Northeast Fisheries Science Center Reference Document 19-11

38th Milford Aquaculture Seminar
January 8-10, 2018

edited by Patricia Widman

October 2019

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NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Ave, Milford CT 06460

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Monday, January 8th

Registration opens at 5:30pm
Opening reception and poster session 6pm

Tuesday, January 9th

8:00-9:00	Continental Breakfast and registration opens		
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10:30	Brian C. Peterson	ATLANTIC SALMON BREEDING PROGRAM AT THE NATIONAL COLD WATER MARINE AQUACULTURE CENTER	p.20
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11:15	Anoushka Concepcion	EXPANDING SEAWEED CULTIVATION IN CONNECTICUT – ADDRESSING BARRIERS RELATED TO PUBLIC HEALTH	p.21
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11:45	Scott Lindell	NEW TOOLS FOR SELECTIVELY IMPROVING STRAINS OF SUGAR KELP FOR FOOD AND FUEL	p.23
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12:15	John Roy	TWENTY YEARS OF COOPERATION; THE SOUND SCHOOL REGIONAL AQUACULTURE CENTER & THE NMFS LABORATORY IN MILFORD	p.25

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2:00	Darien Mizuta	MUSSEL AQUACULTURE TAKES A DEEP BREATH: STRATEGIES AND CHALLENGES OF OFFSHORE FARMING IN THE NE US	p.27
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2:45	Jang Kim	DEVELOPMENT OF AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM FOR KOREA WATERS	p.30
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Session Moderator: Dylan Redman			
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4:15	Yuan Liu	ASSESSMENT OF FISH COMMUNITIES ASSOCIATED WITH OYSTER CAGES USING ENVIRONMENTAL DNA METABARCODING	p.33
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3:45	Kristin DeRosia-Banick	IMPLEMENTATION OF A SATELLITE VESSEL MONITORING SYSTEM FOR SHELLFISH RESOURCE MANAGEMENT	p.54

OYSTERS, HERE WE GROW AGAIN:

HOW LOCATION AND NITROGEN LOADING AFFECT THE HEALTH OF THE EASTERN OYSTER, *CRASSOTREA VIRGINICA*

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Coastal ecosystem health is becoming increasingly threatened by anthropogenic activities, notably nitrogen loading. To maintain the environmental and economic benefits of marine resources, a more thorough understanding of how organisms will respond to changing environments is essential. The eastern oyster, *Crassostrea virginica*, supports a thriving aquaculture industry in Rhode Island. The goal of this study is therefore to test how dynamic estuarine conditions (temperature, salinity, chlorophyll a, dissolved oxygen, pH) affect the impact of nitrogen loading on the growth and survival of *C. virginica*. A two- factor experiment (location x nitrogen treatment) was utilized in order to test nitrogen loading effects on growth rates of *C. virginica.* Oyster cages were placed on opposite ends of the estuarine gradient of Point Judith Pond, Narragansett, Rhode Island to utilize natural coastal and inland tidal cycles. Cages were either enriched with organic nitrogen (Milorganite) or left at ambient conditions. The enrichment goal for each location, Billington Cove Marina (BC) and Bluff Hill Cove (BHC) was 20 µM of nitrogen. Results show that location is a significant factor in oyster growth, in terms of length ($F_{8,11} = 2.74$, $p = 0.0255$) and biomass ($F_{8,11} = 3.07$, $p = 0.0154$). Nitrogen enrichment within each location had no significant impact on growth, likely due to the relatively small level of enrichment reached compared to the large effect of location. Future studies will examine the microbial gut communities and how it impacts the function and ecosystem services of the oysters. Results from this study can aid in coastal resource management within Rhode Island, as well as offer insight as to how changing marine environments affects the health of *C. virginica*.

A PRELIMINARY HEALTH ASSESSMENT OF FARMED BLUE MUSSELS (*MYTILUS EDULIS*) IN CASCO BAY, MAINE

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Blue mussels (*Mytilus edulis*) farmed in Casco Bay, Maine are a high quality, profitable product in high demand on the East Coast. However, due to the effects of climate change; increased water temperature and ocean acidification, mussel farmers are questioning the health, size and stability of their rope grown populations. For example, our partner farm experienced an unexplained mass mortality event after an out of season spawning during the summer of 2016. This study provides a preliminary health assessment of farmed blue mussels in the Gulf of Maine by quantifying reproductive and storage tissue structures as well as identifying pathogens through the use of histology and image analysis. Mussel health is compared between and within inshore and offshore farm sites based on biophysical parameters (salinity, dissolved oxygen, temperature). This project will lend mussel farmers valuable insight to how a changing ocean will affect their mussel yield as well as establish a correlation between environmental conditions and blue mussel health.

COMPARISON OF SHELL DNA EXTRACTION METHODS IN BIVALVE SPECIES

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Bivalve shell DNA is a newer genetic source of material for taxonomic, diversity and molecular differentiation. Shell analysis could provide valuable information such as population history, species type, lineage, relationships, and other important characteristics in regards to genotypes and phenotypes of living and extinct wild and aquaculture populations. After mortality, the shell is all that remains of bivalve species. Finding a protocol that yields the best quality and quantity of DNA could be crucial to advancing future bivalve genetic studies. In this study, extraction protocol methods are being compared to determine which method will yield the best quantity and quality of DNA from shells. In addition, these samples will be compared with reference tissue samples that usually are used for DNA analysis and have demonstrated success in preliminary trials. The DNA concentration will be quantified by using a nanodrop spectrophotometer. Samples will be run through gel electrophoresis to separate and identify the DNA. The extractions are being conducted and compared on three economically and ecologically valuable species: the blue mussel *Mytilus edulis*, the eastern oyster *Crassostrea virginica*, and the bay scallop *Argopecten irradians.*

SEARCHING FOR NUCLEAR INCLUSION X, A POTENTIAL PATHOGEN OF PACIFIC RAZOR CLAMS

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The Pacific razor clam, *Siliqua patula*, is an important recreational fishery species that lives in the intertidal zone of sandy beaches from Alaska to central California. Populations have had periodic, but significant, declines over the past 30-40 years. These declines have correlated with an increase in the presence of an intranuclear bacteria known as Nuclear Inclusion X (NIX). NIX, first identified in 1986, has traditionally been detected using a time and labor intensive histological method. We have developed a quick and easy PCR- based screening protocol that streamlines the diagnosis of NIX infection. Our protocol includes a general DNA extraction and a specific PCR amplification of NIX DNA. Use of our protocol on razor clams harvested in 2015 have resulted in DNA samples that, when sequenced, match with high similarity (≥97%) to each other and to the previously published sequence for the NIX 16S rRNA gene. Preliminary razor clam sampling and screening in 2015 along the Oregon and Washington coasts revealed a very large infection rate averaging 97%; sampling locations with 100% infection were not uncommon. Follow-up screening in 2016 indicated a decline in infection rate to an average of 74%, though all sampling locations still had infected clams. Furthermore, PCR screening conducted on bacterial DNA extracted from sand samples from multiple locations along the Oregon and Washington coasts have confirmed NIX DNA presence in the sand, serving as the first reported evidence that NIX may be transmitted environmentally. Interestingly, NIX DNA was identified in sand samples from beaches both with and without razor clam populations, suggesting that NIX bacteria may be ubiquitous in the environment. This is the first reported data on NIX infection rates in the state of Oregon, and suggests a significant, wide-scale infection.

IMPORTANCE OF BEHAVIOR AS AN INDIRECT INDICATOR OF TEMPERATURE OPTIMUM IN NEOTROPICAL FRESHWATER CRABS

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Behavioral analysis of stress is often the best acute measure of maladaptive conditions. Animals have sensory systems that react to aversive conditions and allow the animal to mount an appropriate behavioral response. In the case of the endemic Colombian freshwater crab, *Neostrengeria macropa*, the effects of temperature over the next century combined with increased risk from human development on the Bogotá Plain could have an impact on its long-term survival. Additionally, there are over 100 other freshwater crabs in the country that could be at risk if the RCP 8.5 IPCC scenario comes to pass. As our group did a large amount of behavioral work with this species, we wanted to evaluate whether evaluating these species' temperature range optima would be best assessed by activity level or respirometry at those temperatures, or both methods. From preliminary respirometry results, significant differences exist between approximately the same treatments as the behavioral evaluation. This method is effective with small groups of individuals, whereas the behavioral results may have a higher resolution, but with a higher number.

HATCHERY REARED MUSSEL SEED STOCK FOR OFFSHORE MUSSEL FARMS

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Seed stock for mussel aquaculture primarily relies on wild seed collection for socking onto growlines. It is not clear that for offshore mussel farms there will be ample wild settlement on spat lines to supply the necessary seed stock for this operation. To supplement seed production, hatchery reared seed will almost certainly be needed. To this end, the NEMAC shellfish hatchery facility at the Salem State University, Cat Cove Marine Lab, began exploring hatchery techniques for spawning and collecting seed to stock the NEMAC offshore mussel farm.

Mussels were obtained from the NEMAC offshore farm site and brought back to the Cat Cove Lab for conditioning and spawning. During the summer, there were two controlled spawnings producing about 3 – 4 million larva each. Larva were fed cultured phytoplankton until settlement on lines and then placed in ambient flowing seawater for natural feeding. Various settlement lines were tried to determine the best settlement.

ICHTHYOTOXIC DINOFLAGELLATES INDUCE DNA DAMAGE, LIPID PEROXIDATION, AND ANTIOXIDANT RESPONSE IN THE GILL TISSUE OF RED SEABREAM *PAGRUS MAJOR*

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Ichthyotoxic dinoflagellates pose a significant threat to aquaculture and fisheries. In this study, we employed several molecular and biochemical response systems of the gill tissue of red seabream *Pagrus major* to understand potential mode of actions of two dinoflagellates, *Cochlodinium polykrikoides* and *Karenia* sp. after exposure to different cell concentrations for 24 h. Overall, both dinoflagellates dose-dependently increased DNA damage, lipid peroxidation (intracellular malondialdehyde; MDA), and glutathione (GSH) depletion/synthesis during both exposure (24 h) and depuration (3 h) phases. We also analyzed enzymatic activities of antioxidant defense systems such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Both dinoflagellates tested in this study significantly modulated enzymatic activity of antioxidant defense system with strong inductions of SOD and CAT, and the levels were maintained during depuration period. Principle component analysis (PCA) showed potential correlations between molecular markers (i.e. DNA damage, MDA, and GSH) and enzymatic responses by cell concentrations and time-courses. Taken together, our results indicate that representative dinoflagellates have potential hazardous effects on the gill of red seabream within relatively short time period, as the gill is the first organ exposed to water and diverse environmental factors including dinoflagellates. Our results also suggest that analyzing a series of molecular and biochemical parameters can be a way of understanding and uncovering the mode of action of ichthyotoxic dinoflagellates.

EPIPHYTES AND EPIFAUNA ON AQUACULTURED SUGAR KELP *SACCHARINA LATISSIMA* ON THE CONNECTICUT COAST

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Preliminary studies of epibiota, including epiphytes and epifauna, were conducted on sugar kelp collected in March and July of 2017. Although commercial collection of kelp occurs earlier in the season before July, the sampling serves as a positive control for the study. Kelp had very few epibiotic organisms in March, but on July samples, severe fouling was observed. Up to 60% of kelp blade surface area was covered with various macroscopic organisms, with a clear gradient of severity from the tip (being the most severe) to the stipe end. Colonial hydroids were the dominant epibionts large enough to be detected by visual inspection. The microscopic-sized organisms were mostly pennate diatoms with a small percentage of dinoflagellates. TCBS (Thiosulfate-Citrate-Bile-Sucrose Agar) culture revealed 0 to 2.2.2 x 10^5 cfu cm^{-2} of *Vibrio* bacteria on various parts of blades (with the highest counts on the tip); the ambient water had substantially lower *Vibrio* counts than the tip of blades. Additional studies with molecular techniques will provide detailed species information on *Vibrio* spp., as well as other pico- and micro-sized organisms.

In the coming year, the full seasonal pattern of epiphytic succession will be described. Specific surveillance will focus on potentially harmful species known to be present in the area that may attach to kelp, including toxin-producing blue green algae *Lyngbya* spp., some bacteria in the genus *Vibrio*, and *Prorocentrum lima.* Information will inform kelp crop management.

FOOD WED STRUCTURE AT *MYTILUS EDULIS* FARM SITES IN CASCO BAY, ME

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Aquaculture farms are becoming an integral part of the coastal marine ecosystem and associated food webs. Bivalve shellfish grown on farms filter feed ambient seston and in turn contribute biodeposits that are recycled back into the food web. In this study, we looked at blue mussel (*Mytilus edulis*) farms and if they alter the food web structure of a coastal ecosystem in Casco Bay, ME. Rope culture farm sites were compared to floating dock sites. Species composition and abundance of fouling benthic organisms (e.g., skeleton shrimp, anemones, amphipods, periwinkle snails) as well as macroalgae was quantified to determine biodiversity. Energy flow through the associated food web will be analyzed using stable isotope analysis ($\delta^{13}C$ & $\delta^{15}N$). This will identify trophic interactions of fouling species and any differences in the food web structure between farm sited and dock sites. Phytoplankton, zooplankton, and particulate organic matter was also analyzed at farm and dock sites to determine the stable isotope signatures of the primary producers of the food web. This study will provide baseline values for long-term primary consumers that will be critical in assessing future interactions of shellfish farms with coastal food webs. In addition, this information will help us better understand how coastal food webs support shellfish farm production.

DEVELOPMENT OF A MIGRATION MODEL FOR *MORONE SAXATILIS* BASED ON THERMAL SHIFTS IN LONG ISLAND SOUND

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Morone saxatilis (Striped bass) follow a historical migration pattern along the East Coast from North Carolina to Maine. Since 1960, average water temperatures of Long Island Sound have risen 5℃ potentially impacting the migration patterns. In an effort to predict the environmental impacts of these migration shifts, historical angler survey data has been analyzed to develop a representation model of the migration timing throughout the years. Historical water temperatures have also been correlated to formulate a prediction model of migration as impacted by temperature shifts in Long Island Sound. With the prediction model in place a location will be selected to deploy an artificial reef. The artificial reef will enhance the surrounding habitat for *M. saxatilis* by introducing rigid structure consisting of reef balls as well as cinder block. Overtime as the reef develops baitfish productivity will rise creating an ecosystem that caters to the need of *M. saxatilis.* The presence of baitfish in deeper, cooler water will allow the *M. saxatilis* to limit exposure to their zone of stress while preying on baitfish species. Eventually due to the ripple effect the fish will be able to migrate alongside of the baitfish in turn leading to a positive feedback loop.

FINDING EFFICIENT GROWING TECHNIQUES FOR SURFCLAMS (*SPISULA SOLIDISSIMA)*

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The introduction of surfclams (*Spisula solidissima*) into New Jersey's aquaculture sector will help farmers diversify and build resilience into their farm plans. In order for a bivalve species to be cultivated on a commercial scale, producers must understand how different conditions affect a species' growth and survival during different stages of its development. This study looks at how the efficiency of gear types used in surfclam production during the late nursery phase. The three gear types tested include an upwelling system—the industry standard, a shallow raceway with sand to mimic surfclams' natural habitat, and a shallow raceway without sand. Efficiency was defined as the greatest production with the least amount of maintenance, specifically survival, growth, and the accumulation of sediment from raw seawater and clam excrement. Sediment accumulation limits efficiency of nursery gear type by limiting water flow that passes by the clams leading to low-oxygen conditions. The upwelling systems required little maintenance and promoted the greatest survival ($p<0.002$). There was no significant difference in clam growth across the treatments. The shallow raceway showed survival similar to that of a sand-filled raceway, but the sand treatment was the most laborious to clean and was more prone to hypoxia. The addition of sand and accumulation of sediment limited the efficiency of each gear type, and therefore the implementation of the natural habitat of surfclams did not provide any advantage.

MORPHOLOGICAL CHARACTERIZATION VIA LIGHT AND ELECTRON MICROSCOPY OF ATLANTIC JACKKNIFE CLAM (*ENSIS DIRECTUS*) HEMOCYTES

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The Atlantic jackknife clam, *Ensis directus*, is currently being researched as a potential species for aquaculture operations in Maine. The goal of this study was to describe the hemocytes of this species for the first time and provide a morphological classification scheme. We viewed hemocytes under light microscopy (using Hemacolor, neutral red, and Pappenheim's stains) as well as transmission electron microscopy (TEM). The 2 main types of hemocytes found were granulocytes and hyalinocytes (agranular cells). The granulocytes were subdivided into large and small granulocytes while the hyalinocytes were subdivided into large and small hyalinocytes. The large hemocytes had both a larger diameter and smaller nucleus to cell diameter ratio than their smaller counterparts. A rare cell type, the vesicular cell, was also observed and it possessed many vesicles but few or no granules. Using TEM, granulocytes were found to contain both electron-lucent and electron-dense granules of various sizes. These numerous granules were the only structures that took up the neutral red stain. Hyalinocytes had few of these granules relative to granulocytes. Large hyalinocytes had both various organelles and large vesicles in their abundant cytoplasm while small hyalinocytes had little room for organelles in their scant cytoplasm. Total hemocyte counts averaged 1.96 × 106 cells mL−1 while differential hemocyte counts averaged 11% for small hyalinocytes, 12% for large hyalinocytes, 59% for small granulocytes, and 18% for large granulocytes. The results of this study provide a starting point for future studies on *E. directus* immune function.

THE EVOLUTION OF POINT-OF-VIEW VIDEO CAMERA MOUNTING SYSTEMS TO IDENTIFY THE ROLE OF AQUACULTURE GEAR AS FISH HABITAT

Dylan Redman[1], Pete Auster[2], Paul Clark[1], Erick Estela[1], Yuan Liu[1], Lisa Milke[1], Gillian Phillips[1], Julie Rose[1] and Renee Mercaldo-Allen[1]. [1]NOAA Fisheries, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460; [2]University of Connecticut at Avery Point, Department of Marine Sciences & Mystic Aquarium, 1080 Shennecossett Road, Groton, CT 06430
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Many commercial oyster growers in New England use bottom cages to grow their product. Anecdotal evidence from the aquaculture industry suggests that fish use oyster cages as habitat. We sought to document habitat services provided by oyster cages to compare with services provided by natural habitats, such as rock reef. Fish abundance and behavior observed in video will be used to assess fish interactions with cages for comparison with fish activity in natural habitats. Mounting systems were designed for GoPro® cameras to enable attachment to oyster cages and also capture video on a rock reef. Minimizing the complexity of mounting structures that could function as habitat was a primary design consideration.

On the oyster cages, a GoPro Hero 3+® video camera along with a CamDo Blink Time Lapse Controller® was mounted to one corner using rubber hose and a GoPro clip mount to record two sides and the opening of a tunnel created by the "legs" of the cage. Initially, another camera with a semi-fisheye lens was mounted on a bridle facing down to record the entire upper surface of each 2' x 3' cage. However, limited water clarity required a different approach to visualizing the top of the cage. To achieve this goal, while reducing camera distance from the cage, a camera was mounted to the upper corner pointing across the horizontal cage surface. This afforded optimal visibility both in and around the cages.

A "T-platform" was fabricated to deploy cameras using minimal structure to capture fish behaviors in natural rock reef habitat. An X shaped base, constructed of fiberglass Aickinstrut® channel, was attached to a zinc plate for negative buoyancy and stability. A threaded flanged union was bolted to the zinc plate; this allowed a threaded section of black pipe in the vertical direction. A cross-fitting or Tee-fitting was threaded onto the vertical section of pipe allowing us to mount GoPro cameras at various heights and widths of our choosing by substituting different length pipes. Magenta filters were added to all cameras to improve video quality. Combinations of GoPro mounting systems and materials readily available from any hardware store allowed for flexibility of mounting options for optimization of the recording system. The final hardware and camera placements worked best for the low visibility conditions at the study sites, provided comparable views across mounting platforms, and can be used in a wide range of estuarine environments throughout the Northeast.

*Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

COMPARATIVE ANALYSIS OF MICOCYSTIN-LR BIOCONCENTRATION DYNAMICS AND SUBSEQUENT ANTIOXIDANT RESPONSE IN THE GILL AND DIGESTIVE GLAND OF TWO MARINE BIVALVES

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Microcystins (MCs) are a major group of potent cyanobacterial toxins foundin freshwater and even brackish waterbodies. To understand the putative correlation between bioconcentration of MCs and antioxidant responses of the gill and digestive gland of bivalves, Pacific oyster *Crassostrea gigas* and blue mussel *Mytilus edulis* were exposed to different concentrations (0.1, 1, 10 and 20 μg L-1) of MC-Leucine-Arginine (LR) for seven days. MC-LR dose-dependently bioconcentrated in the gills and digestive glands of both bivalves during exposure period with different dynamics. The levels were slightly reduced when the bivalves were exposed to seawater during depuration (7 days), while approximately 0.1 μg L-1 of MC-LR was observed in the 10 and 20 μg L-1 exposed bivalves at the end of depuration. Intracellular malondialdehyde (MDA) and glutathione (GSH) levels were tissue-specifically elevated in the 10 or 20 μg L-1 exposed bivalves at 7 day, and the levels were maintained during depuration in both bivalves. Overall, significant higher levels of enzymatic activities of antioxidant defense systems such as glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were observed in the 10 and 20 μg L-1 exposed bivalves. Overall, tissue-specific and species-specific sensitivities upon MC-LR were analyzed with different MC-LR dynamics in the same experimental conditions. These patterns were correlated with the bioconcentration patterns of MC-LR. Our results will be useful to understand species-specific bioconcentration of MC-LR in bivalves and their effects on intracellular oxidative status via accumulation.

DEVELOPMENT OF A LOW ENERGY-INPUT MICRO-ALGAL CULTURE SYSTEM TO SUPPORT AQUAFEED MARKETS

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Increasing algal culture density is critical for enhancing feed capacity and efficiency in traditional shellfish hatcheries as well as providing biomass for high value product targets such as carotenoids, lipids, and various nutraceuticals. Traditional, low-cost algal culture systems, such as Kalwalls, bag and cage, and open pond, provide low-cost and low-tech methods of culturing microalgae, but can be prone to labor intensive protocols, low culture densities, and contamination. In an effort to increase algal culture efficiency, for both the hatchery setting as well as the high-value product market, while also maintaining a low cost of setup, the Rutgers Aquaculture Innovation Center has developed an integrated, high-efficiency, horizontal glass tube photo-bioreactor (PBR), and electro-flocculent harvester system to increase micro algal culture densities and purity. The culture vessel consists of several 2.5 m long horizontal glass tubes (4.9 cm diameter), designed to significantly reducing self-shading and increase availability of photosynthetically active radiation, connected in a sealed recirculating system that allows for controlled, continuous, high-density, axenic culture. *Nannochloropsis* sp. was chosen as the test species due to its increasing hatchery usage as well as its significant potential for high value product production. Direct growth comparisons were conducted between the glass tube PBR and a traditional Kalwall setup. Replicated growth trials show that the glass tube PBR system reaches and maintains peak densities up to three times denser (average of 318 million cells mL^{-1} versus 120 million cells $\cdot$ mL^{-1}) and can maintain prolonged growth and continual (daily) partial harvest at densities twice (about 200 million cells $\cdot$ mL^{-1}) that of a traditional Kalwall system. The low utility-input integrated electro-flocculent harvesting system, coupled with the increased densities and potential for continual harvest, provides an opportunity to increase algal culturing capacities in hatchery settings with limited resources, as well as provides a low-cost platform for culturing algal biomass for potentially high value products.

NOAA'S ECOSYSTEM APPROACH TO AQUACULTURE

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This presentation will provide an overview of the Office of Aquaculture's Ecosystem Approach to Aquaculture (EAA) project, including a definition of EAA, the purpose and rationale for development of the document, and some of the benefits of EAA. NOAA is developing an Ecosystem Approach to Aquaculture that will integrate environmental, social, and economic considerations related to aquaculture as a "fishery" regulated under the *Magnuson-Stevens Act* (MSA). The EAA will also serve to reaffirm that aquaculture is an important component of NOAA's efforts to provide sustainable seafood and maintain healthy, productive marine ecosystems. This includes aquaculture's role in protecting special marine areas, rebuilding overfished wild stocks, restoring populations of endangered species, and restoring and conserving marine and coastal habitats. The definition of EAA is the first step in a wider conversation about ecosystem-based management of aquaculture. Implementing the EAA will involve balancing competing uses of the marine environment, creating employment and business opportunities in coastal communities, and enabling the production of safe and sustainable seafood.

LAYING DOWN GROWTH RINGS FOR CONNECTICUT SHELLFISH

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The Connecticut Shellfish Initiative is a broad-based effort to grow commercial, recreational, and natural shellfish populations, and increase public awareness about their importance for maintaining a healthy Long Island Sound and providing maritime jobs.

Connecticut shellfish are an important part of our environment, culture, tradition, and economy. Shellfish depend upon, and also contribute to, clean water and a healthy Long Island Sound. There are forty-six companies with over 300 people employed in shellfishing-related jobs. Commercial shellfish harvests contribute in excess of $30 million dollars annually, while sales of recreational harvest permits generate over $110,000 annually in direct revenue to local communities.

Connecticut citizens, shellfishermen, regulators, scientists, and other interested parties collaborated to develop and publish the *Connecticut Shellfish Vision Plan* (see http://shellfish.uconn.edu). Announced in October of 2016, the plan contains specific recommendations and sets targets to track short- and long-term outcomes.

Key successes include:

1) Eight new/expanded commercial shellfish operations
2) Approval of hiring plan for three new staff at state Bureau of Aquaculture
3) An alternative route for industry to access shellfish grounds in Branford
4) Expanded public outreach on Connecticut's shellfish heritage (including five community shellfishing days, numerous festivals, and site visits organized by industry members, shellfish commissions, and state agencies and organizations)
5) Bioextraction coordinator position announced by the New England Interstate Water Pollution Control Commission

The next phase will include a focus on:

1) Additional staff and infrastructure for state Bureau of Aquaculture
2) Streamlining shellfish-related laws, regulations, and policies
3) Strategies to address water quality impairments in shellfish harvest areas
4) Establishing restoration priorities
5) Expanding public outreach on the regulatory process for aquaculture
6) Setting specific research goals for high priority issues

These efforts will result in both near- and long-term benefits towards improved recreation, economic development, and a healthier marine ecosystem.

ECSGA POLITICAL AND REGULATORY PRIORITIES

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Presentation will review the priorities for regulatory reform and political advocacy that have been identified by the East Coast Shellfish Growers Association board of directors and our membership. The ECSGA represents commercial shellfish growers from Maine to Florida and works to ensure that policies and regulations developed by federal and state agencies are workable.

Top priorities for the association include:

- Restoring access to European Union markets for US shellfish producers.
- Improving access to effective and affordable crop disaster assistance programs.
- Securing an exemption to the Jones Act for aquaculture operators in state waters.
- Ensuring that hatcheries, wet storage facilities and remote setting operations remain exempt from requirements for National Pollution Discharge Elimination Permits.
- Retaining and restoring access and working waterfront facilities for aquaculture.
- Advocating for funding for critical research needs in various federal agencies.

HIGHLIGHTS FROM THE INTERSTATE SHELLFISH SANITATION CONFERENCE

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Presentation will review top issues and actions taken at Interstate Shellfish Sanitation Conference held in October 2017.
Highlights:

- A new aquaculture chapter was adopted for the Model Ordinance. Impacts will be described.
- States need to collect harvest data from harvesters (or dealers) at least monthly, and report annually. States are encouraged to provide a breakdown of whether the product was shucked, pot-harvest processed or served raw.
- Dealers need to educate employees about hand washing, keeping shellfish cold and how they can't be handling food if they are sick.
- Shellstock received by dealers shall be iced or refrigerated within 2 hours of receipt.
- The Conference voted to appoint a Workgroup to amend the *Vp* Illness Response Guidance document for anticipated interim approval by the Executive Board.
- New shellfish tag requirements will go into effect on Jan. 1, 2019.

Presentation will also describe emerging issues that could have profound impacts on industry such as:

- Whether mooring fields are “marinas” requiring prohibited closure areas.
- Implications of the FDA’s definition of “adulterated”.
- Implications of increased use of “Culture Independent Diagnosis Testing” by hospitals.

ATLANTIC SALMON BREEDING PROGRAM AT THE NATIONAL COLD WATER MARINE AQUACULTURE CENTER

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The USDA-ARS National Cold Water Marine Aquaculture Center (NCWMAC) in Franklin, ME has been supporting the U.S. coldwater marine aquaculture industry for the past thirteen years by developing a genetically improved North American Atlantic salmon. The St. John's River stock was chosen as the focal strain because of fast growth, certification of North American origin, and widespread utilization by industry. The objectives of the program have been to: 1) develop a selection index for carcass weight, cold tolerance, fillet color, fat content, and sea lice resistance, 2) evaluate and validate the usefulness of incorporating genomic information into the salmon breeding program, and 3) evaluate the usefulness of a lumpfish (*Cyclopterus lumpus*) selective breeding program.

Our selected and unselected (control line) Atlantic salmon are evaluated with the assistance of industry partners in net pens to simulate commercial conditions. We have observed an increase in growth by approximately 15% for each generation while survival has been similar between groups of fish. Two of the most important traits for consumers are omega- 3 fat content of the fillet and the color of the fillet. We have observed that crude fat, as measured in the Norwegian prime cut (just behind the dorsal fin) ranges from 7 to 13% (wet weight) for all measured year classes. Total omega-3 fatty acid content in families ranges from 5 to 15% of the total fat with an average of 12.8%. The amount of EPA and DHA in a single portion of the fillet averaged 2-3 g, which is the industry standard. Astaxanthin and canthaxanthin concentrations ranged from 1.1 to 4.1 μg/g of fillet. Future year classes will be evaluated to assess progress in these two traits.

Selection for resistance to sea lice has been an important component of the breeding program since 2015. Evaluations of phenotypic family based resistance were standardized and conducted across all families in the breeding program. Currently, evaluations are based on replicated small scale infections and the heritability seen across our populations is 0.20. In the fall of 2017, the first year class of families screened under the new program were spawned. The offspring of this spawning will be evaluated in 2019. In addition to the current challenged based screening, efforts are being made to develop genetic markers and tools to estimate the genetic breeding values. Furthermore, a lumpfish selective breeding program has also been developed as a means to control sea lice infection.

EXPANDING SEAWEED CULTIVATION IN CONNECTICUT – ADDRESSING BARRIERS RELATED TO PUBLIC HEALTH

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As interest in seaweed cultivation is growing in Connecticut, questions surrounding how to regulate this new industry continue to emerge. Lack of Federal guidelines on the production and processing of seaweed make it difficult for Connecticut producers and processors to develop state-required food safety management plans for seaweed produced for human consumption that are based on Hazard Analysis and Critical Control Point (HACCP) principles. Potential public health hazards related to open water cultivation and dehydration of native *Gracilaria tikvahiae,* an edible species of red seaweed, are being investigated to provide recommendations for regulators and industry. Data collected will be included in a Connecticut-specific seaweed hazards guidance document. In addition, results of a comprehensive survey of Connecticut consumers on seafood with specific questions related to seaweed and interest in local seaweed products will inform efforts to determine potential markets for locally produced seaweed.

THE EVOLUTION OF KELP HATCHERY TECHNOLOGY – FROM ACADEMIA INTO COMMERCIALIZATION

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The goal for UCONN and our non-profit GreenWave partner this season was to enhance the kelp hatchery production capacity to produce 120 sugar kelp spools for farmers in southern New England from locally sourced material. Collections were made using SCUBA in New London, CT and Newport, RI. The growing seaweed industry is pushing for organic certification across all sectors and this season we investigated organic seed production. Experimental treatments were set up at NOAA NMFS Lab to test the effects of several nutrient and seedstring types on sugar kelp development. An update of these results will be presented. Running an industrial kelp hatchery presents higher risks because of the farmers who are dependent on the kelp seed for their next crop. Overall however, the transfer on technology and knowledge from academia into the commercial sector has been successful. Some concerns still remain about how increases in water temperatures will affect wild sorus collection and are driving the industry to research, develop and test methods for induced sporulation and kelp gametophyte culture work.

NEW TOOLS FOR SELECTIVELY IMPROVING STRAINS OF SUGAR KELP FOR FOOD AND FUEL

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As a part of ARPA-E's MARINER program, a team of scientists and entrepreneurs is pursuing a selective breeding program to improve the productivity and composition of sugar kelp, *Saccharina latissimi*, which could serve as feedstock for biofuels. ARPA-E's and our goal is to develop tools and a pathway toward low-cost (< $100/DWT) seaweed feedstock that could supply 10% of US transportation fuels. Current markets are human and animal food and ecosystem services.

Our project will develop several complementary tools to reach this objective. To facilitate high-throughput creation of family crosses, the NOAA Milford Lab and UCONN are developing cell sorting methods to efficiently isolate and clone gametophytes. USDA/Cornell and HudsonAlpha will employ PacBio and Illumina sequencing to create a deep-sequenced reference genome and establish a variant catalog for our founding populations and families. WHOI, UCONN and GreenWave will oversee field trials of 144 families (from 12 different 'strains') planted in triplicate plots on two farm sites (nearshore and offshore) over two growing seasons. The resulting family phenotypic data will be associated with genetic markers (GWAS), and we will identify variants significantly associated with primary productivity and composition traits. A goal is to develop methods to predict offspring (SPs or sporophytes) performance based upon genotype and breeding values of parents (GPs or gametophytes) as a short cut around extensive and expensive field testing (Figure 1). A separate ARPA-E project will test the potential labor-saving use of WHOI's autonomous underwater vehicles and sensors for conducting nutrient, acoustic, and optic measurements of macroalgae plots. These will be compared to conventional hands-on field measurements.

Ultimately our project goal is to select sugar kelp best suited genetically to offshore farm environments and possessing qualities of increased dry matter yield per unit area (up to 10% per generation) and improved composition for use as a bioenergy feedstock.

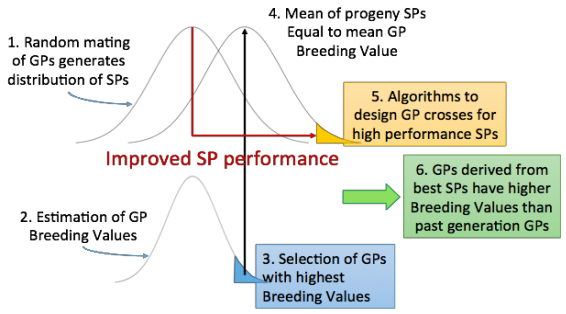

Figure 1. There are breeding advantages that we derive from kelp's biphasic life history. In blue and yellow are the critical elements to improve SP performance within one life cycle. In green is the critical element to improve performance across multiple cycles.

THE DEVELOPMENT AND DEPLOYMENT OF A COMMERCIAL-SCALE KELP FARM IN NANTUCKET SOUND

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A system for the farming of sugar kelp (*Saccharina latissima*) and other macroalgae in offshore waters has been developed and deployed on Horseshoe Shoals south of Cape Cod. The system is a rectangular array that accommodates 2.2 km of longline in an area of 0.22 Ha. This very favorable culture density will allow a high level of productivity in a given lease area and facilitate innovative seeding and harvest techniques to make kelp farming more profitable. This Phase 2 project is being supported by NOAA under their SBIR program following a successful Phase 1 project that demonstrated the viability of the approach through tank testing.

The presentation will explain the rationale for the design and describe the engineering of a site-specific prototype and its fabrication. The permitting process associated with the novel system will presented along with the challenges associated with its assembly and

launching in Woods Hole, MA and the deployment steps from anchor placement to seeding. The system is currently being evaluated for its performance during the 2017-18 growing season.

While the present design is aimed at deep-water locations where anchoring costs can be significant, other variations of the design principles will be explained for shallow waters and for situations where multi-array configurations enable true industrial-scale operations.

TWENTY YEARS OF COOPERATION: THE SOUND SCHOOL REGIONAL AQUACULTURE CENTER & THE NMFS LABORATORY IN MILFORD

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The researchers at from the National Marine Fisheries Service in Milford, Connecticut have been longtime supporters of both the students and staff from the Sound School Regional Aquaculture Center in New Haven, Connecticut. We have worked with many individuals and groups from the Milford Laboratory. Our interactions have always been positive, rich in in educational outcomes and hands-on experiences. Recently, after reviewing some material for an upcoming project I realized that another joint venture with the folks from Milford was probably in the making. This prompted me to begin to write a list of past projects that had cooperative components with my students. The list of projects and support began in 1998, twenty years ago. Time passes rapidly, staff changes and people retire; it seems appropriate that we take a moment to both acknowledge and thank those who have provide so much support and influenced so many of the students in the Sound School's Fish Production Laboratory for the last two decades.

FIRST YEAR RESULTS FROM OFFSHORE SHELLFISH AQUACULTURE IN FEDERAL WATERS OF THE ATLANTIC

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In the summer of 2016, we deployed the first longline as a pilot study to establish a commercial scale (33 acre) offshore mussel farm 7 nautical miles off the coast of Cape Ann Massachusetts (NAE-2012-1598 NEMAC Aquaculture). The purpose was to explore best practices for offshore shellfish aquaculture as well as monitor for potential habitat effects or protected species interactions attributed to shellfish aquaculture in offshore waters. This study also monitored for oceanographic parameters, harmful algal blooms (HABs) and shellfish growth dynamics. This report will focus on the process of setting out our first longline, reporting on our activity to date to disseminate what was learned about this process to others wishing to pursue offshore shellfish aquaculture.

Our initial results indicate that mussels grow as well if not better than they do coastally with little fouling at a depth of 50 – 75 feet. Throughout the summer, we monitored for harmful alga using the sampling protocol established by the state coastal shellfish division at Massachusetts Division of Marne Fisheries (MA DMF) and collected mussels from the site for periodic saxitoxins testing by MA DMF.

Based on the observation of mussel spat settling on the header and growlines, spat collection lines were deployed on site to collect seed stock during the summer. The initial growlines were harvested in September 2017 and after clearing saxitoxin testing were released for non-commercial consumption. Mussels grown offshore were of good quality and taste.

After a successful pilot research study, we plan to modify our permit to expand the number of longlines to a commercial scale farm with 32 – 400 ft. longlines. Our ultimate objective is to refine and enhance offshore shellfish aquaculture as an alternative fishing option for fishermen and lobstermen currently displaced or negatively impacted by current fishery restrictions by providing an incubator farm site for interested parties to try offshore aquaculture. This expansion of shellfish aquaculture from already crowded coastal areas into offshore federal waters will increase revenue streams, producing more US local grown shellfish, reducing the heavy reliance on imported product in the market.

MUSSEL AQUACULTURE TAKES A DEEP BREATH: STRATEGIES AND CHALLENGES OF OFFSHORE FARMING IN THE NE US

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To satisfy the increasing national demand for fisheries products, domestic aquaculture is to be expanded offshore to the U.S. Exclusive Economic Zone (EEZ). For this relocation several constraints need to be overcome such as technical and social issues, including legal ones, and establishment of environmentally-based planning. The choice of suitable sites for the target species in the exposed, high-energy environment is key for success. Mussel offshore culture is to be performed with submerged long-lines, however submersion depths are set based only on ship traffic rather than mussel ecology. To determine suitable depth submersion of mussel ropes in mooring systems, NOAA's open source climatologies and remote sensing data of temperature and chlorophyll *a* from 2005 to 2012 were used to construct a "habitat suitability" analysis of southern New England EEZ areas. Special importance was given to temperature, which affects mussel feeding, triggers reproduction, and indirectly affects byssus effectiveness to adhere to farming ropes. Considering ecological factors at present and anticipating temperature increases, our results suggest mussel ropes be submerged during summer at a minimum of 15 m depth in northern and 20 m depth in southern areas of New England, where temperature is between 10-14 °C and phytoplankton biomass abundant. Promising areas are where the thermocline is shallow such as offshore Long Island, Cape Ann, and offshore New Hampshire, although mussels can be placed elsewhere but deeper in water column during warmer months. Suitability of selected sites will be further validated by assessing water quality and the performance of mussels *in situ* with a flow-through device. The biodeposition measurements also will allow estimation of feeding patterns that may have potential local effects. Future steps are to estimate the availability (sufficient quantity), quality (mortality, free of diseases), and timing of spat offshore, as nearshore larvae are dispersed offshore by currents. These larvae have no chance to encounter a place to settle and currently can be considered "lost at sea", i.e., unable to contribute to natural recruitment of mussels. Mussel spat collectors will be deployed at trial farms at pre-defined seasons. Larval collection offshore will satisfy legal requirements of "locally collected" seeds using larvae that would otherwise be lost. Lastly, as endemic harmful algal blooms poses risks to the productivity of future sites, a review of the historical bloom database will allow the understanding of bloom features. This research will contribute to advancing knowledge for the sound management of American offshore aquaculture.

INCREASING NORTHEAST US MARINE AQUACULTURE PRODUCTION BY PRE-PERMITTING FEDERAL OCEAN SPACE

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Marine aquaculture production in the US lags behind that of other major seafood producing countries. One of the most frequently cited reasons for this is the complexity and cost of the process for obtaining permits to farm seafood in US waters. Obtaining a permit to farm seafood in federal waters requires an extensive process of review and consultation with several agencies. A team of researchers from the Woods Hole Oceanographic Institution, the New England Aquarium, the University of Massachusetts (Boston), and the Massachusetts Aquaculture Association has received funding from the NOAA Sea Grant Program to identify suitable areas in federal waters off New England and pre-permit these for a range of aquaculture activities, with the goal of easing the regulatory burden for aquaculture ventures to increase production of seafood products in US waters. The project team will work with the New England aquaculture industry and with federal and state agencies to identify promising areas of federal waters and then pre-permit these areas for broad categories of marine aquaculture, including longline and on-bottom shellfish culture and large-scale kelp culture with a focus on native species and low-impact growout technologies. We will establish a mechanism for current and prospective aquaculture operators to qualify to use portions of these pre-permitted areas for farming operations, and transition that mechanism entirely to NOAA Fisheries Office of Aquaculture (or another appropriate agency) at the conclusion of the project. Members of the New England aquaculture community who are interested in participating in the process should contact Hauke Kite-Powell at the address above.

MASSCOAST: A NEW SHELLFISH AQUACULTURE SITING AND PERMITTING TOOL FOR MASSACHUSETTS

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Siting and permitting of shellfish aquaculture operations is a key hurdle that prospective growers must overcome to start or expand operations. Permitting in Massachusetts is particularly difficult, as shellfish aquaculture licensing is under municipal jurisdiction, and growers must obtain a range of approvals. The Massachusetts Coastal Oyster Aquaculture Siting Tool (MassCOAST) was developed to assist prospective growers with the siting and permitting process. This GIS-based, online tool provides key environmental and legal data for consideration by prospective growers, local officials, and state regulators, as well as a step-by-step explanation of how to use these data during site selection. This presentation will introduce the tool and consider how it may be a useful model for other states, regions, and industry sectors.

DEVELOPMENT OF AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM FOR KOREA WATERS

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Integrated multi-trophic aquaculture (IMTA) combines the fed aquaculture (e.g., fish or shrimp) with the extractive aquaculture (seaweed and shellfish) to create a more balanced ecosystem. In Korea, most open water fin- and shell-fish aquaculture practices occur in the southeast coastal areas of the country while the seaweed aquaculture mostly occurs in the southwest of the country. This disagreement of cultivation locations between organisms has caused environmental problems, such as harmful algal blooms near finfish farms and nutrient depletion at seaweed farms. A pilot scale IMTA system was introduced to Tongyoung, Gyeongnam, Korea. Sea cucumbers (*Apostichopus japonicus*), oysters (*Crassostrea gigas*) and seaweed (*Gracilariopsis lemaneiformis*) were cultivated with fifty thousand young *Pagrus major* (average weight of 2.2 g) in this IMTA system (1 hectare). Growth, biomass yields and tissue nitrogen contents of each organism were analyzed to evaluate nitrogen (N) discharge from the fish aquaculture and N extraction by the extractive aquaculture (sea cucumbers, oysters and seaweed) during the summer–fall growing season in 2016. Based on the findings in this study, a model IMTA system was developed for environmentally sustainable aquaculture in Korea.

USE OF POINT-OF-VIEW VIDEO CAMERAS TO DOCUMENT FISH INTERACTIONS WITH OYSTER CAGES

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We recorded interactions of fish with oyster cages using point-of-view video cameras to provide data for regulators and fishery managers who make decisions about aquaculture practices, and to document habitat services provided by aquaculture gear. A series of field trials were conducted to 1. Determine how density of oyster cages influences fish abundance and behavior in and around aquaculture gear and 2. Compare fish interactions with oyster cages to fish activity at other habitats without cages. Study sites, located off Milford CT, included an inshore oyster cage farm containing 40+ commercial cages, a deeper (25-30 feet) shellfish lease with generally featureless seafloor, a natural rock reef, and an inshore on-bottom oyster culture area. Oyster cages, measuring 4 x 3 x 2 feet, contained three shelves, each holding two mesh bags, for a total of six bags per cage. Each bag was stocked with 200 oysters. For the cage density comparison, three cages were placed 50 yards apart in close proximity to the farm while at the deeper lease and on-bottom oyster culture sites, three cages were placed at discrete locations 90+ yards apart. Cages were equipped with two Go Pro Hero 3+ cameras. One camera was mounted to view the horizontal surface across the cage top, while a second camera placed at one corner captured activity along two sides and the cage bottom. Cameras began recording the day after deployment for 8 minutes every hour from 7 am to 7 pm over a complete tidal cycle using Blink intervalometer controllers. To assess fish activity at habitats without cages, three minimal T-platforms, each mounted with two cameras, were used. Platforms were surface deployed on featureless and shell bottom. On the rock reef, divers were used to position T-platforms adjacent to boulders to provide a field of view similar to cage-mounted cameras. Video was collected for a two hours after deployment. One cage per site was outfitted with a Hobo Temperature/Light Data logger and Lowell TCM-1 tilt current meter during trials. Video was collected from two of the four sites during any given trial. Magenta filters attached to cameras removed some of the green water coloration. Abundance of fish associated with oyster cages will be determined using MaxN counts (video frame with maximum number of fish of any species observed within a selected time interval) while fish behavior will be scored using Observer XT software.

BEHAVIORS OF FISH ON AQUACULTURE GEAR

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Oyster cages used to contain, handle, and protect oysters in culture, deployed on relatively flat ocean floor, provide three dimensional habitat structure for the local wild fish community. Understanding the use of aquaculture gear and the ecosystem services it provides may inform regulatory decisions and increase social license for shellfish farming. Quantifying the behavioral attributes of fish-habitat interactions is one approach for understanding the functional ecosystem role that oyster cages can play. The use of point- of-view (GoPro) cameras attached to oyster cages facilitated visualization and quantification of the behavioral interactions between animals and these artificial habitats. For comparison, GoPro cameras were also placed on natural rock reefs using a platform designed specifically to add minimal structure in the area surveyed. Video analysis will be conducted using Observer XT (version 14.0) to observe and quantify the range of fish behaviors, including: station keeping, feeding (based on bites in water column and on cage surfaces), flight response (fear or predator avoidance response), and flow refuge (position with refuge from the current). Initial findings suggest that the structure provided by oyster cages provides services similar to those afforded by natural rock reefs.

*The Federal Government does not endorse the use of GoPro™ cameras

ASSESSMENT OF FISH COMMUNITIES ASSOCIATED WITH OYSTER CAGES USING ENVIRONMENTAL DNA METABARCODING

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Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from organisms into the environment. In aquatic systems, analysis of eDNA allows us to understand community composition, as well as relative abundance and distribution pattern of taxa. eDNA was extracted from water samples collected at 4 sites: an inshore oyster cage farm, two shellfish leases with featureless seafloor and different water depths, and a natural rock reef. Mitochondrial 12S rDNA amplicon-based next generation sequencing was conducted to characterize the finfish communities at each site. By generating finfish taxonomy tables using the aforementioned DNA metabarcoding, we aim to thoroughly depict finfish diversities at different habitats to better understand ecological services provided to fish by oyster cages and how that compares with natural structured habitat. This has been the first attempt to survey the finfish diversity in central Long Island Sound using eDNA metabarcoding, and the resulting amplicon sequence data will be deposited in a next generation sequencing database that is currently being constructed to promote the application of eDNA technology in conducting fisheries surveys in the Northeast region.

DIAMOND BACKED TERRAPIN (*MALACLEMYS TERRAPIN*) BLOOD SERUM ANALYSIS OF HEAVY METALS COLLECTED FROM A SALT MARSH SYSTEM IN MADISON, CT USA

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The shellfish industry and public seafood concerns have been expressed about the bioaccumulation of heavy metals into marine food webs for decades from pollution/dredging. Key to this concern has been the identification of long-lived, site specific, non-migrating species (indicators) that show heavy metal pollution entering aquatic food webs.

The East Neck River estuarine system between the towns of Guilford and Madison, CT contains a residual population of the diamond backed terrapin (*Malaclemys terrapin*) that inhabit areas known to contain oysters and clams. It is also recognized by a lack of industrial runoff and salt marshes protected by conservation organizations, including Audubon Connecticut, which maintains a salt meadow sanctuary and the towns of Guilford and Madison with local preserves and Land Trusts.

In June of 2008 as part of a Senior SAE-ISSP Supervised Agriculture Experience project, more recently termed the Capstone Project, I sought to learn more about this terrapin population, locate and identify nesting areas and determine any background contamination. Career Service information was provided as to the process to seek approvals from state and local agencies, including an effort to gain baseline heavy metal data from sampled terrapin blood serums. Permission was obtained from the Madison Land Trust and the Madison Conservation Commission, and a collection permit was issued by the Connecticut Department of Environmental Protection for this project.

A total of five adult terrapins were collected from the Neck River and blood drawn under protocols by the Branford Veterinary Hospital and delivered to the Connecticut Agriculture Experiment Station for testing.

The East River is considered to be largely contaminate-free. It is hoped that blood serum results will provide important baseline data for estuarine researchers and/or studies looking at heavy metals and the shellfish industry.

STORIES BEHIND THE HEADLINES: AN UNLIKELY LOCALE AS GROUND ZERO FOR MARINE CONFLICTS WITH GLOBAL IMPLICATIONS

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Seals/sharks/fish/fishermen; marine animal strandings; half the world population of North Atlantic right whales; over 200 right whales spotted in a single day; concentration of humpback whales; whales/people interactions; the Gulf of Maine heating up faster than anywhere in the world (with one exception)/species disruption/change and human interactions; high sea level rise anticipated/planning for resilience; the Gulf Stream slowing down/unknown consequences. None of these issues takes place in a vacuum and each has a story behind it. Is it an accident of geology and geography that a sand bar sticking 40 miles east and an additional 35 miles north is the primary reason that Cape Cod is ground zero for the concentration of these issues? While not all of these issues are unique to this one area and are more regional, the high number of them as a group is highly unusual and serves as a focal point for discussion. It is also unusual that these conflicts and issues have global implications. This paper will present an introduction to the back-story of as many interactions as time allows.

DID YOUR SHELLFISH DIE FROM AN ALGAL BLOOM, OR HYPOTHERMIA, OR BOTH?

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Episodes of elevated shellfish mortality are often associated with algal blooms. Many algal species produce toxins or other substances that can be lethal to shellfish when cell counts exceed specific densities and exposure times are sufficient. But some algal species co- occurring with shellfish mortality or fishkills have no prior scientifically demonstrated lethal connection; or the conditions present at the time do not conform to the criteria enabling their lethal potential. Under these circumstances one might consider seawater cooling as a possible causal agency. Numerous cases of elevated mortality, of both shellfish and finfish, have been reported following episodes of rapid cooling. However, from a forensic perspective, the lag phase of several days to two weeks can obscure the connection between cooling and mortality. Cases of decreasing temperature associated with high mortality of shellfish and planktivorous fish, including the role of microalgae lipids, will be discussed.

USE OF NATURAL TROPHIC RESOURCES BY DIPLOID, TRIPLOID, AND TETRAPLOID EASTERN OYSTERS

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We compared filtration, ingestion, and assimilation of natural seston by diploid, triploid, and tetraploid oysters at two Chesapeake Bay sites during three seasons using *in situ* biodeposition measurements. Clearance, filtration, ingestion, and assimilation rates and efficiencies were not statistically different between ploidy types at a site at a sampling time. Seston quality (organic/inorganic content, chlorophyll, phytoplankton community composition) differed between sites and between seasons, and oysters modified feeding variables in response to environmental conditions similarly for all ploidy types. Differences in performance of diploid and triploid oysters growing side-by-side at commercial sites in the Chesapeake Bay do not appear to be mainly attributable to differences in acquisition of trophic resources. We hypothesize that metabolic differences between ploidy types may contribute to performance differences.

THE EASTERN OYSTER AS A BIOINDICATOR FOR NITROGEN SOURCES IN THE DELAWARE INLAND BAYS

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The Delaware Inland Bays are three shallow coastal bays located in southern Delaware. The three bays, Rehoboth, Indian River, and Little Assawoman, are surrounded by highly developed areas and have low flushing rates. These characteristics have caused anthropogenic activities to degrade water quality. Ongoing degradation of the bays since the late 1800s has led to a dramatic decline in local *Crassostrea virginica* populations. Oysters are a keystone species, which provide habitat for organisms and help to improve water quality and also act as bioindicator for the ecosystem health. The goal of this research is determine the sources of nitrogen pollution in the bays using oysters as a bioindicators and determines the ability of oyster in the improvement of water quality. A study was conducted in the Rehoboth, Indian River, and Little Assawoman Bays over the course of two years. Aquaculture gear was placed at one location in each bay and oysters were collected at three points throughout the season and were analyzed for stable isotope ratios of carbon and nitrogen. Water samples from throughout the three bays and soil samples from surrounding areas were also collected and analyzed for comparison. This research is expected to help better understand the sources of nitrogen pollution and the role of oyster aquaculture restoring the viability in natural habitat of the Delaware Inland Bays.

EXAMINING *CRASSOSTREA VIRGINICA* DENITRIFICATION RATES: HOW DOES LOCATION, SHORT TERM NITROGEN LOADING, AND RISING TEMPERATURES IMPACT VALUABLE ECOSYSTEM SERVICES?

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As anthropogenic activities continue to add excess nitrogen into the marine environment, understanding how marine organisms will be impacted becomes vital for sustaining coastal resources. The eastern oyster, *Crassostrea virginica*, is valued for performing the ecosystem service of nitrogen removal by facilitating the microbial process of denitrification (conversion of nitrate to dinitrogen gas). Denitrification rates associated with oysters are not well constrained at this time, but are likely influenced by dynamic environmental conditions. It is possible, that in combination with nitrogen loading, rising estuarine temperatures may further alter these rates. This becomes a growing concern, as incomplete denitrification can lead to an accumulation of Nitrous Oxide (N_2O), a potent greenhouse gas. The goal of this study was therefore to examine how denitrification potential of *C. virginica* is influenced by location within a coastal estuary and/or experimental nutrient enrichment. In a two-factor experiment (site x nitrogen treatment), we maintained juvenile oysters at contrasting ends of the estuarine gradient within Point Judith Pond in Narragansett, Rhode Island. Oysters were exposed to nitrogen – enriched or ambient conditions at each location. Upon completion of this four-month field manipulation, the experimental organisms were incubated in a controlled environmental chamber (Hollman Engineering) at the Graduate School of Oceanography, under current and projected temperatures (18°C and 24°C, respectively). Enriched water was pumped into each oyster tank with a target of three times the background of dissolved inorganic nitrogen levels of the field sites (100µM). A three-way ANOVA tested the effects of field location, field treatment, incubation temperature, and the interactions between these factors on denitrification potential. Results indicate that higher temperatures significantly increase N_2O production ($p=0.0123^*$) and also caused oysters who experienced short term nitrogen loading in the field, to switch from a source of dinitrogen gas (denitrification) to a sink ($p=0.0100^*$), thereby eliminating the valuable ecosystem service. These findings can aid in decision making regarding aquaculture and restored oyster reef restoration, as well as offer insight on how to maximize environmental benefits from *C. virginica*.

EVIDENCE FOR NITROGEN REMOVAL VIA PHYTOREMEDIATION WITH PHRAGMITES

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As shellfish restoration biologists, nitrogen eutrophication is one of the biggest challenges we face. Excessive nutrients cause micro- and macro- algal blooms, subsequent oxygen deprivation as the bloom dies, loss of biodiversity, seagrass beds and ecosystem resilience. On Martha's Vineyard, MA, most of the controllable nitrogen inputs originate in residential wastewater. The high costs of construction and operation of conventional tertiary sewage treatment systems make them impractical for most rural watersheds. Furthermore, the decadal timescale at which groundwater moves towards an estuary means that the impacts of nitrogen will continue for years after the installation of treatment systems. This delayed delivery of N-rich ground water makes on-site mitigation an added necessity. Among the options are bioextraction using oysters, seaweed and plants.

Phragmites australis is a cosmopolitan species that is highly invasive in North America. Despite its invasive nature, there is scientific evidence that Phragmites provide important ecological services, especially sequestration of nitrogen, carbon and phosphorus. This strong affinity has been exploited for nutrient management in eutrophied estuaries and lakes in other parts of the world, as well as and in wastewater treatment applications. Science supports the concept of cutting and removing Phragmites as a means to reduce soil and water contamination.

This project focused on the potential to harvest existing Phragmites as a means to remove nitrogen from the adjacent estuary, yet it had many components. We collected bimonthly data on reed height and mass in 8 stands on Martha's Vineyard, during the 2016 and 2017 growing seasons and these tissues were also made available for %N analysis. A stalk density survey was conducted each year, and Phragmites area was estimated at 3 estuaries. Using these data, we calculated kgN m^{-2} in aerial Phragmites biomass, on average, on Martha's Vineyard. Groundwater wells were used to measure nitrogen uptake by a stand of Phragmites. Inspection of native species, permitting regulations and the use of cut Phragmites for marketable products were included to support the vision of widespread, annual harvest of the invasive reeds for bioextraction. Although the study sites were focused on Martha's Vineyard, the results of this study may be applied to Phragmites across the region.

COASTAL ACIDIFICATION AMPLIFIERS ALONG THE US EAST COAST: CONCERNS FOR SHELLFISH PRODUCTION

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In coastal systems endangered by acidified water, it becomes paramount to understand the link between acidification and its environmental drivers. Bays along the US Mid-Atlantic coast are particularly vulnerable to local amplification of ocean acidification due to highly eutrophic conditions, low alkalinity freshwater input, and episodic upwelling of acidified water. To better understand these drivers two research studies were conducted along the NJ coast. The first study was conducted during the summers of 2014 and 2015 at the Aquaculture Innovation Center (AIC) of Rutgers University located in Cape May, NJ. The second study began in the summer of 2017 and focused on elucidating the range of pH and aragonite saturation (Ω_{arag}) conditions experienced in Little Egg Harbor Bay, NJ. Temperature, salinity, dissolved oxygen (DO), and pH were continuously monitored at both locations. In addition, partial pressure of carbon dioxide (pCO_2) was monitored in Little Egg Harbor. At both locations, pH showed diurnal variations that tended to mirror the DO signal. The lowest pH values were measured at the AIC in July of 2014 and again in July of 2015. These pH drops were decoupled from the DO signal. The occurrence of consistent Southwesterly winds and cooler surface water temperatures along the coast during both time frames indicated that upwelling was occurring. The pH drop in 2014 resulted from a complex interaction of wind shifts and high river discharge. Validated data from Little Egg Harbor showed a strong correlation between pH and pCO_2. The Ω_{arag} for these data tended to range between 1 and 1.5 with excursions below 1 at night.

EFFECT OF OCEAN ACIDIFICATION ON THE PHYSIOLOGY OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*

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Marine shelled mollusks are among some of the most sensitive marine organisms impacted by ocean acidification (OA). Exposure to elevated pCO_2 is known to negatively impact growth, calcification, and survival, suggesting potential alterations to energy allocation; however, these mechanisms are poorly understood for many species. We assessed the physiological impacts of OA on a marine bivalve, the northern quahog (*Mercenaria mercenaria*), through several laboratory experiments. Clams were reared in ambient conditions ($pCO_2 \approx$ 400, pH≈8.2) and elevated conditions (pCO_2 ≈1000, pH≈7.5). Larval clams reared in elevated pCO_2 were significantly smaller than those reared in ambient conditions at 48 hours post fertilization. Juvenile clams in elevated pCO_2 had larger shell height at one month and larger length at two months as compared to clams reared under ambient conditions. Respiration rates were reduced under elevated pCO_2, possibly indicating lower metabolism. When challenged with bacterial pathogens, larval and juvenile clams in the elevated treatment had significantly higher mortality as compared to clams in the ambient treatment, suggesting a reduced immunity in larval and juvenile clams at elevated pCO_2. Clams reared under elevated pCO_2 conditions for 1 year and transferred to ambient conditions for six weeks showed higher survival following bacterial challenge as compared to clams continuously exposed to acidified conditions, suggesting recovery of immune performances. In contrast, a subsample of clams reared in ambient conditions for one year and exposed to elevated pCO_2 for six weeks had increased mortality when exposed to bacterial pathogens as compared to clams continuously maintained in ambient conditions. Bacterial counts in clam tissues mirrored the mortality data. Finally, we tested the survivorship and growth of bacterial pathogens (*V. coralliilyticus, V. splendidus,* and *Listonella anguillarum*) used in the exposure experiments to assess if mortality was caused by increased virulence of the pathogens or decreased immunity of the clams. Our preliminary results suggest that physiological responses of *M. mercenaria* to changes in elevated pCO_2 are caused by energy reallocation, although future studies will attempt to determine any genetic contributions.

LOCATION, LOCATION, LOCATION: POPULATION DIFFERENCES IN RESPONSE TO OCEAN ACIDIFICATION IN BLUE MUSSELS

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Recent information all suggests that bivalved molluscs are particularly sensitive to the impacts of ocean acidification (OA). However, we do not know whether differences among local environmental conditions has selected for animals with different sensitivities to stressors, or whether responses to environmental stressors are phenotypically plastic, allowing animals with broad physiological tolerances to be robust to environmental stress. Traditional metrics of the effects of stress integrate over long periods of time (e.g., growth), are a snapshot of animal state (e.g., condition index), or require isolation of animals from their environment (respiration rate). However, infrared and Hall-Effect sensors allow monitoring heartbeat and valve gaping (time spent filtering) with high temporal resolution over extended periods of time, allowing us to detect the immediate metabolic and behavioral responses changes in environmental conditions. Blue mussels, *Mytilus edulis*, were collected from sites around Long Island Sound (LIS) with different water quality conditions to test whether mussels from more stressful environments are more resilient to the impacts of OA. We found that mussels from different populations show different stress responses to OA (manipulating aragonite saturation). Mussels from eastern and western LIS had elevated heartbeat rates in response to OA, while animals from central LIS showed no response to even extreme OA conditions, suggesting site-specific resilience to the environmental stress of OA.

UTILIZING A MEDICAL RESEARCH TOOL, SIMMONS-AMMONS VIDEO ANALYSIS (SAVA) SYSTEM, TO DETERMINE CILIA BEAT RATES OF BLUE MUSSELS, *MYTILUS EDULIS*, EXPOSED TO INCREASED CARBON DIOXIDE

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Cilia in molluscs are responsible for activities such as pumping, feeding, gas exchange, digestion, and reproduction. Cilia found on gill filaments create and control the current that allows water and particles to flow over the gills, and gill cilia also are responsible for the capture and handling of food particles. There are three types of gill cilia: frontal, latero-frontal, and lateral. The lateral cilia are responsible for creating a water current for gas exchange, food intake, and waste removal, and both frontal and latero-frontal cilia move particles along the gill for ingestion or rejection. Feeding rates and ciliary movement can be affected by a variety of environmental factors, including temperature and salinity. Recent studies of biological consequences of increased carbon dioxide in the marine environment, referred to as ocean acidification (OA), have found that feeding behavior of bivalves is more affected by the carbon dioxide concentration than by the calcium carbonate saturation state, suggesting that altered ciliary activity may be involved in bivalve responses to OA.

The Sisson-Ammons Video Analysis (SAVA®) system is used widely in the medical industry to determine cilia beat rates in clinical applications. Currently, gill cilia beats are measured in marine bivalves by using a strobe, which is very time consuming. The SAVA system provides an instant measurement of the beat frequency of a sample using live video, and video samples can be stored for more detailed analysis. We are using the SAVA system to determine if carbon dioxide levels result in changes in gill cilia beat rates of the blue mussel. Preliminary results show that the software is able to detect different beat rates of the lateral and frontal-lateral cilia in blue mussel. The addition of serotonin causes the frequency of both the lateral and frontal-lateral cilia to increase as observed with a strobe. The adaption of SAVA from the medical industry to measure gill cilia beat rates in blue mussels will enable us to determine if gill cilia beat rates change as a result of increased environmental carbon dioxide.

EFFECTS OF SURFACE SURFACTANTS ON PARTICLE CAPTURE EFFICIENCY AND CLEARANCE RATES OF THE BLUE MUSSEL (*MYTILUS EDULIS*) AND THE MARSH MUSSEL (*GEUKENSIA DEMISSA*)

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The capture of particles by bivalve molluscs is dependent on particle encounter and retention on the gill filaments, with several factors influencing this process. Generally, smaller particles (i.e., 0.1 to 4 micrometers) are more difficult for most bivalves to capture, but species-specific differences in gill structures can mediate capture efficiencies. Over the past 30 years, different types of microspheres have been used to examine aspects of particle capture and ingestion by bivalves. Critics have posited that manufactured particles may contain surfactants, chemicals commonly used in the manufacturing of plastics to reduce surface tension, that could produce spurious capture and ingestion rates. The goal of this work was to experimentally assess whether the presence of different types of surfactants on manufactured polystyrene particles can result in altered capture efficiencies (CE) and clearance rates (CR) in the blue mussel *Mytilus edulis* and the marsh mussel *Geukensia demissa*. The effects of three different types of common surfactants (Sodium dodecyl sulfate, Benzalkonium chloride, and Triton-x; 0.2 mg/mL concentration) and a control (no surfactant) were tested. There was no effect of surfactant treatment on CR. Treatment with the nonionic surfactant Triton-X significantly lowered CE for all microspheres tested (3, 6, 10 µm), but there were no significant differences in CE for the other types of surfactants tested compared to the control treatment. These data add to our understanding about particle handling by these bivalves, and suggest that residual amounts of surfactants found on commercially available microspheres have little if any effect on feeding processes.

USING A RECIRCULATING AQUACULTURE SYSTEM FOR CONTROLLED LABORATORY STUDIES TO IMORVE FISHERIES MANAGEMENT IN RESPONSE TO CLIMATE CHANGE

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In recent history, ocean surface temperatures in the United States Northeast Shelf have warmed at a substantially higher rate than the global average, while continuing to absorb atmospheric CO2. This will inevitably lead to warmer more acidic waters in Northeast. The productivity and/or distributions of many living marine resources have been changing in concert with shifting ocean conditions. The James J. Howard Marine Sciences Laboratory through federal mandate has created two experimental systems to assess the regional impacts of these potential environmental changes on economically relevant species. We have designed two experimental systems for this purpose. The first is a flow through system with the capacity to manipulate carbonate chemistry, temperature, salinity, flow rates and, dissolved oxygen. It has been used to expose sensitive early life stages of marine species to ocean acidification and temperature changes. The second is a newly designed Recirculating Aquaculture Research System (RARS) to conduct experiments under controlled laboratory conditions to examine the impacts of a variety of temperatures on the metabolic scope of marine fish species. The intent of these experiments is to determine the capacity as measured by metabolic rate of marine species to temperature and oxygen changes and use that to predict future species success and distribution. In future, improved and expanded versions of these systems could be used to manage targeted fisheries adaptively, or to find optimal aquaculture conditions for selected species, or even to address specific problems or questions facing a specific aquaculture industry.

CONNECTICUT'S RESPONSE TO THE MANAGEMENT OF PATHOGENIC *VIBRIO PARAHAEMOLYTICUS*

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Vibrio parahaemolyticus is a naturally occurring marine bacterium in the same family as those that cause cholera and *Vibrio vulnificus*. Since 2012, the Northeast region of the U.S. has experienced a sharp increase in the number of illnesses linked to *Vibrio parahaemolyticus.* During 2013 the State of Connecticut shellfish control authority closed shellfish harvest areas after an outbreak of illness was linked to oysters harvested from growing areas in Norwalk and Westport. Beginning in 2014, Connecticut's *Vibrio parahaemolyticus* control program managers have worked with industry to incorporate more 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. Post-harvest time and temperature controls as required by Connecticut's *Vibrio parahaemolyticus* Control Plans are evaluated by using continuous temperature data loggers to determine the effectiveness of post-harvest temperature controls, and correlated to impacts on *Vibrio* levels in shellfish and the associated risk of consumer illness.

In order to gain a better understanding of *Vibrio parahaemolyticus* levels in Connecticut shellfish, the State's monitoring plan includes the collection of environmental parameters such as water temperature, air temperature, salinity and depth that may correlate to levels of *Vibrio* bacteria in shellfish. Program managers are working with researchers to analyze this expanded dataset of environmental variables and *Vibrio parahaemolyticus* concentrations in shellfish tissue, to determine the Long Island Sound-specific *Vibrio parahaemolyticus* vs. temperature relationship following methods in the FDA pre-harvest risk model. This information is combined with output from a high-resolution hydrodynamic model of LIS to make daily forecasts of *Vibrio parahaemolyticus* levels available to industry and managers.

DO FREEZE-DRIED AND SPRAY-DRIED FORMULATIONS OF PROBIOTIC STRAIN OY15 PREVENT VIBRIOSIS AND IMPROVE SURVIVAL OF LARVAL OYSTERS (*CRASSOSTREA VIRGINICA*)?

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The Milford Laboratory's research, development and application of probiotic bacterial strain OY15 (*Vibrio alginolyticus*) to eastern oyster larvae (*Crassostrea virginica*) have shown that this benign, naturally-occurring bacterium isolated from the digestive glands of adult eastern oysters (*Crassostrea virginica*), has significant positive effects upon the survival and disease resistance of larvae. OY15 improves survival of oyster larvae by 20- 35% when challenged with a known larval shellfish pathogen (*Vibrio corallilyticus*) by stimulating hemocyte immune defense functions and providing larvae protection from bacteriosis. The Milford Laboratory collaborated with Envera LLC through a Cooperative Research and Development Agreement (CRADA), utilizing Envera's specialized expertise in manufacturing and providing beneficial microbial products to the aquaculture industry, to determine if OY15 can be mass-cultured effectively and produced economically in a stable formulation for commercialization and marketing to commercial oyster growers. Envera was successful in large-scale production of OY15 and provided the Milford Laboratory with stable, freeze-dried and spray-dried bacterial formulations. In May of 2017, the probiotic team conducted a larval oyster bioassay to determine if the effects of the OY15 preparations were similar to the beneficial probiotic effects of live OY15 bacterial cells in protecting larvae from bacteriosis and improving larval survival through metamorphosis. These results will provide the insight needed to move forward toward commercialization of Milford Probiotic Strain OY15 and subsequent marketing to commercial shellfish growers in the United States.

TOWARDS THE DEVELOPMENT OF QPX-RESISTANT CLAM STOCKS

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The hard clam or northern quahog, *Mercenaria mercenaria*, is one of the most valuable seafood products in the Northeast representing the first marine resource in several states. In addition to their economic value, hard clams, like other suspension feeding bivalves, play an important ecological role in benthic-pelagic coupling by transferring energy to the benthos and cycling large amounts of particulate matter. Since the 1990's, several Northeastern states have suffered severe losses in aquacultured and wild hard clam stocks due to a fatal disease caused by a protistan parasite called Quahog Parasite Unknown (QPX). In this framework, the development of disease mitigation strategies has become a primary research priority. This study compared the performance of genetically distinct clam stocks and aimed at the identification of genetic markers associated with disease resistance. Clam seeds were deployed in field sites in New York (5 clam strains) and Massachusetts (2 strains) and regularly sampled for the assessment of growth performance and QPX disease prevalence. Results showed the presence of QPX in the deployed clams in both field sites but higher prevalence was noted in the MA site. Significant differences between strains were noted in the MA site with disease prevalence (and mortality) being markedly (5 fold) higher in one of the 2 tested strains as compared to the other commercial strain. In parallel, we identified single nucleotide polymorphism (SNP) markers in a total of 373 immune-related genes and used a subset of these to individually genotype clams sampled before and after QPX-related mortality events in five distinct strains. Analysis revealed consistent allele frequency shifts in seven SNPs, which represent promising markers for QPX resistance. These seven SNPs are being tested for marker-assisted selection of resistant clams.

A PARASITE IN MAINE – THE STORY OF A MSX OUTBREAK

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In 2010, the Damariscotta Estuary in Maine experienced an outbreak of the oyster parasite MSX (*Haplosporidium nelsoni*). The outbreak caused significant mortalities. As a result the industry implemented changes to reduce the impact of the parasite, including changing the strain of oyster being cultivated. In 2012 we began monitoring the prevalence of the parasite in both cultivated and natural bed populations and showed prevalence levels over 50%. We continued this analysis in 2014 and 2016 and showed that while prevalence has fallen the parasite still has a substantial presence. In addition, we looked at the prevalence of the parasite in potential reservoir species found around the oyster farms. This showed that tunicates harbored high levels of parasite and may act as a reservoir species for the parasite. Consequently, we are building a picture of the aftermath of the parasite outbreak within the estuary and the story associated with it.

MEASURES OF DERMO RESISTANCE IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

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Wild and cultured populations of the eastern oyster, *Crassostrea virginica*, suffer extensive mortalities in response to the protozoan parasite *Perkinsus marinus*, causative agent of Dermo disease, throughout the Northeast and Mid-Atlantic. Several management strategies have been implemented to minimize the negative impact of Dermo, including the establishment of selective breeding programs targeting disease resistance. Yet genetic improvement with respect to disease resistance, which relies on survival data from field performance trials, has been slow. Our work aims to enhance selective breeding for Dermo resistance by identifying potential phenotypic targets of selection in addition to survival. Through a series of controlled, laboratory disease challenge experiments leveraging oysters derived from a family-based selective breeding program, we characterized patterns of survival, parasite proliferation within host tissues, responses to mode of parasite exposure and parasite dose, and feeding behavior to better understand traits associated with overall 'resistance' and how they vary across families. Significant effects of treatment (control, exposure via feeding, exposure via injection) and family on survival and the change in parasite load over time were detected in all challenge trials, indicating genetic variation for quantitative resistance to the parasite. We also found that oysters modify their feeding behavior in the presence of *P. marinus* (evidence for qualitative resistance, or parasite avoidance) and that the change in behavior varies by family. The methods we have developed to understand and more precisely measure Dermo resistance have identified two additional targets for selection, ability to minimize parasite proliferation and ability to minimize parasite ingestion, that can contribute to accelerated germplasm improvement for the eastern oyster.

DEPURATION OF MALE SPECIFIC COLIPHAGE (MSC) BY BLUE MUSSEL (*Mytilus edulis*) SEED

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Several experiments were conducted to document the rates of viral depuration in blue mussel seed. Male Specific Coliphage (MSC) is used widely as a proxy to model depuration of harmful human enteric viruses such as Norovirus and Adenoviruses. MSC depuration has been studied in many mature bivalves of commercial importance; however, few studies have been performed on viral clearance in submarket-sized bivalves growing in highly productive but closed areas. A major regulatory concern is identifying relay times required for depuration of transferred seed.

All experiments were conducted in standalone, 50-L recirculating tank systems, each with its own circulating pump, u/v, and filtration units. Tanks were filled with 10−µm, 1.0-µm, and 0.35-µm filtered and u/v-treated seawater. The tanks were jacketed with recirculating fresh water chilled to 10°C and recirculated overnight prior to studies to ensure sterilization. The mean size of mussel seed used in all experiments was < 30 mm. Initial doses of MSC ranged from 10^4-10^5 plaque forming units (pfu)/ml. Sample sizes were 10- 25 shucked mussels.

To test the systems without mussels, the tanks were inoculated with high doses of both MSC and coliform bacteria, and these were effectively sterilized within a few hours. Mussels then were exposed to MSC, but were not fed and had no water changes for 6 days. These mussels exhibited only a slight decrease (< 1 log) in viral load.

Next, depuration rates were compared between fed and unfed mussels. Over a period of 8 days, the water was changed in both treatments every other day, and algae were added to the fed tanks every other day. Mussels were tested for MSC at days 0, 1, 2, 5, and 8. Both fed and unfed mussels showed nearly identical, 2-log, or 99.00% decreases in viral titers.

A longer, 5-week depuration study was performed next. Weekly water changes and feedings were done for both MSC dosed and un-dosed mussels. Samples for MSC in meats were taken on days 0, 1, 2, 3, 8, 15, 23, and 37. In the first week, there was a rapid drop off in virus titers from the dosed meats, then a slow 4 log final reduction to approximately 20 pfu/100g at the end of the study.

RATIONALIZING INTERSTATE SEED TRANSFERS WITH HATCHERY CERTIFICATION AND DOCUMENTING REGIONAL DISEASE PREVALENCE

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Aquaculture is the fastest growing segment of food production with molluscan shellfish aquaculture leading the way. Oyster aquaculture alone has doubled production in just the past five years leading to seed shortages in many states and subsequent increases in requests for seed importation across state lines. The East Coast Shellfish Growers Association estimates there are around 50 shellfish hatcheries along the East Coast producing a dozen or more different species that support more than 1,400 shellfish farms with production growing at 8-10% annually. Several species are impacted by pathogens that decrease survival and production while also threatening native stocks. Knowing which pathogens pose risks and which do not in any particular situation is key to protecting cultured stocks and wild populations. Without access to such information, regulators are pushed toward more precautionary measures, up to and including bans on transfers.

Recently, a series of facilitated workshops supported by National Sea Grant, several state Sea Grant offices and the USDA APHIS Veterinary Services identified the development of a hatchery certification program as a key need to facilitate transfers of shellfish seed. Hatchery certification is a strategic action that can reduce the need for expensive surveillance and costly batch certifications while providing the biosecurity necessary to minimize disease transfer risk. Developing a viable hatchery certification system will increase regulatory confidence and promote industry growth. The concept of convening a Hatchery Certification Working Group to develop a hatchery certification program was universally accepted by industry and regulators alike. The goal is to develop protocols that are both protective of resources (cultured and wild stocks) and practical for both users and regulators. To that end, a working group was created and the group seeks input to identify needs and concerns as they work to create a viable program for implementation in the near future.

With support from NOAA's Saltonstall-Kennedy Program and the 2017 Aquaculture Initiative, this talk will present progress toward developing a shellfish health management database and a hatchery certification program for the East Coast. The authors seek feedback to identify weaknesses and oversights on their plan as well as suggestions for features that would encourage and facilitate use and participation by industry, managers and academics.

IMPLEMENTATION OF A SATELLITE VESSEL MONITORING SYSTEM FOR SHELLFISH RESOURCE MANAGEMENT

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Connecticut's shellfish program relies heavily on the relay of hard clams and oysters from growing areas classified as Restricted or Prohibited; these waters are often heavily impacted by human pathogens or industrial contamination. Management of relay and seed oyster activities in these impacted areas requires extensive oversight to ensure that contaminated product does not intentionally or inadvertently reach the consumer. Oversight of relay activities is jointly managed by both the Connecticut Department of Agriculture (DAG) and the Department of Energy and Environmental Protection (DEEP) and to-date has relied on a combination of specific licenses, mandatory daily call-in to the DEEP dispatch when performing any relay activity, and the use of random patrols and spot checks.

Increased utilization of the public natural seed oyster beds in Connecticut has necessitated the expansion of shellfish resource management by the Authorities in order to ensure sustainability of harvest effort and to protect stocks in these areas. Effective management of critical seed oyster resource requires knowing where seed oystermen are harvesting and the intensity of that effort. Resource management decisions may then be made in order to determine if an area requires the planting of shell or placement of spawning stock for recruitment purposes in order to maintain productivity.

The State of Connecticut implemented a pilot vessel monitoring system (VMS) in 2017 in order to provide more effective oversight of transplant activities in the Housatonic River prior to and during the Housatonic River navigational channel dredging project. More than 75% of commercial oyster seed harvested for market in Connecticut is initially gathered from these public natural beds, and monitoring technology will allow the State to better protect this critical natural resource while protecting public health.

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