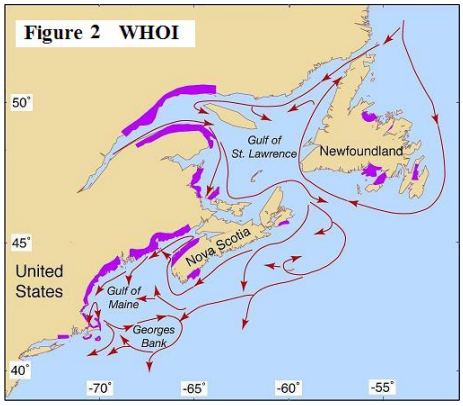
2.2 Background on U.S. Atlantic sea scallop resource

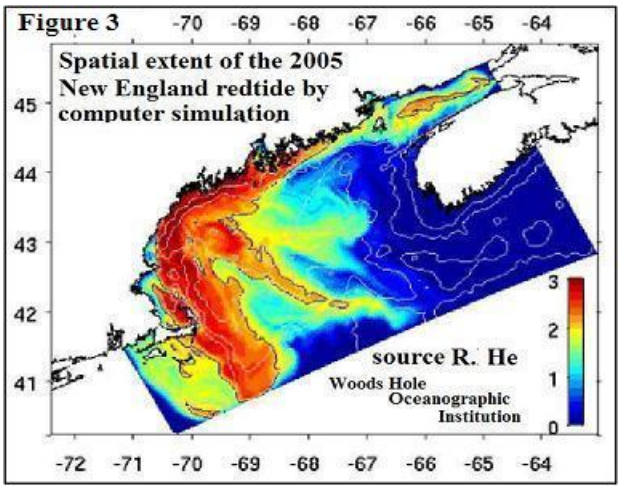
Amendment 10 to the Scallop Fishery Management Plan (FMP) institutionalized the process of identifying and closing areas where large sets of juvenile scallop are found, allowing them to grow significantly beyond the ring size of the fishing gear before the areas are reopened. Whether due to fortuitous environmental conditions for spawning, or to the success of the closure method, the Mid-Atlantic scallop growout areas currently contain more than half of the harvestable scallop resource. Unlike the closure areas on Georges Bank, which also contain large numbers of larger scallops, what we see in the Mid-Atlantic is large sets of new animals settling directly downcurrent from the densely populated growout areas. (Figure 1, red and blue areas)

While the Mid-Atlantic area has been considered to be biotoxin free, testing of scallops has been limited, with the primary concern being the large surf clam fishery. No toxins have been found there, so the area is open for the harvest of whole molluscan shellfish including -whole or roe-on scallops. There has been no significant commercial-scale effort to develop markets for live or whole scallops or other value added scallop products for export, in part because Mid-Atlantic surface water temperatures make it exceedingly difficult to land a live whole scallop during much of the year. In the other half of the northeast region's scallop resource, Georges Bank and Great South Channel, the landing or sale of whole scallops or scallop roe is currently prohibited as a result of federal offshore closures due to PSP established in 1989 and more recently for the more inshore waters in 2005.

2.3 Background on marine biotoxins

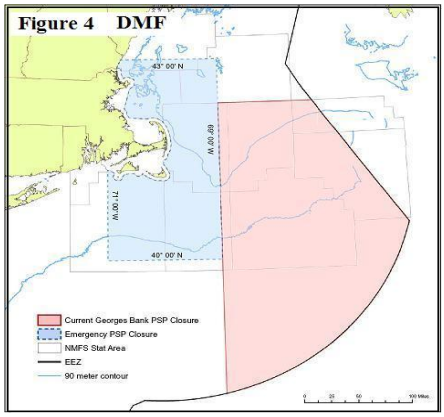
High concentrations of toxic marine algae, known as harmful algal blooms or HABs, are often referred to as "red tides" as concentrations of algae can sometimes become so dense they can cause waters to appear red. In many cases, however, water can be clear and uncolored, yet shellfish can still be toxic due to the potent toxins they have concentrated while filter feeding. The term "red tide" can thus be misleading. Nevertheless, given the popular use of the term in the context of regional PSP issues studied here, this term will be used hereafter.





Alexandrium fundyense, a single celled marine algae, is the causative organism for PSP. A second group of algae, *Pseudo-nitzschia (spp)* are the causative organisms for ASP. When sufficient quantities of *Alexandrium* or *Psudeo-nitzschia* are consumed by shellfish, toxins build up in shellfish tissues, which if consumed by humans, can cause illness or death. Safe harvest of valuable nearshore species, such as clams, mussels, and oysters, both wild and cultured, has been made possible through routine monitoring and periodic closures by state and federal regulatory programs.

In the spring of 2005 a large bloom of toxic phytoplankton developed in the western Gulf of Maine (Figure 3). By June, shellfish toxicity had increased sharply causing the FDA to close shellfisheries from New Hampshire to Martha's Vineyard and out to 69ºW, about 40 miles east of Cape Cod. While the inshore areas were subsequently reopened as the "largest red tide in over 30 years" passed by and shellfish toxicity fell below federal action levels in many species, the landing of whole or roe-on sea scallops from this 16,000 square mile emergency closure area remains prohibited. East of 69º is similarly closed due to toxins found there in 1989 and 1991.



The well-documented events of the PSP poisoning of fishermen and the long distance movement of the algal blooms from the northerly inshore waters southward along the coast (Figure 3), has led to the closing of two large contiguous areas, Georges Bank (in 1989) and the more inshore area from Jeffreys Ledge to the Great South Channel (2005) for the landing of whole molluscan shellfish (Figure 4). These blooms appear to be forced by a combination of events: fresh water runoff, the strong currents in the Gulf of Maine, and the variable influence of wind direction and intensity (Anderson et al., 2005).

The presence and uptake of these toxic algae is the critical issue to development of markets for scallop tissues other than the adductor muscle. Unlike the meats, the sea scallop viscera and roe are known to retain harmful toxins, often for an extended period of time after a bloom. Although there have been many studies of marine biotoxins in scallop tissue (Nassif, et al 1993, Shumway 1993, 2006), prior to this study little was known about the presence or absence of harmful algal toxins in offshore scallop tissues throughout the U.S. range of the Atlantic scallop resource, i.e. there has been no effort to produce an annual, synoptic view.

Worldwide, toxin-free harvest and export of roe-on scallops is permitted by public health officials using a combination of methods: water monitoring, tissue screening, lot testing, and source control - each implemented to meet internationally recognized and standardized shellfish sanitation protocols. However, U.S. producers cannot meet E.U. health and quality standards as sufficient protocols necessary to allow safe harvest are not in place. The E.U. nations have gone through a lengthy process of "harmonization" whereby the various national standards were brought in line with a common approach, satisfying all participants. E.U. / U.S. "harmonization" would simplify export of U.S. shellfish, yet is not likely to occur without specific protocols and testing in place.

We believe the key factors limiting development of U.S. scallop products for export are:

1). Worldwide, whole or roe-on scallops are classified as "whole molluscan shellfish", as they are capable of retaining marine biotoxins which could put the consumer at risk for shellfish poisoning. This classification calls for tissue testing to insure roe-on scallops taken from closed areas are non-toxic (CFSAN 2001).

2). Protocols and testing necessary to meet shellfish safety and import requirements required by other countries have historically not been in place or readily available. A critical factor is the AOAC mouse bioassay which is not currently available to the US industry.

3). The federal Scallop Fisheries Management Plan does not currently allow for the extra labor (in open areas) or weight (in closed areas) necessary to process and land roe-on scallops in a competitive manner, i.e. to offset the extra time it takes to process a roe-on product, to make it immediately economically attractive.

2.4 Project Approach

We sought to address these issues by collecting and testing sea scallops over the U.S. Atlantic range in order to document the present extent of marine biotoxins and the location of market-acceptable meats and roes. We sought to accomplish this primary goal while evaluating two new technologies to test for the presence of the two major biotoxins found in the region. Lastly we planned to bring industry and regulatory stakeholders together in a meeting to explore the opportunities and problems related to the development of a roe-on scallop fishery.

To accomplish these goals, this project set out to meet four objectives:

1). Document to the distribution and abundance of the two known marine biotoxins (PSP and ASP) found in scallop tissues in the Northwest Atlantic using samples from vessels of opportunity.

2). Explore the utility of and results from newly available biotoxin testing methods.

3). Monitor gonad and meat condition over the course of the year in various fishing areas to identify where and when market-ready roe-on harvest might occur.

4). Conduct a workshop with scientific, regulatory and industry partners to present project findings, and explore the next steps for the development of a safe roe-on scallop fishery.

Beginning in July 2004 and over the next 15 months, project vessels collected over 6,000 animals from 500 sample locations across much of the U.S. range of the species, from the waters off Virginia to the International Court of Justice boundary line on Georges Bank. Testing of samples for marine biotoxins was accomplished using two newly developed methods, Jellett Rapid Test (JRT) and Receptor Binding Assay (RBA), with portions crosschecked with High Performance Liquid Chromatography (HPLC) and the ISSC approved Mouse Bioassay (MBA). Roe and meat weights were recorded and plotted by area and season to estimate when and where a market-ready roe-on product was available.

3. Project Team

Donald M. Anderson, Ph.D., Senior Scientist, Woods Hole Oceanographic Institution
Anderson provided guidance in the proposal, testing and reporting phases, HPLC testing, interpretation, and insight into Harmful Algal Blooms.

Geoffrey Day, Seafood Research Associates, Cambridge, Mass., led the project with Taylor. Day gathered initial research, recruited team members, collaborated on the proposal, worked with vessels and partners to collect, process and analyze tissue, conducted the Jellett tests, and collaborated on reporting.

Mike Hopper, TransOceanic Seafoods, Cambridge, Mass, provided initial inspiration for the project as his customers in Europe expressed interest in buying roe-on scallops from the U.S. Hopper also provided the New England Shellfin facility as workspace for the project.

Richard Taylor, www.seascallop.com, Gloucester, Mass., collaborated on the proposal and reporting, and arranged sample collection with the commercial scallop vessels and R/V Albatross IV. Taylor also arranged the funding trips, processed samples, mapped and analyzed the data.

Frances Van Dolah, Ph.D., Research Biochemist and Algal Biochemistry Project Leader, Marine Biotoxins Program, Center for Coastal Environmental Health and Biomolecular Research, NOAA/NOS, Charleston, S.C. Van Dolah provided the lab that conducted the Receptor Binding Assay and LC-MS testing as well as provided guidance and insight into marine biotoxins.

Vessels Kathy Marie, Guidance, Atlantic, Westport, Christian & Alexa, Kathy and Jackie, Elizabeth and Nicki, conducted RSA funding trips and brought in offshore scallop samples throughout the year. Three Graces, Alison Lee, Betsy Gals and many others provided inshore samples. R/V Albatross contributed samples from the 2004 and 2005 scallop survey trips.

New England Shellfin, Inc provided access to the day-boat scallop fleet, storage and processing space, transportation, day labor and ongoing support throughout the project.

4. Description of how the work was accomplished

4.1 Sample collection

In the fall of 2004 twelve commercial scallop fishing vessel Captains were engaged to bring in multiple samples from all fishing operations conducted during the course of the 2004 – 2005 project year with the incentive provided by participation in the Scallop Research Set Aside program (RSA). Additional samples were collected by the NOAA Fisheries R/V Albatross IV during the annual scallop survey cruises conducted in the summers of 2004 and 2005, providing an opportunity to obtain samples from areas that were not necessarily open to commercial vessels. In June of 2005 after the MDMF / FDA / NMFS PSP closure area went into effect, 18 General Category vessels were recruited to sample in that area as it was not well covered by the RV Albatross and the original 12 vessels.

Figure 5

4.2 Sample handling and initial processing

Samples were fresh frozen at sea aboard participating vessels or delivered directly to the freezer at Bergies Seafood, New Bedford, or New England Shellfin, Falmouth, for storage. As the vessels had highly variable schedules, groups of samples were held at these locations, until sufficient samples were on hand to provide a full day's work. Samples were then processed at the New England Shellfin facility in Falmouth. Scallops were shucked, shell heights measured and recorded. Soft parts were separated, rinsed, mascerated, labeled and refrozen in 100 ml replicate sample tubs for local testing, shipping to project partners, and archiving. Meat, roe and viscera weights were recorded to calculate gonadalsomatic index and roe to meat ratios. To insure there was no possibility of mixing potentially toxic tissues with product going into the food supply, processing was done in an isolated area when the plant was not in use.

Figure 6

4.3 Conducting the workshop

A workshop entitled Increasing the Economic Return from the US Atlantic Sea Scallop Fishery was presented at WHOI, Woods Hole Mass on 4 November 2006. Invitations were sent out to license holders, resource managers, regulators, and the scientific community. 35 individuals attended to explore project findings to that time and discuss regulatory issues.

4.4 Unanticipated factors affecting data collection

The 2005 emergency closure precipitated the need for the FDA to conduct comprehensive tissue sampling in the newly closed area. Many of the smaller general category vessels fishing in more inshore waters were landing whole scallops, thus the June closure prohibiting this practice had a particularly strong impact on that segment of the scallop fishery. After receiving biological sampling permits, these fishermen contributed over a thousand additional sample animals to this study in an effort to provide data sufficient to reopen the area. Additional samples from within the Emergency Closure area were shipped to CFSAN for analysis after following the same procedures outlined above.

In October 2005, new enforcement of a longstanding but previously overlooked Multispecies Fishery Management Plan regulatory closure in the Great South Channel caused many of the general category scallop vessel captains to move into different waters, switch over to a different fishery, or sell their vessels. The area reopened in August 2006. In November 2005, just after the end of the first project year, project partner New England Shellfin, Inc. ceased operations, in great part because of the inability to land whole scallop from the closure area. These factors affected data collection after that time as each contributed substantially to our efforts.

5. Methods of testing for marine biotoxins

5.1 Overview

Initial proof of concept of at-sea testing for PSP using the Jellett Rapid Test was conducted by Taylor aboard F/V Westport in 2004. The key question was testing the suitability for use aboard a commercial vessel while fishing. While the initial tests were concluded successfully, all further Jellett testing was conducted at the Shellfin plant as a method of achieving more time-efficient and standardized testing procedures. At the Shellfin facility, Day tested both tissue compartments separately, roes and remaining viscera (eye rings, vellum, and hepatopancreas combined), using the Jellett Rapid Test.

Replicates of samples were shipped to the NOS Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) facility in Charleston for Receptor Binding Assay (RBA) tests. Additional testing using High Performance Liquid Chromatography (HPLC) was conducted at the Woods Hole Oceanographic Institution. Subsamples from the Emergency Closure Area were tested by the FDA Center for Food, Safety and Nutrition using the RBA, and by Mouse Bioassay (MBA) at the Massachusetts Division of Marine Fisheries Lab in Gloucester.

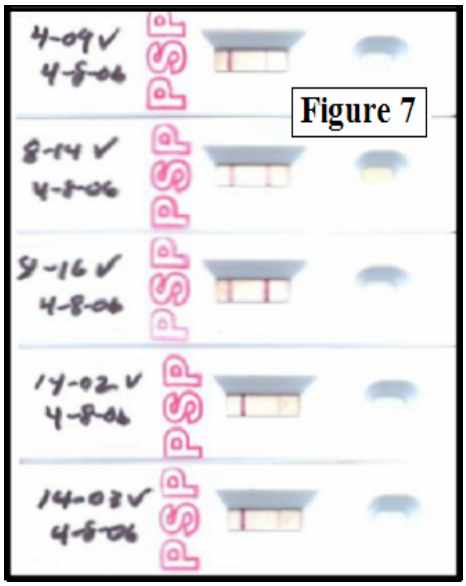


Figure 7

5.2 PSP - Jellett Rapid Test (JRT)

Samples were selected for testing with the JRT for PSP based on three initial factors: time of the year, location, and history of PSP in an area. Tests were spread out through the year and across the range of areas represented in an effort to observe temporal and spatial trends. Gonads were selected for testing more frequently than viscera. Upon preliminary analysis of results, additional tissues were selected to represent areas untested, to test toxicity over time and to test for consistency of test results within groups of samples.

Viscera and gonad tissue fractions were tested although not every sample had both tissue fractions tested. 10 gram tissue samples were extracted according to procedures established by Jellett Rapid Testing Ltd. using the 70% isopropyl alcohol - white vinegar rapid extraction method as described in their literature (Appendix # A.1).

Figure 8

After waiting 35 minutes scoring was determined by comparing colorimetric results visually (Figure 7) with the laminated PSP Color Line Interpretation Sheets provided by Jellett Rapid Testing Ltd. (Figure 8). Results were scored on an improvised 1 to 5 scale, with 1 being negative and 5 being positive, and recorded in an Excel spreadsheet. JRT cartridges were photocopied and cartridges and photocopies were stored for later verification. When colorimetric results did not match guidelines, additional tests were run. Most tests were photocopied in black and white with later tests scanned using a flat bed color scanner (Figure 7).

The JRT for PSP is set to detect PSP at 40µg / 100g STX equivalents (half of the federal standard) using the standard dilution. In some cases, when tests showed positive results, a further 1:2 dilution was done to determine if the viscera or gonads were positive at 80 µg / 100g following the JRT Ltd instructions (Appendix # A.4). In this case, 100 µl of filtered sample extract was added and mixed with 100 µl of filtered extract made from adductor muscle tissue. From this 200 µl mixture, 100 µl was then mixed with the standard 400 µl of the buffer solution, and then 100 µl of this diluted buffered shellfish extract was pipetted into the JRT cartridge well, and results recorded.

5.3 PSP - Receptor Binding Assay (RBA), NOS and FDA

Scallop tissues were extracted for PSP analysis using the standard AOAC procedure for regulatory testing. Extracted scallop tissues were analyzed using the RBA (See Appendix B). All tissues were sent to NOS, however not all samples were tested. Staffing and time constraints limited testing to representative areas and periods.

FDA RBA methods are similar to NOS RBA methods, however the FDA chose to utilize gonad, viscera and meat weights to calculate a “whole animal toxicity”. Although these results are outside the scope of this project, this information may become useful for later discussion with regulators. The FDA “red tide” closure in early June 2005 and subsequent request for samples from the new closure area provided a specific geographic area to concentrate our sample collection.. Representative samples were selected from vessels fishing for scallop meats in that area during the closure. A group of these samples was selected from a broader area near where positive tests had been found previously and to the north. Replicate samples were sent to both the FDA (for RBA) and Mass Division of Marine Fisheries for testing with the Mouse Bioassay.

5.4 PSP - Mouse Bioassay (MBA), Mass DMF

The Mouse Bioassay was conducted using the AOAC standard method (AOAC 1990).

5.5 PSP - High Performance Liquid Chromatography (HPLC), WHOI

Tissue extracts from selected samples were sent from NOS to WHOI for HPLC analysis to confirm this subset of RBA and JRT findings. Samples of both viscera and gonad found toxic in 2004 were initially selected for HPLC analysis.

5.6 ASP - Jellett Rapid Test (JRTA)

Tissue samples to be tested with the JRT ASP test kits were selected from Georges Bank where ASP was suspected to be present, with additional samples selected from Mid Atlantic areas where ASP was not known to be found. Georges Bank was of particular interest because domoic acid (the toxin that causes ASP) was identified in 23 whales found dead off the coast of New England (NOS 2003). In some cases both scallop viscera and gonad tissue from the same sample were tested, however for the majority either the viscera or gonad was tested alone.

The extraction method for ASP is the same as for PSP, but the buffering method uses a different ASP buffer and dilutions are different. Scoring was determined by matching results with the laminated colorimetric ASP Color Line Interpretation Sheet provided by Jellett Rapid Testing Ltd. This sheet shows four variations of results and results were scored using a 1 to 4 system, with 4 being a strong positive. (See Appendix A.3)

5.7 ASP – Receptor Binding Assay (RBAA) and LC-MS for Domoic Acid

Initially, ASP toxins were to be analyzed by NOS using the RBA, but early results were unreliable and NOS later substituted LC-MS (Liquid Chromatography - Mass Spectrometer) as their chosen method for ASP testing. Initial random samples from Georges Bank were selected, and later samples testing positive with the JRT for ASP were identified and tested. LC-MS testing was available on a limited basis (Methods are fully described in Appendix B)

5.8 R / M (roe to meat ratio) and GSI (gonadalsomatic index)

503 samples were dissected with meats, roes, and viscera weighed, and shell heights measured to establish time and place specific relationships. The gonadalsomatic index or GSI is commonly used to determine the reproductive state of shellfish, and consists of the weight of the roe as a fraction of whole animal soft tissue weight. While this ratio is useful to gauge overall reproductive status, we found it more useful to consider the ratio of roe to meat weight (R / M). This ratio gives a direct estimate of the increase in saleable poundage possible in a specific area at a given time of the year. As more data are collected, this dataset could be used to forecast both where and when harvest is optimal.

6. Results and discussion

The map plots below depict the spatial distribution of samples test results for all testing methods in this study. For each group of two, the first plots the values for the roe, the second, the values for the viscera. One complication is that the test for the first group, JRT, is unique in that the test is set to read positive at 40 ug/100g and so the visually estimated strip results judged as positive represent a value of 40. In the other quantitative test methods, RBA, MBA, and HPLC, values between 40 and the federal standard of 80 are represented by yellow markers. Overall there were relatively few values in the range of 40 to 80, the samples either did not register as toxic or were well above 80. With the exception of HPLC, there were 8 samples with roe values over 80.

6.1 PSP – Jellett Rapid Test (JRT)

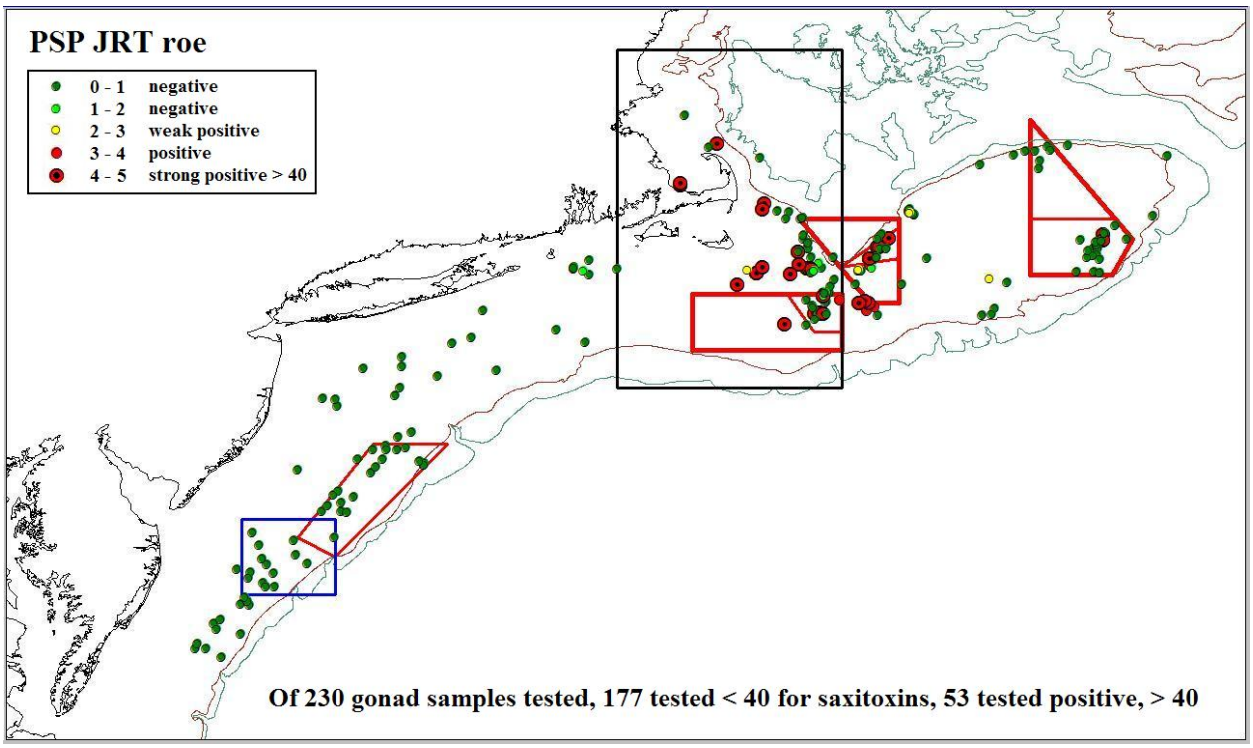


Figure 9. PSP JRT roe

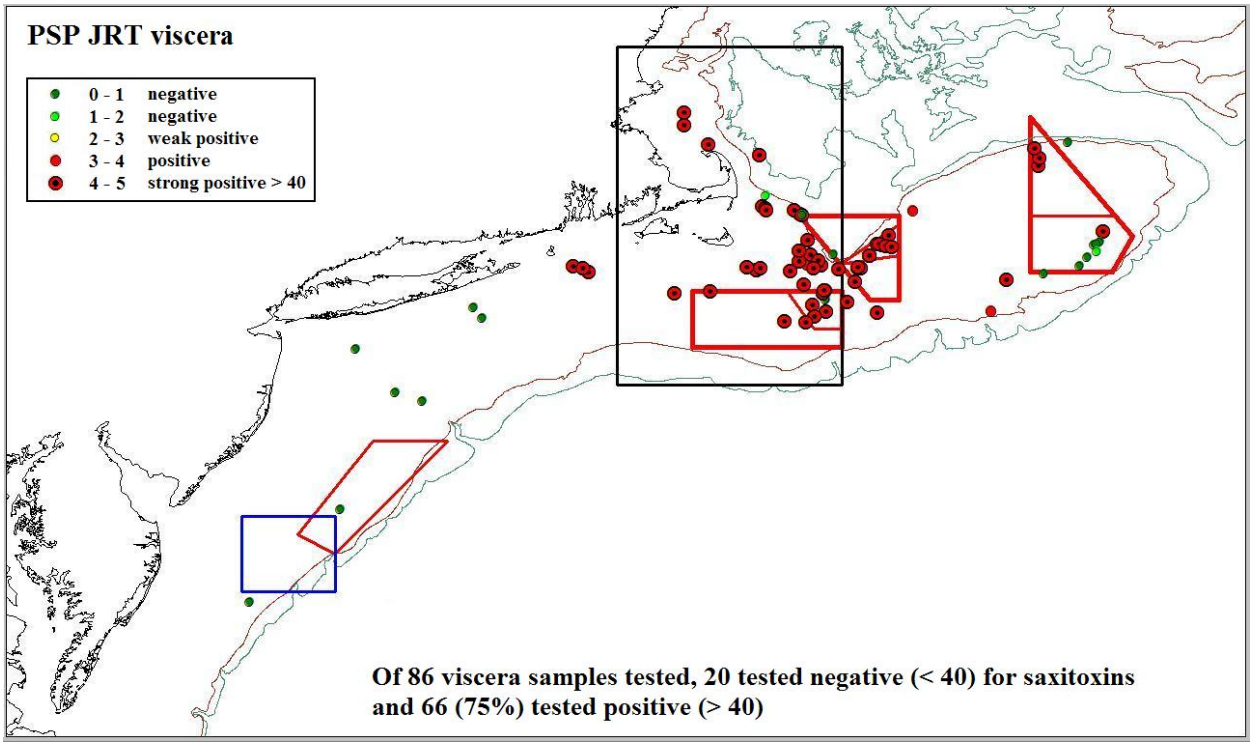


Figure 10. PSP JRT viscera

6.2 PSP - NOS Receptor Binding Assay – NOS RBA, CCEHBR

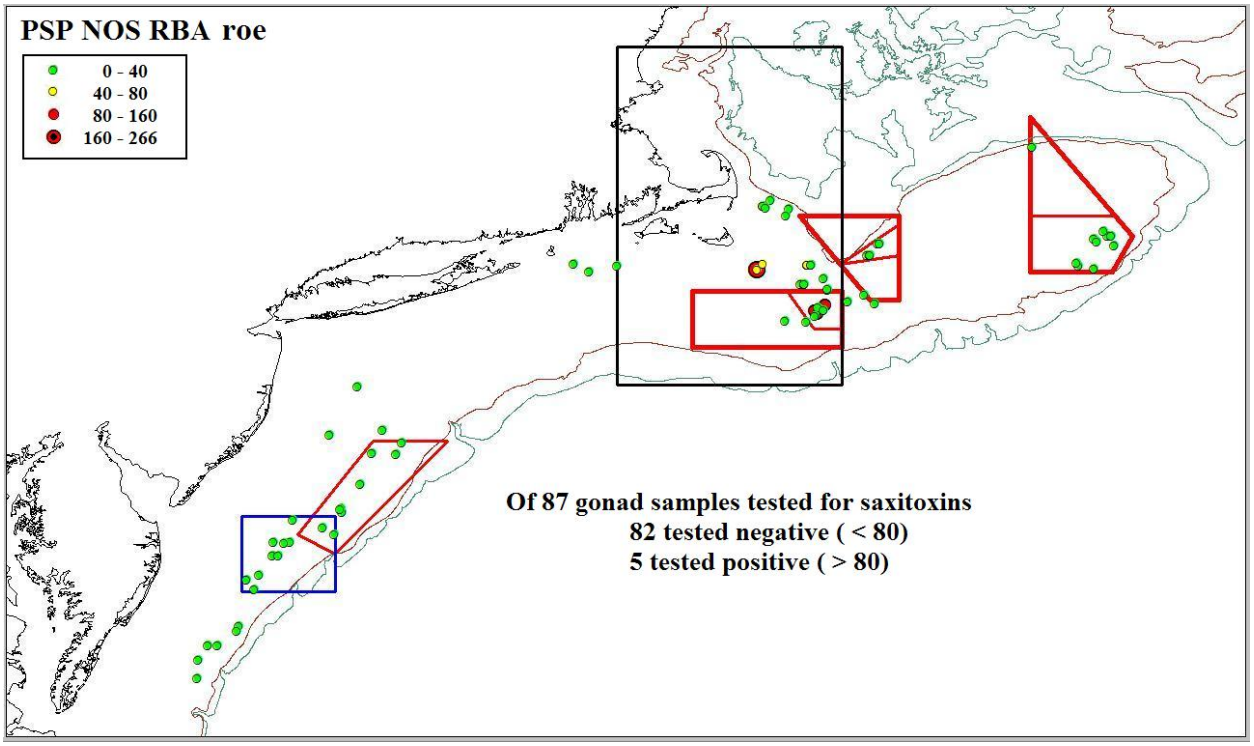


Figure 11. PSP NOS RBA roe

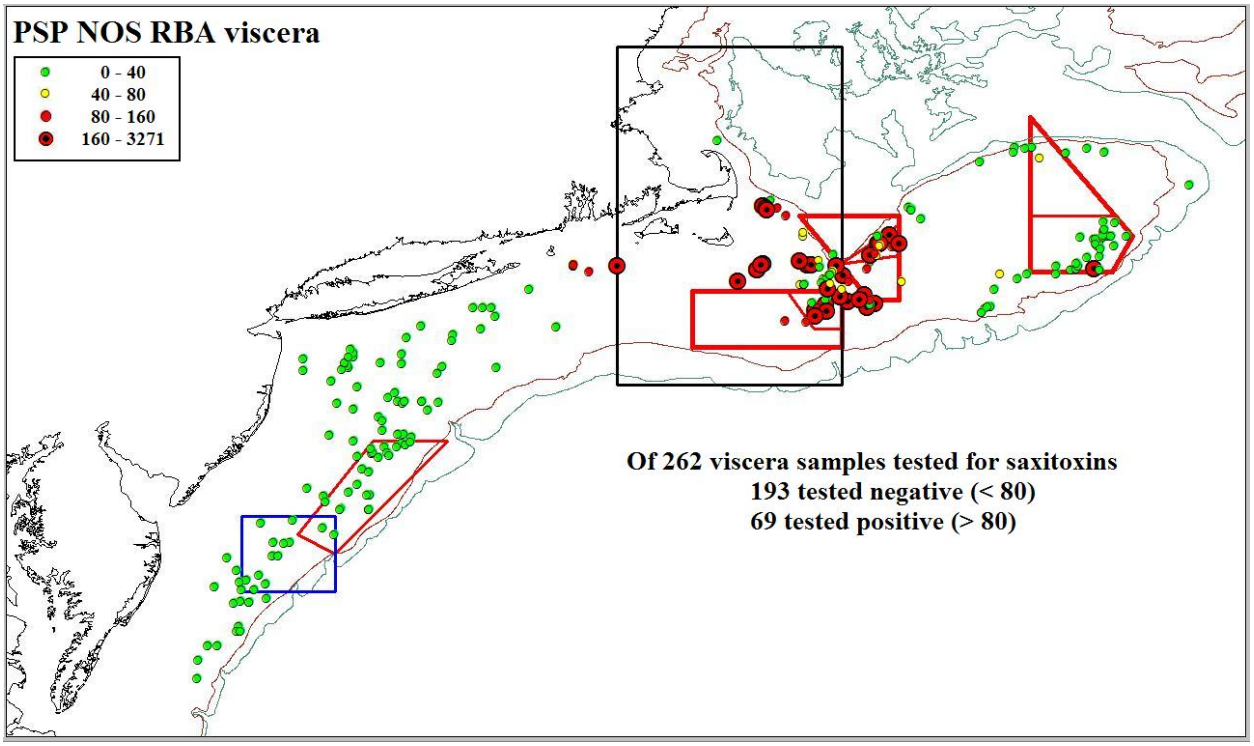


Figure 12. PSP NOS RBA viscera

6.3 PSP – FDA Receptor Binding Assay - FDA RBA

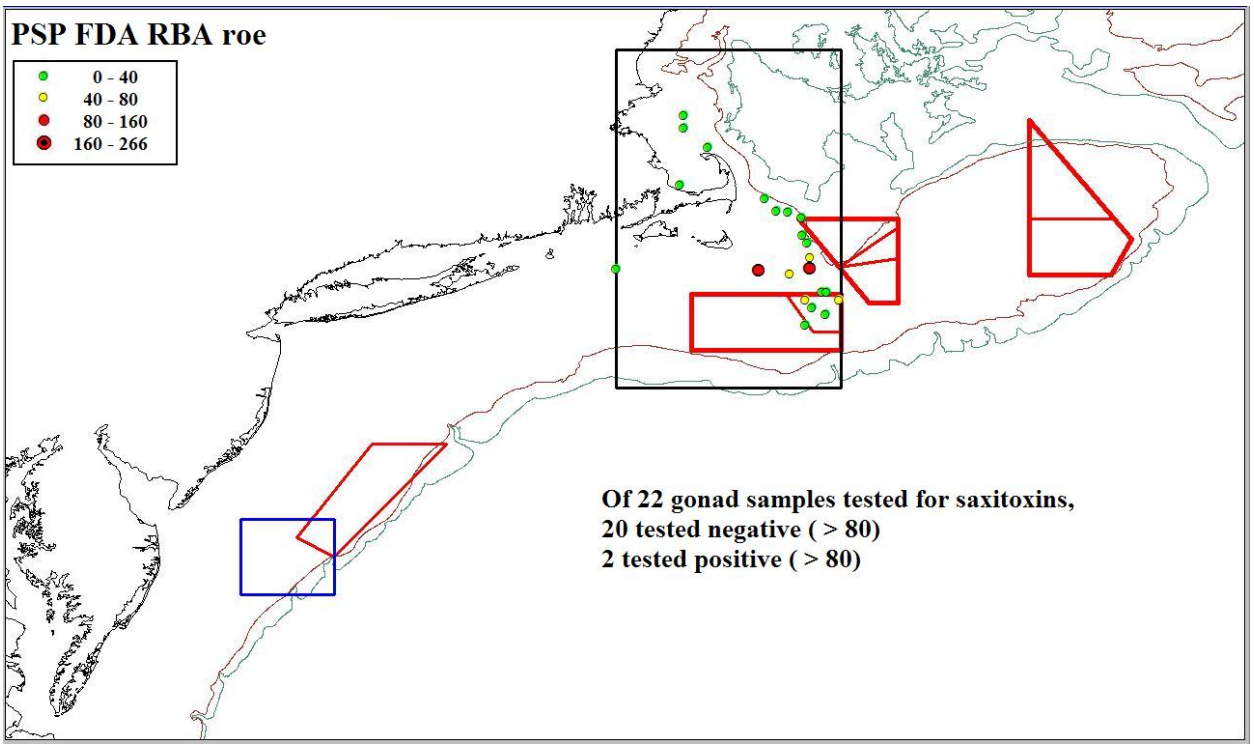


Figure 13. PSP FDA RBA, roe

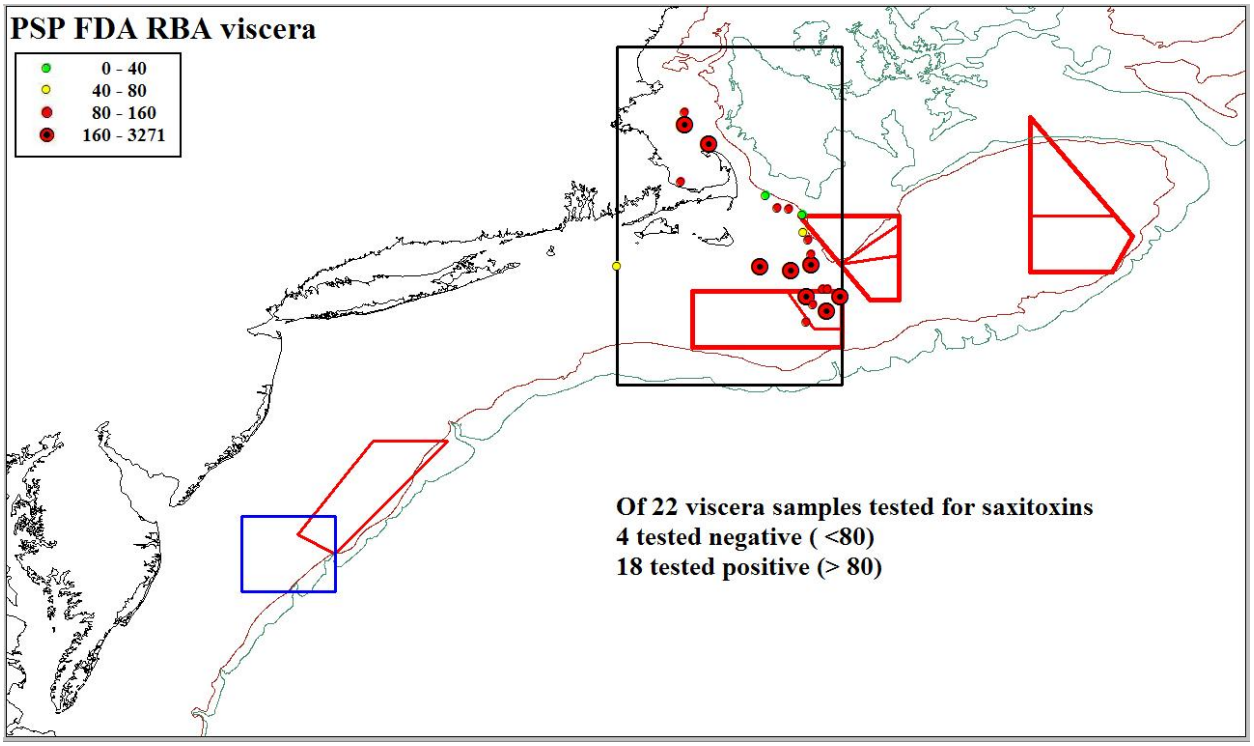


Figure 14. PSP FDA RBA, viscera

6.4 PSP – Massachusetts Division of Marine Fisheries Mouse Bioassay - DMF MBA

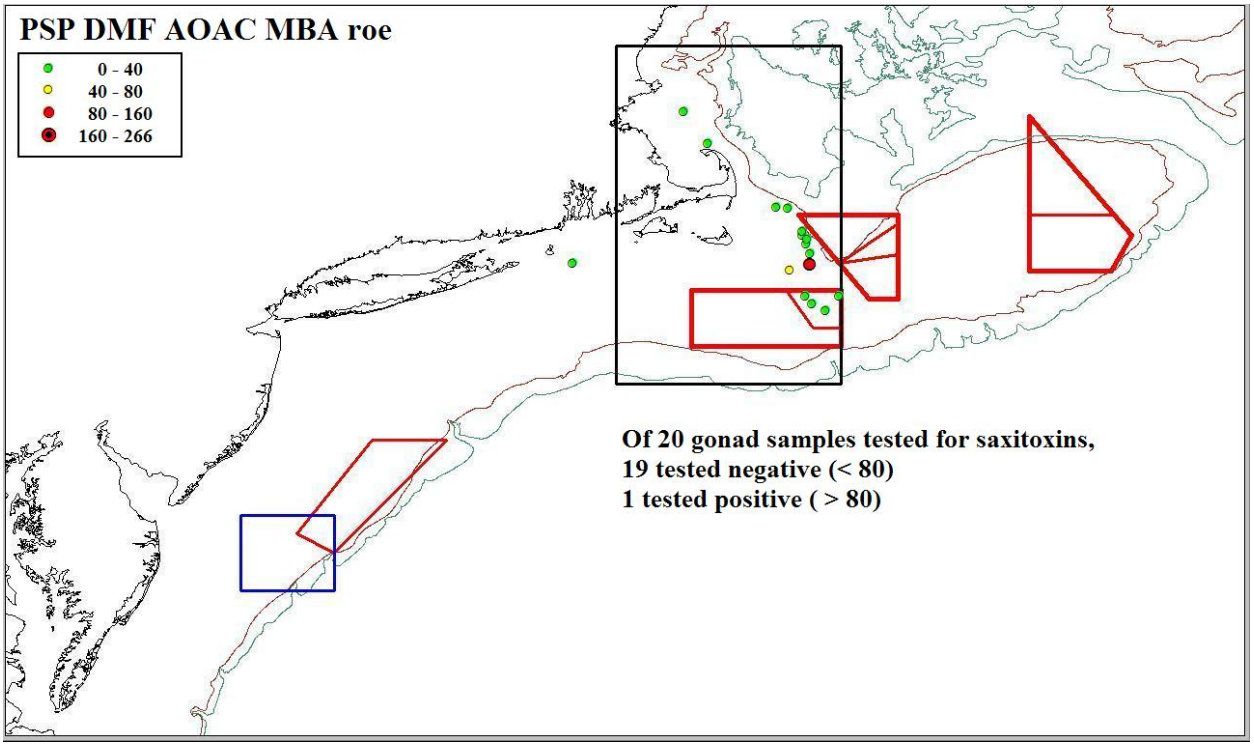


Figure 15. DMF MBA, roe

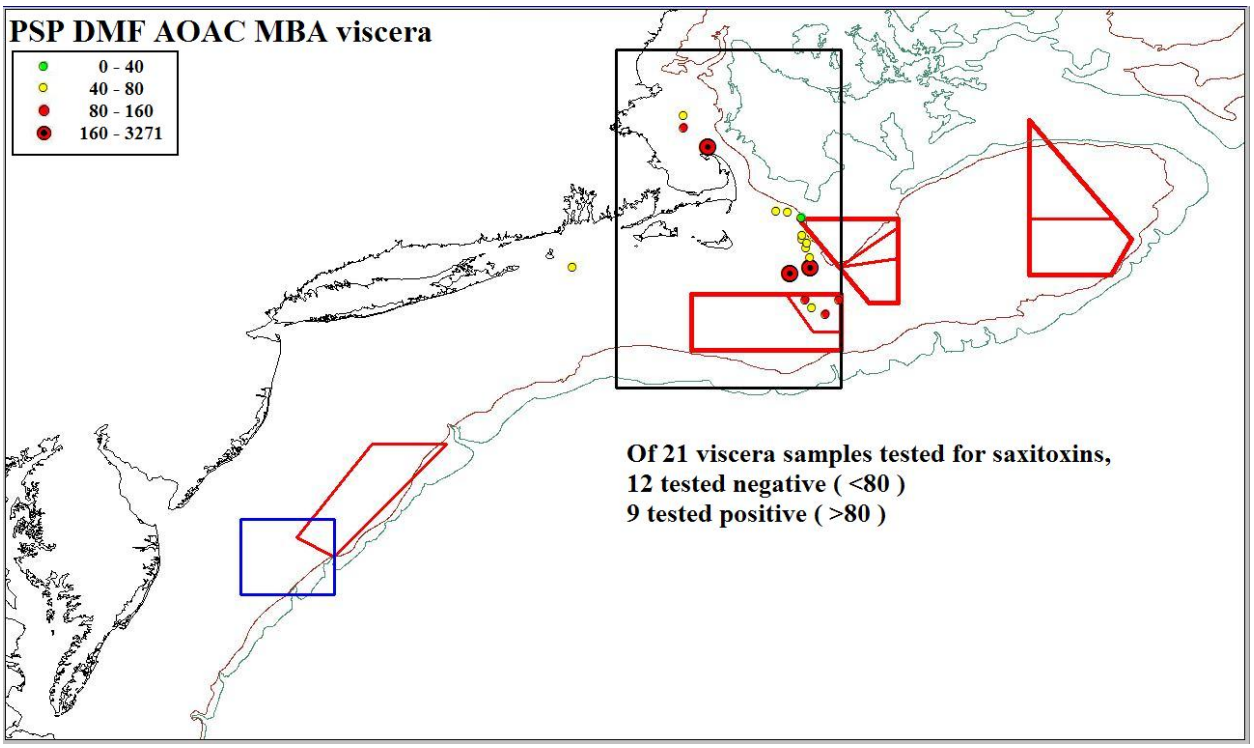


Figure 16. DMF MBA, viscera

6.5 PSP - High Performance Liquid Chromatography (HPLC), WHOI

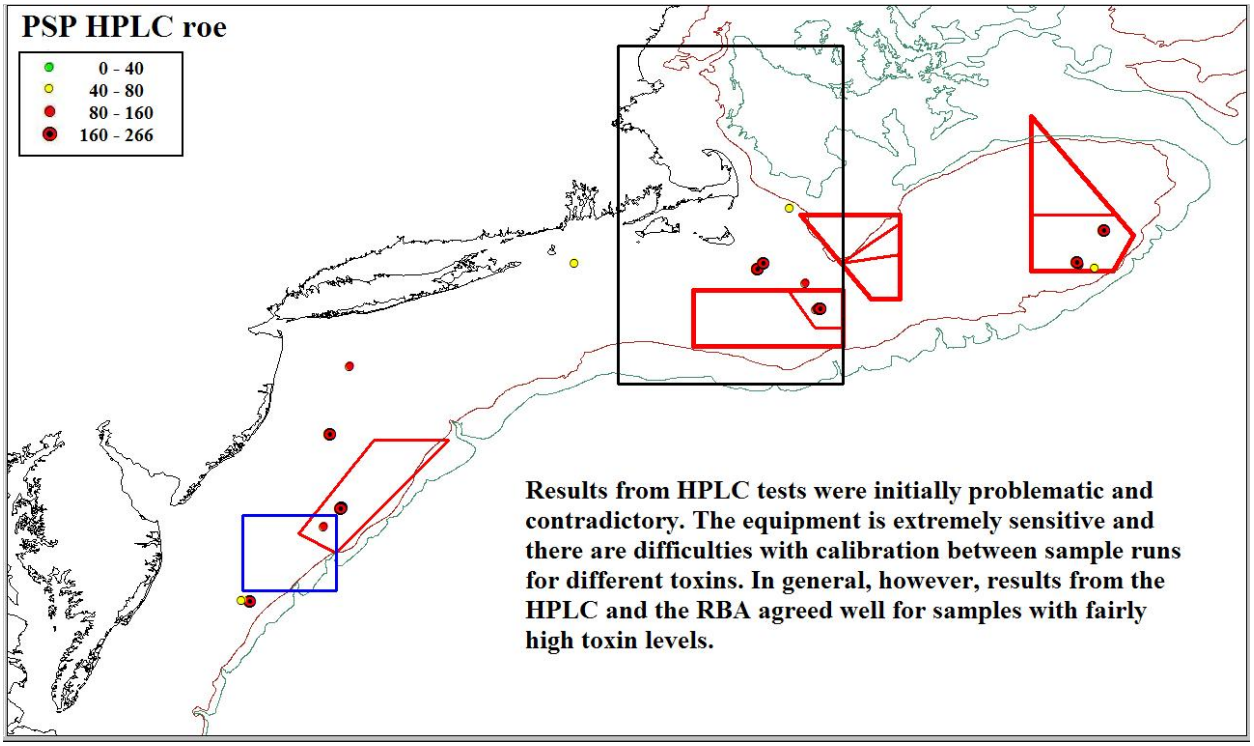


Figure 17. HPLC roe

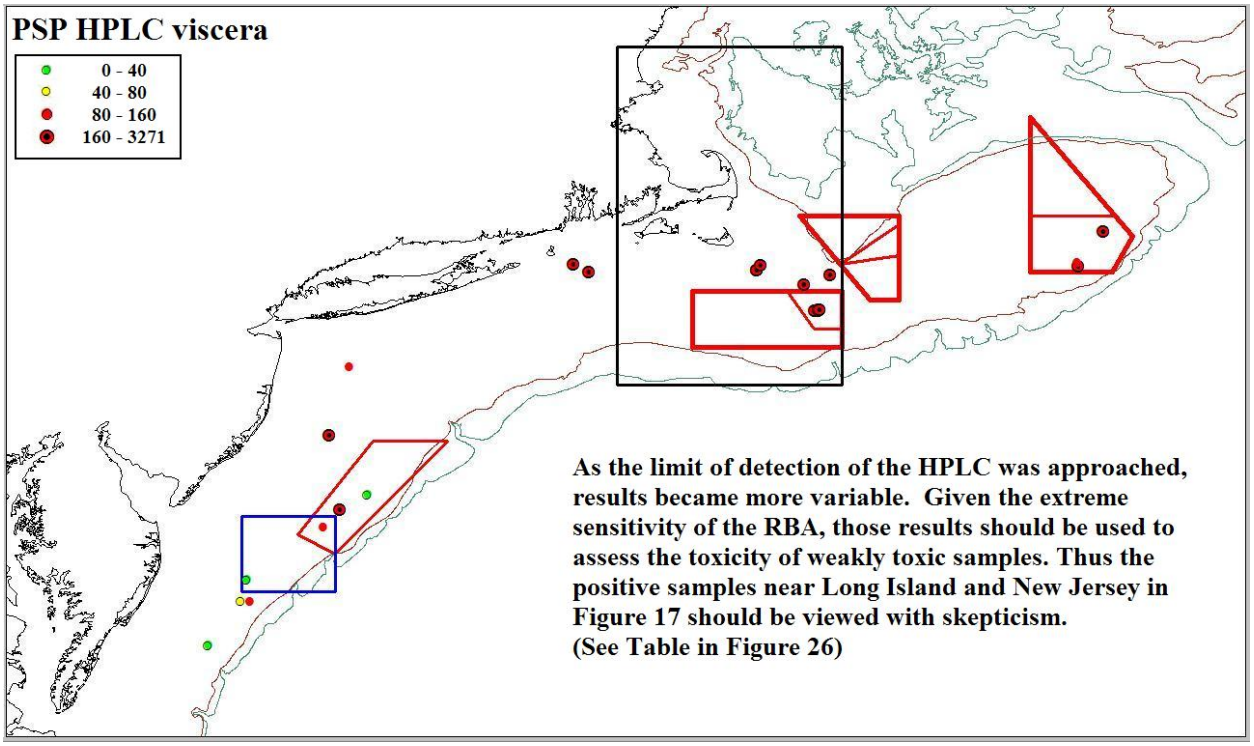


Figure 18. HPLC viscera

6.6 ASP – Jellett Rapid Test (JRTA)

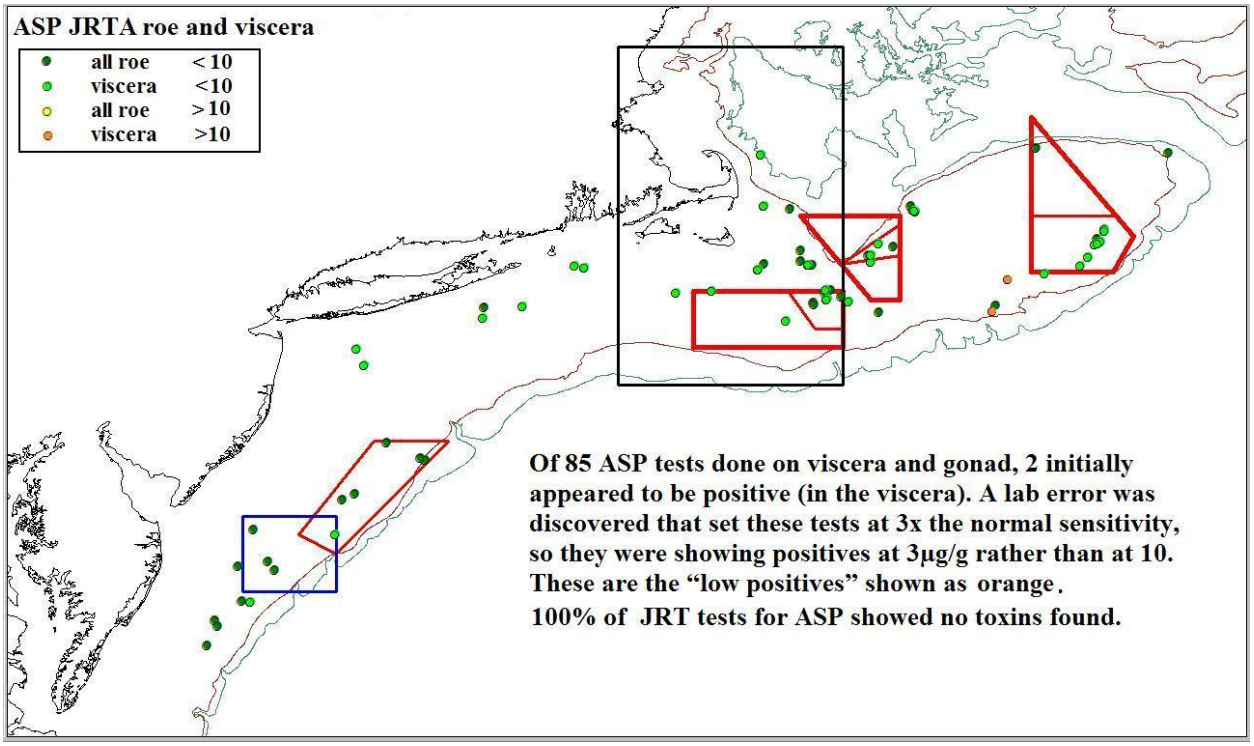


Figure 19. ASP JRT, roe and viscera

6.7 ASP – Liquid Chromatography – Mass Spectrometer (LC-MS)

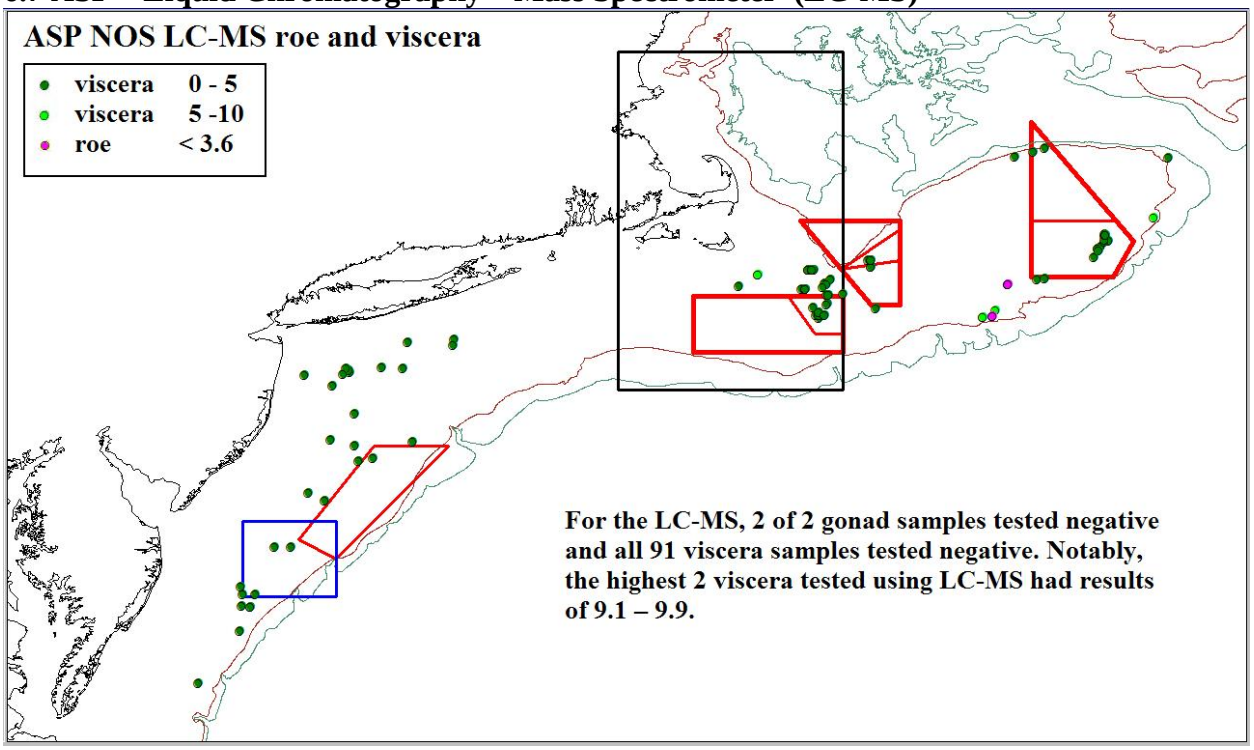


Figure 20. ASP LC-MS, roe and viscera

6.8 Roe to Meat ratio (R / M)

503 samples were dissected with meats, roes, and viscera weighed, and shell heights measured to establish time- and place-specific relationships. Condition of the roes varied markedly over the year from near zero (<1 g) to as high as 160% of meat weight. Notably absent is data between the months of August 2004 (Albatross scallop survey) and November 2004 when RSA awards were announced after the delay caused by the lawsuit regarding Framework 17.

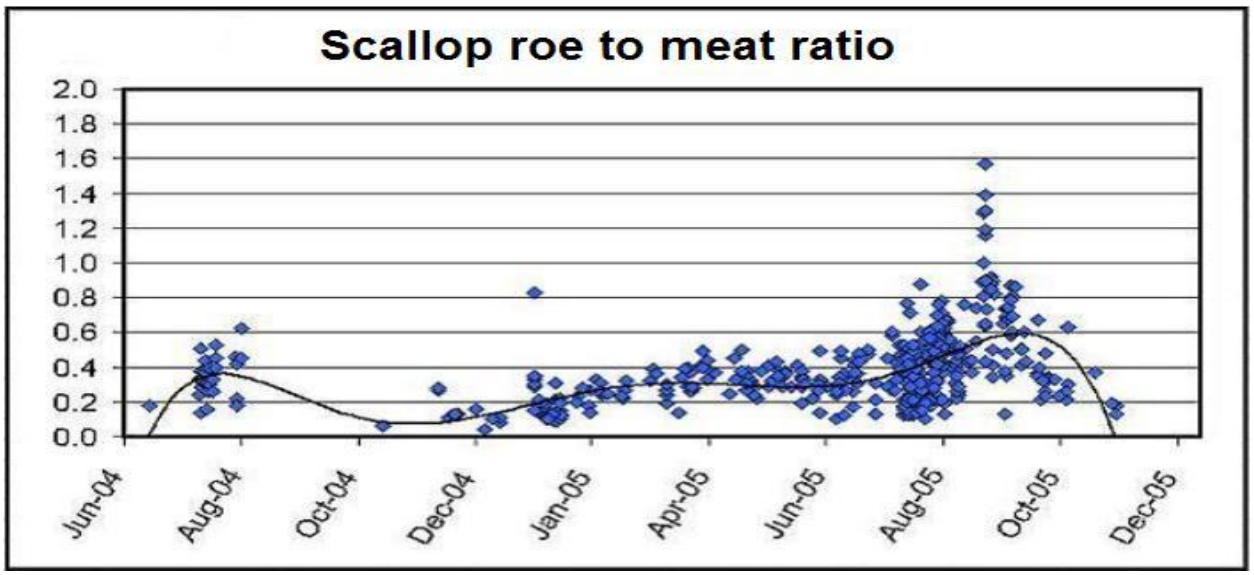


Figure 21.

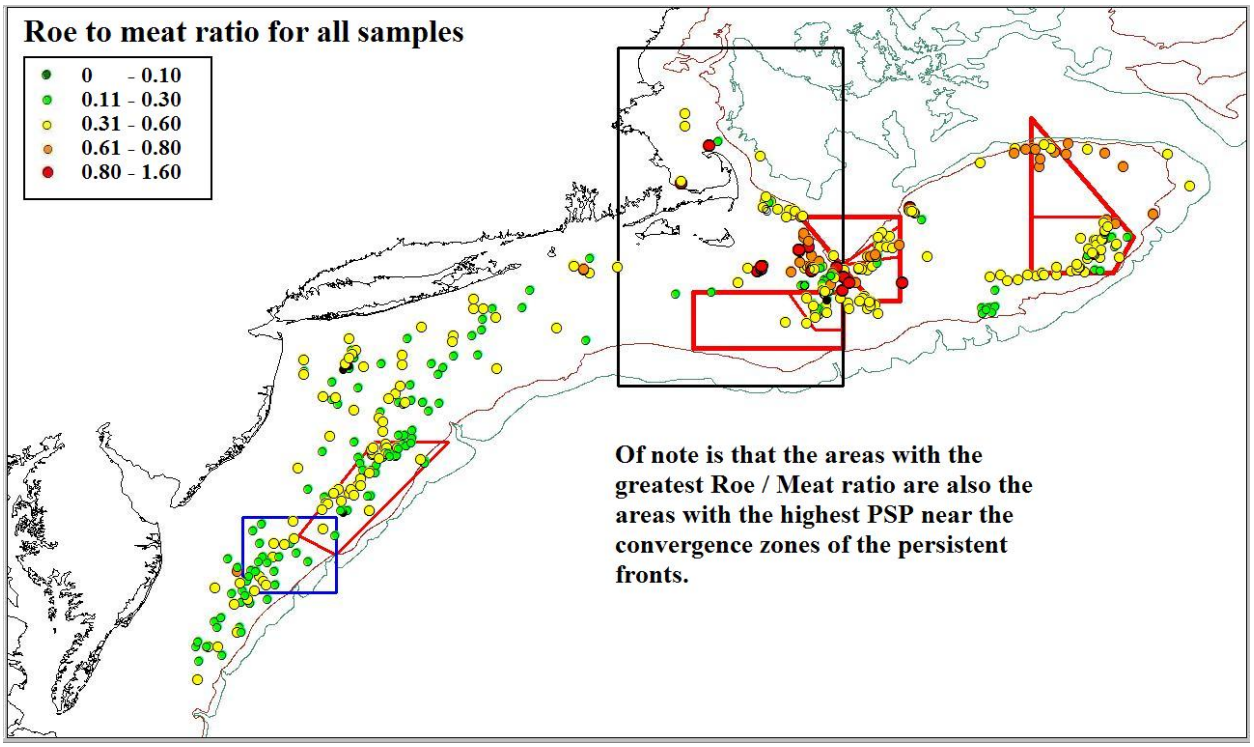


Figure 22. Spatial plot of all samples with the roe fraction

6.9 Summary Tables of results

Scallop RSA 008		Georges Bank - Eastern					G South Channel - Central					Mid-Atlantic - Western					TOTAL
	Samples in Area	112					199					180					491
PSP	Roe	total	>80	80-40	<40	%	total	>80	80-40	<40	%	total	>80	80-40	<40	%	
	NOS RBAPG	10	0	0	10	16%	50	5	3	42	22%	27	0	0	27	27%	87
	FDA RBAPG	0				0%	22	2	4	16	10%	0				0%	22
	DMF MBAPG	1	0	0	1	2%	19	1	4	14	8%	0	0	0	0	0%	20
	JRT_PG	46		4	42	75%	120		49	71	54%	64		0	64	65%	230
	HPLC_PG	4	3	1	0	7%	13	10	3	0	6%	8	6	1	1	8%	25
	Number of Tests	61	3	5	53	100%	224	18	63	143	100%	99	6	1	92	100%	384
PSP	Viscera																
	NOS RBAPV	56	3	5	48	74%	103	66	16	21	46%	103	0	0	103	85%	262
	FDA RBAPV	0				0%	22	18	2	2	10%	0				0%	22
	DMF MBAPV	1	0	1	0	1%	20	9	11	0	9%	0	0	0	0	0%	21
	JRT_PV	16		7	9	21%	63		59	4	28%	7		0	7	6%	86
	HPLC_PV	3	3		0	4%	14	14	0	0	6%	11	5	1	5	9%	28
	Number of Tests	76	6	13	57	100%	222	107	88	27	100%	121	5	1	115	100%	419
ASP	Roe		>20	20-10	<10	%		>20	20-10	<10	%		>20	20-10	<10	%	
	NOS RBAAG																
	NOS LCMSAG	11	0	0	11	55%	20	0	0	20	44%	5	0	0	5	25%	36
	JRT_AG	9		0	9	45%	25		0	25	56%	15		0	15	75%	49
	Number of Tests	20	0	0	20	100%	45	0	0	45	100%	20	0	0	20	100%	85
ASP	Viscera																
	NOS RBAAV	2	0	0	2	5%	1	0	0	1	2%	7	0	0	7	17%	10
	NOS LCMSAV	24	0	0	24	65%	37	0	0	37	64%	29	0	0	29	71%	90
	JRT_AV	11		4	7	30%	20		1	19	34%	5		0	5	12%	36
	Number of Tests	37	0	4	33	100%	58	0	1	57	100%	41	0	0	41	100%	136
TOTAL TESTS		194	9	22	163	4	549	125	152	272	4	281	11	2	268	4	1024

Figure 23. Test results and counts by method and area

Scallop RSA 008		Georges Bank - Eastern					Great South Channel - Central					Mid-Atlantic - Western					TOTAL
	Samples in Area	112					199					180					491
PSP	Roe	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Total
	NOS RBAPG	26.7	1.0	4.7	7.9	10	266	1.0	32.9	48.9	50	8.0	1.0	2.6	2.2	27	87
	FDA RBAPG					0	137	3.1	31.9	33.1	22					0	22
	DMF MBAPG	37.0	37.0	37.0		1	98	30.0	42.1	14.5	19					0	20
	JRT_PG	4.9	1.0	1.3	0.9	46	5	1.0	2.5	1.8	120	1.0	1.0	1.0	0.0	64	230
	HPLC_PG	250.4	44.8	156.8	86.8	4	608	48.4	204.2	158.4	13	318.9	0.0	165.1	113.1	8	25
	Average	79.8	21.0	50.0			223	16.7	62.7	51.3		109.3	0.7	56.2	38.4		
	Total Count					61					224					99	384
PSP	Viscera	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Total
	NOS RBAPV	305.7	1.0	23.6	44.2	56	3271	1.0	259.0	518.4	103	22.8	1.0	3.7	3.6	103	262
	FDA RBAPV					0	1199	28.7	213.8	252.0	22					0	22
	DMF MBAPV	66.0	66.0	66.0		1	915	46.0	150.4	208.3	20					0	21
	JRT_PV	4.9	1.0	2.6	1.8	16	5	1.0	4.6	0.9	63	1.0	1.0	1.0	0.0	7	86
	HPLC_PV	285.0	119.9	201.5	82.6	3	3053	176.8	979.8	1060	14	691.0	0.0	130.7	203.7	11	28
	Average	165.4	47.0	73.4			1689	50.7	321.5	408.0		238.3	0.7	45.1	69.1		
	Total Count					76					222					121	419
ASP	Roe	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Total
	NOS RBAAG																
	NOS LCMSAG	4.0	1.0	1.7	1.2	11	2.0	1.0	1.1	0.2	20	1.0	1.0	1.0	0.0	5	36
	JRT_AG	1.0	1.0	1.0	0.0	9	1.0	1.0	1.0	0.0	25	1.0	1.0	1.0	0.0	15	49
	Average	2.5	1.0	1.4	0.6	10	1.5	1.0			23	1.0	1.0			10	
	Total Count					20					45					20	85
ASP	Viscera	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Max	Min	Mean	Stdev	Count	Total
	NOS RBAAV	1.2	1.1	1.2	0.1	2	0.4	0.4	0.4		1	1.1	0.3	0.6	0.3	7	10
	NOS LCMSAV	9.9	0.1	2.5	2.9	24	6.0	0.0	0.6	1.2	37	2.0	0.1	0.4	0.4	29	90
	JRT_AV	4.0	1.0	1.7	1.2	11	2.0	1.0	1.1	0.2	20	1.0	1.0	1.0	0.0	5	36
	Average	5.0	0.7	1.8	1.4	12	2.8	0.5			19	1.4	0.5			14	
	Total Count					37					58					41	136
TOTAL TESTS						194					549					281	1024

Figure 24. Summary statistics by method and area

Figure 25. Comparison of representative results using different testing methods

SAMPLE	DATE	R/M	RBAPG	RBAPV	MBAG	MBAV	FDAPG	FDAPV	HPLC_G	HPLC_V	JRTP-G5	JRTP-V5
840008	07/14/05	0.77	266	3271					608	2790	4.8	4.8
840010	07/16/05	0.71	197	2916					291	2504	4.8	4.7
840017	07/21/05	0.88	54	2763					164	3053	4.8	
812007	09/12/05	0.51	61.4	1039					426		5	
814001	01/01/05	0.83		702.3						1794		
813014	09/20/05	0.67		648.1							4.9	4.9
A04282	07/19/04	0.45	136.6	510.5					143	638		
840011	07/15/05	0.47	14	350.8							4.9	4.9
830030	06/17/05	0.37		220.1							4.8	5
830031	06/18/05	0.31	13	202.9							4.9	5
808005	08/14/05	0.51	1	196.3			6.4	145.7			1	
808007	08/08/05	0.31	1	181.5			3.1	46.8			1	
860014	07/26/05	0.43		179.8	40	114	29.8	207.9			1	4.1
860006	07/26/05	0.47		168.0	40	150	45.8	248.5			4	
808002	08/11/05	0.48	11	159.1							4.9	5
808004	08/11/05	0.50	4	159.1			17.9	149			1	4.9
830020	06/10/05	0.49	68	139.8							4	4
860009	08/04/05	0.56		120.0	37	59	34.6	99.5			1	
840005	07/10/05	0.53	12	119.5							4.8	4.8
800302	01/03/05	0.14	30.1	115.7					239	352	4.9	
800314	01/05/05	0.14	30.7	114.8					183	306	4.9	
800223	01/06/05	0.17	25.9	110.4					75	196	4.9	
830009	07/06/05	0.31	4	96						513	1	5
800247	01/04/05	0.15	14.8	90					105	263	4.9	
800058	12/01/04	0.16	26.7	83.1					190	200	4.9	4.9
860002	08/05/05	0.40	1	66.4	40	47			74	451	1	
800262	01/05/05	0.15	21.9	65.1					164	353	4.9	
860010	08/04/05	0.66		61.4	38	53					1	
860011	08/04/05	0.67		42.3	38	46	8.4	76.9			1	
816004	10/01/05	0.23		30.7						177		
820040	05/20/05	0.29	1	10.7					263		1	
A04164	07/14/04	0.31	5.7	7.9					131	146		
810034	01/30/05	0.14		7.9					135	329		
800014	11/12/04	0.27		4.4					232	128	1	1
815011	09/24/05	0.48	1	4					142	285		
810013	02/04/05	0.31	1	3.5					236	691		
800045	11/22/04	0.13		3.4					58	76	1	
830026	06/08/05	0.25	1	3.4					319	245		1
816022	09/23/05	0.32	5	3.4					250	120		
810021	02/17/05	0.27		3.1					81	148		
860001	08/01/05	0.78			98	915	137	1199			4.9	
860008	07/26/05	0.46			39	89	47.4	259.6			1	
860012	08/04/05	0.57	8.1		38	53	12.2	83	48		1	
860015	07/26/05	0.45			39	50	27.1	83.7			1	4.8
860016	07/27/05	0.54			30	128					1	5
860017	07/26/05	0.61			38	60	44.7	98.4			1	4.8
811001	09/06/05	0.59				135	10.8	347.6				5
811002	09/06/05	0.58			38	75	7.4	127.5			1	5
811003	09/06/05	0.87			38	222	12.8	231.2			1	5
811005	09/04/05	0.48				38	8.4	35.7			1	5
811007	09/04/05	0.73					99.4	439				
811009	09/04/05	0.74			58	511	67.2	469.7			5	5
811010	09/04/05	0.69			37	66	23.5	89.4			1	5

Figure 26. HPLC results table

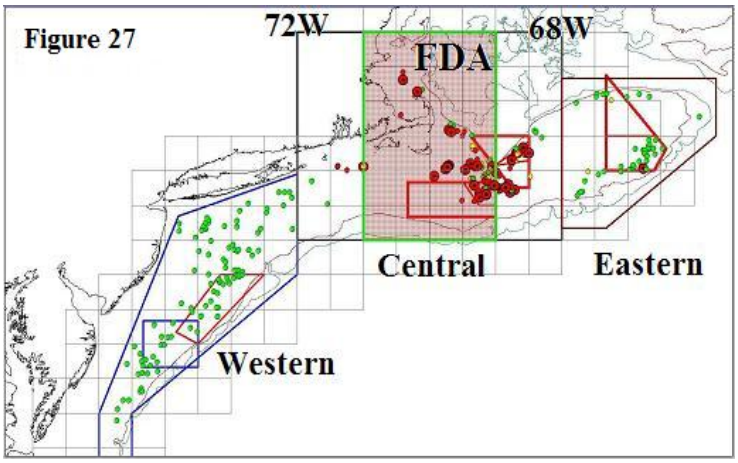
SAMPLE	DATE	AREA	RBAPG	RBAPV	JRTP-G5	JRTP-V5	JRTP-G5-80	JRTP-V5-80	HPLC_G	HPLC_V	G_Notes	V_Notes
860012	20050804	Central	8	-	1	-	-	-	48	-	MLNT	
840008	20050714	Central	266	3271	4.8	4.8	4.9	4.9	608	2790		
840010	20050716	Central	197	2916	4.8	4.7	4.9	-	291	2504		
840017	20050721	Central	54	2763	4.8	-	4.9	-	164	3053		
812007	20050912	Central	61	1039	5	-	-	-	426	-		
814001	20050000	Central	-	702	-	-	-	-	-	1794		
ALBA04_282	20040719	Central	137	511	-	-	-	-	143	638		
810002B	20050728	Eastern	4	306	1	-	-	-	45	-	MLNT	
800302	20050103	Central	30	116	4.9	-	-	-	239	352		JFP
800314	20050105	Central	31	115	4.9	-	-	-	183	306		JFP
800223	20050106	Central	26	110	4.9	-	-	-	75	196		JFP
830009	20050706	Central	4	96	1	5	-	-	-	513		
800247	20050104	Central	15	90	4.9	-	-	-	105	263		JFP
800058	20041201	Eastern	27	83	4.9	4.9	-	-	190	200		JFP
860002	20050805	Central	1	66	1	-	-	-	74	451	MLNT	
800262	20050105	Central	22	65	4.9	-	-	-	164	353		JFP
816004	20051001	Central	-	31	-	-	-	-	-	177		
820040	20050520	Western	1	11	1	-	-	-	263	-		
810034	20050130	Central	-	8	-	-	-	-	135	329		
ALBA04_164	20040714	Western	6	8	-	-	-	-	131	146	MLNT	
ALBA04_088	20040710	Western	7	8	-	-	-	-	-	0		
ALBA04_178	20040714	Western	-	5	-	-	-	-	-	1		
ALBA04_127	20040712	Western	1	5	-	-	-	-	-	1		
ALBA04_150	20040713	Western	8	5	-	-	-	-	0	-		
800014	20041112	Western	-	4	1	-	-	-	232	128		
815011	20050924	Eastern	1	4	-	-	-	-	142	285		MLNT
ALBA04_229	20040716	Western	1	4	-	-	-	-	-	0		
ALBA04_091	20040711	Western	3	4	-	-	-	-	-	2		
810013	20050204	Western	1	4	-	-	-	-	236	691	MLNT	
800045	20041122	Western	-	3	1	-	-	-	58	76		
816022	20050923	Eastern	5	3	-	-	-	-	250	120	MLNT	
830026	20050608	Western	1	3		-	-	-	319	245	MLNT	
810021	20050217	Western	-	3	-	-	-	-	81	148		
MLNT = Most Likely Non Toxic.			JFP = Jellet False Positive - JRTG Showed Positive but RBAG Showed Negative									

6.10 Discussion of results

The overarching goal of this project was to determine if roe-on scallops could be harvested and marketed without risk to public health. Our findings are that in 2004 and 2005, roes collected by the 37 vessels involved in this project were toxin free for almost all samples tested across the range of the scallop resource. The few roe samples testing positive for PSP with quantitative methods were confined to a single area, approximately a 1° square on the southernmost part of Nantucket Shoals. The implication is that safe commercial harvest of roes would have been possible in well over 80% of the most productive Atlantic sea scallop resource areas, even though 2005 experienced one of the largest red tides in New England history. In the area south of Nantucket Shoals, toxins above the federal action levels were found in both roe and viscera during 2004, a year when no toxic bloom was observed. In 2005 high levels of toxins were found in the viscera over a much broader area (Figure 12), however in the roe levels above the federal action standard of 80, were confined to a 1° square near the original site (Figure 11).

Initial analysis of PSP test results identified three distinct areas: Western, Central and Eastern. The Western area extends from 71.5ºW near Block Island to the west, and includes the area south of Long Island and the Mid-Atlantic as far south as the DelMarVa Peninsula. The Central area spans from 71.5ºW to 68.5ºW near Cultivator Shoals, 30 minutes longitude east of the FDA closure area (green rectangle Figure 27). The Eastern area extends east from 68.5ºW across the US portion of Georges Bank to the US - Canada boundary. The Western section is already open for whole molluscan shellfish and our data confirms no ASP or PSP toxins were found there approaching federal action levels. By definition, an open status infers that shellfish may be taken without testing, or risk to public health.

The Central area, a large portion of which is closed to the harvest of roe-on scallops, contained a few samples with PSP toxin levels in the viscera forty times federal standards and three times federal standards in the roe. The toxic roe samples were localized to a smaller area of about 2,700 nautical miles^2, about 25% of the Emergency Closure area of 2005. No ASP toxins of any significance were found in the Central area. The Eastern section had three viscera samples with results above 80, however all roes tested with quantitative methods were below 40. When testing for ASP, the highest two samples from Georges Bank tested just under 50% of the federal action levels.

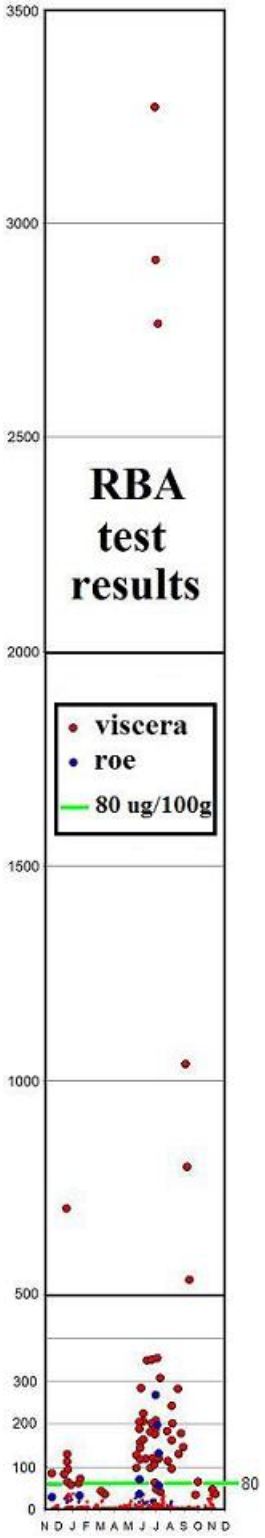


As a preliminary investigation, no single area was sampled systematically throughout the entire year. The use of vessels of opportunity did not allow a preplanned more comprehensive sampling strategy. However this project did produce data over a wide area over for much of the year and allows some insights not otherwise available. The simple conclusion is that while marine biotoxins are a valid reason to control the harvest of whole molluscan shellfish, there are large portions of the scallop grounds that could have supported a significant harvest during 2004 and 2005. The biggest surprise was not that there were toxins to be found in the roe, but that when we did find them, they were localized to a 2,700 square mile area.

A secondary goal was to learn if new biotoxin testing methods were practical, and to cross-test tissues using multiple methods to determine if newer methods, the Jellett Rapid Test and RBA, correlated with the ISSC accepted MBA test.

Examination of both data and plots reveals that overall the JRT had many more positive samples over a larger area indicating toxins were present than the other, more quantitative methods, such as the RBA. This is logical, however, since the JRT will indicate a level as low as 40 µg/100g, whereas the closure level is twice this value. Using the JRT alone, and targeting animals identified as negative by the JRT could have provided reasonably safe harvest in the Western and Eastern areas, and clearly identified the Central area as toxic. 100% of all tests by all methods (excluding the HPLC which was near its detection limits) confirmed there were no toxins found above or near federal levels in either PSP or ASP in the western area. This not only confirms the animals are below 50% of the federal standard, it also initially confirms that the JRT negative result can identify areas where harvest can be expected to be safe using any testing method.

The AOAC MBA results provided by Massachusetts DMF and two other experimental methods, the NOS RBA and the FDA RBA, provided a consistent picture of the spatial extent of toxicity over all areas sampled. In the cases where we tested the same tissue with multiple testing methods, the consistency of results was generally confirmed. (See table 25). Infrequently discrepancies occurred when toxicities were close to the detection limits of the particular testing methodology.



The figure to the left illustrates an important aspect of the project data. The great majority of test results fell toward the bottom of the scale, with only a few test results above 400 STX. While the toxins were found in few samples, the strength of toxicity indicates the public health risk is great.

6.11 Comparison of results

A secondary overarching goal was to evaluate two new technologies used to test for the presence or absence of the two major biotoxins found in the region. Our comparison of multiple testing methods, although performed on a small number of samples, is a preliminary investigation, and may be expanded upon in Phase II of this study.

Our findings confirm that toxicity results qualitatively agree across multiple testing methods. Notably, both experimental method results largely agreed with AOAC Mouse Bioassay results, with both experimental methods proving more sensitive in detecting lower levels of toxicity than the Mouse Bioassay. Further, we found that the JRT could be deployed on land in a commercial shellfish operation and operated by non-laboratory personnel to produce results similar to the standard Mouse Bioassay.

In all cases, the JRT PSP test correctly identified toxic viscera and roe above and below 40 ug / 100g STX when compared to the AOAC MBA. Where results differed widely, the JRT erred on the side of safety by signaling that toxins were present where the MBA indicated there were no toxins present above detection limits of approximately 39 µg / 100 g STX equivalents.

While useful in the sense of having few failures or false negative results, the Jellett Rapid Test strips proved to be significantly more sensitive than advertised when used for gonad tissue testing. Consistently positive test strip results were found in the range of 12 to 40 STX equivalents when gonad tissue was tested by both the JRT and the quantitative RBA. (See Utility of Jellett Rapid Test, Section 7.2 below). Despite the extra margin of safety provided by the JRT showing positive at 40, and the tendency of the JRT strip to produce a high rate of "false positives" for roe tissue, the areas represented by red in the map plots (figure 9 and 10), though more widespread, are not radically different from those mapped using the quantitative RBA and MBA tests.

When comparing results from the FDA RBA and the ISSC approved MBA, there was general agreement when testing the roe, but not when testing viscera. All 13 roe tests compared favorably when tested by both methods. One sample tested over 80µg/100g STX, the remaining 12 samples were negative. Viscera test results showed 8 out of 15 tests agreed, while 7 disagreed widely. Future efforts will explore this issue in greater depth as the second phase of this project has funding dedicated to MA DMF for the specific purpose of testing tissues with the MBA.

JRT ASP tests were compared with the LC – MS test results provided by the NOS project partner lab. All test results from NOS indicated extremely low concentrations of toxins, as did the JRT ASP tests. We initially concluded the ASP JRT tests produced results comparable the NOS results, but this is not a fair evaluation as there were no positives reported by either method. Additionally, in part because of the uniformly negative initial results, and in part because of the immediacy of the major *Alexandrium* bloom we chose to concentrate our efforts on PSP testing.

As to be expected when starting off with a new technology, there were numerous issues to resolve. These included invalid test strips, incorrect lab methods, and a buffer that had not been well calibrated specifically to the scallop roe tissues that we were testing.

While not an explicit initial goal, a new approach to understanding the relationship between toxicity in different tissue compartments appears to offer promise towards increasing the utility of existing and experimental tests. By analyzing the ratio of toxicity between 2 tissue fractions, it may become possible to project the toxicity in one tissue from the toxicity measured in the other. (Section 7.2)

7. Conclusions

Despite the largest New England red tide in many years, only 4 roes of the 57 tested by quantitative methods were found to be above the federal standard of 80µg/100grams tissue within the study area in 2005. All of these samples with high results were in the Great South Channel, Nantucket Shoals area. Roes from animals on Georges Bank were below the federal standard as tested by all methods. All sample groups west and south of Block Island were well below the federal standards for marine biotoxins using all testing methods. While it is clear that a short term study cannot be considered definitive, these test results give us a clear indication that the presence of toxins in scallop roe is not as widespread as previously thought. The fact that this can be stated for a year with such a massive red tide event lends greater credence to the observation.

Broadscale findings of our project include:

1) The area affected by the 2005 bloom (Figure 3) went considerably to the east of the FDA/DMF/NMFS Closure Area, spanning Closed Area 1, into the existing Georges Bank closure (east of 69W) in effect since 1989. The viscera in a few samples west of the Closure Area near Block Island, tested positive. Examination of the data from this project reveals that the eastern U.S. portion of Georges Bank was not seriously impacted.

2) While low levels (<10µg/g) ASP toxins were found in some scallop viscera, no ASP toxicity above federal regulatory action levels was found throughout the study area by any testing method.

3) General category vessels working in the Great South Channel provided the majority of toxic samples. These vessels were not originally planned as project participants nor were they funded as such.

4) The Jellett Rapid Test strips show good agreement with standard testing methods (MBA), but there is room for improvement with respect to false positives in gonad tissue when compared with the quantitative results of RBA and MBA. We are working with Jellett Rapid Test Ltd to test a new scallop roe-specific buffer solution.

5) Initial examination of the data suggests that, at least for this time period, if there were to be selective "openings" for roe scallop harvest, they follow different lines than currently in place (DMF, 2005, Figure 4). Oceanographic features (Bisagni, Mavor, 1996) show a correlation between PSP toxicity in roes and viscera in the area around the Great South Channel. With these features in mind a central closure area extending east at least to 68.5º W rather than 69º W is indicated. This closure may need to be extended west as well, at least to Block Island, and possibly as far west as 72º W longitude.

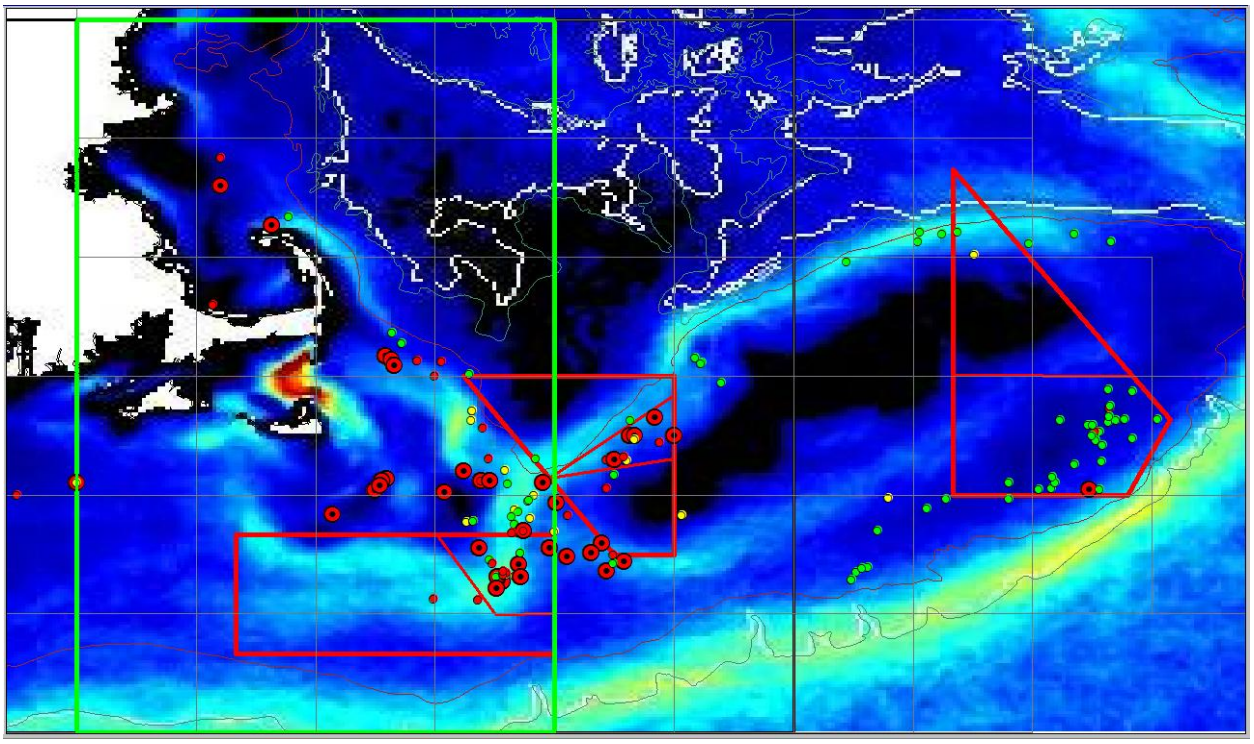


Figure 29. PSP results and persistent fronts in the G. South Channel (Bisagni, Mavor GLOBEC)

Coincident to this study industry and regulators began working together to develop protocols to allow for safe harvest of roe-on and whole scallops as surf clams and ocean quahogs from areas closed for PSP (Appendix E or see URLs in Reference Section 10).

7.1 Lessons learned and need for further work

The primary goal of this effort was to collect data from vessels while in the course of their normal activity during their fishing year. A short 15 month project is a snapshot and cannot determine the safety of scallop roe over time, though the ongoing second RSA-sponsored sampling effort will help us begin to understand year-to-year issues.

The largest challenge was in streamlining the collection, storage, initial processing, and JRT testing of this many samples in order the speed up the turnaround time. The learning curve and developing familiarity with new testing methods are seen as one time startup issues and have improved over time.

Several other areas needing improvement are noted:

1) The amount of time taken from sample collection to producing final data is long. Producing the replicate samples only starts the chain of testing with the project partners, with the parallel tests using MBA and HPLC occurring only on those samples testing positive using JRT and/or RBA (with the exception of a few baseline known negative samples for calibration).

2) The difficulty obtaining samples from specific areas (whether closed or open) over time in order to produce a time series. This issue has two aspects: the first is the concept of "samples of opportunity", that is samples were to be obtained from vessels going about their normal fishing activities. Although we can see the utility of revisits over time, we had no direct control of sampling locations with the exception of a very few fortuitous tows made while transiting. Vessels have little incentive to travel outside of their economic "hot spots" as determined by resource conditions in the open areas, timing and availability of the rotational management areas, weather, etc.

3) Additional delay and resource issues result from the backups that come when staff at collaborating partner labs are already at capacity and suddenly are newly tasked with performing additional work due to the "the largest red tide outbreak in 30 years".

4) We need the services of a knowledgeable contact in the E.U. and to that end MC Monfort has been contracted and will research the size and scope of the roe-on market in France in detail in Phase 2 of this project, funded by scallop RSA NA05NMF4541291.

5) Some of the information derived from the data generated by this project can be more effectively communicated by animations rather than a series of static plots. We plan to illustrate the dynamics of toxicity and roe / meat ratio over time within webpages reached via http://www.seascallop.com/RSA008/, where many of the plots in this report are currently posted.

6) We need to improve collaboration with other scientists and other research projects having related data, such as the larger GOMTOX efforts to study Harmful Algal Blooms in the Gulf of Maine. While outside of the scope of this project it may prove productive to compare toxicity results with red tide modeling methods or oceanographic frontal data to determine if there are patterns that can tell us more about transport pathways affecting scallop toxicity off the coast of New England (Figure 28).

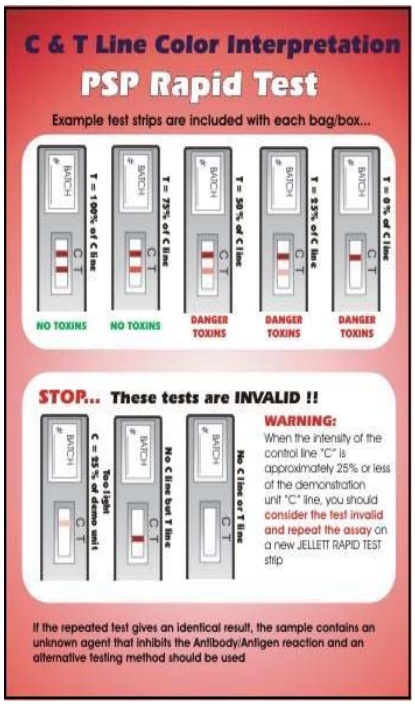
7) No sampling was conducted south of DelMarVa Peninsula, however all other Mid-Atlantic samples were negative for biotoxins.

8) We need to integrate a project partner that will provide the statistical expertise for analysis of this data in order to get the full value from it.

Phase 2 of this project continues sample collection and tissue testing. We foresee that another year and a half's data will let us resolve some of the questions that this first effort has provoked.

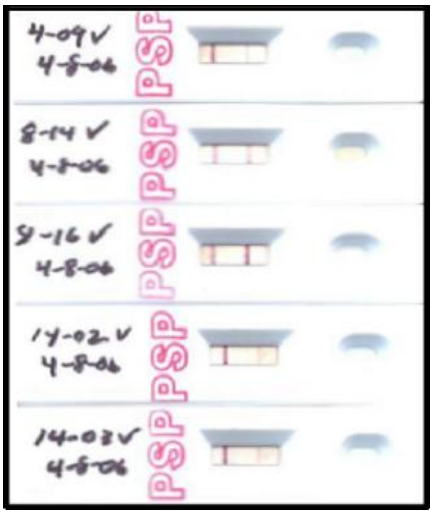
7.2 Efficacy and utility of Jellett Rapid Test

This screening test performed adequately with few outright failures of the test strips. Only one of these failures was in the form of a false negative results, but more often occurred where the control marker band was either weak or absent entirely, thus readily apparent. In both cases a second strip was used with the same tissue extraction and produced a clear result. There were issues of interpretation of results that diminished over time with familiarity, with the primary concern being the variability in strip intensity, compared to the illustration provided by Jellett Rapid Testing, Ltd. Compare the figures on this page.



In samples that were tested by both JRT and confirmatory tests, 53% (17 of 32 positives out of 114 total tested) gonad samples gave a strong positive JRT reading yet had results below 40 STX equivalents using the RBA method. 10 of those 17 (59%) had RBA results below 20. Notably none of these JRT "false positives" came from samples in the Mid-Atlantic.

One "false positive" was found in JRT viscera results (of 66 tests with positive results) out of 86 tests total. However since there were no viscera tests showing results from both RBA and JRT in the range of 15 to 40, the relative sensitivity of the JRT test in this range is unknown at this time. The "false positive" for viscera was "below detection levels" via the MBA and at 35.7 by the FDA RBA so it is right at the "detection levels". The JRT did correctly identify positive viscera in 65 out of 66 cases and in 11 out of 12 cases it did correctly identify negative viscera (see above false positive for viscera). It also correctly identified 5 out of 5 positive gonads (NOS), and in 3 out of 5 gonad positives for FDA. The 2 outliers tested below detection levels with MBA, and 44 and 47 via FDA RBA.



JRT strips are well known to exhibit "false positives". Jellett (2002) reports an average false positive rate of 14%, and, Mackintosh (2002) reported up to 25% false positives. After all tests had been completed and the false positive patterns identified, discussions with JRTL staff identified that the original test had been standardized with whole mussel tissue and custom buffer solutions were available for unique tissues such as scallop gonads. Mackintosh (2002) confirms false positives are common with King scallop gonad tissue; Future testing will be conducted with new buffers as they are made available. These findings plus the results of a linear regression (see below) may affect thinking on the appropriateness of the JRT for use with scallop gonads.

Larger issues regarding Jellett Rapid Test

Given the larger issues of shellfish safety, one-off testing using the JRT (or even at sea testing of multiple samples) is a secondary objective to advance development of a roe-on scallop product for the following reasons:

1). The JRT product is not yet a mature technology.
--Shellfish specialists in the area have not yet built confidence in results.
--No formal or third party training is yet available
--No comprehensive "user's manual" is yet available
--No user-community has developed to foster learning and interpretation

2). Results are somewhat ambiguous
--High rate of false positives when used with gonad tissue
--As yet unknown false positive rate when used with viscera tissue
--Scoring of tests is highly subjective until 100's of tests have been interpreted

3). Opportunity for error is high
--Multiple samples increase chance for error
--New test kits are occasionally substantively different than earlier products shipped – this could be differences in methodology (dilutions), buffers or lab method, but indicates a developing technology. JRT Ltd has been effective at resolving difficulties and replacing test strips inappropriately used due to ineffective communication around changing technologies.

The JRT is marketed as simple, rapid device useful for screening shellfish toxicity. Despite our proof of concept that the test can be conducted on a vessel, conducting multiple on-board tissue extractions and tests without a special facility is likely to be difficult and could be dangerous. The practical experience of running and interpreting hundreds of test cassettes in a Quality Control "kitchen" at New England Shellfin, shows us JRT is both simple and rapid, yet numerous false positives in gonad tissue cast doubt on the test results until the rate can be reduced. Any "positives" from tests conducted outside of a lab should be regarded as "preliminary" until later validated by more rigorous methods. Anyone not previously trained in lab techniques using these tests needs more training than can be provided with a video-cassette. Colorimetric result interpretation should be conducted by two trained individuals, and Jellett Rapid Testing Ltd.'s 2006 distribution of "scoring tests" to improve the detection of invalid tests is a very positive step in this regard.

JRT for ASP is even more sensitive in that the toxins level targeted is 10µg/g and the range of colorimetric results is 4 rather than 5. Furthermore the lab method is more prone to error.

7.3 Deriving more from the data

In the present study we examined a possible relationship between toxicity in viscera and gonad tissue fractions. When following Canadian CFIA methods, a relationship using a multiplier of 12.5 (1000 / 12.5 = 80) for PSP exists for predicting gonad toxicity from viscera toxicity (see Appendix D, Roe Attached Scallop Protocol, CFIA 2002). The CFIA protocol routinely considers gonads safe for harvest and export if the scallop "liver" (hepatopancreas) toxicity is

< 1,000 µg /100g STX equivalents. For example, viscera testing >1,000 for PSP on the Canadian side of Georges Bank in summer of 2006 caused industry to voluntarily close their roe-on season in that area as they knew roes were likely to be toxic (personal communication). Similarly CFIA test the liver to 100µg/1g for ASP implying a multiplier of 5 for ASP (see Appendix D).

Because our study uses the combined hepatopancreas, eye ring, and vellum tissue rather than the "liver" (hepatopancreas) only, we did not replicate their approach. CFIA uses Mouse Bioassay with the standard AOAC extraction, where we have used the RBA with a similar extraction method.

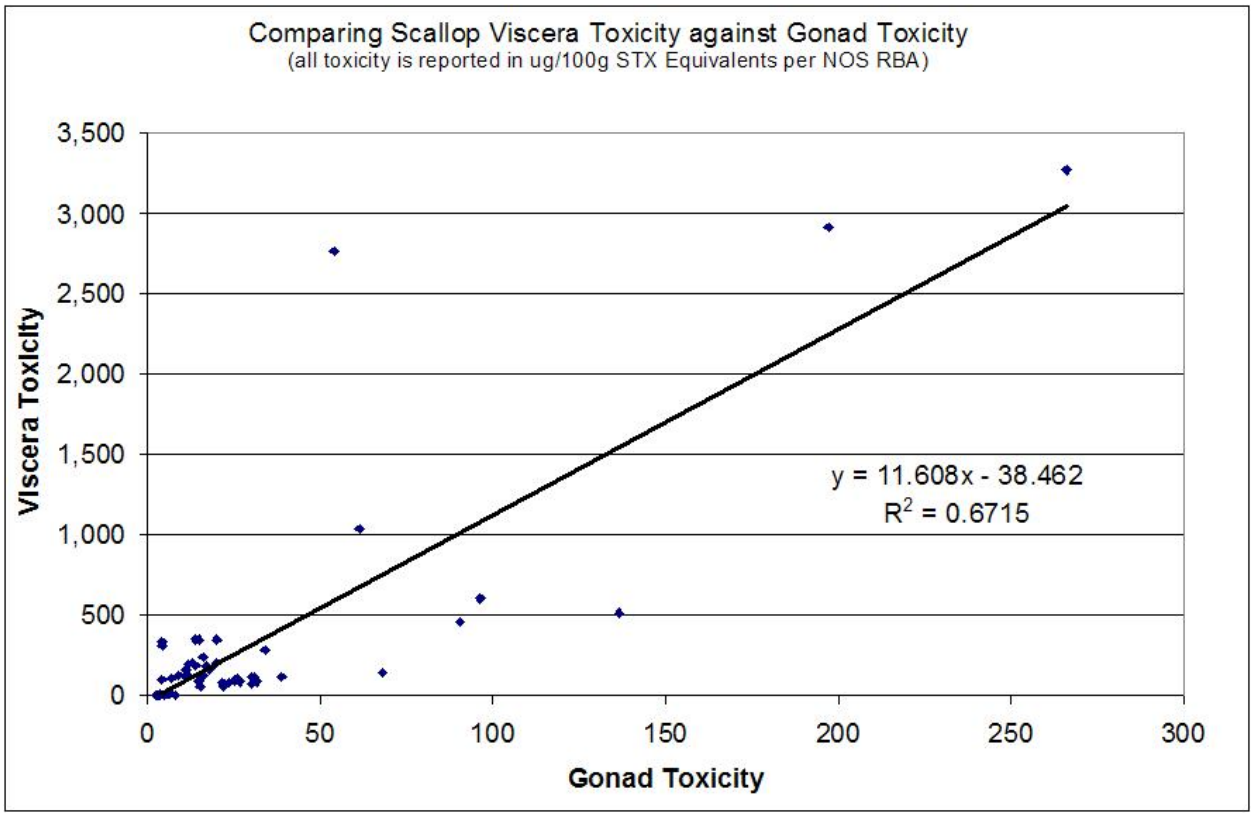


Figure 32

By plotting scallop viscera toxicity values, from NOS RBA tests, against scallop gonad toxicity results, and taking a linear regression, the results suggests that a useful relationship between viscera and roe toxicity might be inferred. Yet because the correlation coefficient is less than 1, additional data points and analysis would be necessary to create a more reliable relationship. Roes testing at 80 which correlate with viscera testing at 928 could imply a mathematic relationship where viscera testing below 928 could indicate associated roes are safe for harvest and export.

Alternatively, selecting 400µg /100g STX equivalents as a simple value with a healthy safety margin (which also is easy to test for using the JRT strips), we reexamined our data. Samples testing 400µg /100g or below for the viscera using the RBA compared with gonad RBA results indicates that **100%** of gonads (n = 103) had results in the gonad below 80, with all but one exhibiting values below 40, or half the federal standard. Using this relationship, in seven

additional samples where only the viscera was tested, and had a value over 400 and under 800, all roe tissue from the same animal would likely register below 80µg /100g STX equiv. The utility of the relationship, if it can be further verified, is that testing could be simplified to measure only that tissue fraction with the strongest signal, the viscera. The implication is also that for an additional 177 samples where the RBA test on the viscera was the only quantitative test made on the sample, and where results for the viscera were below 400µg/100g STX, that the toxicity values for roe only would fall **below** the federal standard.

7.4 Development of a safe roe-on product

From a public safety perspective, product from an area with no history of toxins in scallop gives the highest certainty of safety. Individual results from areas with a mixed history of toxicity, even when backed up by shoreside testing are problematical in that product from a nearby area may contain toxins above the federal action level.

Accordingly, our primary conclusion is the there are no substantive impediments to immediate development of a roe-on fishery in the mid-Atlantic scallop resource areas, and that the economic benefits to the fleet could be substantial. While the need for continued testing is evident, at this time all samples tested (n=180) in the area are free of the toxins that serve to constrain or prohibit development of this market in the other high biomass resource areas, e.g. the Great South Channel, Nantucket Lightship, Closed Area I, and Closed Area II.

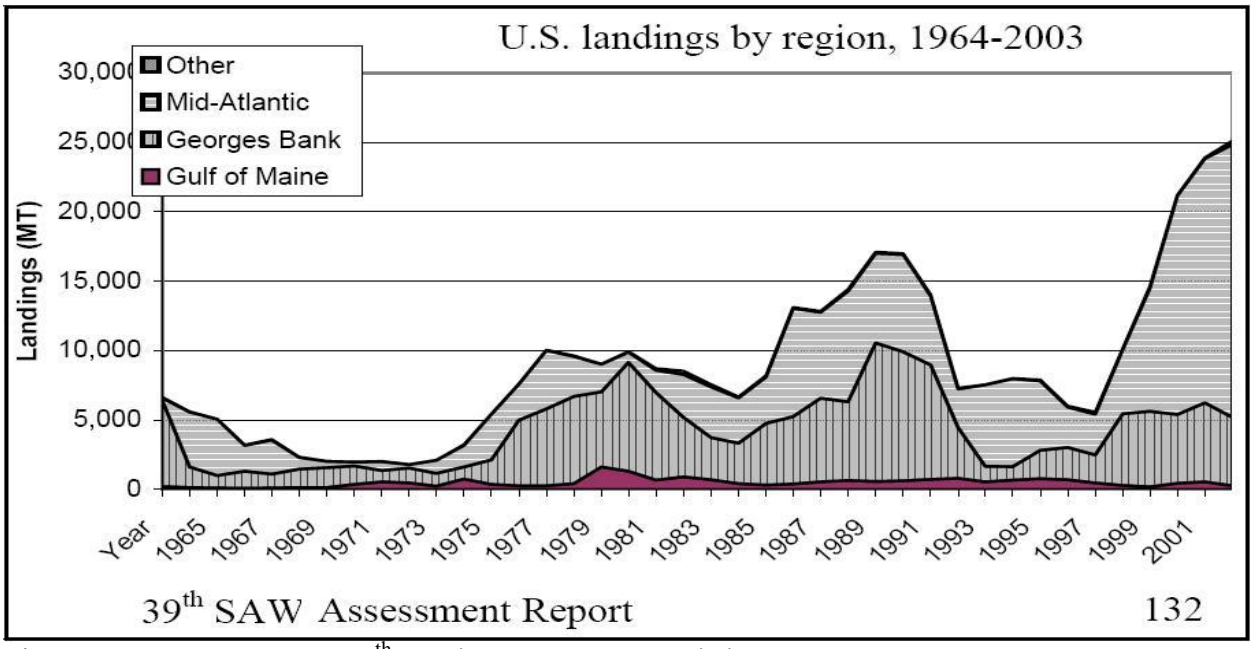


Figure 33. Source: NMFS 39th Stock Assessment Workshop Report

This opportunity is especially evident given the high proportion of scallop biomass in the mid-Atlantic resource areas, recently over 50% of total landings (Framework 18, NEFMC). Additionally there is the favorable spring timing of roe maturation, and a prime market acceptable roe fraction and size relative to the other US sources of this product. Given the scale of the landings from the mid-Atlantic scallop fishery and the portion of the year where the roe is within the sizes deemed acceptable to the E.U. market, Mid-Atlantic production could supply a significant fraction of the entire current E.U. demand.

8. Contribution to decision making and management
While the New England Fishery Management Council has no direct input into the testing or safety of seafood or seafood products, it does have the ability to adjust the landing allowances from the scallop growout areas to reflect the additional poundage of roe as an addition to the current level of 18,000 pounds per trip. This would serve to allow and encourage development of the roe-on fishery. For example, if a vessel is allowed 18,000 pounds of scallop meats, and the roe-to-meat ratio is a measured 33%, then the allowance for vessels landing a mixed product should be 24,000 pounds. This situation would require no more individual animals, thus no increase in the fishing mortality, number of tows or swept area. However, the benefits are more difficult to realize in the open areas within the current management approach of Days At Sea as participating vessels would likely require additional time to cut out the product, a near certainty at startup. The same concept concerning poundage limits that reflect only meat weight would apply to a General Category scallop vessel regarding the existing 400 pound trip limit.

Beyond public health issues and necessary changes in the current iteration of the law concerning landings, such a scenario would present other regulatory issues, with the primary concern being enforcement. While this aspect of the problem is important, it is beyond the scope of this project to address these issues.

Implications concerning offshore shellfish regulatory areas suggested by these data
Documentation of the progressive southerly movement of the toxic bloom, from the inshore Jeffreys Basin area where it was first detected in April, across Massachusetts Bay and southward was excellent. Scientists from the UNH COOA program and from the Woods Hole Oceanographic Institution provided ample warning that shellfish resources were soon to be impacted. Closures were put in place and the public health was effectively protected. The 2005 toxic bloom was the strongest by far of the last 30 years with very high cell densities observed in the water column and shellfish toxicity in Mass Bay measuring up to 4 times greater than the previous maximum in 1978 (Anderson 2005). These events were an important issue to us because directly downcurrent were the high biomass scallop resource areas in the Great South Channel, Nantucket Lightship and Closed Area I.

Before the 2005 bloom the U.S. northwest Atlantic offshore area was effectively divided into just two regulatory zones for the harvest of shellfish such as scallops and clams (Figure 33). The eastern portion (69ºW and to the east – pink) had been under a closure for whole molluscan shellfish for 16 years. The remainder of the entire region (Mid-Atlantic, Southern New England, and Gulf of Maine) was considered toxin free. A third area, the Emergency Closure (light blue), was added in June 2005, spanning from the western boundary (69°W) of the existing Georges Bank PSP closure west to 71°W, from 40° to 43°N.

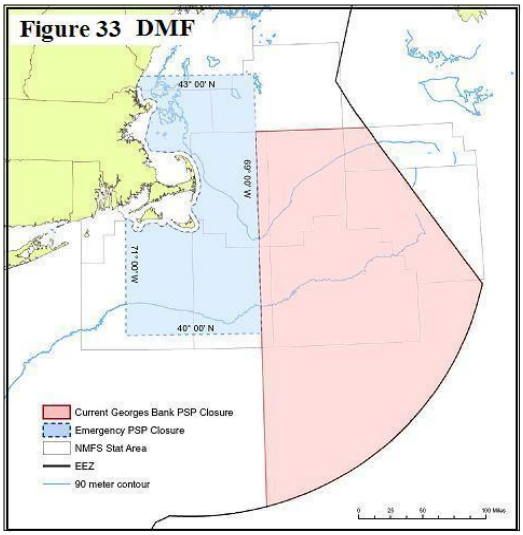
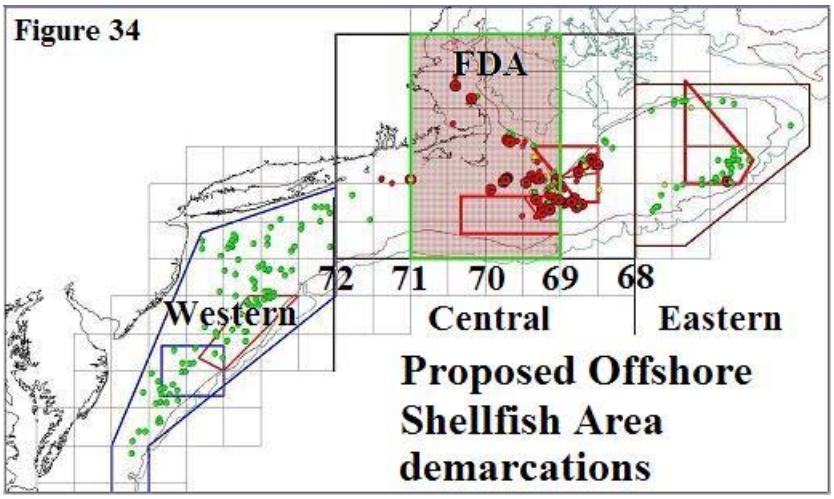


Figure 33 DMF

The area between the Georges Bank PSP Closure and the Mid-Atlantic had been considered free of toxins until the 2005 FDA/DMF/NMFS Emergency Closure. It is in this area, specifically in a small part of the Nantucket Lightship Groundfish Closed Area that we first found toxin in samples collected in July of 2004, well before the bloom of 2005.

Data from this project clearly documents that the area with highest PSP results spans the defacto boundary between the Georges Bank (pink) and FDA closure (light blue) at 69°W. This suggests that the area of greatest concern, this Central area, should be enlarged to the eastwards so that regulatory measures may be selectively applied to areas of the shelf that are experiencing the episodic effects of biotoxins, and not to those areas that remain benign. This Central regulatory area could also be expanded to the west. Though scallop resources are generally sparse to the immediate west of the Emergency Closure area, a limited number of samples were found to be toxic near Block Island, an area that has supported a low volume scallop fishery for many years.

Three spatial regions are readily apparent in the structure of the data: Western, Central, and Eastern, with the dividing lines of longitude at 71.5º and 68.5º. In the Mid-Atlantic, the western-most region, 100% of test results for all samples west of 71.5ºW were negative, i.e. showed no presence of PSP or ASP. The Central region, from 71.5ºW eastward to 68.5ºW, contained the overwhelming majority of the toxic samples for PSP. The easternmost region had three samples with RBA results for viscera over 80 ug/100g, (83, 112, and 305) however the tests results for the roe were low (27, 1, and 4.3). Therefore, we propose the consideration of a new flexible regulatory structure, as indicated in Figure 34, in which there are zones where a roe-on, and potentially a whole animal fishery, can be considered completely safe (Western), and other areas where the risk is low (Southeast Part), and high (the Central area, the Great South Channel).



In order to give a greater level of protection the areas on the map below have been drawn to give the Central area a 1 degree buffer both east and west of the Emergency Closure, from 68°W to 72°W. This gives a 30 minute buffer beyond where toxins were identified. While we realize that this dataset reflects only the situation from mid 2004 and until late 2005, we believe that the underlying physical forcing factors will continue to produce similar events where areas of toxicity are not well matched to the existing closure area boundaries. The data indicates that the scallop resource area on the Southeast Part of Georges Bank within Closed Area 2 was free of significant toxins, and where a PSP closure encompassing the entire bank, while justifiably precautionary without data, would not be warranted with sufficient information.

Overall the situation emphasizes the need for continued collection of samples by industry, continued testing, continued close collaboration with regulators, and greater flexibility in setting closure boundaries as necessary throughout the northern range, particularly since the path of these blooms can be so variable.

9. Acknowledgements

This project results from the efforts of many who helped in the years before the RSA funding. Those include Paul Anderson, (Maine SeaGrant), Sherwood Hall (FDA CFSAN), Paul Tierney (MA DPH), and Sean Cone (F/V Lady Luck).

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Susan Olsen for her outstanding ability to navigate to workable solutions getting the project started, and for her patience with us in seeing it through.

Thanks go to Catherine Tadema-Wielandt and Pete Christopher of NMFS NERO for timely responses to the many questions and for the rapid turnaround on the funding trip LOAs.

Thanks go to Bergies Seafoods in New Bedford for handling the samples before processing.

Many samples were contributed by the General Category scallop fleet without compensation, especially important for the inshore areas most affected by the "Red Tide" event in 2005. Special thanks goes to Captains Lee Jackson, Bruce Gibbs, Joe Smith and many others out of Gloucester, Chatham, Harwich, Hyannis, Provincetown, and Woods Hole.

The NMFS Regional Office and the Massachusetts Division of Marine Fisheries provided timely support in permitting for the sample collection program, especially Kevin Chu and Brian Hooker from NMFS, and Melanie Griffin from DMF who helped by writing the LOAs to allow sample collection from PSP closure areas.

Considerable unfunded effort in testing samples was provided by the NOS CCEHBR lab in Charleston, S.C., the FDA CFSAN Lab in Silver Spring, Md., and the Massachusetts Division of Marine Fisheries lab in Gloucester, Mass. Particular thanks go to Tod Leighfield at CCEHBR, Florence Pettengill, Dave Whitaker, and Mike Hickey at Mass DMF, and Lisa Horn at Jellett Rapid Testing Ltd.

Thanks also to Judy Kleindinst at WHOI for her administrative support of this project.

Special thanks go to Cheryl Correia, Edie and Maria Boat Settlements, and F/V Kathy Marie owner Arnie DeMello, and Captain Paul Rosonina for bringing in samples for 5 years before this project began.

Over the course of the project it became clear that each of these participants served an absolutely necessary function within the larger team, and that no combination of fewer partners could have produced these results.

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11. Appendices

A. Jellett Rapid Testing Ltd. literature, scanned
A.1 Shellfish Tissue Preparation and Extraction Methods (Isopropyl Alcohol and Vinegar)
A.2 Lab Instruction Sheet and Color Line Interpretation for PSP Rapid Test
A.3 Lab Instruction Sheet and Color Line Interpretation for ASP Rapid Test
A.4 Lab Instruction Sheet for Quantitative Results for JRT Sampling Dilutions
B. RBA / LC-MS (Fran Van Dolah, NOS, CCHEBR)
C. HPLC (Dave Kulis, WHOI)
D. Canadian Dockside Protocol (2002)
E. Protocol for Onboard Screening and Dockside Testing for PSP Toxins in Molluscan Shellfish in Federally Closed Waters (2006)

12. Presentations of materials from this project

Geographic Information Systems and Ocean Mapping in Support of Fisheries Reseach and Management, Massachusetts Institute of Technology, Cambridge, Mass., 10 April 2006

3rd International Symposium on GIS/Spatial Analyses in Fisheries and Aquatic Sciences, Shanghai Fisheries University, Shanghai, China, 25-28 August 2005