NJDEP Bureau of Marine Water Monitoring

Proposal for Techniques and Practices for Vibrio Reduction

Submitted to the ISSC Interstate Shellfish Sanitation Conference

July 31, 2014

EXECUTIVE SUMMARY

Aware of the relationship between temperature and prevalence of pathogenic strains of *Vibrio parahaemolyticus* and *V. vulnificus*, the Bureau of Marine Water Monitoring of New Jersey Department of Environmental Protection is proposing a study that will identify and evaluate various oyster handling practices/techniques that could potentially reduce the risk of Vibrio illnesses. The handling methods that we shall be looking at are as follows:

a. Immediate cooling on ice after harvesting before analysis;
b. Shading of oysters for 6-7 hours before analysis;
c. Shading of oysters for 6-7 hours and refrigerated overnight before analysis;
d. Analysis of oyster from harvester; and
e. Tracking and analysis of same oyster lot from retail establishment.

Little is known regarding the fate of Vp and Vv after it leaves the certified dealer. This study will continue to look at the fate of Vp and Vv post-harvest, but also gather info regarding Vp and Vv growth after it leaves the certified dealer en route to the consumer by tracking product to a retail establishment. We will collect samples of oysters from commercial harvesters after harvest, collect samples prior to shipment from the certified dealer (same lot if practical), and work with the FDA to track and collect samples of the lot (same lot if practical) at its final destination (retail establishment). If the same lot is not available, we will analyze retail samples and compare them to data collected on the nearest date.

All samples will be tested for total V.p (tlh+), pathogenic V.p (tdh+ and trh+) and *V. vulnificus* (vvh) using Official FDA BAM chapter 9 and Nordstrom *et al*., 2007. We plan to start the execution of this proposal from August 2014 for handling methods a-c above while sampling from commercial harvesters d-e will commence from May/June 2015 through August 2015.

APPROACH AND METHODOLOGY

The study aim is to look at the relationship between water temperature, air temperature and salinity as it affects the total Vp (tlh+) and pathogenic Vp (tdh+ and trh+) strains. We will also be looking at the levels of total Vp and pathogenic strains along the distribution chain (harvest to retail).

The collection time frame and sample sites for Vibrio parahaemolyticus (Vp) presence in the oyster tissue will specifically take place in the month of August 2014, and from May through August 2015 at various Delaware subtidal commercial harvest locations. Although June through August are traditionally considered the months for Vp bacterial sampling, May has been added in in an attempt to analyze and compare various parameters along with Vp presence in oyster tissue, during time frames preceding and following oyster spawning.

Subtidal collection is proposed to take place four times during each month for Delaware Bay. Two of the sampling events will be performed by the Bureau's staff, from active commercial harvest areas, this sampling is to continue previous analyses on New Jersey's Harvest Practices. The other two sampling events will require the collection of Oysters from a commercial harvester, that will be used to monitor the effects of handling, and will cover from harvest to retail. Each sampling event will require one field day for collection, and three consecutive days of lab time for preparation and analysis. During the study period, animal [Oyster – Crassostrea virginica (Cv)] will be analyzed for Vp using Official FDA BAM chapter 9 and Nordstrom *et al*., 2007 for the enumeration of genes, specific for total and pathogenic Vp

The acquisition of oysters will require the collection of sample sets when visiting each site. For a subtidal site in Delaware Bay, one of nine sites (harvest/quota dependent) will be gathered each week from May to August. Each visit requires the collection of 45 larger oysters, which will be used for three different harvest/post-harvest handling method comparisons. Each method will examine 15 oysters. The exact methodology for collection in subtidal waters is detailed below:

1) Label all plastic shellfish bags prior to arriving at sample location(s) with station ID's and handling method letters. Use an indelible marker (e.g., Sharpie) on Autoclave Tape (striped tape acquired from bacteriological lab) that is placed on the outside of plastic bag.

2) Prior to oyster collection you will have acquired an insulated ice chest/cooler and placed approximately two inches of ice on the bottom. On top of that ice you will place a raised rack (e.g., a sample bottle rack from bacteriological lab) where you can place your site collected oysters' samples. Oysters should be covered with bubble wrap and layers of ice packs placed over top of the bubble wrap.

3) Verify site location w/ GPS and attempt to sample within a half mile of the general GPS coordinates shown on lab/field sheets for subtidal locations.
4) Oysters can be harvested by dredging or tonging with subsequent hand culling for placement in plastic bags for subtidal waters.

5) Cull oysters, selecting best size for lab processing (attempt to select larger specimens).

6) For each site, collect 15 larger species for each required handling method.

7) Oysters should be cleaned, removing mud, macro algae, debris, etc.

8) Place oysters in a sealed plastic bag and place in cooler as noted in step 2 above.

9) Plan to collect shellfish for delivery to process lab within five hours for Delaware Bay.

Sample collection and analysis will also require acquisition of air temperature, water temperature, DO, salinity, pH, and out of water shell temperature from the collection site. Subtidal waters require the recording of water temperature, DO, salinity, and pH from surface and bottom locations in the water column. For handling methods requiring shellfish shading after field acquisition, a pre-process shaded shell temperature and pre-process shaded air temperature is required. Additionally, for Delaware Bay oysters that are put on ice immediately, a pre-process meat temperature should be taken.

In order to fully understand what happens along distribution chain to the retailer, we will also be getting samples of oysters from commercial harvesters immediately after harvesting and put on ice or in refrigeration, and will collect samples before the same lot of oyster is shipped when loading into the truck. We will track the lot to its final destination and collect additional sample for analysis. This will be done in conjunction with USFDA. Prior to collection of samples, air temperature and shell temperature will be taken using calibrated hand held meter.

PROJECT DELIVERABLES

The deliverables from this project will be as follows:

1. We will capture air, water and out of water shell temperatures at every sampling location to better understand how this affect presence of pathogenic strains of V.p and V.v
2. Effects of cooling immediately after harvesting using ice chest or refrigerated chamber will also be examined. The levels of total (tlh+) and pathogenic strains (tdh+ and trh+) as well as vvh from samples that were cooled immediately after harvesting will be compared to those stored under a tarp (shaded) at ambient air temperatures for 6-7 hours before processing as well as those refrigerated overnight after left in the shaded air for 6-7 hours. Ambient air temperatures and shell temperatures will be taking prior to sample analysis.
3. This study will provide better understanding of how handling and shipping processes can affect pathogenicity of *Vibrio parahaemolyticus* and *V. vulnificus* before it get to the final consumer.
4. We will serotyped all pathogenic strains isolated during this study to give us the prevalence as well as to better understand if they are temperature dependent.
5. Statistical analysis of the results obtained and recommendations shall be developed and submitted to ISSC.

PROJECT MANAGEMENT APPROACH

The project will be managed as described below:

Project Director: Bruce Friedman, Chief, Bureau of Marine Water Monitoring, will provide the overall management of the project.

Principal Investigator (PI): Robert Schuster, Interim Section Chief, Bureau of Marine Water Monitoring, will manage the data, perform the assessment, and manage the execution of the project.

Field officer: Marc Resciniti will be the lead field officer to collect field data, shellfish, and oversee other field staff needed to perform the study.

Laboratory Technicians: Three Laboratory Technicians will be available to assist in the analysis of samples under the supervision of PI.

DETAILED AND ITEMIZED PRICING

Materials and Supplies

Supplier	Catalog Number	Item	Amt.	Total Cost
VWR	BDH8014	Sodium Chloride	3	$181.47
VWR	90000-744	Nutrient Agar	2	$258.22
VWR	61001-506	Peptone	2	$179.74
VWR	95022-388	TCBS Agar	3	$443.70
VWR	95020-770	CPC Agar	2	$2,039.80
VWR	95057-782	CPC Supplement	2	$168.80
VWR	95060-676	API Kit	1	$1,306.17
VWR	90003-676	Voges-Proskauer A	1	$65.00
VWR	90003-678	Voges-Proskauer B	1	$76.50
VWR	95060-952	TDA Reagent	1	$39.07
VWR	95060-956	NIT 1/NIT 2 Reagent	1	$70.30
VWR	95060-946	Mineral Oil	1	$31.26
VWR	95060-974	James Reagent	1	$39.07
VWR	10052-582	Vibrio Antisera Kit K-Set	1	$6,041.58
VWR	10052-578	Vibrio Antisera Kit O-Set	1	$858.90
VWR	25388-581	50x9mm Tight Fit Plates	2	$308.04
VWR	25384-252	100x15mm Petri Plates	4	$849.52
VWR	12000-806	1uL Inoculating Loops	10	$1,287.90
VWR	12000-814	Inoculating Needles	5	$643.95
VWR	89003-420	100-1,000uL Pipette Tips	4	$582.28

VWR	89092-962	0.1-10uL Pipette Tips	4	$615.80
VWR	53510-012	1-40uL Pipette Tips	1	$175.65
VWR	53510-070	1-100uL Pipette Tips	1	$161.30
VWR	53510-106	1-200uL Pipette Tips	2	$319.48
VWR	33503-136	70% Alcohol Wipes	1	$559.45
VWR	414004-429	Small Latex Gloves	2	$334.60
VWR	414004-430	Medium Latex Gloves	1	$167.30
VWR	21150-478	0.6 mL Amber Microcentrifuge Tubes	1	$39.44
VWR	22179-004	1.5mL Clear Microcentrifuge Tubes	6	$472.68
Life Technologies	10977-015	PCR-Grade Water	1	$29.00
Life Technologies	10966-034	Platinum Taq Polymerase	10	$4,590.00
Life Technologies	4316034	Custom TaqMan Probe MGBNFQ trh_133-23 –Vic Seq: 5'-AGAAATACAACAATCAAAACTGA-3'	1	$255.00
Life Technologies	4316034	Custom TaqMan Probe MGBNFQ Tdh_269-20_FAM Seq: 5'-TGACATCCTACATGACTGTG-3'	1	$255.00
BioGX	760-0001	VP IAC DNA	1	$550.00
IDT	Custom tl_884F	5'-ACTCAACACAAGAAGAGATCGACAA-3'	1	$8.75
IDT	Custom tl_1091R	5'-GATGAGCGGTTGATGTCCAAA-3'	1	$7.35
IDT	Custom trh_20f	5'-TTGCTTTCAGTTTGCTATTGGCT-3'	1	$8.05
IDT	Custom trh_292R	5'-TGTTTACCGTCATATAGGCGCTT-3'	1	$8.05
IDT	Custom tdh_89F	5'-TCCCTTTTCCTGCCCCC-3'	1	$5.95
IDT	Custom tdh_321R	5'-CGCTGCCATTGTATAGTCTTTATC-3'	1	$8.40
IDT	Custom IAC_46F	5'-GACATCGATATGGGTGCCG-3'	1	$6.65
IDT	Custom IAC_186R	5'-CGAGACGATGCAGCCATTC-3'	1	$6.65
IDT	Custom vvhF	5'-TGTTTATGGTGAGAACGGTGACA-3'	1	$8.05
IDT	Custom vvhR	5'-TTCTTTATCTAGGCCCCAAACTTG-3'	1	$8.40

IDT	Custom IAC_109	5Cy5'-TCTCATGCGTCTCCCTGGTGAATGTG-3'-BHQ_2	1	$315.00
IDT	Custom vvh Probe	56-FAM-5'-CCGTTAACCGAACCACCCGCAA-3'-BHQ_2	1	$195.00
IDT	Custom tl_1043	56-JOEN-5'CGCTCGCGTTCACGAAACCGT-3'-BHQ_2	1	$420.00
		Total Costs		$24,419.32

*Cost does not include shipping and handling.

APPENDIX: REFRENCES

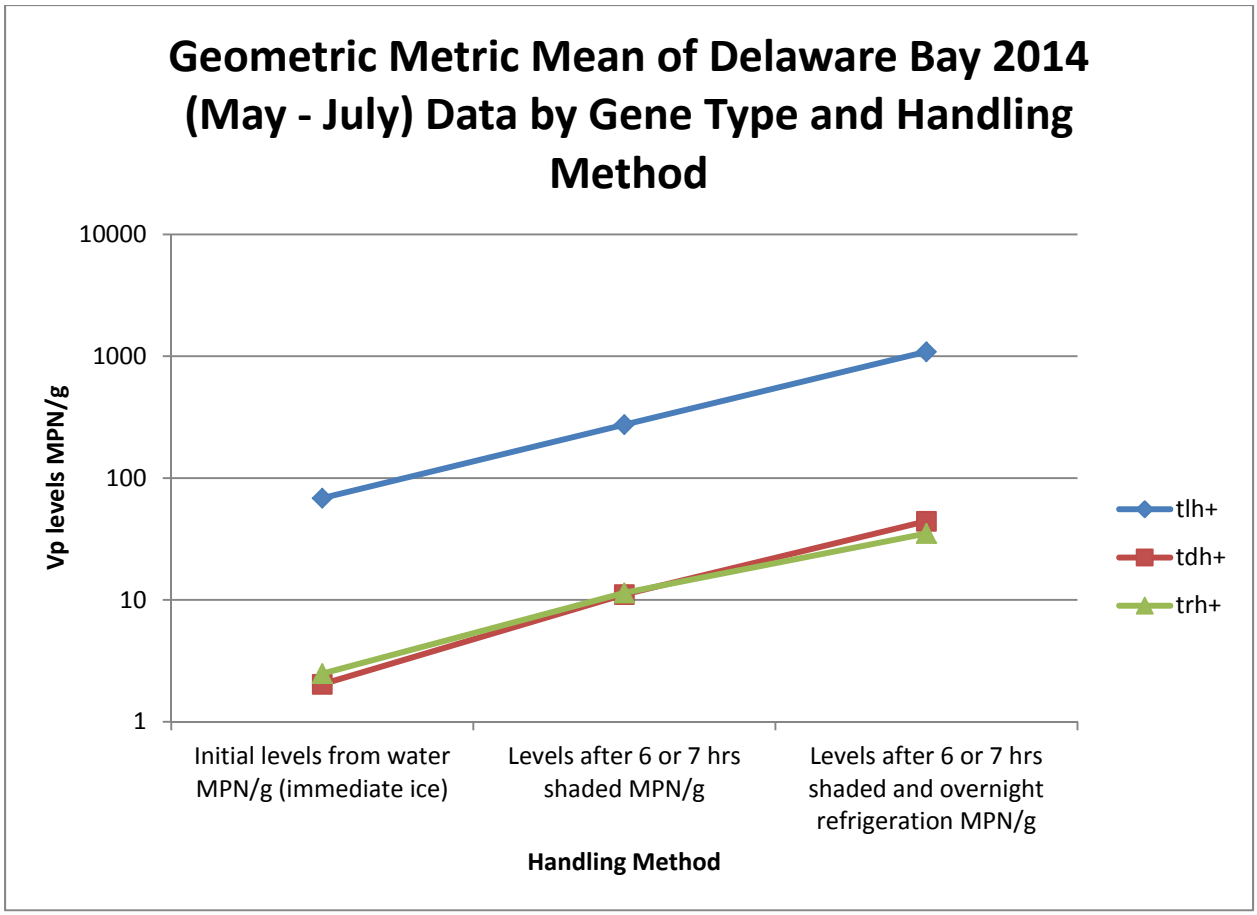
As part of our Vibrio sampling and monitoring plan, the Bureau of Marine Water Monitoring has been studying the relationship between water, air and out of water shell temperatures and pathogenic strains of Vp for the past three years. We also mimic three handling methods:

a. Immediate cooling on ice after harvesting before analysis
b. Shading of oysters for 6-7 hours before analysis;
c. Shading of oysters for 6-7 hours and refrigerated overnight before analysis.

Our preliminary results showed that the level of pathogenic strains (tdh+ and tlh+) from samples that were put on ice immediately after harvesting were little to none. We noticed an increase of these genes from oysters that were shaded for 6-7 hours while the levels either increased or remained constant for the overnight refrigerated samples.

2014 Delaware Bay Vp Sample Results (Preliminary)

	Date	Initial levels from water MPN/g	Levels after 6 hrs shaded MPN/g	Levels after 7 hrs shaded MPN/g	Levels after 6 or 7 hrs shaded and overnight refrigeration MPN/g
tlh+	5/12/2014	<3		0.92	0.92
tdh+	5/12/2014	<3		0.36	0.92
trh+	5/12/2014	<3		0.36	0.92
tlh+	5/19/2014	9.3		4.3	15
tdh+	5/19/2014	4.3		0.74	9.3
trh+	5/19/2014	1.5		1.5	7.5
tlh+	5/28/2014	93		210	2400
tdh+	5/28/2014	0.36		11	2.3
trh+	5/28/2014	9.3		15	14
tlh+	6/2/2014	200		1500	1500
tdh+	6/2/2014	<3		<3	<3
trh+	6/2/2014	0.3		<3	<3
tlh+	6/9/2014	93		93	15,000
tdh+	6/9/2014	3.6		43	15,000
trh+	6/9/2014	7.4		43	9,300
tlh+	6/16/2014	43		75	1,500
tdh+	6/16/2014	<0.3		15	430
trh+	6/16/2014	<0.3		15	230
tlh+	6/23/2014	240		2,900	4,300
tdh+	6/23/2014	<3		43	43
trh+	6/23/2014	3.6		43	43
tlh+	6/30/2014	240		24,000	4,300
tdh+	6/30/2014	<3		120	210
trh+	6/30/2014	<3		110	210
tlh+	7/7/2014	21	4,300		15,000
tdh+	7/7/2014	<3	43		93
trh+	7/7/2014	3.6	23		23
tlh+	7/14/2014	930	930		7,500
tdh+	7/14/2014	3.6	23		93
trh+	7/14/2014	9.2	23		23


Geometric Metric Mean of Delaware Bay 2014 (May - July) Data by Gene Type and Handling Method
10000
1000
100
10
1
Vp levels MPN/g
Initial levels from water MPN/g (immediate ice)
Levels after 6 or 7 hrs shaded MPN/g
Levels after 6 or 7 hrs shaded and overnight refrigeration MPN/g
Handling Method
tlh+
tdh+
trh+

APPENDIX: PROJECT TEAM STAFFING

Management Personnel:

1. Bruce Friedman, Chief

   Bruce Friedman has been with the New Jersey Department of Environmental Protection for 26 years. He has extensive experience with Water Enforcement and Compliance and NJPDES Permitting. Bruce helped develop, implement and manage USEPA's Phase II stormwater program in New Jersey, regulating the discharge of stormwater from municipal separate storm sewers. He currently manages NJDEP's Leeds Point Labratories. He has been involved in source track down and restoration efforts within the Wreck Pond Watershed. Bruce is a graduate of Stockton State College with a Bachelor of Science Degree in Environmental Studies and is a member of New Jersey's Water Monitoring Council, Barnegat Bay Science and Technical Advisory Committee, Interstate Environmental Commission, Interstate Shellfish Sanitation Conference, and the Wreck Pond Watershed Committee.

2. Robert Schuster, Interim Section Chief

   Robert holds a Bachelor of Science Degree in Chemistry with 24 years of experience in both the Chemical and Bacteriological analyses in Marine waters, for the NSSP, USEPA ambient monitoring programs, and the implementation of new technologies, which includes real-time water quality data from buoys, and the development of New Jersey's program for aircraft remote sensing of chlorophyll *a*. He is currently in charge of the laboratory, assessment, and field sections of the NJDEP's Bureau of Marine Water Monitoring.

Laboratory Personnel:

1. Abolade Oyelade – Research Scientist 3.

   Abolade holds Master's degree in Microbiology with over 14 years' experience in research and teaching. He is currently in charge of Advanced Microbiology Laboratory that oversees Vibrio analysis using Multiplex Real-Time PCR Assay as well as Direct Plating Techniques.

2. Elena Heller – Environmental Specialist 3

Elena holds Bachelor of Arts degree in Environmental Science with over 24 years' experience in performing special projects involving bacteriological analyses of shellfish and marine waters.

3. Carrie Lloyd – Environmental Specialist 2

   Carrie holds Bachelor's Degree in Biology with over 9 year experience at NJDEP Bureau of Marine water monitoring. Techniques and skills acquired during time of employment are membrane filtration, multiple tube analysis, qPCR analysis, direct plating analysis, and other basic laboratory skills such as media preparation and quality assurance.

4. Eric Feerst – Section Chief (Retired)

   Eric has Bachelor's Degree in Biology with 37 years' experience in Shellfish sanitation, lab methods, Vibrio monitoring. Presently he is a part-time consultant in Vibrio monitoring program.

Field Personnel:

1. Marc Resciniti – Captain State Boat

   Marc has a Bachelor of Science Degree in Environmental Science with 10 years' experience in fisheries sampling and management, and 4 years' experience with the collection of shellfish and water samples for the Bureau's NSSP compliance.

2. Rodney Sloan – Captain State Boat

   Rodney has 4 years of experience with the collection of shellfish and water samples for the Bureau's NSSP compliance.

3. Lonnie LeVance- Captain State Boat

   Lonnie has 2 years of experience with the collection of shellfish and water samples for the Bureau's NSSP compliance.

4. Keith Murphy - Captain State Boat

   Keith has 12 years of experience with the collection of shellfish and water samples for the Bureau's NSSP compliance.

5. Ken Hayek – Principal Environmental Technician

Ken has 17 years of experience with the collection of shellfish and water samples for the Bureau's NSSP compliance, and water quality sampling techniques for EPA Ambient monitoring projects.

6. Rich Rand- Principal Environmental Technician

   Rich has 15 years of experience with the collection of shellfish and water samples for the Bureau's NSSP compliance, and water quality sampling techniques for EPA Ambient monitoring projects.