

## **THERMAL TOLERANCE OF JUVENILE ATLANTIC SURFCLAMS (*SPISULA SOLIDISSIMA*): A STEP TOWARDS DIVERSIFYING THE NEW JERSEY SHELLFISH AQUACULTURE SECTOR**

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New Jersey shellfish aquaculture is currently limited to two species: the northern quahog (=hard clam) (*Mercenaria mercenaria*) and the eastern oyster (*Crassostrea virginica*); however, shellfish farmers eager to diversify have expressed interest in culturing new species. The Atlantic surfclam (*Spisula solidissima*) represents an ideal target species for diversification because it is native, grows rapidly, and fits into the established farming framework. To optimize the husbandry techniques required for sustainable and profitable farming, it is necessary to gain a thorough understanding of how temperature impacts the performance of the surfclam throughout its different developmental stages. This study examined the effects of five different temperatures ( $\approx 18^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ ,  $23^{\circ}\text{C}$ ,  $24^{\circ}\text{C}$ , and  $26^{\circ}\text{C}$ ) on the growth and survival of juvenile surfclams (shell length = 0.69–3.00 mm). Three independent cohorts were tracked for several weeks and cultured using downweller and upweller systems. Shell length and survival estimates were collected 2–3 times per week. Results suggest that colder temperatures reduce clam mortality, while temperatures between 20 and  $24^{\circ}\text{C}$  promote the greatest growth. The parentage of each cohort also had a significant impact on growth and survival, suggesting there is a genetic component to surf clam thermal tolerance. These findings and the results from our on-going surfclam aquaculture optimization studies will be incorporated into a manual of best practices. This manual will be made accessible to the state's local shellfish farmers. Moreover, these results can be incorporated into species distribution models, and further refine the management of the lucrative wild surfclam fishery.

## **SEASONAL FEEDING AND GROWTH OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) IN THE DAMARISCOTTA RIVER ESTUARY, MAINE**

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Production on oyster farms depends on the quantity and quality of the planktonic food, including phytoplankton and detritus, available at the site. This research, part of the Sustainable Ecological Aquaculture Network (SEANET) program, is investigating seasonal variation in the nutritional quality of suspended particulates and associated feeding and growth responses of eastern oysters (*Crassostrea virginica*). From May through October 2016, bi-weekly water sampling was conducted in an intensively farmed segment of the Damariscotta River Estuary. Multiple water quality parameters were measured, including temperature, salinity, turbidity, chlorophyll  $\alpha$ , pheophytin, total particulate matter, particulate organic matter, particulate inorganic matter, phytoplankton community composition, phytodetritus, and enzymatically hydrolysable amino acids (EHAA, which quantifies bioavailable protein). Seasonal variation in food quantity and quality data were compared to water clearance, filtration, ingestion, and absorption rates in *C. virginica*; these, in turn, were compared to seasonal variation in growth of individual oysters. Preliminary results show that clearance rate scales with temperature, and absorption efficiency and growth are lower when there is an increase in particulate inorganic matter and pheophytin concentrations. Although absorption efficiency and growth were expected to be highest at times of increased chlorophyll  $\alpha$  concentrations, they remained low when high chlorophyll  $\alpha$  concentrations coincided with high concentrations of inorganic matter and pheophytin. These results will be used to improve oyster growth modelling and site selection for oyster farms in Maine.

## **DEVELOPMENT OF A QPCR ASSAY TO DETECT *PROCTOECES MACULATUS* IN THE WATER COLUMN**

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The digenetic trematode *Proctoeces maculatus* is known to infect the blue mussel *Mytilus edulis*. Although little is known about the overall health effects these pathogens have on the blue mussel, it is hypothesized that severe infections can be related to mass mortality events. *P. maculatus* is known to have a multi-host life cycle, starting as miracidia which produce sporocysts that infect their intermediate host *M. edulis*. The sporocysts produce daughter sporocysts, which progress to the production of cercaria. The cercaria exit the intermediate host and move on to infect their second intermediate host or their definitive host (Stunkard and Uzmann, 1959). Theoretically, at the time when the cercaria leave their intermediate host there should be an increased presence of *P. maculatus* in the water column due to the migration of the pathogen to its next host. The ability to detect the presence and relative abundance of *P. maculatus* in the water column could make it possible to identify seasonality trends, and could also be used to relate the departure of these parasites from blue mussels to spawning or mass mortality events within populations. The goal of this research is to create a SybrGreen quantitative polymerase chain reaction (qPCR) assay that is specific and capable of detecting the presence of *P. maculaus* in the water column. The preliminary research for creating the SybrGreen qPCR will be presented, including the creation of plasmids that form reliable and accurate standard curves, and the specificity and lower detection limit of the assay.

## A MULTI-OMIC APPROACH TO REVEAL QPX-CLAM INTERACTIONS

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QPX is a protistan parasite that infects northern quahogs (=hard clams), *Mercenaria mercenaria*, often leading to the development of inflammatory masses (nodules) that result from massive hemocyte infiltration to the infection site in an attempt to isolate and encapsulate parasite cells. Inside nodules, active host-pathogen interactions take place leading either to the death of the parasite or invasion of surrounding tissues and infection worsening. This study was designed to investigate host-parasite interactions using a complementary set of *in vivo* and *in vitro* approaches in conjunction with transcriptomic and proteomic methods. *In vivo*, dual gene expression of QPX and clams to characterize molecular host-parasite interactions was studied. This was done via RNA sequencing of nodule biopsies and contrasting these with RNA sequences generated from uninfected tissues from infected clams or from healthy clams, as well as genomic and transcriptomic information generated from cultured parasite cells. Analyses allowed the identification of 900 QPX transcripts that are only produced in clam tissues during infection. These included genes and molecular processes implicated in the secretory pathways of the parasite and secreted proteases which we suspect to play a primary role in QPX virulence towards clams. In parallel, proteomic methods were used to identify host plasma factors that recognize and bind parasite cells *in vitro*. These included prominent pattern recognition receptors (PRR) such as complement c1q-domain containing proteins and lectins. Results further showed that these PRR are induced upon infection. Altogether, these results provide valuable information on the molecular crosstalk between QPX and its clam host.

## CARBON MINERALIZATION AND EFFLUX IN CLAM AQUACULTURE

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Molluscs mineralize carbon as  $\text{CaCO}_3$  in shells. Shell is a long-term reservoir for carbon but mineralization of dissolved bicarbonate results in an initial efflux of  $\text{CO}_2$ . In Florida, USA, northern hard clams, *Mercenaria mercenaria*, are cultured in mesh bags in coastal habitats. Carbonate content of shells was quantified by coulometry, and shell production was estimated per harvested clam, and per unit area per year.

About 91% of the shell was *M. mercenaria* and the rest were mostly oysters (*Crassostrea virginica* and *Ostrea equestris*) on culture bags, though there were 37 other shelled taxa. Each harvested clam represented a mean of 2.93 g of mineralized carbon, including shell from associated taxa. Clam culture produced about 10,030 kg of mineralized carbon  $\text{ha}^{-1} \text{y}^{-1}$ , and the Florida clam industry produced about 534 tons of mineralized carbon in 2008.

This mineralization resulted in an atmospheric efflux of up to 374 tons (70% of fixed) of carbon as  $\text{CO}_2$ , using a model that predicts 30% dissolution of  $\text{CO}_2$  back into seawater. Models for  $\text{CO}_2$  dissolution vary, however, and clams can use metabolic  $\text{CO}_2$  instead of seawater bicarbonate for up to a third of shell production, reducing net  $\text{CO}_2$  efflux from shell production. In addition, clams use a carbonate pathway for some shell production, which does not have  $\text{CO}_2$  as a byproduct. Values for some of the above variables are still unknown, but they mean that  $\text{CO}_2$  efflux from clam aquaculture is likely much lower than 70% of fixed carbon.

## **APPLE SNAILS: A REVIEW AND INTRODUCTION TO THE SPECIAL SESSION**

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Apple snails (genus *Pomacea*) have received global attention in the areas of invasion biology, aquatic ecology, gastropod physiology, and agriculture. The snails have been introduced around the world through a variety of mechanisms. Like many invasive molluscs, *Pomacea* spp. have high fecundity, grow rapidly, reach high densities, and tolerate a wide range of environmental parameters. As prodigious consumers of aquatic vegetation, they can have significant ecological and economic impacts. This introduction to a special session on apple snails at the 109<sup>th</sup> Annual Meeting of the National Shellfisheries Association reviews the invasion history, distribution, biology, ecology, impacts, and control measures of *Pomacea* spp.

## DEVELOPING A PREDICTIVE MODEL OF ENVIRONMENTAL CONDITIONS FOR THE MANAGEMENT OF CLAM FARMS ON THE FLORIDA GULF COAST

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To maintain and grow the economic impact of the hard clam aquaculture industry of Florida, to meet the increasing national and global demand for aquaculture products, and to respond to global climate change, the industry needs a practical tool for understanding and predicting the risk of crop loss for use in decision-making and farm management. Hard clam aquaculture research and monitoring activities have amassed a large quantity of under-utilized data related to the environment, clam mortality, and productivity in Florida. Available environmental and clam productivity data were 1) identified and formatted, 2) then synthesized and a predictive model of crop loss risk was developed with the intent of 3) working toward provisions of a preliminary decision tool to growers.

Water quality was used to identify periods of time that corresponded to events that negatively influenced the well-being of the northern quahog, *Mercenaria mercenaria*. Optimal ranges led to the development of an Environmental Assessment Index (EAI), which considered relationships between water quality variables and the duration of a suboptimal water quality event. The relationships between water quality variables and a suite of regional environmental variables were used to develop mathematical models for predicting temperatures, salinities, and dissolved oxygen concentrations at clam aquaculture use areas, and the EAI for three subsequent days from a selected start date of a suboptimal water quality event. Ultimately, water quality predictions are intended to enhance current online water quality information made available to clam farmers.

# DELETERIOUS EFFECTS OF HARMFUL DINOFLAGELLATES AND RAPHDOPHYTES ON JAPANESE PEARL OYSTER EGG VIABILITY AND SPERMATOZOA SWIMMING VELOCITY

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Several species of harmful algal blooms (HAB) are known to affect shellfish and an extensive body of literature has reported several inimical effects in bivalve molluscs. Reports of the impacts of HAB species on the reproduction of shellfish, including bivalves, remain, however, very limited. Cosmopolitan species of the genus *Alexandrium* are known to produce paralytic shellfish toxins (PST) and other bioactive compounds, and cosmopolitan species of ichthyotoxic raphidophytes, such as *Heterosigma akashiwo* and *Chattonella marina*, are known to affect mainly fish, with episodic reports of invertebrate mortalities generally associated with hypoxic condition caused by the blooms.

In previous studies, we reported inimical deleterious effects of *A. affine*, *A. catenella*, *Heterosigma akashiwo* and *Chattonella marina* on the fertilization, embryo development and larvae of Japanese pearl oyster. In the present study, we further investigated the effects of laboratory exposure to these HAB species on the quality of gametes of the Japanese pearl oyster, *Pinctada fucata martensii*. Spermatozoa and eggs were exposed to several cell densities of each HAB species. The swimming velocity of spermatozoa and the viability of the eggs were determined. All HAB species caused significant reduction in the swimming velocity of spermatozoa and the most severe effects were induced following exposure to realistically low cell densities of *A. affine*. Eggs were not affected by exposure to the harmful raphidophytes, but their viability was significantly reduced following exposure to *A. affine* and *A. catenella*.



**CYTOTOXICITY AND OXIDATIVE STRESS IN ADULT JAPANESE SCALLOPS,  
*PATINOPECTEN YESSOENSIS*, FED ON A CULTURE OF NON-DSP PRODUCER,  
*DINOPHYSIS CAUDATA***

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The widespread seafood-born poisoning in human, diarrhetic shellfish poisoning (DSP), and is induced by the consumption of bivalve molluscs that accumulate diarrhetic shellfish toxins (DST), oakdaic acid (OA) and dinophysistoxins (DTX), produced by species of the genus *Dinophysis*. Mollusc bivalves are known to accumulate DST at differential rates and are not known to be affected by DST toxins.

Feeding experiments with a cultured strain of *Dinophysis caudata* isolated from western Japan resulted in mortalities of three species of bivalve molluscs, including Japanese scallop, *Patinopecten yessoensis*. The strain of *D. caudata* does not produce DST but pectenotoxins (PTX-2) which are no longer considered part of the DSP old-complex. The results were the first report of lethal effects of a *Dinophysis* species in shellfish. The lethal effects were not associated with PTX-2, strongly suggesting the implication of unknown toxins and / or other metabolites. Further experiments were conducted with adult Japanese scallops. The pathological alterations and oxidative stress response in the digestive organs of Japanese scallops are reported.

## **SPATIAL VARIATION IN SOFTSHELL CLAM (*MYA ARENARIA*) RECRUITS IN A SOUTHERN MAINE TIDAL ESTUARY**

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Softshell clams rank second in commercial landings in Maine, USA, behind only lobster with a dockside value in 2015 of \$22.5 million. Between 2012 and 2015, coastwide landings decreased by nearly 20%, and, in the southern Maine town of Freeport, landings decreased nearly 70% over that same period with collective economic losses to clambers totaling \$680,000. These declines in commercial stocks have prompted shellfish managers to question whether populations are recruitment-limited, or whether other factors (e.g., predation, coastal acidification, disease, habitat loss) may be responsible.

During 2014 and 2015, a series of manipulative field experiments was initiated in a tidal estuary, the Harraseeket River (Freeport, ME), to assess whether softshell clam populations were recruitment-limited. In 2014, an 8-month comparative field experiment at intertidal sites ca. 600 m apart on either side of the river demonstrated a relative lack of wild 0-year class juveniles in protected (netted:  $74.0 \pm 9.9$  ind.  $m^{-2}$ ,  $n = 40$ ) and control ( $18.5 \pm 7.9$  ind.  $m^{-2}$ ,  $n = 20$ ) plots at one site, while at the other site, densities of recruits in protected plots was  $14,835.5 \pm 3,596.2$  ind.  $m^{-2}$  ( $n = 40$ ) and  $16.5 \pm 16.9$  ind.  $m^{-2}$  ( $n = 20$ ) in control plots. Similar results were observed in 2015 using wooden recruitment boxes (0.3 m x 0.6 m x 0.07 m) with 1.9 mm or 6.4 mm mesh tops and bottoms. Relative to other factors that may be responsible for declines in wild clam populations, predation appears to best explain these losses.

## **GROWTH AND SURVIVAL OF CULTURED JUVENILES OF THE ARCTIC SURFCLAM, *MACTROMERIS POLYNYMA*: INTERTIDAL FIELD EXPERIMENTS IN EASTERN MAINE**

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Adult Arctic surfclams, *Mactromeris polynyma*, are highly valued for their distinctive, flavorful foot that, when cooked or processed, changes color from dark purple to reddish-orange. This product is sold as 'hokkigai,' and mostly used for sushi or sashimi dishes. *M. polynyma* ranges from Rhode Island to Labrador in the northwest Atlantic, but 99% of the commercial harvest originates from deep water (> 30 m) in Maritime Canada where, annually, the fishery is estimated to be worth over \$60 million.

Since 2011, cultured juveniles of *M. polynyma* have been reared at the Downeast Institute (Beals, ME) in an attempt to increase the supply, quality, and diversification of domestic seafood. Hatchery and nursery trials have been conducted so that it is possible to produce millions of juveniles (3-10 mm SL) from a single spawning event. Field trials began during the spring of 2012 to examine factors regulating growth and survival in the extreme lower intertidal at several locations in eastern Maine. At two sites, the interactive effects of stocking density (55-1,320 ind. m<sup>-2</sup>) and predator exclusion (plastic, flexible netting – 4.2 mm aperture) on the fate and growth of juveniles (6-10 mm SL) were examined in experimental units using a completely factorial design over 195 days. Clam survival at both sites ranged from 0% to 1.3% in unprotected units vs. 30% to 64% in protected units. Clams added new shell at a rate of 0.06 mm to 0.14 mm/day that was not affected by stocking density. Results from additional studies will be discussed.

## VARIATION IN PARASITE AVOIDANCE BEHAVIOR AMONG SELECTIVELY-BRED OYSTER FAMILIES

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Parasites are everywhere. Owing to their ubiquity, there are clear fitness advantages for hosts to reduce the risk of infection. Here it is reported that filter feeding oysters respond to the presence of a water-borne parasite by modifying their feeding behavior and that the extent of behavioral modification can vary with evolutionary history of parasite exposure. Six selectively-bred oyster families were exposed to seawater containing microalgae and increasing concentrations of *Perkinsus marinus*, the agent of Dermo disease. While some families exhibited little to no alteration in their feeding behavior when the parasite was present, others markedly reduced the rate suspended particles were cleared when concentrations of the parasite increased in the surrounding seawater. In separate laboratory studies, the oyster families that best modified their feeding behavior left the highest concentrations of *P. marinus* remaining in the water column and exhibited the lowest densities of invading *P. marinus* in sampled tissues immediately after exposure. The density of invading parasites, however, did not reflect the parasite densities measured in each family over the time course of our experimental period. The oyster families therefore varied not only in their capacity to reduce initial parasite exposure, but also in their ability to resist established *P. marinus* infections. Behavioral avoidance of *P. marinus* appears to be a first line of defense in the repertoire of immunological, physiological and behavioral responses of oysters used to combat infecting parasites. Since this behavior varied among the oyster families we hypothesize that parasite avoidance behavior is influenced by evolutionary history. Understanding behavioral responses to parasite exposure will refine our understanding of disease resistance strategies in wild and cultured oysters.

## **A HIERARCHICAL BAYESIAN MODEL ESTIMATING MICROBIAL CONCENTRATIONS FROM MOST PROBABLE NUMBER DATA**

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The most probable number (MPN) method is an effective tool for quantifying low concentrations of microbial contamination in food and water; however, it requires often prohibitively large numbers of replicates, subsamples within replicates, and dilutions of each subsample to sufficiently narrow the confidence intervals of estimated microbial concentrations, particularly when testing for differences across predictor variables. A hierarchical Bayesian model that combines data from replicates collected using the MPN method to increase the precision of estimated microbial concentrations will be presented. This model was applied to data collected from an ongoing project investigating the effects of intertidal exposure on concentrations of *Vibrio parahaemolyticus* in oysters grown across a salinity gradient in Delaware and Chesapeake Bays. The hierarchical model decreases uncertainty and improves inference of the effects of ecologically-relevant predictor variables such as intertidal exposure, salinity and region on estimates of microbial concentration.

## CHARACTERIZING THE INTERACTION OF *MYTILUS EDULIS* FOOT PROTEIN-5 WITH HY80 STEEL

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Foot protein-5 (Mefp-5) from *Mytilus edulis* is an adhesive protein found in the adhesive plaque of the common blue mussel *Mytilus edulis* (L), contains high amounts of amino acids L-3,4-dihydroxyphenylalanine (L-dopa, 27 mol%) and lysine (20 mol%). While Mefp-5 has been shown to provide significant corrosion inhibition to a high strength low alloy steel (HY80), the mechanism of inhibition remains elusive. It has been suggested that the close proximity of L-dopa and lysine in Mefp-5 results in a synergy that plays a vital role in the adsorption of the protein to solid substrates. This proposed synergy and its effect on iron oxide films has yet to be elucidated. To characterize how Mefp-5 interacts with the HY80 steel, a variety of analytical spectroscopy techniques were implemented. Solutions of Mefp-5, L-dopa, or lysine dissolved in deionized water, 5% acetic acid, 0.05 M potassium phosphate buffer (pH 5.5), or the same buffer containing mushroom tyrosinase were adsorbed onto HY80 and glass substrates. Raman spectroscopy suggests that adsorbate, solution composition and pH play a role in the type of iron oxide formed and how the protein orients itself on the HY80 surface. At a pH of 5.5, the isoelectric point (pI) of the Fe<sub>2</sub>O<sub>3</sub> oxide suggests the negatively charged oxide surface attracts lysine's positively charged ε-amine group. Infrared spectroscopy indicates that L-dopa is also intimately involved in the adsorption of the protein on HY80 steel. Overall, the data suggest there is synergy between L-dopa and lysine, where lysine aids in iron availability for L-dopa complexation.

## PHYSIOLOGICAL RESPONSES OF EASTERN OYSTERS TO ACUTE AND CHRONIC EXPOSURE TO SUSPENDED SEDIMENTS

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Louisiana estuaries provide habitat for some of the most productive populations of eastern oysters within the United States. Coastal alterations, including changes in Mississippi River flow, sediment diversions, and climate change, are likely to impact areas supporting oyster production. In particular, predictions of extended periods of low salinities and increased total suspended sediments could significantly impact oyster populations by affecting basic physiological functions. In this study, we measured effects of acute (1 hour) and chronic (8 weeks) exposure of total suspended sediment concentrations on eastern oyster oxygen consumption and clearance rates using a series of controlled laboratory experiments. Acute exposure to six sediment concentrations (0, 10, 50, 200, 500, 1000 mg L<sup>-1</sup>) at a salinity of 15 and temperature of 25°C indicated sediment concentration significantly affected oyster clearance but not oxygen consumption rates. Chronic exposure at two salinities (6 and 15) and three sediment concentrations (none, 50, 400 mg L<sup>-1</sup>) at 25°C, indicated that clearance rates were not significantly affected by sediment concentration or salinity. Oxygen consumption rate was not significantly affected by sediment concentration but was negatively affected by reduced salinity. Total suspended sediment concentrations greater than 500 mg L<sup>-1</sup> may affect eastern oysters, but overall, reduced salinity associated with sediment diversions may have greater impacts on oysters, particularly if salinities are reduced for extended periods of time. Understanding consequences of altered salinities and high suspended sediment exposure on the eastern oyster is critical to predicting future oyster production potential, impacts from proposed restoration activities, and climate change.

# **THE INFLUENCE OF HYDRODYNAMICS (ADVECTION AND VERTICAL MIXING) ON DISEASE TRANSMISSION TIME IN BENTHIC METAPOPOPULATIONS DISTRIBUTED AMONG REEFS**

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Disease transmission in benthic filter feeders often occurs directly via filtration of microparasitic pathogens from the water column during feeding. Consequently, models developed to understand this type of transmission must represent not only biological processes occurring during the progression of infection in the host but also hydrodynamic processes involved in the movement and/or retention of pathogens. The contribution of the latter on disease transmission using a single population disease model incorporated into the Regional Ocean Modeling System (ROMS) is explored. A number of simulations considering different scenarios of vertical diffusion and advection in a generic shallow estuary with a benthic filter feeder metapopulation distributed among reefs were run. The results show that the pathogen advection/diffusion processes have some influence on the disease transfer time among reefs with higher advection resulting in slower transfer and higher diffusion creating faster developing infections. If the salinity variation is strong enough to cause deep circulation reversal (surface outflow and bottom inflow), the level of vertical mixing changes the upstream and the downstream transport of pathogens. Low diffusion keeps pathogens near the bottom allowing an upstream infection while higher diffusion moves pathogens through the water depth moving them downstream and towards the bottom between reefs.



## **INFLUENCE OF *PERKINSUS MARINUS* INFECTION AND OYSTER HEALTH ON LEVELS OF HUMAN-PATHOGENIC *VIBRIOS* IN OYSTERS**

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*Vibrio vulnificus* and *Vibrio parahaemolyticus* are naturally occurring marine bacteria that can accumulate in oysters and other bivalve shellfish. These bacteria are responsible for the highest rates of mortality and gastrointestinal illness related to seafood consumption in humans, but do not harm their hosts. In the eastern oyster *Crassostrea virginica*, *Vibrio* species and the important pathogen *Perkinsus marinus* co-occur, yet little is known about potential interactions between the bacteria and this oyster parasite. Answering the fundamental question of whether *P. marinus* and *V. vulnificus* and *V. parahaemolyticus* levels in oysters are correlated was the focus of this research.

Oysters were deployed in the York River, Virginia, where both *Vibrio* species and *P. marinus* are endemic, and sampled from August-October of 2014 and 2015 when levels of all three species were highest. Abundance of the three organisms and pathogenic strains of *V. parahaemolyticus* were determined in individual oysters using quantitative PCR assays. Histopathology was used to gain broader perspective on potential interactions between *Vibrio* levels and oyster health. No relationship was observed between total abundance of *P. marinus* and either *Vibrio* species, and histopathological analyses did not reveal any correlations between *P. marinus*-associated pathology and *Vibrio* levels. Though few in number, oysters infected by *Haplosporidium nelsoni* had higher levels of *V. vulnificus* than uninfected oysters, and oysters of peak gametogenic development had significantly higher levels of pathogenic strains of *V. parahaemolyticus*, both results warranting further study as we seek to understand factors influencing *Vibrio* interactions with oyster hosts.

## MUTUALISM BETWEEN *GEUKENSIA DEMISSA* AND *SPARTINA ALTERNIFLORA* ENHANCES SALT MARSH NITROGEN REMOVAL: IMPLICATIONS FOR LIVING SHORELINES

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Salt marshes have declined globally and are increasingly threatened by erosion, sea level rise, and development. These highly productive, physically demanding ecosystems are populated by core species groups that often have strong trophic interactions with implications for ecosystem function and service provision. One positive interaction occurs between *Geukensia demissa* (ribbed mussels) and *Spartina alterniflora* (cordgrass). This study investigated *G. demissa* facilitation of two functions in salt marshes that relate to N removal (denitrification and water filtration) and the potential implications for living shoreline ecosystem service provision. Microcosm experiments revealed that the highest rates of N<sub>2</sub> production and nitrification occurred when mussels were present with marsh vegetation, suggesting mussels enhanced coupling of the nitrification-denitrification cycle. Surveys spanning the York River Estuary, Chesapeake Bay showed the highest densities occurred in the first meter of marshes with mainstem fringing marshes being the most densely populated ( $1207 \pm 265$  mussels m<sup>-2</sup>). The mussel population was estimated to be ~197 million animals with a water filtration potential of 90–135 million L hr<sup>-1</sup>. Erosion simulation models demonstrated that suitable marsh habitat for mussels would be reduced by 11.8% after 50 years resulting in a 15-16% reduction in mussel abundance, filtration capacity, and denitrification potential. Gains in marsh acreage through the use of living shoreline strategies may help offset these projected losses. The comparative abundance of ribbed mussels in natural fringing marshes and living shoreline marshes and suggest design modifications to enhance mussel recruitment are described.

## **EFFECTS OF SESTON COMPOSITION ON THE BIODEPOSITION OF PARTICULATE NITROGEN BY NORTHERN QUAHOGS (*MERCENARIA MERCENARIA*)**

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Benthic shellfish populations serve as important links between the water column and the sediments in many estuarine and coastal marine ecosystems. Shellfish have been shown to directly facilitate the movement of particulate organic matter from the water column into the sediments through suspension feeding and the subsequent biodeposition of pseudofeces and other wastes. It has been hypothesized that the movement of this material into the sediments may facilitate coupled nitrification/denitrification by providing nitrogen- and carbon-rich organic substrates to benthic microbial communities. The purpose of this project was to examine how seston concentration and composition impact the movement of particulate organic matter and total particulate nitrogen from the water column onto the seafloor surface by Northern quahogs (*Mercenaria mercenaria*). To address this issue, a series of controlled laboratory experiments were completed in which clams were exposed to varied concentrations of total particulate nitrogen, chlorophyll *a*, suspended inorganic solids, and suspended detritus. All of these factors significantly affected the mass of particulate organic matter and total particulate nitrogen that was removed from the water column and deposited onto the surface of the chamber floor as pseudofeces and other wastes.

## **TRANSCRIPTOMIC AND GENOMIC APPROACHES OF ENDOCRINE DISRUPTION SUGGEST NON-GENOMIC ESTROGEN SIGNALING IN *MYTILUS EDULIS***

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Transcriptomic and comparative genomic approaches have become invaluable for understanding metabolic pathways in non-model organisms including shellfish. In particular, the endocrine system in bivalves is poorly understood despite the potential for endocrine disruption to occur in these species. Endocrine Disrupting Compounds (EDCs) mimic natural estrogen in wildlife species and humans which can lead to devastating reproductive and developmental impacts such as gender reversal. Despite increasing evidence for reproductive impairments in bivalves from EDC exposure, current research largely focuses on vertebrates since the nuclear estrogen receptor (ER) is unresponsive to estradiol in mollusks. To investigate a possible mechanism EDCs play in bivalves and to develop biomarkers of endocrine disruption, we exposed *M. edulis* to two concentrations of EE2 (5 and 50ng/L) and 4NP (1 and 100µg/L), sampled mussels at 4wk and 5wk, and performed gene expression analysis on digestive gland tissue using a *M. edulis* microarray. Preliminary results of this experiment have found high elevation of female-specific transcripts, suggesting that EDCs may cause sex reversal in male *M. edulis*, a finding that has not been previously linked in this species. Additionally, the non-genomic estrogen signaling pathway was up-regulated suggesting that despite the evolutionary divergence of hormonal systems reproductive impairments may occur in mollusks but through a different mechanism than vertebrates.

## **AN UPDATE ON THE STATUS OF OYSTER AQUACULTURE (*CRASSOSTREA VIRGINICA*) IN GEORGIA AND AN OVERVIEW OF THE BLUEPRINT FOR GEORGIA OYSTER AQUACULTURE**

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The University of Georgia, Georgia Department of Natural Resources and Georgia Department of Agriculture are partnering to expand the Georgia aquaculture industry. Aquaculture in Georgia is currently dominated by the northern quahog (= hard clam) (*Mercenaria mercenaria*) and the development of sustainable single oyster (*Crassostrea virginica*) is of highest importance to diversify the shellfish industry. Nationally, consumer demand for high-quality, raw-bar-grade oysters is rising and to increase production a Blueprint for Georgia Oyster Aquaculture has been developed that outlines critical needs to grow the industry from its current state. Additional investment in oyster research, training for shellfish growers, resource management and consumer safety is needed to sustain continued growth and realize the goals and actions outlined in the collaborative Blueprint for Georgia Oyster Aquaculture. An update on the current status of the Georgia aquaculture industry and an overview of the Blueprint for Georgia Oyster Aquaculture will be provided as Georgia transitions from an oyster industry based on wild harvest to an aquaculture based industry.

## **PRELIMINARY RESULTS OF A SURVEY FOR THE OYSTER PARASITE *BONAMIA* SP. IN TEXAS BAY SYSTEMS**

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Texas Parks and Wildlife Department has been working on expanding the existing private oyster lease program and developing an Oyster Aquaculture Plan for Texas. Movement of oysters from one Texas bay system to another is restricted because of potential disease transfer and genetic differences in natural oyster stocks. Except for Dermo, oyster diseases such as Bonamiosis, which was found serendipitously in 2007 in Florida waters, have not been well characterized in Texas bays. Therefore, the objective of this project is to examine American oysters, *Crassostrea virginica*, from different Texas bays for the presence of *Bonamia*. Thirty oysters were collected from four different bays during October-November 2016: Copano Bay, San Antonio Bay, Galveston Bay, and Sabine Lake (Matagorda Bay will be collected in December). Oysters were weighed and shell parameters (height, width, thickness) measured. Oysters ranged in shell height from 30.5 to 140.3 mm. No measurement was significantly different, e.g., shell height ( $P=0.11$ , mean =  $72.5 \pm 0.1$ mm), total weight ( $P=0.08$ , mean =  $76.6 \pm 0.1$ g), or meat content (% of total weight:  $P=0.15$ , mean =  $0.09 \pm 0.01$ %) among the sampled bays. A small sample of tissue was taken for future PCR analysis for *Bonamia* sp. as well as a 5-10mm thick cross-section of the oyster meat for future histological evaluation. The project data will assist in guiding science-based policy for movement of oysters between Texas bay systems as well as potentially extending the known global range of *Bonamia*.

## STANDARDIZATION OF MICROSATELLITE MARKERS TO EVALUATE GENETIC VARIABILITY IN THE WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

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The white shrimp (*Litopenaeus vannamei*) is the species that is more cultivated worldwide, because it has characteristics such as rapid growth, tolerance to wide ranges of salinity, and adaptability to captivity among others. The production of white shrimp at the global and national level has shown a loss of up to 100% in production due to the presence of diseases. The evaluation of genetic variability of batches of broodstocks allows for the genetic state of these and implement strategies of selective breeding to be known in order to produce progeny with better productive characteristics (growth, survival and disease resistance). Microsatellite markers are one of the most useful and informative tools to evaluate genetic variability, these are short DNA sequences repeated in tandem, with them it is possible to determine allelic difference between organisms, to estimate genetic parameters that characterize a population.

The objective was standardization of twelve microsatellite markers to evaluate genetic variability in the white shrimp (*L. vannamei*). Fifty wild shrimp were collected from the coast of Sonora, Mexico. Twelve microsatellite markers (*Lv23, Lv25, Lv28, Lv29, Lv35, Lv55, Lv56, Lv72, Lv76, Lv89, Lv96 y Lv512*) were amplified (PCR) and the products were visualized on agarose gels at 1.5%. Of the list of markers only six were standardized (*Lv23, Lv25, Lv35, Lv55, Lv72 y Lv76*) and these were genotyped by fragment analysis (QIAxcel). Genetic parameters were calculated using CREATE1.35, HW-QuickCheck, ARLEQUIN v3.11 and FSTAT 2.9.3. The results of genetic variability using these markers will be discussed.

## WAVED WHELK (*BUCCINUM UNDATUM*) IN THE MID-ATLANTIC BIGHT: LIFE HISTORY TRAITS AND POPULATION STRUCTURE

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Recent expansion of the unmanaged waved whelk (*Buccinum undatum*) fishery in the Mid Atlantic Bight has initiated investigation into local life history parameters of this species, which vary with location. Limited adult dispersal and a lack of planktonic larval stage create spatially distinct populations with regional variation in size at sexual maturity and shell morphology. This current study presents the distribution and density of the stock in the Mid Atlantic, size at onset of sexual maturity, and an age length curve for the south portion of the population. In the summer of 2015, waved whelk samples (n = 231) were collect in the mid-Atlantic, from Georges Bank to DelMarVa, using a modified scallop dredge. All whelk (n = 3877) were sexed, weighed, measured, and assessed for maturity. Size at maturity varied between sexes and sites and ranged from 54 to 72 mm. The largest whelk measured 103 mm shell length (SL) (mean = 69 mm) from the Georges Bank region with the smallest animal from the New Jersey region, measuring 15 mm SL (mean = 65 mm). Abundance estimates of whelk were also made from counts of bottom images taken in the HabCam survey conducted by NEFSC. Driving forces behind local adaption might include, depth, temperature, habitat, predation, and prey availability. This evidence for local adaptation in whelk means that if fishing was to continue and management necessary, regional management rather than national management would best protect the Mid Atlantic whelk fishery.



# HEALTH SURVEY OF *CRASSOSTREA VIRGINICA* ALONG A NORTH TO SOUTH GRADIENT IN THE GUANA TOLOMATO MATANZAS NATIONAL ESTUARINE RESEARCH RESERVE, FLORIDA, USA

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Oyster populations in the Guana, Tolomato, Matanzas National Estuarine Research Reserve (GTMNERR) have been noted by locals to exhibit a north-south gradient in condition. The aim of this study was to assess the health of oysters along this gradient, to determine whether disease plays a role in observed differences in condition.

A total of 270 oysters were collected from three sites (n = 30/site) within three regions (north, central, south, n = 90/region) of the GTMNERR in June 2015, cleaned, weighed and measured. Oysters were examined for the presence of pests (i.e. *Polydora*, etc.), *P. marinus* (using RFTM media), *Bonamia* spp. (using PCR).

Oysters collected from the south differed significantly from those collected in the north and central regions with respect to height and width. Presence of pests were similar between regions, except for *Polydora*, in which prevalence was higher in the south (68%) compared to the central (13%) and north (19%). Prevalence of *P. marinus* did not differ significantly between regions, but was high, averaging 65% at north and central sites and 58% at southern sites. *P. marinus* intensity did not differ significantly between regions and intensity was generally low, though higher at north (1.16) and central sites (0.91) than at southern sites (0.75). *Bonamia* spp. was not detected. This was the first study to characterize oyster disease in the GTMNERR and results suggest that the presence of disease causing organisms do not explain the observed north to south oyster population decline within the reserve.

## PATHOGEN SOURCE OR SINK: THE POTENTIAL ROLE OF BIVALVE AQUACULTURE IN MITIGATING DISEASE RISK

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Rapid anthropogenic change has intensified disease impacts on marine and freshwater environments. Understanding the ecology of these diseases is essential to mitigating their effects, and is particularly urgent for diseases related to aquaculture, which is growing rapidly worldwide and important for the sustainability of human coastal communities. Intensive aquaculture can exacerbate disease impacts on nearby natural host populations. However, it is far from certain that this is universally the case, and we question whether aquaculture may on the contrary be a sink for some directly transmitted pathogens. *Perkinsus marinus* is a major pathogen of the oyster *Crassostrea virginica* along the Atlantic and Gulf coasts. Dermo disease caused by *P. marinus* typically reaches peak severity after two growing seasons, and in areas where this disease is prevalent aquaculturists hedge against disease risks by getting oysters to market before large losses occur. Parasite transmission occurs when infected oysters die and release infective parasite stages into the water column, which are then filtered by uninfected oysters nearby. Oyster aquaculture may be a sink for *P. marinus* given that cultured oysters are growing and filtering parasites from water bodies shared with wild populations but harvested before large numbers die and release parasite stages into the environment. Here we modeled interactions between oyster aquaculture and wild populations, showing how the scale of aquaculture and the extent of harvest can be optimized to minimize disease impacts on surrounding wild populations. The results are immediately relevant to oyster aquaculture in areas where wild oysters are present.

## **INTERANNUAL SEASONAL PATTERNS OF *PERKINSUS MARINUS* INFECTIONS AND OYSTER MORTALITY IN DELAWARE BAY**

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The etiological agent of Dermo disease, *Perkinsus marinus*, has been a major source of oyster mortality in Delaware Bay since 1990. The oyster population is also challenged by *Haplosporidium nelsoni* (the etiological agent of MSX disease) but MSX disease has not been a problem since the late 1980s following the spread of resistant oysters throughout most of the bay. The MSX parasite is still widely prevalent in the Bay and quickly attacks naïve oysters whereas native oysters survive well. Monthly monitoring of *P. marinus* infection prevalence and intensity has occurred since 1999 with corresponding levels of mortality, temperature and salinity and provides a unique opportunity. Temporal (seasonal) and spatial patterns show the typical cycle of infection intensification during summer followed by remission over the winter and spring with a gradient of increasing infection intensity as salinity increases. Interannual variation in the time series includes periods of drought contrasted by periods of flooding, as well as periods of varying duration of temperature thresholds. These data provide a unique opportunity to investigate relationships of environmental controls on disease status and impact which will be explored in this presentation.

## THREATENED BIRDS ENDANGER OYSTER FARMS

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The lower Delaware Bay shoreline in Cape May County, New Jersey, provides important habitat for migratory shore birds including the Red Knot (*Calidris canutus rufa*), which was recently listed as Threatened under the Endangered Species Act (ESA). This shoreline also serves as the center of the New Jersey oyster aquaculture industry. Since the listing, the interplay between conservation measures aimed at protecting the red knot and their effect on the oyster aquaculture industry has taken on critical focus. Several non-governmental conservation organizations have petitioned state and federal agencies to stop shellfish aquaculture growth and curtail existing activities as they are apprehensive that oyster farming may pose disturbances to Red Knots during their annual spring migration to arctic breeding grounds. Concern has also been expressed that intertidal oyster farms may negatively impact horseshoe crabs, whose eggs serve as the primary food source for the red knot during its Delaware Bay stopover. As required by ESA consultation provisions, the U.S. Fish and Wildlife Service has developed a *Programmatic Biological Opinion for Structural Aquaculture* (PBO). The PBO prescribes risk-averse conservation measures to reduce potential impacts of oyster farming activities in the lower Delaware Bay, NJ on the Red Knot population and their critical habitats. Outcomes of this process will likely be precedent setting. An overview of the ESA experience with respect to Red Knots and oyster farms in Delaware Bay will be discussed.

## **DESIGN, BUILDING, AND PERFORMANCE OF A SIMPLE YET FUNCTIONAL AMPHIBIOUS FARM VEHICLE**

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Near-shore intertidal environments often present ideal conditions for cultivating shellfish, providing both the appropriate environmental conditions and reducing travel time and costs to access farms; however, the dynamic topographies of alternating sand bars and sloughs, tide-dependent work windows, and environmentally sensitive habitats characteristic of intertidal locations also present challenges to developing environmentally sound, optimal and efficient farm operations. Focusing their efforts during the low tide, many oyster farmers who operate intertidal farms have at best two hours on either side of a low tide to carry out daily husbandry and harvest tasks. This limited period heightens the need for equipment and practices that maximize efficiencies while minimizing environmental impacts. A critical problem is the lack of a low impact versatile vehicle that allows oyster growers to efficiently transport oysters and gear to and from, and within the farm, and provides a platform for production activities, such as harvesting and sorting stocks. An Amphibious Farm Vehicle (AFV) was developed for working an intertidal oyster farm. Central to the design was the desire for the vehicle to be low impact—quiet and environmentally friendly. The resultant manually operated amphibious farm vehicle has increased the efficiency of farm operations, while minimizing environmental impacts on a small scale oyster farm in the lower Delaware Bay, NJ.

## **EFFECT OF SUBSTRATE AVAILABILITY ON THRESHOLDS IN MUSSEL BED ESTABLISHMENT**

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Mussels organize in mussel beds that exert positive feedbacks on themselves, which enhances mussel bed stability. In harsh environments positive feedbacks are lacking in the establishment phase and substrate availability might be conditional. It was studied how substrate availability on soft substrate in the form of coarse shell material affects thresholds to prevent mussel (*Mytilus edulis*) dislodgment at different current speeds, mussel densities, aggregation states, and how it affects mussel patch morphology. Unravelling this mechanism is relevant in understanding the ecology of establishment, for restoration purposes and for commercial culture. Mechanisms were studied in a lab experiment using a large racetrack flume and results were validated in a field experiment. Two different experiments were performed in the flume: 1. effect of coarse shell material on mussel dislodgement at high and low flow speed at two aggregation levels and three mussel density levels and 2. effect of patch morphology on mussel dislodgement with and without coarse shell material. In the field experiment mussels were placed on plots with and without coarse shell material, mussel losses, aggregation behaviour and differences in attachment strength of mussels were analysed after one month. Results show that presence of attachment substrate stabilized mussel patches in low energy environments and was conditional in high energy environments for mussels to persist. Aggregation into clumps enhanced dislodgement, and dislodgement was reduced with mussel density and patch weight. Coarse shell material reduced mussel losses (by a factor 3), aggregation (by a factor 2.4) and increased attachment strength (by a factor 2.4).

## **MATERNAL AND SALINITY EFFECTS ON BLUE CRAB (*CALLINECTES SAPIDUS*) LARVAL MORPHOLOGY: IMPLICATIONS FOR BIOPHYSICAL INTERACTIONS**

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Adult blue crab (*Callinectes sapidus*) population abundance is highly variable, and may be related to variability in larval recruitment. Larval success partially depends on morphology, as it affects predation risk, feeding and swimming ability, and ultimately dispersal capability. The purpose of this experiment was twofold: 1) To determine the degree of inter-brood and intra-brood variability in morphology in blue crab larvae, and 2) to determine whether maternal effects or salinity influence the morphological development of larvae. Salinity varies in coastal environments, and is a key environmental parameter in crab larval development. To test maternal effects, larvae from six ripe adult female Delaware Bay crabs were reared in laboratory cultures. Larvae were sampled at hatching and the morphology of 125 individuals was measured microscopically. Additionally, larvae from two females were split and reared two separate salinity treatments (28 and 33), and sampled regularly for morphological measurements (348 larvae overall). Measurements for all larvae included size, shape, spine, and swimming appendage dimensions. Reynolds number and drag were estimated from measured morphology. Morphology was highly variable both within and between broods, and most metrics were significantly influenced by maternal identity. Therefore, maternal effects should be controlled for in future developmental studies, and morphological variability should be considered in biophysical modeling. After 22 days of culture, larvae from lower salinity (28) were larger, experienced higher drag force, and had longer swimming appendages. This suggests that morphological development is sensitive to abiotic factors. Additionally, larvae may be triggered by an estuarine environment to accelerate their development.

## PROGRESS TOWARD STREAMLINING SHELLFISH HEALTH MANAGEMENT

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The rapid expansion of shellfish aquaculture along the US East Coast has occurred against a significant backdrop of parasitic diseases, and generally reflects successful management of these diseases. Nonetheless, difficulties remain with regard to shellfish health management. Uncertainty concerning pathogen distributions, prevalences and impacts challenges state regulators to make informed decisions quickly regarding interstate seed transfers. This has contributed to establishment of zero-tolerance policies with regard to pathogens in seed proposed for transport, to the detriment of aquaculture commerce and, potentially, regional biosecurity of the industry. The increasing numbers of disease analyses required with increasing volume of interstate transfers stresses limited diagnostic resources and produces additional expenses for industry in both time and the cost of these analyses. With collaborators from industry, regulation, and the extension and scientific communities coastwide and with support from the NOAA Aquaculture Research Program, NJ and VA Sea Grant, and the USDA APHIS Veterinary Services, we have been working to streamline disease management by making improvements in several areas, including the resolution of pathogen distributions and guidelines for hatchery certification for expedited transport of seed. An update will be provided on progress on these fronts and perspective on the future directions of this initiative.



## EVIDENCE FOR RESISTANCE EVOLUTION AS A COMPONENT OF THE RESPONSE OF *CRASSOSTREA VIRGINICA* TO *PERKINSUS MARINUS*

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Persistence of dermo disease caused by *Perkinsus marinus* in *Crassostrea virginica* in eastern US estuaries has long suggested that oysters are incapable of developing resistance to this pathogen. Indeed, *P. marinus* prevalences and weighted prevalences among wild oysters in Chesapeake Bay remain high. Oyster populations are nevertheless beginning to recover, which may reflect tolerance evolution: the oyster not limiting the parasite by inhibiting colonization or proliferation within tissues—i.e., through resistance—but rather minimizing physiological consequences of infection. The interpretation is being reassessed, however, that resistance evolution is not occurring, particularly because of insight gained through disease monitoring in Virginia since 1988. Annual spring deployments of naïve sentinel oysters have revealed chronically intense *P. marinus* infection pressure over the period. Yet despite high prevalences and weighted intensities of *P. marinus* in wild oysters, a closer look at peak-season *P. marinus* infection intensities has revealed a marked decrease in the percentage of oysters with more intense *moderate* and *heavy* infections. The peak annual percentage of oysters with heavy infections at Wreck Shoal in the James River, for example, was similar to the percentage in sentinels from 1988-2002 (95% confidence interval about mean:  $27.7 \pm 5.8\%$  versus  $28.8 \pm 8.8\%$ , respectively). It has been significantly lower since 2003 ( $3.8 \pm 3.3\%$  versus  $16.2 \pm 4.8\%$ ) at this and other locations. While tolerance adaptation may be significant with regard to oyster interactions with *P. marinus*, resistance appears to be playing a subtle role in keeping proliferation within oyster tissues at tolerable levels.

## PHYSIOLOGICAL EFFECTS OF SALINITY ON EASTERN OYSTERS FROM THE NORTHERN GULF OF MEXICO

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The eastern oyster, *Crassostrea virginica*, is characterized by its broad tolerance to temperature and salinity. Accurate characterization of physiological rates under the range of temperature and salinity oysters are exposed to is essential for developing individual performance and population models. While there are abundant physiological data for eastern oysters from temperate regions physiological data from subtropical northern Gulf of Mexico are limited. The objectives of this study were to estimate the rates of clearance and oxygen consumption of Gulf oysters under a broad range of salinities (3, 6, 9, 15 and 25) in winter (17°C) and summer (27°C). Clearance rate was similar in winter and summer with highest rates in the 9 to 25 salinity range, reduced by 50-80% at a salinity of 6, and by 90-95% at a salinity of 3. Overall, differences in clearance rate between winter and summer were not found. Oxygen consumption rates in winter had limited variation and were similar between salinities. In contrast, oxygen consumption increased about four fold from winter to summer, and summer rates decreased with decreasing salinities and showed large variation between oysters likely due to varying gonadal volume. Valve movements measured at the five salinities indicated oysters remained opened between 50 and 60% of the time in the 6 to 25 salinity range and about 30% of the time at a salinity of 3. The valve opening amplitude was also significantly narrower in oysters at salinities of 3 and 6.

## PHOTORECEPTION AND GILL LATERAL CELL CILIA ACTIVITY IN BIVALVES

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Gill lateral cell cilia (GLCC) activity in bivalves has been well studied for many decades. In *Mytilus edulis* and other bivalves gill lateral cells are innervated from their ganglia and this innervation regulates the beating rates of GLCC. The neuronal mechanisms of how GLCC respond to environmental sensory cues has not been well studied. Various sensory inputs to mantle rim tentacles, including light, were found to alter GLCC activity in *M. edulis* and *Crassostrea virginica*, suggesting a sensory-motor integration mechanism involving the visceral and possibly cerebral ganglia. More recently we found shining light on the cerebral ocelli, a structure whose physiology has not been well studied, also modified GLCC activity. In *C. virginica*, shining light on mantle tentacles or cerebral ocelli decreased GLCC beating rates, while in *M. edulis* light had the opposite effect, increasing GLCC beating rates. Histamine is the neurotransmitter of photoreception in many invertebrates. Previously, it was found that histamine and a histamine H<sub>2</sub>-like receptor present in mantle rim tentacle of both *C. virginica* and *M. edulis*. It was also found that when famotidine, a histamine H<sub>2</sub> receptor antagonist, was applied to mantle rim or ocelli, the sensory effect of light on GLCC was blocked in both bivalves. These findings strengthen the existence of a sensory-motor integration mechanism in the regulation of the activity of GLCC, as well as expand the role of histamine as the neurotransmitter of photoreception to bivalves. This work was supported by 690340047 of PSC-CUNY, 2R25GM06003 of the NIGMS Bridges Program and a Carnegie Foundation award.

## PHENOTYPIC PLASTICITY IN GASTROPOD SHELL MICROSTRUCTURE: LACK OF RESPONSE TO A NOVEL PREDATOR AND REDUCED GROWTH

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Inducible defenses in shell thickness, shape, and growth are common in marine gastropods and these traits are often correlated with each other. This plasticity is often a byproduct of predator-induced refuge seeking behavior that causes decreased feeding rates and reduced growth. Few studies, however, have examined the possibility of shell microstructure as a phenotypically plastic trait, and those that did measured the microstructure at or near the aperture only, ignoring shell remodeling or thickening of the body and apical whorls. The various types of molluscan microstructures have different biomechanical properties which can alter the defensive role of the shell. Phenotypic plasticity was tested for a species of marine snail, *Littorina littorea*, to the nonnative predatory crab, *Hemigrapsus sanguineus*, and in response to a reduced growth rate. Snails were collected from three Long Island Sound populations to test for population differences in plastic response. The thickness of the inner crossed lamellar layer, outer irregular prismatic layer, and total thickness near the aperture and throughout the body and apical whorls was measured. Results suggest that microstructure in *L. littorea* is not plastic in response to *H. sanguineus* or reduced growth rate; however, differences among sites in microstructural thickness of the apical whorls that are not attributable to experimental treatments may suggest plasticity in microstructure in response to an environmental factor not captured by the scope of the experiment.

**EXPERIMENTAL TRANSPLANTATION OF MANILA CLAM *RUDITAPES PHILIPPINARUM* FROM AN AREA OF LOW *PERKINSUS OLSENI* INFECTION TO AN AREA OF HIGH *P. OLSENI* INFECTION ON THE WEST COAST OF KOREA: DRAMATIC INCREASE IN THE PREVALENCE AND INFECTION INTENSITY**

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Clams were experimentally transplanted from an area of low *Perkinsus olseni* infection to an area of higher infection, to understand the infection dynamics. In June 2008, adult clams (30-35mm) were transplanted from Padori to Hwangdo, with 3 different experimental setups (Exp-A, 184 clams/m<sup>2</sup>, Exp-B, 396 clams/m<sup>2</sup>, and Exp-C, 488 clams/m<sup>2</sup>). The Exp-A only included lightly infected transplanted clams as a control while the others are mixed with the heavily infected local clams in Hwando. *P. olseni* infection level was determined using RFTM and 2M NaOH digestion. Twenty-three days after, the prevalence of transplanted clams increased markedly, from 30% to 60-70%, regardless of the experimental design. At 49 days, the prevalence and the intensities of transplanted clams increased dramatically; the prevalence and intensity reached 100% and ~10<sup>6</sup> cells/g tissue, respectively. From day 49 to the end of the experiment (day 107), the prevalence of transplanted clam remained 100% and the intensity varied from 1.0 to 1.5x10<sup>6</sup> cells/g. In contrast, the prevalence and intensity in Manila clams in Padori remained 30-70% and 50,000-110,000 cells/g tissue. No clear difference in the infection level was found among 3 experimental setups. Despite the high level of *P. olseni* infection, condition index and glycogen levels in the transplanted clams increased significantly, and mortality was low (10% at the end). It was believed that the level of available food in Hwangdo was sufficient enough to meet the level required by the clams to grow and reproduce, and by *P. olseni* in the clam tissues, resulting in net growth.

# AN EXAMINATION OF THE USE OF SEASCAPE SCALE HABITATS INCLUDING OYSTER AQUACULTURE AND *ZOSTERA MARINA* BY FISH AND CRAB IN US PACIFIC NORTHWEST ESTUARIES

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Understanding the ecological role of shellfish aquaculture (*Crassostrea gigas*) and eelgrass (*Zostera marina*) as important habitat in US Pacific Northwest estuaries is important for management decisions. The aquaculture industry is currently restricted by regulations concerning impacts of their activities on *Z. marina* as this seagrass is designated as Essential Fish Habitat (EFH) for federally managed fish species. In this study, abundance, species diversity, and behavior activities of fish and crab in Pacific Northwest estuaries were quantified using underwater GoPro® video surveys. The particular interest were landscape scale features that might influence behavior of these more mobile species and here, longline oyster aquaculture, eelgrass beds and the edge between these two habitats was evaluated. Predation tethering units (PTUs) were also deployed in each of these habitats to evaluate predation risk and refuge value and temperature, relative light, and salinity measurements were taken to characterize the physical environment. Habitat complexity represented as seagrass density and epiphyte cover were also measured within each habitat. Preliminary results suggest there are important differences in use of these habitats by some fish and invertebrates that could be associated with predation and refuge value.

# **ELEVATED TEMPERATURE HAS ADVERSE EFFECTS ON A GABA-MEDIATED ADAPTIVE RESPONSE TO SEDIMENT ACIDIFICATION IN JUVENILE SOFT-SHELL CLAMS**

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Sediment acidification can influence the burrowing behaviour of juvenile marine bivalves such that fewer bivalves burrow into more acidified sediments. Unlike maladaptive behavioural responses to ocean acidification (OA) in other marine organisms, reduced burrowing in acidified sediments is likely an adaptive trait that allows these organisms to avoid shell dissolution and subsequent mortality. While neurotransmitter interference has been linked to maladaptive behavioural responses to OA, the mechanism(s) controlling the adaptive burrowing responses of juvenile bivalves in response to sediment acidification remain unknown. Further, the consequences of global change stressors on this adaptive burrowing response have yet to be tested. Whether GABA<sub>A</sub>-like receptor interference could act as a potential biological mechanism underpinning the adaptive burrowing responses (measured as the proportion of clams burrowed into sediment) of juvenile soft-shell clams to sediment acidification using the GABA<sub>A</sub> antagonist, gabazine was investigated. Also, the combined effects of elevated seawater temperature (+3°C) and sediment acidification on juvenile clam burrowing behaviour was tested for effects of ocean warming on bivalve burrowing responses to sediment acidification. Results showed that significantly fewer clams burrowed into low pH sediments when gabazine was not administered and under ambient temperatures; however, gabazine administration and elevated temperature each reduced the proportion of clams that avoided burrowing into low pH sediments. These results suggest that CO<sub>2</sub> effects on neurophysiology (GABA<sub>A</sub>-like receptors) can mediate an adaptive response to high CO<sub>2</sub> in juvenile marine bivalves by allowing them to avoid more acidic sediments; however, this response may be adversely affected by ocean warming.

## **THE WARBURG EFFECT IS THE METABOLISM THAT PLAYS IN FAVOR OF THE HERPESVIRUS OSHV-1 IN OYSTER *CRASSOSTREA GIGAS***

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Since 2008, mass mortalities of juvenile *Crassostrea gigas* affect all rearing sites along coasts of France when seawater temperature exceeds 16°C. Recent mortality events are also reported in UK, Australia and New Zealand. Results of diagnostic tests show that they are associated with the detection of the particular genotype of the ostreid herpesvirus 1 (OsHV-1). The aim of this study was to better understand the pathogenesis of OsHV-1 in oyster. Eight month-old *Crassostrea gigas* were anesthetized and injected in the muscle with high load (challenged oyster) or low load (control oyster) inoculum of OsHV-1. At 2 days post-injection, quantity of OsHV-1 DNA in challenged oysters was 10.000 times higher than in that of control oysters. Then, for the first time, a two-dimensional (2-D) proteomic approach was used to identify metabolic signatures of OsHV-1 infection between challenged *versus* control oysters. Ten proteomes obtained from individuals that exhibited contrasted infection status, as measured by their individual OsHV-1 DNA level were analyzed. The results identified 25 abundant protein spots that showed a marked change in accumulated levels. Challenged oysters exhibited an increased glycolysis and mitochondrial activity combined with an accumulation of a specific protein, the porin VDAC, which reflects a “Warburg effect”. The Warburg effect is an atypical metabolism that favors OsHV-1 by providing cellular energy and building blocks during viral genome replication in oyster, and was initially reported in cancer cells and more recently in shrimp infected with virus. Ongoing studies in our lab and in the field investigate how environmental factors and farming practices could control the Warburg effect in *Crassostrea gigas* to delay or protect from disease mortality risk.



## **FATE AND PERSISTENCE OF CONTAMINANTS OF EMERGING CONCERN IN OYSTERS GROWN IN A WASTEWATER-IMPACTED COASTAL POND**

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Several towns on Cape Cod (Massachusetts) are incorporating shellfish propagation into wastewater management plans as a way of removing nitrogen from coastal waters. The shellfish are transferred to a grow-out site from which they are then harvested for consumption. Although the ability of shellfish to improve water quality is well documented, there have been few studies to investigate the impact on the shellfish of being grown in polluted waters. Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products, are typically found in higher concentrations in wastewater-impacted waters. Since 2010, the Center for Coastal Studies has been monitoring the coastal waters of Cape Cod for CECs. Several of the CECs found in coastal water samples were also found in shellfish grown in these waters.

This study used the eastern oyster (*Crassostrea virginica*) to examine the fate and persistence of CECs. Oysters are known to filter large volumes of water. Although this makes them attractive as an alternative method of improving water quality in degraded areas, it also has the potential to expose them to high concentrations of anthropogenic contaminants. Therefore, the ability of oysters to bioaccumulate these contaminants has implications for wastewater management and ecological rehabilitation methods that incorporate oysters in mitigation efforts to address water quality standards. This study measured CEC levels in oysters grown in a wastewater impacted coastal pond and at set intervals after transfer to the grow-out site to determine purging rates of detected contaminants.

## **IMPACT OF MUSSEL CULTURE IN MALPEQUE BAY ON LOBSTER CONDITION AND ON NUTRITIONAL QUALITY OF THEIR MAJOR PREY SPECIES, THE ROCK CRAB**

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Recent studies suggest that the distribution of American lobster (*Homarus americanus*) is affected by the presence of mussel aquaculture facilities. Lobsters are commonly more abundant inside of than outside of farm sites, possibly in response to increased density of one of their preferential prey, the rock crab (*Cancer irroratus*), which are attracted by mussels fall-off. A field study was initiated to assess the effects of mussel culture in Malpeque Bay, Prince Edward Island, Canada, on the nutritional quality of rock crab and lobster condition. Fat, protein, calcium, phosphorous, astaxanthin, water, and ash content were measured in male and female crabs of multiple size classes, captured under mussel lines or at nearby reference sites in June 2015. Caloric and carbohydrate contents were calculated. The concentrations of fat, protein and glycogen were measured in the digestive gland of male and female lobsters (60-89.9 mm, carapace length), captured at the same sites. Preliminary results indicate that caloric, protein, and astaxanthin content of certain crab categories (sex and size) are higher at the farm sites, possibly in association with a higher proportion of soft crabs (post-molt) there. The condition of lobster did not differ between farm and reference sites. A more exhaustive sampling campaign is underway to assess the population structure of lobsters and crabs, their nutritional quality and condition. Acoustic tagging studies are being conducted to study the movements of these crustaceans and their degrees of association with the mussel culture facilities.

## VARIABLE PARTICLE RETENTION EFFICIENCY BY *MYTILUS EDULIS*: FACT OR FICTION AND WHY IT MATTERS

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Particle retention efficiency (*RE*) in *M. edulis* has been reported to decrease rapidly for particles smaller than 4  $\mu\text{m}$ . Although this relationship is firmly established in the literature, recent research has shown that the minimum particle size for efficient retention by mussels can change seasonally between 4 and 35  $\mu\text{m}$ . Although these data have been interpreted as indicating a high degree of physiological control of particle capture by mussels, they have also been construed as an indication of the presence of methodological errors in traditional approaches used to measure bivalve feeding rates and efficiencies.

The possibility that variable physical properties of seston particles and/or miscalculations can result in erroneous *RE* measurements was examined using three methodologies and three particle sources (natural seston, algal cell monocultures and clay). Comparable *RE* measurements were obtained with all methodologies and diets when routine precautions were taken. In all cases, the mussels exhibited maximum *RE* for particles greater than 8–11  $\mu\text{m}$ . The *RE* for smaller particles declined gradually with 50–60 % retention of 4- $\mu\text{m}$  particles and 30–40 % retention of 2- $\mu\text{m}$  particles. Differences in the *RE* size spectra of mussels and tunicates (*Ciona intestinalis*) further indicated that *RE* was not influenced by methodological artifacts that, if present, would result in similar responses by both species. Additional studies were conducted to assess the effects on *RE* of changes in water viscosity associated with temperature seasonality.

Assumptions regarding the *RE* spectrum of mussels has contributed to many conclusions on the environmental effects and provision of ecological services by dense populations. The accuracy of these conclusions can only be assured if the effective retention of food particles is measured under relevant conditions and is not simply assumed.

## **WOMEN, A CRITICAL LINK IN SHELLFISHERIES AND FOOD SECURITY IN WEST AFRICA**

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In West Africa, shellfish habitats in coastal estuaries and mangrove wetlands are under increasing pressure from population growth, urbanization and development, overexploitation for fuel wood, destruction for agriculture, inundation from sea level rise and aquifer salinization from climate related changes in precipitation patterns and from disruption of fresh and saltwater flows due to human activity. Women dominate shellfish harvesting enterprise in these ecosystems, largely due to its seasonal nature, proximity to shore, and relatively low monetary returns. Their household food security is frequently impacted when these women, who have few other economic opportunities, are faced with potential decline of the shellfisheries that are an important element of their family income. The status of shellfish stocks and local socio-economics of shellfisheries in three West African nations of Ghana, Senegal, and The Gambia is discussed, along with individual efforts in these countries to increase economic opportunities for these shellfishers in the three countries through participatory, community-based, ecosystem-based co-management approaches. Specific outcomes of these efforts include increased direct participation in data-driven shellfish co-management decision making by the shellfishers themselves, improving product quality and consumer confidence in shellfish product safety through multi-agency shellfish sanitation programs, programs to develop value-added marketing of shellfish products, and efforts to provide greater economic resiliency through diversification of family income opportunities, including aquaculture, in addition to their traditional seasonal shellfishery harvests.

## **SHELLFISH ASSEMBLAGES AS HOTSPOTS FOR BIODIVERSITY**

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Shellfish have a wide global distribution, and abundance of both wild and cultured stocks is significant. Shellfish assemblages are also known to provide habitat for many other species and this engineering function plays an important role in many ecosystems. Though aquaculture is often judged for its ecological impacts, it is increasingly recognized that cultured shellfish stocks can also provide a variety of ecosystem services. From a biodiversity perspective, fisheries of natural shellfish stocks can negatively impact biodiversity, while at the same time biodiversity can be high at culture plots, suggesting that ecosystems may also benefit from aquaculture activities.

In this presentation a review will be presented on the drivers for biodiversity, in order to evaluate the key factors for biodiversity development in shellfish assemblages. We will also review, where relevant, the trade-offs between biodiversity reduction and biodiversity enhancement for (1) natural banks, (2) natural banks that are fished, (3) culture plots or suspended cultures and (4) shellfish restoration areas. A case-study for an important mussel cultivation area in the Waddensea nature reserve (The Netherlands) will highlight the trade-offs between the impacts and benefits at the scale of an entire ecosystem.

## **SPATIAL AND TEMPORAL ANALYSIS OF QPX DISEASE WITH ENVIRONMENTAL CONDITIONS IN RARITAN BAY, NY**

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QPX disease has been monitored in Raritan Bay NY since hard clam mortalities were discovered in 2002, resulting in a long-term data set that includes disease prevalence as well as environmental parameters. Additional data sources (oceanographic buoys and meteorological stations) have been utilized to provide context of climate trends in relation to QPX disease dynamics. Data clearly show that infection is limited and nearly absent in certain areas of the bay. Temporal variability of infection across the fishery area showed relationships with environmental factors in common with the spatial patterns. Warmer years tend to have lower disease levels across the monitoring stations and the warmest areas of the fishery are typically without QPX infections. Drought and high salinity preceded the reported QPX disease related hard clam mortalities and other years of low precipitation have surges of QPX infections. The lowest salinities areas are consistently the least infected. Other environmental monitoring parameters (e.g., D.O., sediment type, clam size) were examined and a positive trend of disease prevalence does emerge with clam density. Multiple regression models showed strong relationships between infection and the combination of temperature and salinity conditions. QPX infection has a seasonal pattern in Raritan Bay with peaks observed during the summer that in more recent years have shifted toward spring which corresponds to shifts observed in the temperature record. Continued warming trends with predicted intense precipitation events that can drive low estuarine salinity phases may actually provide these hard clams with some relief from QPX disease pressure.

## **OYSTER FARMING IN SOUTH CAROLINA: INDUSTRY DEVELOPMENT, CHALLENGES, AND OPPORTUNITIES**

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South Carolina has a long history of oyster harvesting and strong cultural ties to oysters, with oyster roasts that feature wild-harvested clusters being a fixture during the fall and winter months. Wild-harvested singles, while always coveted, were harder to come by and in recent years are even more difficult to find. This led a handful of enterprising growers to turn to using mariculture techniques, namely off-bottom methods, to produce premium single oysters to meet the demand of Charleston's high-end raw bars. Recently, more growers are entering the industry and in-state capacity to provide seed is increasing to meet the demand. Technology transfer efforts have focused on hatchery and nursery capacity development and evaluation of floating culture gear. This presentation will provide an overview of the status of oyster farming in South Carolina and challenges and opportunities the industry is facing as it continues to grow.

## **OXYGEN CONSUMPTION IN THE ISLAND APPLE SNAIL, *POMACEA MACULATA* IN WATER, IN AIR, AND DURING ESTIVATION**

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The island apple snail, *Pomacea maculata*, is a recent successful invader of southern U. S. freshwater habitats. These snails have gills and a “lung” formed from mantle tissue. They also have an extendable siphon that enables them to breathe air or inflate the lung while remaining below the water surface. Inflation of the lung allows the animals to float in the water column. The animals are also able to estivate for long periods when exposed to air. We measured the rate of oxygen uptake of animals in water, in air, and during estivation. The  $VO_2$  for animals in water was  $22.1 \pm 19.3 \mu\text{l g wet wt}^{-1}\text{hr}^{-1}$  ( $n=27$ ). The rate of oxygen uptake in air was  $55.0 \pm 51.0$  ( $n=54$ ). The rate of uptake for 43 animals during estivation ranged from 0 to 3.5. During 126 days of estivation, the snails appeared to breathe in pulses every few days. Heart rate fell from 25 to below 5  $\text{beats min}^{-1}$  during estivation. Snails maintained in air of 90% relative humidity on a wet substrate for several weeks were active, eating and crawling with no signs of entering estivation. Snails confined in aerated water but unable to breathe air showed no mortality or abnormal behavior for several weeks. The rates of oxygen uptake in air and water are consistent with values reported for other gastropods. These experiments show that the animals are able to breathe air, but are not obligate air breathers. The biochemical changes associated with this estivation are under investigation.



## **GONADAL TRANSCRIPTOME ANALYSIS OF THE PACIFIC GEODUCK, *PANOPEA GENEROSA***

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The Pacific geoduck, *Panopea generosa* Gould, 1850, endemic to the eastern Pacific coast and estuaries from 29-57 N latitude (Punta Canoas, Baja California, Mexico to Kodiak Island, Alaska, USA), represents a valuable export fishery for Mexico, USA and Canada, primarily to markets in Asia. To meet demand, mostly from China and Hong Kong, aquaculture production of this valuable clam has increased considerably in Washington State, USA and British Columbia, Canada over the last two decades, and there have been some efforts in order to cultivate this species in Mexico. Despite the economic importance of the Pacific geoduck little is known about its reproductive biology. The main objective of this work was to generate a transcriptomic resource for Pacific geoduck, *P. generosa*, using RNA-seq.

More than 443 million reads were obtained and the de novo assembly resulted in more than 153,000 transcript contigs with a mean contig length of 660 bp and an N50 value of 1015 bp and a GC content of 37%. A total of 25,963 contigs matched the consulted databases (Swiss-Prot/Uniprot, Gigaton and RUPhibase). While only 148 and 198 contigs were expressed only in male and female gonads, respectively; however, only 36 and 44 contigs were annotated, respectively. A total of 1366 microsatellites were obtained, of which 97.4 % had a dinucleotide motif. This work is considered to be a line base for further transcriptomic analysis of the *P. generosa* gonads.

## **EVALUATION OF A SUBMERSIBLE MUSSEL RAFT FOR USE IN SEMI-EXPOSED SITES: FIELD STUDY**

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Risk in mussel farming using floating rafts is due, in large part, to drop-off from storm waves and damage from drift ice. A novel submersible mussel raft was developed by Pemaquid Mussel Farms to reduce wave forcing and to avoid contact with floating ice during winters in northern waters. When submerged, the raft pontoons are flooded, and it is held vertically by lines attached to surface floats and horizontally by a spread mooring. Field tests on a full-scale prototype, each about three weeks long, provided 6-degree-of-freedom motion and mooring load data for a variety of wave and current forcing conditions. This data set showed that the submerged raft oscillates with significantly smaller heave and pitch amplitudes than the surfaced raft for wave periods of interest (generally between 2 and 8 seconds). The submerged configuration greatly decreases vertical velocities and accelerations of the mussel rope attachment points, reducing feeding interruptions and mussel drop-off in storms. Consequently, harvest from the submerged raft was approximately 300% of typical harvests from surface rafts.

## **EVALUATION OF A SUBMERSIBLE MUSSEL RAFT FOR USE IN SEMI-EXPOSED SITES: NUMERICAL MODELING**

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A novel submersible mussel raft was developed by Pemaquid Mussel Farms. Numerical models of the surfaced and submerged raft configurations were developed using a finite-element approach with hydrodynamic forces based on a modified Morison equation formulation. Models developed in the software programs Aqua-FE and Orcaflex showed good agreement with field measurements of raft motion, particularly for wave periods associated with storm energy at the semi-exposed test site. The reduced motion of the submerged configuration was shown to result from the decreased waterplane area and increased inertia, which reduce the heave and pitch natural frequencies so that they are below the frequencies associated with the highest wave energy.

## ASSOCIATION BETWEEN $\alpha$ -AMYLASE GENE AND GROWTH TRAITS IN THE ASIATIC CLAM *MERETRIX MERETRIX*

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The enzyme,  $\alpha$ -Amylase, that catalyzes the hydrolysis of the  $\alpha$ -(1,4) glycosidic linkages in starch and related compounds in carbohydrate metabolism, is highly important for the utilization of energy sources in animals. Although the  $\alpha$ -Amylase gene of several species in molluscs, such as *Pecten maximus*, *Corbicula fluminea*, *Crassostrea gigas*, *Pinctada maxima*, and *Pteria penguin*, has been characterized and their expression in various adult tissues has been examined, its gene structure, function and association with growth traits are not well understood.

Here, the cDNA and introns of  $\alpha$ -Amylase gene from the Asiatic commercially important clam *Meretrix meretrix* (*MmAmy*), quantitative expression profiles in adult tissues and embryos/larvae and association with growth traits are reported. The genomic DNA sequence of *MmAmy* was 6689bp, which contains 7 exons and 6 introns. The full length cDNA was 1783bp (GenBank accession no. KP250879), with a 1566bp of ORF encoding 522 amino acids, including a 19-amino acid signal peptide. qRT-PCR of tissue expression profiles showed that *MmAmy* was enriched in viscera and no expression in other five tissues. The expression was not detected during the early embryos and trochophore, and was detected since D-shaped larva while the highest expression was observed in the umbo larvae, being consistent with other molluscs.

The analysis of mRNA and enzyme activity of *MmAmy* of clams with four shell colors indicated that DF clams were the highest and the RS were the lowest ( $P < 0.05$ ), which was consistent with the fact of growth comparison, suggesting that there is an association between *MmAmy* gene and growth traits.

## **CHARACTERIZING THE ROLE OF DNA METHYLATION AS A MECHANISM FOR RAPID ADAPTATION UNDER GLOBAL CHANGE IN A COASTAL MARINE INVERTEBRATE**

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Rapidly shifting environments are exposing marine invertebrates to environments not previously experienced in recent history, leading to phenotype-environment mismatches and potential reductions in overall fitness. At the organismal level, phenotypic plasticity may help buffer against decreases in fitness. Mechanistically, there is increasing evidence that some phenotypic plasticity is regulated by epigenetic modifications – potentially heritable modifications that induce changes in gene activity without altering the underlying DNA sequence. To elucidate the role of this mechanism in mediating phenotypic plasticity under two prominent global change related stressors (warming and ocean acidification), an exposure experiment was performed using the eastern oyster (*Crassostrea virginica*). Using a fully factorial exposure design (two temperatures x three  $p\text{CO}_2$  levels), phenotypic responses were measured (i.e. growth and respiration rate) at multiple time points along the exposure duration and tissue samples were collected immediately following each phenotyping event. Data from this experiment will be used to test the hypotheses that (i) DNA methylation, induced in the tissue of shell formation (i.e., mantle tissue), is correlated with changes in transcription and phenotypic response to environmental change, and (ii) that methylation changes induced in the mantle tissue are also induced in the germline — indicating that such changes are potentially heritable. Results from this research will help characterize the role of epigenetic modifications in regulating phenotypic responses under multiple experimental scenarios, clarify the potential heritability of these epigenetic marks, and provide insight into mechanisms that underlie the capacity of marine invertebrates to respond and potentially adapt to global changes.

## **EFFECTS OF TiO<sub>2</sub> NANOPARTICLES ON OYSTER HEMOCYTES: *IN VITRO* STUDIES UNDER LIGHT AND DARK CONDITIONS**

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In marine bivalves, physiological responses to environmental stresses are mediated, in part, by hemocytes that are found in the open circulatory system. These cells are critical for the innate immune response and act to recognize, phagocytize, and oxidize nonself particles. The purpose of this study was to examine the immunological response of oyster hemocytes (*Crassostrea virginica*) following exposure to bulk (>1 μm) and nanoparticulate (<90 nm) titanium dioxide (TiO<sub>2</sub>). Two different types of nanoparticles (NP), both commonly found in consumer products, were used in the experiments: 1) UV-Titan M212 (93% rutile) and 2) 100% anatase form. Hemolymph was withdrawn from the adductor muscle of oysters and exposed to the aforementioned particles at concentrations of 0.1, 0.5 and 1.0 mg/L, for two and four hour periods under both dark and environmentally-relevant light conditions (350-800 nm). At the end of the incubation period, the mortality, phagocytosis, and oxidative stress of hemocytes were evaluated using flow-cytometric assays. Results indicated that under dark and light conditions, exposure to bulk or NP TiO<sub>2</sub> had minimal apparent effects on oyster hemocytes. Under some experimental conditions, NP caused significant changes in phagocytosis under both dark and light conditions, compared to hemocytes exposed to larger “bulk” particles and un-exposed control hemocytes. Photoactivation of TiO<sub>2</sub> did not enhance the effects of NP. Findings of this work demonstrate that oyster hemocytes are relatively insensitive to TiO<sub>2</sub> NP following acute, *in vitro* exposures of up to 4 hours under dark and light conditions.

# LARVAL RECRUITMENT PATTERNS DRIVE BURROWING SHRIMP (*NEOTRYPAEA CALIFORNIENSIS*) POPULATIONS IN US WEST COAST ESTUARIES AND THREATS TO SHELLFISH AQUACULTURE

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The ghost shrimp, *Neotrypaea californiensis* is an important member of estuarine intertidal communities along the US Pacific Coast, but its burrowing behavior causes significant problems for shellfish culture. Populations of these shrimp in Willapa Bay, Washington were monitored for two decades and Yaquina Bay, Oregon, since 2005. Ghost shrimp density increased dramatically in the 1990s in Willapa Bay, and then declined almost as precipitously through 2010. Similar, though less dramatic declines occurred in other estuaries from 2005-2010. These shrimp have pelagic larvae which develop in the coastal ocean, so the question was posed to whether these population changes were related to estuarine recruitment which were also monitored. Relatively high recruitment was observed in Willapa Bay through the mid-1990s but a period of about 10 years lapsed with very low recruitment. Significant relationships were found between the number of recruits and the number of larger 1-year-old shrimp present a year later in both estuaries. Recruitment events since 2010 have resulted in recent population increases in both of these estuaries which are of concern to shellfish growers especially in Willapa Bay where a control program was recently suspended. Population simulations of Willapa Bay shrimp were conducted using a size-structured model to determine the relative effect of recruitment and mortality rates on population abundance. Results suggest that frequency and magnitude of recruitment events drive population fluctuations. These examples are used to suggest thresholds of concern for shellfish bed management.

## CHANGES IN GENETIC COMPOSITION OF PACIFIC OYSTER (*CRASSOSTREA GIGAS*) LARVAE REARED IN HIGH *pCO2* CONDITIONS

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Since 2006, the West Coast oyster industry has been threatened by the effects of ocean acidification (OA) that has reduced the supply of “eyed” larvae and spat to commercial growers. While much research has focused on the effects of acidified seawater upon phenotypic characteristics of *Crassostrea gigas* larvae, effects on their genetic composition under these conditions have not been described.

Experiments conducted in 2015 and 2016, under both laboratory and commercial hatchery settings, indicated that high *pCO2* conditions (~1600 ppm) resulted in poorer early larval development when compared to larvae reared under ambient *pCO2* conditions (~400 ppm); however, larvae from selectively bred oysters from Oregon State University’s Molluscan Broodstock Program (MBP) appeared more tolerant of acidified seawater than larvae from wild stocks. During the 2015 laboratory experiment, samples were collected to examine changes in gene frequencies during development of larvae from these two stocks cultured under high and ambient *pCO2* conditions. Approximately 10,000 genome-wide Single Nucleotide Polymorphisms (SNPs) were identified from the genomic DNA extracted from these larval pools and these were analyzed for changes in allele frequencies between age classes of larvae that were concordant across experimental replicates.

Initial results suggest that genetic changes in larval pools across the 22 day culture period are distinct for all groups but that the allele frequencies of numerous loci appear to be uniquely changed in larval groups that have been exposed to high *pCO2* conditions, suggesting a significant contribution of genetics to the larval fitness of oysters cultured in high *pCO2* seawater.



# **INFLUENCE OF DIET ON ELEMENT INCORPORATION IN THE SHELLS OF TWO BIVALVE MOLLUSCS: *ARGOPECTEN IRRADIANS CONCENTRICUS* AND *MERCENARIA MERCENARIA***

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Biogenic carbonates have received much attention as potential proxies of environmental change; however, a major pathway of elemental incorporation is often overlooked when making interpretations or designing experiments. This research experimentally examines the influence of diet on elemental shell composition in juvenile shells of the bay scallop, *Argopecten irradians concentricus*, and the northern quahog, *Mercenaria mercenaria*.

Experiments were conducted using *Argopecten irradians concentricus* juveniles fed *Isochrysis* sp., *Chaetoceros* sp., *Pavlova* sp., *Tetraselmis* sp., or a mix of all four in a 2:1:2:2 ratio. Experiments with juvenile *Mercenaria mercenaria* compared shell chemistries among clams fed unicellular diets of *Isochrysis* sp. (CCMP1324), *Pavlova pinguis* (CCMP609), *Chaetoceros mulleri* (CCMP1316), *Isochrysis* sp. (CCMP1611), *Pavlova* sp. (CCMP1209), or *Chaetoceros galvestonensis* (CCMP186), a mixed diet of all species in equal ratios (Mixed), or no provided algae.

The results indicate that diet can influence shell chemistry either directly or indirectly, with degree of influence varying by diet and mollusc species. The present research provides valuable information concerning shell dynamics and potential diet-associated fluxes, thus demonstrating the need to consider the composition of dietary inputs when assessing environmental associations with elemental shell chemistries.

## **TAURINE PREVENTS THE NEUROTOXIC EFFECTS OF MANGANESE ON THE PHYSIOLOGICAL RESPONSE OF A CILIO-INHIBITORY DOPAMINERGIC SYSTEM**

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Manganese, a neurotoxin causing Manganism a Parkinson's-like disease, disrupts dopaminergic neurotransmission. The mechanism is unknown. Unlike Parkinson's, reports postulate the mechanism is more related to downstream neuronal pathways than deficits in nigrostriatal function. Gill lateral cell cilia of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations. Dopamine causes cilio-inhibition; serotonin cilio-excitation. Previous work showed acute and short-term manganese treatments block cilio-inhibition by dopamine. Recent reports suggest the amino acid taurine is worth investigating as a neuroprotective agent because of its efficacy in other neurodegenerative diseases. It was hypothesized that taurine would reverse neurotoxic actions of manganese on cilio-inhibitory effects of dopamine in *C. virginica*. Acute experiments testing dopamine dose responses on gills treated with manganese with/without taurine were conducted. Cilia of control cells responded normally to dopamine. Manganese treatments disrupted the dopamine induced cilio-inhibition and taurine treatments prevented this disruption (ED<sub>50</sub> = 10<sup>-6</sup>M). Short-term experiments where *C. virginica* were treated for 3 or 6 days with manganese with/without taurine were also conducted. Taurine prevented the effect of treating whole animals with manganese on disrupting the cilio-inhibitory dose response of dopamine in gill lateral cells. This study shows that taurine effectively prevented manganese neurotoxicity on the cilio-inhibitory dopaminergic system in *C. virginica*. These findings are helpful in furthering the understanding of the mechanism of manganese neurotoxicity and provides evidence suggesting taurine needs to be further investigated as a potential therapeutic agent for Manganism. This work was supported by grants 690340047 of PSC-CUNY, 2R25GM06003 of the Bridge Program of NIGMS and a Carnegie Foundation award.

## **ROLE OF CARBOHYDRATE-PROTEIN INTERACTIONS IN MEDIATING FOOD CHOICE IN SUSPENSION-FEEDING BIVALVES**

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Suspension-feeding bivalves (SFB) are able to sort and select food particles from a complex mixture. While this process has been described for decades, the fine mechanisms of particle sorting continue to remain ill-defined. This presentation will summarize findings on some of the molecular interactions mediating particle selection in these animals. Results demonstrated the presence in mucus covering SFB feeding organs of lectins that bind microalgae cell surface carbohydrates (CSC), providing a molecular mechanism for particle sorting via strong bonds between mucus and specific food particles. Results from feeding experiments, using either engineered microspheres with tailored CSC or microalgae whose CSC were blocked, showed that bivalves preferentially ingested carbohydrate-rich particles while they rejected others. The latest investigations evaluated the likelihood for a given microalga to be ingested or rejected based upon its cell-surface carbohydrate signature. In this context, the carbohydrate signatures of 16 microalgae species were characterized using 10 different lectins. A subset of these microalgae (12 species) was then used in feeding experiments where different pairs of microalgae were presented to oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*) to evaluate selection. Results show that cell-surface carbohydrates are good predictors for particle fate. Specifically, microalgae rich in glucose/mannose residues were preferentially selected by both oysters and mussels. These findings not only unravel aspects of the mechanism used by SFB to sort their food particles, but also provide a promising predictive tool that could be used to assess bivalve performance and benthic-pelagic coupling under ecological or aquaculture contexts.

## GENETIC TOOLS FOR DECODING *PERKINSUS MARINUS* GENOME

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The protozoan parasite *Perkinsus marinus* is the etiological agent of Dermo disease in oysters. Countries reporting the presence of *Perkinsus* spp. continue to increase highlighting the ubiquitous distribution of the parasites and the immense importance for aquaculture, especially in the absence of effective intervention strategies. Compared to other protozoan parasites of molluscs, advances in *Perkinsus* spp. culture methodologies put them in the front seat for understanding the parasite's biology and ecology, which may result in novel disease intervention strategies, and biotechnological applications as well. A plasmid-based transfection system by fusing the Green Fluorescent Protein to a highly expressed intron-less *P. marinus* open reading frame, flanked by endogenous genomic sequences (*p*MOE[MOE]:GFP) is presented. Integration of this plasmid into the genome by non-homologous recombination yields stable green *P. marinus*. Recently, based on *p*MOE[MOE]:GFP, a drug selection cassette for *P. marinus* has been developed using *Sh-ble* fused to mCherry, which confers resistance to the glycopeptide antibiotic bleomycin; however, to address complex hypothesis driven questions and decipher the function of most of the *P. marinus* genes, it is necessary to fine-tune the available tools and to develop/adopt genome editing tools (CRISPR/Cas 9). How to expedite *Perkinsus* research by developing new transfection vectors and protocols will be discussed. The quality and accessibility of genomic and transcriptomic datasets ensuring access by the global research community to jump-start research on this important pathogen are being improved. Finally, the *Perkinsus* model system has proven very successful to expose undergraduate community college students to scientific research.

## SEX DETERMINATION/DIFFERENTIATION GENES IN THE FUNCTIONAL HERMAPHRODITE SCALLOP, *NODIPECTEN SUBNODOSUS*, IDENTIFIED IN A *DE NOVO* TRANSCRIPTOME ANALYSIS

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The lion-paw scallop is a functional hermaphrodite Pectinid species that represents an important fishery resource along the Pacific coast of the Baja California Peninsula in Mexico. Because nothing is known about hermaphroditic scallop sex determination-differentiation, the aim of this study was to establish whether genes known to be involved in sex differentiation in other organisms could be identified in the scallop, and to establish their expression pattern during gonad development. A transcriptomic approach (MiSeq-Illumina) was used from two early life stages, eyed-larvae and spat, and three juvenile tissues: inactive gonad, adductor muscle, and digestive gland. End-point PCR and qPCR were performed evaluating expression patterns for the identified genes in somatic tissues and different gonad gametogenic stages. Genes identified as *dmrta2*, *sox9*, *ovo* and *sfrp2* were specific for the testis and not the ovary gonad region. In agreement with the findings, those genes have been described as playing a role in vertebrate male sex differentiation. Other genes found that are known to play roles in sex differentiations were *Virilizer*, *tra2a*, *foxl2*, *wnt4* and *pbx1* among others. Finally, a gene known to be involved in the sex determination pathway of the hermaphroditic model organism *Caenorhabditis elegans*, *sex-1* was annotated. Its protein contains a DNA binding and zinc finger domains and it was found only expressed by mapping reads back to the spat stage, but not in somatic tissues or during gonad gametogenesis by PCR. This study represents the first work aimed at understanding the genetics of sex determinations/differentiations in hermaphrodite scallops.

## **AN ON-LINE, SEARCHABLE DATABASE FOR IDENTIFICATION OF BIVALVE LARVAE USING SHELLBI: MACHINE VISION ANALYSIS OF BI-REFRINGENT PATTERNS IN LARVAL SHELLS**

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A searchable, interactive database containing bi-refringent patterns of larval shells and their classifications using the software package ShellBI will allow for rapid identification of unknown larvae using the polarization technique. The PostGres, HTML-cgi driven database provides species-specific galleries of images of larval shells from many sources, including both historical and present day, that have been classified by ShellBi, a software package that uses polarization patterns to characterize shells to species. One feature of the database is the ability to view these galleries and visually compare them to unknown samples. Another powerful feature allows users to upload their own image files of larvae and run ShellBi directly, first extracting the color and texture patterns and then classifying them remotely and producing a detailed classification report. The interactive use of the website, the optical setup, and the software necessary to perform these classifications will be described.

## **EVALUATING COST EFFECTIVE STRATEGIES FOR MITIGATING MUD BLISTER WORM INFESTATIONS IN OFF-BOTTOM OYSTER AQUACULTURE**

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In recent years, the strong demand and increased specialization of the half-shell oyster industry has given way to an increasingly competitive market. While farmers explore various grow out techniques to ensure product consistency and marketability, much of the cultured oyster industry remains victim to mud blister worm, *Polydora websteri*, infestation. Mud worms gain access to oysters as larvae and as they grow they begin to burrow into the shells creating ‘mud blisters’, which can negatively impact the perception, flavor and overall marketability of cultured oysters destined for the half-shell market.

This study took place over a three-month period in Portersville Bay, Alabama. The goal was to determine a cost-effective and widely applicable method for addressing mud worm infestation on cultured oysters grown in the OysterGro™ floating cage system. The overarching question was to determine how the factors of ploidy (triploid, diploid), stocking density (125, 150, 175), and flip regime (weekly, biweekly) impact mud worm abundance. Overall, the frequency of desiccation ‘flip regime’ had the most significant effect on mud worm abundance, with weekly flipped oysters showing very low or no signs of infestation; however, diploid bi-weekly flip oysters showed a significantly higher infestation when compared to triploid bi-weekly flipped oysters. This finding suggests that without a weekly desiccation regime, diploid oysters may be more prone to mud worm infestation when compared to faster-growing triploids.

## **CTENOPHORE (*MNEMIOPSIS LEIDYI*) PREDATION ON BAY SCALLOP (*ARGOPECTEN IRRADIANS*) LARVAE**

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Bay scallops were once an important component of the New Jersey Barnegat Bay-Little Egg Harbor (BB-LEH) ecosystem and closely associated with the seagrass meadows; however, beginning in the 1970s, the New Jersey bay scallop population declined and has remained low. Recruitment limitation may play a role in preventing the population from recovering. It is not known if planktonic predators such as ctenophores (*Mnemiopsis leidyi*), which have been found in high densities in BB-LEH, act as important predators on bay scallop larvae. In order to assess potential predation pressure by ctenophores on bay scallops, predation rate of individual ctenophores on high densities of hatchery-reared bay scallop veliger larvae was observed. Each ctenophore was then transferred to filtered seawater at hourly intervals to measure egestion rate. Egested living larvae were placed in beakers with food to determine survival after 24 hours. Ctenophores ingested 23% of bay scallop larvae. A small proportion of those larvae were egested live (<1%) and survived for 24 hours post-egestion. The results of this study suggest that ctenophores can contribute to predation on bay scallop larvae and may negatively impact bay scallop populations.



## CLIMATE CHANGE TRADEOFFS: THE IMPACT OF ACIDIFICATION AND WARMING ON JUVENILE BLUE CRAB, *CALLINECTES SAPIDUS*, PHYSIOLOGY

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Future climate scenarios predict increases in both oceanic temperature and dissolved carbon dioxide ( $p\text{CO}_2$ ) over the next century. Calcifying invertebrates, which depend on specific conditions of temperature and carbonate chemistry for many biological processes, may be especially impacted by these climate changes. The results of a study on the combined effects of increased water temperature and  $\text{CO}_2$  on various physiological metrics of juvenile blue crab, *Callinectes sapidus*, will be reported. Crabs were exposed to one of four temperature/ $p\text{CO}_2$  treatments (26°C/800ppm, 26°C/8,000ppm, 32°C/800ppm, 32°C/8,000ppm) for two complete molts (approximately 30 days). Crab growth rate (length of inter-molt period), food consumption (% of crab mass), shell thickness (mm), carapace chemistry ( $[\text{Ca}^{2+}]$  and % $\text{CaCO}_3$ ), energy storage (MJ/kg dry tissue), and metabolic rate ( $\mu\text{mol/g/min}$ ) were quantified in individual crabs. In warmer water, crabs grew significantly faster, ate significantly more food, but did not change their energy storage patterns. Crabs in warm water had thinner shells with lower  $[\text{Ca}^{2+}]$  and lower % $\text{CaCO}_3$ . Conversely, crab growth was not significantly impacted by more acidic water, but energy storage in these crabs decreased in addition to a decrease in shell thickness. Increased temperature and acidity did not affect the metabolic rate of crabs due to a significant acclimation effect from being held at experimental conditions for approximately 30 days. Results of this study indicate that, while there are tradeoffs in the physiological response to juvenile blue crabs to climate change stressors, these animals may have the ability to acclimate to these changes given sufficient time.

## **HYBRIDIZATION BETWEEN *HALIOTIS RUFESCENS* X *HALIOTIS FULGENS* *GUADALUPENSIS*: PRELIMINARY RESULTS**

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Worldwide, the lack of selective breeding programs in abalone culture means that crossbreeding between related organisms generates high levels of inbreeding. Negative effects of inbreeding result in decreased fertilization, hatching, and survival rates, as well as increased deformities at larval stages. In contrast, it is well documented that some interspecific crosses between abalone showed *hybrid vigor* in commercial traits, such as growth, survival, as well as an increase in the immune response and thermal tolerance. The hybridization process is considered as a viable tool for genetic improvement in abalones. For a successful interspecific cross, there are two critical factors that affect fertilization and hatching success: the sperm concentrations (cell/mL) and the oocyte-age (min). In this work, two independent tests were carried out to generate information on these two factors to obtain viable offspring between the red abalone *H. rufescens* and the green abalone from Guadalupe Island *H. fulgens guadalupensis* (RG). During the first test, six sperm concentrations were used to determine the optimal concentration for a successful fertilization. The second test evaluated four oocyte-ages to maximize offspring. In both cases, the progeny were evaluated in terms of fertilization, hatching, and larval survival. Hybrid larvae were obtained and the optimal sperm concentration and oocyte-age were assessed. The next step will be to test both optimal factors at the same time to evaluate their synergistic effects and RG progeny performance under different temperature regimens to assess thermal tolerance.

## **INTERACTION OF POLYSTYRENE NANOPARTICLES WITH PACIFIC OYSTER (*CRASSOSTREA GIGAS*) GAMETES**

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Several million tons of plastics are produced yearly in the world and it was estimated that 4.8-12.7 million tons of plastic waste ended up in oceans in 2010. Nanoplastic debris in marine environment is expected to occur as a result of the breakdown of large plastic items and also from direct inputs of nanoparticles from urban and industrial processes. The Pacific oyster, *Crassostrea gigas*, accounts for a large part of shellfish aquaculture production worldwide. They have an external fecundation making this life stage very sensitive to pollutants, and presumably nanoplastics, present in the water column. The aim of this study was to investigate the toxicity of 100 nm carboxylated (PS-COOH) and amine (PS-NH<sub>2</sub>) polystyrene nanoplastics (NPs) on oyster gametes. Spermatozoa and oocytes were exposed to 0.0001, 0.001, 0.01 and 0.1 mg mL<sup>-1</sup> of PS-NH<sub>2</sub> or PS-COOH for 1, 3 and 5 hours.

Results evidenced higher toxic effects when gametes were exposed to PS-COOH and spermatozoa were more affected. Spermatozoa exposed to both types of NPs at the highest concentration 0.1 mg mL<sup>-1</sup> showed time dependent changes in cell morphology and an increase in mortality. At this concentration, PS-COOH induced the most severe effects on oyster gametes: sperm cells showed an increase in ROS production and in motility after 5h exposition, while oocytes evidenced morphological changes and an increase in mortality. PS-NH<sub>2</sub> did not show any effect on oocytes at the range of concentrations tested emphasizing the need of knowledge on fate and impact of surface charges of nanoplastics on live cells.

## HISTONE VARIANT EXPRESSION AND DNA METHYLATION MODIFICATIONS IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* DURING SHORT-TERM RESPONSES TO FLORIDA RED TIDES

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The increase in the frequency and toxicity of episodes of massive algal proliferations known as Harmful Algal Blooms (HABs) represents one of the most important threats to fisheries, aquaculture-based industries, and human populations in coastal areas. The southeastern U.S. is particularly affected by the so-called Florida Red Tides [blooms of the dinoflagellate *Karenia brevis* and associated brevetoxins (PbTx)], causing high mortality rates of marine organisms and annual losses in excess of \$40 million. Eastern oysters (*Crassostrea virginica*) are particularly affected by Florida Red Tides, experiencing DNA damage and apoptosis, alterations in larval development and mortality. It is known that responses to adverse environmental conditions such as those imposed by HABs are largely dependent on epigenetic regulatory mechanisms. The study of the epigenetic mechanisms mediating exposure-response relationships constitutes the basis for environmental epigenetic analyses, providing information about how different environmental factors influence phenotypic variation. Among marine invertebrates, bivalve molluscs constitute emerging models in environmental epigenetics; however, studies examining cause-effect relationships between environmental stressors, specific epigenetic mechanisms and subsequent responses in marine invertebrates are still lacking. The present work provides the first insight into the role of histone variants and DNA methylation during the exposure of eastern oysters to the brevetoxin-producing dinoflagellate *K. brevis*. Results support the role of histone H2A.X and its phosphorylation during oyster responses, along with a genome-wide reduction in DNA methylation that is concomitant with the increase in *K. brevis* concentration.

**CALCULATING TIME-DEPENDENT CONNECTIVITY OF NORTHERN QUAHOG  
(*MERCENARIA MERCENARIA*) AND EASTERN OYSTER (*CRASSOSTREA VIRGINICA*)  
LARVAE IN BARNEGAT BAY LITTLE EGG HARBOR ESTUARY**

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Understanding bivalve larval connectivity can improve management of bivalves by identifying areas best suited for sanctuaries and harvest. Modeling that combines particle attributes (e.g. buoyancy or behavior) coupled with physical processes of water movement has been used to estimate the transport and connectivity of marine species; however, until recently, these types of models were used to evaluate connectivity in only two dimensions. Recently, a retention clock matrix (RCM) concept was introduced to add the temporal dimension to the evaluation of connectivity between user-defined areas. Numerical particles simulating the behavior of *Mercenaria mercenaria* and *Crassostrea virginica* populations were released from points in Barnegat Bay Little Egg Harbor (BBLEH) and connectivity was estimated using a Lagrangian particle-tracking model (LTRANS), offline ROMS output, and RCM. Results show similar transport patterns for both species despite behavioral differences of particles. The RCM connectivity matrices provide management guides for the larval connectivity of both *C. virginica* and *M. mercenaria* populations in BBLEH for various pelagic larval durations that could vary annually or during decadal oscillations of climate.

## **CREATING A LIBRARY OF HISTORICAL LARVAL SHELLS FOR SPECIES-SPECIFIC IDENTIFICATION OF EXTANT AND FUTURE COLLECTIONS**

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A library of larval shells developed from historical samples that provide species-specific classification would allow for rapid identification of unknown larvae from a variety of sources. Numerous bivalve larvae were reared from known adult specimens by scientists around the world from 1977-1995. These bivalve larvae were stored in vials and preserved in 95% EtOH. The EtOH in many of these vials evaporated over the years. Molecular (PCR) techniques were used in an effort to sequence many of the dried-out specimens, while optical (polarized light and a full wave compensation plate) techniques were used to image each of the species patterns. Molecular techniques successfully identified 22% (10/46) of the larval bivalve species, with matches in GenBank. Representative stages of 72% (39/54) of the larval bivalve species in the collection were successfully imaged. Similarities across birefringence patterns were observed at the ordinal and familial taxonomic level. These results show the sequences for 10 bivalve species and illustrate that optical techniques are efficient for imaging dried-out shells for classification purposes.

## **COUPLED REMOTE SENSING AND *IN SITU* MONITORING TO ENHANCE SHELLFISH PRODUCTION AND IDENTIFY NEW HABITATS IN MAINE**

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Maine has more than 3,000 miles of coastline and there is great interest in utilizing this region for aquaculture. Because this area is so vast, many of the bays and estuaries throughout Maine have not been evaluated for their suitability to support aquaculture. The Sustainable Ecological Aquaculture Network program of the University of Maine was established in part to evaluate and identify shellfish habitat 'hot spots' that could later be developed by the aquaculture industry. Using a combination of monitoring tools including *in situ* growth studies, land/ocean biogeochemical observation (LOBO) buoys, satellite imagery, and shellfish growth models data are now emerging that provide detailed information on the available area for shellfish cultivation in Maine. In addition to novel sites, LOBOs were deployed in the Damariscotta River, an estuary that is currently home to >70% of the shellfish aquaculture in Maine. Deployment of LOBOs within the Damariscotta has proven to be valuable to aquaculture researchers as well as industry members (e.g. hatchery managers, field managers, etc.) to help understand estuarine biogeochemical trends and processes and to improve production. These studies illustrate the value of both remote sensing and real-time environmental monitoring to improve culture practices and understand constraints of aquaculture in Maine.

## **THE EFFECTS OF ALLELOCHEMICALS FROM BLOOM-FORMING SEAWEEDS (*ULVA COMPRESSA* AND *ULVA RIGIDA*) ON SHELLFISH LARVAE**

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Seaweed blooms have been increasing in frequency and severity worldwide due to anthropogenic activities. These blooms can disrupt recreational activities, interfere with commercial fisheries, and deplete oxygen during decomposition. Narragansett Bay has experienced seaweed blooms dominated by blade-forming *Ulva* for over a century. Evidence from other systems has suggested that *Ulva* can negatively impact other organisms through allelopathy (i.e. the production and release of chemical compounds that suppress growth, development, or reproduction of other organisms). The objective of this study was to determine whether the dominant bloom-forming species of *Ulva* in Narragansett Bay affect survival or behavior of oyster larvae, through a series of laboratory challenge experiments. Oyster larvae (2-11 days old) were exposed to crude extract from cultured *Ulva compressa* or *U. rigida* (5 g/L) that was either nutrient replete or deplete, and their survival was determined periodically over one week. It was found that there was a significant negative effect of *Ulva* extract on oyster survival, which depended on both the *Ulva* species and the nutrient condition. Oyster survival in larvae exposed to nutrient replete *Ulva compressa* extract dramatically decreased over time, with less than 25% survival (% of control) after one week. Larval survival was not significantly affected by nutrient deplete extract (without *Ulva*), although larvae exposed to this extract had a slower swimming speed than larvae in natural seawater (control). Results suggest that *Ulva* blooms may cause oyster larval mortality under eutrophic conditions, which are common in coastal ecosystems.



## **PLANNING NATIVE OYSTER RESTORATION THROUGH A HABITAT SUITABILITY ANALYSIS**

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Native oysters were historically abundant throughout the California coast and provided many natural ecosystem services such as shoreline stabilization, water quality improvement, and habitat for fish and invertebrates. Due to overharvesting and habitat modification in the early 1900s, native oyster populations were severely depleted and have since then struggled to recover for a variety of reasons. As a result, only a fraction of their historical numbers exist today and society is generally unaware of their historical presence, thus resulting in a shifting baseline syndrome. For this reason, native oysters are typically not included restoration plans in California.

In order to motivate the inclusion of native oysters in additional restoration plans in Southern California, we aim to create an interactive story map depicting: 1) historic and current native oyster distribution; 2) recommendations for potential restoration sites; 3) areas not suited for restoration. These recommendations will be developed by analyzing data from natural history museums, CSU Fullerton, public access water quality data, and available site information. Sites will include bays, estuaries, and lagoons in Southern California. Currently in California, native oyster restoration projects have been implemented in San Francisco Bay, Elkhorn Slough, Alamitos Bay, Newport Bay, and San Diego Bay. These projects represent the first steps in restoring this native species to the California coastline. It is hoped that through this project there will motivate and guide increased restoration to enhance the abundance of the Olympia oyster in Southern California.

## **NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) TO DETERMINE COMPOSITIONAL ANALYSIS IN OYSTERS: PRESENT AND FUTURE**

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NIRS technology provides a fast, safe, and eco-friendly alternative to traditional analytical methods which may necessitate use of chemicals that are toxic to humans and the environment. The principle of NIRS is based on absorption of the energy from the infrared spectrum by the target samples. The reflected light provides a spectral “fingerprint” of the composition of a sample and can be used to create quantitative models. Last year, Aquaculture Genetics and Breeding Technology Center (ABC) developed two models suited for compositional analysis of moisture and glycogen in the eastern oyster, *Crassostrea virginica*. These models were developed using homogenized tissues and showed a high correlation between the predicted and measured data ( $R^2 = 0.97$  for moisture and  $R^2 = 0.94$  for glycogen).

In order to improve the accuracy and reproducibility of our NIRS glycogen model, we developed a new glycogen model using 381 individually freeze-dried oysters. Special attention was given to assessing a wide range of glycogen concentrations, thereby oysters of different size, age class, ploidy, sampling time, and condition were assessed. This data set included the 138 oysters previously used and 243 new samples. The resulting calibration and validation for freeze-dried tissue had a higher correlation ( $R^2 \geq 0.96$ ) than for homogenized tissue. Long-term storage of freeze-dried tissue is deemed more stable than homogenized samples.

One of the other advantages of NIRS technology is the potential for multi-component analysis. In order to increase the utility of NIRS, additional models are currently in the building stage, all based on freeze-dried material.

## **FACULTATIVE PERIOD OF SYMBIONT ACQUISITION AND DEVELOPMENTAL EFFECTS OF SYMBIOSIS IN THE GIANT CLAM *HIPPOPUS HIPPOPUS***

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Giant clams have a specialized tubular system that houses their obligate symbionts, *Symbiodinium*. Since symbionts affect development in other organisms, they may be involved in giant clam tubular system development. To determine whether symbionts influence development in *Hippopus hippopus* giant clams, growth patterns and cell proliferation were compared in two groups of juveniles either inoculated with *Symbiodinium* or not. *Symbiodinium* uptake occurred from day 8 to 26, with on average ~5% of individuals infected per day. The inoculated culture grew significantly faster ( $2.91 \pm 0.37$   $\mu\text{m}/\text{day}$ ) than the control (uninoculated) culture ( $1.03 \pm 0.41$   $\mu\text{m}/\text{day}$ ); however, both treatments grew, and daily shell length measurements did not significantly differ until day 22. Consistent with this, at day 13, proliferating clam cells were randomly distributed and not correlated with the number of symbionts. At day 26, proliferating clam cells were more likely to occur within ~25  $\mu\text{m}$  of a symbiont and were correlated with the number of symbionts in an individual. These results indicate that *H. hippopus* has a facultative period over which juveniles can acquire *Symbiodinium* but still grow without them. Symbionts have a delayed influence on host growth and cell proliferation, only detectable in the fourth week. Increased cell division in the immediate vicinity of *Symbiodinium* implies that symbionts may be influencing local host cell proliferation, possibly of the tubules. These findings suggest that *Symbiodinium* acquisition is facultative in *H. hippopus* until at least week 4, with not all individuals needing symbionts by day 10 as is commonly recommended.

## SUPERIOR TRIPLOID EASTERN OYSTERS PRODUCED BY SELECTING TETRAPLOIDS

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Because of their sterility, superior growth, and improved summer meat quality, triploid oysters have become an important part of oyster aquaculture, accounting for 30-50% of productions in the US and France. Triploids are produced by crossing diploids and tetraploids, and the sterility of triploids, while beneficial for aquaculture, poses a challenge to further improvements. Tetraploid genomes are unstable, and it is unclear whether superior characteristics in tetraploids can be faithfully transmitted to their triploid progeny.

To determine if triploid eastern oysters (*Crassostrea virginica*) can be improved by selecting tetraploids, two classes of triploids were produced by crossing the same diploid females with the largest (L) or smallest (S) tetraploid males from the same cohort. Two experiments were conducted with two different sets of parents. The two classes of triploids and control diploids from each experiment were deployed in triplicate for field evaluation.

At 6-month post-fertilization and in both experiments, L-triploids were significantly ( $p < 0.001$ ) larger than S-triploids, and both triploids were significantly ( $p < 0.001$ ) larger than diploids. In Experiment 1 where the largest and smallest 10% tetraploids were used, L-triploids were 79% heavier than S-triploids, which in turn were 50% heavier than diploids. In Experiment 2 where the largest and smallest 20% tetraploids were used, L-triploids were 21% heavier than S-triploids, and the latter were 54% heavier than diploids. These results show that despite genome instability tetraploids have a large influence on the performance of their triploid progeny, and superior triploids can be produced by selecting and using the best-performing tetraploids.

## **HATCHERY-INDUCED GENETIC CHANGES DURING PRODUCTION OF RESTORATION SEED: HOW EXTENSIVE ARE THEY AND DO THEY MATTER?**

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Growing oyster seed to market size is an aquaculture goal that benefits from careful selective breeding. For oyster restoration, however, ‘best practices’ maintain that leveraging wild recruitment is preferable to planting hatchery-produced oysters, and when necessary, hatchery production should use wild broodstock and minimize genetic bottlenecks. Unfortunately, few data exist to document the magnitude of genetic bottlenecks typically accompanying single-generation hatchery production of eastern oysters, and even fewer data are available on the causal link between genetic diversity and fitness-related performance in restoration contexts. The theoretical justification for these best practices is strong, but theory will not drive the spawning practices in resource-limited production hatcheries or cash-strapped restoration projects. To the contrary, one of the only demonstrations of spat recruitment from a “restoration” planting of eastern oysters came from planting disease-resistant seed from a selected strain carried out in our laboratory. Available data on genetic bottlenecks and domestication selection of eastern oysters during single-generation hatchery production will be reviewed and microsatellite and genomic data bearing on hatchery-induced genetic changes will be provided.

## GENE EXPRESSION IN MANILA CLAM *RUDITAPES PHILIPPINARUM* HAEMOCYTES AND IN THE PARASITE *PERKINSUS OLSENI* - IN VIVO AND IN VITRO CHALLENGES

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The infection with the protozoan *Perkinsus olseni* is one of the most serious diseases of Manila clam *Ruditapes philippinarum*, one of the major species in global bivalve production. A study was conducted to understand the molecular mechanisms underlying *R. philippinarum*-*P. olseni* interaction. One hundred-bp PE Illumina RNA-Seq reads were used to construct a *de novo* transcriptome of *R. philippinarum* haemocytes after three ways of challenge, i) field exposure (FI), ii) laboratory *in vivo* (IVV) challenging clams with *P. olseni* zoospores, and iii) *in vitro* (IVT) challenging haemocytes with trophozoites, zoospores, and parasite extra-cellular products. Additionally, a *de novo* transcriptome of *P. olseni* trophozoites challenged with Manila clam plasma was assembled. The haemocyte transcriptome consisted of 33,079 transcripts of which 7,300 were annotated, while the *P. olseni* transcriptome included 47,590 unique transcripts of which 23,505 were annotated. Two oligo-microarrays (host and parasite) were designed and used to evaluate gene expression in the above mentioned experimental conditions. Clams showed higher number of up-regulated genes in FI (many of them linked to anti-oxidation process and immunity), while higher number of down-regulated genes were identified in IVV and IVT (many of them related to immunity and proteolysis). The designed *P. olseni* trophozoite oligo-microarray revealed up-regulation of vesicle-mediated transport genes but down-regulation of genes linked to proteolysis. Particularly, the regulation of genes encoding glutathione peroxidase, glutathione-S-transferases and cathepsins in haemocytes and trophozoites indicated their prominent roles in this host-parasite interaction. This study will contribute to design new strategies for controlling perkinsosis in Manila clam.

## **PROTOCOL DEVELOPMENT FOR SPERM CRYOPRESERVATION IN THE NORTHERN QUAHOG (=HARD CLAM), *MERCENARIA MERCENARIA***

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Cryopreservation is a technology to preserve structurally intact living cells or tissues by using low temperatures. Over past decades, cryopreservation has addressed research-scale protocol development in many fish and shellfish species; however, no research has been reported on the northern quahog *Mercenaria mercenaria*, a major shellfish aquaculture species in the U.S. The goal of this study was to establish a basic protocol for sperm cryopreservation through systematic evaluation of factors in the process of cryopreservation. The objectives were to evaluate the: 1) effects of centrifugation for sperm condensation at speeds of 1000, 2000, 3000, and 4000 rpm on sperm viability; 2) toxicity of cryoprotectants including dimethyl sulfoxide (DMSO), propylene glycol (PG), ethylene glycol (EG), glycerol at concentrations of 5, 10, and 15% on fresh sperm viability; 3) effects of cooling rates (10, 20, 30, 40°C/min from 5°C to -80°C) with selected cryoprotectants on post-thaw sperm viability; 4) effects of thawing temperatures (30, 40, 50°C) on post-thaw sperm viability, and 5) reliability of cryopreservation through assessing viability and fertility of post-thaw sperm from individual males ( $n \geq 20$ ). Centrifugation at 2000 rpm for 5 min could allow sperm cells concentrated without affecting quality, thus sperm concentration can be standardized  $4 \times 10^8$  cells/mL for protocol development. DMSO or PG (5 and 10%) showed less toxicity on fresh sperm with exposure time of 15 min. Currently, data collection on cooling rate, thawing rate, and cryopreservation reliability is ongoing. This study will be useful in hard clam aquaculture for preserving valuable germplasm and hybrid breeding.

## **HEMOCYTIC NEOPLASIA IN NORTHERN QUAHOGS (=HARD CLAMS), *MERCENARIA MERCENARIA*: TRANSMISSIBILITY OF NEOPLASTIC CELLS**

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In the summer of 2009, numerous 2-3 year old adult northern quahogs (=hard clams) (*Mercenaria mercenaria*) in Wellfleet, MA began to surface and die. Pathological evaluation identified a new neoplastic disease, termed hemocytic neoplasia (HN). Examination of a sample of hemolymph (blood) from the pericardial sac of affected animals showed few to abundant large, unusual cells. These tumorous cells caused significant obstruction of the vascular system and a loss of normal hemocytes. There was no histological or epidemiological evidence that suggested the causative agent was bacterial, fungal, or toxic in origin leading to the hypothesis that it is caused by a viral agent. Preceding studies in soft clams and cockles have successfully been able to transmit a similar neoplastic disease present in each species into naïve animals of the same species by injecting them with abnormal circulating cells from the affected animals (Oprandy et al, 1981 and Taraska and Böttger, 2013). This study attempted to repeat the transmission experiment in *M. mercenaria*; however, while a change in cell characteristics was observed, the disease itself was not successfully transmitted. Knowledge of transmissibility of the disease in northern quahogs will be vital to its containment which could significantly impact the fishery and aquaculture industries.



## **UPDATE FROM THE KEN CHEW CENTER FOR SHELLFISH RESEARCH AND RESTORATION**

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This presentation is an update on the Kenneth K. Chew Center for Shellfish Research and Restoration (K. Chew Center). The K. Chew Center, opened in 2014, is located at the National Oceanic and Atmospheric Administration Manchester Research Station, and operated wholly by the Puget Sound Restoration Fund (PSRF). PSRF, established in 1997, is a non-profit organization with the mission of restoring critical, keystone marine species and habitat in Washington State.

The K. Chew Center is a hatchery-style facility dedicated to the research and restoration of native marine species. At its opening, the main activity was the production of restoration-grade Olympia oysters (*Ostrea lurida*), but the K. Chew Center has since become home to research and restoration projects on species such as the pinto abalone (*Haliotis kamtschatkana*), California sea cucumber (*Parastichopus californicus*), purple-hinged rock scallop (*Crassadoma gigantea*), and kelp (various species). Not only has the K. Chew Center diversified in the number of marine species worked with since doors first opened, but it has also expanded in aquaculture capabilities such as improved water filtration, and microalgae production.

This presentation will provide an overview of the Chew Center's restoration and research goals, the production and research that has taken place there, and its ever-improving aquaculture capabilities.

## **ASSESSMENT OF TROPHIC RESOURCES OF *CRASSOSTREA VIRGINICA* IN THE TEN THOUSAND ISLANDS MANGROVE (FLORIDA, USA) USING STABLE ISOTOPE AND LIPID BIOMARKERS**

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A study of stable isotope and fatty acid compositions was completed on the eastern oyster, *Crassostrea virginica* from the Ten Thousand Islands (Florida, USA). Freshwater inflow from watershed management is affecting this region, greatly modifying the salinity gradients. Oysters and POM (Particulate Organic Matter) and BMA (Benthic microalgae) were sampled during two contrasted period: wet season (August 2012) and dry season (February 2013). Sampling was done in three bays at 3 stations from upstream to downstream. Stable isotopes showed that oysters fed more on POM than on BMA. Principal component analyses on fatty acids for POM reveal that during wet season POM was enriched in 16:1n-7, 20:5n-3, 16:2n-4 (diatoms biomarkers) and 18:3n-3 (green microalgae biomarker). But during the dry season, POM was dominated by 18:4n-3, 18:5n-3 and 22:6n-3 markers of dinoflagellates. In the oyster digestive glands, a similar pattern was found confirming that dinoflagellates constitute a part of oyster diet during dry season and that diatoms and green microalgae mainly contributed to oyster diet during the wet season. This study showed that the oyster diet is mainly influenced by seasonal fluctuations in salinity, potentially due to seasonal changes in freshwater inflows.

## **THE ROLE OF LIFE CYCLE ASSESSMENT (LCA) IN EVALUATING ENVIRONMENTAL COSTS OF ANIMAL SOURCE FOODS**

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A meta-analysis of 136 assessments of animal source foods (ASF) was conducted that measured four environmental impact metrics (energy use, greenhouse gas emissions, and the release of nutrients and acidifying compounds into ecosystems). The studies reviewed used life cycle assessment (LCA) to quantifiably measure these impacts at given stages of production. There are notable differences between production methods; the lowest impacts were seen in capture fisheries for small pelagics and aquaculture for mollusks. The highest impact methods were industrial beef production and catfish aquaculture. Regulatory restrictions on ASF production methods, as well as consumer guidance, should consider their relative environmental impact, and currently there appears to be little relationship between regulatory restrictions and impact in most developed countries.

The goal of this presentation will be to 1) highlight shellfish production, in particular bivalve aquaculture, as a viable source of animal protein that is also low impact to the environment; 2) demonstrate the usefulness of LCA as a valuable tool for measuring sustainability and efficiency, and; 3) discuss the methodology of applying LCA to oysters farms on the west coast.

# **TEMPERATURE EFFECTS ON BIOFOULING: IMPLICATIONS FOR THE AQUACULTURE INDUSTRY**

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The global demand for protein and food security underlies a rapid increase in the production of fisheries resources. Aquaculture is, in fact, one of the fastest growing food producing sectors on the planet and now provides the majority of fish and shellfish for human consumption. Common place are open ocean and coastal culture operations that employ suspension technologies that facilitate faster growth of target species; however, many of the same environmental conditions that facilitate increased production of cultured species can and do lead to increased fouling by non-target organisms. Removal of fouling organisms represents a substantial cost to the aquaculture industry and, at present, accounts conservatively for 5-10% of total operational costs. Increases in sea surface water temperatures associated with global climate change are likely to increase rates of biofouling and associated maintenance. The effects of temperature on the accumulation of fouling organisms as a logical first step toward better understanding the potential economic consequences of climate change and temperature related effects on the aquaculture industry were assessed.

## ISOLATION AND UTILIZATION OF PROBIOTICS TO MANAGE EPIZOOTIC SHELL DISEASE IN AMERICAN LOBSTERS, *HOMARUS AMERICANUS*

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Epizootic shell disease (ESD) in the American lobster, *Homarus americanus*, has caused major challenges in the southern New England lobster industry. The lobster fishery generates over \$600 million per year in the U.S., yet there are no tools for managing ESD in wild lobster populations. The goal of this study is to identify bacterial probiotics that could be used as a tool to reduce prevalence of ESD in wild lobster populations. The objectives of this project are 1) to isolate and characterize bacteria from lobsters that act as probiotics against ESD-associated bacteria, and 2) test the effect of probiotic treatment on the progression of ESD in live lobsters. So far, 24 candidate bacterial isolates have been identified from lobsters in Narragansett Bay as having probiotic characteristics against ESD-associated bacteria, *Thalassobius* sp., *Aquimarina homaria*, or fish pathogen *Vibrio anguillarum*. Ninety-six lobster postlarvae were treated with six of these candidate probiotic strains, in addition to one probiotic isolated from the eastern oyster, *Crassostrea virginica* (*Phaeobacter inhibens* S4), and challenged the postlarvae with *Thalassobius* sp. After 3 weeks, all postlarvae lobsters experienced some degree of shell disease except those exposed to two different candidate *Pseudoalteromonas* sp. strains and the negative control. Increased lesions were observed with one isolated candidate, indicating a potential pathogen. These results indicate that postlarvae challenges could be used to screen for potential probiotics that could delay the progression of ESD. Utilizing probiotics to combat ESD could have significant applications fisheries management, maintaining economic viability of a cherished food source.

## **MODELING MARINE BIVALVE POPULATIONS: APPROACHES AND CHALLENGES**

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Marine bivalves acquire infectious diseases either through direct contact or by filtering pathogens during feeding. Once acquired, the progression of infection in the host is mediated by environmental conditions, the host condition, host defense system, and pathogen proliferation. Consequently, models developed for disease effects on host population dynamics must represent this range of individual responses to infection and relate these to population responses. Also, pathogen transmission between hosts is an important challenge for understanding how epizootics wax and wane and how disease influences host population dynamics. The details of host population processes and pathogen transmission dynamics are blended in models that evaluate the effects of natural selection and/or genetic modification in developing disease resistance in the host population. Application of this type of model to marine diseases is only now beginning and represents a promising approach that may provide a mechanistic basis for managing marine diseases and their host populations. Marine diseases pose additional challenges for the management of marine resources and mathematical models provide one approach for quantifying population and environmental effects and addressing the competing goals of managing the diseased population versus managing the disease.

# **FACTORS AFFECTING DISTRIBUTION OF THE ATLANTIC SURFLAM (*SPISULA SOLIDISSIMA*), A CONTINENTAL SHELF BIOMASS DOMINANT, DURING A PERIOD OF CLIMATE CHANGE**

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The Atlantic surfclam (*Spisula solidissima*) is a dominant member of the biological community of the Middle Atlantic Bight continental shelf and also a commercially harvested species. Climate warming is affecting the biology and distribution of this species, which provides an opportunity to investigate the processes and conditions that are restructuring this fishery and the implications for ecological and socio-economic systems. The Management Strategy Evaluation (MSE), which is a system of linked models, developed for the surfclam fishery is an attempt to provide a comprehensive mechanistic description of the surfclam's response to climate change and understand the cascade of effects initiated by changes in oceanographic conditions that ultimately appear as social and economic effects, which in turn inform development of management policies for the resource. This study provides an overview of the components of the surfclam MSE, relevant results, and implications for management and policy. The lessons learned from the surfclam MSE provide a basis for applying similar approaches to other ecologically important species that are also commercially exploitable resources.

## **PARENTAGE ANALYSIS REVEALS LOSS OF GENETIC DIVERSITY IN HATCHERY PRODUCED COHORTS OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA***

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Large-scale hatchery supplementation of marine fisheries increases abundances but poses risks to population genetic diversity and resilience. Eastern oyster (*Crassostrea virginica*) populations in Chesapeake Bay have declined over 99% since peak harvests in the 1880s and in 2011, a large-scale hatchery supplementation program was initiated in the Choptank River and sub-tributaries. Although this restoration program has increased abundances, the genetic effects on the long-term viability and resilience of oyster populations remain unknown. To examine the effects of hatchery propagation on genetic diversity, parentage analyses were performed on six hatchery-produced cohorts (mass spawns) of spat using up to nine microsatellite markers, to estimate changes in diversity, variance in parental contribution, and the number of effective breeders ( $N_b$ ). It was found that the  $N_b$  was significantly reduced from the total number that spawned ( $T_s$ ) and offspring showed significantly reduced allelic richness and observed heterozygosity compared to adults in all cohorts. The tendency for individuals to dominate spawning events decreased with the number of individuals that spawned for both sexes, while the relative reduction in  $N_b$  (compared to  $T_s$ ) decreased as sex ratios approached one. Using these relationships, we attempt to estimate the cumulative  $N_b$  in one sub-tributary over five years of stocking. Overall, hatchery production of *C. virginica* reduces genetic variability relative to parents, but yearly rotation of hundreds of broodstock may mitigate this effect. Future oyster restoration efforts in the Choptank should focus on maximizing diversity during hatchery propagation, possibly through increasing broodstock numbers and attempting to maintain a balanced sex ratio.



## THE EFFECTS OF OCEAN ACIDIFICATION ON LARVAL OYSTER SWIMMING RESPONSES TO A SETTLEMENT CUE

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Increasing concentrations of atmospheric CO<sub>2</sub> are driving changes in the carbonate chemistry conditions of the world's oceans by lowering pH, a phenomenon known as ocean acidification. This study examined how low pH influences the swimming behavior of larval eastern oysters, *Crassostrea virginica* in response to a chemical settlement cue. Competent-to-settle larvae were exposed to one of four seawater treatments: filtered seawater (no settlement cue) at an ambient pH (~8.1), filtered seawater (no settlement cue) at low pH (~7.3), oyster bathwater (a chemical settlement cue) at ambient pH (~8.1), and oyster bathwater (a chemical settlement cue) at low pH (~7.3), and recorded and analyzed videos of larval motion. Low pH did not influence the proportion of larvae that come back up off the bottom—i.e. low pH did not impair the ability of larval oysters to interpret and respond to a chemical settlement cue; however, low pH did have an effect on the average track length of larvae that come back up off the surface; low pH induced a higher level of activity and more exploratory swimming behavior of larvae in the water column.

## DOES TOLERANCE EVOLUTION UNDERLIE AN OYSTER REVIVAL IN CHESAPEAKE BAY?

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The decimation of *Crassostrea virginica* in Chesapeake Bay due to intensified *Perkinsus marinus* and *Haplosporidium nelsoni* parasitism in the 1980's left little reason to expect that populations would recover. Rather than developing resistance against diseases to which they had long been exposed, the oysters were in historically poor condition. Today, *C. virginica* populations are expanding, a feat often credited to widespread restoration activity. While oyster abundance is increasing in restored populations, it is matched by an increase in unrestored areas— an indication that the recovery may have some basis in oyster biology. Resistance to *H. nelsoni* has been documented, but *P. marinus* abundance remains historically high, suggesting that resistance to *P. marinus* does not explain recent oyster success. Furthermore, anecdotal observations of increased fecundity in contemporary oysters raised the alternative possibility that tolerance evolution may be occurring. A retrospective analysis of histological samples of infected oysters from 1988-2014 was begun to investigate this possibility, with data collection on oocyte densities and diameters as well as gonadal area fractions in ripe oysters from summer samples. The latter two analyses are ongoing, but results on oocyte densities revealed a significant increase in the density of oocytes per field (at 100X), from a mean of 138.5 oocytes from 1988-2002 to 231.2 from 2003-2014 (Welch's t-test,  $p < 2.2e-16$ ,  $n=660$ ). Improving fecundity in moderately infected oysters suggests diminished physiological consequences of *P. marinus* infections, which indeed would conform to the definition of a tolerance adaptation.

## **BEYOND THE CLUMP: IDENTIFYING OPTIMAL SHORELINES FOR RIBBED MUSSEL (*GEUKENSIA DEMISSA*) RECRUITMENT**

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The ribbed mussel (*Geukensia demissa*) is an integral member of the salt marsh community along the east coast of the United States. Ribbed mussels improve salt marsh resilience by reducing erosion, enhancing growth of *Spartina alterniflora*, and enhancing habitat for other marsh fauna. Like most species, the ribbed mussel is not homogeneously distributed either within a marsh or among marshes. Despite their importance to the marsh, no studies to date have attempted to identify factors influencing their distribution on both a local and regional scale. To accomplish this, 30 marshes were surveyed in Chesapeake Bay along environmental exposure clines for ribbed mussels. A combination of local and regional factors, notably stem density, marsh type (platform or sloped), and energy regime, was found to explain most of the variation (>70%) in mussel density within our study area. These results allow for the identification of segments of shoreline that are optimally suited for ribbed mussels, and provide guidance for designing marsh restoration and living shoreline projects to facilitate recruitment of this species.

## IMMUNOHISTOFLUORESCENCE LOCALIZATION OF BIOGENIC AMINE RECEPTORS IN GANGLIA AND TISSUES OF THE BIVALVE MOLLUSC, *MYTILUS EDULIS*

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Biogenic amines are neurotransmitters in sensory and motor systems in invertebrates. In bivalves, particularly *Mytilus edulis*, physiological roles of serotonin and dopamine have been well studied, especially in gill and heart. Recently, in the related bivalve *Crassostrea virginica*, other biogenic amines, including GABA and histamine along with their receptors, were demonstrated to be present. These amines are being shown to have physiological roles. It was hypothesized that *M. edulis* has receptors for the biogenic amines, serotonin, dopamine, histamine and GABA. Immunohistofluorescence techniques were used to test this. Primary antibodies to serotonin, dopamine, histamine and GABA receptors; and secondary FITC-labeled antibodies were used to visualize these biogenic amine receptors in cerebral ganglia, visceral ganglia, gill and mantle. Tissues were dissected, snap frozen, cryostat sectioned, fixed with EDAC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride), treated with blockers, and incubated with primary and secondary antibodies. Results show serotonin 2A-like, dopamine D2-like, histamine H2-like and GABA R1-6-like receptors present in cerebral ganglia, visceral ganglia, gill and the sensory tentacles of the mantle rim. This comparative study provides new knowledge of the neurobiology of *M. edulis* by confirming the presence of these biogenic amine receptors in the animal nervous system and innervated organs, in a comparable manner to what was reported in *C. virginica*. It also provides a foundation to further study the physiological roles for histamine and GABA in *M. edulis*. This work was supported in part by grant 2R25GM06003 of the Bridge Program of NIGMS, grant 690340047 of PSC-CUNY and a Carnegie Foundation award.

## COMPARATIVE TOXICITY OF COREXIT® 9500, OIL, AND A COREXIT®/OIL MIXTURE IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

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Following the explosion of the British Petroleum Deepwater Horizon exploration platform in 2010 and subsequent release of 4.9 million barrels of crude oil into the Gulf of Mexico, an unprecedented 1.84 million gallons of chemical dispersant, predominantly Corexit® 9500, was used to help control the spill. Given their particle feeding behavior, abundance in coastal zones, and wide distribution, bivalves are at significant risk for exposure to contaminants such as oil and oil dispersant. Furthermore, oysters are an economically and ecologically important species in the Gulf of Mexico. Eastern oysters (*Crassostrea virginica*) were exposed to Corexit® 9500, crude oil (high-energy water accommodated fraction; HEWAF), and a Corexit®/oil mixture (chemically-enhanced water accommodated fraction; CEWAF) to evaluate potential toxic effects on innate immune functions (phagocytosis and respiratory burst) and clearance rates. Respiratory burst appeared more sensitive than phagocytosis to the effects of Corexit® alone, and had a significant negative correlation with Corexit® water and tissue concentrations. Phagocytosis appeared more sensitive than respiratory burst to the effects of HEWAF and CEWAF. Oyster clearance rates were more sensitive to CEWAF than Corexit or HEWAF alone. Clearance rates had a significant negative correlation with Corexit® concentration, but not with HEWAF or CEWAF. Our experiments have shown that subtle, sub-lethal effects on water filtration rates and innate immune functions occur following acute *in vivo* exposure to Corexit®, HEWAF, and CEWAF, with different sensitivities. Data from this study can be used for more accurate risk assessment concerning the impact of oil and Corexit® on the health of oysters.

## COMPARATIVE STUDY OF THE RESPONSE TO LIGHT ON THE SENSORY MOTOR INTEGRATION OF GILL LATERAL CELL CILIA IN BIVALVE MOLLUSCS

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Gill lateral cells (GLC) of bivalves *Crassostrea virginica* and *Mytilus edulis* are innervated by serotonin/dopamine nerves. Motor aspects of GLC innervation are well studied, but not the sensory. It was found that sensory cues, including light, algae and crab extract initiated sensory-motor integration responses between mantle rim sensory tentacles (MRST) and GLC cilia. Histamine, a neurotransmitter in photoreceptors, also initiated a response. Both bivalves have cerebral ocelli (eyespots) that have rarely been studied other than histological/anatomical reports. It was hypothesized that ocelli are light sensitive and involved in histamine mediated sensory-motor integration effecting GLC. Animals with intact innervation to access actions of light and a histamine H<sub>2</sub> receptor antagonist on ocelli were studied. Stimulating ocelli with an eleven lumen, ¼ inch diameter spotlight decreased cilia beating in *C. virginica*, but increased beating in *M. edulis*. Excising ocelli prevented the responses. Famotidine, a histamine H<sub>2</sub> receptor antagonist, blocked responses to light. Effects of light on MRST in *M. edulis* were also studied. Light caused cilio-excitation, opposite to cilio-inhibitory in *C. virginica*. Since *C. virginica* is nocturnal, while *M. edulis* is not, opposite responses are expected. The study demonstrates sensory-motor integration between MRST and motor responses of GLC, and adds new knowledge of the role of ocelli in a class of animals where it has not been well studied. It supports the hypothesis histamine is the neurotransmitter for photoreceptors in ocelli and mantle rim in these bivalves. This work was supported by 690340047 of PSC-CUNY, 2R25GM06003 of the Bridge Program of NIGMS and the Carnegie Foundation.

## **GENOMIC VARIATION AND LOCAL ADAPTATION BETWEEN CANADIAN AND LOUISIANAN POPULATIONS OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*)**

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Both genetic variation and physiological plasticity facilitate the broad environmental tolerance and expansive geographic range of eastern oysters (*Crassostrea virginica*). Changes in temperature and salinity independently and synergistically influence the physiology of marine organisms by affecting metabolic rates, respiration, energy acquisition, and growth rates. Changes in gene expression are the most rapid and versatile reaction that an organism has when it experiences stress, so this research aims to find a unique genetic biomarker of combined salinity and temperature stress in *C. virginica* that can be used to easily identify variation in tolerance between populations. Transcriptomes of individual oysters from New Brunswick and Louisiana that were exposed in the laboratory to fully crossed temperature (20°C and 30°C) and salinity (15 and 25) treatments will be sequenced, assembled, and compared. The transcriptomes will be sequenced using RNA-seq methodology and *de novo* assembled using the Trinity platform to compare variation in gene expression. After several potential biomarkers of stress have been identified samples will be collected from the field to assay Louisiana and New Brunswick populations during periods of low salinity and high temperature stress in order to validate the hypothetical biomarkers via qPCR. This approach will allow identification of transcriptional differences that could be used to quickly assess a wild population stress levels, increase the predictability and production of commercial oyster industries, and allow for more accurate predictions of how climate change will differentially impact geographically separate populations of eastern oysters.

**DETERMINING ECOLOGICAL BASELINES FOR THE RIBBED MUSSEL (*GEUKENSIA DEMISSA*) AND THE POTENTIAL HARVESTING IMPACTS OF ITS EMERGING COMMERCIAL FISHERY IN SOUTH CAROLINA, U.S.A.**

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Commercial landings of the ribbed mussel, *Geukensia demissa*, have increased approximately twenty-fold in South Carolina over the past 5 years, yet little is known about the sustainability and ecological impacts of this harvest. The ecological importance of *G. demissa* within South Carolina estuaries centers on its role as a secondary foundation species that promotes ecosystem functioning through its interactions with the dominant salt marsh vegetation, *Spartina alterniflora*. Current research objectives in support of improved management for this species are: 1) to characterize *G. demissa* habitat (elevation and salinity) and demography (density and size-frequency) in the high marsh; 2) to gain a better understanding of the role that *G. demissa* plays in facilitating the transient nekton community; and 3) to investigate how harvesting practices affect juvenile recruitment of *G. demissa* and *S. alterniflora* productivity. The demography of *G. demissa* occurring in harvested and unharvested areas will be discussed. Furthermore, data comparing nektonic community assemblages associated with *G. demissa* aggregations and control plots (*S. alterniflora* plots lacking *G. demissa*), gathered through the development of a novel, small-scale, drop-net sampling technique, will be presented.



## **A MODEL TO SIMULATE THE EFFECTS OF HYDRODYNAMICS AND PARTICLE REMOVAL ACROSS A RESTORED OYSTER REEF**

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Oyster reefs create complex habitats that allow for a variety of biogeochemical processes. Few models have been developed that describe these complex transformations at the spatial and temporal scales that they occur. For example, elevated rates of denitrification have been recently observed on restored oyster reefs, and have been attributed to the reef community itself, as opposed to the underlying sediment. Here we present a three-dimensional modelling tool of nitrogen dynamics that incorporates the complex hydrodynamic and ecological processes specific to restored oyster reefs. This modeling tool is aimed at enabling managers to quantify the ecosystem services associated with oyster reefs of varying size, shape, and age. The model is comprised of three major components: an oyster filtration model, a biodeposition model, and a sediment nutrient flux model. The filtration model describes advection and diffusion of chlorophyll concentration across a reef, as well as chlorophyll removal through oyster filtration. The biodeposition model simulates biodeposit production, resuspension, settlement, and nutrient content. Accumulations of biodeposits will serve as the substrate for the sediment nutrient flux model. Preliminary model simulations are presented at a large restoration site at Harris Creek, a Chesapeake Bay tributary.

## **MILFORD PROBIOTIC STRAIN OY15 MOVES TOWARD COMMERCIALIZATION: RESULTS OF A COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA) BETWEEN THE MILFORD LABORATORY AND ENVERA LLC**

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In an effort to improve hatchery production of eastern oyster (*Crassostrea virginica*) seed for aquaculture and restoration, the Milford Laboratory has isolated and evaluated a naturally occurring beneficial bacterial isolate, Milford probiotic strain OY15 (*Vibrio alginolyticus*) from the digestive glands of adult eastern oysters. This probiotic strain has demonstrated significant protective effects against a shellfish larval pathogen B183 (*Vibrio corallyliticus*) in experimental larval trials and can improve survival by 20-35%. The Milford Laboratory has entered into a formal cooperative agreement with Envera LLC, which provided specialized expertise to determine if OY15 can be mass-cultured effectively and economically and produced in a stable formulation for commercialization and marketing to oyster growers. During this collaboration, Envera was successful in large-scale production of OY15 and has provided the Milford Laboratory with stable, freeze-dried and spray-dried formulations of this probiotic strain. The Milford Laboratory has performed flow-cytometric analyses to determine immune stimulation of oyster hemocytes by ten *Bacillus* strains sold commercially by Envera as probiotic strains for aquaculture. Flow-cytometric, immune-function assays were employed as a potential method for screening unknown bacterial isolates for probiotic candidates, specifically looking for stimulation of phagocytosis and reactive oxygen species release, two critical components of the innate immune system of shellfish required for successful pathogen elimination. Both hemocyte functions are stimulated by Milford probiotic strain OY15. Of the ten Envera *Bacillus* strains, ENV375 and ENV401 significantly stimulated phagocytosis and reactive oxygen species release, and show promise as potential probiotic strains, individually or combined, for use with oyster larvae.

## CELLULAR RESPONSES TO BETA BLOCKER EXPOSURES IN MARINE BIVALVES

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Beta ( $\beta$ ) blockers are prescription drugs used for medical treatment of hypertension and arrhythmias. They prevent activation of adenylate cyclase and increases in blood pressure by limiting cAMP production and protein kinase A activation. After being taken therapeutically,  $\beta$  blockers may make their way to coastal habitats via discharge from waste water treatment plants, posing a potential risk to aquatic organisms. The aim of our research is to evaluate cellular biomarkers of  $\beta$  blocker exposure using two drugs, propranolol and metoprolol, in three commercially important marine bivalves - *Crassostrea virginica*, *Mytilus edulis* and *Mercenaria mercenaria*. Bivalves were obtained from Narragansett Bay (Rhode Island, USA) and acclimated in the laboratory. Following acclimation, gills and hepatopancreas tissues were harvested and separately exposed to 0, 1, 10, 100 and 1000 ng/l of each drug for 24 hours. Samples were preserved for cellular biomarker assays. Elevated cellular damage and changes in enzymatic activities were noted at environmentally relevant concentrations, and *M. mercenaria* was found to be the most sensitive bivalve out of the three species tested. These studies enhance our understanding of the potential impacts of commonly used prescription medication on organisms in coastal ecosystems, and demonstrate that filter feeders such as marine bivalves may serve as good model organisms to examine the effects of water soluble drugs. Evaluating a suite of biomarkers allows us to better define molecular initiating events and subsequent key events that might be used to develop adverse outcome pathways (AOPs) for unintended environmental exposure to  $\beta$  blockers.

## **EVALUATING OYSTER REEF- AND NATURAL FIBER-BASED LIVING SHORELINE STRATEGIES IN SOUTH CAROLINA, USA**

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Oyster reef restoration practitioners in South Carolina, USA have been constructing intertidal living shorelines since 2001, primarily focusing on the essential fish habitat value of these reefs. Many of these reefs, however, by virtue of their provision of physical structure, have also facilitated significant sediment accretion and subsequent marsh re-colonization, highlighting their potential to counteract salt marsh erosion. Further, private property owners are increasingly interested in using living shorelines as a preferred alternative to bulkheads and seawalls. The state regulatory agency permitting process does not, however, currently address living shoreline approaches and therefore serves as a regulatory barrier. This presentation will provide an overview of an ongoing research project funded by the NOAA NERRS Science Collaborative that is generating the science-based information necessary to guide policy development regarding living shorelines. This project is evaluating the ability of oyster-based and natural fiber-based living shoreline approaches to provide structure that facilitates sedimentation, protects adjacent marshes, and reduces erosion across a broader range of habitats than has previously been explored. This presentation will give an overview of field efforts to create and monitor new living shorelines and to re-visit existing living shorelines created over the past 15 years to quantify their shoreline protection services over longer time frames than will be available from structures installed during this project. Ultimately the findings from this project will be developed into a guidance document that will be utilized in the development of a comprehensive regulatory framework and in future implementations of living shorelines in South Carolina.

# **DEEP ECOTYPE-SPECIFIC LINEAGES OR PARALLEL EVOLUTION IN NORTH AMERICA? THE RELATIONSHIP BETWEEN PHENOTYPIC AND GENOTYPIC DIVERGENCE IN *LITTORINA SAXATILIS* IN THE GULF OF MAINE**

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Distinct ecotypes of the intertidal snail *Littorina saxatilis* have been studied extensively in European populations in order to explore the underlying genomic architecture of parallel evolution. Although *L. saxatilis* is also native to the Gulf of Maine in North America, despite being well represented in the ecological literature, the link between phenotypic and genotypic divergence is less well studied in the New World. Snails were collected at 5 regions across the Gulf of Maine (Casco Bay to the Bay of Fundy) with a wave-protected and wave-exposed locality represented in each region (N=328). Shell phenotypes were quantified using a landmark methodology; highly multi-locus genotypes were quantified via a genome reduction technique on a next-generation sequencing platform. Significant phenotypic and genotypic differences were observed between protected and exposed ecotypes; however, geographic region was also an important component in the observed variance at both phenotypic and genotypic levels.

# **HARNESSING ENVIRONMENTAL DNA TO ASSESS THE ENVIRONMENTAL SUSTAINABILITY OF COMMERCIAL SHELLFISH AQUACULTURE IN THE PACIFIC NORTHWEST**

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Commercial shellfish aquaculture makes significant contributions to the economy and culture of the Pacific Northwest. The industry faces intense pressure to minimize environmental impacts as a result of Federal policies like the Magnuson-Stevens Fisheries Conservation and Management Act and the Endangered Species Act. These policies demand the protection of essential fish habitat and declare several salmon species as endangered. Consequently, numerous projects related to the protection and rehabilitation of eelgrass beds, a crucial ecosystem for countless fish species, have been proposed at both state and federal levels.

Eelgrass beds and commercial shellfish farms occupy the same physical space; therefore understanding the effects of shellfish aquaculture on eelgrass ecosystems has become a priority of both government and industry. This study evaluates the organismal communities that eelgrass and oyster aquaculture habitats support. Water samples were collected from Willapa Bay, Washington; Tillamook Bay, Oregon; Humboldt Bay, California; and Sammish Bay, Washington to compare species diversity in eelgrass beds, oyster aquaculture plots, and boundary edges between these habitats. Diversity was assessed using a novel technique: environmental DNA (eDNA). All organisms shed DNA through the constant loss of tissues and waste. This material was collected and sequenced, revealing the species composition of these habitats.

Industry professionals and government officials should consider these findings to better inform policies regulating eelgrass beds and oyster aquaculture. Furthermore, this information may be used to improve the environmental sustainability of commercial shellfish aquaculture while simultaneously enhancing its growth and profitability in the face of ever-changing political and ecological landscapes.

## **EFFECT OF DIATOM DIET ON PINTO ABALONE (*HALIOTIS KAMTSCHATKANA*) POSTLARVAL SURVIVAL, GROWTH, AND RADULA MORPHOLOGY**

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The pinto or northern abalone, *Haliotis kamtschatkana*, restoration efforts currently rely on hatchery production of juveniles. Hatchery managers report a bottleneck in production at the benthic postlarval stage, in the first six months after metamorphosis from a lecithotrophic and planktonic larval stage. Postlarvae suffer high mortality, and slow growth inhibits efficient production of restoration-grade juveniles. Other abalone species have shown correlations between radular morphology, diet type, and feeding efficiency. Growth and survival of postlarval *H. kamtschatkana* was measured under six monospecific diatom diets, and these performance indicators correlated with physical characteristics of the diatoms. The diatom species used were *Achnanthes brevipes*, *Amphora salina*, *Amphiprora paludosa*, *Cylindrotheca closterium*, *Navicula incerta*, and *Nitzschia laevis*. A starvation treatment was included. Changes in radular morphology in response to the different diets were also monitored. Postlarval growth and survival were measured for eight weeks post-settlement, and radula samples were taken weekly and analyzed using SEM. Results of the experiment will be discussed.

## **DETERMINING THE CONDITION INDEX AND CARBONATE PRODUCTION OF *CRASSOSTREA VIRGINICA* IN THE MISSISSIPPI SOUND**

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The eastern oyster, *Crassostrea virginica*, is a keystone organism that provides habitat complexity in the form of oyster reefs that support a diversity of estuarine life, a primary source of calcium carbonate in most estuaries, and other ecosystem services such as biofiltration and nutrient cycling. The eastern oyster also supports a valuable commercial fishery along the Gulf of Mexico. The health of an oyster in terms of yield and meat quality can be estimated by a metric called the condition index. The condition index has historically been calculated using a variety of methods, all of which estimate the extent of utilization of the shell cavity by an oyster, with maximum utilization indicating optimal health and circumstances. Evaluations of carbonate production depend on the estimate of shell weight from population size-frequency and mortality measures, yet variation in shell weight with height among populations is poorly known. The condition indices of oysters from three reefs of increasing distance from freshwater input in the Mississippi Sound were calculated using four combinations of wet meat weight and shell parameters. The most accurate model was identified and was then used to calculate the condition indices for oysters from the three reefs. The indices were then examined to elucidate differences in condition of oysters between the reefs. Shell height-to-weight relationships were also developed and compared for the three reefs.



## MONITORING DERMO DISEASE IN THE MISSISSIPPI SOUND AND OBSERVATION OF A MASS MORTALITY EVENT

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Dermo disease (pathogen *Perkinsus marinus*) has played a significant role in the mortality of the eastern oyster, *Crassostrea virginica*, in the Gulf of Mexico where waters remain relatively warm throughout the year. A Dermo monitoring program has been established for the oyster reefs near Pass Christian, MS. A total of five stations located on Pass Christian Tonging Reef, Pass Marianne Reef, Telegraph Reef, Henderson Point Reef and a reef called Between Bridges were sampled monthly from February to November of 2016. A target of 20 live oysters were processed each month for the detection of Dermo using Ray's fluid Thioglycollate medium (RTFM) assays. Oysters were selected so as to represent a range of sizes encompassing the size frequency distribution from 40 mm to the largest animal measured. During the later phase of the monitoring program, data from a mass mortality event producing upwards of 95% mortality on the primary productive reefs off Pass Christian was captured. Evidence suggests that the combined effect of high Dermo infection intensity and low oxygen likely was responsible. Survival, though scant, was much higher for submarket-size oysters than for market-size oysters.

## ***BONAMIA OSTREAE* IN THE NEW ZEALAND FLAT OYSTER *OSTREA CHILENSIS*: UNDERSTANDING DISPERSAL OF THIS IMPORTANT MARINE PARASITE**

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In January 2015, the haplosporidian parasite *Bonamia ostreae* was reported for the first time in New Zealand infecting the New Zealand flat oyster *Ostrea chilensis* where it was associated with high mortalities. In New Zealand, *B. ostreae* has only been detected in the Marlborough Sounds. Until this report, *B. ostreae* was restricted to the European flat oyster *Ostrea edulis* within the Northern Hemisphere. Therefore, this report represents a significant geographic range extension for this parasite that raises questions of dispersal. A new internal transcribed spacer (ITS) rDNA sequence of New Zealand *B. ostreae* is presented in order to address whether *B. ostreae* is a contemporary arrival to New Zealand and from which Northern Hemisphere population it originated. The analysis of ITS rDNA sequences of New Zealand and Northern Hemisphere *B. ostreae* reveal low genetic diversity of New Zealand *B. ostreae* which suggests a contemporary arrival, whereas high genetic diversity from a small number of Northern Hemisphere isolates prevent inferences of dispersal origins. Dispersal hypotheses of *B. ostreae* will be discussed including the risk of finer scale dispersal of *B. ostreae* to other New Zealand flat oyster beds via natural dispersal of *O. chilensis*.

## **SPAWNING TRIALS WITH CULTURED SUNRAY VENUS CLAMS, *MACROCALLISTA NIMBOSA*, CONDUCTED OUTSIDE THE NATURAL SPAWNING SEASON**

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Research initiated in 2006 concluded that the sunray venus clam could be cultured similar to the hard clam; however, hatchery operators in Florida report problems with synchronous spawning of females and males outside the natural presumptive fall spawning season. In order to address these concerns, spawns were attempted in winter (February), spring (March) and summer (August) of 2015 with two lines of cultured stock. Conditions leading to successful spawns were recorded and larvae produced were followed through to field nursery.

Spawning was induced in five of seven attempts using thermal stimulation, combined with algae addition. Season had no bearing on spawning success, but conditioning did. A minimum of two weeks of conditioning was necessary for successful spawning to occur and additional conditioning resulted in a greater percentage of clams that spawned. All spawns occurred during the heating phase of the cycle. Three of the spawns resulted in successful larval production with season having no bearing on larval production. Larvae set in 5-7 days. Survival from D-stage veliger larvae to post-set was 28-37.5% and survival of post-set to a 1.2 mm screen was 93-100%. Post-set reached hatchery seed size in 2-3 months. Hatchery seed transferred to the UF land-based nursery at Cedar Key held on a 2.0 mm screen (94%) at 30 days and a 3.3 mm screen (46%) at 60 days. These trials resulted in successful seed production conducted outside of the natural spawning season of sunray venus clams, which may lead to reliable, consistent availability of seed.

## **REPRODUCTIVE PATTERNS AND FATTY ACID COMPOSITION OF WILD AND CULTURED SUNRAY VENUS CLAMS, *MACROCALLISTA NIMBOSA*, ON THE FLORIDA WEST COAST**

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The sunray venus (SRV) clam has been advocated as an alternate species to hard clam aquaculture in Florida and three cultured lines have been developed and distributed to Florida hatcheries. However, hatcheries continue to report problems with synchronous spawning outside the presumptive natural fall spawning season. In an effort to determine whether gametogenesis occurs asynchronously, a study was conducted comparing gametogenesis and the gonadal fatty acid profile of a wild SRV population to a cultured line from November 2014 through April 2015.

Gametogenesis was significantly impacted by sex, location and time ( $P < 0.0001$ ), sex and location ( $P < 0.0001$ ), sex and time ( $P < 0.0001$ ), and location and time ( $P = 0.0067$ ), but not the interactions of all three. Spawning activity of males and wild females peaked in the fall. Spawning activity in cultured females was protracted (January to April), which fits the purported asynchronous spawning reported by shellfish hatcheries.

The gonadal fatty acid profile was significantly impacted by time, sex and location. All four categories of fatty acids (SFAs, MUFAs, n-6 PUFAs, n-3 PUFAs) were significantly affected by month. Seasonal changes appeared to be impacted more by food availability than temperature. Sex was a factor in all fatty acid types except for PUFA n-6, and likely reflect differences in the composition of eggs and sperm. Population influenced n-3 PUFAs, but not SFA, MUFA or n-6 PUFAs, but only in the spring, which likely reflects variation in the amount of phytoplankton or its composition at collection locations.

**ARE JUVENILE MANILA CLAMS, *RUDITAPES PHILIPPINARUM*, FREE FROM *PERKINSUS OLSENI* INFECTION? A CASE STUDY FROM A CLAM POPULATION ON THE WEST COAST OF KOREA**

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Previous studies performed in Korean waters revealed that most of adult Manila clams on tidal flats on the west coast heavily infected with *Perkinsus olsenii*. In contrast, *P. olsenii* infection in young and juvenile clams is poorly known in Korea, while juvenile clams are often transplanted to the other area for aquaculture. Accordingly, *P. olsenii* infection among juvenile clams in Boryeong on the west coast was examined, where the young clams have been harvested and transplanted to other clam beds for the culture. In August 2016, juvenile clams of 5-25 mm SL (6-12 month old, N=325) were collected and *P. olsenii* infection level determined using RFTM and 2M NaOH digestion. *P. olsenii* infection in the juveniles were compared with the adults (25-40 mm, N=84) collected from the study site. The assay revealed that the juveniles of SL 5-10 mm, (less than 4 month old) were confirmed to be infected with *P. olsenii* with prevalence and infection intensity of 75% and 110,000-3,000,000 cells/g tissue (N=16). The juveniles of 10-15 mm SL (4-6 month old, N=180) also showed high prevalence (76.7%) and infection intensity (32,000-3,100,000 cells/g tissue). Adult clams were also heavily infected with *P. olsenii* with prevalence and infection intensity of 95-100% and 7,000-1,300,000 cells/g tissue. Our data indicated that the young clams heavily infected with *P. olsenii* similar with the level of adults. It is believed that on the west coast of Korea, *P. olsenii* could be transmitted by transplanting the infected juveniles from area of high level *P. olsenii*.

## **FAIR WEATHER FRIENDS ONLY: DO BIVALVES FAIL AS ECOSYSTEM ENGINEERS IN CONTROLLING TEMPERATE-BOREAL SPRING ALGAL BLOOMS?**

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Dense populations of suspension feeding bivalves are often described as ecosystem engineers, partially because of their potential to (a) crop phytoplankton and therefore remove nitrogen ultimately inputted from a regional water shed and (b) increase water transparency by removing algae from the water column. This “engineering” power is restricted to shallow well-mixed water columns. Seasonality has been argued to be a strong limiting factor in this component of ecosystem engineering since bivalve feeding rate may be strongly reduced in cold seasons. In Jamaica Bay we have found that the major phytoplankton bloom of the year is typically in mid-winter, when feeding of the oyster *Crassostrea virginica* is shut down. This local anecdote may apply widely to the temperate-boreal zone. Studies of Long Island Sound and the boreal Atlantic show that the so-called spring diatom increase usually occurs in February-March, when the water column is nearly isothermal (and therefore well mixed) and very cold (e.g. ca. 6°C in Long Island Sound), perhaps also when bivalve feeding is strongly reduced by cold temperature. A model conceived by Michael Behrenfeld takes this newly appreciated reality into account and argues that the Sverdrup model of spring phytoplankton blooms is flawed and conditions set up in winter with low zooplankton grazing allows phytoplankton to rapidly increase in late winter in very cold and nearly isothermal water. If true, the Jamaica Bay anecdote has great import because bivalves would in effect have little access to the strongest bloom of the year. It is hard to be an ecosystem engineer when the engines can't run. Of course even if they are inadequate as processors of nitrogen input, bivalves could act as feeding ecosystem engineers in spring-summer and help increase water transparency.

## **QUANTIFYING BIVALVE SHELLFISH FOOD AVAILABILITY BY CHLOROPHYLL-A WITH *IN VIVO* FLUORESCENCE-BASED SENSORS – WHAT NEEDS TO BE CONSIDERED?**

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Food availability is one of the most important factors in bivalve shellfish aquaculture site selection, whether the goal is food production or restoration of ecosystem functions. Although bivalve shellfish consume bacteria, detritus, and small-sized zooplankton, phytoplankton typically is the most abundant and important food available. In addition to being food for shellfish, phytoplankton is also one of the key variables characterizing the interactions between shellfish aquaculture and the environment. *In vivo* fluorescence-based estimates are used widely for quantifying chlorophyll-*a* as an alternative to chemical measurements. The method is fast and convenient, and allows unattended, continuous data collection. *In vivo* fluorescence-based chlorophyll-*a* estimates, however, have inherent problems converting fluorescence to chlorophyll-*a*. For example, we obtained inaccurate chlorophyll-*a* estimates by fluorescence-based sensors at multiple coastal sites with a wide range of chlorophyll-*a* -- there were persistent diel patterns with apparent high values at night and low values during the day.

Laboratory studies with cultures of *Tetraselmis chui* and *Chaetoceros neogracile* showed that the diel cycle of chlorophyll-*a* from fluorescence sensors were responses to light, not circadian rhythms. Comparison between true values of chlorophyll from the extraction method and fluorescence-based estimates showed that the fluorescence-based method overestimated chlorophyll-*a* during the dark periods and underestimated chlorophyll-*a* during the light phase. These results will be used to construct a detailed algorithm correcting fluorescence signals to more realistic chlorophyll values.

## EASTERN OYSTER REEFS FACILITATE SEAGRASS BEDS

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In summer 2011, 24 experimental oyster reefs were constructed of oyster shell on muddy sand bottom without seagrass in two tributaries of Chesapeake Bay, the Great Wicomico and Piankatank Rivers. In each river, there were two sites, one on the north shore and one on the south shore. Each site had six 0.4-m-high reefs (5-8 m<sup>2</sup> in area) of three different reef types that mimicked the shape and orientation of historic reefs in Chesapeake Bay. Prior to reef construction there was no seagrass adjacent to some sites and sparse patches of seagrass at other sites. Each site had a paired control site without oyster reefs or seagrass. All 24 reefs were colonized in 2011 by wild larvae of the eastern oyster *Crassostrea virginica* at high densities; juveniles and adults were also at high density and biomass in 2011 and 2013, and have persisted through 2016. By 2014, all 24 reefs also had a 1-2 m border of seagrass, mostly widgeon grass *Ruppia maritima* with some eelgrass *Zostera marina*, whereas paired control sites had no seagrass. The seagrass has persisted through 2016, and seems to be a permanent addition to the oyster reefs. To our knowledge, this represents the first experimental field demonstration of seagrass facilitation by oyster reefs.



## IDENTIFICATION OF CANDIDATE DISEASE-RESISTANCE GENES IN THE EASTERN OYSTER UTILIZING GENOMIC RESOURCES FROM THE PACIFIC OYSTER

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The Pacific oyster (*Crassostrea gigas*) genome project has generated rich genomic resources including a reference assembly and extensive transcriptomes that provide expression profiles of all genes at all developmental stages, in different adult organs and under various environmental conditions. These resources are useful for inferring gene function and comparative genomics. In this study, *C. gigas* genomic resources were explored to identify candidate disease-resistance genes in the eastern oyster (*Crassostrea virginica*), a sister species that was separated from *C. gigas* 83 mya. Despite extensive divergence in nucleotide sequences, protein sequences and gene structure are highly conserved between them allowing easy identification of homologs. A set of 1002 *C. virginica* genes potentially involved in immune and stress responses were identified based on annotation and transcriptome profile in *C. gigas* and sequenced in 7 pairs of before- and after-mortality *C. virginica* samples with AmpliSeq™ to detect single-nucleotide polymorphism (SNP) frequency shifts. The 7 populations were Rutgers NEH™ disease-resistant lines where mortalities were caused by *Perkinsus marinus* and/or environmental stress. Consistent SNP frequency shifts were found in several genes including a Death-associated protein related to apoptosis and up-regulated by stress in *C. gigas*, a heat shock protein and a G-protein coupled receptor that are up-regulated by stress and OsHV-1 infection in *C. gigas*, and an acetylcholine receptor that is down-regulated by stress and OsHV-1 infection in *C. gigas*. These and other genes provide good candidates for further functional studies and as target genes for editing or marker-assisted selection for disease/stress resistance in oysters.

## DOES CSDE1 DRIVE POST-TRANSCRIPTIONAL STRESS RESPONSES IN MOLLUSCS?

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RNA binding proteins are poorly understood regulators of translation with the capacity to greatly influence cell physiology. In bacteria, proteins with RNA-binding cold shock domains (CSDs) regulate the response to cold temperature. In vertebrates, the Cold Shock Domain Containing Protein E1 (CSDE1) regulates translation of target mRNAs associated with disease and environmental stress. For the first time, the full-length mRNA and protein for CSDE1 from the bay scallop, *Argopecten irradians*, and the eastern oyster, *Crassostrea virginica* have been characterized. In both species, the mRNA encodes an 815 bp protein containing five well-conserved CSDs. Bioinformatic approaches were used to identify 12-14 bp CSDE1 binding sites on target mRNAs from all available scallop and oyster sequences. Consistent with studies from vertebrates, putative binding sites on many target mRNAs locate to the 3' UTR. Putative targets, often shared-between oysters and scallops, include mRNAs encoding proteins related to muscle function, innate immunity, environmental stress, metal binding, and metabolism. Many targets are homologs of established CSDE1 targets in vertebrates and some are known to be upregulated in response to thermal or hypoxic stress. Preliminary expression data show that CSDE1 is upregulated in *A. irradians* adductor muscle and gill tissues in response to hypoxia ( $p < 0.05$ ). Recombinant CSDE1 proteins from both *A. irradians* and *C. virginica* are being used to verify CSDE1 binding to candidate genes and to examine translational regulation of the hypoxia response.

## **THE NEUROTOXIC EFFECTS OF MANGANESE ON DOPAMINE POST-SYNAPTIC RECEPTORS ARE REVERSED BY TAURINE**

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Manganese, a neurotoxin causing Manganism a Parkinson's-like syndrome, disrupts dopamine neurotransmission in brain. The neurotoxic mechanism is unknown. Reports postulate it is more related to post-synaptic dopamine receptors (D2DR) dysfunction, than degeneration of dopamine neurons. Gill lateral cells of *Crassostrea virginica* are innervated by cilio-inhibitory dopamine nerves and have D2DR receptors. Previous work found manganese blocked cilio-inhibition of dopamine on lateral cells and manganese reduced D2DR fluorescent intensity in the cells, suggesting manganese causes a loss of D2DR number or a structural disruption of receptor integrity. The amino acid taurine alleviates symptoms in several neurodegenerative diseases. It was hypothesized that taurine would reverse manganese neurotoxicity on gill lateral cell. Oysters (*C. virginica*) were treated for 3 or 5 days with manganese followed by 3 or 5 days with taurine. Gills were excised and treated for immunohistofluorescence as previously described. Fluorescence intensity was quantified using ImageJ from NSF. Manganese treatments caused a 66% decrease in fluorescence compared to controls. Animals treated with 3 days of manganese followed by 3 days of taurine showed only an 18% reduction. Animals treated 5 days with manganese then 5 days with or without taurine gave similar results. The study indicates taurine reversed manganese induced loss of D2DR fluorescence. The neuroprotective mechanism of taurine needs to be further investigated. This study suggests taurine may be a promising therapeutic agent for treatment of Manganism. This work was supported by grants 690340047 of PSC-CUNY, 2R25GM06003 of the Bridge Program of NIGMS and a Carnegie Foundation award.

## SEXUAL DIMORPHISM IN MARINE BIVALVES AND EMERGING THOUGHTS ON THE LONG LIVED *ARCTICA ISLANDICA*

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Sexual dimorphism in the Bivalvia, as distinct from protandry and protogyny, is quite rare and often dramatic, for example that of parasitic males in the Teredinid *Zachsisia*. A brief review of sexual dimorphism in bivalves and a recent data set describing the demographics of 231 male and 266 female *Arctica islandica* of shell lengths ranging from 19 to 121 mm, collected on the Mid Atlantic shelf and Georges Bank during the 2014-2016 time period will be provided. Male clams exhibited a mean length of  $79.9 \pm 14.7$ mm (st.dev); females were  $86.7$ mm  $\pm 14.3$ mm; with a combined population value of  $83.6$ mm  $\pm 14.9$ mm. This data set will be expanded. The emerging sex specific frequency distributions are suggestive of sexual dimorphism in this long lived species. The question “what would be the advantage, if any, of sexual dimorphism in such a long lived, sedentary species?” is posed.

## INTERACTIONS BETWEEN POLYSTYRENE MICROPLASTICS AND MARINE PHYTOPLANKTON LEAD TO SPECIES-SPECIFIC HETERO-AGGREGATION

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This study assessed the potential for phytoplankton cells to form hetero-aggregates with small micro-polystyrene (micro-PS) according to their physiological status. A prymnesiophyceae *Tisochrysis lutea*, a dinoflagellate *Heterocapsa triquetra* and a diatom *Chaetoceros neogracile* were exposed to micro-PS (2 µm; 3.96 µg PS.L<sup>-1</sup>) during their growth culture cycles. Micro-PS were quantified using an innovative flow-cytometry approach, which allowed the monitoring of the micro-PS repartition in microalgal cultures and the distinction between free suspended micro-PS and hetero-aggregates made of micro-PS and microalgae. Hetero-aggregation was only observed for *C. neogracile* during stationary growth phase. Highest levels of “lost” micro-PS stuck on flasks were observed with *Tisochrysis iso* and *H. triquetra* and this proportion increased with the age of the culture for both species. Finally, no effects of micro-PS were observed on microalgal physiology in terms of growth and chlorophyll fluorescence. Overall, these results highlight the potential for single phytoplankton cells and residual organic matter to interact with MP, and thus potentially influence their distribution as well as their bioavailability in water column for filter feeders.

## **ISSUES AND SOLUTIONS IN USING GENOME SCANS TO UNDERSTAND THE GENETIC BASIS OF LOCAL ADAPTATION**

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Local adaptation occurs when individuals within a species have higher fitness in their home environment than they do in a foreign environment. Due to the recent ability to obtain massive amounts of sequence data, there has been an increasing interest in studying the genetic basis of local adaptation of shellfish populations from genomic data. This increase in the ability to ascertain genomic data has also been paralleled by a rapid increase in the number of statistical methods that can be used to analyze that data, broadly termed genome scans. The application of these methods, however, can be sometimes frustrating to the investigator because different methods give different results. This presentation will review some of the reasons for these disparities, and also present some potential solutions including better calibrated test statistics, better sampling designs, and integrating signals across multiple tests.

## **SNP DISCOVERY AND VALIDATION USING HRM (HIGH RESOLUTION MELTING) FOR THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA***

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Medium- to high-throughput (HTP) Single Nucleotide Polymorphism (SNP) genotyping assays are invaluable for many genetic and genomic applications including pedigree analysis, QTL mapping, and genome-wide association studies. Although allele-specific, PCR-based, genotyping platforms such as Fluidigm<sup>TM</sup> are reliable and cost-effective, assay design is challenging for highly polymorphic species. Here an approach to identify and validate SNPs in the eastern oyster, *Crassostrea virginica*, for inclusion in an HTP genotyping assay intended to isolate markers associated with disease resistance is described. Over 3000 SNPs were detected in RNA transcripts differentially expressed among disease resistant and susceptible eastern oyster families. SNP-containing transcripts were assigned a gene ontology (GO) term and those with stress and immune-related GO terms were targeted for validation in a panel of individuals with disparate genetic backgrounds. For each SNP, the validation process involved genotyping by High Resolution Melt (HRM) analysis and subsequent cloning and sequencing of amplicons with distinct melting profiles to assign melting curves to a specific genotype. In many cases, more than three melting profiles (two homozygote and one heterozygote) were detected and sequence data confirm the presence of additional SNPs/insertions/deletions within the amplicons. Short sequences containing more than one polymorphism cannot be genotyped accurately with HRM or HTP methods. Thus far, 54 SNPs within 36 unique transcripts have been screened and validated, but only seven are suitable for HTP genotyping. Results caution that genotyping assay design and the interpretation of genotypic data must account for the extensive polymorphism within the eastern oyster genome.

# MORPHOLOGY AND MORPHOMETRY OF LARVAL AND POST-LARVAL BIVALVE MOLLUSCS FROM FRESHWATER, ESTUARINE AND MARINE HABITATS: A SYNTHESIS

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A monograph on the identification of larval and post-larval bivalves is in its final stages of completion and will contain 192 plates of scanning electron micrographic sequences of the larval and post-larval stages of the following 54 species: *Anomia simplex*; *Ameritella mitchelli*; *Arca noae*; *Arctica islandica*; *Arcuatula papyria*; *Argopecten irradians*; *Argopecten irradians concentrius*; *Astarte castanea*; *Bankia gouldi*; *Brachidontes exustus*; *Chione cancellata*; *Corbicula fluminea*; *Crassostrea virginica*; *Cyclocardia borealis*; *Cyrtopleura costata*; *Dinocardium robustum*; *Diplothyra curta*; *Dreissena polymorpha*; *Dreissena bugensis*; *Ensis directus*; *Gemma gemma*; *Geukensia demissa*; *Hiatella arctica*; *Ishcardium recurvum*; *Laevicardium mortoni*; *Leiosolenus bisulcatus*; *Limecola balthica*; *Lunarca ovalis*; *Lyonsia hyaline*; *Mercenaria texana*; *Mercenaria mercenaria*; *Mesodesma arctatum*; *Modiolus americanus*; *Modiolus modiolus*; *Mulinia lateralis*; *Mya truncata*; *Mya aranaria*; *Mytilopsis leucophaeata*; *Mytilus edulis*; *Noetia ponderosa*; *Ostrea edulis*; *Ostrea equestris*; *Pecten maximus*; *Periploma leanum*; *Petricola pholadiformis*; *Pitar morrhuanus*; *Placopecten magellanicus*; *Rangia cuneata*; *Ruditapes philippinarum*; *Solemya velum*; *Spisula solidissima*; *Tagelus plebeius*; *Tellina agilis*; *Teredo navalis*.

This multi-authored monograph will synthesize a body of research conducted over a period of more than 40 years by the principal investigator and his collaborators/co-authors on the morphology and morphometry of the shells of larval and post-larval bivalve molluscs from a wide spectrum of freshwater, estuarine, and marine habitats. This widely accessible and broadly disseminated product is envisioned as being a classic reference for current and future students and colleagues conducting a myriad of biodiversity, systematic and environmental studies that would not otherwise be possible in a spectrum of freshwater, estuarine and marine environments.



## ASSESSING LINKS BETWEEN GENOMIC AND ENVIRONMENTAL VARIATION IN A POPULATION OF BIVALVES FROM THE GULF OF MAINE

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Bivalves are integral members of the benthic community in both estuarine and oceanic environments. Changes in water quality and chemistry can impact population health, in particular, because these bivalves are marine calcifiers. In the Gulf of Maine, bivalves are important fisheries (both wild and aquacultured) and assessing the impacts of the effect water quality has on bivalve populations is imperative. In coastal Maine, bivalves were sampled from Boothbay Harbor to the Kennebec River region using a grab sampler, and a water quality Sonde assessed spatial variation in water quality. Linear models were applied to determine correlations between environmental variables (salinity, pH, temperature, and dissolved oxygen) and bivalve abundance; the abundant species are *Nucula proxima* and *Arctica islandica*. Further, a Next Generation Sequencing assay was applied across *N. proxima* populations to investigate correlations between highly multilocus genotype and environmental parameters. This powerful genome survey will help to assess the impact variation in water quality has on the population composition of these bivalves.

## SEASONAL DISEASE CYCLES OF THE PARASITE *HEMATODINIUM PEREZI* IN THE MARYLAND AND VIRGINIA COASTAL BAYS

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The blue crab makes up one of the largest commercial crustacean fisheries in the US yet recent population levels have fluctuated and annual catch has seen a general decline. These fluctuations are likely the result of many factors including mortality due to infections by *Hematodinium perezii*. In conjunction with the National Park Service and the Maryland Department of Natural Resources, a two year study in 2014 and 2015 was conducted to monitor the presence of *H. perezii* DNA within the Maryland Coastal Bays, comparing seasonal cycles in the abundance of parasite DNA in environmental samples to parasite presence in host blue crabs. It was hypothesized that the peak in water column occurrence would coincide with high infection intensities. Through the use of quantitative PCR, parasite DNA can be identified in environmental samples and crab tissues. A late summer to early fall peak in *H. perezii* infection prevalence and intensity was observed in both years, roughly matching the peaks in environment presence of parasite DNA, consistent with a release of the parasite during sporulation events. An additional peak in environmental detection occurred in both years during early spring months, the cause of which is currently unknown but may be related to infections in overwintering crabs or alternate hosts, particularly those that are more active in colder months. Several new crustacean hosts were identified within this estuary, including the sand shrimp (*Crangon septemspinosa*). A complete understanding of the disease system may allow for prediction of disease mortality and support management of the blue crab fishery.

## **EFFECTS OF OCEAN ACIDIFICATION ON GONAD DEVELOPMENT OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*)**

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Atmospheric carbon dioxide (CO<sub>2</sub>) concentrations have been rising during the past century leading to increase in oceanic dissolved inorganic carbon and ocean acidification. Recent observations demonstrate that some ecosystems already experience annual pH variability that vastly exceeds the magnitude of long-term projections in open ocean regions (~0.2 units). Estuarine waters are characterized by extreme fluctuations in pH levels, from the values of 8.2 down to value lower than pH 7, and periods of low pH can persist for weeks during spring, summer and fall.

Estuarine ecosystems provide habitat to ecologically and economically important marine species such as the eastern oysters (*Crassostrea virginica*). The sustainability of oyster populations depends upon gametogenesis and reproductive success – activities that coincide with periods of low pH occurrence in estuaries.

Oysters were exposed to low levels of pH (7.5, 7.1 and 6.7) for 5 weeks during gametogenesis (n = 115). Exposure at pH 7.1 and 6.7 resulted in a delay and inhibition of gamete development, respectively. Oogenesis appeared to be more sensitive to low pH conditions compared to spermatogenesis. This study provides evidence that exposure at low pH during gametogenesis cause reproductive disruption in oysters. A temporal change in gametogenesis may result in reduced gamete quality, fertilization success, or fitness of larvae and would have profound negative implications on the natural oyster population.

## **SUB-ANNUAL SIGNATURES IN THE VALVES OF JUVENILE *ARCTICA ISLANDICA* AS HISTORICAL RECORDS OF INTRA-ANNUAL CHANGING ENVIRONMENT**

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The long-lived *Arctica islandica* has been used extensively in reconstructing climate data at the annual growth increment level over periods extending back several millennia. The question “can sub annual growth signatures be used as proxies of intra-annual environmental data over extended periods of decades, or even centuries?” was posed. Collections of small *Arctica islandica* on the inner continental shelf south of Long Island in August 2014 afforded the opportunity to examine the coherence between sub-annual signatures in the growth record in the valves with notable environmental cues such as absolute temperature (the annual bottom water temperature cycle and water column mixing associated with seasonal thermocline degradation is well documented for the Southern New England Shelf), and phytoplankton blooms. This temporal “snapshot” of data in small clams in the context of a substantial time series of early life history records from individual clams from the Mid Atlantic Bight and Georges Bank with birthdates ranging as far back as the middle 19<sup>th</sup> Century is discussed.

## **VIRUSES OF THE PROTOZOAN PARASITES *PERKINSUS* SPP.**

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Commercially valuable bivalves are important members of coastal ecosystems where, through filter-feeding, they remove particulate matter thus enhancing water quality, and can form “reefs” that provide habitats for a myriad of organisms. “Dermo” disease caused by the protozoan parasite *Perkinsus marinus* severely affects farmed and wild populations of oysters. Improved knowledge of *Perkinsus* ecology is of utmost importance to aid in the design of therapies and management strategies to minimize its detrimental economic and ecological impacts. Most of the studies aimed to understand and intervene against Dermo have centered in a game of two: protozoan parasite and bivalve host. Viruses that propagate in the nucleus of trophozoites of both *P. marinus* TXsc ATCC# 50983 and *P. olseni* ALG-1 ATCC# 50984 have been found and isolated. The discovery of these 40-50 nm virions adds a new dimension to the investigation and management of Dermo disease. *Perkinsus* virulence factors are typically associated with: (i) Cell propagation and differentiation; (ii) Protection against the bivalves immune response; (iii) Nutrient uptake via enzymatic break down of bivalve tissues into transportable components. While research on other parasitic protozoa of human relevance has shown that viruses have a profound effect on their propagation, life style, and virulence, the role of viral infection has not yet been investigated as a virulence factor of *Perkinsus* spp. In addition to ultrastructural analysis, genomic characterization of the *Perkinsus* viruses to help understand their ecology, infection strategies and virulence toward their bivalve hosts are underway.

## AN OVERVIEW OF APPLE SNAIL RESEARCH IN LAKE SEMINOLE, GEORGIA

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Apple snails are among the most successful invasive species across tropical and subtropical freshwaters. In Lake Seminole, a shallow reservoir in the Apalachicola-Chattahoochee-Flint (ACF) watershed, *Pomacea maculata* was first observed in 2003 and *P. paludosa* is present within its native range. Previous research in Lake Seminole has focused on regional hydrology, submerged aquatic vegetation (SAV), and biogeochemical cycling and water quality. It was hypothesized that *P. maculata* introduction and expansion will alter biological, chemical, and physical processes within the lake over time. Surveys have shown *P. maculata* are present throughout large sections of the Flint River and Spring Creek arms of the lake, with rapid dispersal throughout the lake since 2013. Potential limiting factors of *P. maculata* dispersal include low temperature and spatial calcium variability.

In Lake Seminole, water column total nitrogen (TN) and phosphorous (TP) can be drastically reduced by SAV beds, including *Hydrilla* which covers ~50% of the lake. Experimental mesocosms containing *P. maculata* and SAV were used, and observed significant increases in water column TN and TP, and reduction in *Hydrilla* coverage. This provides evidence for grazing potentially altering biogeochemical cycling as *P. maculata* populations expand. Significant changes in water quality may have implications in the ACF, where water quality and quantity are concerns to stakeholders. Research on the impacts of *P. maculata* in Lake Seminole, focusing on bottom-up/top-down trophic dynamics and on biogeochemical cycling in the system, using a combination of population surveys, monitoring, and *in situ* experimentation is continuing.

## **INTERACTIONS BETWEEN A THREATENED SHOREBIRD AND OYSTER AQUACULTURE IN NEW JERSEY: DO FARMS IMPACT RED KNOT HABITAT USE AND FORAGING?**

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Oyster farming along lower Delaware Bay New Jersey, USA is growing with a rising public demand and readily available source of disease resistant oysters. The region is also an important migratory stopover site for the Rufa Red Knot, a threatened shorebird, during its spring migration from wintering grounds in Argentina to breeding grounds in the Canadian Arctic. This energetically taxing journey requires Red Knots to periodically stop and 'refuel'. In Delaware Bay, they rely heavily on the lipid-rich eggs deposited by spawning horseshoe crabs to rapidly gain enough weight to complete their migration. Horseshoe crab spawning peaks during a 3- to 7-week window each spring, and the timing of the red knot migration typically allows them to take advantage of this energetically rewarding food source during the brief period it is available. Intertidal oyster aquaculture occurs within portions of the same tidal flats, challenging regulatory agencies to develop oyster farming guidelines that minimize potentially negative impacts to red knots without compromising farm viability. How oyster aquaculture activities affect Red Knot foraging and habitat use are being assessed. Preliminary research suggested that knots were least likely to be present during low tide while farms were exposed and tending can occur. There was no statistically significant effect of untended racks, but tending oysters did correspond to a reduction in red knot presence. This work was funded by NJ Bureau of Shellfisheries, NJ Endangered and Nongame Species Program, and NFWF. Continuing work is funded by New Jersey Sea Grant.

## **THE EFFECT OF PLOIDY AND BROODSTOCK ORIGIN ON SPRING MORTALITIES OF COMMERCIALY RAISED OYSTERS, *CRASSOSTREA VIRGINICA***

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Maximizing the survival of commercially produced triploid *Crassostrea virginica* oysters is a matter of significant economic importance to the state of Virginia. Over the last few years, commercial oyster growers in Virginia have reported significant mortality events of oysters during spring months. The spring of 2014 was particularly bad for several growers. The most severe mortality occurred on the eastern shore and in some cases as high as 85% of the crop. Triploids were often exclusively grown on these farms, leaving it unclear if these mortality events were specific to triploids. Additionally, many of the affected Virginia farmers were growing oysters produced by crossing broodstock from Maine to broodstock from Virginia. To investigate the effect ploidy and broodstock origin have on spring mortality events, four triploid and four diploid groups were produced by crossing different combinations of Virginia, Louisiana, and Maine broodstock and deployed the groups to three commercial oyster farms that experienced unusually high mortality in 2014, as well as to a control site lacking reports of unusual spring mortality. Throughout the spring and summer of 2016, the survival and growth of oysters from each group were measured. A spring mortality event was observed at only one of the four sites, in which a range of cumulative mortality between 7% and 24% was observed among triploid groups between April and June. Survival of diploids during this time period at the same site ranged from 2% to 7%, suggesting ploidy and broodstock origin may be important in spring mortality events.



## **TESTING FOR GENETIC EFFECTS ON OYSTER STRESS TOLERANCE AND FITNESS-RELATED PERFORMANCE**

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New York coastal waters, once a booming oyster fishery, are now nearly void of live oyster reefs; however, recent surveys have documented substantial remnant populations of adult oysters in the upper estuarine zone of the Hudson River Estuary (HRE) near Tarrytown, NY. This portion of the estuary frequently has prolonged periods with salinities below 5, near the lower threshold typically reported for *Crassostrea virginica*. Evidence for the population robustness comes from consistent annual recruitment relative to other parts of the estuary since 2012. Motivated by intense interest in oyster restoration in the more populated lower parts of the HRE, where waters are closed to harvest, we are testing the relative growth and survival of wild Tarrytown spat to hatchery-produced spat from moderate-salinity wild broodstock. Hatchery-produced oysters with high vs. low genetic diversity were outplanted at 11 sites along the HRE gradient from Tarrytown to Jamaica Bay (high salinity) to test for a link between genetic diversity and fitness related performance measures. Highest growth was observed near Tarrytown and in Jamaica Bay with low growth at the mid-estuary New York Harbor sites. Wild spat were transplanted to 5 sites and showed faster growth compared to hatchery-produced oysters where conditions were marginal (low salinity or low water quality), suggesting that hatchery bottlenecks may be more of an impediment for spat under non-ideal conditions. Hatchery spat also were segregated into fast and slow-growing larvae for field monitoring and laboratory challenge experiments. Differential stress tolerance will be related to genetic by environment interactions.

## **BIVALVE MOLLUSCS AS FOOD, STRUCTURE, HABITAT, AND ECOSYSTEM ENGINEERS**

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Bivalve molluscs provide many biological and economic benefits to humans and ecosystems. In the last three decades scientists have been studying the ecosystem services provided by bivalves and have come to a greater understanding of, and appreciation for, these services, which include water filtration, denitrification, sequestration of carbon, and habitat for many other organisms. More recently, bivalves have been used in estuarine restoration projects such as “living” or “green” shorelines, many of which have been constructed in combination with various marsh plants (e.g., *Spartina alterniflora*), to stabilize shorelines and reduce erosion. Some of the services provided by bivalves have very practical value, and economists and biologists have been collaborating to establish the monetary value of some of them. In addition, bivalve shells are also of economic value as a commodity in restoration projects and aquaculture and are also used in many different products and for various purposes (e.g., road construction). The resulting loss of shell from oyster reefs due to harvest practices and lack of shell recycling has thus become an important consideration for resource managers trying to maintain the sustainability and biological integrity of reefs; therefore shell budgets are being used in some states to insure no net loss of shell on public reefs. This session will include presentations on these topics and also techniques for studying and monitoring the effects of bivalves on ecosystems.

## **STUDYING THE MECHANISM OF ACTION AND EVALUATING THE EFFICACY OF FORMULATED PROBIOTICS FOR *CRASSOSTREA VIRGINICA* LARVAE AGAINST INFECTION OF *VIBRIO CORALLIILYTICUS* RE22**

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The eastern oyster *Crassostrea virginica* is an ecologically and economically important species in the east coast of the United States and the Gulf of Mexico. The rearing of oyster larvae in hatcheries can be impacted by mortalities caused by bacterial pathogens such as *Vibrios*. Probiotics are an inexpensive, practical, and natural method of disease control. Two probiotics, *Bacillus pumilus* RI0695 and *Phaeobacter inhibens* S4, were previously shown to protect oyster larvae from infection of *Vibrio coralliilyticus* RE22. A stable and easy to use formulations of these probiotics is needed for practical use in hatcheries. Several probiotic formulations were tested by comparing their efficacy in protecting larvae as compared to fresh cultures using laboratory and hatchery trials. It was shown that formulations of these probiotics are as effective as fresh probiotics in protecting the larvae from a *V. coralliilyticus* RE22 infection, providing a relative increase in percent survival ranging from 21%-83% for *Bacillus pumilus* RI0695 and 11%-39% for *Phaeobacter inhibens* S4. Formulations were also shown to be safe for use in bivalve shellfish hatcheries. Additionally, an attempt to understand the mechanism of action of these probiotics was made by studying the immunological response of larvae treated with probiotics. This knowledge could be useful in improving the efficacy of these probiotics.

## **TRACKING THE FOOTPRINTS OF ATLANTIC SURFCLAMS AND OCEAN QUAHOGS FROM A 30-YEAR SURVEY IN GEORGES BANK: A TALE OF TWO SPECIES?**

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The Atlantic surfclam (*Spisula solidissima*) and the ocean quahog (*Arctica islandica*) are commercially important bivalves in U.S. Atlantic waters. Over the last two decades, the range of surfclams has been contracting offshore and towards the northeast due to rising bottom water temperatures. Changes in distribution and depth range of the long-lived quahogs are less understood. Using clam tow surveys conducted by the National Marine Fisheries Service in the Georges Bank region from 1978 to 2011 and GIS techniques, the long-term spatial patterns in occurrence of these two species based on location of shell and live animals were examined. Live surfclams and shell co-occurred throughout the region, except in the western region where only live surfclams were detected indicating a recent shift into deeper waters. A more pronounced distinction between location of shell and live animals occurred for ocean quahogs: shell was primarily found in the central region and live quahogs in deeper waters surrounding the northern and western boundaries. Both of these shifts are consistent with increasing water temperatures, for ocean quahogs since the end of the Little Ice Age early 1800s and for surfclams since the 1980s. These findings can help establish climate change vulnerability indices for surfclams and ocean quahogs in the Georges Bank region. Following a similar approach with long-term surveys conducted in the Mid-Atlantic Bight would allow establishing vulnerability indices for the entire range of the stocks.

## **DOWN(EAST) ON THE SCALLOP FARM IN MAINE**

### **Dana L. Morse**

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The state of Maine has the most significant inshore fishery for sea scallops (*Placopecten magellanicus*) in the US, and the value in its 'dayboat' quality has spurred development of aquaculture production for this species. Prior work has demonstrated that Maine producers can acquire seedstock, produce a quality live product in a reasonable time frame and with reasonable cost, and can capture significant value in the market. Live sales are presently stalemated however, because of the high costs of testing for phycotoxins such as saxitoxin and domoic acid. Meanwhile, the process known as 'ear-hanging' is showing promise in Maine waters, and strong technology transfer efforts have greatly increased the interest of commercial fishermen to try this production method. Through a pilot project funded by the USDA NESARE program, early estimates of growth and mortality appear quite favorable, and many partners in the state have contributed toward strong relationships with Japanese gear manufacturers, scientists and others. Maine is well poised to capture some of the half-billion dollar scallop market, and to more fully integrate aquaculture as an income diversification opportunity for Maine fishermen. This presentation will review the equipment, techniques and husbandry considerations for scallops in Maine, focusing on the ear-hanging approach.

## **DETERMINING INCIDENTAL DISCARD MORTALITY OF SEA SCALLOPS IN THE DREDGE FISHERY IN THE MID-ATLANTIC BIGHT**

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Incidental fishing mortality, also known as non-capture mortality, is the mortality associated with individuals contacted by the fishing gear, but not caught. The most recent sea scallop assessment set this rate at 10% and 20% of the fully recruited fishing mortality in the Mid-Atlantic region and on Georges Bank, respectively; however, these rates are based on experiments conducted in the 1970s and 1980s using a dredge configuration that longer complies with regulations in the commercial fishery. To obtain up-to-date estimates on incidental mortality, we sewed a twine mesh bag (1 7/8") into the top a dredge configured to current regulations and conducted a total of thirty-nine tows with this gear near the Hudson canyon access area. For all scallops collected in the twine mesh bag that would have normally passed through the dredge rings, shell damage was assessed, and for a subset of scallops in each injury category, post-release mortality was monitored. No scallops categorized as undamaged died after being returned to the sea floor and monitored at one- and two-week intervals. A large fraction of those scallops categorized as having sub-lethal and lethal damage were still alive after two weeks of post-release monitoring. When mortality rates by injury category, from the sub-sample of scallops that were monitored post-release, were applied to the entire incidental catch, total incidental mortality rate was low in both 2015 (3%) and 2016 (1%). These results are consistent with previous estimates from the same region, but lower than what is currently assumed in the assessment.

# **SUPERNUMERARY CENTROSOMES ASSOCIATED WITH ABNORMAL CHROMOSOME SEGREGATION IN TRIPLOID AND TETRAPLOID EMBRYOS OF *CRASSOSTREA VIRGINICA***

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Chromosome instability (CIN) seems to be a regular feature in oysters, more so in polyploids and especially in tetraploid *C. virginica*. In diploid oysters CIN manifests as aneuploidy, in polyploid oysters – aneuploidy and mosaicism, the latter characterized by multiple cell ploidies in the same individual. It was hypothesized that supernumerary centrosomes may be involved in CIN. Centrosomes are the main microtubule organizing center of the cell and too many of them – supernumerary – can cause disruption mitosis. Supernumerary centrosomes are associated with cancer in humans.

Two sets of crosses were produced using either non-mosaic (no apparent CIN) tetraploids or mosaic tetraploids. For each set, the crossing pattern was  $2n\text{♂} \times 4n\text{♀} \times 4n\text{♂} \times 2n\text{♀}$ . The diploids were crossed to themselves as a control. Developing embryos were sampled at 0, 10, 30, 60, 80, and 100 minutes PF, encompassing early division until about the 8 cell stage. Eggs and embryos were fixed and stained with either of two primary antibodies for microtubules and centrosomes in combination with secondary staining for chromosomes with DAPI.

Survival rate was lower in embryos from mosaic parents. Development rate lagged in polyploid versus diploid embryos. A number of chromosomal aberrations were observed during embryogenesis in all ploidies, but at higher frequency in polyploids. Abnormalities included abandoned chromosomes, supernumerary centrosomes, and abnormal chromosome segregation. The frequency of abnormalities was higher when mosaic parents were used and female tetraploid parents produced a higher frequency of abnormalities than male tetraploid parents. Results suggest supernumerary centrosomes are the driving force for CIN in oysters.

## **CALCIUM SPHERULES IN THE MANTLE TISSUE OF THE ISLAND APPLE SNAIL, *POMACEA MACULATA***

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The island apple snail, *Pomacea maculata*, breathes air with a lung formed from modified mantle tissue. Scanning electron microscopy of the mantle revealed the presence of spherical inclusions with diameters of 5 to 40 microns. These spheres were extremely abundant in the mantle tissue that forms the lung. Energy dispersive spectroscopy analysis of the spheres suggests that the primary constituent was calcium carbonate; there was also a strong magnesium signal. The spherules contained only trace amounts of phosphorus. Spherules were found in the hemolymph. Few calcium containing spherules were found in gill tissue. During prolonged estivation, the number of large spherules in the mantle decreases markedly. The spherules probably serve as temporary stores of mineral for shell growth, repair and formation of egg shells. During estivation, mobilization of the carbonate in the spheres may serve to buffer acidic metabolic products.



## **THE ROLE OF FISHERY ENHANCEMENT IN THE SUSTAINABLE OYSTER FISHERY IN DELAWARE BAY**

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Wild harvest of Eastern oysters (*Crassostrea virginica*) has been culturally and economically important to the Delaware Bay for well over a century. This fishery has remained relatively stable over the past two decades with an average of 76,085 bushels (1 NJ bushel = 35 L and yields about five pounds [2.27 kg] of meat) of oysters harvested annually since 1996. Quotas are set using an exploitation-based reference point system that uses data from catch and annual assessment of oyster abundance. Additionally, fishery management employs enhancement strategies that include habitat improvement (planting shell) and transplanting of oysters from lower to higher productivity regions. The effect of these strategies was analyzed using repeat observations in space and time of oyster abundance on these areas relative to adjacent unenhanced areas in the central region of the stock. Results show that on average, without enhancement, oyster abundance remains constant; however, with enhancement, oyster abundance is significantly increased. On average, enhanced grids (~25 acres each) saw an increase of 20 oyster/m<sup>2</sup> (> 2 million oysters per grid) over a three-year period following the enhancement activity. Oyster enhancement on a scale of relevance to the fishery not only contributes to the sustainability of the stock, but is also of great importance to the ecology of the bay.

## **HORSESHOE CRAB ACTIVITY AND INTERACTIONS ON RACK-AND-BAG OYSTER FARMS**

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Concern has recently been raised about the ability of horseshoe crabs (*Limulus polyphemus*) to navigate safely in and around intertidal oyster farm gear, and how farms may change shorebird foraging activity. During the 2016 crab spawning season, a series of experiments were conducted in the Delaware Bayshore region to assess the ability of crabs to move around and among oyster farms to mate and spawn, and to survey the spatial distribution of dislodged eggs upon which Red Knots feed. These experiments included (1) testing rack heights for impairment of crab passage, (2) repeated crab census on paired farm/control sites to test if crabs avoid farms, and (3) spatial survey of dislodged egg distribution along the wrack zone. All crabs, regardless of size, passed easily beneath racks 10 cm (4 inches) or more above the bottom. Thus, regulated rack height of 30.5 cm (12") should be sufficiently precautionous to allow crab movement beneath racks. The crab census observed 853 crabs total, with no evidence of a difference in crab numbers among farmed and control transects ( $p=0.3$ , paired Wilcoxon signed-rank test). In total, 2 out of 853 (<0.5%) crabs were observed to be impeded by racks. Crab eggs washed up in the wrack zone were distributed unevenly throughout the survey region. Dislodged eggs were observed most frequently in the central portion of the survey area, and were not concentrated in the area of farms suggesting that in 2016, Red Knot foraging opportunities were not spatially coincident with farm locations.

## **ANCIENT CLAM GARDENS AS NURSERY GROUNDS: HOW DO SHELL HASH ADDITIONS AFFECT SETTLEMENT AND SURVIVAL?**

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At least 2000 years ago, coastal First Peoples from the North Pacific began constructing and actively managing “clam gardens”. These features are characterized by rock walls built at the lower tidal limit of beaches, which effectively back-filled the intertidal, decreased beach slope, and extended the area of optimal clam habitat. In intertidal surface sediments, grain size and inorganic carbon content are important characteristics controlling settlement and post-settlement survival. These sediment characteristics were tested as a driving mechanism for increased clam productivity observed today in clam gardens relative to non-walled beaches. Survey data from Quadra Island, British Columbia, reveal that clam gardens have (1) on average four times more calcium carbonate, which likely buffers pH at the sediment-water interface and increases the chances of settlement, and (2) more coarse-grained sediment (particles >1mm), which may increase post-settlement survival. These qualities correlate positively with surveyed abundance of adult clams. Furthermore, a sediment transplant experiment was run to test how changes in sediment characteristics may alter settlement and early survival. Three treatments (clam garden sediment, non-clam garden sediment, 100% shell hash) were deployed at randomly stratified intertidal plots on a clam garden site. Plots were sampled over 14 months and examined for differences in larval settlement and juvenile survival. Early results suggest an ontogenic trend such that larger juveniles (>1000µm) are found in higher abundance in clam garden sediments.

## GENETIC DIVERSITY AND PARENTAL CONTRIBUTION OF THE RED OCTOPUS, *OCTOPUS MAYA*, WILD BROODSTOCK IN CAPTIVITY

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The genus *Octopus* includes ~200 species, many of which are commercially important resources worldwide. In America, the endemic red octopus *O. maya* has supported a fishery since 1949. Catches varied from 4,500 to 20,000 t between 2001-2015. This fishery generates 15,000 direct jobs and an estimated annual income of 27 million dollars. After 14 years of research, baby *O. maya* (250 g) can now be produced on a semi-pilot scale using wild and hatchery-reared animals. Studies on other octopus species have demonstrated that captivity reduces animal sizes after several generations and multiple paternity has been observed; however, the genetic diversity of *O. maya* wild-broodstock is unknown and there is a lack of parental studies to assess multiple paternity. The objective of this study was to evaluate the genetic diversity of one batch of wild broodstock and one batch of newly-hatched octopus *O. maya*. Wild-broodstock were collected from Sisal, Yucatán; juveniles were produced using one female and three males. Eight microsatellite loci (*Omy2-0*, *Omy2-07*, *Omy2-37*, *Omy4-01*, *Omy4-11*, *Omy4-18*, *Omy4-20*, *Omy4-40*) were amplified (PCR) and genotyped by fragment analysis (Qiagxcel). Genetic parameters were calculated using GenALEx 6.5 and FSTAT 2.9.3; while parental contribution was done using COLONY 2.0. Broodstock showed moderate genetic diversity, with 7-15 alleles/locus and new alleles were found from previously reported. *Ho* ranged from 0.40-0.88, while *He* varied from 0.73-0.88. Two loci were in *HWE*. Five of the rest showed heterozygote-deficit and one heterozygote-excess. *Fis* average value of 0.28 ( $\pm 0.19$ ) shows a moderate endogamy. Parental contribution will be discussed.

# **INTERACTIONS AMONG CULTIVATED EASTERN OYSTERS *CRASSOSTREA VIRGINICA* AND THE UPPER DAMARISCOTTA RIVER, MAINE ECOSYSTEM: A COASTAL MONITORING AND MODELING STUDY**

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As part of the University of Maine NSF EPSCoR SEANET project, hourly LOBO buoy measurements, estuarine transects and water samples were taken every two weeks in fall of 2015 and throughout 2016 to better understand the factors which control oyster growing area productivity (and makes one site better than another), and factors which control fluxes, benthic impacts and nutrient recycling rates among the estuary, the phytoplankton, and the bivalves. Oyster growth was dependent on phytoplankton and detritus biomass, primary productivity, water temperature, and water velocity. Oysters influenced the ecosystem by recycling nitrogen and increasing ammonium fluxes, grazing the smaller size fractions of the phytoplankton and providing habitat for numerous species of invertebrates and fish. Farm-scale interactions will be presented in a GIS format (ShellGIS).

## **SIZE AT MATURITY AND REPRODUCTIVE BIOLOGY OF JONAH CRABS, *CANCER BOREALIS*, IN THE MID-ATLANTIC BIGHT**

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Jonah crab, *Cancer borealis*, landings have increased 5-fold since 1990. Historically, Jonah crabs are not a targeted species, but rather caught as by-catch in both lobster and Black sea bass traps; however, the Atlantic States Marine Fishery Commission (ASMFC) approved an Interstate Fishery Management Plan (FMP) for Jonah Crab which commenced June 2016. The FMP includes a minimum legal size (4.75") based on scarce and dated information. This research focuses on updating fundamental biological and reproductive information on Jonah crabs in the Mid-Atlantic Bight necessary to manage this species with an unknown stock status. It was hypothesized that sexual maturity in males will occur at carapace widths greater than 4.75". Generalized linear models based on morphometric analyses are used to determine the size at 50% sexual maturity. Both chela height and abdomen width are sexually dimorphic traits in Jonah crabs. Gonadal somatic indices (GSI) were generated to validate morphometric analyses and identify reproductive cycles. Expanding available information will allow for accurate appointment of minimum legal sizes, minimization of discard mortality, and increase understanding of stock reproductive capacity, thus improving management and long-term sustainability for the Jonah crab fishery.

## **EVIDENCE OF MULTIDECADAL RECRUITMENT IN THE OCEAN QUAHOG, *ARCTICA ISLANDICA*, IN THE WESTERN ATLANTIC OCEAN**

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Ocean quahogs (*Arctica islandica*) are the longest-lived, non-colonial animal known today, with maximum life span estimates exceeding 500 years. Ocean quahogs are a commercially important bivalve, inhabiting the continental shelf of the North Atlantic basin. Although considerable information exists on the growth and physiology of *A. islandica*, limited information is available regarding recruitment and age demographics; accordingly how the stock will respond to fishing is uncertain. A consequence is uncertainty in the biological reference points used to manage the fishery. The age of ocean quahogs that were fully recruited to the commercial fishery (>80 mm shell length) were examined from four sites covering the range of the stock within the US exclusive economic zone (EEZ) through analysis of annual growth lines in the hinge plate. Population age frequencies from each of the four sites were used to develop an age-length key for each site enabling reconstruction of population age frequencies. All four sites experienced an increase in recruitment beginning between approximately 1855 and 1905, depending upon site. Few quahogs were present in prior years. Thereafter, at each site, the populations reached carrying capacity through more or less continuous recruitment. The growth of several clams from each site was also assessed using three models (von Bertalanffy, Gompertz, and Tanaka's ALOG curve). This information will be used to develop a long-term recruitment index that will reduce uncertainty in the status of the stock and fishery by establishing a basis for improved biological reference points.

## DEVELOPMENT OF A FLAT OYSTER *OSTREA EDULIS* TRANSCRIPTOMIC DATABASE AND AN OLIGO-MICROARRAY TO EVALUATE GENE EXPRESSION PROFILES OF HAEMOCYTES IN RESPONSE TO BONAMIOSIS

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The production of the European flat oyster *Ostrea edulis* has declined mostly due to bonamiosis. Genomic resources, needed for genetic improvement of the species, are still scarce. A sequence database (OedulisDB) including haemocyte transcriptomic resources (7,159 unique sequences; 78.5% annotated; 984 specific immune-genes) was developed and used to design and validate the first immune-enriched oligo-microarray, which was used to assess the response of oyster haemocytes to bonamiosis. Gene expression of haemocytes from two strains of *O. edulis* (bonamiosis-tolerant and naïve) was evaluated at 1, 30 and 90 days after challenge (DAC) with *Bonamia ostreae*. The challenge involved submersing oysters in aerated seawater with high parasite load for 24 h. The narrow clustering of the five biological replicates at each experimental condition supported the consistency of the technical work performed and the cohesive response of oysters to the parasite. The tolerant strain showed much stronger response than the naïve at 1 DAC, and several apoptotic, cytoskeletal and immune-related genes were up-regulated, along with nine G protein-coupled receptors. This suggests that the tolerant strain, deriving from a bonamiosis endemic area, was selected for a quicker response to the parasite. Conversely, the naïve strain response at 1 DAC was weak, while a higher number of modulated genes were found at 90 DAC, some of them shared with those of the tolerant strain at 1 DAC, possibly reflecting a delayed response. The results contribute to a better understanding of the genetic response to this disease, providing key information for future studies aiming bonamiosis control.



## **ARE WE GETTING THE REAL PICTURE? TECHNICAL ARTIFACTS THAT BLUR THE CHARACTERIZATION OF SYMBIOTIC MICROBES IN BIVALVES**

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There have been a growing number of studies focusing on the characterization of microbial inhabitants of bivalve tissues, and on the role of these microbes in bivalve health and immunity. Prior investigations showed marked changes in the diversity and composition of microbial communities in conjunction with spatial, temporal, and environmental variables. Most previous work targeting the identification of microbes in the circulatory system of oysters was performed by collecting hemolymph from the adductor muscle via a notch made at the ventral margin of the shell. This collection technique, while practical and easy to implement, involves inserting a needle through soft tissues naturally exposed to seawater, and therefore has the potential to contaminate target samples. To test this hypothesis, the microbial make-up of hemolymph samples collected using this classical “notch” technique was contrasted with samples collected from a “hole” drilled through the right valve directly over the adductor muscle. Aliquot samples from each oyster collected using both techniques were grown (marine agar and TCBS) or submitted to bacterial community characterization using 16S rRNA amplicon sequencing on an Illumina MiSeq platform. Culture results showed no bacterial growth in all “hole” hemolymph samples, while cultures of “notch” hemolymph samples were positive. Further, the diversity and composition of bacterial communities in both sample types were markedly different within individual oysters. Finally, significant shifts in hemolymph microbiome composition were noticed based on the order of sampling using both techniques (e.g. “notch” first and “hole” second, or vice versa). These results highlight the importance of sound sampling methods for the characterization of symbiotic microbes, especially when highly sensitive techniques are used.

## **COLLAPSE OF THE OYSTER FISHERY IN APALACHICOLA BAY, FLORIDA**

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Apalachicola Bay is a highly productive estuarine system that has supported a successful commercial oyster fishery in the Florida Panhandle for decades. In past years, the fishery accounted for over 90 percent of the Florida annual oyster harvest but in late 2012, landings declined sharply. This decline coincided with decreased freshwater inputs to the estuary resulting from drought-related low river flow and increased water withdrawals from upstream metropolitan areas. The associated increases in estuarine salinity likely caused physiological stress in the oysters as well as increased disease and predation rates. Simultaneously, fishing pressure increased due to limited availability of oysters from other Gulf Coast fisheries following the Deepwater Horizon oil spill in 2010. The resultant collapse of the fishery prompted Florida officials to request a declaration of commercial fishery failure which was granted by the United States Department of Commerce on August 12, 2013. This declaration included funding for restoration of oyster habitat and monitoring of existing oyster resources. One component of that monitoring effort was a fisheries independent survey of oysters throughout Apalachicola Bay. Survey locations were randomly selected from areas defined as potential oyster habitat in a variety of existing shapefiles and datasets. A total of 161 stations were sampled; 91 of those stations had live oysters or hard substrate available for colonization. Live oysters were present at 66 of those 91 stations at a mean density of 17 oysters/m<sup>2</sup>. Results indicate that many areas originally considered potential oyster habitat have disappeared due to loss of substrate.

## **OYSTER REEF RESTORATION IN HARRIS CREEK, MARYLAND: A DIVER'S PERSPECTIVE**

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Oyster reef restoration has been ongoing in Harris Creek, MD, for 5 years. Hatchery-produced spat-on-shell have been planted on specifically targeted historical oyster bottom in high densities (5 million/acre). Drought and relatively calm winds provided remarkably clear water in late 2016. Pictures and video provide a rare look into estuarine oyster restoration.

## **HOW WILL THE EMERGING HARMFUL ALGAL BLOOM SPECIES *ALEXANDRIUM MONILATUM* IMPACT *CRASSOSTREA VIRGINICA* IN CHESAPEAKE BAY?**

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The toxin-producing harmful algal bloom (HAB) species *Alexandrium monilatum* has been associated with finfish and shellfish mortalities in the Gulf of Mexico. In the lower Chesapeake Bay, this dinoflagellate is an emerging HAB species; over the last decade, its range has expanded almost annually, with accompanying record-high cell densities. Bottom sediment samples were collected in southwestern Chesapeake Bay from 2014-2016 to map and quantify overwintering resting cysts of *A. monilatum*. These cysts were found to be widespread in the region, with densities as high as 90,000 cysts/cm<sup>3</sup>. Laboratory bioassays were used to investigate potential toxicity of *A. monilatum* to adult eastern oysters, *Crassostrea virginica*. Oyster valve gaping and mortality were monitored and histology was used to assess tissue damage. Oysters did not exhibit mortality or tissue damage in a 48-hour toxicity bioassay, however a 96-hour bioassay led to 67% mortality with erosion of gill and mantle epithelial tissues apparent in 94% of oysters exposed to *A. monilatum*. Oysters were deployed in the field in 2015 to assess effects of exposure before, during, and after a natural *A. monilatum* bloom. There was no mortality of deployed oysters, but minor epithelial erosion of the mantle was seen in half of the oysters sampled during the peak of the *A. monilatum* bloom. This dinoflagellate is clearly established in Chesapeake Bay and, at least under laboratory conditions, can adversely affect oyster health; however, the potential impacts of *A. monilatum* on oysters in natural waters require further investigation.

## **HIGH GENETIC LOAD IN MARINE ANIMALS: A REVIEW OF THE EVIDENCE**

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Marine invertebrates and fish are well known for their remarkable genetic diversity, which is commonly explained by high connectivity and large population sizes. Other potential sources of diversity, such as a higher mutation rate, have been much less considered, though evidence for a high genetic load in marine bivalves has been accumulating for nearly half a century. In this presentation, the results of a recent literature review of genetic load in marine animals is discussed, which includes molecular evolution approaches to examine mutational load ( $dn/ds$  ratios), genome-wide marker segregation datasets, and inbreeding load data. Overall, marine animals appear to have higher genetic load than terrestrial animals (higher  $dn/ds$  ratios, inbreeding load, and segregation distortion), though results are mixed for marine fish and data are lacking for many marine animal taxa. Bivalves demonstrate significantly increased marker segregation distortion compared to marine fish, though this may reflect a reporting bias and relatively larger maps or increased linkage group number in fish. Bivalves (oysters) appear to have the highest loads among marine animals, though more data are needed from other bivalve species and more marine invertebrate taxa generally. For oysters, a high load may be related to a chronically low effective population size that, in concert with a higher mutational rate, elevates the number of segregating deleterious mutations. Approaches to advance this area of research using high-throughput sequencing technologies are discussed, as well as strategies to distinguish genotype or sequence error from the true signal of marker segregation distortion in genome-scale linkage datasets.

## MECHANISTIC MODEL OF PARALYTIC SHELLFISH TOXIN ACCUMULATION IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*)

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The Pacific oyster (*Crassostrea gigas*) can be affected by various agents such as pathogens, parasites, xenobiotics, and toxic algae. This filter-feeder can accumulate phycotoxins while feeding on harmful algal blooms (HAB), including paralytic shellfish toxins (PST). To protect human health, closures of shellfish harvesting are declared during HAB, impacting the aquaculture industry.

Dynamic energy budget models (DEB) were demonstrated as powerful tools to quantify energy flows in bivalve species. Coupling bio-accumulation to DEB models has allowed to simulation of the dynamics of contaminants in organisms, taking account of uptake from environment, decontamination, and by assessing the influence of physiological variables on the contaminants kinetics.

To calibrate a mechanistic DEB model of accumulation/detoxification of PST, two sets of experiments were performed. Exposures of *C. gigas* to the toxic dinoflagellate *Alexandrium minutum* were run. As first experiment, the toxin accumulation was monitored at the individual scale. A significant relationship between PST concentrations in oyster tissues and individual ingestion rate was observed. Individuals that accumulated high concentrations also exhibited a high respiration rate. Damages caused by the toxins may account for this increase in the respiration rate of oysters, *i.e.* in their maintenance costs. Secondly, juvenile oysters were exposed over 2 months to *A. minutum*. Their growth, gametogenesis, and toxin contents were estimated weekly to quantify the effects of PSTs on the maintenance costs. Thus, the dose-response relationship was established. A DEB-based model will be produced from experimental data, and a validation will be performed with wild oysters.

## OYSTERS, SUSTAINABILITY, AND THE WORLD OF REFERENCE POINTS

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The use of biological reference points to sustainably manage eastern oyster *Crassostrea virginica* stocks has proven challenging due to the mortality inflicted by its pathogen *Perkinsus marinus*. A range of reference points has been evaluated in practice; only one has reference to Magnuson-Stevens maximum sustainable yield (*msy*) goals. Though not a statutory requirement for oyster fisheries, demonstrating sustainability would be much advanced by *msy*-based goals. Constant-abundance reference points have received most attention. These do not provide *msy*-based relationships, although their implementation has proven to be useful in management. Constant-abundance reference points stabilize the stock at a known (usually the present) state. The alternative *msy* approach is used to stabilize the stock at a state maximizing sustainable production. Formulation of *msy* reference points based on surplus production models has received considerable attention in federally-managed fisheries. The *msy* reference points based on surplus production formulations for oyster management and include in these formulations mortality from Dermo disease was evaluated. The  $A_{msy}$  (abundance at maximum sustainable yield) and  $F_{msy}$  (fishing mortality rate at maximum sustainable yield) was also calculated. Results suggest that *msy*-based reference points may be feasible and that this approach could then be used to identify management options that would support federal sustainability goals. Most state survey designs do not provide datasets consistent with surplus production estimates; in particular, the relationship between broodstock and recruitment and a good estimate of natural (non-disease) and disease mortality is required. Careful attention to survey design is an important first step in achieving improved management goals.

## INVASION DYNAMICS OF THE OCEAN QUAHOG, *ARCTICA ISLANDICA*, IN THE WESTERN ATLANTIC OCEAN

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The ocean quahog, *Arctica islandica*, is the oldest common non-colonial animal on earth. The species is pan-boreal, with maximum life span exceeding 500 yr. The ocean quahog supports a major east coast shellfish fishery in a latitudinal range unusually southern for the species as a whole. Recently developed age frequency data show that the animal began recruiting towards the end or shortly after the end of the Little Ice Age in the mid-Atlantic Bight to Georges Bank region. The population trajectory includes an extended time of low abundance, followed by a rapid increase in abundance over about a 30-yr period, thereafter capped. The suggested population dynamics explaining the three phases include a long period of low abundance during which initial recruits grew to maturity and population abundance rose to overcome an Allee effect, followed by a dramatic population expansion due to locally supported recruitment, after which the population equilibrated at carrying capacity. A population dynamics model of the process provides a satisfactory fit based on a specified age-at-maturity, a Tanaka growth curve, a logistic broodstock-recruitment relationship, and a Lorenzen mortality relationship. Inference from the modeling experience is that ocean quahogs, while living to very old age with the required low adult mortality rate, nevertheless, unlike other long-lived species, have a high (and very bivalve like) recruitment capacity which, however, is rarely observed as the species is typically at carrying capacity.



## USE OF RNASEQ TO IDENTIFY GENE EXPRESSION DIFFERENCES BETWEEN DERMO-RESISTANT AND -SUSCEPTIBLE EASTERN OYSTER FAMILIES

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Dermo resistance has long been a priority for eastern oyster breeding programs, yet the lack of simple methods to directly measure resistance limits genetic improvement of the trait. Laboratory challenge experiments were conducted to characterize Dermo-resistant and -susceptible phenotypes among selectively-bred oyster families. Oysters were injected with either a standardized dose of cultured *Perkinsus marinus* cells or basal salt solution and survival and parasite load were monitored over a six-week time period. Six oysters per family, per treatment were sub-sampled at each of 4 time points and mantle tissues were collected for both parasite quantitation via qPCR and RNA extraction. Forty-eight indexed RNAseq libraries were constructed from individuals belonging to the two most divergent families (3 individuals per family, per treatment, per time point). Libraries were pooled and sequenced on two Illumina HiSeq lanes, resulting in between 20 and 30 million reads per individual. Following QC, reads from each individual sequenced were mapped to a reference eastern oyster transcriptome assembly and transcript abundances were quantified. Results of differential gene expression analyses comparing treatments within a family, time points within a treatment within a family, and families within a treatment will be presented and the potential for using gene expression differences to further characterize Dermo-resistant and –susceptible phenotypes will be discussed.

**EFFECTS OF TUMBLING AND REFRIGERATION ON THE ABUNDANCE OF *VIBRIO VULNIFICUS* AND *VIBRIO PARAHAEMOLYTICUS* IN CULTURED OYSTERS (*CRASSOSTREA VIRGINICA*)**

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Off-bottom culture of oysters frequently involves routine handling (e.g. grading, splitting) that exposes oysters to elevated temperatures. Exposure to elevated temperatures and emersion from the water can cause growth of *Vibrio vulnificus* and *V. parahaemolyticus*, which are bacteria that are a public health concern when oysters are consumed raw. The effect of two common types of handling (tumbling and refrigeration) on *Vibrio* levels in oysters both initially and then over time after resubmersion were examined. Tumbling is used to grade oysters, improve shell appearance, and reduce biofouling, while refrigeration is used to suppress bacterial growth while emersed from the water. Tumbling (tumbled or not) and refrigeration (refrigerated overnight or not) for four different experimental treatment combinations during a 24-hr removal from the water, and examined the recovery of *Vibrio* spp. back to ambient levels following resubmersion were tested over 14 days. Preliminary results from two trials performed during the summer 2016 season will be presented.

## **EXPRESSED EXOME CAPTURE SEQUENCING (EECSEQ): A METHOD FOR COST-EFFECTIVE EXOME SEQUENCING OF NON-MODEL ORGANISMS**

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Exome capture is an effective tool for surveying the genome for loci under selection; however, traditional methods require *a priori* knowledge of exon sequences from previous genome and/or transcriptome methods. Here, a new method is presented that uses cDNA directly to enrich and capture genomic DNA for expressed exon sequences, eliminating the need for previous bioinformatic analysis and costly probe synthesis. To empirically test the efficiency and accuracy of EecSeq, 4 adult eastern oysters (*Crassostrea virginica*) were heat shocked at 36° C for 1 hour along with four control oysters kept at ambient temperature (20° C). Stranded mRNA libraries were prepared for two exposed and two control individuals; half of the libraries were sequenced and the other half used for EecSeq probe synthesis. Genomic DNA was extracted from all eight individuals, enriched via captured probes, and sequenced directly. Genotypes from individuals sequenced via both methods were compared for accuracy and congruence and the percent cover of the expressed transcriptome was assessed for all EecSeq individuals. Preliminary results will be discussed.

## THE POTENTIAL FOR ACCLIMATIZATION TO OCEAN ACIDIFICATION THROUGH EPIGENETIC MEMORY IN GEODUCK CLAMS

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Shellfish are a major contributor to marine aquaculture production. Shellfish production, however, is reduced by changes in seawater chemistry driven by ocean acidification (OA). There is a growing rationale for examining the potential for acclimatization to OA, where exposure to stressful environments can prepare organisms for future stress. The sensitivity of early life stages and the potential for geoduck clams to display acclimatization to ocean acidification were tested, and the role of DNA methylation has in the physiological response and environmental memory in a series of experiments. First, larval geoducks were exposed to ambient (~8.0) and low pH (~7.4) for 10 days and it was found that larval mortality decreased and shell size increased in low pH conditions. Second, juvenile geoduck were exposed to ambient (~8.0), low (~7.4) and lower (~7.0) pH for 23 days, placed in ambient common garden for several months, then re-exposed to ambient (~8.0) pH and low pH (~7.4) for another 23 days. In geoduck juveniles there was a size benefit of preconditioning to low pH. Juvenile growth initially declined at pH ~7.4 and 7.0 in the first exposure, but when replaced in the ambient conditions, the initial exposure to low pH resulted in compensatory growth, such that the juveniles grew larger. Growth in the pre-exposed juveniles was also more resistant to low pH in the second exposure. This suggests that acclimatization to OA can result in benefits to geoduck growth, with exposure memory that is potentially linked to epigenetic mechanisms such as DNA methylation.

## **PREVENTATIVE TREATMENTS FOR THE CONTROL OF BLISTER WORM *POLYDORA WEBSTERI* IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA***

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The polychaete *Polydora websteri*, or “blister worm”, burrows into the shells of several commercially important shellfish species, including those of the eastern oyster *Crassostrea virginica*. Infestations of blister worm can impact the marketability of oysters and the reputation of a farm and once established, it is very difficult to rid oysters of infesting adult blister worms. The life history of *P. websteri*, however, includes a dispersive planktonic stage; larval blister worms initially settle and form a mud tube on the external surface of the oyster shell. Recently settled worms do not have the protection of the burrow and are more vulnerable to harsh conditions than are adult worms. Over the past several years, we have worked with three oyster farms in Maine that employ to surface culture to test treatments, such as air-drying and washing of oysters, to reduce blister worm settlement in their cultured oysters. Results indicate that the intensity of infestation on even a single farm can vary 10-fold or more across years. Air-drying resulted in a substantial drop in the number of new blister worm burrows and washing in combination with air-drying may result in even greater reductions. The effect, however, was highly dependent on the timing of treatment application relative to local weather patterns, annual variation in the settlement of blister worms, and other factors external to the experiment and in no case was a 100% reduction in the occurrence of blister worm on cultured oysters observed.

## GAUGING THE CURRENT IMPACT OF BLISTER WORM ON THE ATLANTIC AND GULF COAST OYSTER CULTURE INDUSTRIES

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The blister worm, *Polydora websteri*, is a polychaete worm that burrows into the shells of several commercially important shellfish species, including those of the eastern oyster *Crassostrea virginica*. As oysters deposit new shell material over *P. websteri* burrows, the buildup of mud and detritus inside of the worm burrow creates unsightly blisters that can impact the marketability of oysters and the reputation of a farm. Historical records indicate a long history of *P. websteri* impacts on oyster populations and culture on both the US Atlantic and Gulf Coasts. Anecdotal information suggests that blister worm continues to be a significant pest in these regions and that the impact of blister worm has increased as oyster culture operations have expanded. In addition, there have been reported cases of substantial crop losses from heavy blister worm infestation. There are no reliable estimates, however, of the number of farms impacted by this pest, the severity of these impacts, and potential remedies that have been applied by oyster farms. Results will be presented of a survey of oyster farmers intended to gather first-hand information on the geographical scale over which problems with blister worm occur, the impact this pest species has on individual farm operations, how culture practices and farm location affect levels of blister worm infestation, and remedies that farmers have applied. This survey was first distributed to shellfish farmers in the northeastern US and recently extended to include growers in the mid-Atlantic and Gulf of Mexico regions.

## **MORE THAN TWENTY YEARS OF UNLOCKING THE MYSTERIES OF THE GENUS *PERKINSUS* USING MOLECULAR TOOLS**

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For more than twenty years molecular detection tools including polymerase chain reaction (PCR), in situ hybridization (ISH) using probes to target parasite cells in host tissues, and sequence-based phylogenetic analyses have been used to help identify new *Perkinsus* species around the world, synonymize species, and more accurately determine host and geographic ranges. These parasite species cannot be confidently identified and discriminated based on morphological characters alone, and some morphometrics such as size seem to vary within a particular *Perkinsus* species based on the host species. In 1995 three described *Perkinsus* species were recognized and two of those, *P. atlanticus* and *P. olseni*, have since been synonymized. Today there are seven described species, and the host and geographic ranges of many of these have been greatly expanded in recent years largely based on molecular data. At this time *Perkinsus olseni* is the most cosmopolitan and has been found in almost 20 different host species from five continents. Both *P. chesapeaki* and *P. beihaiensis* have been detected on three continents and in at least 8 and 15 hosts, respectively. *Perkinsus qugwadi*, *P. mediterraneus* and *P. honshuensis* appear to have more restricted host and geographic ranges, although these could expand with additional studies. Phylogenetic analyses based on DNA sequences indicate that *P. qugwadi* is the most divergent and basal of the *Perkinsus* species. *Perkinsus mediterraneus* and *P. honshuensis* sequences are very similar, although in phylogenetic analyses the clades have demonstrated reciprocal monophyly, and they are currently found only in Europe and Asia, respectively.

## **GENETIC VARIATION AMONG US ATLANTIC AND GULF COAST POPULATIONS OF THE OYSTER BLISTER WORM, *POLYDORA WEBSTERI***

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The blister worm, *Polydora websteri*, is a polychaete species that burrows into the shells of several commercially important shellfish species, including those of the eastern oyster *Crassostrea virginica*. The worm is a cosmopolitan species and historical records indicate that infestations of blister worm have impacted oyster culture worldwide. Blake and Hopkins described the life cycle for blister worms; females deposit a series of egg cases containing up to 50 eggs along their burrow. Larvae remain in the egg cases until they reach 3 segments in length; after exiting the burrow they complete development in the water column. These planktotrophic larvae are ready to settle onto calcareous substrates and metamorphose when they reach 17 segments, upwards of 45 days post-hatch. An extended planktonic period for this species can lead to high dispersal and high levels of connectivity and low levels of genetic differentiation among populations. In contrast, Haigler observed that some early hatching larvae consume undeveloped “nurse” eggs and may complete development to the 17-segment stage within the burrow. A protracted period of local larval development could limit dispersal and lead to higher levels of genetic differentiation among populations. Results of a preliminary investigation of the patterns of genetic variation among *P. websteri* sampled from the Atlantic and Gulf Coasts of North America will be presented. In addition, morphological and genetic evidence for the presence of other burrowing polychaete species among oysters and scallops from Northeast shellfish farms will be provided.



## **REDUCTION OF *VIBRIO CORALLIILYTICUS* AND *VIBRIO TUBIASHII*-INDUCED MORTALITIES OF LARVAL OYSTERS USING BACTERIOPHAGE THERAPY**

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The oyster pathogens, *Vibrio coralliilyticus* and *Vibrio tubiashii*, impact larval oysters and occasionally contribute to major mortality events in shellfish hatcheries. *Vibrio coralliilyticus* is also a significant coral pathogen causing coral bleaching and the loss of coral reefs worldwide. The LD<sub>50</sub> of four strains of *V. coralliilyticus* and two strains of *V. tubiashii* in 1-2 week-old eastern oyster (*Crassostrea virginica*) and Pacific oyster (*C. gigas*) larvae were determined. The LD<sub>50</sub> values, calculated at 6 days post challenge with *V. coralliilyticus*, ranged from  $1.1 \times 10^4$  to  $4.0 \times 10^4$  cfu/mL seawater. In contrast, the *V. tubiashii* strains were highly infectious toward eastern oyster larvae (LD<sub>50</sub> =  $3.8 \times 10^3$  to  $1.2 \times 10^4$  cfu/mL), but were noninfectious toward healthy Pacific oyster larvae.

Over the past few years, we have been isolating and characterizing phages against these vibrios with the goal of developing an effective treatment to reduce mortalities of larval shellfish in hatchery settings. Tailed, lytic phages specific for *V. coralliilyticus* and *V. tubiashii* were isolated from Hawaiian seawater, characterized for host specificity and morphologically (by scanning electron microscopy), and some have been DNA sequenced. To date, phages against both *Vibrio* species have been isolated and have shown that a mixture (cocktail) of these phages are an effective treatment to reduce mortalities in larval eastern and Pacific oysters by over 70% after challenge of the larvae with high levels of both *Vibrio* species. This bacteriophage-based therapy is being commercialized with industry and university partners for use in hatcheries.

## **USING NEXT GENERATION SEQUENCING TO IDENTIFY LOCAL ADAPTATION TO SALINITY IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*, IN THE LOUISIANA GULF COAST**

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Salinity plays a key role in the distribution of American oysters, *Crassostrea virginica*, an estuarine species that provides food, shelter, and nursery habitat for numerous species in the Louisiana Gulf Coast. Climate change can affect salinity by altering precipitation patterns which in turn alter the frequency, location, and volume of freshwater inflow to estuaries. To improve accuracy of predicted responses of *C. virginica* to climate change, we want to test if oyster populations are locally adapted to salinity. Restriction Site Associated DNA Sequencing (RADseq) will be used to identify potential SNPs responsible for differences in salinity tolerance between two wild populations of oysters: Vermilion Bay (average salinity of 5.6) and Lake Fortuna (average salinity of 14.9). The STACKS software will be used to align fragments and then compare SNP frequencies across populations. From the RAD sequencing data, it is expected that the SNP's responsible for variation in salinity tolerance across populations will be identified. A one-year reciprocal transplant experiment will identify potential alleles that are under selection in the field. Identifying markers associated with salinity tolerance will assist in the selection of breeding stocks for use in restoration or aquaculture.

## **UNLOCKING THE SECRETS IN LARVAL SHELLS: LINKING LARVAL EXPOSURE TO PERFORMANCE *IN SITU***

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The larval shell of the California mussel, *Mytilus californianus* contains a record of seawater pH during dispersal in its trace element biogeochemistry. This proxy tool allows us to evaluate for the first time in the field the role of environmental exposures of larvae in shaping the performance of individuals later in life (carry-over effects). *M. californianus* larvae disperse along coastal California throughout the summer as upwelling events occur and relax. Juvenile mussels were collected as they recruited to shore, and their condition and shell traits were compared in relation to the oceanographic conditions that the larval stages experienced. The U/Ca in the larval shells of the individuals was used as a proxy for seawater pH exposure, in addition to regional oceanographic data. With more cumulative exposure to upwelling conditions, the shape of the larval shell changed, and juvenile shell area decreased, while juvenile shell thickness increased. These properties more loosely correlated with seawater pH, approximated with larval shell U/Ca. These correlations provide insight for interpreting longer time series of recruit biological data and direct measurements of seawater pH. This evidence of carry-over effects related to upwelling exposure provide clues towards the role of larval experience in shaping pH and temperature tolerance in benthic *M. californianus* populations.

## TISSUE-SPECIFIC EXPRESSION OF A NOVEL EXPANDED IMMUNE GENE FAMILY IN THE EASTERN OYSTER

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The population of the eastern oyster, *Crassostrea virginica*, along the east coast of the United States has suffered precipitous declines in both the natural environment and in the setting of oyster hatcheries due to bacterial and protozoan pathogens. Different populations face diverse pressures by the microscopic communities with which they interact, and oysters are unique in their ability to dynamically respond to these environments in terms of gene expression. Transcriptome analysis has revealed several diversified immune gene families in the eastern oyster. The GTPase of the immunity-associated proteins (GIMAP) is a differentially expanded immune gene family in the eastern oyster estimated to have ~26-28 genes, that has been found to play a critical role in B-cell survival and apoptosis-mediation in humans. Understanding intra-tissue variation in GIMAP gene expression would provide enhanced insight into the role this gene family plays in immunity. The present study performed q-PCR analyses of varied tissue samples from resistant and susceptible families of eastern oysters challenged with either *Perkinsus marinus* or *Vibrio anguillarum* in order to evaluate this intra-tissue variation in GIMAP gene family expression. Characterization of tissue-specific variation in GIMAP expression has provided valuable insight regarding the potential role of this gene family in the innate immune response to disease. Future research will complete comparative phylogenetic analyses to analyze the structure, composition, and patterns of GIMAP gene family organization in the eastern oyster as compared to closely related bivalves, such as the Pacific oyster, and distantly related species, such as humans and zebrafish.

## **COORDINATED BEATING OF GILL LATERAL CELL CILIA OF *MYTILUS EDULIS* AND *CRASSOSTREA VIRGINICA* INVOLVES NEURONAL INNERVATION AND FUNCTIONING GAP JUNCTIONS**

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Gill lateral cell (GLC) cilia of bivalves drive pumps providing water currents for feeding, respiration and waste elimination. GLC in *Mytilus edulis* and *Crassostrea virginica* are innervated by serotonergic/dopaminergic nerves. It is unclear if each GLC is innervated. GLC have gap junctions (GJ). Questions arise if GJ coordinate beating. It was hypothesized that GJ coordinate GLC cilia beating in bivalves. Using immunohistofluorescence we found GJ present. To determine if GJ are involved in coordinating cilia beating, preparation where filaments were divided into proximal and distal sections by petroleum jelly that prevented diffusion of drugs from side to side was used. The branchial nerve entering the proximal ends was stimulated. Drugs were applied to distal ends. Cilia beating was measured at both ends. Lindane caused dose-dependent decreased beating. Lindane reduces GJ permeability and increases intracellular Ca<sup>2+</sup>, so diltiazem was used, a Ca<sup>2+</sup> channel blocker, and found diltiazem did not alter beating, nor the actions of lindane. It was observed in *M. edulis* and *C. virginica* that clusters of GLC on the same gill filaments have cilia beating at coordinated rates, but at disorganized rates with 10<sup>-5</sup>M lindane, suggesting, like cardiac tissue, there are pacemaker and follower cells. Results show the physiological functioning of GJ involved in coordinating GLC cilia beating in two bivalve species and neuronal innervation in conjunction with functioning GJ are necessary to coordinate beating rates. Disrupting either one interferes with normal responses. This work was supported by 690340047 of PSC-CUNY, 2R25GM06003 of the Bridge Program of NIGMS and the Carnegie Foundation.

## **ASSESSMENT OF PLOIDY ON NEUROTOXIC SHELLFISH POISONING TOXINS IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) BY LABORATORY EXPOSURE TO *KARENIA BREVIS***

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Brevetoxins (BTXs) produced by the dinoflagellate, *Karenia brevis*, can accumulate in eastern oysters (*Crassostrea virginica*) and cause neurotoxic shellfish poisoning in humans. Eastern oysters destined for human consumption in the US originate increasingly from commercially produced seed and production has improved with the incorporation of triploids. The metabolic activity of triploid oysters may differ from that of diploids affecting their potential to accumulate and depurate toxins such as BTXs. The aim of this study was to determine the effect of ploidy on BTX accumulation and elimination in *C. virginica*. In October 2016, individual adult diploid and triploid *C. virginica* (Cedar Point, AL) were exposed daily to whole cells of *K. brevis* at a high bloom concentration (1500 cells mL<sup>-1</sup>) for four days. Three individuals per ploidy were sampled following each day of exposure to *K. brevis* and one individual per ploidy was sampled on days 4, 7, and 14 after exposure had finished. The clearance rate of *K. brevis* cells (between 0-2h) was significantly reduced between the first and last day of exposure, although no differences were observed between ploidy. Cytotoxic activity was recorded in oysters after 1d of exposure (6-8 mg.kg<sup>-1</sup>) and increased to 45-70 mg.kg<sup>-1</sup> after 4d exposure to *K. brevis*. The concentration of BTX biomarkers in oysters (BTX-B2 and desoxy BTX-B2) increased throughout exposure, reaching greater than 1.6 mg.kg<sup>-1</sup> after 3d of exposure (guidance level, 0.8 mg.kg<sup>-1</sup> BTX-2 eq). Results will be used to assess regulatory monitoring and management of BTXs in this commercially significant species.

## **ONTOGENETIC TRANSITIONS IN FEEDING MODES OF THE BLUE MUSSEL *MYTILUS EDULIS* AND THE RIBBED MUSSEL *GEUKENSIA DEMISSA***

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Most molluscs, including bivalves and gastropods, have a very small (~100-200µm) planktonic feeding larval stage (veliger), with specialized structures for capturing algae. Larvae undergo a radical metamorphosis that produces juveniles (~300-500µm) with structures similar to adults, but much smaller, and generally do not attain full adult form until animals are often more than a year old. Currently, the general assumption is that of a veliger suspension-feeding mode being immediately replaced by the adult feeding mode, with the ctenidium (= gill) as the main particle-collecting organ. However, simple calculations of the nutritional needs of bivalves and the particle capturing capability of small sized gills make this unlikely. Thus animals, particularly in low food environments, may have an enhanced foot for pedal feeding, allowing them to gain nutrition from the benthos. The objective of this project is to compare, contrast, and determine the relative importance of different feeding modes during early ontogenetic stages of several species of suspension-feeding molluscs. To this end, scanning electron microscopy (SEM) will be used to determine patterns of ciliation and morphology of the developing foot and developmental morphology of the gill across ontogeny of newly metamorphosed juveniles of two bivalve species. Both soft tissue (gill and foot) and shell morphological changes when larvae are grown under different feeding environments (planktonic versus benthic food) will be examined. These examinations will allow assessment of performance in early juveniles when given different food choices, links between functional morphology and performance, and the relative contributions of pedal versus suspension feeding at early life stages.

## **EFFECTS OF OCEAN ACIDIFICATION ON LARVAL DEVELOPMENT AND SURVIVAL OF *MYTILUS EDULIS***

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Ocean acidification (OA), specifically the decreasing saturation state of aragonite associated with decreasing pH, has been shown to decrease survivorship and growth, and impair shell building in the larvae of calcifying organisms. An experiment was conducted to assess OA resilience in an ecologically and commercially important species, *Mytilus edulis*, across early life history stages and among offspring from populations with different environmental histories. This experiment is part of an on-going study testing for acclimation and cross-generational adaptation to OA as well as tradeoffs in growth and development.

Mussels were sampled from populations on the New York and Connecticut shores of the Long Island Sound. Mussels were conditioned under three OA treatments (ambient, present extreme, and future  $\Omega$ /pH) and induced to spawn. The resulting larvae from each population were divided among the three OA treatments and raised at their respective OA conditions throughout development in a static system. The effects of OA on survivorship, growth, and development will be compared as a function of OA treatment and source population. Preliminary results and conclusions will be presented.



## **LEGISLATIVE UPDATES FOR THE TEXAS OYSTER FISHERY, RESTORATION, AND AQUACULTURE**

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The Texas oyster fishery covers about 39,000 acres of public reefs from primarily Copano Bay to Galveston Bay. Harvest occurs from November through April. In addition, about 2,300 acres of private leases for cultch planting or depuration in only Galveston Bay are harvested year-round by leaseholders. Over the past decade, oyster harvesters on public grounds have not typically reached daily sack limits. Independent sampling by Texas Parks and Wildlife Department (TPWD) found catch per unit effort rates diminishing since 2002. Sustainability is understood by commercial oyster industry members, which led to an agreement of a sack tax that has assisted, along with federal support, with placing cultch on approximately 435 acres from 2009-2014. Non-governmental organizations, such as Galveston Bay Foundation, The Nature Conservancy, and TAMU-CC have been conducting oyster reef restoration projects in Galveston Bay, Matagorda Bay, and Copano Bay, respectively. All private leases agreements expire in early 2017, which has allowed TPWD and the Texas General Land Office to examine and suggest changes to regulations for the fishery, restoration efforts, and aquaculture. Potential changes included the expansion of private leases beyond Galveston Bay, the utilization of private industry to assist with reef restoration, and restoration leases being off-limits to harvesting for 15 years. Stakeholder meetings from spring through fall resulted in TPWD proposing an increase in lease fees to \$60/acre but then reduced, active use criteria, new TGLO fees, but no restoration or aquaculture changes. Texas Parks and Wildlife Commissioners will meet in January 2017 for final approval.

## TEMPORAL DISTRIBUTION OF L-DOPA-CONTAINING PROTEINS INVOLVED IN OYSTER SHELL FORMATION

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Marine bivalve organisms such as *Crassostrea virginica* (eastern oyster) produce structural proteins that are critical in adhesive strategies and shell biomineralization. The unique properties of these proteins derive from the amino acid composition. L-3,4-dihydroxyphenylalanine (L-dopa), which is a unique key amino acid in the cross-linking of these proteins, can be considered a biomarker for identification and localization of shell formation proteins. The focus of this research was to determine the distribution of L-dopa-containing proteins involved in the process of biomineralization in the eastern oyster, *Crassostrea virginica*. Three organismal compartments were identified as sources of L-dopa protein pre-cursors: hemocytes, cell-free hemolymph, and mantle tissue. Hemolymph was harvested from the adductor muscle of notched oysters and hemocytes were subsequently collected via hemolymph centrifugation. Mantle tissue was collected from either side of the notch area. The product of repair, nascent shell deposited in the notch, was collected from the notch site at discrete time points post-notching. Amino acid composition was determined via anion exchange HPLC with pulsed amperometric detection. L-dopa concentration was related to the time since notching: higher levels of L-dopa were found in hemocytes collected at 24-28 hours post-notching and in hemolymph at 96 hours post notching, indicating an induction of resources for notch repair. L-dopa was found in higher levels in nascent repair shell and declined as the shell aged indicating protein cross-linking. These data support the premise that L-dopa-containing proteins are involved in oyster shell formation and that they are distributed among several components and products within the system.

## **THE APPLICATION OF OPERATIONAL NATIONAL WEATHER SERVICE PRECIPITATION DATA TO SHELLFISH HARVESTING MANAGEMENT**

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The National Oceanic and Atmospheric Administration Southeast River Forecast Center (SERFC), a field office of the National Weather Service, monitors hydrometeorological conditions and prepares streamflow and level forecasts for rivers in the Southeast U.S. and Puerto Rico. To accomplish this primary mission, SERFC hydrometeorologists continually process hourly Multi-sensor Precipitation Estimate (MPE) data, a program that combines weather radar rainfall estimates and rain gauge data to produce calibrated precipitation estimates for use in river forecasting.

Recently, the SERFC has been working with the Division of Aquaculture, Florida Department of Agriculture and Consumer Services, and the Shellfish Sanitation and Recreational Water Quality Section in the North Carolina Department of Environmental Quality, agencies who are charged with monitoring and closing shellfish harvest areas in their respective states for the protection of public health. Precipitation data is a key variable in making harvest area closure decisions and this collaborative effort is leveraging SERFC's continuously calibrated precipitation estimates to provide more reliable 24-hour rainfall totals, an indicator of the potential for pollution runoff, to decide if shellfish harvesting waters should remain open or be closed.

This presentation will describe the production of MPE data, the variety of formats that MPE data is packaged in, and how MPE is applied operationally in harvest area closure decisions. Additionally, topical forecast data will be described that may be applicable for future shellfish closure decision and resource management.

# **INFLUENCE OF REEF HEIGHT AND OYSTER AGGREGATION ON ESTIMATES OF *CRASSOSTREA VIRGINICA* CLEARANCE RATES IN THE GUANA TOLOMATO MATANZAS NATIONAL ESTUARINE RESEARCH RESERVE (GTM NERR), FL**

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Oysters have been lauded for being “ecosystem engineers” and providing a variety of ecosystem services. Some of these services, like improved water quality and control of harmful algal blooms, are closely linked to oyster filtration. Frequently, clearance rates attributed to oysters have been the result of laboratory studies. These observations may be incorporated into models meant to simulate the system-wide level impact of oyster reefs. There is controversy as to how well lab data can be applied to systems of interest, and the studies that have extended this exploration to field-based measurements are limited.

This work was focused on characterizing in-field *Crassostrea virginica* clearance rates in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR) in the St. Augustine region of Florida. Along with this objective, two-subquestions were explored to support better clearance estimates: 1) how do clearance rates differ on higher and lower points on reefs? Prior studies suggest reef elevation may affect certain characteristics of oyster success such as growth and mortality; filtration may show similar patterns, and 2) how do clearance rates attenuate as oysters are aggregated? As oysters become clustered on reefs, re-filtration occurs and should be taken into account when upscaling study results to reef-scale estimates.

Biodeposition field methods employing sediment traps were adapted from those in Yu and Culver (1999) and Sroczynska et al (2012) to explore reef height. To explore the effect of oyster aggregation, a flow-through seawater system comprised of upwelling columns was constructed. Data and conclusions from these experiments are presented.

## **VIBRIO SP. ABUNDANCE IN OYSTERS, WATER AND SEDIMENT SAMPLED FROM VARIOUS TREATMENT/CULTURE CONDITIONS**

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Both *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) can cause gastroenteritis in humans when oysters are consumed raw. With increasing water temperatures, the concentration of *Vibrio* sp. bacteria increases in estuarine environments and can accumulate in the tissues of Eastern oysters as part of their filter feeding process. As water temperatures continue to rise due to climate change, predicting and mitigating risks of *Vibrio* outbreaks has become an important public health issue. Varying harvesting methods were used to give insight on whether certain techniques can reduce the concentrations of Vp within oysters. Four oyster farms at two growing areas, one subtidal and one intertidal, were sampled at two-week intervals between April and October 2015 in southeast Massachusetts. This study used a quantitative multiplex polymerase chain reaction (qmPCR) test method to assess Vp and Vv levels in the sediment, water column and oyster. The samples were also evaluated for occurrence of two pathogenic genes associated with the ability of Vp to cause the disease in humans. Vp was found to have a strong positive correlation with water temperature. Placing oysters in trays within the water column of the subtidal growing area depurates some of the total and pathogenic bacterial load. Growing out the oysters in trays along the bottom of the intertidal flat had reduced bacterial burdens; however, due to significantly different concentrations of Vp in the water and sediment between farms, the reduced bacterial levels cannot be fully credited to harvesting method (work funded by NOAA NAOAR4170071, WHOI Subaward #A101190).

## USING LONG-TERM MONITORING DATA TO DERIVE REGIONALLY SPECIFIC GROWTH AND MORTALITY RELATIONSHIPS FOR LOUISIANA OYSTERS

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In Louisiana, eastern oysters (*Crassostrea virginica*) deliver a suite of valuable ecosystem services and support the largest commercial oyster fishery in the United States. Current management and restoration advice is based on natural mortality and growth assumptions derived from work performed outside the Gulf of Mexico; however, compared to Atlantic populations, Louisiana's oyster reefs experience lower salinities (< 15), a shorter overwintering period, and prolonged exposure to high summer temperatures. Additionally, changes in coastal and riverine management have resulted in significant shifts in the timing and variation of salinity and temperature over important oyster areas. To better manage Louisiana oysters in the face of these changes, our research aims to update environmentally-driven mortality and growth assumptions for Louisiana oysters. Data derived from two large-scale, long-term (1988-2013) reef monitoring projects conducted by Louisiana Department of Wildlife and Fisheries on public oyster reefs were analyzed. Finite distribution mixture modeling of monthly dredge samples with directed tray studies to quantify growth and mortality rates across spat ( $\leq 25$  mm), seed (25-75 mm) and sack ( $> 75$  mm) oyster size classes were combined. Generalized Additive Model and response surface-model frameworks were used to examine the non-linear relationship between estimates of mortality and growth and long-term trends in salinity and temperature. Louisiana oysters were found to perform better at lower salinities than previous models have indicated. The results are currently being used to better parameterize stock assessment and habitat suitability models for the management and restoration of Louisiana oysters.

## **OYSTER COMMUNITIES ON ARTIFICIAL SUBSTRATES OF VARYING COMPLEXITY IN CHESAPEAKE BAY**

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Shoreline development has increased the presence of vertical seawalls in urban coastal areas, but green engineering techniques can be used to enhance inhospitable seawall structures and restore natural communities. Oysters serve as ecosystem engineers and can enhance associated communities. The effects of artificial concrete substrates deployed as a “green engineering” technique along seawalls at two field sites in Chesapeake Bay that differed in water-flow rates were investigated. The substrates included concrete tiles with three different complexities (surface rugosity), two different densities of seeded oysters, unseeded tiles, and a control tile of the existing seawall. Differences in community structure as well as oyster mortality and settlement were assessed to examine the effectiveness of these alternative substrates. After three months of deployment, oysters on flat tiles had greater mortality than those on complex tiles, and at one site, survival tended to increase at higher oyster densities. There was higher oyster spat settlement on more-complex tiles, due to oysters surviving in crevices. Community structure was similar among treatments, but there tended to be more mobile species on complex compared to flat tiles. There was higher barnacle and algal settlement at the field site with higher flow rates.

## **UNDERSTANDING *POLYDORA WEBSTERI* INFESTATION IN *CRASSOSTREA VIRGINICA*: APPLICATIONS FOR OFF-BOTTOM OYSTER FARMING**

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The mud worm, *Polydora* spp., settle into the shell of oysters, creating mud filled burrows within the valves. The oyster responds by secreting a nacreous layer, creating a “mud blister”. The damage caused by mud worms degrades the overall condition of the eastern oyster (*Crassostrea virginica*). Oysters containing mud blisters are not favorable for the lucrative half shell market, as they are not as aesthetically appealing. Techniques are known to remove the worms, but the blisters remain.

The aim of this study was to test techniques that favor mud blister healing in eastern oysters for oyster farmers. Tidal heights, ploidy, and combinations of the two were tested as different factors that increase mud blister healing. Mud worms were removed from oysters using a brine dip and then the oysters were sorted by ploidy into mesh BST bags to grow at intertidal and subtidal heights for 6 weeks. One oyster per bag was collected each week.

Subtidal oysters were consistently found to have significantly higher amounts of re-infestation than intertidal oysters, regardless of the ploidy of the oyster. Amongst intertidal oysters, there was no significant effect of ploidy or time on percent change in visible mud worm burrows and blisters over the experiment. Further research is necessary to understand the temporal dynamics of oyster shell recovery and treatments that may affect that rate.



# PHENOTYPIC, GENETIC, AND EPIGENETIC POPULATION STRUCTURE OF OLYMPIA OYSTERS IN PUGET SOUND, WASHINGTON

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Olympia oysters (*Ostrea lurida*) have received considerable attention with regard to conservation and restoration along the west coast of the United States; however there is limited information on local population structure. Using oysters raised under common conditions for up to two generations (F1s and F2s), this project tested for evidence of adaptive differentiation among three populations of in Puget Sound, Washington; Oyster Bay, Dabob Bay, and Fidalgo Bay. Reproductive output and growth rate were used as proxies for fitness. Adult F1 oysters from Oyster Bay were significantly larger than those from Dabob Bay and demonstrated earlier timing of reproduction. In F2 oysters, protoshell length in hatchery-raised larvae varied significantly among populations, with larvae from Oyster Bay significantly larger than those from Dabob Bay. To measure juvenile growth rate, larvae from each population of the same size and age cohort were established on 10 cm x 10 cm PVC tiles and grown out in Clam Bay for 10 weeks. By 6 weeks, a similar pattern was observed where oysters from Oyster Bay were significantly larger. To understand how such phenotypic variation is inherited (i.e. genetic or epigenetic), reduced-representation genomic sequencing (2bRAD) and bisulfite sequencing (BS-Seq) was conducted on adult F1 oysters from Oyster Bay and Dabob Bay. Using a preliminary draft of the Olympia oyster genome, these data indicate methylation patterns are distinct among populations, even after a generation in a common environment.

## POPULATION GENOMICS AND PHYLOGEOGRAPHY OF THE OLYMPIA OYSTER (*OSTREA LURIDA*)

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Understanding the evolutionary processes that cause population divergence is crucial to predicting how species will respond to rapid global environmental change. The Olympia oyster, *Ostrea lurida*, is patchily distributed from California to the central coast of Canada. As an ecologically and commercially important shellfish species, understanding the underlying pattern of demographic population structure is critical for informed management practices. This pattern could be consistent with a null model of no significant population structure, a continuous isolation-by-distance (IBD) model, or contain regional blocks of genetic similarity separated by barriers to gene flow. It was hypothesized that, by using thousands of genetic markers, evidence of regional population structure and IBD would be observed across the species range. DNA was extracted from adult Olympia oysters sampled across 20 sites from Klaskino Inlet, Vancouver Island (50° 17'55") to San Diego Bay, CA (32° 36'9"), as well as 15 individuals from the sister species, *O. conchaphila*. Reduced representation Genotype-by-Sequencing libraries (GBS) were constructed to genotype thousands of single nucleotide polymorphisms (SNPs). A Mantel test using  $F_{ST}$  and water distance rejected the hypothesis of pure isolation by distance. Using a new method to visualize spatial population structure called EEMS (Estimated Effective Migration Surfaces), a phylogeographic divide was identified between Puget Sound, WA and Willapa Bay, WA. Although this method cannot distinguish between different scenarios that could produce the observed spatial structure, it supports the rejection of both a continuous IBD model and the null model of no significant genetic structure.

## **CRYOPRESERVATION OF TROCHOPHORE LARVAE IN THE NORTHERN QUAHOG (=HARD CLAM), *MERCENARIA MERCENARIA***

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Cryopreservation technology can advance and improve the sustainability of fish and shellfish aquaculture and fisheries. Studies on germplasm cryopreservation in fish and shellfish have been performed in over 200 species with a focus on sperm cryopreservation in aquaculture fishes such as salmonids, carps, and catfish. For shellfish, trochophore cryopreservation has been reported in two oyster species (the Pacific and eastern oysters). To our knowledge, no study has been reported for the hard clam, *Mercenaria mercenaria*, an important aquaculture species in the U.S. The goal of this study was to develop a preliminary protocol for larval cryopreservation in the hard clam. The objectives were to evaluate the: 1) toxicity of cryoprotectants on trochophore larvae at exposure time of 1, 15, 30, 45, 60, and 75 min; 2) effect of cooling rates (5, 10, 20, 30°C/min from 5°C to -80°C) on post-thaw trochophore viability and D-stage survival. Cryoprotectants including dimethyl sulfoxide (DMSO), propylene glycol, ethylene glycol, and glycerol at final concentrations of 5, 10, 15, 20% (v/v) were examined, and the results indicated that DMSO and propylene glycol showed less toxicity at concentrations of 5 and 10% (v/v). Data about trochophore survival after cryopreservation are currently in the collection process. The viability of post-thaw larvae was estimated using neutral red stain immediately after thawing and assessing the survival to D-stage. This study is the first report on larval cryopreservation for *M. mercenaria* and will have wide application for germplasm preservation and breeding for aquaculture and genetic programs.

## **MULTIPLE USES OF MOLLUSCS**

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Marine molluscs, bivalves in particular, play a dominant role in coastal ecosystems. Besides the economic value for fishery and aquaculture, they provide food for birds and benthos, a habitat for a large number of species, they regulate water quality and sequester carbon and nitrogen, and they are a rich source for human nutrition. As eco-engineers, epibenthic bivalve beds are used for coastal defence and nature conservation. They also produce significant amounts of shell material that has many applications, not only as bulk product but also for cultural applications.

These functions can be defined as goods and services. This concept provides a framework for description and analysis of the role of molluscs in the ecosystem, and a basis for addressing a wide range of topics related to the use of molluscs for exploitation, restoration and coastal management.

Understanding goods and services may improve management decisions. A way to improve decision making is to valorise the goods and services. The economic revenues of aquaculture are based on the market value of harvestable products, while the economic values of ecosystem services are often not adequately quantified and not fully captured in commercial markets. Both for market and political decisions, techniques are needed that can be used to make economic values of mollusc ecosystem services explicit, provided full knowledge of the goods and services is available.

In the presentation, state of the art in-multiple uses of molluscs will be reviewed.

## **TETRODOTOXIN (TTX) IN MUSSELS AND OYSTERS IN THE NETHERLANDS**

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TTX is known as a highly potent neurotoxin that occurs in a range of marine species, of which various species of puffer fish of the family *Tetraodontidae* are most prominent. The spread of the neurotoxin, reported as a threat to human health in Asian countries, is observed in the Pacific and Mediterranean. In 2015 and 2016 TTX was first analysed and detected in mussels and oysters in culture areas in the Oosterschelde SW Netherlands. In July 2016 concentrations were detected up to levels of 253 µg/kg and 101 µg/kg in shellfish meat of oysters and mussels respectively. Prior to 2015 no data are available.

As a response the national food safety authority (NVWA) prepared a Risk Assessment to determine a safe standard. Due to low data availability of dose-response relationships, a provisional standard for consumption was set at a level of 20 µg/kg shellfish meat (LOQ). No common safety levels exist yet at European level as is the case for other biotoxins.

Bivalve harvesting from areas with TTX occurrence above action limit was closed for about 5 weeks in 2016. As this was just at the opening of the season, considerable socio-economic damage occurred as consumers refrained from bivalve consumption, even though control was intensified and mussels on the market did not contain TTX.

The event raised discussion about toxicological risk assessment and management, given the low safety standard and the economic impact. A research program was initiated to detect sources and to analyse dose-effect relationships. First results will be discussed.

## **HARVESTING OYSTERS FOR FOOD WHILE CONSERVING REEFS AS HABITAT: A SHELL-BUDGET MODEL APPROACH**

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Although ecosystem services of oyster reefs are well-described and threats to their existence well-documented, methods for restoring oyster reefs in the face of environmental impacts and as a component of a sustainable fishery are only now emerging. A numerical model of oyster population dynamics, which defines sustainability as no-net-loss of shell, has been applied to public oyster grounds in Louisiana. The model is used to simultaneously and minimally estimate a fishable abundance of oysters (*Crassostrea virginica*), while maintaining a constant surficial shell resource. Model inputs are oyster abundance and size, reef size, oyster density, and substrate density. Oyster growth and mortality rates, and cultch loss rate are parameterized. Model outputs include estimates of sustainable harvest and reef mass loss or gain. Thus, the model simultaneously balances recruitment, natural mortality (= shell addition) and shell loss, determines the population requirements to achieve this balance, and allocates the remainder of production to the fishery. For exclusive restoration efforts (no fishing allowed), shell accretion is a metric for restoration success. For an oyster fishery, shell is invariably removed, but sustainable harvest is estimated as one that maintains the surficial shell of the reef itself.

# **EVOLUTION OF MITOTIC INSTABILITY IN TRIPLOID AND TETRAPLOID PROGENY OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* OVER TIME USING CYTOGENETIC TECHNIQUES**

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The commercial production of triploids, and the creation of tetraploid brood stock to support it, has become a successful technique in aquaculture of the eastern oyster, *Crassostrea virginica*. Triploid oysters are valued for their sterility that generates several advantages for oyster culture, such as reduced gonadal development, allowing for higher growth rates and superior market quality during the reproductive season. Nevertheless, tetraploids undergo chromosome loss becoming unstable (mosaics), causing two principal concerns: the fate of the commercial triploid seed produced from mosaic tetraploid brood stock and the fate of future generations of tetraploid brood stock if mosaics are used to create them.

In previous work at ABC, the extent of aneuploidy in triploid embryos, produced from both non-mosaic and mosaic male and female tetraploids, showed non-statistically significant differences for chromosome loss (aneuploidy). In the present work, the level of aneuploidy from triploid and tetraploid juveniles, produced from both mosaic and non-mosaic male and female tetraploids, were analyzed. The main objective of this work was to affirm the tendency for polyploid oysters to lose chromosomes over time.

Similar to embryos, non-statistically significant differences for chromosome loss in triploid juveniles produced from mosaic and non-mosaic tetraploid x diploid crosses, from either male or female tetraploids, were observed; however, there were statistically significant differences for chromosome loss in tetraploid juveniles produced from mosaic and non-mosaic tetraploids. These results confirm that chromosome instability, as manifest in mosaic tetraploids, are of little concern for producing triploids, but are clearly problematic for tetraploid breeding.

## **PLEASE DON'T GO: AN ASSESSMENT OF SIMULTANEOUS SEEDING OF ADULT AND JUVENILE MUSSELS AS A METHOD TO INCREASE SPAT RETENTION**

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The New Zealand mussel industry is worth around NZ\$250 million and accounts for over 70% of the total export earnings from aquaculture. This important industry is heavily reliant on natural seed supply and, as a result, can be heavily impacted by fluctuations in rates of settlement. In addition, the early production cycle is inefficient and massive amounts of seed are lost. Some studies have indicated that a likely cause of poor retention of juvenile mussels is secondary settlement, the process by which juvenile mussels can move to optimise their position and chances of recruiting to an adult population. The factors that trigger secondary settlement and promote successful recruitment to a wild population of mussels are not well understood and likely not replicated in an aquaculture setting. Single cohorts of juvenile mussels are deployed at very high densities for aquaculture purposes whereas in the wild, mussels appear to recruit or at least survive better among adults. A factorial field experiment was used to test whether the addition of live adult mussels or their shells increased retention of juveniles compared to standard industry seeding techniques after one and five months. In addition the responses of biofouling organisms to our experimental conditions were assessed. Overall, fewer than 80% of juveniles were retained during the experiment, but there were no effects of adding adult mussels. By contrast, adding live adults more than doubled the number of the problematic biofouling mussels, *Mytilus galloprovincialis*. The importance of these findings for mussel aquaculture in New Zealand will be discussed.



## **TOLERANCE OF *CRASSOSTREA VIRGINICA* TO COMBINATIONS OF LOW SALINITY AND HIGH TEMPERATURE**

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The upstream distribution of oysters in the Chesapeake Bay subestuaries is limited by a combination of low salinity and high summer temperature. Salinity in these estuarine reaches is marginally increasing with sea level rise and channel maintenance dredging; however, increasing frequency of high volume summer freshets with storm events drives short duration (days) periods of unusually low salinity in summer months. The tolerance (cumulative mortality over time) of two sizes (small = <35 and large = >35 mm shell length) of *Crassostrea virginica*, collected from a low salinity reef in the upper James River, Virginia, were examined where extended summer salinities of 4-6 are commonplace, to sustained salinities of 4, 3, 2, and 1 at ambient (therefore seasonally fluctuating) summer temperatures of 28-30 °C for a 30-day trial. At 1 salinity, <7 days at <28 °C indicates approximately 5 and 10% mortality of small and large oysters, respectively. At  $\geq 28$  °C and >7days, mortality at 1 salinity increases substantially: 30%, 65% and 100% at 14, 21 and 25 days for small oysters; 40%, 80% and 100% at 14, 21 and 25 days for large oysters. Mortality at 2 salinity was 20%, 30% and 40% at 14, 21 and 30 days for small oysters, and 25%, 35% and 55% for large oysters. At 3-4 salinity mortality of small oysters was <15% at 21 days and <20% at 30 days; for large oysters variable ranges were observed in replicates and treatments with 10-25% at 21 days and 10-35% at 30 days.

## **HOLDING MIRRORS UP TO NATURE: THE STRUCTURE, FUNCTION, AND EVOLUTION OF THE EYES OF SCALLOPS**

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The eyes of animals come in a variety of forms and some of the most unusual designs are found in certain types of molluscs. Scallops, for example, have dozens of eyes arrayed along the edges of their valves. These are among the only eyes known to use a concave mirror to focus light for image-formation, they are one of the very types of eyes to contain two separate retinas, and they provide scallops with visual acuity that far exceeds that which is observed in other bivalves. This session will include: 1) new evidence that the eyes of scallops are dynamic structures that demonstrate a light-evoked pupillary response and may be able to change shape voluntarily in ways that influence the qualities of the images that fall on the two separate retinas; 2) a discussion of the wide range of visually-influenced behaviors demonstrated by scallops, some of which are quite dramatic because scallops – unlike other bivalves – are able to swim for short distances using a form of jet-propulsion; and 3) a review of evidence that the scallop nervous system is complex and centralized relative to that which is observed in other bivalves and that the optic nerves traveling from the dozens of image-forming eyes on the mantle may project to the lateral lobes of the parietovisceral ganglion (PVG) in a spatiotopic manner. These considerations will build to a discussion of how scallops and their relatives may be useful for studying the co-evolution of sensory structures, forms of locomotion, and centralized nervous systems.

## GEODUCK AS INDICATORS OF ENVIRONMENTAL CHANGE

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The Pacific geoduck (*Panopea generosa*) is the largest clam native to the Pacific Northwest and is a burgeoning aquaculture species due to growing export demands from Asia. In Washington State, geoduck support important commercial fisheries via farmed and wild populations in Puget Sound. As a sedentary, calcifying bivalve occupying mostly subtidal sediment, geoduck are likely to be impacted by climate stressors, which have already been documented as trending towards warmer, more acidic marine conditions. In summer 2016, two trials were performed in which sibling juvenile geoduck were out-planted in five sites throughout Washington State, each site containing cohorts placed inside and outside eelgrass beds. Geoduck were enclosed to minimize predation, water chemistry was continuously monitored, and after four weeks of exposure geoduck gill tissue was taken for proteomic analysis. The gill proteome was sequenced using tandem mass spectrometry to reveal expression pattern associated with local conditions. Together these results demonstrate that protein profiles can provide valuable information on local conditions including how environmental change can influence bivalve physiology.

## **ASSOCIATION OF A VIRUS PATHOGENIC TO ATLANTIC BLUE CRABS (*CALLINECTES SAPIDUS*) WITH FISHING AND AQUACULTURE PRACTICES**

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Atlantic blue crabs (*Callinectes sapidus*) are ecologically important benthic grazers that support fisheries throughout the Americas. In internationally sampled locations, crabs exhibited a mean prevalence level of ~20% infection by a pathogenic reovirus *Callinectes sapidus* reovirus 1 (CsRV1). Immature pre-molt blue crabs are often maintained in short-term aquaculture until they molt to produce value-added soft shell crabs. Mortality in this artisanal industry is 10-40%. Most dead crabs carry high CsRV1 loads and are frequently discarded in nearby estuarine waters. This study investigated whether virus-related mortality was associated with specific culture conditions and practices, and if discarded virus-infected crabs posed a threat to crabs in the natural environment. A quantitative polymerase chain reaction (qPCR) assay for CsRV1 was used to confirm that most crabs dying in soft crab production have high virus loads across production systems in Maryland and Virginia, but not Louisiana. Concurrent experiments investigated the potential for dead crabs to transmit the virus to cohabiting or cannibalistic non-infected crabs. A short-term cohabitation experiment produced no detectable transmission of virus by crabs exposed to heavily infected dead crabs. A cannibalism transmission trial did not demonstrate significant transmission of CsRV1 from infected crab muscle to live crabs by ingestion. Ongoing studies will measure virus transmission by long-term cohabitation of uninfected live and CsRV1-infected dead crabs. Quantitative data for transmission and mortality rates obtained will be used to estimate the risk of infection that discarded dead crabs pose to the wild fishery and to better understand disease dynamics in wild crab populations.

## PROBIOTIC-DRIVEN CHANGES IN REARING WATER MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN AN OYSTER HATCHERY

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Larval oysters in hatcheries are susceptible to diseases caused by marine bacterial pathogens, including *Vibrio* spp. These diseases may be prevented by daily addition of probiotics like *Bacillus pumilus* RI06-95 to water in rearing tanks. It is proposed that the presence of these probiotics may change how the larvae respond to pathogens, regulate their environment, and recruit beneficial microbes. Rearing water samples (1-2 liters) were collected from control and probiotic-treated tanks in an oyster hatchery at four timepoints between Day 0 (spawning) and Day 12. Water samples were filtered onto 0.22µm Sterivex filters and DNA was extracted directly from the filters, then prepared for sequencing. All tanks were analyzed using 16S rRNA sequencing of the V6 region, in order to determine microbial community structures. Whole microbial community metagenomes in select tanks were determined using whole genome shotgun sequencing on the Illumina NextSeq platform. Metagenomes were compared between control and probiotic-treated tanks by sorting assembled contigs on the basis of GC content, tetranucleotide frequency, and genome coverage. Genes that are abundant in these communities were annotated and characterized for ecological function in the hatchery system. Lastly, bacterial pathogens were identified and their abundances were correlated to microbial community structures. Shifts in the diversity of the microbial community were seen in probiotic treated tanks compared to non-treated tanks, including the presence of a higher proportion of *Phaeobacter* spp. This early-stage project will inform interactions between probiotics and microbial communities in oyster hatcheries, and how new probiotics may be designed.

## **THE ROLE OF SCIENCE IN SHELLFISH MANAGEMENT: THE SEA SCALLOP RESOURCE IN A CHANGING ENVIRONMENT**

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At a time when there is increasing concerns about the negative effects of the changing ocean environment, the sea scallop (*Placopecten magellanicus*) fisheries in the United States and Canada have experienced unprecedented rebuilding. Several factors have led to the successful rebound of these industries, including revised management approaches, investments in improved survey technologies, data-rich stock assessments, favorable environmental conditions, and some luck. These conditions may have led to two extremely large scallop recruitment events. In 2003, about 12 billion recruits were observed in the Mid-Atlantic, while the total population was about 21 billion scallops. This resulted in the Elephant Truck closed area, which sustained the fishery for the next 6 years. In 2014, about 31 billion recruits were observed on Georges Bank, the largest recruit abundance ever recorded, while the total population was about 39 billion scallops. Similar recruitment events have been observed in Canadian waters. Through the use of spatial and temporal management strategies, large recruitment events have been protected until the scallops reach a harvestable size contributing to the rebuilding of these stocks. However, natural mortality rates for these recruitment events are not well defined; occurring from several sources and vary spatially. Although there is no comprehensive understanding of density-dependent relationships for scallops, which complicates management of the species, these stocks are being monitored using absolute density estimate techniques (numbers of scallops per m<sup>2</sup>), enabling comparisons between different areas over time, and giving insight into population dynamics and how they might change in a shifting environment.

## EXAMINING THE POTENTIAL OF PONDEROUS ARK (*NOETIA PONDEROSA*) CULTURE IN FLORIDA

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The ponderous ark, *Noetia ponderosa*, has been considered a potential aquaculture species in Florida. Recent hatchery advances in determining optimal settlement conditions resulted in sufficient seed production to conduct replicated trials and document growth and survival in land-based nursery, field nursery, and growout systems using methods similar for hard clam culture. Over 100,000 seed sieved on 1.2-2.0 mm screens (6.4-7.9 mm shell length [SL]) were nursed in downwellers for two months. During that period, 86% of the seed were retained on a 5.0 mm screen with an average size of 9.9 mm SL, 5.9 mm shell width (SW), and 0.3 g total weight (TW). Juveniles were planted in 4 mm mesh polyester (soft) bottom bags and polyethylene (hard) bags at 6,640/m<sup>2</sup>. After 5.5 months, survival was similar ( $P=0.20$ ) for both bag types (soft, 95.5±4.7%; hard, 66.5±28.9%), but growth was greater ( $P<0.05$ ) in the soft bags (average 17.6 mm SL, 10.8 mm SW, 1.9 g TW). In growout trials using 9 mm mesh soft bags, several stocking densities (540-800/m<sup>2</sup>) and harvest times (12-24 months) were evaluated. At 12 months, average size of arks was 43.6mm SL, 27.3 mm SW, and 26.2 g TW; survival averaged 89.3%. At 18 months, arks reached an average size of 51.9 mm SL, 32.4 mm SW, and 44.2 g TW; survival averaged 67.7%. After an additional six months, growth increased only by 2.5% for SL, 3.1% for SW and 9.7% for TW; survival was similar (67.4%). No differences ( $P>0.05$ ) due to stocking densities were determined. These results suggest that commercial production of ponderous arks may be feasible.

## **TRANSCRIPTIONAL PATTERNS OF CHROMATIN-ASSOCIATED GENES IN RESPONSE TO ENVIRONMENTAL STRESS REVEALED BY NETWORK ANALYSIS**

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Environmental changes may elicit an epigenetic response, often through structural modifications in the chromatin, playing a critical role in gene expression regulation. These complex mechanisms involve the action of specific proteins such as histone variants and histone-modifying enzymes among others. The expression patterns of these chromatin-associated genes has the potential of mirroring processes of environmental stress, thus representing a promising approach for the development of biomarkers; however, in order to develop highly selective biomarkers, the relationships and dynamics of expression profiles must be considered.

The analysis of co-expression profiles obtained from data series provides insights into the gene interactions that mediate an epigenetic response. Pairwise correlation values are calculated to build graphical undirected graphs (networks) comprising information about the expression levels of individual genes as well as their inter-relationships. Networks can be subsequently compared to find both common and unique patterns to pinpoint mechanisms triggered by a specific environmental stressor. To test this idea, bivalves such as oysters represent ideal models given their ecological and economical value, sessile lifestyle as filter feeders, and their relatively high tolerance to environmental stress.

The present work describes the gene co-expression networks activated in oysters under conditions of stress, revealing transcriptional patterns associated with an epigenetic response to specific stressors. Particularly, considered were environmental factors of concern such as temperature, salinity or heavy metal pollution. Apart from the potential development and applicability of biomarkers, these patterns convey a better understanding about the organization of chromatin-associated proteins in the regulation of gene expression.



## **SPATIAL DISTRIBUTION OF ATLANTIC SURFCLAMS (*SPISULA SOLIDISSIMA*) IN THE MIDDLE ATLANTIC BIGHT AND GEORGES BANK**

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The Atlantic surfclam (*Spisula solidissima*), a commercially important species, supports one of the largest fisheries on the northeast coast of the United States. Most of the surfclam population is found on the Delmarva Peninsula, along the New Jersey shelf, and on Georges Bank. Using data from ~30 years of surfclam stock surveys, variance-to-mean ratios were calculated for a range of size classes (lower boundary: 64, 80, 93, 104, 120 mm) of surfclams both temporally and spatially. The variance-to-mean ratio is a measurement of the spatial distribution of an organism, indicating degree of patchiness. Analysis of the surfclam dataset showed that the variance-to-mean ratio declined over the decades from the 1980s to the present in all assessed regions (Delmarva, New Jersey, Long Island, Southern New England, Georges Bank). A possible explanation for this decline is that the fishery targets the patches and consequently the patchiness in the stock is declining; however, the effect was also observed on Georges Bank, a region closed to fishing over much of this time. Additionally, size classes were distributed differently over each of the regions, typically with larger animals more patchy. These differences could be attributed to the fact that small animals recruit over a much broader region, some locations offering suboptimal habitat with low survival. The effect of these trends on the population dynamics of the stock require further investigation particularly regarding the stock recruit relationship of the surfclam, as some portion of the recruits appear not to support the spawning capacity of the stock.

## **GROWTH AND SURVIVAL RESPONSES OF RED ABALONE, *HALIOTIS RUFESCENS*, TO VARIOUS LEVELS OF DIETARY PROTEIN**

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The red abalone is one of the most valuable abalone species in the international market. In Mexico, red abalone production by aquaculture is 20-50 tons yearly. The abalone industry is a high-risk and costly industry, as abalone require 3-5 years to reach the market due to slow growth rates. Feeding strategy is based on their preferred natural diet (*Macrocystis pyrifera*), and the quality varies seasonally. To improve abalone industry competitively, producers use formulated feeds. It is important to maintain a diet that provides constant protein content and improves growth. The aim of the present study was to test the effect of a formulated diet with various levels of dietary protein on growth and survival of juvenile red abalones. The experiment was set up in two stages: first was focused on conditioning the juveniles to the formulated diet, and secondly to evaluate their growth. Three-month old abalones (6 mm, n=100) were fed with three experimental diets of the commercial feed ABKELP with 25, 30 and 35% of protein and one control diet (*M. pyrifera*), for three weeks at 19°C (five replicates/treatment). Behavior and survival was evaluated at this stage. Diet with 25% of protein showed the greater mortality. For the second stage, survived abalones of each treatment were divided into three replicates (n=95), and feeding strategy follows as established in the first-stage for three months. A significant higher growth rate was observed in the 35% protein-diet. The results of the present study will contribute to increase growth rates in aquaculture facilities.

## GENETIC SIGNATURE OF INTERANNUAL OYSTER RECRUITMENT EVENTS IN THE LOWER CHESAPEAKE BAY

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Single nucleotide polymorphism markers (SNPs) were developed for genetic analysis of the eastern oyster, *Crassostrea virginica*. In collaboration with the Chesapeake Bay Foundation (CBF) these markers were used to test a hydrodynamic connectivity model intended to predict where oyster larvae produced in the Lafayette River, Virginia would settle. In 2013 wild oysters from Tangier Island, VA and a disease-resistant hatchery strain were planted on two different reefs in the Lafayette River with the intention of testing the model by genetically tracking offspring. Baseline samples were collected from the Lafayette, as well as from nearby river systems and more distant sites in Chesapeake Bay. Oyster spat were sampled from the Lafayette River in 2013 and 2014. The samples were genotyped and assignment tests were performed to identify source population(s) for the spat. A weak pattern of isolation by distance among the lower Chesapeake Bay oysters was observed, suggesting that oyster reef connectivity is high. Hatchery oysters were significantly different from each other and from wild oysters. A genetic signal from oysters that CBF planted in the Lafayette River in 2011 and 2012 was evident. While the data supported the overall circulation patterns predicted by the model, the level of connectivity between the Lafayette and nearby river systems varied between years. A Bayesian-based clustering algorithm demonstrated that oysters near the mouth of the Lafayette were genetically distinct from those further upriver. Results indicate that these SNP markers are useful for population genetic studies and for discriminating among hatchery strains.

# SEAWATER AS AN ALTERNATIVE TO RAY'S FLUID THIOGLYCOLLATE MEDIUM (RFTM) TO DETECT AND QUANTIFY THE INFECTION OF *PERKINSUS OLSENI* IN THE MANILA CLAM *RUDITAPES PHILIPPINARUM*

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Ray's fluid thioglycollate medium (RFTM) assay has long been the most common and convenient method to detect and quantify the infection of *Perkinsus* species. Trophozoites of the parasite in host tissue enlarge in this medium, and differentiate into prezoosporangia. In the natural environment, however, trophozoites have to develop into prezoosporangia without any medium after their host dies. In this study, whether seawater can be an alternative to RFTM for the differentiation of trophozoites of *Perkinsus olseni*, and for the detection and quantification of the infection in the Manila clam *Ruditapes philippinarum* was examined. Tissues of naturally infected clams were minced, divided into two aliquots and incubated in filtered seawater or in RFTM for a week. The infection intensities determined from these two cultures showed no significant difference, and were strongly correlated to each other. A known number of *in vitro*-cultured trophozoites of *P. olseni* were incubated with uninfected host tissue in filtered seawater adjusted to different salinities. The highest number of trophozoites was observed in the group of salinity 30. These results showed that in presence of host tissue, trophozoites of *P. olseni* can differentiate into prezoosporangia in seawater as well as in RFTM, and suggest that incubation in seawater can be an alternative to the RFTM assay at least in case of Manila clams. This simple and costless method will facilitate the diagnosis of *Perkinsus* infection and will help to understand the life cycle and spread of the infection of *Perkinsus* species in the natural environment.

## **INVESTIGATION OF THE POPULATION GENETIC STRUCTURE OF THE INVASIVE ISLAND APPLE SNAIL, *POMACEA MACULATA*, IN SOUTH CAROLINA, USA**

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Freshwater gastropods in the genus *Pomacea* have proven to be successful invaders in the United States and in other regions around the world. The island apple snail (*Pomacea maculata*), native to South America, is currently established in several southeastern states, including South Carolina, where it was first observed in 2008. *Pomacea maculata* is considered to be an invasive species due to the negative impacts associated with its intense grazing, high fecundity, ability to out-compete native species, and potential to serve as a host for the rat lungworm nematode parasite (*Angiostrongylus cantonensis*), which can cause eosinophilic meningitis in humans. Despite these negative impacts, knowledge of *P. maculata* specific to South Carolina is limited.

This presentation will focus on one component of an ongoing research project to improve our understanding of the *P. maculata* invasion in South Carolina. DNA samples were extracted from lung tissue of 100 *P. maculata* ranging in size from 25 to 80 mm in shell height collected from three distinct locations in South Carolina. These samples were analyzed using a suite of 15 microsatellite markers and molecular diversity indices were calculated for each microsatellite locus. Determining the population genetic structure of *P. maculata* in these locations is an important step in understanding the different aspects of this invasion, including founding population size, sources of the invasion, dispersal of the species, predicting the success of a population, and the likelihood that secondary introductions have occurred following the initial introduction.

## **THE DEVELOPMENT AND APPLICATION OF A GENE BASED qPCR ASSAY FOR EARLY DETECTION AND MONITORING OF PSP-ASSOCIATED BLOOMS**

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There is now a good understanding of the genetic basis for toxin production by a number of groups of microorganisms, including dinoflagellates. The discovery of these toxin biosynthetic pathways has enabled the development of genetic screening tests to identify the presence of toxin producing organisms.

Specifically the synthesis of saxitoxin in dinoflagellates has been found to be catalysed by a group of enzymes encoded by sxt genes, beginning with the unusual gene, sxtA. A quantitative Polymerase Chain Reaction (qPCR) assay targeting the sxtA gene to detect saxitoxin-producing dinoflagellates in marine environmental samples has been developed as a tool for marine environmental management. The abundance of sxtA correlates with the abundance of the saxitoxin-producing species of *Alexandrium* species. Using this assay, detection and quantification of sxtA has also been correlated with saxitoxin uptake in shellfish.

The Phytoxigene™ DinoDTec assay is specific to the saxitoxin synthesis gene (sxtA) and can detect low concentration of PSP producing species in seawater column. Identifying the biosynthetic pathway of toxin production has long been cited as being critical for the utilisation of better tools in the management of water assets impacted by algal blooms.

# **HOW TO MEASURE MUSSEL VITALITY: EVALUATION OF SURVIVAL IN AIR AND NEUTRAL RED ASSAY AS A STRESS RESPONSE INDICATOR FOR THE BLUE MUSSEL (*MYTILUS EDULIS*) PROCESSING INDUSTRY**

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A high quality product and a long shelf life are crucial for the shellfish industry. From the moment the shellfish are fished until the moment they are sent to retail, they undergo several forms of stress. Stressed shellfish may have a reduced vitality, and potentially lower shelf life. The aim of the present study was to test survival in air (SIA) and the neutral red assay (NRA) for identifying stress in mussels. Commercial size mussels were exposed to different levels of mechanical and thermal stress, and mussel vitality was determined by SIA and NRA methods. For mechanical stress, recovery from the stressor was also studied. After stress exposure, the mussels ( $n \geq 30$  mussels per group;  $N=4$  groups per treatment) for SIA analysis were placed in a climate room and daily checked for dead mussels in order to establish  $LT_{50}$ . The NRA is a cell viability assay based on the ability of viable cells to incorporate neutral red (NR) within lysosomes. The NRA protocol was based on Lowe et al. (1995). Hemolymph was sampled from each individual mussel ( $n=10$  per treatment) and incubated with NR solution. Cells were examined under the microscope, up to a period of 3 hours. Observations were categorized into four stages indicating the subsequent cell degradation stages, to define retention time. Results will be presented from a range of experiments. SIA and NRA methods will be compared in their sensitivity and response to the different stressors, and commercial applicability will be discussed.

## **THE REGULATORY BURDEN: TYPES OF ECONOMIC EFFECTS ON WEST COAST SHELLFISH FARMS**

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A survey to assess the costs and impacts of regulations affecting shellfish aquaculture on the west coast was conducted in the fall of 2016. Producers widely acknowledged the importance of regulations, specifically with regards to environmental protection and food safety; but many producers reported indirect costs of regulatory compliance in their business. Detailed analysis of this dataset is still ongoing, but there are several economic effects that were observed during interviews and from initial reviews that are worthy of note. These included significant impacts to business development and planning resulting from permit and licensing delays, cash flow interruptions from missed planting or harvest windows, increased legal fees and administrative costs, and lost sales or missed opportunities for expansion and diversification. In addition to these impacts, some producers also reported lost investment capital due to regulatory uncertainty, and the management and labor challenges of ensuring regulatory compliance as a small business. These indirect factors are likely to account for the majority of the regulatory costs affecting the sector, as was concluded during a recent, similar study on baitfish and sportfish farms.



**PREVALENCE AND INTENSITY OF THREE OYSTER PARASITES (*PERKINSUS MARINUS*, *HAPLOSPORIDIUM NELSONI* AND *BONAMIA* SP.) IN WILD AND CULTURED OYSTERS (*CRASSOSTREA VIRGINICA*) ON AN OYSTER FARM IN SOUTHEASTERN NORTH CAROLINA**

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Populations of the eastern oyster *Crassostrea virginica* along the Atlantic coast of the United States have been severely impacted by a variety of parasites over the past 60 years. This study was undertaken to monitor the prevalence and intensity of three protozoan parasites of *C. virginica* – *Perkinsus marinus* (Dermo), *Haplosporidium nelsoni* (MSX), and *Bonamia* sp. – in wild and cultured oysters using quantitative PCR (qPCR). Monthly samples consisted of 100 wild oysters collected from Masonboro Sound, NC starting in February 2016. A line (HC5-9) of cultured oysters was sampled prior to deployment on the farm in July 2016 and monthly thereafter. In the wild oysters, peak prevalence for *P. marinus* occurred in early fall (85%) and in early spring for *H. nelsoni* (51%). *Bonamia* sp. was detected in only two of the samplings, with peak prevalence observed in March (16%). Intensity of infection (estimated by Ct value) was greatest for *P. marinus* in late fall and in late summer for *H. nelsoni*. Cultured oysters initially tested negative for all three parasites, but by early fall *P. marinus* and *H. nelsoni* were detected. The influence of environmental factors such as temperature, salinity, and chlorophyll *a* on the occurrence of these parasites was also examined.

## **A COMPARATIVE MOLECULAR, BIOCHEMICAL, AND STRUCTURAL ANALYSIS OF GALECTINS FROM AQUATIC MOLLUSCS: THEIR ROLE(S) IN INNATE IMMUNITY**

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Galectins constitute a widely distributed lectin family characterized by their binding affinity for b-galactosides and a unique binding site sequence motif in the carbohydrate recognition domain (CRD). Galectins display a remarkable functional diversity, by participating in developmental processes, cell adhesion and motility, regulation of immune homeostasis, and recognition of glycans on the surface of viruses, bacteria and protozoan parasites. Most of the studies have focused on the identification and domain organization of galectin-like transcripts or proteins in diverse tissues and cell types, including hemocytes, and their expression upon environmental or infectious challenge. Lectins from the eastern oyster *Crassostrea virginica*, however, have been characterized in their molecular, structural, and functional aspects and some notable features have become apparent in the galectin repertoire of aquatic molluscs. These include different domain organizations relative to those observed in vertebrates, carbohydrate specificity for blood group oligosaccharides, and upregulation of galectin expression by infectious challenge. Although galectins from some aquatic molluscs have been shown to recognize microbial pathogens and parasites and promote their phagocytosis, they can also selectively bind to phytoplankton components, suggesting that they also participate in uptake and intracellular digestion of microalgae. Experimental evidence suggests that the protozoan parasite *Perkinsus marinus* is selectively recognized by the oyster hemocyte galectins over algal food or bacterial pathogens, thereby subverting the oyster's innate immune/feeding recognition mechanisms to gain entry into the host cells [Supported by grants IOS-0822257 and IOS-1063729 from NSF, and grant 5R01 GM070589-06 from NIH to GRV]

## USING SHOTGUN PROTEOMICS AS AN INDICATOR OF ENVIRONMENTAL VARIABILITY

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Oysters are important species, as they provide crucial ecosystem services and are the basis for aquaculture operations. Like other marine invertebrates, oysters are susceptible to changes in environmental conditions. Increasingly acidified waters pose a threat to larval development and adult shell integrity. Wild oysters also face a second threat: increasing temperature regimes. There is a limited set of experiments studying oysters' response to multiple stressors that directly reflect the suite of conditions and variability experienced in the wild. To examine the physiological effects of multiple stressors, including acidification, dissolved oxygen content, and temperature in the wild, 150 sibling *C. gigas* specimens were outplanted in five different study sites in and around the Puget Sound, WA. Gill tissue was collected after a month and shotgun proteomic analyses were performed with distinct differences in expression observed based on site characteristics. These data provide insight into how oysters are impacted by different environmental conditions and offer a framework for future lab experiments.

## TRANSCRIPTOMIC INFORMATION FROM PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) OVARY AND EYESTALK, AND EXPRESSION PATTERNS FOR GENES PUTATIVELY INVOLVED IN THE REPRODUCTIVE PROCESS

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In order to further investigate the genes involved in *Litopenaeus vannamei* reproduction, cDNA and SSH libraries derived from female eyestalk and gonad were produced, allowing the identification of expressed sequences tags (ESTs) that potentially have a role in the regulation of gonadal maturation. In the present study, different transcripts involved in reproduction were identified and a number of them were characterized as full-length. These transcripts were evaluated in males and females in order to establish their tissue expression profiles during developmental stages, and in the case of females, their possible association with gonad maturation was assessed through expression analysis of vitellogenin. The results indicated that the expression of *vitellogenin receptor* (*vtgr*) and minichromosome maintenance (*mcm*) family members in the female gonad suggest an important role during previtellogenesis. Additionally, the expression profiles of genes such as *famet*, *igfbp* and *gpcr* in brain tissues suggest an interaction between the insulin / insulin-like growth factor signaling pathway (IIS) and methyl farnesoate (MF) biosynthesis for control of reproduction. Furthermore, the specific expression pattern of *farnesoic acid O-methyltransferase* suggests that final synthesis of MF is carried out in different target tissues, where it is regulated by esterase enzymes under a tissue-specific hormonal control. Finally, the presence of a vertebrate type steroid receptor in hepatopancreas and intestine besides being highly expressed in female gonads, suggest a role of that receptor during sexual maturation.

## **IMPACTS OF *DEEPWATER HORIZON* OIL AND DISPERSANTS ON VARIOUS LIFE STAGES OF OYSTERS *CRASSOSTREA VIRGINICA***

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The 2010 Deepwater Horizon Oil Spill released oil into the Gulf of Mexico. Many of the polycyclic aromatic hydrocarbons and other oil constituents found in oil are toxic. The oil spill, which continued for three months and which resulted in extensive exposure of nearshore habitats well beyond the period when oil was being discharged from the wellhead, coincided with the spawning season of the Eastern oyster (*Crassostrea virginica*), an environmentally and commercially important shellfish species in the Gulf of Mexico. The long planktonic nature (14 to 21 days) of oyster larvae, sedentary nature of adult oysters, high filtration rates and their micro algal/particulate diet make them vulnerable to acute exposure to contaminants both in solution and bound to suspended sediment, and adsorbed onto algal and other particles.

A series of experiments were carried out to examine the effects of the *Deepwater Horizon* (DWH) oil and dispersants on various life stages of oysters ranging from gametes to adults. Oysters were exposed to water accommodated fractions of oil (with and without dispersant), sediment elutriates, contaminated sediment, and contaminated algae. Fertilization success, morphological development, growth, survival and settlement success after these exposures were measured. Fertilization success decreased, developmental abnormalities increased, and larval growth, survival and settlement, and reproductive development of adults decreased in a dose-dependent manner relative to oil exposure and exposure duration.

Results suggest that exposure of various life stages of oysters to oil and/or dispersants will have a negative impact on overall growth and survival and may have implications on the population structure.

## **OYSTER SOUTH: SUPPORTING OYSTER FARMING IN THE US SOUTH**

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Oyster aquaculture is an opportunity in the southern United States (from North Carolina around to Texas) for a viable near-shore domestic aquaculture industry that can provide a large economic boon to the coastal communities in the region (both to producers as well as local supporting industries). Oyster aquaculture may help watermen maintain traditional ways of life, keeping them working on the water. Oyster South, a non-profit 501(c)(3), is dedicated to supporting the sustainable development of oyster aquaculture in the southern US, to help the regional economy, improve the coastal environment, and preserve the coastal culture and traditions ([www.oystersouth.com](http://www.oystersouth.com)). Membership consists of both oyster consumers and industry members. Industry members draw from a broad base, including growers, wholesalers, restaurateurs, chefs, media and educators, all striving to advance oyster aquaculture in the southern US. We encourage this through information exchange, fund-raising, and highlighting the region's world-class oysters and oyster farmers in the media. Oyster South will provide a regional network for Southern oyster farmers to engage with the public as well as potential buyers.

## **FIELD TESTING OF NOVEL ANTIFOULING COATINGS FOR THE AQUACULTURE INDUSTRY**

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Results from research designed to develop a non-toxic and bio-based solution to biofouling through the development of novel, soy-based polymers that release hydrogen peroxide when exposed to visible light will be presented.

Biofouling resistance of photoactive soy-based polymers was demonstrated through a battery of panel tests conducted with industry partners globally. Soy-based photoactive release coatings reduced biofouling on aquaculture gear (oyster bags), and thus improved growing conditions. Oysters grew significantly larger in treated bags over the three month grow-out period. No differences in oyster mortality were observed between treated and untreated bags. Treated bags required less maintenance and could be reused without cleaning.

Soy-based photoactive release coating technology is a viable solution to the biofouling problem experienced by shellfish farmers who rely upon gear changes and cleaning to control biofouling. Eliminating costs associated with cleaning or changing gear by application of the proposed non-toxic antifouling technology will increase profitability of these farms, and improved animal health and increased growth rates will result from clean nets and cages. This non-toxic approach to prevent biofouling of aquaculture gear will improve production efficiency, and thus increase profitability of aquaculture in the US.

Potential commercial applications of the proposed non-toxic biofouling release coating include netting used for fish farming (barrier and market), cages and bags used for oyster farming, lantern nets used for scallop farming, buoys and lines used for mussel and algae farming, barrier nets, anti-predator nets, and bags used for clam farming, floating upweller systems (FLUPSY), fishing nets and traps, and oceanographic equipment.

## INGESTION AND REJECTION OF MICROPLASTICS BY SUSPENSION-FEEDING BIVALVES: IMPLICATIONS FOR EXPOSURE AND ENVIRONMENTAL FATE

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Suspension-feeding bivalves are exposed to a manifold of natural and anthropogenically-derived particles in the aquatic environment. Which particles are actually captured and ultimately ingested depends on several physicochemical and biological factors including limitations of the feeding structures and particle-selection capabilities of the species. In a series of experiments with blue mussels (*Mytilus edulis*) and eastern oysters (*Crassostrea virginica*) the capture, selection, and ingestion of several different types of microplastics with different shapes, sizes and surface properties (wettability, surface charge) was examined. Particles < 2 µm are captured at a low efficiency unless they are incorporated into marine aggregations. Large (> 100 µm) fibers and angular / spherical particles are captured at a high efficiency but are rejected in pseudofeces and thus ingested in low proportions. Particles between 10 and 20 µm are more likely ingested if they have more hydrophobic surfaces or possess surfaces with a lower charge (Zeta potential < ca. -8 mV). These results advance the understanding of the types of microplastics that are ingested by bivalves and those that remain in the environment. Developing physicochemical profiles of different types of microplastics to predict which are rejected versus ingested is important for assessing internal exposure and environmental fate.



## **DEVELOPMENT AND EXPANSION OF SHELLFISH AQUACULTURE IN NORTH CAROLINA: CHALLENGES AND OPPORTUNITIES**

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Although New England has historically been the center of cultured shellfish production on the east coast, in recent years the industry is expanding in other states along the Atlantic seaboard. Most notable is Virginia, which has undergone phenomenal growth in its shellfish aquaculture industry over the last decade. In 2014, the farm gate value of clams and oysters cultivated in Virginia waters was \$56 million, supporting over 500 jobs. Despite sharing a common border and similar coastal resources, growth of the shellfish aquaculture industry has been minimal to date with a 2014 farm gate value of only \$532,000.

Over the course of the last several years, interest in shellfish aquaculture – especially water column farming of oysters has increased greatly in North Carolina, which has been reflected by a steady increase in leases and acreage devoted to this practice. In addition, at the urging of the NC Shellfish Growers Association, the North Carolina General Assembly has begun easing regulatory barriers to entry, state marine institutions are devoting more resources towards research to improve industry efficiencies and output, technology transfer and training efforts have been increased, and strategies are being developed to expand markets for shellfish.

An overview of the present shellfish aquaculture industry in North Carolina including production practices that are employed will be presented. Challenges facing the industry including regulations, user conflict and public perception, seed supply, biofouling of gear, and disease issues will also be discussed. In addition, the growth potential of the industry will be examined.

## SUSTAINABILITY OF SPANISH CLAM FISHERIES IN A CHANGING CLIMATE

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Low salinity events and heat waves cause catastrophic mortality in commercial shellfish beds in Galicia, NW Spain. In order to predict catastrophic events, models of mortality and physiology in response to salinity and temperature fluctuations were developed. Laboratory relationships among scope for growth, mortality rate and thermal and salinity stress were measured in four commercially important bivalves, *Ruditapes philippinarum*, *Ruditapes decussatus*, *Venerupis corrugata*, and *Cerastoderma edule*. Regional ocean forecasts provided hourly salinity time series within shellfish beds which were used to calculate daily mortality, based on the laboratory relationship between mortality and salinity. Modeled cumulative mortality reached 100% in January 2014 in shellfish beds in the upper Ría de Arousa, coincident with total loss of *V. corrugata*. Scope for growth was greatly reduced during low salinity events in all four species. *V. corrugata* is also sensitive to thermal stress, with 30% mortality after 3 low tide exposures to 30°C in the laboratory, corresponding to field mortality. Weather and ocean forecasts were used to simulate temperature time series in shellfish beds and predict cumulative mortality during heat-waves. Over multi-decadal time scales all four species are likely to suffer reduced physiological performance, and *V. corrugata*, which attracts high prices, is likely to become extinct in the intertidal in a changing climate. In Galicia, women harvest clams exclusively in the intertidal, and men harvest exclusively in the subtidal, so the loss of *V. corrugata* from the intertidal will have negative economic impact on women and a positive economic impact on men.

## **INCENTIVIZING NATIVE OYSTER RESTORATION THROUGH ECONOMIC VALUATION OF ECOSYSTEM SERVICES IN SOUTHERN CALIFORNIA**

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The Olympia oyster, *Ostrea lurida*, is the only oyster native to the west coast of the United States. Due to over-harvest, shoreline development, and the introduction of non-native species, Olympia oysters were virtually depleted by the mid-twentieth century. Incorporating native oysters into restoration projects in Southern California has thus been largely ignored due to the shifting baseline syndrome; restoration managers do not realize the historic presence or associated ecosystem benefits provided by oysters because they have been functionally absent for so long. Oyster reefs provide ecosystem services such as small-scale shoreline stabilization, habitat for economically viable fish and invertebrates, and water quality improvement.

Oysters on the east coast, *Crassostrea virginica*, have been economically valued and the aim of this study was to produce a similar valuation of invertebrate production through restored *Ostrea lurida* reefs in Southern California. This session will include: 1) benefit cost ratios for restored oyster reefs as a means of local shoreline stabilization along various sites in Southern California, and; 2) a bioeconomic model valuing the increase in biomass of commercially important marine species on restored oyster reefs. There are already data supporting an increased abundance of polychaetes, amphipods, isopods, and other bivalves as determined by Danielle Zacherl and collaborators (currently unpublished).

This valuation is hoped to provide an economic incentive for the incorporation of native oysters into future restoration plans and mitigation projects in Southern California.

## SPATIAL VARIATION IN ADULT SEX RATIO ACROSS MULTIPLE SCALES IN THE INVASIVE GOLDEN APPLE SNAIL, *POMACEA CANALICULATA*

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Adult sex ratio (ASR) has critical effects on behavior and life history, and has implications for population demography, including the invasiveness of introduced species. ASR exhibits immense variation in nature, yet the scale-dependence of this variation is rarely analyzed. In this study, using the generalized multilevel models, the variation in ASR across multiple nested spatial scales was investigated and analyzed the underlying causes for an invasive species, the golden apple snail *Pomacea canaliculata*. The variance in ASR to describe the variations at different scales was partitioned and then included the explanatory variables at the individual and group levels to analyze the potential causes driving the variation in ASR. A significant female-biased ASR for this species was determined when accounting for the spatial and temporal autocorrelations of sampling. Counter to nearly equal distributed variation at plot, habitat and region levels, it was found that ASR showed little variation at the town level. Temperature and precipitation at the region level were significantly positively associated with ASR, whereas the individual weight, the density characteristic and sampling time were not significant factors influencing ASR. This study suggests that offspring sex ratio of this species may shape the general pattern of ASR in the population level while the environmental variables at the region level translate the unbiased offspring sex ratio to the female-biased ASR. Future research should consider the implications of climate warming on the female-biased ASR of this invasive species and thus on invasion pattern.

## GENOMIC RESPONSES OF MOLLUSC BIOMINERALIZATION TO OCEAN ACIDIFICATION

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Biom mineralization is a common biologically controlled process in many molluscan species. As the product of biomineralization, the shell secreted by molluscs provides a supportive and protective structure for their soft bodies. The biomineralization conducted shell formation is an essential step during the larval development of molluscs. Recent studies have shown that the biomineralization processes are heavily affected by stresses of environmental factors. Of particular interest are the influences of ocean acidification on the molluscan biomineralization at the genomic level.

The goal of this study is to understand the alteration of genomic regulation in molluscan shell formation in response to low pH environment caused by increased  $p\text{CO}_2$ . A genome comparison was conducted between two molluscan species: one with shell (*Biomphalaria glabrata*) and the other one without shell (*Aplysia californica*) in their life spans. Functional annotation and clustering with these identified genes further specified a group of genes that were unique in mollusk biomineralization. Functional studies on several identified genes were performed with a *B. glabrata* embryonic cell line. The expression patterns of these genes and their roles in biomineralization related calcium uptake were also identified. Finally, the biomineralization related genes were further annotated on the genome of *Crassostrea gigas* and the homologous genes were cloned from *C. virginica*. Further functional analyses with annotated genes were performed with the larvae of *C. virginica* under the stresses of increased  $p\text{CO}_2$ .

The results of this study will contribute to understand molecular mechanism of molluscan biomineralization under the stresses of ocean acidification.

## TESTING PLASMA SUBTILISIN INHIBITORY ACTIVITY AS A SELECTIVE MARKER FOR Dermo RESISTANCE IN EASTERN OYSTERS

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Recent findings suggest inhibitors of the serine protease subtilisin from eastern oyster plasma (e.g., cvSI-1) play a role in host defense against the protist parasite *Perkinsus marinus* that causes dermo. A study was therefore conducted to determine whether plasma subtilisin inhibitory activity (PSIA) could be used as a marker for selecting broodstocks in breeding programs to produce progenies with increased dermo resistance. Oysters from two wild populations that have previously been shown to differ in dermo resistance were collected from the Louisiana coast. The PSIA of 500 oysters from each population were measured and three groups of 50 oysters each were established to spawn. The first group was composed of randomly sampled oysters, the second was composed of oysters with the highest PSIA and the third was composed of oysters with the lowest PSIA. After spawning, the progenies were deployed in a dermo endemic area and sampled quarterly for two years to measure their mortalities, growths, *P. marinus* infection intensities, condition indexes, PSIA, and cvSI-1, cvSI-2 and cvSI-3 gene expressions. Oyster cumulative mortalities and *P. marinus* infection intensities of the progenies of all groups increased from April to October of 2015 and 2016 as temperature increased and following expected seasonal trend. While the progenies of the two populations differed markedly in their mortalities and *P. marinus* infection intensities, differences between the progenies of the unselected and selected groups were not found. Measurements of PSIA and cvSI-1, cvSI-2 and cvSI-3 gene expressions are being completed and will also be presented.

## **SPATIO-TEMPORAL VARIATION IN *PERKINSUS OLSENI* INFECTION IN MANILA CLAM *RUDITAPES PHILIPPINARUM* IN KOREAN WATERS**

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Previous studies have demonstrated that most of Manila clams in Korean waters are infected with *Perkinsus olseni*, although the level varies spatially and temporally. In 2007 spring and fall, *P. olseni* infection was surveyed in Manila clams in Korean waters collected from 23 locations. The infection prevalence and intensity was determined using RFTM and 2M NaOH digestion. The survey indicated that clams from river mouth areas were free from *P. olseni*. The prevalence ranged 80-100% in most of the sampling locations on the west and south. The population mean infection intensity of clams from tidal flats on the west and south coast varied 50,000 to 200,000 cells/g tissue in case of light infection, while the intensity ranged 1,000,000 to 5,000,000 cells/g tissue in case heavy infection in November. The infection intensity determined in November was significantly higher than May. The infection intensity was closely related to latitude, bottom type, and density of clams. The infection intensity of clams collected from intertidal areas in small bays on the south coast was significantly higher than the level determined from the north-west coast (Incheon Bay) due to the higher surface seawater temperature in south coast than in west coast during winter period. Prevalence and infection intensity of *P. olseni* in clams from Jeju Island, where the clam density was 5-10 times lower than the density on the west and south coast, and the SST stayed over 13°C during winter, was lower than the level recorded from the south coast and Incheon Bay.

## HEALTH OF OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS IN NATURAL AND RESTORED REEFS ALONG A NORTH TO SOUTH GRADIENT IN THE INDIAN RIVER LAGOON (IRL), FLORIDA

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As a sessile, sentinel species, oysters are used to evaluate ecosystem health. Due to the ecosystem services they provide oyster restoration is of prime importance in the Indian River Lagoon (IRL). The aim of this ongoing study is to investigate the seasonal health (Summer, Fall, Spring) of natural and restored oyster reefs in the IRL along a north to south gradient (Mosquito Lagoon to the Jupiter Inlet). A total of 540 oysters were collected from three natural and three restored sites ( $n = 30/\text{site}$ ), within three regions (north, central, south,  $n = 180/\text{region}$ ) in summer of 2016. Health indices compared included size, physiological condition, sex, prevalence of shell abnormalities, pests and parasites.

Oyster size did not differ significantly between region or reef type, although tissue weight was lower and oysters were in poor physiological (watery, digestive tubule atrophy) condition in the north. Sex ratio was approximately 2:1 females to males with restored and northern reefs having higher percentages of males and indeterminate sex. Pea crabs and mud blisters were more prevalent in the south, and boring sponge in the north. Restored southern reefs had significantly more pea crabs (28%) than did natural reefs (6.7%). *Perkinsus marinus* was more prevalent in north and central regions and in natural reefs. *Bonamia* spp. was only detected in natural reefs in the south. Initial assessment (summer 2016) indicate that oysters in the central IRL are healthier and that this region has the greatest potential for successful restoration efforts.



# THE OYSTER MICROBIOME: INTERRELATIONSHIPS AMONG HOST GENOTYPE, MICROBIOME COMPOSITION, AND DISEASE RESISTANCE IN *CRASSOSTREA VIRGINICA*

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The eastern oyster, *Crassostrea virginica*, is a keystone species in estuarine environments of the eastern United States. Unfortunately, overfishing, habitat degradation, and infectious disease have decimated the *C. virginica* population. Efforts to strengthen them against these diseases have relied upon cultivation of disease-resistant strains. The emerging field of microbiome research offers the potential to understand the role of the *C. virginica* microbiome in disease resistance. Research on the human gut microbiome has linked it to mediation of the host immune response (e.g. promotion of pro-inflammatory host defense factors). Our previous research examined the microbiome composition of *C. virginica* extrapallial fluid over the course of one year and established that *C. virginica* plays host to a core microbial community that is distinct from that of its environment and exhibits seasonal variations. This study further examines the interplay between host and microbiome in *C. virginica*, as well as the influence of host genotype on microbiome composition and disease resistance. Wild and disease resistant oysters have been deployed at estuarine locations along the Delaware Bay and the Delaware Inland Bays and are being sampled at two month intervals across an annual cycle. At each time point, twenty-four oysters are genotyped, tested for the presence of disease, and their microbiome is characterized via bacterial 16S rRNA and viral marker gene sequencing. These genotypic, gene expression, and microbiome “profiles” build a multi-tiered systems biology view enabling exploration of the interrelationships between *C. virginica* genotype, disease resistance, and microbiome composition.

## **TRANSCRIPTOME ANALYSIS AND DISCOVERY OF GENES INVOLVED IN IMMUNE RESPONSE OF HYPOXIA/THERMAL CHALLENGED SMALL ABALONE *HALIOTIS DIVERSICOLOR***

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The deteriorating environmental conditions have threatened the abalone aquaculture industry in the southern coasts of China for a long time, especially in the summer period. It's therefore necessary and urgent to understand the mechanism of Abalone defense against environmental challenge. However, studies on its immune system are limited by the lack of genomic resources. In this study, abalones were exposed to a range of environmental stressors (hypoxia, thermal and hypoxia plus thermal stress) and sequence haemocytes of stressed and non-stressed at a particular time point by transcriptome sequencing. Then a reference transcriptome was generated which represented haemocytes responding to multiple environmental stressors. A total of 103,703,074 clean reads were obtained and 99,774 unigenes were annotated through the NCBI database. Among these unigenes, 47,154 and 20,455 had homologous sequences in the Nr and Swiss-Prot protein databases, while 16,944 and 10,840 unigenes could be classified by COG and KEGG databases, respectively. There were 24,189 genes that were significantly differentially expressed after hypoxia challenge. And the differentially expressed genes (DEGs) number after thermal challenge and hypoxia plus thermal challenge was 29,165 and 23,665, respectively. Among which more than 3,000 genes were involved in at least 230 pathways, including several classical immune-related pathways. These findings may provide information on *H. diversicolor* innate immunity. Also, the further analysis on the potential immune related genes in different signaling pathways and the obtained transcriptome data may provide an invaluable genetic resource to study the genome and functional genes of abalones.