

**34<sup>TH</sup> MILFORD AQUACULTURE SEMINAR AGENDA  
FEBRUARY 24-26<sup>TH</sup> 2014**

**COURTYARD BY MARRIOTT, SHELTON, CT**

**Monday, February 24th**

6:30 PM	Registration Opens		
7:00- 10:00		POSTER SESSION AND OPENING RECEPTION	
p1	Joseph Buttner	LIBERIA AND NORTHEASTERN UNITED STATES AQUACULTURE INITIATIVE	Northeastern Massachusetts Aquaculture Center, Cat Cove Marine Laboratory and Salem State University
p2	Carter Cyr	EFFECTS OF STOCKING DENSITY AND CULTURE VOLUME ON POPULATION DYNAMICS OF <i>PARVOCALANUS</i> SP. COPEPOD CULTURES	Roger Williams University
p3	Matthew Dicostanzo	DEVELOPMENT OF A RESTOCKING SYSTEM FOR STAGE IV <i>HOMERUS AMERICANUS</i>	Bridgeport Regional Aquaculture Science and Technology Education Center
p4	Timothy Gerson	MODULAR LARVAL REARING SYSTEM FOR INTEGRATING AQUACULTURE INTO PUBLIC AQUARIA	Roger Williams University
p5	Leana Gomes	DEVELOPMENT OF THE APPLICATION OF <i>MYTILUS EDULIS</i> BYSSAL THREADS AS A BIOLOGICAL THREAD	Bridgeport Regional Aquaculture Science and Technology Education Center
p6	Hannah Jaris	ASSESSING THE CONTRIBUTION OF AQUACULTURE AND RESTORATION TO WILD OYSTER POPULATIONS IN RHODE ISLAND	Columbia University
p7	Eilea Knotts	CONDITION INDEX OF NORTHERN QUAHOGS, <i>MERCENARIA MERCENARIA</i> (LINNAEUS, 1758), IN NARRAGANSETT BAY IN RELATION TO SEDIMENT TYPES	Department of Biological Sciences, University of Rhode Island
p8	Kelly Markowitz	THE TREMATODE <i>PROCTOECES MACULATUS</i> IN THE BLUE MUSSEL <i>MYTILUS EDULIS</i> : MOLECULAR IDENTIFICATION, PREVALENCE AND INTENSITY	Hofstra University

p9	Renee Mercaldo-Allen	A FIELD-BASED NURSERY SYSTEM TO "HEAD START" LOBSTERS FOR IMPROVED POST-RELEASE SURVIVAL IN POTENTIAL STOCK ENHANCEMENT EFFORTS: A PROGRESS REPORT	NOAA, Milford Laboratory
p10	Diane Murphy	<i>VIBRIO PARAHAEMOLYTICUS</i> AND SHELLFISH IN SOUTHEASTERN MASSACHUSETTS	Woods Hole Sea Grant & Cape Cod Cooperative Extension
p11	Alisha Patel	A RECIPE FOR SUCCESSFUL CULTURE OF FIRE SHRIMP, <i>LYSMATA DEBELIUS</i> IN RECIRCULATING AQUACULTURE SYSTEMS	Roger Williams University
p12	Joshua Reitsma	SHELLFISH NITROGEN CONTENT FROM COASTAL WATERS OF SOUTHEASTERN MASSACHUSETTS	Cape Cod Cooperative Extension & Woods Hole Sea Grant
p13	Jennifer Savicky	PHYSIOLOGICAL EFFECTS OF ORGANIC NUTRIENTS, TEMPERATURE AND PHOTOPERIOD ON <i>GRACILARIA TIKVAHIAE</i> , MCLACHLAN (GRACILARIALES, RHODOPHYTA)	University of Connecticut
p14	Ariel Smith	THE EFFECTS OF INCREASED ACIDITY ON LIPID STORES IN <i>MYTILUS EDULIS</i>	Bridgeport Regional Aquaculture Science and Technology Education Center
p15	Sheila Stiles	DEVELOPMENTAL AND GENETIC EVALUATION OF PH EFFECTS ON SELECTED LINES OF BAY SCALLOPS	NOAA, Milford Laboratory
p16	Michael Taylor	PATHOGENIC <i>VIBRIO</i> OCCURRENCE IN NORTHEAST US SHELLFISH AND STRATEGIES FOR THEIR REMOVAL	University of New Hampshire
p17	Ami Wilbur	PERFORMANCE EVALUATION OF NEWLY ESTABLISHED NORTH CAROLINA OYSTER ( <i>CRASSOSTREA VIRGINICA</i> ) LINES UNDER AQUACULTURE CONDITIONS	UNCW Shellfish Research Hatchery
p18	Sarah Woolley	EFFECTS OF DIFFERENT HARVEST RATES ON THE POPULATION DYNAMICS OF THE CALANOID COPEPOD <i>PARVOCALANUS SP.</i>	Roger Williams University

## Tuesday, February 25th

	8:00-8:30	Continental Breakfast		
	8:30	Chris Brown & Walter Blogoslawski	WELCOME AND OPENING REMARKS	NOAA, Milford Laboratory
p19	8:45-9:15	Michael Rust	ARE WE DOING THE RIGHT RESEARCH RIGHT?	NOAA, Office of Aquaculture
p20	9:15	Tessa Getchis	THE CONNECTICUT SHELLFISH INITIATIVE	Connecticut Sea Grant/UCONN Extension
p21	9:30	Robert Rheault	THE EAST COAST SHELLFISH GROWERS ASSOCIATION CELEBRATES TEN YEARS OF ADVOCATING FOR THE SHELLFISH AQUACULTURE INDUSTRY	East Coast Shellfish Growers Association
p22	9:45	Gef Flimlin	COMMUNITY SUPPORTED AGRICULTURE: ADDING SEAFOOD TO THE RECIPE. A TWO YEAR PILOT PROJECT ANALYSIS	Rutgers Cooperative Extension
	10:00-10:30	Break		
p23	10:45	Nathaniel Mulcahy	CULTIVATED SHELLFISH BASED CARBON OFFSETS: MITIGATING CLIMATE CHANGE, IMPROVING THE ENVIRONMENT AND PROVIDING ECONOMIC INCENTIVES FOR GROWERS	WorldStove/MeasurableOffsets
p24	11:00	John Brawley	A STUDY OF THE EFFECTS OF CULTURED VS. WILD OYSTERS (CRASSOSTREA VIRGINICA) ON NITROGEN REMOVAL AND ECOSYSTEM ENHANCEMENT IN TWO SIMILAR EUTROPHIC ESTUARIES ON CAPE COD, MA	Woods Hole Group, Inc.
p25	11:15	Gary Wikfors	WHAT WE LEARNED GROWING RIBBED MUSSELS AT HUNTS POINT	NOAA, Milford Laboratory
p26	11:30	Mason Silkes	PRACTICAL MUSSEL FARMING IN SOUTHERN NEW ENGLAND	American Mussel Harvesters
p27	11:45	Scott Lindell	DO MUSSEL FARMS NEED MUSSEL HATCHERIES?	Marine Biological Laboratory
p28	12:00	Edward (Ted) Maney	AN UPDATE ON ESTABLISHING AN OFFSHORE MUSSEL FARM IN FEDERAL WATERS IN THE GULF OF MAINE	NEMAC Cat Cove Marine Lab
	12:15-1:45	Lunch		
p29	1:30	Joseph Choromanski	GROWTH STUDY OF BAY SCALLOPS IN A FLOATING UPWELLER SYSTEM	NOAA, Milford Laboratory
p30	1:45	Nathaniel Mulcahy	IMPROVED TECHNIQUES FOR DEPURATION AND DEGRITTING OF ENSIS DIRECTUS	WorldStove/MeasurableOffsets
p31	2:00	Lisa Calvo	NATURAL HISTORY AND CONTROL OF THE MUD WORM, POLYDORA CORNUTA ON A DELAWARE BAY, NEW JERSEY OYSTER FARM	Haskin Shellfish Research Laboratory, Rutgers University
p32	2:15	Jason Krumholz	MEASURING CHANGES IN TOTAL PHYTOPLANKTON-SIZED PARTICLE VOLUME OVER TIME AS A PROXY FOR THE PRIMARY PRODUCTION AND FOOD AVAILABILITY IN NARRAGANSETT BAY, RI	NOAA, Milford Laboratory

p33	2:30	Yaqin Judy Li	THE IMPORTANCE OF CONSIDERING PRIMARY PRODUCTIVITY IN BIVALVE AQUACULTURE SITE SELECTION – AN EXAMPLE FROM THE BRONX, NEW YORK	NOAA, Milford Laboratory
p34	2:45	Shannon Meseck	IS THERE A LINK BETWEEN SHELLFISH DREDGING, CARBONATE CHEMISTRY, AND BIVALVE SETTLEMENT ON THE SURFACE OF MARINE SEDIMENTS?	NOAA, Milford Laboratory
	3:00-3:15	Break		
p35	3:15	Mark Dixon	MOVING BIODEPOSITION MEASUREMENTS OF SHELLFISH FEEDING FROM A SHORE-BASED TO A SHIPBOARD TECHNOLOGY	NOAA, Milford Laboratory
p36	3:30	Barry Smith	DAY AND NIGHT PH CYCLING OF ALGAL CULTURES; WHAT OUR ALGAE ARE DOING WHILE WE SLEEP	NOAA, Milford Laboratory
p37	3:45	Cori Rose	COLLABORATING TO DEVELOP A REGULATORY PROCESS FOR SEAWEED AQUACULTURE IN CONNECTICUT	US Army Corps of Engineers
p38	4:00	Jianheng Zhang	THE WORLD'S LARGEST MACROALGAE BLOOMS IN THE YELLOW SEA, CHINA: EVALUATION OF BIOLOGICAL BLOOM PROCESSES	Shanghai Ocean University
	4:15	Adjourn		

### Wednesday, February 26th

	8:00-8:30	Continental Breakfast		
p39	8:30	Genevieve Bernatchez	NUTRITIONAL ANALYSIS OF STORE-BOUGHT SCALLOPS FROM DIFFERENT COUNTRIES	NOAA, Milford Laboratory
p40	8:45	Derrick Chelikowsky	PAVLOVOLS, STEROLS UNIQUE TO THE GENUS PAVLOVA, ARE THE BIOACTIVE COMPOUNDS THAT INDUCE METAMORPHOSIS IN BAY SCALLOP PEDIVELIGERS FED CULTURED PAVLOVA	NOAA, Milford Laboratory
p41	9:00	April Croxton	SEASONAL VARIATION IN IMMUNOLOGY OF LONG ISLAND SOUND BIVALVE SPECIES	NOAA, Milford Laboratory
p42	9:15	Thomas Howell	SEASONAL DYNAMICS OF MALE SPECIFIC COLIPHAGE AND ENTERIC VIRUSES IN BIVALVE SHELLFISH WITHIN WASTEWATER TREATMENT FACILITY SAFETY ZONES	Spinney Creek Shellfish Inc.
p43	9:30	Marta Gomez-Chiarri	DISEASE MANAGEMENT STRATEGIES FOR SHELLFISH AQUACULTURE: THE IMPORTANT ROLE OF HATCHERIES	University of Rhode Island
p44	9:45	Inke Sunila	THE FIRST COMPREHENSIVE SURVEY FOR MSX, <i>HAPLOSPORIDIUM NELSONI</i> , IN CONNECTICUT	State of Connecticut, Bureau of Aquaculture
	10:00-10:30	Break		
p45	10:30	Gabriel Geist	PREVALENCE OF DISEASE, GROWTH ABNORMALITIES AND TISSUE METAL CONTENTS IN EASTERN OYSTERS ( <i>CRASSOSTREA VIRGINICA</i> ) ALONG THE CONNECTICUT COASTLINE	The Sound School
p46	10:45	Michael Rice	PROGRESS IN ESTABLISHING A GAMBIAN NATIONAL SHELLFISH SANITATION PROGRAM (GNSSP)	University of Rhode Island
p47	11:00	Diane Kapareiko	DISCRIMINANT ANALYSIS OF OYSTER HEMOCYTE IMMUNE FUNCTIONS AS A PREDICTOR OF POTENTIAL PROBIOTIC CANDIDATES	NOAA, Milford Laboratory
p48	11:15	Devon Marsden	USDA- FARM SERVICE AGENCY	USDA- Farm Service Agency
p49	11:30	Rob Cerda	AQUACULTURE AND CROP INSURANCE	Crop Insurance Systems
	11:45-1:00	Lunch		
		<i>Joint MAS/NESSA Session on Vibrio</i>		
	1:00	Dorothy Jean McCoubrey	BASICS OF <i>VIBRIO</i> BIOLOGY, NATIONAL VP SUMMARY AND FDA'S 2013 <i>VIBRIO</i> STUDIES	US FDA
p50	1:30	Kristin DeRosia-Banick and Michael Hickey	REGIONAL OVERVIEW OF THE 2013 <i>VIBRIO PARAHAEMOLYTICUS</i> SEASON	CT Department of Agriculture, Bureau of Aquaculture; MA Division of Marine Fisheries

p51	1:45	Laura Wigand	WASHINGTON STATE OVERVIEW OF 2013 <i>VIBRIO</i> SEASON AND RETROSPECTIVE	Washington State Department of Health
	1:55	Bob Rheault	ECSGA PERSPECTIVES	ECSGA
	2:05	Ken Moore (or ISSC designee)	INTERSTATE SHELLFISH SANITATION CONFERENCE AND 2013 BIENNIAL MEETING <i>VIBRIO</i> ACTIONS	ISSC
p52	2:15	Feng Xu	PATHOGENIC <i>VIBRIO PARAHAEMOLYTICUS</i> STRAINS AND TOTAL POPULATION DYNAMICS IN NEW ENGLAND SHELLFISH HARVEST AREAS	University of New Hampshire
p53	2:30	Roxanna Smolowitz	IDENTIFICATION OF <i>VIBRIO</i> SP. ABUNDANCE IN CULTURED OYSTERS FROM NORTHEAST U.S. FARMS	Roger Williams University
p54	2:45	Steven Pitchford	MOLECULAR CHARACTERIZATION OF <i>VIBRIO VULNIFICUS</i> ISOLATED FROM EASTERN OYSTERS ( <i>CRASSOSTREA VIRGINICA</i> ) FROM THE NORTHEASTERN COAST OF THE US AND COMPARISON WITH CLINICAL STRAINS	NOAA, Milford Laboratory
p55	3:00	Kathryn Markey	VARIATIONS IN DATA: THE IMPORTANCE OF SAMPLE PREPARATION AND PROCESSING PROTOCOL OF OYSTER HOMOGENATES FOR THE DETECTION OF <i>VIBRIO PARAHAEMOLYTICUS</i> AND <i>VIBRIO VULNIFICUS</i> IN RHODE ISLAND AND MASSACHUSETTS SAMPLES	Roger Williams University
p56	3:15	Carmelo del Castillo	A COMPARATIVE GENOMICS ANALYSIS OF THE T3SS2 $\alpha$ PATHOGENICITY ISLAND OF <i>VIBRIO PARAHAEMOLYTICUS</i>	Stony Brook University
	3:30-3:45	Break		
	3:45-4:45	<p>PANEL DISCUSSION</p> <p><i>Michael J. Hickey, MA Dept. of Marine Fisheries, Moderator</i></p> <p>Kristin DeRosia-Banick, State of Connecticut, Bureau of Aquaculture  Tessa Getchis, Connecticut Sea Grant/UCONN Extension (p57)  Steve Jones, University of New Hampshire  Dorothy Jean McCoubrey, FDA  Meghan Maloney, CT Dept of Public Health  Ken Moore (or ISSC designee), ISSC  Bob Rheault, ECSGA</p>		
	4:45	Adjourn		

## **LIBERIA AND NORTHEASTERN UNITED STATES AQUACULTURE INITIATIVE**

**Joseph Buttner, Lawrence Gleekia, Thomas Pedulla, Nadine Budrow**

Northeastern Massachusetts Aquaculture Center, Cat Cove Marine Laboratory and Salem State University, Department of Biology, Salem State University, Salem, MA 01970 USA.

Faculty and students from Salem State University (SSU) were recently invited by Liberia to assist with nurturing a sustainable peace. After a brutal, long civil war that ended in 2003, Liberia has entered into a fragile peace. In 2012, acknowledging the potential of a viable, sustainable aquaculture industry to produce critically needed animal protein, jobs and income, we were asked to assist with resurrecting aquaculture expertise and capacity lost during the civil war. Initial efforts have targeted the Ganta Mission where aquaculture had been pursued extensively. In 2006, mission personnel dammed a small stream and created a pond as a water reservoir to irrigate crops. By 2008, mission personnel had observed fish in the pond. Commencing in 2008 an annual harvest has occurred that yields approximately 50 lb of fish. In 2012, we met with our counterparts at the Ganta Mission and observed the pond, more accurately a swamp. It quickly became obvious that we could work together and greatly increase fish production. In 2013, SSU personnel returned and assisted with pond harvest, identifying and quantifying fish collected. The site was surveyed and staked so two additional ponds could be constructed (0.41 and 0.44 acres). A work plan was cooperatively crafted. The plan has been modified, submitted and approved by the Fulbright Program. We anticipate returning this year to assist with pond construction, to instruct locals on pond management, and to move toward a nation-wide aquaculture initiative.

**EFFECTS OF STOCKING DENSITY AND CULTURE VOLUME ON POPULATION DYNAMICS OF *PARVOCALANUS SP.* COPEPOD CULTURES**

**Carter Cyr, Pierce Howell, Bradford Bourque, David Cerino, Andrew Rhyne**

Roger Williams University, Center for Economic and Environmental Development, One Old Ferry Rd, Bristol, RI 02809 USA.

Rearing early life stages of marine fishes requires a suitable feed source. Almost universally, a live, natural food source is considered optimal. *Parvocalanus sp.* copepods are considered a premier larval feed for a wide range of aquacultured species due to their small size and nutritional value. In this study, the effects of initial stocking density and culture volume on the population dynamics of *Parvocalanus sp.* copepods in laboratory cultures were investigated. This project was segregated into two parts. First, five variants of initial stocking density were tested for their effects on population dynamics of *Parvocalanus sp.* copepods. Next, two variants of culture volume were tested for their effects on population dynamics of *Parvocalanus sp.* copepods.



**DEVELOPMENT OF A RESTOCKING SYSTEM FOR STAGE IV *HOMERUS AMERICANUS***

**Matthew Dicostanzo<sup>1</sup>, Holly Turner-Moore<sup>1</sup>, Ronald Goldberg<sup>2</sup>, John Roy<sup>3</sup>, Kirk Shadle<sup>1</sup>**

<sup>1</sup>Bridgeport Regional Aquaculture Science and Technology Education Center, 60 Saint Stephens Rd, Bridgeport, CT 06605 USA. <sup>2</sup> U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA. <sup>3</sup> Sound School, 60 South Water St, New Haven, CT 06519 USA.

The decline of the lobster, *Homarus americanus*, in Long Island Sound has crippled the commercial lobster industry. This decline has led to a moratorium of all commercial harvesting despite management efforts such as the Connecticut V-notch Program. Developing a lobster re-stocking technique could prove to be instrumental in any restoration effort. On site acclimation is the one key factor to address for successful lobster re-stocking. To accomplish this acclimation phase, the stage IV lobsters are individually placed in grow out tubes. The tubes are constructed by attaching two 10cm diameter PVC end caps on to the ends of a 14cm long tube of rigid mesh. The tubes are then placed into an enclosed cage and deployed in a depth of six to nine meters of water. In addition to providing an ease of access, the tubes prevent predation, escaping of lobsters, and provide a natural biofouling food source for lobsters to consume. Preliminary data suggest that when stage IV lobsters are put into these tubes, if provided with a gel diet as initial supplement to biofouling, the lobster will have an initial 80% chance of survival. Visual data show that three to four weeks post deployment, lobsters transition from a light blue color, to a red color, indicating the consumption of the natural sourced biofouling has begun. Use of biofouling tubes provides the opportunity for the lobsters to acclimate to a given environment months before they are released. Ultimately, these lobsters will have a greater chance of survival after release.

## **MODULAR LARVAL REARING SYSTEM FOR INTEGRATING AQUACULTURE INTO PUBLIC AQUARIA**

**Timothy Gerson, Bradford Bourque, David Cerino, Andrew Rhyne**

Roger Williams University, Center for Economic and Environmental Development, One Old Ferry Rd, Bristol, RI 02809 USA.

Public aquaria maintain large collections of fish that are primarily acquired through wild capture. These institutions also often promote environmental conservation as a primary goal. The display exhibits at these institutions are maintained to provide optimal water quality and nutrition for the fish populations. Subsequently, these fish volitionally spawn regularly in captivity. The goal of this project is to provide institutions with a uniform system for raising the offspring to provide an aquacultured alternative to wild caught specimens. The Modular Larval Rearing System (MoLaRS) was developed to streamline and simplify fish production, minimizing space and labor, which are at a premium in aquarium facilities. MoLaRS utilizes recirculating technology for solids capture and removal of nitrogenous wastes. Innovative techniques for live feed management and system hydraulics minimize the labor required for system operation. At Roger Williams University, we demonstrated successful production of settled juvenile fish through the use of MoLaRS. Some of the eggs were collected from public aquarium exhibits, while others were collected from our own broodstock. In a three-month period we were able to rear seven different tropical and temperate fish species, from pelagic and demersal eggs with varying environmental and nutritional requirements. MoLaRS have been constructed and shipped to public aquaria all over the United States and are currently being used to attempt to raise fish species from eggs collected from their own exhibits. MoLaRS have the versatility and potential to raise less challenging species, as well as species never before raised in captivity.

## **DEVELOPMENT OF THE APPLICATION OF *MYTILUS EDULIS* BYSSAL THREADS AS A BIOLOGICAL THREAD**

**Leana Gomes, Kirk Shadle**

Bridgeport Regional Aquaculture Science and Technology Education Center, 60 Saint Stephens Rd, Bridgeport, Ct 06605 USA.

Blue Mussels thrive in the ocean due to an extracellular connective structure called the *byssus*, which is a collection of fibers that adhere to most surfaces. The mussel byssus of *Mytilus edulis*, commonly known as blue mussels measures about 2-5 centimeters, cm, in length and are 50 micrometers,  $\mu\text{m}$ , in diameter. The distal end of each byssal thread attaches to a rock, while the proximal end joins to the living tissue of the mussel. Each byssus has a fibrous core covered by a thin protective cuticle and self-assembles from an ensemble of precursor proteins in the groove of the mussel foot. The application of *Mytilus edulis* byssal threads as a biological thread would be a substantial advancement in the medical and fishing industry. Instead of using the standard stapling or sowing method to stitch patients, doctors could use a thin, pliable thread that would retain its strength. Moreover, if multiple threads were bound together, they could be used for rope in the fishing industry. In this experiment, for a test trial of the mussel suspension, 10 mussels were suspended for about 3 days and about 4 mussels still remained suspended 6 centimeters, cm, above the bottom of the tank. In the second trial, only 4 mussels were suspended for days and 3 mussels remained suspended. In upcoming experimentation, about 3 grams, g, of dry byssal threads will be incubated in a 5 ppm, 10 ppm, and 15 ppm solution of ethylenediamine tetraacetic acid (EDTA) for 24 hours. Furthermore, 3 g of byssal threads will be incubated in a 5ppm, 10 ppm, and 15 ppm solution of Biotin D for 24 hours. Three grams of byssal threads will also be incubated for 24 hours in a 5 ppm, 10 ppm, and 15 ppm solution containing both Biotin D and EDTA. Biotin D treated byssal threads, EDTA treated byssal threads, Biotin and EDTA treated byssal threads, and untreated byssal thread structures will be compared using a Scanning Electron Microscope. The tensile strength of each treated byssal thread will be compared using stress-strain curves.

## ASSESSING THE CONTRIBUTION OF AQUACULTURE AND RESTORATION TO WILD OYSTER POPULATIONS IN RHODE ISLAND

**Hannah Jaris<sup>1</sup>, David Steven Brown<sup>2</sup>, Marta Gomez-Chiarri<sup>3</sup>, Dina Proestou<sup>4</sup>**

<sup>1</sup> Columbia University, 4 Douglass Street Apt 2R, Brooklyn, NY 11231 USA. <sup>2</sup> The Nature Conservancy, University of Rhode Island Narragansett Bay Campus, Narragansett, Rhode Island 02882 USA. <sup>3</sup> University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, Rhode Island 02881 USA. <sup>4</sup> USDA, Shellfish Genetics Program, Kingston, Rhode Island 02881 USA.

The decline of the eastern oyster (*Crassostrea virginica*) has led to renewed interest in restoration and aquaculture efforts. Recent field surveys suggest that wild populations in Rhode Island are increasing, yet the factors contributing to expansion are unknown. We used molecular tools to determine if the expansion of wild populations is a result of self-seeding or a consequence of recruitment from nearby aquaculture and restoration sites. Samples were collected from eight populations within a large coastal lagoon highly influenced by human activity. Three “wild” populations located varying distances from restoration and aquaculture activities were compared to two restored and three aquaculture populations. A ninth population, collected from a site free from intense human activity was included to serve as a control. Adult oysters (Shell Height  $\geq 75$  mm; n=30) collected in early June from each of the nine populations, and oyster spat (SH  $\leq 25$  mm; n=30) collected in late September from the four wild populations were genotyped at 14 microsatellite loci. From the multilocus genotype data we measured the extent of variation within and differentiation among populations by estimating a number of genetic parameters. Assignment tests were also used to assign spat to source populations. These analyses will provide a better understanding of the factors responsible for the enhancement of wild oyster populations in Rhode Island coastal ponds. Furthermore, knowledge of how specific human activities contribute to the genetic diversity of standing wild oyster stocks can help evaluate the success of restoration and aquaculture activities.

**CONDITION INDEX OF NORTHERN QUAHOGS, *MERCENARIA MERCENARIA* (LINNAEUS, 1758), IN NARRAGANSETT BAY IN RELATION TO SEDIMENT TYPES**

**Eilea Knotts<sup>1</sup>, Matthew Griffin<sup>2</sup>, Michael Rice<sup>3</sup>**

<sup>1</sup>Department of Biological Sciences, University of Rhode Island, 9 East Alumni Ave, Kingston, RI 02881 USA. <sup>2</sup>Center for Environmental & Economic Development, Roger Williams University, 1 Ferry Rd., Bristol, RI 02809 USA. <sup>3</sup>Department of Fisheries, University of Rhode Island, 9 East Alumni Ave., Kingston, RI 02881 USA.

To determine the effect of sediment types on the condition of quahogs in areas open or closed to fishing in Narragansett Bay, sediment grain size was compared to the gravimetric conditional index (CI) of quahogs at nine sites (n=5/site) collected September, 2013. Sediments were separated by standard sieve analysis and categorized into sediment types (gravel, sand, mud) by component fraction weight percentages. These component percentages were compared to the CI of quahogs collected from each site. Significant differences in sediment percentages of gravel and sand ( $p < 0.01$ ) along with sand and mud ( $p < 0.01$ ) were found among all sites using Wilcoxon Rank Sum non-parametric statistics. Multiple regression analyses showed that percentage of mud is most strongly correlated with decreasing CI as the percentage of mud increases, with a negative linear correlation of  $r^2 = 0.68$  ( $p < 0.1$ ). When fishing statuses of the nine sites (open, conditional, closed) were correlated with sediment grain sizes, there was a significant difference between closed and conditional sites ( $p < 0.05$ ). With the exception of Bissel Cove (a muddy site that is open to fishing), open fishing sites have the least amount of mud percentage; closed sites had the highest. Possible explanations for decreased mud percentage in open sites could include sediment disturbance due to shellfishing activity or simply that most closed sites are coincidentally in relatively confined coves that experience lower average tidal currents that favor deposition of finer-grained sediments. Mud sediments may be a proxy for lower current speed and therefore lower potential food availability leading to decreased CI.

**THE TREMATODE *PROCTOECES MACULATUS* IN THE BLUE MUSSEL *MYTILUS EDULIS*: MOLECULAR IDENTIFICATION, PREVALENCE AND INTENSITY**

**Kelly Markowitz, Jason Williams, Maureen Krause**

Hofstra University, Department of Biology, Hofstra University, Hempstead, NY 11549 USA.

The digenetic trematode *Proctoeces maculatus* is a pervasive parasite of numerous mollusc and fish species. Infections of sporocyst stage *Proctoeces maculatus*, predominately found in the mantle tissue of mollusc hosts such as *Mytilus edulis*, are highly variable. Intensity can be as low as one sporocyst per mussel, while in others the mantle is saturated with sporocysts. Trematode species have been historically identified and classified using morphological data. However, molecular data have been more recently used to determine the phylogeny of digenetic trematodes using rDNA sequencing. Specifically, genetic data on the genus *Proctoeces* have led to reclassification of some species within the genus. The goals of this research are to determine prevalence and intensity of *Proctoeces maculatus* in *Mytilus edulis* from Long Island and confirm the identification of the trematode species through sequencing of the 18S and 28S rDNA. Preliminary findings have shown highly variable parasitization of mantle tissue up to 350 sporocysts/100mm<sup>2</sup>. Additionally, current sequencing results cannot confirm the parasite as *Proctoeces maculatus*, and may support classification of *Proctoeces maculatus* as a species complex.

**A FIELD-BASED NURSERY SYSTEM TO “HEAD START” LOBSTERS FOR IMPROVED POST-RELEASE SURVIVAL IN POTENTIAL STOCK ENHANCEMENT EFFORTS: A PROGRESS REPORT**

**Renee Mercaldo-Allen<sup>1</sup>, Ronald Goldberg<sup>1</sup>, Catherine Kuopat<sup>1</sup>, Paul Clark<sup>1</sup>, John Roy<sup>2</sup>**

<sup>1</sup>U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA. <sup>2</sup>The Sound School, 60 South Water Street, New Haven, CT 06519 USA.

Early benthic stage lobsters, raised by students from The Sound School, were used to test an experimental nursery system for field grow-out of lobsters as a step toward potential stock enhancement efforts. Postlarval lobsters were individually placed in perforated plastic mesh cylindrical “habitats”; each habitat was housed in an outer protective sleeve with a larger mesh size to facilitate adequate water flow. During June 2013, twenty-four lobsters contained in habitats were transferred into each of 3 metal cages and deployed southwest of Charles Island in Milford, CT. Cages were hauled monthly and lobsters photographed to measure changes in carapace length (CL) using Image J software. Lobsters consumed a naturally occurring diet and many doubled in size over the period June through October 2013, with 75% survival. Culture of lobsters to a larger size may increase the probability of survival during restocking efforts. Lobsters will remain in the field through the winter. In spring 2014, surviving lobsters of 12 mm CL or larger will receive coded-wire tags and be released on a nearby cobble reef. Head-starting of early benthic stage lobsters shows promise as a tool for future stock enhancement in Long Island Sound.

**VIBRIO PARAHAEMOLYTICUS AND SHELLFISH IN SOUTHEASTERN MASSACHUSETTS**

**Diane Murphy, Joshua Reitsma, Abigail Archer**

Woods Hole Sea Grant & Cape Cod Cooperative Extension, PO Box 367, Barnstable, MA 02630 USA.

*Vibrio parahaemolyticus* (Vp) is a naturally occurring, halophilic bacterium that commonly inhabits coastal marine waters. Its growth is regulated by temperature and illnesses are typically associated with consuming raw shellfish harvested from warmer waters. Illness can be prevented by thoroughly cooking shellfish and risk minimized by keeping oysters and clams at a temperature below 50° F after harvest. In recent years Vp has been an emerging concern to the local commercial shellfish industry and in 2007, vibriosis (i.e. Vp illness) was identified as a nationally reportable illness to the Centers for Disease Control and Prevention (CDC). While the bacterium is not new, the reporting of increased incidence rates has prompted the Food and Drug Administration to update shellfish handling requirements. In 2012 the Massachusetts Division of Marine Fisheries (DMF) developed a “*Vibrio* (Vp) Control Plan” which they later modified for 2013. In response to increased shellfish management regulations and uncertainties between the correlation of Vp levels and oysters, studies were initiated in southeastern MA to examine Vp levels in oysters during time-temperature treatments. Studies included 1) *Vibrio Seasonality Study*; oysters were sampled at 4 time periods representative of seasonal changes in air & water temperatures throughout 2013. Samples included oysters immediately iced as well as samples left at ambient temps for 5 hours before refrigeration. 2) *Vibrio Time Interval Study*; examined *Vibrio parahaemolyticus* levels in oysters removed from the water for 5 hours as well as Vp levels in oysters after 1, 5, and 19 hours back in the water.



**A RECIPE FOR SUCCESSFUL CULTURE OF FIRE SHRIMP, *LYSMATA DEBELIUS* IN RECIRCULATING AQUACULTURE SYSTEMS**

**Alisha Patel, Melanie Fuoco, Bradford Bourque, David Cerino, Andrew Rhyne**

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Fire shrimp, *Lysmata debelius* are a highly prized marine ornamental species and are listed as one of the top invertebrates sold in the aquarium trade. Wild capture of Fire shrimp in Sri Lanka is the primary source to the aquarium trade and the population is likely over-exploited. Aquaculture may be a solution to reduce fishing pressure. Methods for the aquaculture of Fire shrimp have been previously developed. Some challenges presented by Fire shrimp are the long larval stage and their aggressive behavior as juveniles during grow-out. We describe the system and methods that produced 934 juvenile Fire shrimp with a survival rate of 72% from day 2 through settlement (day 55-90). This represents the highest published survival rates for larval Fire Shrimp. We evaluated the onset of aggressive behavior among juvenile shrimp and investigated the use of separate enclosures to reduce the mortality from aggressive behavior. We tested the effects of holding space surface area and volume, in addition to stocking density upon the growth and survival of juvenile Fire shrimp. Finally, we provide guidelines for improved Fire shrimp aquaculture.

## **SHELLFISH NITROGEN CONTENT FROM COASTAL WATERS OF SOUTHEASTERN MASSACHUSETTS**

**Joshua Reitsma, Diane Murphy, Abigail Archer**

Cape Cod Cooperative Extension & Woods Hole Sea Grant, PO Box 367, Barnstable, MA 02630 USA.

As nitrogen entering coastal waters of Cape Cod continues to be an issue, much attention has been generated to identify potential options which may help alleviate this stressor to estuaries. With Cape Cod's long history of shellfish harvest, the propagation or culture of filter feeding bivalves represents a unique opportunity to potentially alleviate a growing problem and also generate economic activity. Recognizing the importance of locally derived information, legal sized oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) from various Cape Cod area sources were recently tested for nitrogen content stored in tissues which would represent a net removal from a water body if harvested. Local results were similar to literature values for oysters averaging 0.69% nitrogen by total dry weight (0.28gN/animal), while quahogs were slightly higher than values in the literature averaging 0.67% nitrogen by total dry weight (0.22gN/animal). These values did vary by season (spring less than fall) and to a lesser extent by location or grow out method. The biggest driver of difference in N content among similarly sized shellfish cohorts was the mass of shell or meat tissue contained as opposed to the percent nitrogen in the tissues. The dry weight of meat tissue increased from spring to fall for both quahogs (63%) and oysters (98%), and wild or bottom grown oysters had heavier shells (46g and 47g respectively) than those grown off bottom (36g). Nitrogen isotope data indicate shellfish from certain water bodies in the region are incorporating significant amounts of nitrogen from anthropogenic sources.

**PHYSIOLOGICAL EFFECTS OF ORGANIC NUTRIENTS, TEMPERATURE AND PHOTOPERIOD ON *GRACILARIA TIKVAHIAE*, MCLACHLAN (GRACILARIALES, RHODOPHYTA)**

**Jennifer Savicky<sup>1</sup>, Jang Kim<sup>2</sup>, Scott Lindell<sup>3</sup>, Charles Yarish<sup>1</sup>**

<sup>1</sup>Department of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT, 06901 USA. <sup>2</sup>Department of Marine Sciences, University of Connecticut, 1 University Place, Stamford, CT, 06901 USA. <sup>3</sup>Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543 USA.

Inorganic nutrients have been primarily used in land based *Gracilaria* cultivation systems. We compared an organic fertilizer to inorganic fertilizer for *G. tikvahiae* culture which might enable it to receive an organic food designation. *G. tikvahiae* was cultured at 20 °C and 25 °C in conjunction with long day (16:8 L:D) and neutral (12:12 L:D) photoperiods. The environmental conditions were the following: 20 °C with a long day photoperiod, 20 °C with a neutral photoperiod, and 25 °C with a long day photoperiod. Concentrations of the organic nutrients used were 250, 500 and 1000 µM of nitrogen in comparison to 500 µM of nitrogen supplied by the inorganic fertilizer. *G. tikvahiae* exhibited a dark appearance and a higher growth rate with the addition of organic fertilizer in comparison to inorganic fertilizer. By week four, the average growth rate of *G. tikvahiae* in all temperatures and photoperiods with the inorganic fertilizer was 6.48% for 500 µM N. The *G. tikvahiae* grown in the organic fertilizer by week four in all temperatures and photoperiods had a growth rate of 7.54% for 250 µM N, 10.33% for 500 µM N, and 12.45% for 1000 µM N. In weeks three and four, there was a significant color difference seen in the *G. tikvahiae* grown in the organic fertilizer. The 250µM N was bleached, while the 500 µM N and 1000 µM N were a dark color. If *Gracilaria* is grown in an organic fertilizer it may qualify as “organic” for human consumption.

## **THE EFFECTS OF INCREASED ACIDITY ON LIPID STORES IN *MYTILUS EDULIS***

**Ariel Smith, Christian Foito, Kirk Shadle**

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*Mytilus edulis* (blue mussels) lipid stores are used as long term energy storage and are important in life processes such as over winter survival. The typical pH of Long Island Sound is 7.4 and due to ocean acidification, this pH has the potential to decrease. The lipid stores of *Mytilus edulis* may be affected due to this potential pH fluctuation. To quantify this potential impact, lipid stores of *Mytilus edulis* will be investigated. Nine tanks will be set up to hold the mussels in different acidic environments. Three different pHs will be tested in triplicate. Ten mussels, roughly uniform in size and shape will be placed in each tank, and exposed to pHs of 7.4 (control: standard pH of Long Island Sound), 7.0, and 6.6. The mussels will be exposed to these conditions for two weeks. To determine the change in lipid stores, high performance liquid chromatography (HPLC) will be used. A conclusion will be made based on the amount of lipid the mussels have stored. It is hypothesized that a more acidic environment will result in a difficulty to store lipids over the winter. This experimentation may be indicative of the future as the effects from ocean acidity continue to create a more acidic environment for all sea life. If test results show that lipid production is affected, it could mean permanent and inevitable harm to the *Mytilus edulis* population.

**DEVELOPMENTAL AND GENETIC EVALUATION OF PH EFFECTS ON SELECTED LINES OF BAY SCALLOPS**

**Sheila Stiles, Joseph Choromanski, Dorothy Jeffress**

U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA.

Climate change in atmospheric CO<sub>2</sub> levels over extended periods has contributed to alterations in seawater chemistry, such as lowered pH and calcium carbonate. Economically and ecologically valuable bivalve shellfish like the bay scallop (*Argopecten irradians*), which require calcium carbonate for shell formation, are reported to be especially vulnerable to these changes. Moreover, critical stages in early development from fertilization to larvae appear to be less tolerant of lower pH than later stages. Fertilized eggs and early embryos from two genetic lines of bay scallops selected for growth were exposed for 48 hours to ambient (pH= 7.9– 8.2) and low pH (pH= 7.1 – 7.3) by bubbling CO<sub>2</sub> in triplicate cultures in preliminary experiments to evaluate viability and genetic effects on early development and survival. Results from viability and cytogenetic appraisals demonstrated a significant effect of virtually no development or survival at the end of 48 hours in the low pH seawater and some development in the control of ambient pH seawater. Cytogenetic evaluation at 3 hours post-fertilization revealed more embryos with chromosomal and nuclear abnormalities in the low pH exposure than embryos in the ambient pH seawater. Additional trials are planned.

## **PATHOGENIC *VIBRIO* OCCURRENCE IN NORTHEAST US SHELLFISH AND STRATEGIES FOR THEIR REMOVAL**

**Michael Taylor<sup>1</sup>, Thomas Howell<sup>2</sup>, Steve Jones<sup>3</sup>**

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The shellfish industry in the Northeast US has suffered the consequences of increasingly more frequent *vibrio*-associated disease cases and outbreaks linked to shellfish consumption. *Vibrio* diseases are an important emerging issue. Shellfish harvest timing and post-harvest handling need to be optimized to ensure safety for consumers and economic viability for the shellfish industry. The purpose of this presentation is to provide information on the occurrence and varying population levels of pathogenic *Vibrio* species and potential management strategies to reduce risk in Northeast shellfishing areas. Concentrations of *Vibrio parahaemolyticus* (Vp), *Vibrio vulnificus* (Vv) and *Vibrio cholerae* (Vc) in the oysters, sediments, and water were determined using the 3-tube MPN enrichment method coupled to a cultured based confirmation assay in conjunction with a genetic marker based qPCR. All *Vibrio* species varied seasonally and annually with peaks in warm summer months, yet species differed in detection frequency and duration from January to December. Concentrations are correlated with temperature and other environmental factors. Relaying from lower to higher salinity areas shows promise for decreasing *Vibrio* levels in harvested oysters, with evidence of biological mechanisms for *Vibrio* elimination. This information can inform *Vibrio* surveillance design for other areas in the Northeast and serve as a basis for minimizing risks in harvested shellfish. Ongoing research seeks to better understand the microbial community and physico-chemical conditions that may enhance *Vibrio* removal from harvested shellfish, and the potential for prediction of conditions associated with increased risk associated with shellfish harvest timing and practices.

**PERFORMANCE EVALUATION OF NEWLY ESTABLISHED NORTH CAROLINA OYSTER (*CRASSOSTREA VIRGINICA*) LINES UNDER AQUACULTURE CONDITIONS**

**Ami Wilbur**

UNCW Shellfish Research Hatchery, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K Moss Lane, Wilmington, NC 28409 USA.

A line development program was initiated at UNCW's Shellfish Research Hatchery in 2012. Oysters derived from 3 wild populations (Crab Hole (CH), Stump Sound (SS) and Hewletts Creek (HC)) in North Carolina were deployed on 5 lease sites (Cedar Island (CI), Harkers Island (HI), Stump Sound (SS), Topsail Sound (TS) and Masonboro Sound (MS)) in February 2013. At three sites (HI, TS and MS) two selected lines (Hana and XB) from Virginia were also deployed. Performance (survival, growth, condition and shell shape) at all locations was assessed quarterly. At 18 months (11 months post-deployment), significant differences (ANOVA,  $p < 0.05$ ) in shell height among lines and across farms were observed. Of the NC lines, the HC and SS grew significantly faster than the CH on all but one farm (MS) and grew at similar rates as the Virginia lines. The fastest growth rates were observed in CI and were the lowest in MS. Survival also varied among lines, farms and quarter. With the exception of SS, the lowest survivals were noted in the spring quarter (41-63%) and were higher (>75%) for the summer and fall quarters. In SS, survival was high in the spring (>80% for all lines) but much lower in the summer (40-55%). While the slowest growing, the CH line exhibited significantly lower height to length ratios (less elongate shape) but was similar to the other lines with respect to the depth of the cup.

**EFFECTS OF DIFFERENT HARVEST RATES ON THE POPULATION DYNAMICS OF THE CALANOID COPEPOD *PARVOCALANUS SP.***

**Sarah Woolley, Bradford Bourque, David Cerino, Andrew Rhyne**

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The laboratory at Roger Williams University's (RWU) Center for Economic and Environmental Development raises many types of fish, such as Clownfish, Tautog, Cunner, Black Seabass, and Gobies for the purpose of production, research, and aquaculture education. The main food source for these larval fish are the nauplii of the calanoid copepod *Parvocalanus sp.* In order to maintain a sufficient amount of copepods to feed to the larval fish every day, we must retain sufficient productivity of copepods in established cultures. Previous studies have shown that reproductive rates slow as the copepod densities increase. In this study, nine 200 liter tanks of *Parvocalanus* copepods were established at their optimal stocking density and cultured using established methods determined by previous research at RWU. We compared the productivity of *Parvocalanus* cultures when the populations were harvested daily at 0%, 10%, and 30% of the tank volume. The population dynamics in each of the nine tanks was evaluated with daily visual counts of copepodite and nauplii density. The primary goal of this research is to establish methods to culture these calanoids stabilized at their peak level of productivity. Achieving optimal productivity and density in copepod cultures allows us to maximize our larval fish production with the fewest number of copepod tanks, increasing space and labor efficiency in the laboratory.



Tuesday, February 25<sup>th</sup>, 2014

## **ARE WE DOING THE RIGHT RESEARCH RIGHT?**

### **Michael Rust**

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NOAA's research plan indicates that we should be doing "use inspired" research - so what's the use? And where (and who) does the "use" come in? Scientists are trained in the scientific method to properly design and conduct studies that will lead to high quality objective knowledge about the topics we study (Doing research right). Much less attention is paid to teaching scientists what topics need study (Doing the right research), not to mention making sure that the high quality objective knowledge we develop gets to those who can use it (part of doing research right). Aquaculture research is without exception, focused on application of the findings. Support for aquaculture research ultimately comes from those who gain by application of the information developed by research (industry, regulators, politicians, bureaucrats, NGO's, seafood lovers and others). If they are not gaining, they will not be supporting. How do we develop research programs that are strategic and well supported especially when different user groups want different things, traditional resources are scarce, priorities change faster than labs can, good research takes time, and we have our own favorite rabbits to chase? While I am unlikely to provide all the answers, I hope to give a framework for thinking about strategic planning of research and provide some examples.

## **THE CONNECTICUT SHELLFISH INITIATIVE**

**Tessa Getchis<sup>1</sup>, Julie Rose<sup>2</sup>, Nancy Balcom<sup>1</sup>, Anoushka Concepcion<sup>1</sup>, Sylvain De Guise<sup>1</sup>**

<sup>1</sup> Connecticut Sea Grant/UCONN Extension, 1080 Shennecossett Road, Groton, CT 06340 USA. <sup>2</sup>U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA.

Recognizing the potential for growth among important shellfish sectors, the Connecticut Sea Grant/UCONN Extension Program is facilitating the development of a visioning plan for the State's natural, recreational and commercial shellfish resources. This plan will be developed through a collaborative, proactive and thoughtful public process. The document will include policies and practices that protect and enhance the State's natural shellfish resources, and promote recreational opportunities, agricultural viability and sustainable harvest. In an effort to build a framework for the plan, organizers created a committee comprised of shellfish businesses, industry associations, town, state, and federal government officials, environmental for-profit and non-profit organizations, researchers, extension agents, and service providers. The committee is charged with identifying concerns, sources of conflict, and opportunities for improving shellfish resource use and management. Three public scoping sessions in Fall 2013 asked participants to identify priority issues. Nearly 100 individuals attended the sessions, including business owners, recreational harvesters, regulators, researchers, and environmental organizations. While numerous issues were identified, five prominent themes arose:

1. Understanding risk perception and improved risk communication with respect to public health
2. Transparent and coordinated regulation, management, and enforcement
3. Prosperous and sustainable commercial shellfish industries
4. Improved understanding of and access to recreational shellfish opportunities
5. Protected and enhanced natural shellfish resources

Improving communication among stakeholders was a crosscutting priority. As such, the organizers are planning a conference to begin to generate enthusiasm about all things shellfish in Connecticut and to solicit broad participation in the ongoing Connecticut Shellfish Initiative. To learn more and get involved, visit: <http://smp.uconn.edu>.

Tuesday, February 25<sup>th</sup>, 2014

**THE EAST COAST SHELLFISH GROWERS ASSOCIATION CELEBRATES TEN YEARS OF ADVOCATING FOR THE SHELLFISH AQUACULTURE INDUSTRY**

**Robert Rheault**

<sup>1</sup>East Coast Shellfish Growers Association, 1121 Mooresfield Rd, Wakefield, RI 02879 USA.

The ECSGA was formed by a group of like-minded growers and extension folks at the Milford Aquaculture Seminar in 2003 and has served as a voice for industry concerns ever since. Membership continues to grow and we topped 140 industry members last year. The association has been consumed with *Vibrio* issues as well as issues such as trade disputes with the European Union, shellfish breeding for disease resistance, funding for ocean acidification and *Vibrio* research, specialty crop status and marketing efforts. We work primarily at the national level, however, we often get involved in local issues when we can. I serve as a major conduit to the press whenever issues arise and I am trotted out to give the industry perspective at dozens of conferences and meetings annually. We work closely with our sister associations: the Pacific Coast Shellfish Growers Association and the Gulf Oyster Industry Council. We have an annual budget of just over 50K, and we could certainly accomplish more if we had a larger budget. We have a quarterly newsletter that comes with a complimentary copy of Fish Farming News, and we have an excellent website that serves as a significant resource for information relating to all things shellfish. Our e-mail discussion LIST is followed by over 300 people and is a free flowing font of information and answers to your most vexing questions.

Tuesday, February 25<sup>th</sup>, 2014

## **COMMUNITY SUPPORTED AGRICULTURE: ADDING SEAFOOD TO THE RECIPE. A TWO YEAR PILOT PROJECT ANALYSIS**

**Gef Flimlin<sup>1</sup>, Lianna Bass<sup>2</sup>**

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Rutgers Cooperative Extension with assistance from NJ Sea Grant Consortium partnered with Sea Salt Community Supported Agriculture (CSA) organic farm to develop a small-scale pilot program offering shareholders the option to buy biweekly local seafood shares in addition to farm produce. The program has operated for 2 years. Approximately 30% of the shareholders opted to participate in the pilot project. The program hoped to expand regional markets for local seafood by adapting the success of community supported fishery (CSF) models to the popularity of the local foods movements. The program coordinators chose to source all shares from seafood distributors that sell locally harvested or grown seafood. Every two weeks, seafood shares were distributed out of the farm store, accompanied by information about the product suppliers, methods of harvest or production, a brief natural history of the featured species, instructions for handling and preparation, and recipe suggestions. For the shellfish growers, the return was much greater than their normal wholesale market returns, although some were the same depending on their normal marketing strategy. Customer satisfaction surveys were administered at the end of both the 2012 and 2013 seasons to gauge the program's success among customers and identify areas for improvement. Recommendations were noted regarding whether this pilot project could be expanded to other CSAs without the assistance of the Extension people who did significant coordination and some delivery.

Tuesday, February 25<sup>th</sup>, 2014

**CULTIVATED SHELLFISH BASED CARBON OFFSETS: MITIGATING CLIMATE CHANGE, IMPROVING THE ENVIRONMENT AND PROVIDING ECONOMIC INCENTIVES FOR GROWERS**

**Nathaniel Mulcahy**

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With the acidification of the oceans, warming waters, and rising sea levels, there is little doubt that climate change poses a significant threat to shellfish industries around the world. Following the Kyoto Protocol, several cap and trade plans were put into place to help mitigate these issues. While shellfish industries are at risk, they may also provide a solution. Recently, efforts have been made to have cultivated shellfish recognized as a carbon offset. A joint effort among European certification organizations, shellfish researchers and shellfish growers, has resulted in new protocols for offsets that are being tested. These new protocols will, hopefully, serve the triple functions of reducing the world's CO<sub>2</sub> levels, encouraging more shellfish cultivation, and providing economic incentives for shellfish growers. In this paper we review requirements, options, and verification procedures that are due to go into effect in 2014 for Europe and two pilot programs in the United States.

Tuesday, February 25<sup>th</sup>, 2014

**A STUDY OF THE EFFECTS OF CULTURED VS. WILD OYSTERS (*CRASSOSTREA VIRGINICA*) ON NITROGEN REMOVAL AND ECOSYSTEM ENHANCEMENT IN TWO SIMILAR EUTROPHIC ESTUARIES ON CAPE COD, MA**

**John Brawley<sup>1</sup>, Matt Weeks<sup>2</sup>, Diane Murphy<sup>3</sup>, Josh Reitsma<sup>3</sup>**

<sup>1</sup>Woods Hole Group, Inc., 81 Technology Park Dr., East Falmouth, MA 02536 USA. <sup>2</sup>Town of Falmouth, Department of Natural Resources, 180 Scranton Avenue, Falmouth, MA 02540 USA.

<sup>3</sup>Cape Cod Cooperative Extension/Woods Hole Sea Grant, Box 367, Barnstable, MA 02360 USA.

The removal of excess nitrogen from estuarine systems through the enhancement of shellfish stocks has become a popular activity among coastal scientists and managers. The town of Falmouth, Massachusetts has embarked on a demonstration project to study the extent that the culture of oysters (*Crassostrea virginica*) can enhance water quality in Little Pond, a relatively small estuary receiving a high nitrogen load. Approximately 2.5 million seed oysters are being grown in surface shellfish nursery gear in Little Pond for at least 2 years. A monitoring program is being conducted throughout this project to determine whether measurable changes in water quality and other ecological indicators can be achieved. Hypothesized reductions in nitrogen concentration, phytoplankton standing stock, and the frequency of low DO events are being quantified through the comparison of current data to previous monitoring programs in the estuary. We have also discovered a significant natural set of oysters in a nearby estuary (Salt Pond). We have initiated a water quality monitoring program in Salt Pond, including a stock assessment and genetic studies to determine the degree of relatedness and effective population size. As in Little Pond, previous water quality monitoring data are being used to measure the effect of the oysters on the nitrogen cycle and the potential for cascading ecological responses (e.g., improved water clarity, lower phytoplankton standing stock, and possibly lower nitrogen concentrations). We are also tracking changes in water quality as this large standing stock of oysters matures and/or succumbs to mortality. The two studies are running in parallel and offer a unique opportunity to understand the role that wild and cultured oysters play in nutrient and ecosystem dynamics in the south coastal estuaries of Cape Cod.

## WHAT WE LEARNED GROWING RIBBED MUSSELS AT HUNTS POINT

**Gary Wikfors<sup>1</sup>, Eve Galimany<sup>1</sup>, Julie Rose<sup>1</sup>, Mark Dixon<sup>1</sup>, Yaqin Judy Li<sup>1</sup>, Shannon Meseck<sup>1</sup>, Carter Newell<sup>2</sup>, Lynn Dwyer<sup>3</sup>, Mark Tedesco<sup>4</sup>**

<sup>1</sup>U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA. <sup>2</sup>Maine Aquaculture Research & Development, Damariscotta, ME 04543 USA. <sup>3</sup>National Fish and Wildlife Foundation, 40 West 4th Street, #151, Patchogue, NY 11772 USA. <sup>4</sup>EPA Long Island Sound Office, 888 Washington Boulevard, Stamford, CT 06904 USA.

A two-year demonstration project to evaluate suspension culture of ribbed mussels, *Geukensia demissa*, at the confluence of the Bronx River and the East River tidal strait yielded many insights into the process of implementing shellfish nutrient bioextraction, a new concept gaining momentum in the water-quality management community. Many regulatory and organizational challenges were encountered and overcome, allowing the research team to collect a comprehensive scientific data set on the fundamental aspects of ribbed mussel autecology and nutrient and seston dynamics necessary to evaluate the technical feasibility of using ribbed mussel aquaculture to improve water quality at Hunts Point and elsewhere.

Ribbed mussels appeared to spawn in August or September, but recruitment on various collectors was inadequate to populate a commercial mussel raft; therefore, seed sourcing is a problem that needs to be solved. Young adult ribbed mussels did, however, adapt well to subtidal culture in socks suspended from a commercial Maine blue-mussel raft. Hunts Point seston had low phytoplankton and particulate organic matter, but very high inorganic sediment held in suspension by tidal turbulence. Ribbed mussels modified filtration and selection to maintain absorption efficiency, but were unable to process a sufficient quantity of seston to obtain enough nutrition to sustain rapid soft-tissue growth at the site. These findings highlighted the importance of site evaluation, including both seston analysis and mussel-filtration measurements, in further development of nutrient bioextraction using this non-commercial bivalve species.

Tuesday, February 25<sup>th</sup>, 2014

## **PRACTICAL MUSSEL FARMING IN SOUTHERN NEW ENGLAND**

**Mason Silkes<sup>1</sup>, Bill Silkes<sup>1</sup>, Scott Lindell<sup>2</sup>**

<sup>1</sup> American Mussel Harvesters, 165 Tidal Dr. North Kingston, RI 02852 USA. <sup>2</sup> Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543 02543 USA.

Mussel farming (*Mytilus edulis*) on the east coast of the USA could be a pathway to greater domestic seafood supply, and industrial opportunities. It is currently on a research/small commercial scale. Maine Department of Marine Resources reported a total of 636 metric tons (mt) of farmed mussels for 2010 and the combined landings from experimental farms in Southern New England was approximately 10 mt in 2010 (Silkes, personal communication). The potential to grow is reflected in US imports and the steady growth of the live mussel market. In 2012 live farmed mussel imports from New Zealand totaled 65 mt worth \$304,000 (NMFS). There were 14,941 mt of live mussels imported from Canada worth \$38,500,165 in 2012 (NMFS). American Mussel Harvesters in cooperation with Scott Lindell of the Marine Biological Laboratory and interested fishers has been experimenting with different methods of growing this bivalve on aquaculture leases in Narragansett Bay, Rhode Island Sound, and Vineyard Sound, MA. We have experimented with different seed collection methods, seed processing approaches and grow out materials. The challenges associated with these different approaches will be discussed as well as vessel requirements, the difficulty of finding aquaculture sites and the challenges of moving offshore. Mussel aquaculture is environmentally sustainable; we are working on making it economically, and socially sustainable. We believe the economic success of mussel farming in New England will foster social acceptance providing jobs for displaced fishers and revitalizing working waterfronts.



## **DO MUSSEL FARMS NEED MUSSEL HATCHERIES?**

**Scott Lindell, Emma Green Beach**

Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543 USA.

Mussel culture is one of the most intensively productive sectors of the shellfish culture industry, and historically relies on wild seed. However, limitations of the wild supply of mussel seed pose a substantial impediment to the expansion of mussel culture locally and internationally. Recently several new blue mussel, *Mytilus edulis*, farms have started in New England and currently there are at least 5 pending applications for mussel farming, including 2 in federal waters. Mussel farming regions worldwide are turning to hatchery production to ensure consistent, high-quality seed supply. New Zealand is spending \$50 million dollars over 7-years in R&D. The cost of producing mussel seed may exceed what most growers are willing to pay, but new feeds and breeding technologies may soon make cultured seed more cost-effective than wild. We investigated use of alternative and live diets to culture mussel seed. Broodstocks were conditioned with either live diatoms or a freeze-dried algal diet (Ori-Go™, Skretting Ltd.). 48-hr survival was higher for larvae spawned from broodstock fed live diets. In another feed trial of 10-day post-set mussel spat, one group was fed a mixed live algal diet, and another fed Reed™ Mariculture's Shellfish Diet algae paste. After 7 weeks, spat fed live algae were significantly larger (3.0 mm) than those fed algal paste (2.4 mm); survival was similar. From 3.5 million fertilized eggs, 350,000 eyed larvae were set into two 220L conical tanks filled with setting rope. An estimated 80% survived to a size (> 2mm) suitable for outdoor nursery culture.

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Tuesday, February 25th, 2014

## **AN UPDATE ON ESTABLISHING AN OFFSHORE MUSSEL FARM IN FEDERAL WATERS IN THE GULF OF MAINE**

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Last year, we reported on an effort to establish a demonstration mussel farm in federal waters off the coast of Massachusetts. We have been working through the permitting process to obtain an Army Corps of Engineers (USACE) permit pursuant to Section 10 of the Rivers and Harbors Act of 1899 to establish a commercial scale (33 acre) offshore mussel farm off the coast of Cape Ann, Massachusetts (NAE-2012-1598 NEMAC Aquaculture). Once permitted, the first objective is to set up a small-scale prototype farm with deployment of 2 to 4 longlines to research best practices for offshore shellfish aquaculture as well as monitoring potential fishery and habitat enhancement effects attributed to shellfish aquaculture in offshore waters. Since this site is within the Northern Temporary Paralytic Shellfish Poison (PSP) Closure Area (50 CFR Part 648), the plan is to initially acquire a Letter of Authorization (LOA) to conduct research on controlled experimental harvests to provide samples that would indicate the PSP toxin levels are below the regulatory limit necessary to allow commercial harvests. The second objective is to refine and enhance offshore mussel culture as an alternative fishing option for fishermen and lobstermen currently displaced or negatively impacted by current fishery restrictions. This report will focus on the status of securing a USACE permit to establish this farm and disseminate what was learned about this process to others wishing to pursue offshore shellfish aquaculture. We hope to secure permits and begin to establish longlines this summer.

## **GROWTH STUDY OF BAY SCALLOPS IN A FLOATING UPWELLER SYSTEM**

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A floating upweller system, typically utilized in oyster and clam nursery work, was used in a trial for bay scallop culture, comparing survival and growth to cage culture. A cohort of bay scallops spawned in March of 2013 was stocked at experimentally-varied densities: two wells with smaller scallops at 400 ml (shell-height range 5-8 mm.), and three wells at 500 ml of larger scallops (8.2–17.6 mm.). One well was stocked with 3L of the larger scallops as a high-density test. As controls, wire mesh cages were stocked with the same populations and similar densities. All culture systems were maintained and measured at regular intervals, and stocking densities were modified to maintain equal treatments throughout the experiment. Early indications, at the two month interval, showed significantly better growth ( $p > .05$ ) of the low density groups (0.35mm/day) in the upwellers compared to high density groups (0.24mm/day). At the end of the study period the upwellers performance (0.25 mm/day) showed a trend favoring a difference in comparison to the (0.23mm/day) growth seen in the cages. Results after the four month study period showed favorable growth and survival performance of bay scallops in the floating upweller, encouraging further studies to define proper stocking densities for scallops in upwellers and a timetable to move scallops into cages.

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## **IMPROVED TECHNIQUES FOR DEPURATION AND DEGRITTING OF *ENSIS DIRECTUS***

**Nathaniel Mulcahy**

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Razorfish or razor clams (*Ensis spp.*) are often found to have high levels of grit which may diminish their salability to live markets. Existing degritting techniques are not entirely effective and may impede proper depuration. Finally, stress induced during depuration may prevent the animals from surviving long enough to reach the more lucrative Far East live markets. Our objective was to create a more effective and scalable degritting procedure that would open new markets for growers and harvesters of *Ensis spp.* The current study describes and evaluates this new depuration and degritting technique for *Ensis directus* and compares its effectiveness with respect to two standard depuration procedures. Both grit count and fecal coliform indicators were used to compare and quantify the effectiveness of the different depuration and degritting techniques. The new procedure proved effective in depuration and degritting while the animals tested exhibited fewer indications of stress which may predict a higher survival rate.

**NATURAL HISTORY AND CONTROL OF THE MUD WORM, *POLYDORA CORNUTA* ON A DELAWARE BAY, NEW JERSEY OYSTER FARM**

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Biofouling caused by marine polychaetes belonging to the genus *Polydora* is an important concern for oyster aquaculturists around the world, and particularly challenging for Delaware Bay, New Jersey's oyster farmers. Two species, *Polydora websteri* and *Polydora cornuta* (formerly *ligni*) are present in the Bay. Generally referred to as mud-worms, polydorid worms can kill oysters, reduce oyster growth, weaken the shells, and cause internal shell blisters that decrease product appeal and marketability. Location, local conditions, and cultivation methods play important roles in modulating infestation timing and severity, and the efficacy of control methods. The development of effective strategies to control mud worm fouling is contingent upon an understanding of the ecology of this organism and its interactions with farm and environmental conditions. To elucidate the understanding of *P. cornuta* population dynamics, plankton samples were collected weekly from April through September and settlement and community succession were monitored. Fouling, larvae and worm abundance, and life history characteristics were measured and analyzed. Egg-bearing adult worms were observed in March, indicating the presence of overwintering infestations on the farm. Fluctuations of the condition and intensity of fouling mud tube colonies occurred throughout the spring and summer months. Planktonic larvae abundances peaked every four weeks during the course of the study (May-September), coinciding with the full moon, indicating that spawning events occurred at regular intervals. The largest peak in planktonic larvae occurred in mid-May and the heaviest fouling was observed in July. This information will be used to inform and improve *P. cornuta* control measures.

**MEASURING CHANGES IN TOTAL PHYTOPLANKTON-SIZED PARTICLE VOLUME OVER TIME AS A PROXY FOR PRIMARY PRODUCTION AND FOOD AVAILABILITY IN NARRAGANSETT BAY, RI**

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Changes in total phytoplankton biovolume over time can be used as a proxy for cell growth. This approach was evaluated by measuring the total volume change in phytoplankton-sized particles during 5-hour incubations under simulated sunlight. Surface samples were collected from Narragansett Bay, RI on six dates between June and August of 2012 at five stations spanning Narragansett Bay's chlorophyll and nutrient gradients. Total particle volume was measured using a Sequoia Scientific LISST™100-X Laser *In Situ* Scattering Transmissometer over 32 logarithmically distributed size bins between 2.5 and 500 µm (a size range covering both photosynthetic nano and microplankton species.) Concurrent measurements of chlorophyll concentration, carbon-14 uptake, and taxa enumeration were conducted. An increase in phytoplankton-sized particle volume was detected during the incubations for the majority of samples; however, there were significant challenges with sampling noise in replicate measurements. If sampling noise issues can be addressed satisfactorily, this approach becomes a more rapid and non-destructive favorable method to repeatedly sample size-fractionated phytoplankton growth over time. This has potential application for the siting of aquaculture facilities, as both the absolute phytoplankton biovolume and the rate of change would be indicators of food availability for biological growth.

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## **THE IMPORTANCE OF CONSIDERING PRIMARY PRODUCTIVITY IN BIVALVE AQUACULTURE SITE SELECTION – AN EXAMPLE FROM THE BRONX, NEW YORK**

**Yaqin Judy Li, Shannon Meseck, Mark Dixon, Genevieve Bernatchez, Julie Rose, Eve Galimany, Marguerite Petit, Gary Wikfors**

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The East River tidal strait is located in a highly-urban and industrial section of New York City. The site has high dissolved inorganic nutrient concentrations, with combined nitrate, nitrite, and ammonia of 20-70  $\mu\text{M}$ . This high nutrient level has the potential to support high primary productivity, and thus food for bivalve shellfish. Previous studies, however, showed that ribbed mussels were under nourished because of low seston quality at the site. Chlorophyll *a* at the site ranged 1-2  $\mu\text{g L}^{-1}$ , an order of magnitude lower than the 10-yr average value of  $>10 \mu\text{g L}^{-1}$  in western Long Island Sound during the April-October study period. Dissolved oxygen in the water varied only slightly between day ( $\sim 4 \text{ mg L}^{-1}$ ) and night ( $\sim 3 \text{ mg L}^{-1}$ ) in the summer, indicating low photosynthetic activity. Using variable fluorescence measurements, we tested the hypothesis that the low chlorophyll at the site was attributable to low primary productivity. Experiments employing light manipulation and variable fluorescence fluorometry with the FIRE<sup>TM</sup> (Fluorescence Induction and Relaxation System by Satlantic) were performed. The photosynthetic variable “maximum electron transport rate” (ETR<sub>max</sub>) increased 20% to 120% when water samples were incubated under elevated irradiance (100 to 200% of the *in situ* irradiance). Results indicated primary productivity in this high-nutrient, high-turbidity coastal environment was light limited, likely because of shading by sediment from intense tidal turbulence. Low local primary productivity, therefore, is a contributing factor, along with a high inorganic sediment load, to the poor food quality for bivalve shellfish at the site.

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**IS THERE A LINK BETWEEN SHELLFISH DREDGING, CARBONATE CHEMISTRY, AND BIVALVE SETTLEMENT ON THE SURFACE OF MARINE SEDIMENTS?**

**Shannon Meseck, Renee Mercaldo-Allen, Julie Rose, Catherine Kuropat, Paul Clark, Genevieve Bernatchez, Ronald Goldberg**

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High mortality of settling marine bivalves in near shore sediments is common during the first few days to weeks as they transition from the pelagic larval phase to the benthic juvenile phase. There are many factors during settlement that can lead to high mortality (i.e., predation, low oxygen, starvation). Recent research suggests that the carbonate saturation state at the sediment surface may have a role in this mortality, especially in sediments that are under-saturated with carbonates (low aragonite saturation state). We investigated whether there is a difference in carbonate saturation state between dredged and non-dredged sediments and if the number of settling bivalves differed between the two treatments. The two treatments, dredged (n=9) and non-dredged (n=9), were sampled weekly from July 22<sup>nd</sup>, 2013 (beginning of settlement) to September 23<sup>rd</sup>, 2013. Samples were taken for porewater concentrations of DIC, alkalinity, pH, dissolved oxygen, and for grain size; Benthic grabs were taken for bivalve identification. From alkalinity and pH measurements, the calcite and aragonite saturation states were calculated using CO2SYS. The dredged sites had significantly more calcite and aragonite present and more recently-settled bivalves (*Nucula*, *Yoldia*, *Mya*) than the non-dredged sites, suggesting that dredging enhances larval sediment by increasing dissolved inorganic carbon at the surface layer of the sediments.



## **MOVING BIODEPOSITION MEASUREMENTS OF SHELLFISH FEEDING FROM A SHORE-BASED TO A SHIPBOARD TECHNOLOGY**

**Mark Dixon<sup>1</sup>, Robert Alix<sup>1</sup>, Werner Schreiner<sup>1</sup>, Eve Galimany<sup>2</sup>, Julie Rose<sup>1</sup>, Yaqin Judy Li<sup>1</sup>, Heather Refalo<sup>3</sup>, Gary Wikfors<sup>1</sup>**

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The biodeposition method of quantifying shellfish filtration and feeding can be used to evaluate potential aquaculture sites and to calculate the nutrient bioextraction potential of shellfish aquaculture as part of a broader water quality strategy. This *in-situ*, method gathers data that can be used to calculate clearance rate, filtration rate, absorption rate, absorption efficiency, and rejection proportion of suspension-feeding shellfish. Ambient water flowing at carefully regulated rates through replicate chambers containing individual shellfish is the cornerstone of the physical design of the apparatus used in the biodeposition method. The previous apparatus works well on stable platforms like docks and bulkheads; however, the majority of shellfish aquaculture occurs in areas remote from stable platforms. As aquaculture moves further offshore, capability to do shellfish filtration measurements aboard a vessel is becoming necessary. We have designed and tested a double-gimbaled table to facilitate the use of the biodeposition method shipboard. Land-based comparisons indicate water flow and shellfish behavior were equivalent in the gimbaled and original designs. The gimbaled design is modular and readily transported, and the sampling techniques are easily transferrable. Shipboard trials in Long Island Sound, coastal Massachusetts, and offshore California have yielded preliminary data and acted as proof-of-design. Direct, site-specific measurements of shellfish feeding now can be made directly at existing or prospective farm sites from aboard a vessel.

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## **DAY AND NIGHT PH CYCLING OF ALGAL CULTURES; WHAT OUR ALGAE ARE DOING WHILE WE SLEEP**

**Barry Smith and Alison Tamsett**

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Living cells respire to access stored energy. The algae used in aquaculture are grown autotrophically, typically acquiring solar energy during the day and using part of that energy catabolically when in the dark. As long as the energy absorbed during the lighted period exceeds the energy required for cellular metabolism, the algae can grow and divide. These divisions generate the algal biomass we require to feed aquacultured species.

Photosynthesis assimilates CO<sub>2</sub> during the day to make storable energy and respiration generates CO<sub>2</sub> in the dark. This assimilation and release of CO<sub>2</sub> changes the pH of the algal culture medium. Studying diurnal pH cycles of cultures grown in the Greenhouse for Research on Algal Mass Production Systems (GRAMPS) furthers our understanding of algal culturing.

Nine 400 liter cultures of *Tetraselmis chui* were grown in GRAMPS. Three cultures were not pH controlled, three were adjusted to pH 7.2, and three were maintained at pH 8.2. Daily samples were taken for cell enumeration, temperature, pH, and quality observations. Weekly samples were taken for nutrient levels. The pH control system also continuously logged pH values of each culture.

Algal cultures in a stable repeating cycle of nutrient addition, growth, harvest, repeat, should exhibit a rhythmic pH change without wide fluctuations. If this is found to be true, then logging pH can be used to monitor culture health, production, and stability. Current data analysis suggests pH changes as an indicator of algal culture condition will be a valuable tool.

## **COLLABORATING TO DEVELOP A REGULATORY PROCESS FOR SEAWEED AQUACULTURE IN CONNECTICUT**

**Cori Rose<sup>1</sup>, Kristin DeRosia-Banick<sup>2</sup>, Tessa Getchis<sup>3</sup>, David Carey<sup>4</sup>, Kristin Bellantuono<sup>5</sup>, Anoushka Concepcion<sup>3</sup>**

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The Connecticut Aquaculture Permitting Workgroup (Workgroup) is a partnership among Connecticut Sea Grant, the Department of Agriculture/Bureau of Aquaculture (CT DA/BA), the Department of Energy & Environmental Protection (CT DEEP) and the Army Corps of Engineers to facilitate interagency communication to streamline the often complex regulatory framework for marine aquaculture permits in Connecticut. Currently the Workgroup is developing a regulatory process for seaweed cultivation. It will cover both research and commercial operations of varying scale and integrate “cultivation” and “harvest through market phases” of the business. The new harvest and market application will initiate pre-application review of the cultivation with the federal (Section 10 of the Rivers and Harbors Act) and state (Structures, Dredging & Fill) regulatory agency permit requirements if using structures in coastal, tidal navigable waters. It is envisioned that the Seaweed Producer Application Package, to include applications for growing area and seaweed producer licenses will launch the interagency coordination process. At the CT DA/BA program level, the type of seaweed production permit route will be dependent upon the end product of the crop: 1) non-food production, 2) raw agricultural commodity, or 3) processed seaweed commodity. The Workgroup is also working on new guidance for harvest and post-harvest handling and processing. To date two small scale commercial operations, and one small-scale research operation, have been permitted in the waters of Long Island Sound in the State of Connecticut. This includes what is believed to be the first commercial-scale raw commodity seaweed farm in the continental United States.

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## **THE WORLD'S LARGEST MACROALGAE BLOOMS IN THE YELLOW SEA, CHINA: EVALUATION OF BIOLOGICAL BLOOM PROCESSES**

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Since 2007, the world's largest macroalgal blooms have occurred along the coastal area of the Yellow Sea. In 2012, shipboard surveying and satellite remote sensing were used to monitor the whole bloom process. On April 15, the blooms originated in the Rudong Sea area of the South Yellow Sea where bloom patches were of dark green. The bloom reached its peak size of 219.6 km<sup>2</sup> in Rizhao Sea area of the North Yellow Sea on May 26, and decreased promptly when floated into the Qingdao area, where the blooms turned yellow and the algae were twisted and wrinkled. Meanwhile, vegetative cells of the green tide algae gradually changed into sporangia from which germ cells were released as the blooms drifted northward. In the laboratory, the daily specific growth rate reached up to 82.15±2.13 % for young thalli under 20 °C and 140 μmol·m<sup>-2</sup>·s<sup>-1</sup>. One cm<sup>2</sup> blades (single layer) released 2.84-6.62×10<sup>6</sup> spores or 1.14-2.65×10<sup>7</sup> gametes. Up to 96.4% of the released cells germinated into young thalli. These findings provide critical information to understand the formation of the green tides in the Yellow Sea of China, in 2012.

## **NUTRITIONAL ANALYSIS OF STORE-BOUGHT SCALLOPS FROM DIFFERENT COUNTRIES**

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Both wild-caught and aquaculture-raised scallops are preserved by soaking the meat in phosphate-based compounds; however, this may increase moisture content and weight if misused. In 1992, the Food and Drug Administration (FDA) guidelines stated that the moisture content was not to exceed 80% to be labelled as scallops. Since 2004, this guideline has been rescinded. NOAA Seafood Inspection program continues to monitor moisture content and labeling practices. This survey investigated the moisture, protein, and phosphorus contents of scallops to address three questions: (1) Is there a difference between wild-caught and aquaculture-raised scallops? (2) Is there a difference between the United States, China and Mexico? and (3) Are the bags labelled correctly according to the 1992 FDA guidelines? Moisture and total phosphorus contents were determined using the *Association of Analytical Communities* Official Methods. Protein content was determined using an elemental analyzer. There was a significant difference between scallops of different origins ( $p < 0.05$ ). Scallops from the United States and China were not significantly different from each other, but the one bag sampled from Mexico had higher moisture and lower protein and total phosphorus. No significant differences between aquaculture-raised and wild-caught scallops in moisture or protein ( $p = 0.18$  and  $p = 0.74$ , respectively) were detected. Out of 8 bags sampled, only one bag met the 1992 guidelines for labeling as “scallops” instead of “scallops by-product”. This non-scientific market survey suggests that, if the FDA guidelines were reinstated, there might be changes in how scallop meat products are currently labeled in the USA.

**PAVLOVOLS, STEROLS UNIQUE TO THE GENUS *PAVLOVA*, ARE THE BIOACTIVE COMPOUNDS THAT INDUCE METAMORPHOSIS IN BAY SCALLOP PEDIVELIGERS FED CULTURED *PAVLOVA***

**Derrick Chelikowsky<sup>1</sup>, Diane Kapareiko<sup>1</sup>, Dorothy Jeffress<sup>1</sup>, José-L Giner<sup>2</sup>, Gary Wikfors<sup>1</sup>**

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Previously we demonstrated that feeding northern bay scallop (*Argopecten irradians irradians*) larvae cultured *Pavlova* spp. induced early metamorphosis at relatively small size. Further, preliminary evidence suggested that sterols unique to the Genus *Pavlova*, termed “pavlovols,” were responsible for settlement induction. Structural similarity between pavlovols and the hormone ecdysone, and blocking of *Pavlova*-induced settlement by an ecdysone-blocking insecticide (azadiractin), led to the hypothesis that pavlovols are analogs to the hormone ecdysone and are the compounds responsible for settlement induction by dietary *Pavlova*.

In the present study, we tested this hypothesis by exposing larval bay scallops to combinations of pavlovol synthesized *in vitro*, synthetic ecdysone, and azadiractin at bioactive concentrations. Further, the relative contribution of nutritional benefits of dietary *Pavlova* were investigated by supplementing a monospecific diet of T-ISO (*T-isochrysis lutea*) with cholesterol in a quantity equivalent to the metabolizable sterol content of a *Pavlova* dietary supplement. Timing and size of metamorphosis of larvae were measured.

Significant findings were: 1) addition of cholesterol to T-ISO increased larval growth; 2) the ecdysone-blocker azadiractin inhibited larval metamorphosis under all conditions; 3) both ecdysone and synthetic pavlovol stimulated metamorphosis, unless blocked by azadiractin. These results show that pavlovol is the compound within *Pavlova* that stimulates metamorphosis because it acts as an ecdysone analogue. More fundamentally, this is the first indication that ecdysone is involved in life-history transitions in a bivalve mollusk. We conclude that adding *Pavlova* to a T-ISO diet contributes to larval performance by improving growth (nutritional effect) and by stimulating metamorphosis (hormonal effect).

## **SEASONAL VARIATION IN IMMUNOLOGY OF LONG ISLAND SOUND BIVALVE SPECIES**

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Long Island Sound serves as a habitat for several commercially-important bivalve species. Successful perpetuation of harvestable populations of shellfish in this estuary, whether through aquaculture or natural recruitment, depends upon robust health of spawning stocks. Combined effects of environmental stressors, both natural and human-caused, and opportunistic pathogens and parasites can compromise bivalve health. The ability of bivalve mollusks to withstand stress and disease pressure is dependent in large measure upon cellular immune defense mechanisms accomplished by hemocytes. A preliminary study measuring immune defense functions (adhesion, phagocytosis, the production of reactive oxygen species (ROS), apoptosis) and characterization of hemocytes in eastern oysters, bay scallops, hard-shell clams, soft-shell clams, and blue mussels was conducted over three seasons in 2012 and 2013. Results from these analyses show differences in the unstimulated production of reactive oxygen species (ROS) and phagocytosis over the sampling period. Results from this study have prompted further inquiry applying a more robust, monthly sampling frequency to further understand seasonal cycles in immunity of these bivalve species. Overall, these results will be applicable to other estuaries and the species that live within these systems.

**SEASONAL DYNAMICS OF MALE SPECIFIC COLIPHAGE AND ENTERIC VIRUSES IN BIVALVE SHELLFISH WITHIN WASTEWATER TREATMENT FACILITY SAFETY ZONES**

**Thomas Howell<sup>1</sup>, William Burkhardt<sup>2</sup>, Michael Chambers<sup>3</sup>, Steve Jones<sup>4</sup>**

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Large areas of productive shellfish growing waters in the New England are closed to harvesting due to concerns associated with human fecal pollution from municipal wastewater treatment facility (WWTP) outfalls. The objective of this study is to identify areas that can be reopened for shellfish harvesting, as had occurred in Maine for softshell clams in a previous project. This study's focus is to determine if similar successes are possible with New England oysters and quahogs. Shellfish near WWTFs in the Taunton River (MA), Salem Sound (MA) and the Piscataqua River (NH/ME) were collected monthly and analyzed for male specific coliphage (MSC), fecal coliforms (FC) and limited norovirus and adenovirus detection. In the Taunton River, oysters and quahogs showed relatively low MSC levels and seasonal accumulation rates during cold months compared to softshell clams. In the Salem Sound and the Piscataqua River, relative accumulation rates of the indicator organisms decreased in American oysters and quahogs as water temperature decreased, while softshell clams and European oysters exposed to the same temperatures continued to accumulate and eliminate MSC. FC levels were poor indexes of MSC and virus levels in all shellfish types at all sites. These findings reinforce previous findings that could lead to changes in NSSP guidelines to allow greater harvest of shellfish in outer safety zones during some times of year. The results also suggest harvest management related to WWTF impact may require different approaches based upon shellfish species, and WWTF performance in addressing risk of illness attributed to enteric viruses.



## **DISEASE MANAGEMENT STRATEGIES FOR SHELLFISH AQUACULTURE: THE IMPORTANT ROLE OF HATCHERIES**

**Marta Gomez-Chiarri<sup>1</sup>, David Rowley<sup>2</sup>, David Nelson<sup>3</sup>, Dina Proestou<sup>4</sup>, Anu Frank-Lawale<sup>5</sup>, Standish K. Allen Jr.<sup>5</sup>, Ximing Guo<sup>6</sup>, Paul Rawson<sup>7</sup>**

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Disease management is a critical component of the successful production of bivalves. Infections by bacterial pathogens can cause rapid mortality of shellfish larvae with devastating consequences for both the hatcheries and the farmers that rely upon them. Furthermore, several bacterial and parasitic pathogens can cause large-scale mortalities in juvenile and adult oysters. The impact of these diseases varies regionally and may be exacerbated by changes in water quality and local environmental conditions resulting from climate change. An effective integrated health management plan for shellfish production starts at the hatcheries, which are responsible for producing hardy and healthy seed for grow-out facilities. Key disease management strategies include the use of disease-resistant strains as well as a plethora of husbandry strategies geared to avoid the exposure of shellfish larvae to pathogens or increase the resilience of larvae to environmental stress and pathogen exposure. We will discuss the relevance to shellfish hatcheries of recent research in two areas: 1) breeding for disease resistance in oysters, and 2) probiotics. Recent research on the performance of several disease-resistant oysters in locations from Virginia to Maine suggest local adaptation of selectively-bred oyster lines to the site of origin and a need for the development of regional breeding programs. Probiotics have been shown to decrease the levels of *vibrios* in oyster larval tanks and increase the survival of oyster larvae and juveniles challenged with bacterial pathogens. These strategies could help minimize the impact of infectious diseases in shellfish hatcheries and provide hardy seed for shellfish grow-out.

**THE FIRST COMPREHENSIVE SURVEY FOR MSX, *HAPLOSPORIDIUM NELSONI*,  
IN CONNECTICUT**

**Inke Sunila<sup>1</sup>, Timothy Visel<sup>2</sup>**

<sup>1</sup>State of Connecticut, Department of Agriculture, Bureau of Aquaculture, 190 Rogers Avenue, Milford, CT 06460 USA. <sup>2</sup>The Sound School, 60 South Water Street, New Haven, CT 06519 USA.

Unusually heavy mortalities of eastern oysters, *Crassostrea virginica*, were observed in 1984-85 along Connecticut's shoreline. As a result, Dr. Sung Feng from the University of Connecticut, initiated an extensive collection of oysters from the Hammonasset River, East River, Tom's Creek and Pawcatuck River from June of 1986 until December of 1987. A reference sample was collected from Mecox Bay on the Atlantic shore of Long Island. Oysters were fixed and processed for histological examination. Slide collection consisted of 782 oysters, 59 of which were gapers. The average prevalence of MSX in the CT samples was 17%; and no MSX was present in the reference sample from Mecox Bay, NY. MSX plasmodia were detected in 40% of the gapers. The highest prevalence of MSX occurred in Hammonasset River in June of 1987 with prevalence of 50%. Also SSO (Seaside Organism), *Haplosporidium costale*, occurred in the oyster samples as a coinfection with MSX. Dr. Feng's archived slide collection demonstrates a widespread MSX epizootic and establishes the involvement of MSX in the observed oyster mortalities.

**PREVALENCE OF DISEASE, GROWTH ABNORMALITIES AND TISSUE METAL CONTENTS IN EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) ALONG THE CONNECTICUT COASTLINE**

**Gabriel Geist<sup>1</sup>, Vincent Breslin<sup>2</sup>**

<sup>1</sup>The Sound School, 60 South Water Street, New Haven, CT 06519 USA. <sup>2</sup>Southern Connecticut State University, 501 Crescent St., New Haven, CT 06511 USA.

The eastern oyster, *Crassostrea virginica*, is an important economic resource for the Connecticut shellfish industry. This study examined whether the prevalence of pathological changes and disease in oysters occurs in association with elevated metal concentrations in their tissues or surrounding sediment environments. Fifty oysters of the same size (75-100mm) were collected from seed oyster beds at five locations during late Fall 2008 from three separate rivers/harbors along the CT coast representing a variety of sediment environments. The oysters were examined for condition index, the presence of pathologic changes, and disease. Results were compared with results of a concurrent study examining sediment and tissue metal concentrations of the same respective population of oysters. The prevalence of pathologies and disease in the oysters varied among the sites examined in the Housatonic River estuary, New Haven Harbor and Westbrook Harbor. No straight line association from a pathological response to an injurious agent, in this case metal contamination, can be clearly drawn from the present study.

## **PROGRESS IN ESTABLISHING A GAMBIAN NATIONAL SHELLFISH SANITATION PROGRAM (GNSSP)**

**Michael A. Rice<sup>1</sup>, Foday Conteh<sup>2</sup>, Bamba Banja<sup>3</sup>, and Fatou Janha<sup>4</sup>**

<sup>1</sup>Department of Fisheries, Animal & Veterinary Science, University of Rhode Island, Kingston, RI 02881, USA. <sup>2</sup>Department of Water Resources, Government of The Gambia, Abuko, The Gambia. <sup>3</sup>WASH Program, Coastal Resources Center, University of Rhode Island, Old Jeshwang, KMC, The Gambia. <sup>4</sup>TRY Oyster Women's Association, Old Jeshwang, KMC, The Gambia.

The Tanbi Estuary of Gambia, Africa supports shellfisheries for oysters, *Crassostrea tulipa*, and the senile ark, *Senelia senilis*. In January 2013, the TRY women shellfishers entered into a cooperative shellfish co-management agreement with the Gambian government, empowering the women to co-establish and enforce shellfish management protocols for their fishery. Lack of shellfish sanitation protocols is a key reason for low domestic market prices for shellfish, so a GNSSP was envisioned as a means to boost consumer confidence and access domestic markets with Gambia's robust seasonal tourism trade. Sanitary shoreline surveys were begun to document sources of contamination and establish priorities for remediation. Since September 2010, water is sampled bimonthly for total (TC) and fecal coliforms (FC) at stations near shellfish harvesting areas. Conclusions are: 1) FC is a superior indicator of potential contamination than TC; 2) FC values from most shellfish growing areas meet or exceed a FC standard of 14 MPN/100mL most of the year; 3) highest average FC values correspond to local rainfall maxima from July to October during the traditional off-season for shellfishing; 4) sanitary remediation has the effect of localized water quality improvement (e.g. introduction of sanitary latrines at Old Jeshwang); and 5) there is enough precision and repeatability in the data to establish water quality classification zones. In Wenchong, without sanitation and near a dumpsite, FC values are typical of a prohibited zone, but areas away from settlements could be certified year-around harvest sites. Postharvest sanitation from harvest to market remains as Gambia's key sanitation challenge into the future.

## **DISCRIMINANT ANALYSIS OF OYSTER HEMOCYTE IMMUNE FUNCTIONS AS A PREDICTOR OF POTENTIAL PROBIOTIC CANDIDATES**

**Diane Kapareiko, Julie Rose, Jennifer Alix, Dorothy Jeffress, Gary Wikfors**

U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA.

In an effort to improve hatchery production of Eastern oyster (*Crassostrea virginica*) seed for aquaculture and restoration, the Milford Laboratory has isolated and tested a naturally-occurring strain of *Vibrio* sp., OY15, that has demonstrated significant protective effects against a *Vibrio* sp. shellfish larval pathogen (B183) in experimental larval trials. OY15 was selected as a potential probiotic candidate based upon its ability to inhibit pathogen B183 in Kirby Bauer disc diffusion studies, suggesting competitive exclusion of pathogen B183 as a mechanism by which OY15 exerts its probiotic effects. In larval experiments, we failed to find any evidence to support this mechanism, however, *in vitro* flow cytometry assays on adult oyster hemocytes indicated that probiotic OY15 stimulated oyster hemocyte immune functions-adhesion, phagocytosis and reactive oxygen species release-suggesting an immuno-stimulatory mechanism for the probiotic effects on oyster larvae. Subsequently, we employed these flow-cytometric, immune-function assays as a method for screening unknown bacterial isolates for probiotic candidates. Statistical analyses (ANOVA) of hemocyte immune function results for 11 unknown bacterial strains isolated from the digestive glands of eastern oysters did not yield any promising probiotic candidates such as OY15. Using Discriminant Analysis (DA) methods, however, we characterized and separated the unknown bacterial isolates into groups or classes, based upon linear combinations of their immune function results. In addition, using the hemocyte response profile for probiotic OY15 as a “probiotic indicator” and immune-suppressive profile of pathogen B183 as a “pathogen indicator”, we can employ this statistical method to screen for likely probiotic candidates.

Wednesday, February 26, 2014

## **USDA- FARM SERVICE AGENCY**

### **Devon Marsden**

USDA- Farm Service Agency, 344 Merrow Road, Tolland, CT 06084 USA.

The CT Farm Service Agency will present an overview of the insurance and loan programs that are available to producers through our agency. The Noninsured Crop Disaster Assistance Program (NAP) provides financial assistance to producers of noninsurable crops when low yields, loss of inventory or prevented planting occur due to a natural disaster. For a \$250 service fee, the program covers the amount of loss over 50% of the expected production yield and is based on market prices. FSA is the lender of first opportunity through the USDA for producers. We offer direct, low interest loans to cover operating expenses, equipment, seeds, and infrastructure. The microloan program is a direct loan, of up to \$35,000, with a faster application process for producers.

Wednesday, February 26, 2014

## **AQUACULTURE AND CROP INSURANCE**

### **Robert Cerda**

Crop Insurance Systems, Inc., 1505 Silver Valley Court, Wentzville, MO 63385 USA.

Robert Cerda, President of Crop Insurance Systems, will discuss the substantial business benefits of crop insurance for producers of food including aquaculture. He will provide information regarding the requirements for approval of a Federal Crop Insurance program. Mr. Cerda will also provide a discussion of potential product design strategies including the strengths of various designs. He will also talk about the process of creating an insurance product for oysters including timelines for development of an insurance product.

## **REGIONAL OVERVIEW OF THE 2013 *VIBRIO PARAHAEMOLYTICUS* SEASON**

### **Kristin DeRosia-Banick**

Connecticut Department of Agriculture, Bureau of Aquaculture, PO Box 97, Milford, CT 06460 USA.

*Vibrio parahaemolyticus* is a naturally occurring marine bacterium in the same family as those that cause cholera and sicknesses from *Vibrio vulnificus*. This bacterium lives in brackish saltwater and causes gastrointestinal illness in humans. Prior to 2012, *Vibrio parahaemolyticus* typically caused sporadic food-borne illnesses related to raw molluscan shellfish consumption in the Northeast region. During 2013, a large percentage of *Vibrio parahaemolyticus* illnesses were linked to clams and oysters harvested on the East Coast and subsequently determined by serotyping of clinical isolates to be linked to a specific virulent strain of *Vibrio parahaemolyticus*. According to the Centers for Disease Control, 104 *Vibrio parahaemolyticus* isolates with the same PFGE pattern were reported to PulseNet from persons in 13 states who became ill from May 12, 2013 through August 19, 2013. Of the 104 *Vibrio parahaemolyticus* isolates, 76 have been serotyped and all 76 were found to be serotype O4:K12. This strain was previously unique to the Pacific Northwest region, however was identified in clinical isolates associated with shellfish harvested from Oyster Bay, NY beginning in 2012. In 2013, Connecticut, Massachusetts, and New York closed harvest areas associated with illnesses from August 2 to September 17, August 30 to October 5, and June 29 to September 14 respectively. The Northeast state shellfish regulatory authorities will continue to work with the shellfish industry in 2014 to incorporate stringent time to temperature requirements in order to minimize the proliferation of this virulent strain of bacteria, and reduce the risk of consumer illness associated with molluscan shellfish.



## **WASHINGTON STATE OVERVIEW OF 2013 *VIBRIO* SEASON AND RETROSPECTIVE**

### **Laura Wigand**

Office of Shellfish and Water Protection, Washington Department of Health, PO Box 47824, Olympia, WA 98504 USA.

The Washington State Department of Health (Health) has a long history of *Vibrio parahaemolyticus* (*Vp*) management, beginning in the 1990s. The 2006 outbreak led to expansion of *Vp* control and the development of an illness-based Control Plan. Health currently manages *Vp* through a Control Plan for the commercial industry, outreach with recreational harvesters, and environmental sampling. In 2013 Health collected 311 samples from 24 growing areas, leading to eight growing area closures due to high *Vp* levels. There were an additional nine growing area closures under the Control Plan due to the occurrence of four sporadic illnesses and 18 one-hour reductions in time-to-temperature controls due to the occurrence of two sporadic illnesses. A total of 79 illnesses were potentially associated with commercially harvested oysters in Washington in 2013. There is a tendency to over count illnesses attributed to Washington oysters since Health does not distinguish between oysters traced to one or multiple growing areas in implementing the Control Plan. Among the 79 illnesses, 40 were traced to only Washington growing areas and the remaining 39 illnesses were traced to both Washington and out of state growing areas. Given this high illness occurrence, Health is making recommendations to revise the Control Plan to the State Board of Health in consultation with a *Vp* Advisory Committee. Health and the shellfish industry hope to move towards a preventative, risk-based approach to *Vp* management. This approach should allow a greater number of harvest days while reducing illnesses and protecting public health.

Wednesday, February 26, 2014

**PATHOGENIC *VIBRIO PARAHAEMOLYTICUS* STRAINS AND TOTAL POPULATION DYNAMICS IN NEW ENGLAND SHELLFISH HARVEST AREAS**

**Feng Xu<sup>1</sup>, Ashley Marcinkiewicz<sup>1</sup>, Meghan Hartwick<sup>1</sup>, Vaughn Cooper<sup>1</sup>, Steve Jones<sup>2</sup>, Cheryl Whistler<sup>1</sup>**

<sup>1</sup> University of New Hampshire, Department of Cellular, Molecular and Biomedical Science, Durham, NH 03824 USA. <sup>2</sup> University of New Hampshire, Jackson Estuarine Laboratory, 85 Adams Point Rd, Durham, NH 03824 USA.

The Northeast U.S. has experienced a dramatic increase in the incidence of shellfish-borne vibriosis cases in the past few years. *Vibrio parahaemolyticus* (Vp) is the most prevalent cause of disease, yet little is known about the origins and characteristics of Vp clinical strains. Total and potentially pathogenic Vp populations in oysters, sediments and overlying waters from the Great Bay Estuary have been studied since 2007, while clinical Vp strains from Maine, New Hampshire, Massachusetts and Connecticut have been studied from cases occurring in 2010-13. Vp clinical and environmental strains have been analyzed to determine the presence of an array of genetic markers associated with pathogenic potential to provide genotype differentiation of strains. A subset of isolates has also been analyzed by Multi Locus Sequence Analysis (MLSA) to determine relatedness between regional clinical and environmental strains, and with strains from other areas. As is typical, pathogenic type strains are rarely isolated from oysters. Genotyping and MLSA analysis indicate an array of different types for clinical strains, and that environmental populations are extremely diverse. However, this approach did identify a dominant endemic pathogenic lineage from MA. A few environmental strains were closely related to local and global clinical strains, suggesting the potential endemic emergence of pathogenic strains from local populations. Total Vp populations are now detected for longer duration and at higher levels in some sites compared to the 1990s, which may be a factor contributing to population changes and the potential for further emergence of pathogenic strains.

Wednesday, February 26, 2014

## **IDENTIFICATION OF *VIBRIO* SP. ABUNDANCE IN CULTURED OYSTERS FROM NORTHEAST U.S. FARMS**

**Roxanna Smolowitz**

Roger Williams University, One Old Ferry Road, Bristol, RI 02809 USA.

*Vibrio* sp. bacteria are halophilic, gram negative, are common components of marine environments and increase in concentration with increasing water temperatures. The eastern oyster, a filter feeder, accumulates *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) within its tissues. While these bacteria do not cause disease in oysters, they can cause disease in the humans who consume raw oysters. Data concerning the levels of Vv and Vp in the northeast is lacking and regulations are necessarily based on data collected in other areas of the country. The FDA uses the MPN method to determine *Vibrio* sp. abundance in oysters. Methodology problems are the lack of specificity, costs, and time requirements. With funding from Northeastern Regional Aquaculture Center, our lab has developed a quantitative multiplex PCR which we are using both to identify levels of Vv and Vp in the oyster tissues. We are in the process of establishing the relative ability of our specific, quick test to the currently used MPN method for detection of Vv and Vp in oyster tissues. Sediment and water samples from the same locations as the sampled oysters are being evaluated to identify annual Vv and Vp data relevant to the northeast.

**MOLECULAR CHARACTERIZATION OF *VIBRIO VULNIFICUS* ISOLATED FROM EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) FROM THE NORTHEASTERN COAST OF THE US AND COMPARISON WITH CLINICAL STRAINS**

**Steven Pitchford, Yann Reynaud, Sophie De Decker, Gary Wikfors, Christopher Brown**

U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460 USA.

The marine pathogen *Vibrio vulnificus* is a cause of seafood related sickness and mortalities worldwide. In the US, most illnesses are a result of consumption of raw or undercooked oysters, mainly harvested from the Gulf of Mexico. *V. vulnificus* also can be found in oysters and the environment along the northeastern coast of the U.S. especially in warmer months. Far fewer illnesses, however, have been reported from this area. Accordingly, *V. vulnificus* strains from the northeast have not been studied as thoroughly as those from the Gulf and southern regions. The primary goal of this study was to determine if there are genetic differences between isolates known to be virulent and the strains recovered in oysters sampled from Maine to Virginia. We analyzed eighty-three strains of *V. vulnificus*, including 18 clinical isolates provided by the Centers for Disease Control which are known to be pathogenic. A polyphasic, molecular-typing approach was carried out based upon the three known biotypes of *V. vulnificus* and three other genes possibly associated with virulence. Multi Locus Sequence Typing (MLST) using housekeeping genes was also performed. Phylogenetic analyses of these markers and MLST results produced similar patterns of clustering into two main lineages ('LI' and 'LII'), with the clinical and oyster strains clustering together in both lineages. Lineage LII was comprised primarily but not entirely of clinical isolates. Putative virulence markers were present in both the clinical and oyster strains. These results suggest that some strains of *V. vulnificus* found in northeastern oysters are phylogenetically close to clinical strains and may be capable of virulence.

**VARIATIONS IN DATA: THE IMPORTANCE OF SAMPLE PREPARATION AND PROCESSING PROTOCOL OF OYSTER HOMOGENATES FOR THE DETECTION OF *VIBRIO PARAHAEMOLYTICUS* AND *VIBRIO VULNIFICUS* IN RHODE ISLAND AND MASSACHUSETTS SAMPLES**

**Kathryn Markey, Roxanna Smolowitz**

Roger Williams University, 1 Old Ferry Rd., Marine and Natural Sciences 100A, Bristol, RI 02842 USA.

*Vibrio* species are commonly found in the marine environment. They can be found in the water column, sediments and can accumulate in oysters via filter feeding. Aquaculturists and regulators are increasingly concerned about high levels of *Vibrio parahaemolyticus* (Vp) and *Vibrio vulnificus* (Vv) because of their potential to cause illness in humans after consumption of raw shellfish. Typically, severe and lethal illness is seen in immune compromised individuals after consumption of shellfish with elevated levels of these bacteria. Increased summer water temperatures have heightened this concern since, with an increase in temperatures come increases in the growth of these bacteria. Currently, methods to detect *Vibrio* sp. in oyster homogenates are lengthy processes. The technique involves incubating oyster tissues in alkaline peptone water (APW) for overnight enrichment. Following overnight enrichment the most probably number (MPN) per gram of homogenate are calculated and species are positively identified with Biochemical, DNA probe or PCR analysis of suspect colonies (FDA-BAM). These processes take many days and may artificially enhance the numbers of *Vibrio* sp. by enrichment. This study looks at the MPN process and utilizes a newly developed duplex-qPCR assay to detect *Vibrio parahaemolyticus* (tlh gene) and *Vibrio vulnificus* (ToxR gene). We investigated the population dynamics in the MPN tube for both Vp and Vv along with how different DNA extraction protocols may yield different results in the duplex-qPCR. Future work will focus on optimization of existing pathogenic gene multiplex qPCR assay in our lab and alternative methods for *Vibrio* sp. detection from the MPN method.

## **A COMPARATIVE GENOMICS ANALYSIS OF THE T3SS2 $\alpha$ PATHOGENICITY ISLAND OF *VIBRIO PARAHAEMOLYTICUS***

**Carmelo del Castillo, and Bassem Allam**

Marine Animal Diseases Laboratory, School of Marine and Atmospheric Sciences, Stony Brook University, NY 11794, USA.

There has been an increase in *Vibrio parahaemolyticus* (Vp) infection due to the consumption of raw oysters in the Northeast. Vp is ubiquitous in the environment, but can acquire virulence factors that make them pathogenic. The most well studied of these virulence factors, the *tdh* gene, is contained in a discrete genetic unit called a Pathogenicity Island (PAI). This particular PAI, T3SS2 $\alpha$ , also contains genes for the type 3 secretion system - a virulence factor that enables the transfer of genetic material from the host. PAIs are unstable; their very nature makes them susceptible to insertions, deletions, or mobilization. They can be acquired or transferred, and evolve independently and at a faster rate compared to the host organism, thus PAIs can be potent regulators of the virulence of pathogens and can give a hint to the interactions and evolutionary relationships among these genetic units.

Sequences from selected strains of Vp were mined for similarity to the T3SS2 $\alpha$  of type strain RIMD2210633. The resulting sequences were concatenated, in the case of contigs, and aligned. A phylogenetic tree was created to quantify the evolutionary relationships and a MAUVE alignment was constructed to visualize the interrelatedness of subsets in the sequence pattern. These representations demonstrate the dynamic relationships of the PAI, the rearrangement between strains isolated from different locales, and the conservation of sequences among strains isolated in a specific locale. These visualizations are an important tool to understand the PAIs and can give a hint as to how these sequences are disseminated.

**VIBRIO OUTREACH IN THE NORTHEAST U.S.: WHO'S THE AUDIENCE, WHAT'S THE MESSAGE, AND IS THE MESSAGE GETTING THROUGH?**

Tessa Getchis<sup>1</sup>, Anoushka Concepcion<sup>1</sup>, Steve Jones<sup>2</sup>, Diane Murphy<sup>3</sup>, Joshua Reitsma<sup>3</sup>, Abigail Archer<sup>3</sup>, Lisa Calvo<sup>4</sup>, Tal Ben-Horin<sup>4</sup>, Dale Leavitt<sup>5</sup>, Azure Cygler<sup>6</sup>, Gregg Rivara<sup>7</sup>, Donald Webster<sup>8</sup>, Dana Morse<sup>9</sup>

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<sup>8</sup>University of Maryland, Wye Research and Education Center, PO Box 169, Queenstown, MD 21658 USA.

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*Vibrio parahaemolyticus* (Vp) is a species of pathogenic bacteria that causes gastrointestinal illness in humans. It is naturally-occurring in the marine environment, but is present in higher concentrations during summer. Illness primarily occurs from the consumption of shellfish such as oysters, but skin infections may also occur following exposure. The Center for Disease Control (CDC) investigates thousands of cases of *V. parahaemolyticus* infection occur each year in the United States, and many cases go unreported. In most cases, infections can be prevented by thoroughly cooking seafood, however, as the consumption of raw oysters is a popular trend in this region, the key is to keep shellfish cold. Preventive measures such as time/temperature controls are being implemented at the commercial harvester level, but consumers must also be aware of the importance of keeping product cold. Harvest area closures have been implemented in several states, but only after illnesses have occurred.

Making the issue more complex is the fact that there has been an increase in infections caused by a specific strain of *Vibrio parahaemolyticus* not previously recorded in the northeast U.S. According to the CDC, before 2012, *Vibrio parahaemolyticus* infections of this strain were rarely associated with shellfish from the Atlantic coast.

Extension specialists from throughout the northeast U.S., are developing outreach programs and educational materials for various target audiences including shellfish producers, recreational harvesters, seafood distributors, seafood retail outlets, and shellfish consumers.

While our aim is to provide the most up-to-date and science-based information on Vp, our challenge has been the fact that there exist many questions and far fewer concrete answers. We are working with regulators, researchers, and industry associations to identify information gaps and are striving to better coordinate and evaluate the effectiveness of our Vp messaging.