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STUDY OF SEROTONIN AND DOPAMINE POST-SYNAPTIC RECEPTOR MECHANISMS IN THE BIVALVE MOLLUSC *CRASSOSTREA VIRGINICA*.

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Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations from their ganglia. Serotonin is the neurotransmitter causing cilio-excitation, dopamine cilio-inhibition. Post-synaptic serotonin and dopamine responses are G-protein linked metabotrophic mechanisms involving activation or inhibition of adenylcyclase and changes in ion channel conductance. We studied ions involved in generating the post-synaptic responses by observing membrane potentials of lateral cells with DIBAC, a voltage sensitive fluorescent dye, using artificial sea water with ion replacements based on formulas from *Formulae and Methods VI*, Marine Biological Labs, Woods Hole, MA. Stimulating the branchial nerve at 5 Hz increased cilia rates and caused prolonged increased fluorescence, indicative of membrane depolarization. Stimulating at 20 Hz after exciting cilia decreased beating rates and reduced fluorescence, indicative of membrane repolarization. With potassium free salt water the response to stimulations was reduced. The same was seen with sodium free salt water. No differences were seen with calcium free salt water. The study shows a correlation between changes in membrane potential and beating rates of the cilia, with the activity of effector channels which change ionic fluxes. It provides insights into the serotonin and dopamine post-synaptic receptor mechanisms in the gill of *C. virginica*.

SPATIAL DYNAMICS OF THE BLUE CRAB *CALLINECTES SAPIDUS* FISHERY IN CHESAPEAKE BAY: UNDERSTANDING POST-MATURATION PROCESSES TO IMPROVE EFFICACY OF MARINE STOCK ENHANCEMENT.

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The blue crab *Callinectes sapidus* is one of the most important commercial species in Chesapeake Bay (CB). Nevertheless, over-exploitation and habitat degradation have contributed to precipitous declines in the spawning stock and juvenile recruitment. From 2002–2009, hatchery-reared juveniles were released in several small coves throughout CB to determine the feasibility of stock enhancement. However, in order to better determine the long-term efficacy of stock enhancement efforts an understanding of post-maturation process (e.g., harvest, migration, etc.) is required. Thus, a mark-recapture experiment was conducted in multiple locations in the Maryland portion of CB, during 2007–2010 to estimate: 1) recovery rate; 2) tag-reporting rate; 3) fishery composition; 4) movement patterns. To date, nearly 30% of tags have been reported. Harvest rates were high throughout most of the study period. Tag-reporting rates were also high for all fishery sectors, indicating a high level of cooperation with most fishers. The recreational catch was a considerable component of overall harvest (~20–35% per year), much larger than previous Bay-wide estimates. Differences among years were noted, possibly in response to changes in fisher behavior due to newly imposed management regulations. These data provide an important understanding of the complex nature of the CB blue crab fishery.

THE CHALLENGE OF DOMESTICATING TETRAPLOID BROOD STOCK FOR THE PRODUCTION OF TRIPLOID OYSTER SEED.

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Triploid production has gained a foot hold in many corners of global oyster aquaculture and more recently in the Chesapeake Bay with *C. virginica*. The vast majority of triploids are produced through a tetraploid (male) × diploid cross (genetic triploids). Like their diploid counterparts, tetraploids can be domesticated to produce strains better suited for farming. Unlike diploids, however, there are some additional considerations for breeding tetraploids. For example, because tetraploids have double the number of chromosomes as diploids, chromosome set stability may be compromised, therefore may be a "trait" that affects both chromosome segregation in the gametes as well as instability of somatic chromosome constitution (i.e., reversion)—and both of these may influence the quality of triploids. For selective breeding of more standard traits, such as disease resistance or growth attributes, the issue is whether to select tetraploids directly, or select diploids and then make them into tetraploids. For this latter consideration, new methods of producing tetraploids need to be considered. The attributes (or misattributes) of genetic triploids are determined by various possible causes, including sterility, hybrid vigor (heterosis), selective breeding, and gene dosage. Determining the genetic contribution to these affects to include in a tetraploid breeding program will be challenging.

FIBER DIGESTION IN THE JUVENILE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN.

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Five experiments were performed to determine the importance of chitin and cellulosein the diet of juvenile *C. sapidus*. A compartmentalized recirculating system was established to provide optimal conditions, maintaining the animals with little mortality. The appropriate ration, compartment size, and an adequate baseline diet

were established. We replaced 20% of a commercial diet with varying amounts of chitin and cellulose. Crabs fed the cellulose-containing diet had higher growth rates, conversion efficiencies, molt increments and frequencies than crabs fed the chitin containing diet, but were equal to the control diet. We then assayed for chitinase andcellulase in gut tissues. Chitinase had lower specific activity ($0.072 + 0.159$ mU $mg^{-1}min^{-1}$) than cellulase ($3.52 + 0.16$ mU mg^{1} min^{-1}) in the foregut and hepatopancreas. There was no effect of diet on specific activity. The results show juvenile *C. sapidus* is capable of utilizing cellulose, but not chitin, when delivered as 20% of a diet.

INVOLVEMENT OF PROPHENOLOXIDASE (PPO) ACTIVITY IN THE SHELL HARDENING PROCESS IN *CALLINECTES SAPIDUS*: CLONING OF PPO AND PPO ACTIVATING ENZYME (PPOAE) AND THEIR SPATIAL DISTRIBUTION.

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Arthropods and nematodes undergo molts by which they shed their exoskeleton for somatic growth. In some crustaceans, molting is also essential in the reproductive process. The molt cycle and molting process are orchestrated by various hormones and neuro-hormones that act in concert. Molting involves the synthesis of a new cuticle during premolt, shedding of old exoskeleton (ecdysis) and the hardening of new cuticle. The processes of sclerotization and calcification mediate the shell hardening in crustacean. It has reported that phenoloxidase promotes the initial sclerotization in insects, while it plays an important role in the innate immunity of crustaceans. In order to understand the involvement of pro-phenoloxidase in the shell hardening in *Callinectes sapidus*, we first aimed to characterize two genes: PPO and its activating enzyme (DQ667138). Our preliminary study provides evidence of presence of PPO and PPOAE in different tissues in *C. sapidus* and their possible role in the shell hardening process.

ASSESSMENT OF THE TOXICITY OF OIL SPILL DISPERSANT COREXIT 9500 ON JUVENILE AND LARVAL BLUE CRABS, *CALLINECTES SAPIDUS*.

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Due to the *Deepwater Horizon* oil spill, over 1.5 million gallons of dispersant were applied to areas of the Gulf of Mexico. Due to their complex lifecycle utilizing open waters and estuaries, larval stages of blue crabs, *Callinectes sapidus*, were out in the Gulf of Mexico migrating back to the estuaries during the time of the spill, greatly increasing the likelihood of encountering the dispersant and oil. Temperature and salinity are factors known to effect toxicity in marine invertebrates, and the toxicity of Corexit 9500 had not been examined at Gulf of Mexico summer surface temperatures. The objectives of this study were to determine the toxicity and sub-lethal effects of the dispersant Corexit 9500 on blue crab larvae and juveniles. We found that juveniles tolerated very high (1000 ppm) concentrations of dispersant for 96 hrs, while zoeal stages reached 100% mortality in 100 ppm concentrations after only 48 hrs. All surviving crabs were held to monitor for long-term effects; however, none were seen. At concentrations similar to those believed to be in the Gulf, very little to no mortality occurred. Therefore, the dispersant alone most likely had a minimal impact on the blue crab population in the area.

DYNAMICS OF *VIBRIO VULNIFICUS* STRAINS IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) UNDER REFRIGERATION.

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Vibrio vulnificus is one of the main human pathogens associated to the consumption of raw or undercooked oysters from the Gulf of Mexico. In addition, like many other bacteria, *V. vulnificus* is able to elicit a cold shock response to protect itself when cold temperatures are encountered such as during refrigeration of the oysters that harbor it. The objective of this study was to follow *V. vulnificus* populations in oysters during a two-week period under refrigeration. We compared total numbers of *V. vulnificus* in both laboratory raised and commercially acquired oysters during the refrigeration process at day 0, 7, and 14. To assess if refrigeration selects for specific *V. vulnificus* strains, a total of 300 randomly selected isolates of *vulnificus* were taken during the entire course of the study and typed by 16S-RFLP and AFLP. Both 16S-RFLP types, A (environmental) and B (clinical), were observed through-out the refrigeration process with type B being the most pre-dominant (52%). Ratios in the types showed no significant change during the entire process nor did the number of *V. vulnificus*, although total bacterial numbers actually rose. Relationships between *V. vulnificus* types and changes the in oyster microbial community during refrigeration were also noted.

AN INTEGRATED STUDY OF MARTEILIOSIS IN DIANA LAGOON (CORSICA, FRANCE).

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Diana lagoon, located in Corsica (France) is an interesting site for conducing studies on marteiliosis because of the simultaneous presence of the protozoan parasite *Marteilia refringens* and two

susceptible bivalve species: the flat oysters *Ostrea edulis*, and the mussels *Mytilus galloprovincialis*.

In order to follow the development of the infection within the lagoon, mussels and oysters were collected monthly for one year for histological examination. For each infected bivalve, infection intensity and parasite development stages were recorded as well as their location within the digestive tract. Considering the possible involvement of zooplankton species as intermediate hosts in the life cycle of *Marteilia refringens*, samples of zooplankton were collected every two weeks in the water column surrounding the bivalves. These samples were firtsly tested by PCR and positive samples were subsequently tested by *in situ* hybridization.

Histological observation suggested that *Marteilia refringens* mainly infects mussels in Diana lagoon. Several zooplactonic species appeared positive by PCR and *in situ* hybridization tests helped to clarify the role of these species in the parasite life cycle. Lastly, some parasites detected in mussels and zooplancton species were compared at the molecular level with isolates of *M. refringens* detected in other regions in France.

DETECTION OF *BONAMIA OSTREAE* IN LARVAE OF *OSTREA EDULIS*.

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Bonamia ostreae is an intracellular protistan parasite affecting flat oysters *Ostrea edulis*. It can be detected in juveniles but mortalities mainly affect oysters which are more than two year old. The parasite is usually observed inside hemocytes and sometimes free in gill epithelia suggesting a parasite release through these organs. However, the infective form and ways of entry and release remain undetermined. Flat oysters incubate their larvae in their pallial cavity for 8–10 days before releasing them in the water column. Flat oysters exhibiting larvae during summer time between 2007 and 2009 in Bay of Quiberon were selected for our study and were treated in order to perform some *in situ* hybridization (ISH) and PCR tests on both adults and larvae. PCR tests revealed the presence of parasite DNA in some adults and larvae. Specific labeling could be detected by ISH in connective tissue of gills, mantle and in one case of gonad from spawners and in cells surrounding the visceral cavity of some larvae. Our results suggest that larvae might present some risks in terms of *Bonamia ostreae* spread and their movements should thus be restricted when they are exported from infected zones.

THE RISE AND FALL OF *CRASSOSTREA VIRGINICA* OYSTER REEFS IN THE MID-ATLANTIC.

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A subroutine of the DyPoGEn model designed to simulate the carbonate budget of an oyster reef was used to study rates of reef accumulation or loss under various conditions. For Mid-Atlantic estuarine conditions, model simulations suggest that reef accretion can only occur if oyster abundance is near carrying capacity. Simulations further suggest that reef accretion is infeasible where Dermo is a controlling influence on population dynamics. Dermo causes mortality in older (larger) oysters. As a consequence, population abundance and shell length decline, reducing total shell input. Model simulations suggest that reef recession requires an inordinately unbalanced carbonate budget and that stasis is a preferred state where the exposure of carbonate in the reef framework to the overlying water is limited while the surficial shell layer deteriorates. Reef stasis appears to be the only obtainable restoration goal in Mid-Atlantic estuaries where Dermo holds sway. In this situation, an achievable management goal is a condition in which some moderate portion of surficial shell remains, conducive to adequate recruitment and sufficient to prevent loss of the reef framework, but insufficient for reef expansion.

PRELIMINARY ASSESSMENT OF HIGH SALINITY RELAY AS A *VIBRIO VULNIFICUS* POST-HARVEST PROCESSING STRATEGY FOR CHESAPEAKE BAY OYSTERS (*CRASSOSTREA VIRGINICA*).

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Approved post-harvest processing (PHP) methods for reducing *Vibrio vulnificus* levels in shellfish are capital intensive, have limited throughput and are not readily available to the Virginia shellfish industry. The lack of persistence of *V. vulnificus* under high salinities suggests that high salinity relay might be an effective PHP. During 2010 we initiated two oyster relay experiments to assess the effectiveness of high salinity exposure in reducing *V. vulnificus* densities and potential negative effects of salinity shifts on oyster survival. Oysters from three Chesapeake Bay sites (14–23 psu) were relayed to a high salinity ocean-side Eastern shore

site (>30 psu). Samples were collected at time 0 prior to relay and on days 7 and 14 of relay. Mortalities were recorded at each time point. Oysters were analyzed using a conventional 3-tube most probable number (MPN) approach in conjunction with qPCR to detect *V. vulnificus*. Two to three log decreases in *V. vulnificus* densities in oysters from all sites were observed after 14 days with final densities being <1 qPCR MPN g^{-1} oyster meats. Mortalities remained low even for the oysters from the low salinity site (<4%). Although this method requires further validation, it shows promise as an affordable, alternative PHP.

HARMFUL ALGAL BLOOM-RELATED ILLNESS SURVEILLANCE SYSTEM (HABISS).

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The potential negative health and environmental effects from harmful algal blooms (HABs) are many—HABs threaten beaches, recreation venues, food resources, and drinking water. Mounting evidence indicates global climate changes support increased frequency and geographic extent of HABs. Moreover, each year our desire to live at lakeside and our reliance on large surface waters for recreation and drinking water put more people and animals at risk for exposure to HABs and the toxins they produce. Limited guidance is available for those tasked to protect public health. In response to the need to support public health decision-making, NCEH has developed a Harmful Algal Bloom-related Illness Surveillance System (HABISS). HABISS is a unique surveillance system that includes not only human health data, but also animal health data and environmental data about the harmful algal blooms (HABs) themselves. In the future, data collected and stored in HABISS will be used to assist in predicting local HABs, thus allowing state public health and environmental health prevention activities to be in place not only in response to reports of human or animal illnesses, but also in advance of anticipated public health problems.

EVALUATION OF THE EFFICACY OF NON-LETHAL HEMOLYMPH SAMPLING FOR THE STUDY OF PARASITE INFECTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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Crassostrea virginica is a commercially and ecologically important oyster vulnerable to two major parasites known to cause epizootic events. Currently, destructive sampling methods are generally used to assess disease status of a population but do not provide information regarding the progression of disease in individuals. In the interest of being able to evaluate individual response, this preliminary study investigated the utility of hemolymph sampling from anesthetized oysters as a source of tissue for the molecular detection of the parasites *Haplosporidium nelsoni* and *Perkinsus marinus*. Oysters were collected from Hewlett's Creek and kept in a flow-through outdoor tank for 14 weeks. Oysters were anesthetized in 2% $MgCl_2$ on a biweekly basis. Hemolymph samples were collected from the adductor muscle of 22 oysters using a syringe, and 19 oysters were poked but not bled. Fifteen oysters were anesthetized but subjected to no additional handling. Results indicate that $MgCl_2$ is an effective anesthetic, with nearly half of oysters gaping following exposure. Mortality rates were similar across treatments (bled 54.5%, poked 42.1%, and anesthetized 60%). Growth over the 14 weeks was also similar (bled 1.57 ± 1.62mm, poked 1.41 ± 1.36, anesthetized 2.19 ± 3.17mm). Evaluation of parasite prevalence and intensity is in progress and will be discussed.

FRESH AND FROZEN: IMPACTS OF SEVERE WINTERS ON GREEN MUSSEL, *PERNA VIRIDIS*, DISTRIBUTION IN THE SOUTHEASTERN UNITED STATES.

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The green mussel, *Perna viridis*, is a recent invader in the Caribbean Basin, including the southeastern United States. Green mussels occur in the Gulf of Mexico along west Florida and have been reported in the Atlantic intermittently from Ponce de Leon Inlet, FL, to Charleston, SC. Green mussel distribution is not continuous between the Gulf and the Atlantic, implying multiple human-mediated introductions. Based on field and laboratory data concerning temperature tolerance, we have suggested that green mussels will not persist in natural habitats north of Georgia. January freezes in 2003 killed most intertidal green mussels in their US range, but populations returned to all areas by the end of the same year. More severe freezes in January 2010 killed intertidal and most subtidal green mussels in the same range and recovery was less complete. As of the end of 2010, green mussels remain in Tampa Bay but have not reappeared in areas sampled on the Atlantic coast (northeast Florida). Winter kills support our predictions that green mussels are likely to remain an issue only for subtropical and tropical regions. They may, however, use industrial thermal refuges found in many estuaries, as they have in Japan, where they are also introduced.

LOUISIANA OYSTERS AND THE *DEEPWATER HORIZON* OIL SPILL OF 2010

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The April 20, 2010 explosion of the *Deepwater Horizon* oil rig allowed large volumes of crude oil to enter Louisiana's estuaries. These estuaries support the largest domestic oyster resource,

producing over 14.7 million pounds of oyster meat in 2009. Louisiana opened all Mississippi River freshwater diversions in southern Louisiana to abate oil impacts. These openings, coupled with high Mississippi River discharge, inundated the estuary with large volumes of fresh water in an attempt to keep oil at bay. Sampling of oysters began almost immediately and included contaminant sampling, oyster mortality monitoring, and extensive sampling of oysters through the Natural Resource Damage Assessment (NRDA) process. Data collection continues, and it may be years before true impacts to oysters are ascertained. Preliminarily, no direct oiling of commercial oyster beds has been officially documented and only small amounts of oil contamination have been found in oyster meats. Significant oyster mortalities occurred in August 2010 in some areas and appear to be attributable to depressed salinities and high water temperatures. Additionally, the Louisiana oyster industry was heavily impacted through closures and near collapse of the market. Recovery efforts, therefore, should focus on restoring the oyster market as well as oysters.

CLONING, CHARACTERIZATION OF A GLUTAMINE SYNTHETASE GENE FROM BLOODY CLAM *TEGILLARCA GRANOSA*.

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Glutamine synthetase (GS) is a ubiquitous enzyme with diverse biological functions such as ammonolysis, detoxication, acid-base balance and cell signal transduction. A glutamine synthetase gene of the blood clam *Tegillarca granosa* (*TgGS*) was identified in a cDNA library and cloned by RACE PCR. The full-length cDNA was 1762 bp with a 1104 bp open reading frame encoding 367amino acids, a 65 bp 5'untranslated region, and a 593 bp 3'untranslated region, and a polyadenylation signal (AATAAA) and a polyadenine tail. The calculated molecular mass of the deduced protein was 36.27 kDa, and the theoretical isoelectric point was 5.008. A phylogenetic analysis of GS sequences showed that *TgGS* was clustered with the invertebrate group as expected. The amino acid sequence of *TgGS* showed 70% to 82% homology with that from other species, indicating the GSs are conservative in evolution and may have the same function in diverse species. Quantitative RT-PCR was used to detect the mRNA expression of *TgGS* in five different tissues. High level expression was detected in haemocytes and mantle. *TgGS* was up-regulated after bacteria *Vibrio parahaemolyticus* and lipopolysaccharide (LPS) challenges, which suggest that GS may play a role in anti-bacterium defense in the blood clam.

GENETIC ANALYSIS OF *HAPLOSPORIDIUM NELSONI* FROM FOUR U.S. POPULATIONS WITH INTERNAL TRANSCRIBED SPACER SEQUENCES.

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Haplosporidium nelsoni is a protozoan and the causative agent for the deadly MSX (multinucleated spheric unknown) disease of the eastern oyster (*Crassostrea virginica*). The disease has devastated oyster populations along much of the mid-Atlantic coast, and an outbreak is being observed in Maine. To understand the population genetics of the pathogen and its possible mechanisms of transmission, we cloned and sequenced the highly polymorphic internal transcribed spacers (ITSs) and the 5.8S ribosomal RNA gene of *H. nelsoni* from infected oysters from four populations in Chesapeake Bay (CB), Delaware Bay (DB), New Hampshire (NH) and Maine (ME). The DNA fragment (484 bp) was amplified in 10 infected oysters from each population, and products from each population were pooled for cloning and sequencing. Analysis of 129 sequences from the four populations identified 28 haplotypes. Haplotype diversity is highest in CB (88.3%) and lowest in DB (58.9%). Overall sequence divergence among haplotypes is low, suggesting a recent origin from a common source for all four populations. The frequency of common haplotypes differed among different populations. Such a difference can potentially be used to differentiate populations and infer transmission route. Further analysis of more samples with additional genes is under way.

TEMPERATURE VARIABILITY DRIVES THE INCIDENCE OF WITHERING SYNDROME.

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Withering syndrome (WS) is a chronic wasting disease of California abalones. Controlled laboratory studies have shown that elevated water temperatures play a key role in both the transmission and development of WS, and in the wild, the onset of WS-induced mortalities in intertidal black abalone are universally associated with periods of elevated water temperature. Absolute rate of mortality are decoupled from patterns in water temperature however. During aerial exposure, the flux of heat into and out of intertidal organisms is driven by complex and interacting climatic factors such as solar radiation, air temperature, and relative humidity. As a result, temperature extremes at low tide can far exceed those experienced during submersion, significantly increasing the range of body temperatures experienced by intertidal organisms. We tested the effect of regularly fluctuating temperatures on the incidence of withering syndrome by independently manipulating incoming radiation, tidal height, and water

temperature in controlled laboratory experiments. Our results reveal clearly that infection with the etiological agent of WS increases significantly with regular fluctuations in the thermal environment, regardless of the mean temperature experienced by hosts. This finding highlights an urgent need to develop a better mechanistic understanding of the response of infectious disease to dynamic climate variables.

THE IMPACT OF BAIT ON THE SUSCEPTIBILITY OF AMERICAN LOBSTERS (*HOMARUS AMERICANUS*) TO SHELL DISEASE INVESTIGATED USING NITROGEN ISOTOPE RATIOS.

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Shell disease (SD), has been present in southern New England at low levels for decades and has recently increased in range and occurrence. Large proportions of fish in American lobster (*Homarus americanus*) diet has been linked to increased rates of SD. Therefore, the use of fish as lobster bait may be linked to increased SD rates in lobsters. Lobsters from the western portion of Martha's Vineyard, Massachusetts (WMV, 41°N 71°W) were randomly divided into three groups of 16 and exposed to dietary treatments (100% herring (H), 48% crab, 48%, blue mussel and 4% plant matter (W), or 50% herring, 24% crab, 24% mussel, 2% plant matter (M)) to determine if lobster tissue $\delta^{15}N$ levels reflected diet. The results of the feeding experiment confirmed that differences in diet are observed in the $\delta^{15}N$ levels of the consumer's muscle tissue. The $\delta^{15}N$ levels of tissue samples from 175 wild lobsters with varying degrees of SD were unrelated to SD severity but did indicate lobsters were eating large amounts of fish (bait). This result does not support the speculation that fish used as bait is contributing to SD outbreaks in the southern New England area.

DISSEMINATED NEOPLASIA IN *MYA ARENARIA* LINKED TO CHRONIC HEAVY METAL POLLUTION.

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Disseminated neoplasia a diffuse tumor of the hemic system in bivalve molluscs is characterized by mitotic, rounded hemocytes. This disease is considered to be among the six most destructive molluscan diseases identified in different geographic regions and mollusc species. Efforts to link the onset of this fatal disease to environmental contaminants have depended on data collected following episodic events. To document neoplasia onset during chronic contaminant exposure we examined disease development in the soft shell clam, *Mya arenaria*, at six New England locations of known environmental, contaminant and sediment qualities. Transplants of healthy *Mya arenaria* (22–25 mm) to six sites for four months documented the highest frequency of neoplasia development and decrease of phagocytic ability at sites characterized by silt/clay rich sediments with high frequencies of heavy metal contamination. Sediment levels of heavy metals were significantly correlated with neoplasia development though not with tissue levels of heavy metals. Animal growth at all sites showed no significant relationship to environmental temperatures, sediment characteristics or contaminant levels. These results indicate vulnerability of small clams to environmental stress induced by heavy metal contamination, which decreases their immune defenses. (CASSDA and FDG through WCU to SAB, Saltonstall/Kennedy NA08NMF4270416 to CWW/SAB)

DISSEMINATED NEOPLASIA AND CLAM POPULATIONS IN A CANADIAN NATIONAL PARK—KOUCHIBOUGUAC NATIONAL PARK.

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Kouchibouguac National Park of Canada (KNPC), situated along the Northumberland Strait in South Eastern New Brunswick, encompasses an area of 238 km^2 and allows both commercial and recreational softshell clam (*Mya arenaria*) harvesting. Clam populations at nine different sites of known sediment composition in KNPC were evaluated according to clam densities, sizes and neoplasia occurrence. With up to 34.31% of clams at marketable size and up to 48.36% of 20–50mm the majority of clams are in the size range where neoplasia has been detected in other natural populations. Fully neoplastic and therefore fatally ill animals are found at frequencies between 0–10% while animals without

neoplastic cells were detected at frequencies of up to 67.92%. Though this indicates that populations are impacted by neoplasia there is a downward trend with 0–2.63% frequencies of fully neoplastic animals during most recent detections. Since *Mya arenaria* is one of the most ecologically important bivalve species in Eastern Canada, influencing primary production and nutrient cycling with a long history of exploitation and traditional fishing activity in KNPC the Park's clam management strategies need to consider disseminated neoplasia to assess the health of its clam populations. (Funding to SAB through WCUPA CASSDA and FDG)

FUNCTIONAL GENOMICS OF GAMETOGENESIS AND REPRODUCTIVE ALLOCATION IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*.

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The Pacific oyster is a marine bivalve of major economic and ecological importance. Its massive but also very flexible allocation to reproduction (up to 60% of an individual's flesh weight) makes it an interesting species in which to study factors regulating gametogenesis. Additionally, quantitative genetic studies have revealed tradeoffs between reproductive allocation and survival. In this context, recent developments of genomic resources in *Crassostrea gigas* are being combined with experimental selective breeding, QTL mapping and functional approaches to identify genes involved in the variation of reproductive allocation. Microarray technology is used to identify genes differentially expressed between lines selected to be resistant or, conversely, susceptible to summer mortality, a trait known to be related to reproductive allocation. Similarly, reproductive stage- and sex-specific genes can be identified, providing insights into spermatogenesis and cogenesis in this hermaphroditic species. As no effective mutagenesis method is yet available in oysters, the function of specific genes is being investigated directly by RNA interference and protein neutralization with antibodies. Complementary approaches contribute to identifying genes involved in reproductive allocation in oysters and will ultimately show how evolutionary forces shape their variation.

A REO-LIKE VIRUS ASSOCIATED WITH MORTALITIES OF BLUE CRAB *CALLINECTES SAPIDUS*: DEVELOPMENT OF TOOLS TO IMPROVE SOFT-SHELL CRAB AQUACULTURE.

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Soft shell crabs are a high value commodity often produced in short term aquaculture. Unfortunately, soft-shell aquaculture facilities often suffer >25% crab mortality. To investigate the cause, we analyzed crabs from soft-shell aquaculture facilities and uncovered a reo-like virus (RLV) in most dead and dying animals. Subsequently, RLV was observed in a majority of sampled dead/dying crabs from soft shell aquaculture facilities from Delaware to Forida. Since 2002, IMET (previously COMB) has reared ~1 million blue crabs in a fully-contained recirculating aquaculture system. The aquaculture center has experienced sporadic unexplained mortality of captive broodstock crabs caught from the wild. Virus infections were suspected to be responsible for many mortalities. Using a molecular assay developed to detect RLV, the virus was observed in 44% of archived broodstock mortalities. Additionally, 7 of 8 RLV positive broodstock died within a few weeks based on non-lethal assays. The virus is passed easily by injection and we have evidence that it can be transmitted by cohabitation and cannibalism. We are currently working with local watermen to develop management practices to reduce mortality from RLV in aquaculture facilities, as well as reduce potential virus contamination of waterways from flow-through soft shell aquaculture shedding systems.

THE LOSS OF BAY SCALLOP LARVAE FROM NANTUCKET HARBOR DUE TO TIDAL FLUSHING.

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Nantucket Harbor supports a wild population of bay scallops (*Argopecten irradiams irradians*) which is fished commercially. Recently, the harvest has varied from year to year by nearly a factor of ten, from a high of 32,500 bushels in 2004–5 to 3,850

bushels in 2006–7. Tidal currents in the Harbor can reach almost two knots near the entrance and could provide a mechanism for flushing bay scallop larvae out of the Harbor. To investigate this possibility, tidal drifters were deployed, which indicated that larvae spawned at high tide in the outer 1/3 of the harbor could be lost in just one outgoing tide. During the 2010 spawning season, spat collectors were deployed every two weeks at locations both inside and outside the Harbor. During the June, 2010, spawning event, the collectors (5 replicates) deployed just outside the Harbor entrance on June 16 collected an average of 785 ± 112 seed scallops, while the collectors just inside the Harbor entrance collected 140 ± 42 seed scallops. This would indicate that during the June spawning event 85% were transported outside the Harbor and were lost to the Harbor population.

BREATHLESS NIGHTS: DIEL-CYCLING HYPOXIA AND THE PREVALENCE OF *PERKINSUS MARINUS* (DERMO) INFECTIONS IN CHESAPEAKE BAY OYSTERS.

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Little is known about the consequences of chronic exposure to diel-cycling hypoxia that is common in nutrient enriched shallow waters and is characterized by dissolved oxygen (DO) concentrations that vary from supersaturated during mid-day to between near-zero and about 50% saturation in early morning hours. Laboratory and field experiments both indicated that diel-cycling hypoxia increases acquisition of Perkinsus marinus (dermo) infections by oysters, most likely by reducing immune responses. Oyster filtration rates declined in response to decreasing DO, but rapidly recovered as DO increased. qPCR assays for waterborne P. marinus indicated that non-lethal diel-cycling hypoxia decreases the release of infective cells—possibly because of lower filtration rates and feces production – and therefore may not increase exposure of nearby oysters to P. marinus. Our experiments suggest that diel-cycling hypoxia can create spatial variation in disease dynamics and is important to consider in restoration siting. Nevertheless, the ability of oysters to access the water column in shallow sites may mean that there is great potential for restoration of appropriate scale to create positive feedback favorable for oyster populations at currently degraded sites.

THE ROLE OF SHELLFISH AQUACULTURE AND NUTRIENT REMOVAL AND CREDIT TRADING IN LONG ISLAND SOUND.

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Eutrophication is recognized as a serious threat to the functioning of and services provided by coastal ecosystems worldwide. Recent studies have shown that removal of nutrients through growth and harvest of shellfish can contribute to nutrient reductions, complementing traditional watershed-based management methods. Need for an Ecosystem Approach to Aquaculture has led to development of aquaculture analysis tools that work at different scales of space (farm- to system-level), time (seasonal to annual and/or long-term analysis) and complexity (ease of use to complex process-based modeling). Tools selected for a study in Long Island Sound include a system-scale, process based ecological model (EcoWin2000), a local-scale carrying capacity and environmental effects model (FARM) and a management-level eutrophication screening model (ASSETS). This approach combines field and laboratory studies and multiple models to simulate the role of cultivated species and the overall economic impact of Integrated Multi-Trophic Aquaculture in reducing eutrophication in Long Island Sound. It includes analysis of the relationship between shellfish productivity and food sources, impacts of changes to shellfish stocking densities, and an overall assessment of ecological status. An economic analysis examines ecological services provided by filter feeding aquaculture organisms. Results provide guidance to managers on the best options for integrated nutrient management.

PHARMACOLOGICAL STUDY OF SEROTONIN AND DOPAMINE POST-SYNAPTIC RECEPTORS OF THE LATERAL CILIATED CELLS OF THE GILL AND VISCERAL GANGLIA OF *CRASSOSTREA VIRGINICA*.

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Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic–dopaminergic nerves. Serotonin and dopamine increase and decrease beating rates, respectively. We studied

serotonin and dopamine receptor types. Serotonin receptors are classified as HT1 - 7. All but HT3 are G protein metabotrophic. HT3 receptors are ionotrophic. DA receptors are classified as D1 and D2. D1 receptors are coupled to G protein Gαs and activate adenyl cyclase. D2 receptors are coupled to the G protein Gαi, and inhibit adenyl cyclase. Serotonin and dopamine agonists and antagonists were tested at the gill and visceral ganglia to determine their efficacy in altering cilia beating rates. Analysis of the receptor families and subtypes for the agonists and antagonists indicates that HT4 or HT7 receptors are present in the gill and visceral ganglia, and that the DA receptors present in the gill and visceral ganglia are the D2 type. The study also shows that this preparationis a good model for pharmacological studies of serotonin and dopamine function as well as the pharmacology of drugs affecting biogenic amines in nervous systems.

GENE EXPRESSION AS A METHOD TO DISCRIMINATE SHELLFISH POPULATIONS AND THEIR FITNESS FOR DIFFERENT ENVIRONMENTS: RESULTS WITH MANILA CLAMS AND PLANS FOR EASTERN OYSTERS.

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During the last decade, there has been an increase of the number of genomic studies performed on marine organisms to identify physiological pathways altered by abiotic and/or biotic stress. Bivalves are among the most studied organisms because of their ecological and economical importance. The manila clam, *Ruditapes philippinarum*, is one such economically-important shellfish, but currently few fully-annotated genomic data sets are available in databases such as Genbank™.

Using Suppression Subtractive Hybridization, we generated and identified more than 250 unique sequences from Manila clam hemocytes challenged by the pathogenic bacterium, *Vibrio tapetis*. Then, the expression levels of 22 genes were investigated using Real-time PCR allowing identification of genes showing strong variation in expression during *V. tapetis* challenge.

More than 14,500 Expressed Sequence Tags are available in Genbank™ for the eastern oyster *C. virginica*. Examination of the literature has enabled the identification of 19 genes showing variations according to land practices and dermo disease infection caused by *Perkinsus marinus*, one of the most severe diseases of eastern oysters. Gene expression variations will be investigated in two contrasting populations from Martha's Vineyard and Clinton Harbor.

A NOROVIRUS OUTBREAK IN VANCOUVER, BRITISH COLUMBIA RELATED TO THE CONSUMPTION OF RAW OYSTERS FROM A SPECIFIC HARVEST SITE IMPLICATING AN ILL WORKER

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Late in September 2010, the Canadian Food Inspection Agency received a report of a number of gastro intestinal illnesses related to a catered event which served raw oysters. An intensive investigation was carried out to conduct traceback of multiple oyster lots. The uniqueness of one suspected brand stood out and focused the investigation in the direction of a specific harvest site. Specific distribution/storage practices extended the shelf-life beyond the normal 7–10 days post-harvest and continued consumer exposure remained. A health risk done on the oyster products concluded that a health risk existed and oyster products were recalled. Subsequently, Health Canada laboratory testing confirmed the presence of norovirus in the specific lot implicated in the outbreak.

THE USE OF AERATION AS A SIMPLE AND ENVIRONMENTALLY SOUND MEANS TO PREVENT BIOFOULING.

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Biofouling is a major problem faced by marine industries. Physical and chemical treatments are available to control fouling, but most are costly, time consuming or negatively affect the environment. We examined the use of aeration (i.e., continuous streams of air bubbles) to prevent fouling. Experiments were conducted in Connecticut, Massachusetts and Virginia. Experimental panels (10 × 10 cm; PVC and cement) were deployed facing the seafloor with or without aeration. Aeration flowed continuously from spigots 0.5 m below the panels at a rate of ~3.3 to 5.0 l min^{-1}. After one and four weeks, aerated PVC panels at all locations had significantly less fouling than non-aerated controls. Aeration prevented fouling on both PVC and cement surfaces. Fouling was reduced on panels directly in bubble streams while panels 30 cm and 5 m away had significantly more fouling. Thus, aeration appears to be an effective and simple way to prevent fouling.

WORKING TOWARD CONSENSUS: APPLICATION OF CARRYING CAPACITY IN MANAGEMENT OF BIVALVE AQUACULTURE.

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A framework was developed for determining carrying capacity through mass-balance ecosystem modeling and stakeholder involvement that can be used to guide management of bivalve aquaculture. Two Ecopath models were constructed for Narragansett Bay and coastal lagoons, Rhode Island, USA where aquaculture has doubled in six years and user conflict is high. Cultured oysters were not a significant part of Narragansett Bay or the lagoons, despite rapid increase in the industry. Bivalves are not food limited and are unlikely to become food limited in the distant future in part due to a large detritus pool. Cultured oyster biomass in Narragansett Bay is currently at 0.5MT km^{-2} and could be increased 625 times without exceeding the ecological carrying capacity of 297MT km^{-2}. Cultured oyster biomass in the lagoons is currently at 12MT km^{-2} and could increase 62 times this value without exceeding the ecological carrying capacity of 722MT km^{-2}. Narragansett Bay and the lagoons were more productive and had higher carrying capacity than heavily cultured New Zealand bays. Involving the stakeholders in the modeling process increased understanding and acceptance of the science thereby making the results more likely to be incorporated into future management and policy formulation.

TRANSLATING SCIENCE FOR MANAGEMENT: CARRYING CAPACITY OF BIVALVE AQUACULTURE.

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Mass-balance modeling and stakeholder input was used to calculate the carrying capacity for bivalve aquaculture in two ecosystems where user conflict is high. Oyster aquaculture has effectively doubled in leased area in six years in Narragansett Bay and in coastal lagoons in Rhode Island. The carrying capacity results (297MT $km^{-2}y^{-1}$ and 722MT $km^{-2}y^{-1}$, respectively) indicate that there is potential for 625× increase in biomass of oysters in Narragansett Bay and 62× increase in the lagoons without causing unacceptable ecological impacts. However, precautionary measures should be taken when using these carrying capacity values for management. The modeling approach used does not account for temporal or spatial variability or the dynamic nature of population growth near the carrying capacity limit. Furthermore, physical conditions of these ecosystems are expected to change in the future with global climate change in ways that will undoubtedly influence bivalve population growth. For these reasons, a precautionary approach for the management of bivalve aquaculture is strongly advised. Half of the carrying capacity, equivalent to the Maximum Sustainable Yield in fisheries management, could also be used as a precautionary approach for bivalve aquaculture management. Managing below carrying capacity promotes equity among all stakeholders and activities (i.e. recreation, fisheries, shipping).

THE DEVELOPMENT OF AN ANTIFOULANT FOR USE IN BIVALVE AQUACULTURE BASED ON THE INHIBITION OF METAMORPHOSIS IN ASCIDIAN LARVAE BY SELECTED ALLELOCHEMICALS.

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Ascidian fouling poses a significant threat to aquaculture on a global scale. Several species; including *Ciona intestinalis*, *Didemnum vexillum* and *Styela clava*; are widespread invaders which severely impact the culture of bivalves. A prime example is the Greenshell™ mussel culture industry in New Zealand which has periodically experienced significant decreases in productivity due to these pests. Despite a myriad of techniques being tested with the aim of controlling fouling ascidians, a long-term, cost-effective solution is yet to be found. Our study exploits existing knowledge that a range of allelochemicals are capable of inhibiting metamorphosis in marine larvae. We aim to test these in seeking a high-tech remedy to counter ascidian fouling in the culture of bivalves. To date four compounds [polygodial (IC_{50}: 97.2 hg ml^{-1}, IC_{99}: 712.8 hg ml^{-1}), radicicol (IC_{50}: 45.9 hg ml^{-1}, IC_{99}: 928.0 hg ml^{-1}), muscimol (IC_{50}: 21.0 hg ml^{-1}, IC_{99}: 39.3 mg ml^{-1}), spermidine (IC_{50}: 4.5 mg ml^{-1}, IC_{99}: 45.2 mg ml^{-1})] have been demonstrated to inhibit metamorphosis of *C. savignyi* larvae. Further to these findings, efforts will be made to quantify effects of the most potent contenders on the physiological health of post settlement Greenshell™ mussels, followed by the development of techniques for application in an aquaculture setting.

VARIATION IN LARVAL TRAITS AMONG PAIR-MATED TETRAPLOID EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, FAMILIES.

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Triploid eastern oysters (*Crassostrea virginica*) are the cornerstone of oyster culture in Virginia, comprising 80% of cultured oysters in 2009. Triploids are produced predominantly by tetraploid x diploid mating. While these animals are pivotal to triploid production, there is little documentation of their characters, including larval culture of tetraploid crosses, relative to that of diploid and triploid *C. virginica*. To examine variation in larval traits of tetraploid *C. virginica* four groups of five pair-mated families each were produced from four tetraploid stocks. All 20 families were reared until settlement. From these four groups two trends for larval growth and survival were evident. Two groups displayed linear growth trends culminating with eye-spot development at 14 and 16 days on average. The two other groups exhibited a growth trend characterized by a slow growth during the first week of rearing and longer time to develop eye-spots, 16 and 20 days on average. The average size (±1 S.D.) of first-eyed larvae among the 20 families was varied, ranging 321 ± 18.5μm to 374 ± 7.0 μm. Survival to first-eyed larvae was greater for the two groups with linear growth rates than the two other groups. This study begins a long-term examination of variation in tetraploid characters.

IDENTIFICATION OF GENES AND MOLECULAR MARKERS IN PACIFIC OYSTER (*CRASSOSTREA GIGAS*) LARVAE THAT ARE ASSOCIATED WITH RESISTANCE TO VIBRIO TUBIASHII EXTRACELLULAR PRODUCTS AND HIGH SEAWATER pCO_2.

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Since about 2006, hatcheries on west coast of U.S. have experienced severe mortalities when raising Pacific oyster (*Crassostrea gigas*) larvae. The most likely causes of these catastrophic mortalities are the pathogenic bacterium *Vibrio tubiashii* and high pCO_2 in upwelled seawater, both of which are expected to become more serious with continuing climate change (global warming and ocean acidification). *V. tubiashii* extracellular products have been shown to cause mortality of oyster larvae, and high pCO_2 in seawater favors the dissolution of larval shells. We will address this problem from two angles. First, we will identify families that are resistant or sensitive to *V. tubiashii* extracellular products and compare their transcriptome profiles before and after exposed to *V. tubiashii* extracellular products to identify candidate genes. Second, we will perform bulk segregation analysis using RAD technology to identify genetic markers associated with loci related to tolerance of *V. tubiashii* and high pCO_2 by comparing the allele frequencies before after an exposure to *V. tubiashii* extracellular products and/or high pCO_2 calibrated to kill 70–90% of the population.

RELATIONSHIP BETWEEN THE EXPRESSION OF STRESS-RELATED GENES AND FIELD PERFORMANCE.

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We previously identified genes that respond to heat stress or differ between heat sensitive and tolerant families of Pacific oysters using lethal heat shock assays on juveniles. Here we used adults and conditions designed to approximate summer mortality to extend those results. We exposed oysters from high and low-surviving families to changing temperatures: 1 week at ambient (~12°C), a gradual increase to and a two-week chronic stress at 22°C, and acute heat shock (40°C, 1 hour). We collected gill tissue for expression analysis and to-date have quantified expression levels of 8 genes at the end of the ambient period, at the end of the chronic stress, and 8 hours after acute heat shock. We estimated genetic correlations between gene expression and three production traits (yield, survival and weight at harvest in intertidal and subtidal field trials), individual shell length, width, height, whole animal weight, and time-to-death after heat shock as the correlations among family-specific best linear unbiased predictions (BLUPs) from mixed-model analyses. HSP 27 was the single best predictor of yield. Most correlations were negative, indicating that stress-tolerant families suffer lower levels of cellular damage under stress and thus have reduced requirements for enzymes for protection and repair.

SEASONALITY AND TRANSMISSION OF PARASITES INFECTING *OSTREA EQUESTRIS* IN MASONBORO SOUND, NC.

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Various parasites have decimated oyster populations on a global scale in the past century. Protozoan parasites have contributed to infection and mortality of the commercially and economically important oyster, *Crassostrea virginica*. While several studies have been conducted to determine the seasonality of the parasites infecting *C. virginica*, little is known about how these parasites impact the co-occurring oyster, *Ostrea equestris*. To address this, monthly samples of *O. equestris* were sampled from Hewletts

Creek, NC from January to December 2010 and the prevalence of *P. marinus*, *H. nelsoni*, and *Bonamia sp.* was evaluated using molecular methods. To date, *P. marinus* has been detected in 0/12 samples collected in spring, 8/24 samples collected in the summer and 5/24 samples from the fall. *H. nelsoni* has been detected in 3/12 samples collected in the spring, 0/24 samples from the summer, and 0/24 samples in the fall. *Bonamia sp.* was only present in 7/8 oysters collected in late winter. Additional incubation studies evaluating the role of *O. equestris* in transmitting the parasite *Bonamia sp.* among oysters (including the vulnerable *C. ariakensis* and naïve *C. virginica*) are in progress and will be discussed.

IMPACTS OF *HAPLOSPORIDIUM NELSONI* AND *PERKINSUS MARINUS* ON GAMETOGENESIS AND SPAWNING OF *CRASSOSTREA VIRGINICA* IN CHESAPEAKE BAY.

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Recently, older oysters surviving years of disease exposure have been found to be reasonably abundant in Chesapeake Bay, particularly in sanctuaries from harvest. Restoration interests view them as key broodstock because of their demonstrated disease resistance. Opponents argue that these oysters, while alive, are too diseased to reproduce. Anecdotal hatchery reports have indicated difficulties in spawning wild oysters, again attributed to disease. To what extent does disease impact gametogenesis and spawning in wild oysters? Earlier research indicated that while *Haplosporidium nelsoni* was highly disruptive, *Perkinsus marinus* effects were limited to serious infections and were focused on the period of peak gonadal development. Our evaluation of oysters from four populations in 2007–8 provided contemporary support. Parasitism depressed oyster condition primarily near peak maturity in early summer. *Haplosporidium nelsoni* infections of even light intensity depressed condition, though the prevalence of *H. nelsoni* is generally low. Only heavier *P. marinus* infections affected condition. Gametogenesis and spawning were abolished only by the heaviest early-season *H. nelsoni* or *P. marinus* infections. Generally, most oysters surviving the prior season's epizootic can condition normally and spawn. Hatchery issues may relate more to natural variability in timing of gametogenesis and spawning in wild oysters than to disease.

EVOLUTIONARY ECOLOGY OF EASTERN OYSTER *CRASSOSTREA VIRGINICA* AND ITS PARASITES.

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Crassostrea virginica has long been plagued by protistan parasites *Perkinsus marinus* and *Haplosporidium nelsoni*. In Chesapeake Bay, USA, in 1959, *H. nelsoni* joined an established *C. virginica*-*P. marinus* system. How its arrival perturbed this system is the focus of our study. We proceed by conducting contemporary analyses of wild and sentinel oysters against the backdrop of a sixty-year time series, and by reevaluating archival histological materials to assess changes in parasite presentation and disease. Analyses have revealed decreasing *H. nelsoni* infection in wild oysters despite increasing infection pressure on naïve sentinels, suggesting evolving disease resistance. Some resistance to disease caused by *P. marinus* has also developed, though *P. marinus* impacts remain serious. *Perkinsus marinus* parasitism intensified in the mid-1980s, which has been attributed to drought and warming climate. Retrospective histopathology, however, reveals a fundamental change in parasite presentation since 1986. Large *P. marinus* trophonts at relatively low infection intensities have been replaced by abundant minute parasite cells; large meronts of high nuclear count have largely been lost. We hypothesize that this change relates to the observed disease intensification and an increase in parasite virulence; and that this is a response by *P. marinus* to the introduction of *H. nelsoni*.

RECRUITMENT OF BAY SCALLOPS TO ARTIFICIAL SEAGRASS UNITS AFTER HIGH DENSITY FREE PLANTING TO A SPAWNER SANCTUARY IN HALLOCK BAY, NY.

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Bay scallops once supported a vibrant fishery on Long Island, New York. Populations crashed in the mid 1980s due to a series of brown tides, and while blooms haven't occurred within the Peconic Estuary since 1995, scallops have not naturally recovered. Recent restoration efforts have produced over 6 million scallops for deployment in lantern nets or on-bottom spawner sanctuaries, leading to significant increases in scallop spat recruiting to collectors in the recent years. Hallock Bay, NY, received on-bottom free planted scallops in the winters of 2008 and 2009. These scallops experienced high survival and high densities at spawning. A series of artificial seagrass units (ASUs) were placed in this bay prior to the spawner sanctuary being established to test for recruitment effects. Scallop spat recruitment to these ASUs was monitored via artificial shoot collectors placed at the edge and interior of each unit. Results indicate there was variable spatio-temporal settlement, and no significant trends were observed for patch type or within patch location for recruitment of scallops. However, shoots enclosed in mesh spat bags had significantly higher recruits than exposed shoots, suggesting a high natural mortality rate for post-set seed scallops, likely due to predation.

DEVELOPING GUIDELINES FOR THE CULLING OF EASTERN OYSTERS *CRASSOSTREA VIRGINICA* IN NEW BRUNSWICK (CANADA).

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Many New Brunswick commercial growers claim that 25 to 50% of oyster seed from any spatfall are ''runts'', or genetically inferior in terms of growth potential. Seed removed from collectors are graded to eliminate these ''slow'' growers, without any allowance for differing histories associated with settlement time or density-dependent effects. Likewise, the first grading of seed reared in floating bags is typically severe, as it is assumed that any variance in individual growth performance is genetically-based, rather than being related to variable growing conditions within the bag. This strategy of aggressively removing the smaller oysters from a given year-class effectively increases the cost of seed, as well as the risk of seed shortage should there be a recruitment failure. This project evaluated the growth performance of four size grades of oysters from the same year-class reared using two grow-out techniques (i.e. floating bags vs. rope-grown) under different localized environmental conditions (i.e. offshore vs. inshore). The early growth performance of oysters from the two major New Brunswick seed sources was also compared at two commercial sites. The results of these trials will indicate the prevalence of true ''slow'' growers, elucidate the factors which may be responsible for their occurrence, and assist in developing appropriate culling guidelines.

ASSESSING THE EFFECTS OF NATIVE OYSTER RESTORATION ON CHESAPEAKE BAY WATER QUALITY.

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More than 25 years ago, a link was proposed between decimation of the oyster population and deteriorating water quality in Chesapeake Bay. Since then, restoration of native oysters and introduction of exotic oysters have been proposed as means to reduce eutrophication effects. The Chesapeake Bay Environmental Model Package (CBEMP) was employed to assess potential benefits of oyster restoration. The CBEMP consists of coupled models including a watershed model, a three-dimensional hydrodynamic model, and a three-dimensional biogeochemical model. The effects of a tenfold increase in oyster biomass and restoration of 19th century biomass were examined. On a local scale, oyster restoration enhances the removal of nutrients and solids from the water column. The primary effects, including improvement in water clarity and restoration of submerged aquatic vegetation, are in shallow embayments rather than in the deep mainstem of the bay. In the mainstem, summer-average bottom dissolved oxygen improves by roughly 0.3 mg/L for a tenfold increase in oyster biomass. We recommend that oyster restoration be targeted to specific areas with suitable environments and that resulting environmental improvements be viewed on similar, local scales. Oyster restoration should be viewed as one of many contributions to remediation of the bay's problems.

LOCAL RESOURCE MANAGEMENT GOES DIGITAL: IMPROVING MUNICIPAL SHELLFISHERIES MANAGEMENT WITH INTERACTIVE MAPPING TECHNOLOGY.

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Connecticut's municipal shellfish commissions are responsible for managing shellfisheries within their town waters. Each commission is required to develop a comprehensive management plan that identifies appropriate sites for both recreational and commercial shellfishing activity to avoid potential social (use) conflicts and adverse environmental effects. The use of geospatial information is critical to this decision making process. Previously, data layers including distribution and abundance of marine resources, mooring positions, shellfish classification areas, water quality data and existing harvest areas have been distributed in formats ranging from hand drawn or paper maps to sophisticated GIS datasets. The variety of information and range of technical skill required to utilize these data has been an impediment for municipal managers across the state, most of which are volunteer-based, on a limited budget, and less likely to have training in geospatial technology. With help from the Connecticut Sea Grant Program and the Connecticut Department of Agriculture, Bureau of Aquaculture, the University of Connecticut Center for Land Use Education and Research (CLEAR) has developed an interactive online map viewer to provide the commissions with the tools and information necessary to make informed decision regarding the siting of shellfisheries in their town. The online viewer is built using ESRI's ArcGIS API for Flex which allows for the creation of rich Internet applications on top of ArcGIS Server.

AFTER THE DEGREE: LOOKING BEYOND THE IVORY TOWER.

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Choosing a career after graduate school is utterly personal and requires us to consider a great many things related to field of study, location, personal relationships, and long term goals. Graduate

school prepares students quite well for a multitude of careers in academia, business and government; however, university training often promotes self recruitment to the extent that students are unaware of options outside of this setting. This session will assemble a panel of experienced professionals to answer questions and provide information about the types of positions available outside academia and tips on how to find them. Panel members include Bill Dewey (Taylor Shellfish Farms), Karolyn Hansen (Univ. of Dayton), Barbara Kirkpatrick (Mote Marine Lab), Mike Oesterling (Virginia Sea Grant), and Michael Rubino (NOAA). Panelists will share a brief bio outlining their career path, followed by questions from the audience. The insight gained in this session might additionally be useful to locate jobs in a recession, and facilitate connections across academia, agency and industry. Students at any stage in their degree or postdocs are encouraged to attend.

PATTERNS OF GENE EXPRESSION ASSOCIATED WITH ENVIRONMENTAL MORTALITY IN PACIFIC OYSTERS.

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The Pacific oyster under aquaculture conditions succumbs to periodic mortality during the summer resulting from an inability to overcome physiologically challenging abiotic stresses and pathogens during a time of reproductive and metabolic strain. To better understand the physiological demands for survival during summer reproduction, we followed three hundred oysters through a mortality event in the summer 2008. Hemolymph was periodically sub-sampled from each individual in the field through the summer, which allowed their survival to be determined by environmental factors. We then selected samples on two days preceding observed mortality, and probed a *Crassostrea gigas* microarray to measure gene expression. Examination of gene expression patterns in hemolymph from oysters that would later die revealed increased expression of genes possibly involved in cell signaling, wound repair, angiogenesis, protein metabolism, transport, and transcriptional activation when compared to their surviving counterparts. These data reveal that it might be possible to predict oyster mortality events using gene expression data.

OFF-BOTTOM OYSTER FARMING IN THE GULF OF MEXICO: EVALUATION OF START-UP COSTS AND LABOR REQUIREMENTS OF DIFFERENT AQUACULTURE GEAR IN A SIDE-BY-SIDE COMPARISON.

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A study of the most viable oyster aquaculture practices is currently being conducted at coastal sites in Alabama. Data will be used to provide guidance to current and prospective oyster farmers in the North Central Gulf of Mexico region. Here we report on the per acre start-up costs of four different types of gear: OysterGro™ cages, floating bags, LowPro™ cages, and an adjustable long line bag system. Data are derived from purchase, installation and maintenance of this gear at a commercial oyster farm in Sandy Bay, Bayou la Batre, AL. In addition, labor requirements and gear failure rates will be reported based on two field seasons of commercial production. Finally, we will provide a qualitative evaluation of the field performance of each of the gear types.

MONITORING POTENTIAL IMPACTS OF OCEAN ACIDIFICATION ON OYSTER LARVAE SURVIVAL AND RECRUITMENT ON THE U.S. WEST COAST.

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For the past four years water quality conditions in the northeastern Pacific Ocean have significantly impacted commercial hatchery production of oyster seed upon which oyster farms are dependent. Simultaneously, natural oyster sets in Puget Sound and Washington coastal estuaries may also have been affected. It is believed changes in water chemistry due to higher than normal upwelling, coupled with an increase in the prevalence of a pathogenic bacterium are key factors affecting the survival of oyster larvae and juveniles. Monitoring and experimentation around specific water quality parameters were completed in 2009–10 to investigate these changes and their impacts upon shellfish and coastal/Puget Sound ecosystems. Water quality analyses focused on a suite of inorganic carbon parameters—pH, pCO_2, aragonite saturation state, carbonate ion concentration, and others—with high-quality data for dissolved inorganic carbon and alkalinity. This report summarizes key elements of that work in the following areas: 1) overview of current projects and key players from research, industry and government; 2) relationships between upwelling and wind events, water quality, larval recruitment and spatfall in Willapa Bay and Puget Sound, Washington, with an emphasis on pCO_2 and carbonate saturation levels; and 3) shellfish larvae production, water quality and water treatments at Oregon and Washington hatcheries.

RIBBED MUSSELS AS SENTINELS FOR DERMO DISEASE.

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The oyster pathogen *Perkinsus marinus* is planktonically transmitted among hosts. Non-host filter-feeders should therefore capture transmissible stages and may provide a mechanism for

measuring rates of transmission. We sampled ribbed mussels for the oyster pathogen *Perkinsus marinus* at three sites in Delaware Bay. Thirty mussels were collected from each site. Gills and remaining soft tissues were processed separately using a standard RFTM *Perkinsus* burden analysis. Gills contained up to 27,719 cells g^{-1} whereas remaining tissues contained less than 31 parasites g^{-1} indicating that infections were not becoming established in the body. Results showed significant differences among sites: Maurice River > Cape Shore = Money Island (means of 1110, 14 and 15 cells g^{-1}, respectively). These data contrast the expectation of highest level at Cape Shore where salinities are highest, and may indicate that factors other than salinity are responsible for pathogen distribution. Before results can be broadly applied, genetic analysis is needed to confirm the species of *Perkinsus* detected, histology is needed to confirm a lack of gill infections, and experiments are needed to quantify rates of acquisition and elimination by this non-host species. Support for this work was provided by an NSF EID REU Supplement to Award 0622672.

CRACKING UNDER PRESSURE: COMPARING SHELL STRENGTH OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) AND ASIAN OYSTERS (*CRASSOSTREA ARIAKENSIS*).

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A comparative study of Eastern oysters (*Crassostrea virginica*) and the Asian oyster (*Crassostrea ariakensis*) has been undertaken, with the aim of quantifying the susceptibility of these species to predators which crush or break oyster valves to access the soft interior tissues, such blue crabs and cow-nosed rays. We compared the compressive forces needed to produce cracks in the shells of adults, 4 and 6 year olds, of both species. The data show that *C. virginica* shells are able to withstand some 250% more force than *C. ariakensis* ($p < 0.001$). In addition, we assessed the shell cracking characteristics by measuring toughness (resistance to crack propagation, kJ/m^2) and hardness (resistance to irreversible deformation, GPa). These metrics may shed light on similarities and differences in predator vulnerabilities between *C. ariakensis* and *C. virginica*, while contributing to an understanding of underlying microscopic mechanisms by which oyster valves form and enable survival.

GAINING GROUND ON ACIDIFICATION – A DISCUSSION BETWEEN SCIENTISTS, GOVERNMENT, AND INDUSTRY TO INCREASE COLLABORATIVE RESEARCH EFFORTS.

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Following a series of regional ocean acidification workshops with the seafood industry this past year, SeaWeb identified shellfish producers as the sector most concerned and most willing to actively engage on ocean acidification research and policy. This two-hour applied science seminar will identify collaborative opportunities for research & data collection and policy engagement on ocean acidification between NOAA, scientists, shellfish producers, and other stakeholders. Bringing to bear the full range of discussions and information presented throughout the Ocean Chemistry and Shellfish session, a blue ribbon panel will comment on opportunities for partnerships, incorporating their own experiences when relevant. Following these remarks the conversation will be opened up to everyone participating in the session.

PHYSIOLOGICAL AND ENDOCRINOLOGICAL RESPONSES OF *CALLINECTES SAPIDUS* TO *LOXOTHYLACUS* INFECTION.

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Loxothylacus texanus, a rhizocephalan barnacle, seems to manipulate the endocrine system of the host blue crab, *Callinectes sapidus*. Infected individual crabs undergo sexual changes and a precocious prepubertal molt (from a small juvenile to a correspondingly small-sized adult crab with female characteristics by skipping many juvenile molts). Determination of the cause for these parasite-induced changes in the host endocrine system would certainly provide information useful to manipulate the molting activity of the blue crab. The transition period during which those parasite-induced changes take place is crucial. Consequently, a preliminary attempt was made to evaluate expression-levels of important neuropeptide genes in the crab nervous system (eyestalk ganglia and thoracic ganglia complex) between infected and uninfected individuals. Evaluation focused on tissues, including hemolymph, to detect differences in gene expression as well as concentration levels of glucose, lactate, and ecdysteroids (Ecd) in the hemolymph. Our data demonstrate differences between infected and non-infected animals in terms of the transcription levels of neuropeptide gene expression and heat shock protein (HSP) 90 using quantitative RT PCR and Ecd in the hemolymph. Funding from NOAA NA17FU2841.

MULTIFUNCTIONALITY OF CRUSTACEAN HYPERGLYCEMIC HORMONE NEUROPEPTIDE FAMILY IN MOLTING AND REPRODUCTION OF THE BLUE CRAB, *CALLINECTES SAPIDUS*.

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Induction of molting and reproduction after eyestalk ablation promoted the presence of regulatory substances within the eyestalk ganglia: medulla terminalis X-organ and the sinus gland (MTXO-SG). The hormonal profiles present in the MTXO-SG may differ among decapod crustaceans, but they can be largely sub-grouped on the basis of structural homology: 1) crustacean hyperglycemic hormone (CHH) and 2) molt-inhibiting hormone (MIH)/ mandibular organ-inhibiting hormone (MOIH)/ vitellogenesis/ gonad-inhibiting hormone (V/GIH). CHH with adaptive roles by elevating the glucose level from animals experiencing stressful conditions (hyper- and hypothermia, hypoxia, and low salinity) has multiple target tissues and functions including ecdysteroidogenesis, osmoregulation, and vitellogenesis. Recently, MIH, known for exclusively suppressing ecdysteroidogenesis in Y-organs, is also reported to have an additional role in vitellogenesis of adult female, *Callinectes sapidus*, suggesting that some CHH neuropeptides may acquire an extra regulatory role at adult stage. This paper reviews the regulatory roles of CHH and MIH at the levels of specific functions, temporal and spatial expression, titers, their binding sites on the target tissues, and the second messengers of *C. sapidus*. It further discusses the diverse regulatory roles of these neuropeptides and the functional plasticity of these neuropeptides in regard to life stage and species specific physiology.

THE EFFECT OF DIEL-CYCLING HYPOXIA ON EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) CLEARANCE.

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Oysters in shallow water can be exposed to oxygen concentrations that fluctuate between hypoxic and supersaturated conditions on a day-night cycle. To assess the effect of diel-cycling hypoxia on clearance rates of *Crassostrea virginica*, oysters in two size ranges were placed in tanks on a flow-through system and exposed to three different cycling oxygen treatments. Two of these cycles mimicked the low nightly dissolved oxygen (DO) concentrations experienced in shallow, eutrophic waters, and consisted of gradually dropping DO concentrations to 1.5 or 0.5 mg/L, holding those concentrations for three hours, and gradually raising them to normoxia again. The third treatment continuously held DO concentrations at normoxic levels. Oyster clearance rates calculated from *in vivo* fluorescence and flow rates were similar across all three treatments immediately before DO concentrations were dropped and shortly after DO concentrations returned to normoxia. Oysters exposed to the 0.5 mg/L diel-cycling hypoxia treatment cleared significantly less chlorophyll a then those in the other treatments while the DO levels were held at the 0.5 mg/L target concentration. These findings indicate that oysters respond to diel-cycling hypoxia by reducing feeding during periods of low DO but feeding rates quickly recover following short exposures to hypoxia.

EVALUATING THE COSTS AND BENEFITS OF A MECHANICAL GRADER TO IMPROVE AQUACULTURE PRODUCTION OF OYSTERS, *CRASSOSTREA VIRGINICA*: AN EXPERIMENTAL TEST OF GEAR TYPE AND TUMBLING.

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Although mechanical graders are intended to sort oysters by size, industry members have anecdotally reported improved shell morphology and reduced fouling. To evaluate these potential effects, and potential costs (e.g., reduced growth), four types of commercial gear (OysterGro™ cages, floating bags, LowPro™ cages, and an adjustable long line bag system) were stocked with oysters (mean shell height = 57.2 ± 1.3 mm) and subjected to three tumbling treatments: untreated, tumbled monthly and emersion/ handling controls from June to November, 2010 in Sandy Bay, Mississippi Sound, AL. Mortality, shell metrics, shell shape, degree of fouling and sensory characteristics were measured. Although preliminary results showed that gear type significantly affected most of the response variables (e.g., mortality, degree of fouling, shell metrics, weights, etc.), there were also significant effects of the tumbling treatment. Tumbled and emersed oysters were significantly less fouled than the untreated oysters, but shell length of tumbled oysters was significantly shorter than untreated oysters. Significant interactions occurred among gear type and tumbling treatment (e.g., shell height was significantly shorter in the tumbled and emersed oysters than in the untreated oysters only in the adjustable long line system). Implications of these results to the oyster aquaculture industry will be discussed.

RESTORATION OF OYSTERS AND ADJACENT VEGETATED HABITATS: METHODOLOGIES, REEF SUCCESS ASSESSED AND POTENTIAL INDIRECT EFFECTS IN THIS RECENTLY RECONNECTED AND SUBSTRATE-LIMITED AREA ON SANIBEL, FL.

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Clam Bayou and its 470 acres were recently reconnected (2006) through the additional of culverts to the adjacent waters thereby restoring tidal hydrology. Immediately after freshwater impacting

the isolated area and its habitats (mangroves, seagrasses, and oysters) was released and within two days natural, daily tidal flows were re-established, along with more natural salinities. Along with water flow and water quality improvements, wading and shorebirds, including several listed species have returned in large numbers to Clam Bayou. Oysters and SAV are performance measures and part of the overall Comprehensive Everglades Restoration Plan (CERP). Through a TNC-NOAA grant, we have constructed intertidal reefs near existing oysters/seagrasses. A related NOAA-National Association of Counties grant is focusing concurrently on mangroves, seagrasses and oysters. In addition to habitat restoration, we are assessing interactions among these adjacent habitats. The project is greatly enhancing available substrate for oyster 'spat' recruitment. Reef development, associated organisms and other ecosystem services (seston removal) are being monitored with appropriate reference areas. The restoration of mangroves and the development of prop roots will also be monitored as potential oyster settlement substrate. To date hundreds of volunteers have participated in reef construction (>580 m^2) and red mangrove (>1.25 acres) plantings.

TRAP DESIGN FOR ASSESSMENT OF PRE-RECRUIT LOBSTER (*HOMARUS AMERICANUS*) POPULATIONS.

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The Maine Department of Marine Resources has conducted a Sea Sampling Program (SSP) on commercial lobster boats using lobster catch carapace length (CL) for stock assessment. In county catch 1985–2003, number market size catch/trap haul was positively associated with same year landings while increased proportion premarket size catch/trap haul was negatively associated with same year landings. Catch in trap surveys using commercial traps with escape vents for pre-recruit lobster removed have caught few juvenile lobster. Small volume traps constructed of 9 mm mesh were designed to catch pre-recruit size lobster (<82.5 mm CL). Ventless commercial traps and small volume traps with different trap heads were fished near Southwest Harbor, Maine. Ventless commercial traps did not catch lobster <50 mm CL. Small traps made with hard wire mesh heads with 38 mm openings did not catch lobster. Small traps with soft woven entry heads with either a wire ring or woven 64 mm head opening did catch larger juvenile lobster (45 mm < CL < 82.5 mm) Few lobster < 45 mm CL were caught in wire traps. For population assessment, traps can be used to sample larger juveniles >45 mm CL and adults.

SHELLFISH RESTORATION AND AQUACULTURE: NITROGEN SEQUESTRATION, REMINERALIZATION AND DENITRIFICATION.

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The improvements in water quality attributed to bivalve restoration and aquaculture include water filtration of algae and other particulates, sequestration of nitrogen and phosphorus in biomass, and microbial denitrification. Assessment of these water quality attributes can be difficult in the field, and much current information is based on laboratory studies. Our approach for determining rates of nitrogen recycling and denitrification uses incubation cores in sediments with bivalves and stirred microcosms for more complex reef structures. Because of the focusing of organic matter by clams and oysters, bivalve habitats have high rates of metabolism and nitrogen remineralization than other benthic habitats. In this presentation we compare denitrification and nutrient flux results from Chesapeake Bay oyster habitats and Maryland coastal bay clam aquaculture. Oyster habitats are more complex, focus more organic matter, and in general have a more positive water quality impact than do clam habitats.

VARIABILITY AND LIMITS OF THE AQUACULTURE BIOFILTER: A META-ANALYSIS OF PUBLISHED BIVALVE CLEARANCE RATES.

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A fundamental knowledge of bivalve feeding behavior is a minimum requirement for understanding how aquaculture interacts with the surrounding ecosystem. The potential environmental effects and ecological services of bivalve culture are related in large part to how the cultured population interacts with the ecosystem by means of suspension feeding. Uncertainty or inaccuracy in feeding rate estimates strongly influence predictions of bivalve growth, site carrying capacity and ecological effects. Bivalve feeding has been studied across a wide range of laboratory and natural conditions and there is a vast literature on clearance rate, which is the volume of water cleared of particles of a certain size in a period of time. Despite this large research effort, there remains uncertainty in clearance rate measurements and methodologies, which reduces confidence in predictions of community level food utilization. A literature review and statistical synthesis of published average clearance rate data (1975 to 2010) was conducted from pooled data for mussel (n = 401 mean values reported), scallop (n = 123), oyster (n = 123) and cockle (n = 111) species. The primary objectives were to investigate central tendencies and maximum capacity in bivalve feeding rates and to compare methodologies and interspecies responses.

ASPECTS OF THE BIOLOGY OF THE SOFT-SHELL CLAM, *MYA ARENARIA*, IN THE IRISH SEA.

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The first study of the soft-shell clam, *Mya arenaria*, in the Irish Sea area is being undertaken at Bannow Bay, Co. Wexford, Ireland. This study is part of a larger collaborative project assessing the impact of climate change on aquaculture and fisheries in the Irish Sea. Since March 2010, 30 *M. arenaria* have been collected each month. The reproductive biology and development of the clams is being assessed using histology. Clams are being screened for pathogens also. In addition, one-off collections of 100 individuals per year are being carried out at four sites in the Irish Sea, two on the Irish coast and two in Wales to assess the genetic variation in this species in this area. Previous work using the "universal" DNA primers for amplification of the mitochondrial cytochrome c oxidase subunit I gene *(CO1)* with Western Atlantic and Northern European members of this species has shown a low level of genetic variability and a lack of genetic differentiation among the populations studied across the range. We will investigate whether Irish *Mya arenaria* fit into the current model of soft-shell clam genetic variation demonstrated using this marker.

RECOVERY RATES OF BIVALVE HEMOCYTE INTRACELLULAR pH FOLLOWING *IN VITRO* ACID EXPOSURE

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Projections of ocean acidification effects upon carbonate shell-forming species have caused great concern for the future of shellfisheries. Bivalve species inhabiting estuarine environments, however, have evolved in environments with fluctuating pH levels. We hypothesized that hemocytes, cells directly involved in calcification, would possess homeostatic mechanisms capable of maintaining intracellular homeostasis under acidic external conditions. A previous experiment showed that intracellular granular hemocyte pH in eastern oysters exposed *in viv*o to low pH seawater (6.7) was the same as in oysters in the ambient pH of 7.8. *In vitro* assays showed the response to external acidification to be linear recovery from an initial drop within 10 minutes. These results indicate that granular hemocytes are able to re-establish intracellular pH on a time scale of minutes, despite acidic external conditions. In the current study, we proposed to determine if other molluscan shellfish species also possess these homeostatic mechanisms. An *in vitro* hemocyte pH-recovery-rate assay was applied to additional species of aquaculture interests, including the northern quahog *Mercenaria mercenaria*, the blue mussel *Mytilus edulis*, and the softshell clam *Mya arenaria*. Preliminary results indicate that time scale responses to external acidification following exposure to low pH seawater were comparable to responses observed in oysters.

MONITORING DERMO INFECTIONS IN TEXAS OYSTER POPULATIONS AND THEIR RESPONSE TO CHANGES IN FRESHWATER INFLOWS.

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Oysters are essential components of Texas coastal estuarine ecosystems. Their role as a biological indicator for determining minimum freshwater inflows is the basis of several ecological evaluations where water rights and allocations are being evaluated. Increased salinity regimes resulting from decreased freshwater inflows to bays and estuaries favors the growth of Dermo (*Perkinsus marinus*), a parasite that infects and decimates Texas oyster populations. Texas Parks and Wildlife Department has successfully applied and standardized a rapid, accurate, and quantitative molecular method (quantitative polymerase-chain reaction, or QPCR) to detect and monitor Dermo infection in oysters. QPCR Dermo results were comparable with conventional Ray's fluid thioglycolate method (RFTM), which uses a modified Mackin Scale of Intensity. QPCR was able to detect Dermo with greater frequency than RFTM during colder winter temperatures. Dermo infections detected by both methods positively corresponded to decreased monthly salinities in individual bay systems. QPCR was found to be an effective tool for quantitatively monitoring Dermo infections in oyster populations and identifying their response to changes in freshwater inflows.

DERMO (*PERKINSUS MARINUS*) INFECTION AND FRESHWATER INFLOWS INTERACTION WITH TEXAS OYSTER POPULATION DYNAMICS.

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Our study developed a deterministic model to simulate oyster population dynamics and the interaction of Dermo (*Perkinsus marinus*) infection and freshwater inflows on three reefs along a salinity gradient in West Matagorda Bay. Results showed the relationships of growth, spawning, spat set and Dermo infection were positively correlated with distance from Colorado River, timing and duration of freshwater inflows, in addition to direction of tidal amplitude. Direct gradient analysis of simulation results showed distance from freshwater source, in addition to salinity and temperature conditions thirty days prior to the sample date accounted for 36 percent of the total variation in Dermo infection

levels. Although typical seasonal fluctuations in freshwater inflows from the Colorado River provided decreased proliferation of Dermo infections in up-estuary populations, sustained reductions in freshwater inflows resulted in lethal infection levels at all reefs. Simulation results demonstrated the three reefs in West Matagorda Bay function as a metapopulation which require discrete timing and duration of freshwater inflows.

SUSFISH: SHELLFISH PRODUCTIVITY IN THE IRISH SEA- WORKING TOWARDS A SUSTAINABLE FUTURE.

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The SUSFISH project is a collaborative interdisciplinary projectbetween Irish and Welsh universities looking at the potential effects of climate change on aquaculture and fisheries in the Irish Sea area. Within Ireland the research is concentrating on looking at aspects of the health status of a range of shellfish and how climate change may influence host-pathogen interactions. Someof the data being generated is being modeled mathematically to determine how various factors may influence dynamics currently at play.

QUAHOG SHELLS AS REUSABLE AND BIODEGRADABLE LARVAL LOBSTER GROW OUT FOR USE IN OPEN WATER.

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Seeding lobster larvae enhances the resident lobster population and increases the yields of the local lobster fishery. The low survivability of larvae in laboratory culture causes this process to be both time consuming and costly. Our laboratory has developed a biodegradable lobster grow out system for use in open water employing recycled quahog (*Mercenaria mercenaria*) clam shells. This method aims to raise newly hatched and second stage lobsters larvae to a viable release age while drastically decreasing the cost associated with such seeding efforts. By raising larvae in such an enclosure in open water, the larvae have access to naturally occurring food sources, yet are protected from predation. In preliminary studies several lobsters were placed in a clam shell assuming cannibalism as initial feed. Use of these enclosures has been shown to be more cost effective that the traditional batch process of larval seeding.

DO SCAVENGERS INFLUENCE DERMO DISEASE TRANSMISSION? EXPERIMENTS IN OYSTER PARASITE TROPHIC INTERACTIONS.

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Perkinsus marinus is responsible for Dermo disease in oysters from Maine to Texas. Passive shedding of parasites from infected and moribund hosts is considered the primary mechanism by which the parasite spreads. Few studies have investigated how trophic interactions may affect transmission. This study investigates how predators and scavengers influence transmission of *Perkinsus*, and focuses on the actions of several common oyster-associated species: the blue crab (*Callinectes sapidus*), small xanthid crabs, mud snails, and small fish (mummichogs). These species can vary in abundance on natural beds, oyster leases and around oyster aquaculture. As they tear flesh to feed they may liberate some parasites while destroying others that they consume. Some parasites may survive digestion and be passed on to a new host. The balance of these interactions will determine the net impact of predators and scavengers on rates of transmission. In laboratory experiments, specific pathogen free (spf) oysters were repeatedly exposed to infected oyster tissue in tanks with scavengers (experimental) or without (control). After two months, spf oysters were assayed for differences in parasite burden. Initial data from the first replication of experiments indicated that scavengers increased parasite burden relative to oysters exposed to infected tissue alone.

INTERACTIVE EFFECTS OF SALINITY AND ELEVATED CO_2 LEVELS ON JUVENILES OF THE EASTERN OYSTERS *CRASSOSTREA VIRGINICA* (GMELIN).

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The potential effect of ocean acidification on marine calcifiers has been well established. For estuarine organisms, fluctuations in environmental parameters such as salinity may exacerbate the effects of ocean acidification. Here, we test the combined effect of salinity and CO_2 level on the Eastern oyster, *Crassostrea virginica* (Gmelin), using physiological and shell mechanical assessments. Seven week old juveniles were exposed to normocapnia or hypercapnia (380 or 800 ppm) at high or low salinity (30 or 15 ppt) for 10 weeks. Compared to controls (380 ppm, 30 ppt), mortality was significantly higher, and tissue mass significantly lower in oysters exposed to either low salinity or hypercapnia. Carbonic anhydrase activity positively correlated with mRNA expression for both

salinities under normocapnia, but did not correlate with mRNA levels under hypercapnia. pCO_2 significantly affected concentrations of ADP and AMP at low salinity, and glycogen and lipid only at high salinity, suggesting an interactive effect of pCO_2 and salinity on these energy related indices. When combined, hypercapnia and low salinity negatively affected shell mechanical properties, resulting in significantly lower hardness and crack resistance. Such interactive effects may compromise survival of Eastern oysters under future ocean conditions. Authors gratefully acknowledge NSF support (IOC 0951139 to IMS/EB).

DOES DREDGING DURING THE HARVEST OF SHELLFISH EFFECT SEDIMENT BIOGEOCHEMISTRY?

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Most studies designed to investigate effects of hydraulic shellfish harvest focus on benthic organisms with little research being done on biogeochemical changes to the sediment. A field experiment was conducted in Long Island Sound with an industry partner on leased shellfish beds where hard clams, *Mercenaria mercenaria*, are cultivated. Sediment-core samples were collected from non-dredged and dredged 1-hectare plots over a six month period. Sediment pore-water porosity, hydrogen-sulfide concentrations, dissolved total ammonia, pH, and dissolved oxygen were measured. We also collected particulate samples for grain-size analysis and particulate carbon, nitrogen, and sulfur. Preliminary results indicate that there were significant differences in pH, dissolved oxygen, and hydrogen sulfide concentration between dredged and non-dredged plots; however, there was also significant seasonal variability in these parameters. The chemical data indicate that the seasonal variability in this system is much larger than the effects of hydraulic dredging.

VIBRIO VULNIFICUS CONTROL PLANS: ASSESSMENT OF FIRST SEASON OF IMPLEMENTATION IN GULF STATES

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States bordering the Gulf of Mexico implemented control plans on May 1 2010 to reduce to risk of *Vibrio vulnificus* illness among consumers of raw Gulf oysters. The controls were based on a "risk calculator" that was developed by FDA at the request of Gulf oyster industry and regulators. Individual states customized time temperature controls according to their industry practices including reductions from baseline values for time from harvest to first refrigeration and times to cool to 13°C after refrigeration. Evaluating the effectiveness of these controls was complicated by extended closure of most of the shellfish growing areas in Louisiana, Mississippi and Alabama resulting from the BP oil spill. Preliminary reports indicate oyster harvests equal to or greater than normal in Florida and Texas to make up for lost production in neighboring states and that most of the cases reported through November were from oysters harvested in these two states. Preliminary illness reports indicate fewer cases than in most recent years. The ISSC will meet in January with more up to date illness data and will attempt to sort out possible effects of reduced harvest and to determine whether adjustments are needed in state control plans for 2011.

QUANTIFYING PHYTOPLANKTON CONSUMPTION BY FLUPSY-HOUSED OYSTERS.

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A wealth of high-quality, bench-top data is available on all aspects of oyster, *Crassostrea virginica*, biology and physiology. This is especially true for feeding-rate and metabolic activities. The metabolic activities of large groups of oysters in natural settings are less well quantified. Specifically, the interactions of nursery-cultured oysters in a floating upwelling system (FLUPSY) with the local environment are poorly documented. Extrapolating the existing data, gathered under highly controlled settings, is not an ideal approach to fill the knowledge gap.

Direct measurements of dominant phytoplankton taxa, particle size, chlorophyll, dissolved oxygen, and pH were used to evaluate oyster feeding activity in a commercial FLUPSY. Discrete water samples were taken as estuarine water entered and left the FLUPSY for phytoplankton taxonomic evaluation and size distribution analysis. Long-term, real-time data on water-quality parameters were recorded using data logging SONDES. Results indicate that oyster feeding is intermittent and often dependent upon food availability, water quality and tidal and diel influences. A mass balance approach provides insight into the metabolic activity of the FLUPSY as a system. A picture of a dynamic interaction between the FLUPSY-housed oysters and the environment emerges.

FUNCTIONAL MODIFICATIONS IN MITOCHONDRIA OF OYSTER *CRASSOSTREA GIGAS* UNDER CONTROLLED FEEDING CONDITIONS.

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Effects of modifications of mitochondrial membrane lipids on functional capacities of gills mitochondria in the oyster *Crassostrea gigas* were studied by comparing 3 groups of oysters fed T-*Isochrysis* aff. Galbana (T.*iso*), *Chaetoceros calcitrans*, or a mix of both. In parallel, oysters were kept in the rearing area for the same duration (5 weeks).

Mitochondria purified from gills were analyzed and no change neither in the protein level nor in the citrate synthase activity were detected among dietary treatments Despite the differences in fatty acid composition of *C. calcitrans* and T.*iso*, mitochondrial oxidative capacities and cytochrome c oxidase activity were not significantly different according to experimental diets. These results suggest that oysters could possess high homeostatic capabilities to minimizing the effect of dietary fatty acids and related membrane lipid modifications on mitochondrial functionalities.

Interestingly, higher mitochondrial activities were found in oysters kept in natural environment compared to dietary conditioned oysters suggesting that other environmental parameters, e.g. other nutrients found in their natural food, could modulate more drastically mitochondrial capacities.

OYSTER RESTORATION GOALS FOR WILLAPA BAY, WASHINGTON SHOULD REFLECT THE PROMINENT ROLE OF AQUACULTURE.

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Two well documented ecosystem services for native oysters on the US east coast are their capacity to filter large quantities of phytoplankton and their ability to form extensive reefs that provide habitat for other fish and invertebrates. The role of the native oyster, *Ostrea lurida* in US West coast estuaries, however is less clear because they are no longer present in sufficient quantities to quantify these services and instead have been replaced with actively cultured Pacific oysters, *Crassostrea gigas*. We used information on filtering rates, as well as the present role of live oysters and shell as habitat for juvenile Dungeness crab (*Metacarcinus magister*), to contrast these two ecosystem services in Willapa Bay, Washington. Native oysters were estimated to have covered 12% of the low intertidal area in this estuary which contrasts favorably with the current estimate of 20% of the estuary now used for aquaculture; however reef morphology and location of these two oysters in the estuary differ substantially. These differences result in potentially greater levels of these two ecosystem services provided by aquaculture than those historically provided by native oysters, which should be considered when setting oyster restoration goals for this and other estuaries along this coast.

THE ABILITY OF PAS, ACETYLSALICYLIC ACID AND CALCIUM EDTA TO PROTECT AGAINST THE TOXIC EFFECTS OF MANGANESE ON MITOCHONDRIAL RESPIRATION AND MEMBRANE POTENTIAL IN THE GILL OF *CRASSOSTREA VIRGINICA*.

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Manganese is an essential metal that at excessive levels in brain causes Manganism, which is similar to Parkinson's disease. The mechanism of action is not completely understood but may be due to mitochondrial damage and resulting dysfunction of the brain's dopaminergic system. Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations from their ganglia. Manganese treatments disrupt the cilio-inhibitory dopaminergic system. Here we studied effects of manganese on mitochondrial respiration and membrane potential. We prepared mitochondria from gill and studied respiration and mitochondrial membrane potential using the cationic dye TMRM (Tetramethylrhodamine, methyl ester, perchlorate). We took time lapse micrographs of gill filaments and mitochondrial smears. Manganese caused dose dependent decreases in O_2 consumption, which was blocked by calcium disodium EDTA, p-aminosalicylic acid (PAS) or acetylsalicylic acid (ASA). Manganese decreased mitochondrial membrane potentials and this was partially blocked by PAS, but not caEDTA. The study demonstrates that manganese reduces oxygen consumption and disrupts the mitochondrial membrane potential. PAS and ASA protected against both toxic effects and may be better therapeutic agents than caEDTA in the treatment of Manganism.

EFFECTS OF PROLONGED FRESHWATER EXPOSURE ON EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS IN A NORTHERN GULF OF MEXICO ESTUARY.

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With several large-scale freshwater diversion structures across coastal Louisiana, it is important to know the effects of prolonged freshwater exposure on economically and ecologically valuable oyster populations. Seed and market sized oysters in cages resting on the bottom, as well as recruitment plates suspended in the water column, were monitored at four sites in Breton Sound, LA, along what is typically a salinity gradient ranging from ~5 to ~20. In April 2010, the Caernarvon Freshwater Diversion was fully opened with the goal of minimizing the impacts of the *Deepwater Horizon* oil spill on wetlands, resulting in extreme low salinity (<2) at all sites through August 2010. High seed and market-sized mortality and reduced condition were observed in oysters at all sites. Only the site that normally has the highest salinity showed any recruitment as of October 2010. *Perkinsus marinus* infection prevalence in surviving market oysters was low at all sites and all infection intensities were light. While low salinity may be beneficial to oyster populations by reducing *P. marinus* infection levels, prolonged extreme low salinity through spring and summer appears to cause heavy mortality and negatively impact recruitment in the short term.

BLUE CRAB POPULATION DYNAMICS, STOCK ENHANCEMENT AND AQUACULTURE IN NORTH CAROLINA.

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Unprecedented low levels of spawning stock biomass (SSB) in NC since 1999 appear to have been sustained by correspondingly low recruitment of megalopae and juveniles. We summarize results of field and lab studies during 2001-2008 aimed at assessing the feasibility of (1) stock enhancement and (2) spatial closures to rebuild SSB, as well as the (3) efficacy of freshwater pond grow-out of hatchery-reared (HR) blue crabs to provide soft-crabs for increasing market demand. The level of SSB and frequency of storms explained over 80% of the annual variation in megalopal settlement. Wild-caught early juveniles stocked in recruitment-limited coves enhanced local densities over controls; however, stocked crabs appeared to emigrate via pelagic dispersal within 5 weeks of stocking. Field surveys and analyses of fishery-independent data indicated that current spawning sanctuaries in NC protect less that 1% of the blue crab spawning stock. HR crabs stocked in freshwater ponds grew a record rates, demonstrated ~15% survival, and show strong economic potential if (1) a reliable source of seed crabs can be identified, and (2) an efficient means to remove peelers from ponds is developed. Fishery managers must re-build blue crab SSB and protect key settlement and nursery areas to ensure population growth.

DETERMINATION OF THE EFFECT DIET HAS ON THE ELEMENTAL COMPOSITION OF THE JUVENILE SHELLS OF THE HARD SHELL CLAM, *MERCENARIA MERCENARIA*.

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Biogenic carbonates have received much attention in the recent past due to their potential as proxies of environmental change; however, due to the multiple influences on the chemical composition of these carbonates, it is vital to understand the degree to which each effector has in order to accurately interpret collected data. This research examines the elemental composition differences of the juvenile shells of the northern quahog *Mercenaria mercenaria* as compared to diet. All specimens were reared under strict salinity (30 ± 2) and temperature (28°C) parameters, but received different uni-algal species diets, a mixture of all algal species, or were not fed (starvation control). Shells were randomly selected at different growth intervals and, after preparation, analyzed using inductively coupled plasma—optical emission spectrometry (ICP-OES) to obtain elemental concentration data. The concentration values derived were analyzed both as element/Ca ratios as well as straight concentrations as compared to valve length, valve mass, time, and diet received. Results indicate trends in shell dynamics related to physiological condition as well as diet received.

QUANTIFYING OYSTER REEF LOSS AND FUNCTIONALITY AT ESTUARINE AND ECOREGIONAL SCALES: TOWARDS QUANTITATIVE GOALS FOR RESTORATION IN THE US.

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Globally it has been estimated that some 85% of oyster reefs and beds have been lost in the past two centuries (Beck et al. 2011). Through an extensive new review we have developed quantitative

data on the current and historic extent of oyster reef habitat in ~40 US bays. In addition we have collated data on oyster density and size-class distribution, enabling the estimation of biomass. Such data enable us to better characterize changes in habitat quality over time.

As oyster reef restoration in the US has gained momentum, and success has been achieved at ever-larger scales, there is a growing need to define meaningful restoration goals. Oyster reefs provide a range of ecosystem services including water filtration, enhanced denitrification rates within surrounding sediments and the provision of fish habitat. We argue that ecosystem services can provide an ecologically meaningful framework for setting oyster restoration goals, and use water filtration as a case study.

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MUCOSAL C-TYPE LECTINS IN *CRASSOSTREA VIRGINICA* AND *MYTILUS EDULIS*: POTENTIAL INVOLVEMENT IN PARTICLE CAPTURE AND MUCOSAL IMMUNITY.

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Lectins are known to participate in the defense functions of bivalves where they play an important role in the recognition of foreign particles. They also contribute to other processes requiring carbohydrate-protein interactions such as symbiosis and fertilization. Our recent work has demonstrated the involvement of lectins present in pallial mucus in particle sorting in suspension-feeding bivalves. Here we describe two mucosal C-type lectins from the oyster *Crassostrea virginica* (CvML) and the mussel *Mytilus edulis* (MeML). The sequences of these lectins present a signal peptide, a single carbohydrate recognition domain, and two putative conserved sites for calcium binding. Both CvML and MeML transcripts were expressed in mucocytes lining the epithelium of the digestive gland and the pallial organs but were not detected in other tissues including hemocytes. Further investigations demonstrated that the expression of CvML and MeML were significantly up-regulated following starvation. Additionally, the expression of CvML was up-regulated after bacterial bath exposure but not after injection of bacteria into oyster's adductor muscle. These results highlight the potential role of CvML and MeML in the interactions between suspension-feeding bivalves and waterborne microorganisms at the pallial interfaces with possible involvement in primary physiological functions such as particle capture or mucosal immunity.

SEPARATION OF HEMOCYTES FROM *CRASSOSTREA VIRGINICA* USING PERCOLL GRADIENTS AND LABELING WITH METALLOPROTEINASE ANTIBODY

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The Eastern Oyster *Crassostrea virginica* is a benthic organism that is constantly presented with innumerable immune challenges such as parasites, bacteria and viruses. As such, innate immunity is extremely important with the shell functioning as the first line of defense. Hemocyte-driven molluscan shell growth and repair are crucial functions needed to protect vulnerable tissues. Few molecular tools are available to isolate and study hemocytes and relatively few immune markers have been identified. Furthermore, little is known about the overall cellular mechanisms that drive shell repair. In this study, a protocol for separating hemocytes by density on percoll gradients was developed to determine whether there were observable differences in enriched fractions of hemocytes of varying density. Only the 30% and 50% percoll gradients contained bands of hemocytes. Next, these hemocyte populations were labeled with Cv-MMP, an antibody against oyster metalloproteinase. As modulators of innate immunity in mammals, little is known about the role of metalloproteinases in invertebrate host defense. Metalloproteinase was observed in large aggregations of hemocytes, but not in smaller aggregations or single cells. The shell matrix protein Folian was also observed in these plated hemocytes. This data indicates a possible role for MMP and Folian in encapsulation or shell repair, both functions of hemocyte aggregation.

MICROSCOPIC OBSERVATIONS OF JUVENILE OYSTER DISEASE SHELLS FROM *CRASSOSTREA VIRGINICA*

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The Eastern Oyster *Crassostrea virginica* is a keystone species that occupies estuarine ecosystems. As such, they are preadapted to survive extreme variations in temperature and pH. Environmental conditions are expected to worsen with global climate change and ocean acidification may further stress the immune response. This will especially impact juveniles, which are particularly susceptible to juvenile oyster disease (JOD). One hallmark of JOD infection is the presence of brown conchiolin deposits on the shell. Conchiolin is a secreted by bivalve cells on the edge of the mantle epithelium, and is often secreted during shell repair, encapsulating foreign

pathogens. This poster reports observations of brownish conchiolin regions of shell repair in adult oysters, conchiolin patches from juvenile oysters affected by JOD, and normal shell. Under scanning electron microscopy, the conchiolin samples appeared less ordered and more fleshy and organic than the ordered calcite structures of normal adult shell, although EDS data revealed that these shell types were comprised of the same elemental composition.

A PROFITABILITY ANALYSIS OF BOTTOM CAGE OYSTER AQUACULTURE IN THE PATUXENT RIVER, MARYLAND

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The state of the Oyster Fishery in the Chesapeake Bay is in a prolonged state of collapse. A relatively new idea aimed at increasing the oyster population is aquaculture. The aquaculture model involves a transition from the traditional wild harvest model to an agricultural model. This paper provides the theoretical background to oyster aquaculture, case studies of successful transitions to aquaculture and analysis of a current oyster aquaculture businesses or demonstration projects. Specifically, this paper focuses on an active demonstration project on the Patuxent River run by the Morgan State University Estuarine Research Center in St. Leonard, MD. Through such analysis one can gain an idea of the policies and preconditions necessary to a successful transition to aquaculture. These include continued research and feasible changes in state regulations, specifically to allow a transferable lease system that allows the market to allocate the best areas for aquaculture to those with the ability to exploit them. With the proper preconditions a small scale bottom cage aquaculture business is a viable business model. Within an aquaculture business, there are three main factors influencing the outcome of any aquaculture activity on this scale; growth rates, survival rates, and price. Growth rates and survival rates both influence the profitability of any aquaculture venture, but in the sensitivity analysis neither variable caused farm survival to drop below 90%. A change of price however, by $0.05 in either direction made an incredible difference in whether the aquaculture project would fail or be successful.

SUCCESSFUL IDENTIFICATION AND DISCRIMINATION OF HATCHERY REARED BLUE CRABS RELEASED INTO THE CHESAPEAKE BAY USING A GENETIC TAG.

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Before animals reared in captivity can be used as a model for study or to supplement wild populations it is important to demonstrate that they can integrate into wild populations with high success and without adversely affecting the natural population. For the blue crab *Callinectes sapidus*, coded radio wire tags have been used to positively discriminate hatchery raised individuals from wild. However, this technique can be prohibitively expensive due to high startup costs and manpower needs during implantation; also the mortality rate during implantation is a consideration. In order to reduce costs and increase the number of possible individuals monitored per unit effort, the suitability of the mitochondrial gene ND2 as a genetic tag to identify hatchery crabs was tested. Batches of juveniles approximately 20 mm in carapace width were implanted with wire tags and released into a variety of habitats in the southern Chesapeake Bay. Juveniles were later sampled from release sites and scanned for wire tags and sequenced with ND2 specific primers. Sequence comparison to batch mothers showed 92.6% congruence in positive identification through wire tagging and ND2 sequencing. Thus, use of genetic tags is as successful as wire tags with lower costs and mortality.

HETEROPLASMY IN THE MITOCHONDRIAL GENOME OF THE BLUE CRAB *CALLINECTES SAPIDUS* DEMONSTRATED IN MULTIPLE LOCI ACROSS GENERATIONS.

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Heteroplasmy is the existence of multiple genomic haplotypes within a single individual and has been demonstrated in many organisms including humans. Although frequency is often low, even slight genomic heterogeneity can have profound effects on the health of the organism. Sequence chromatographs of mitochondrial markers from *Callinectes sapidus* often contain double peaks indicating the presence of multiple different sequence products and possibly heteroplasmy. In order to elucidate the nature of these observations a male and female blue crab as well as their megalopal offspring were tested for heteroplasmy by amplification and cloning of PCR products using ND2, ND4, and COXI specific primers. Sequencing comparison of clones revealed as many as 24 haplotypes from one gene in a single individual, and in some cases, premature stop codons. However, sequence identity to offspring was exclusive to the mother indicating that maternal inheritance of mitochondria was conserved and heteroplasmy was not a result of contaminated sperm. There were also significant differences in haplotype diversity between genes indicating that some loci are more plastic than others. These results call into question assumptions of linear inheritance in this species and encourage the use of *Callinectes sapidus* as a model for future studies of mitochondrial heteroplasmy.

MODELLING THE ROLE OF BIVALVE SHELLFISH IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) - EXAMPLES FOR SHRIMP PONDS AND OFFSHORE FINFISH CAGE CULTURE.

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There is a lively discussion in the US and EU at present regarding the role of Integrated Multi-Trophic Aquaculture (IMTA) in the development of sustainable aquaculture, both in terms of optimizing production and reducing negative externalities. Simulation models can inform decision-making on these issues, by exploring changes in harvest potential and in environmental emissions from fed aquaculture due to bioextraction of organic particles by bivalve shellfish. We present an example for pond culture of shrimp, quantifying the significant improvements to water quality in IMTA with Pacific oysters, and the consequences for effluent loading to receiving waters. Carrying capacity optimisation for pond production is determined by means of models, and some practical issues are highlighted with respect to culture cycles and disease risks. In the second case study, we test a large (5 km^2) offshore aquaculture area where Mediterranean mussels are combined with gilthead bream, and illustrate the role that shellfish play in reducing biodeposition of excess feed and finfish waste products. The models are used to study the ideal proportions of the combined aquaculture, in terms of production and environmental effects. The use of quantitative indicators in both examples to calculate indices for sediment organic enrichment and eutrophication is exemplified.

BOX AND SPATIAL MODELS FOR ECOSYSTEM-BASED MANAGEMENT.

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The multiple ecological processes involved in aquaculture sites makes ecological modeling an essential tool for ecosystem-based management, increasing the understanding of and assessing the potential interactions in complex ecosystems. The spatial scale at which the study area is defined is a major distinction between model techniques, ranging from few cells representing a whole bay (box models) to hundreds of cells representing multiple locations on a geolocated map (spatial models). Box models entail coarse spatial resolution, but their strength lies in their computational simplicity and condensed hydrodynamics, resulting in a valuable first approach to culture management. High-spatial resolution models require coupling of a physical model with a biogeochemical one, increasing the time and cost needed to perform the analyses. However, the spatial resolution allows simulation of the effects of farm location on the ecosystem and the interactions between farms. In addition, results can be mapped, which enhances the potential of the model for marine spatial planning. Although spatial models are desirable for their high resolution, we have found multiple box models to be rapid and effective modes of simulation. In this communication we will demonstrate some modeling examples, summarizing the strengths and weaknesses of both approaches.

THE EFFECT OF CAGE SIZE AND RATION ON GROWTH OF THE JUVENILE BLUE CRAB, *CALLINECTES SAPIDUS* (RATHBUN), IN A RECIRCULATING SYSTEM.

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Blue crab (*Callinectes sapidus* (Rathbun)) hatchery technology is being developed at the Center of Marine Biotechnology (Baltimore, MD, USA). To more efficiently produce juveniles and adults for research purposes, the appropriate cage size and ration need to be determined. We performed a 21-week experiment, using a commercial pelleted shrimp diet and three rations representing a range of recommended rations for penaeid shrimp. We developed a compartmentalized recirculating system with two cage sizes ($10 \times 10 \times 10$ cm and $15 \times 15 \times 10$ cm) to determine if smaller cages limit growth. Both mean wet weights (using initial weight as a covariate) and food conversion efficiencies were significantly affected by cage size and ration ($p < 0.05$, Nested ANOVA). Percent of food consumed was not significantly different among treatments, except in the small cages where the intermediate ration was significantly higher than the other two rations ($p < 0.007$). From this data we concluded that small cages inhibit growth. In addition, all the rations are sufficient to maintain basic metabolic functions, but within a range such that crabs aren't being overfed. Genotyping of the brood revealed evidence of multiple paternity, and the food conversion efficiency was significantly different ($p = 0.037$) between crabs with one of two different fathers.

AN ENVIRONMENTALLY–FRIENDLY COATING SYSTEM THAT OFFERS EXCELLENT FOUL RELEASE PERFORMANCE

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Attachment of bio-fouling on ship hulls, off-shore constructions and inland water conservancy facilities has been a serious issue. It lowers the system efficiency, increases operation cost and requires tedious cleaning services. The traditional technology against bio-foul attachment is to apply the anti-foul (AF) coatings

over the surface to be protected. However, it has been realized that AF coatings contain inorganic or organic biocides that pollute the ocean and inland water environment and cause ecological concerns. An environmentally-friendly coating system (called DUPLEX), was therefore developed, which does not contain biocides and other harmful materials. The new novel coating system offers excellent foul release performance. Other unique properties include low VOC, high flash point, easy application, good mechanical durability, etc. Any of related industries, such as ship building/repairing, power plant water cooling intake, fish farming nettings, etc., should be benefitted by using Fujifilm DUPLEX system.

PAYMENT FOR ECOSYSTEM SERVICES PROVIDED THROUGH SHELLFISH AQUACULTURE: CHALLENGES AND OPPORTUNITIES

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Cultured and wild bivalves provide an array of ecosystem services including water filtration, nutrient sequestration and denitrification, and habitat. The ecosystem services provided also have direct human use benefits with commercial, recreational, subsistence and cultural value. While progress has been made recognizing the ecosystem functions of shellfish, the non-commercial values of the benefits accrued are generally provided free of charge, remain largely outside the realm of economic decision-making, are not protected from degradation, and are often only appreciated when it is too late. Payment for ecosystem services (PES) is receiving considerable attention in broader society and has been applied to other activities such as forested watershed and wetland conservation, contaminated site remediation, and insecticide use. However, little traction has been gained in recognizing the values provided by commercial shellfish farming. For commercial operations, the challenge faced in the acceptance of PES relate to validating the benefits of shellfish culture with the episodic pulse disturbances that occur during production. This paper will consider how ecosystem services have been successfully considered in other applications, review potentially available markets for the ecosystem services provided by shellfish, and examine the challenges that the industry must address to receive broader recognition of the services provided.

CHANNELED WHELK (*BUSYCOTYPUS CANALICULATUS*) ASSESSMENT IN THE MID-ATLANTIC: SEXUAL COMPOSITION, SIZE FREQUENCY, AND AGE AT MATURITY.

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Relatively few studies have been carried out on the reproduction of channel whelk despite their importance as a fishery product in the US. An understanding of the sizes at which a fishery becomes reproductively mature is essential for management considerations. This research investigates the reproductive biology of the channel whelk population in the Mid-Atlantic region and in particular calculates the size and age at which the population becomes sexually mature. Spatial variations within the channel whelk Mid-Atlantic resource area may have consequences in the implementation of minimum landing sizes for the fishery. Preliminary results on whelk aging techniques, population size frequencies, and reproductive assessment will be discussed.

OVERVIEW OF SEAFOOD, HARMFUL ALGAL BLOOMS (HABS), AND HUMAN HEALTH.

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Harmful algal blooms (HABs) are caused by exuberant growth of phytoplankton (e.g. dinoflagellates, diatoms and cyanobacteria) which cause harm to humans, other animals and the environment through the production of potent natural toxins and/or anoxia. HABs appear to be increasing in their incidence and geographic distribution worldwide in all aquatic environments. Several diseases have been identified in humans and other animals associated with the consumption of seafood contaminated with these HAB toxins, as well as through other routes of exposure (including direct water contact and aerosols), resulting in acute and chronic illness. Although the majority of the HAB-associated diseases consist of acute gastrointestinal (vomiting and diarrhea) and neurologic symptoms (strange sensations known as paresthesias) lasting a few days, these illnesses can be deadly. Furthermore, in certain cases, these illnesses can continue for weeks to months, causing significant neurologic symptoms (paresthesias, and possibly mood and memory changes). Since these HAB toxins are tasteless and resistant to heat and acidity, cooking and storage methods do not prevent these diseases, only the prevention of exposure through the detection of the organisms or of the toxin contamination. As the HABs increase, the seafood industry will increasingly face their growing negative perception and economic impacts.

REGULATION OF WATER PROCESSING IN SUSPENSION-FEEDING BIVALVE MOLLUSCS

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This study examined modulation of pumping activity under conditions of varying dietary quality and quantity over both long (>300 min) and short (30 min) time periods. Although many studies have shown that changes in seston concentration and quality affect water processing, digestion and absorption rates in suspension feeders, the physiological mechanisms underpinning these responses have yet to be identified. Planar (2D) particle image velocimetry (PIV) was used to visualize and calculate flow fields generated by water

processing activity in two species of bivalves: blue mussels, *Mytilus edulis*; Atlantic bay scallops, *Argopecten irradians*. Two potential loci of control were examined; exhalant velocity (V), and cross-sectional area of the exhalant aperture (Ax). Volume flux (Q) was calculated as the product of V and Ax, and compared to clearance rate (CR) measure during the assays. Major findings included the following: 1) in some cases *in vivo* responses of mussels were different than those of scallops when exposed to the same feeding regimes; and 2) control of water processing and feeding can be decoupled and altered independently in both mussels and scallops. These results imply that both autonomous and physiological regulatory mechanisms of control can be employed in species-specific manners.

UTILITY OF SELECTIVELY BRED LINES OF THE NATIVE OYSTER (*CRASSOSTREA VIRGINICA*) FOR HALF SHELL AND ON BOTTOM GROWOUT IN VIRGINIA.

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Over the past decade genetic improvement of domesticated lines in Virginia was defined by disease resistance to MSX and dermo. With the development and successful dissemination of these animals to the aquaculture industry new markets are emerging with new traits for selection. For grow out, two markets exist. An intensive half shell market where a grower expects to harvest his crop in 18 months and an extensive -on bottom- culture of remotely set eyed larvae where the product is shucked oyster meat and growing time is usually 24 months. For the former fast growth and meat yield are important with disease tolerance less so because the lethal second summer of disease exposure is eliminated. However, with the slower growing conditions of on bottom culture, disease resistance still drives successful production. Accordingly, we developed a mass selection strategy to deliver a product that both farmers could use. This study tests the utility of our lines for each grow out scenario. Survival, growth rate and meat yield were assessed over a 28 month period in various salinities. Selectively bred lines were compared to a wild control. Results showed that our lines were significantly better than controls and useful for both purposes.

A SUBMERSIBLE OYSTER AQUACULTURE SYSTEM FOR THE CHESAPEAKE BAY.

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As an undergraduate senior project, a group of ocean engineering students at the U.S. Naval Academy designed a submersible aquaculture system for grow out of the eastern oyster (*Crassostrea virginica*). The structure components were specified for conditions of the Chesapeake Bay near Annapolis, MD where inland waves, icing and multi-use issues can be substantial. The design process included wave forecasting analysis, mooring system design using catenary-type equations, hydrostatics, system structure analysis and an investigation of fouling resistant materials. An engineering economic analysis was also performed.

BAC HAPLOTYPES SHOW EXTENSIVE SEQUENCE POLYMORPHISM IN THE PACIFIC OYSTER GENOME.

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BAC clones (N = 58) from *Crassostrea gigas* were chosen for sequencing on the basis of hybridization to eight immunity-related genes. Clones were assembled into nine sets representing distinct regions of the oyster genome; for some sets, contigs representing different haplotypes were obtained. Manual gene annotation was undertaken using an extensive *C. gigas* EST database, *in silico* gene prediction programs, haplotype alignments and comparison with gene models from other species. Extensive sequence polymorphism in aligned haplotypes reflected abundant SNPs (1 per ≈30 bp in noncoding sequence, 1 per ≈65 bp in CDS), with amino acid sequences of inferred proteins typically differing among alleles by 0-3%. Clusters of related genes were found in many contigs. Indels were abundant, including micro-indels (<10 bp), diverse transposable elements, and occasionally entire genes. Many transposable elements appear to be recently active, as evidenced by 'empty sites' in haplotype alignments. Dinucleotide microsatellites were the most abundant simple sequence repeat, with (AG/CT)n found every 25 kb, (AT)n found every 83 kb, and (AC/GT)n found every 559 kb; no CG microsatellites were observed. The high level of haplotype variability will complicate assembly of whole genome shotgun sequences from *C. gigas* despite the use of inbred source material.

SUMMER MORTALITY OUTBREAKS OF FRENCH PACIFIC OYSTERS, *CRASSOSTREA GIGAS* SINCE 2008: RESULTS OF THE REPAMO NETWORK SURVEILLANCE.

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Officially created in 1992, the French network for surveillance and monitoring of mollusc health (REPAMO) ensures the survey of shellfish health status along French coasts according to the obligations of the European Directive 2006/088/EC, notably the monitoring and investigations of mollusc increased mortality. In 2008, important increased mortality events were reported in most Pacific oyster producing areas in France during spring and summer. The pattern of the mortality notifications seemed to differ from other years, with a higher number of notifications and higher mortality intensity (up to 80–100% in some areas). The mortalities were sudden and mainly affected 6 to 18 month old *Crassostrea gigas* juveniles. OsHV-1 -especially a new variant OsHV-1 microvar- and bacteria belonging to Vibrionaceae family were frequently detected in affected populations. In 2009 and 2010, recurrent severe mortality outbreaks also occurred with the detection of the variant OsHV-1 microvar in most cases. The 2009 and 2010 mortality pattern was lightly different from 2008 suggesting that a disease was introduced and spread in 2008 and it has become endemic since 2009.

EAST MEETS WEST: THE NOVEL USE OF ROCKY INTERTIDAL BIVALVE RECRUITMENT TECHNIQUES IN A SALT MARSH ECOSYSTEM.

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The ribbed mussel (*Geukensia demissa*) has been hypothesized to generate significant ecological effects on the salt marsh ecosystem by depositing nutrients to the benthos that fertilize marsh grass, *Spartina patens* and *Spartina alterniflora*. Changes in ribbed mussel populations have a direct positive relationship with changes in the salt marsh habitat influencing its stability. We compared mussel recruitment in different regions of a marsh over two years. For recruitment collectors we used a common household scouring pad (Tuffy®, Clorox Corporation), which has been used for bivalve recruitment in other marine studies. We examined recruitment in different regions of the marsh, with 3 replicate collectors per site at 6 different sites. Recruitment was determined by examining all individuals in each Tuffy after ~30 days of deployment. Ribbed mussel larvae counts were higher the summer of 2008 as compared to 2009. Overall, recruitment was significantly different among areas in the marsh ($P < 0.001$) as well as among dates ($P = 0.025$). Different species responded disparately among sites and dates ($P < 0.021$). Ribbed mussel recruitment was highest near creeks as opposed to mid or high marsh areas. In addition, ribbed mussel recruitment was highest in June in both 2008 and 2009.

INVESTIGATING THE ROLE OF DNA METHYLATION AS AN EPIGENETIC MECHANISM IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*).

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Environmentally induced epigenetic changes are increasingly acknowledged as important factors contributing to the physiological and ecological responses of organisms to environmental change. Considering the importance of DNA methylation in gene regulation, its susceptibility to environmental influence, and its potential heritability, this epigenetic mechanism is an ideal candidate for providing insights into how shellfish respond and adapt to their environment. DNA methylation has been well studied in mammals, however the same level of research has not been extended to invertebrates and surprisingly little is known about this mechanism in these taxa. Recently, we have applied *in silico* approaches to characterize DNA methylation in Pacific oysters (*Crassostrea gigas*). Our results suggest that DNA methylation has regulatory functions in *C. gigas*, particularly in gene families that have inducible expression, including those involved in stress and environmental responses. In light of these findings, we are currently adapting a number of new technologies to experimentally investigate DNA methylation in oysters in order to better understand how environmental stressors induce DNA methylation changes and whether these induced patterns are transmissible across multiple generations. Preliminary results and broader implications of these analyses in terms of applications in ecology and aquaculture and will also be presented.

EFFECT OF MENHADEN AND SOY OILS ON JUVENILE GROWTH AND EARLY GONAD PRODUCTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*.

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Dietary lipids are important sources of energy and essential fatty acids for aquatic animals. Sources of animal and plant oils are increasingly limited and expensive, and dietary requirements for

these oils must be carefully considered in diet formulations. High levels of dietary lipids (≥6.3% as fed) from either neutral or phospholipid sources negatively affect growth in adult or juvenile *Lytechinus variegatus*, respectively. Knowledge of essential fatty acid requirements of sea urchinsis limited. In this study, fifteen semi-purified diets that varied in neutral lipid source (menhaden oil, menhaden oil +2% soy lecithin, menhaden oil +1.33% soy oil, or soy oil) and level (0, 2, 4, or 8%) were fed to *L. variegatus* (average initial wet weight 5.68 ± 0.03 g, n = 10 per treatment) for eight weeks. Increasing levels of dietary lipid, regardless of source, negatively affected wet weight gain. Gonad growth was inhibited by increasing levels of soy or menhaden oils. Dry gut weight was positively correlated with level of menhaden oil and negatively correlated with level of soy oil. Results from this study suggest that supplemental dietary lipids may not be necessary for growth in *L. variegatus*. Supported by Mississippi-Alabama Sea Grant and NOAA.

SO HAPPY TOGETHER: WHY SHELL-PLANTING AND SUSTAINABLE FISHING WORK FOR OYSTER POPULATIONS IN THE DELAWARE BAY.

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A shell-planting program was established in the Delaware Bay in 2005 as a means of enhancing recruitment of the native oyster species *Crassostrea virginica*. Maintaining a restoration program as well as a sustainable fishery in this oyster population requires that a quantitative, end-of-season survey be employed in a stock assessment as a primary tool to evaluate them within the context of the entire oyster population. Surplus production, an important product of the stock assessment, is the net increase in the harvestable population, taking into account recruitment into harvestable size classes and natural mortality. An effective shell-planting program has the potential to more than double the local oyster population, thereby buffering the effects of inadequate recruitment and natural mortality as well as increasing surplus production. From 2005 to 2008, shell-plants contributed between 3 and 24% of total spat recruitment, for an average of 15%. The surplus production calculation determines the amount of fishing pressure the population can tolerate; from 1996 onward, fishing has been limited to less than 2% of the total oyster stock. By utilizing the information provided in a comprehensive stock assessment, a restoration program like shell-planting can help support a sustainable fishery.

COMMUNITY SUPPORTED AQUACULTURE: INVESTING IN THE FUTURE OF LOCAL SHELLFISH FARMS.

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According to market research consumers are changing their food buying habits and there is a trend toward purchasing products that are grown locally and by farmers/farms that people are familiar with. Community Supported Agriculture operations or "CSAs" act as a mechanism for community members to invest in a farm prior to the production season, assume a shared risk with the farmer, and receive a return on their investment - usually fruits, vegetables, or other farm products. CSAs has become a popular business structure for traditional land-based agriculture as a means to supply local agricultural products to consumers and also as a way to resolve the general disconnect among consumers, food production and farmers. CSAs have also become trendy among consumers, who are often willing to pay more for CSA products than their conventionally marketed equivalents. This type of business structure is gaining appeal of other food production industries including aquaculture.

A preliminary assessment of shellfish consumers in towns across Connecticut revealed a widespread interest in having access to local shellfish and a desire to support the development of shellfish CSAs. Ninety percent of those surveyed (n = 128) expressed a willingness to pay a premium for shellfish grown locally. Respondents were willing to pay a premium of at least $0.50 to more than $2 per pound greater than the average product price. In addition, a survey of municipal shellfish managers who use shellfish for stock enhancement or restoration efforts revealed that their demand regularly exceeded supply. Respondents stated that they either imported shellfish seed from outside of Connecticut or purchased expensive market-sized product from commercial harvesters. Many of these commissions also expressed interest in investing in a CSA as means of supplying their shellfish demands. These and other data will be used to determine the feasibility of establishing the Connecticut's first shellfish CSA.

IN-VITRO ORGANIZATION OF MINERALIZING LARGE HEMOCYTE AGGREGATES BY CYTOSKELETAL ELEMENT

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Involvement of hemocytes in *Crassostrea virginica* shell formation by deposition of calcium carbonate crystals has been previously elucidated. Here we describe *in-vitro* behavior of oyster hemocytes and the involvement of various structural proteins such

as actin and tubulin and adhesive proteins like E-cadherins using time lapse fluorescence microscopy. Explanted hemocytes formed migratory dynamic aggregates which communicate with each other to form large aggregates which measure about ~300–500 microns in diameter. Structural proteins such as actin and microtubules play crucial role hemocyte migration and aggregate formation. The role of aggregate formation in biomineralization and wound healing is discussed.

VISUALIZING ADRENERGIC RECEPTORS ON THE SENSORY ORGANS OF OYSTER AND BARNACLE SETTLEMENT STAGE LARVAE.

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Marine invertebrate larvae explore surfaces prior to settlement and metamorphosis to juveniles. This process defined in the literature as 'tactile chemical sense', is the ability to recognize specific molecular moieties present on a surface. The choice to attach and metamorphose is contingent upon receiving specific cues through the exploratory organs, such as the setae on the 4th antennular segment of the barnacle cyprid larvae or the foot of the oyster pediveliger larvae. Interfering with this process of surface exploration is believed to be a promising strategy for developing effective yet environmentally benign antifouling coatings. For development of such fouling deterrent coatings it is important to first understand the nature of the receptors present on the larval exploratory organs. Adrenergic receptor ligands have been shown to inhibit larval settlement in four disparate phyla Mollusca, Arthropoda, Bryozoa and Annelida. With the use of fluorescent agonists, antagonists and antibodies we visualized adrenergic receptors using confocal laser scanning microscopy. Our findings suggest that these receptors are present on the foot of the Pacific Oyster (*Crassostrea gigas*) pediveliger larvae and the setae of the 4th antennular segment of barnacle (*Balanus amphitrite)* cyprid larvae.

MEASURING THE ECOLOGICAL EFFECTS OF HYDRAULIC SHELLFISH DREDGING ON BENTHIC BIOTA.

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Harvesting of clams with hydraulic dredges directly affects the benthos, but the rate of ecological recovery is specific to degree of disturbance and many biotic and abiotic factors. A field experiment was conducted in Long Island Sound with an industry partner on leased shellfish beds where hard clams, *Mercenaria mercenaria* are cultivated. We compared abundance and biodiversity of benthic fauna on six 1-hectare plots before a mid-June dredging and then on three dredged and three non-dredged plots for a period of six months. Benthic organisms (>1 mm) were enumerated from Smith-MacIntyre grabs and macrofauna were sampled with a 1-meter beam trawl. Results indicate a high biodiversity that did not change markedly after dredging. During the 6 months after dredging, settlement occurred for many species found in the pre-dredging plots, especially bivalves, and abundance increased greatly. Recently settled *Mya arenaria*, *Mercenaria mercenaria*, and *Mulinia lateralis* were dominant species. Few statistical differences among individual species or assemblages were detected between dredged and non-dredged sites. Beam trawl catches of macrofauna revealed much stronger seasonal effects than from dredge treatments. Results from this study and a companion study on biogeochemical effects of dredging document how hard clam cultivation on leased beds affects habitat ecology.

ASSEMBLY AND ANNOTATION OF THE *CRASSOSTREA GIGAS* TRANSCRIPTOME MATERIALS

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A prerequisite for functional genomics is that pre-existing sequence data are available in the form of either genomic or gene transcript sequences. To complement ongoing efforts to sequence the Pacific oyster genome, we undertook a large-scale EST project to sequence the oyster transcriptome. In the first phase of this project, we collected 200,000 paired-end Sanger sequences from a series of normalized cDNA libraries. The cDNA libraries were prepared from adductor muscle, gill, and mantle tissue isolated from oysters that had been subjected to a series of environmental stressors, and were constructed using a cDNA cap-trapping protocol to ensure that a high proportion of full-length long cDNAs were cloned. Next, a subset of ~8,000 non-redundant cloned cDNAs was identified and shotgun sequenced with Illumina technology. The resulting 24 million 100bp reads allowed a

large proportion of the cDNAs to be assembled into full-length sequences. Finally, two normalized 454 libraries were prepared from larvae which contributed >900,000 more sequences to the assembly. A substantial proportion of the oyster transcriptome is represented by this assembly.

THE ROLE OF RESUSPENSION IN PARTICLE BUDGETS OF MUSSEL CULTURE ECOSYSTEMS.

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Models of shellfish culture ecosystems have been successful in predicting bivalve growth, and criteria for sustainability and carrying capacity have arisen from these efforts. Models are based on seston depletion as a limiting resource, with a dominant focus on the water column. In contrast, the benthos have been less a part of this scenario for various reasons. Benthic diagenetic processes have been long been studied at aquaculture sites with an eye on eutrophication impacts from biodeposition. Sediment and organic resuspension have received less attention, although they are potentially important in particle budgets, especially in shallow waters. We developed a generic modeling approach to resuspension that can be coupled to existing models of carrying capacity. We have an archived data set used for groundtruthing and parameterization which includes vertical particle profiles, sediment trap measurements, current records, and *in situ* flume experiments.Results indicate the extent of contribution of resuspended benthic microalgae and detritus to suspended food relative to exchange and primary production. The model is currently being applied to the Limfjord (Denmark) where there is a study (MUMIHUS) intended to quantify the role of mussel culture in controlling eutrophication on a large scale.

ACUTE PSYCHOLOGICAL IMPACTS OF THE *DEEPWATER HORIZON* OIL SPILL IN TWO COMMUNITIES.

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Although there were many concerns about the environmental impact of the *Deepwater Horizon* Oil Spill, less attention was given to the acute impact on residents of Gulf Coast communities.

Using a community based participatory research model, standard assessments of psychological distress, neurocognition, coping, resilience and risk perception were administered to 94 residents of two Gulf Coast fishing communities with direct (Baldwin County Alabama) and indirect (Franklin County) exposure to the oil spill.

Findings indicated no significant differences between exposure groups on any psychological measures.While members of both communities displayed clinically significant depression, 13% of participants in the exposed community met DSM-IV-TR criteria for a Major Depressive Episode.Persons with economic resource loss had significantly higher scores on Tension/Anxiety, Depression, Fatigue, Confusion and Total Mood Disturbance Scales; were less resilient and more likely to use a less effective coping strategy.

There was a significant psychological impact on Gulf Coast communities during the oil spill, with effects extending beyond areas of direct oil exposure. Economic disruption after the spill may have a greater psychological health impact than the presence of oil on immediately adjacent shoreline.

THE COASTAL COHORT: A WINDOW TO UNDERSTANDING AMNESIC AND PARALYTIC SHELLFISH POISONING.

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The *CoASTAL* (Communities Advancing Studies of Tribal Nations Across the Lifespan) Cohort is a random sample of 687 Native Americans from three tribes in the Pacific NW. The tribes share a problem with the marine biotoxins, domoic acid and saxitoxin,which have been detectable at low levels in their shellfish over several years. In response to animal studies andmedia communications,recreational harvesters visiting the region,raise concerns about the impacts of these low level exposures on their health. They have been particularly concerned about the risk for seizure disorder. Using cohort data, and a variety of research methods, it is possible toaddress some emergentsymptom related questions.

Toward this end, neurologic, medical symptom and history data (e.g. seizures, numbness and tingling) for the cohort was analyzed over 4 years and also compared to established national and regional base rates. Findings indicated that although there were some elevations in neurological symptoms, these could be explained by causes other than shellfish poisoning.

Preliminary findings indicateno evidence for medical symptoms associated with low level domoic acid or saxitoxin exposure in the CoASTAL Cohort. This cohort of high shellfish consumers provides a window to answering emergent questions about the impacts of shellfish consumption on human health.

CAUSES AND CONSEQUENCES OF OCEAN ACIDIFICATION

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The world ocean is currently absorbing over 20 million metric tons of carbon dioxide (CO_2) each day. Addition of CO_2 at this rate is overwhelming the oceans buffering ability and is causing the ocean to become progressively more acidic. The average pH of oceanic surface waters has been lowered by 0.1 units since the pre-industrial period, which represents a 30% increase in hydrogen ion activity. Of particular significance is that the rate of pH decline is nearly 100 times greater than anything experienced in hundreds of millennia. If anthropogenic emissions of CO_2 are continue to increase unabated, pH is likely to decrease by another 0.5 pH units by 2100. This would correspond to an increase in acidity of 300%. The rate of change in ocean chemistry is well beyond the range of natural variability and adaptation for some marine species to these changes may not be possible. Although it is difficult to predict the impact of a rapidly acidifying ocean on marine organisms and their ecological habitats, there are certain to be significant consequences. For example, most indicators suggest that organisms with $CaCO_3$ shells of tests (e.g. corals, pteropods, larval/juvenile bivalves) will likely be most impacted. Reducing CO_2 emissions to the atmosphere is the only known way to mitigate ocean acidification. Even then, ocean pH will take tens of thousands of years to rebound to pre-industrial levels.

POPULATION STRUCTURE OF OYSTERS ON MARTHA'S VINEYARD, MA IN RESPONSE TO SELECTION BY *PERKINSUS MARINUS*, USING MICROSATELLITE MARKERS.

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Neutral genetic markers are routinely used to detect population structure, but this has seldom been done for adaptively relevant markers. This study was initiated to determine whether history of exposure to *Perkinsus marinus* (Dermo disease) creates population structure. Three isolated oyster populations on Martha's Vineyard, MA acquired Dermo disease at different times, spanning approximately a decade, while another has remained free of detectable levels of the parasite. Oysters from each population were genotyped at both neutral and disease resistance-linked microsatellite markers. Divergence among populations was analyzed to test the hypotheses that: (1) isolation has created population structure, as evidenced by differences among allele frequencies at neutral loci, and that (2) Dermo disease has increased population structure by altering the genotypic frequencies of these populations at loci that are structurally linked to host-defense genes. Population structure was detected using both sets of markers, but the patterns were not the same. Using resistance-linked markers, divergence was related to the duration of disease exposure, while for neutral markers it was not, indicating that while reproductive isolation has created divergence among the populations, differential selection by *Perkinsus marinus* has generated additional population structure.

INITIATION OF A LONG-TERM COMMITMENT TO RESTORE OYSTER POPULATIONS IN THE NEW YORK HARBOR REGION.

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The Hudson River Foundation, New York/New Jersey Baykeepers, US Army Corps of Engineers,University of New Hampshire, and other partners are working together on an Oyster Restoration Research Partnership (ORRP) to begin to restore native eastern oyster (*Crassostrea virginica*) populations to the New York Harbor region. Restoration of oysters is a major component in the Hudson-Raritan Estuary Comprehensive Restoration Plan. The Plan calls for 500 acres of new oyster reef habitat by 2012 and 5,000 acres of established reef habitat by 2050. The long-term aim is to restore the eastern oyster to its historic ecological role in the region, with a focus on ecosystem services provided by oysters rather than human harvest and consumption. In summer 2010, implementation of the program was initiated with the construction of five experimental-scale oyster reefs scattered throughout the region. Reef development (survival and growth of the oysters themselves) and performance (ecosystem services provided by the reefs) will be measured over a ~2-year period in order to better design full-scale restoration efforts.

TWO-DIMENSIONAL "ECOMAPS" OF SESTON DEPLETION ZONES OVER AN INTERTIDAL *CRASSOSTREA VIRGINICA* OYSTER REEF.

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Changes in water column chlorophyll *a* (chl-a) were measured with one or more *in situ* fluorometers along multiple transects across intertidal eastern oyster (*Crassostrea virginica*) reefs on Sanibel, Florida by slowly moving back-and-forth across reefs while continuously logging position and other data. Measurements were made over three days (May, June 2010), involving portions of two ebbing and one flooding tide. ArcGIS was used to interpolate

chl-a data over the reefs (384 m^2) and in proximity to it (20 m) allowing a visualization of any changes in water quality parameters versus reef location and adjacent seagrasses. Two-dimensional interpolated maps consisting of contours of changes in chl-a concentrations (by inverse distance weighting) clearly showed decreases in chl-a across reefs, with rapid increases to near ambient non-reef concentrations within several meters downstream of reefs. A negative logarithmic relationship ($r^2 = 0.99$) was detected between water velocity and % chl-a removed. For example, at low flows (0.03 m/sec) chl-a was 2.5× lower (61% filtered) over the reef, whereas at higher flows (0. 21 m/sec), chl-a was 1.5× lower (32% filtered). The visualizations provide empirical data on the spatial extent of the potential effects of oyster reefs on adjacent habitats (seagrasses) potentially impacted by oyster filtration.

SPECIFIC NOROVIRUS BINDING TO OYSTER TISSUES AND BIOACCUMULATIONS.

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Noroviruses (NoVs) are single-stranded RNA viruses, highly diverse genetically, with three human genogroups (GI, GII and GIV). As these viruses are very resistant to inactivation, the sanitary consequences are contamination of the environment and food, including molluscan shellfish. The mechanism of viral accumulation, inactivation and elimination in oyster has not been completely characterized. In human, it has been shown that histo-blood group antigens (HBGA) on cells of the human gastrointestinal tract act as receptors for Nova We showed that oyster digestive cells contain type A-like HBGA, co-localizing in the oyster digestive tubule cells where 90 % of norovirus binding occurs. Norwalk strain (prototype norovirus genogroup I) bind specifically to the oyster digestive tissues through the A-like carbohydrate structure indistinguishable from human blood group A antigen, with a clear seasonal effect. GII strains bind to DT through the same A-like ligand but also via a sialic acid. This sialic ligand is also involved in specific binding of these strains to gills and mantle, without any seasonal effect. We demonstrated that these ligands have an impact of bioaccumulation efficiency of these viruses by oyster, and that carbohydrate ligand specificities of the strains could at least partly explain the strain-dependent bioaccumulation characteristics.

TAKING SHELLFISH RESTORATION TO SCALE: A DECADE OF DEVELOPMENT IN RESTORING SHELLFISH HABITATS.

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Since 2001 TNC and NOAA's Community-based Restoration Program have partnered to restore habitats critical for the marine resources of the U.S., funding over 100 projects including 54 shellfish restoration projects in 16 coastal states. Projects have included clams, oysters, scallops and abalone with the majority focused on restoring habitat of either Olympia (*Ostrea conchaphila*) or Eastern oysters (*Crassostrea virginica*). The partnership has been a major contributor to the development of restoration techniques and methods of measuring success that have propelled shellfish restoration. The partnership's projects have also influenced the trajectory of shellfish restoration by raising the profile of the ecosystem services provided by bivalves, highlighting the poor condition of most exploited shellfish species, and influencing decisions about management of shellfish reefs as a habitat. The partnership's projects were the impetus for a global "Shellfish Reefs at Risk" analysis that identified both problems and solutions and spurred actions to conserve shellfish reefs internationally. The proof-of-concept projects also laid the foundation for larger scale shellfish restoration projects funded through the American Recovery and Reinvestment Act via the NOAA Restoration Center. The success of shellfish restoration and the importance of the ecosystem services that are restored point to the need for restoration at ecologically meaningful scales. This has catalyzed the current shellfish restoration planning being conducted at national and regional scales. A summary of achievements to date, mechanisms for increasing the scale, and possible future directions for shellfish restoration will be presented.

USING MOLECULAR METHODS TO MONITOR PATHOGENS IN EARLY LIFE HISTORY OF BLUE CRABS (*CALLINECTES SAPIDUS*).

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Although diseases have the potential to affect blue crab populations, there have been few studies on the impact of diseases on overall mortality, especially in early life history. The protozoan parasite, *Hematodinium* sp., is a significant blue crab pathogen in Atlantic coastal bays of North America. It has also been recently reported that a reo-like virus (RLV) is associated with blue crab mortality in soft-shell crabs operations in from the Mid-Atlantic to Gulf of Mexico. PCR-based assays for specific diseases can permit disease prevalence assessments of large numbers of very small life stages that would otherwise be impractical to test by histological methods. With support by the NOAA-Living Marine Research Cooperative Science Center, a collaboration involving a NOAA/NCCOS lab, two undergraduate institutions, and a university research center is using specific PCR assays to detect *Hematodinium* sp. and RLV in megalopae and young juvenile crabs in the salt marshes near Savannah, Georgia. Collections of juvenile crabs were made throughout the summer of 2010, and megalopae were collected in the fall of 2010. Preliminary analyses indicate very low prevalence of both diseases. More complete results are expected by the beginning of 2011.

USING PCR METHODOLOGY TO SEARCH FOR AN ENVIRONMENTAL PRESENCE OF *HEMATODINIUM* SP., A LETHAL PARASITE OF BLUE CRAB *CALLINECTES SAPIDUS*.

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Hematodinium sp. is a parasitic dinoflagellate associated with mortalities of blue crabs (*Callinectes sapidus*) along the Atlantic and Gulf coasts of North America. It is possible that over-wintering reservoirs of this parasite may reside in alternate hosts or sediment. To understand disease dynamics and facilitate forecasts of blue crab disease outbreaks, methods to detect *Hematodinium* sp. in environmental samples are needed. While histological methods are impractical for such studies, PCR-based methods can be applied to DNA extracted from gut contents, sediment or water. Because these sources are also likely to contain other dinoflagellate species, it is crucial to use the most specific DNA target available for PCR-based detection. We therefore developed endpoint and quantitative polymerase chain reaction (qPCR) assays, based on the ITS1 and ITS2 sequences of the rRNA gene cluster, to provide the sensitivity and specificity needed to quantify *Hematodinium* sp. DNA in environmental samples. We verified the efficacy of sediment DNA extraction and analysis methods using a surrogate for *Hematodinium*, consisting of *E. coli* carrying the cloned *Hematodinium* sp. rRNA target genes. Using these ITS-based assays and associated methods, we have found evidence of *Hematodinium* sp. in sediment from Maryland and Delaware coastal bays.

DOES LESS BAIT CATCH FEWER LOBSTERS? ASSESSING THE EFFECT OF BAIT REDUCTION ON LOBSTER HARVEST.

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Lobster fishermen may be forced to reduce the amount of bait used to harvest lobsters due to herring quotas or the increased cost of herring. The objective of this study was to determine if reduced amount of bait caused a reduction in lobster harvest. Using reduced bait, three in-shore fishing vessels fished from Belfast and Beals Island Maine, and Morrell, Prince Edward Island, Canada between June to October, 2010 using either north Atlantic herring (*Clupea harengus*) or gaspereau (*Alosa psuedoharengus*) and measuring subsequent lobster harvest. The reduced bait treatment consisted of bait bags filled with half the average bait weight of the control (amount of bait typically used). Bait bags were fished for 3, 5 and 8 days and then weighed to determine bait weight loss. Using a t statistic, assuming unequal variances, reduced bait treatments were assessed to determine whether the difference between the control and the reduced bait catch was statistically different. The difference between the control and the reduced bait catch was not significant. It appears that reduction in bait used does not influence lobster catch.

EVALUATION OF OYSTER (*CRASSOSTREA VIRGINICA*) SEX RATIOS FROM THREE VIRGINIA ESTUARIES WITH REGARD TO SHELL LENGTH, AGE, AND BIOMASS.

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Oyster population reproductive capacity and dynamics are controlled at the most basic level by the observed sex ratios. Since oysters are sequential, protandric hermaphrodites the sex ratio for

a population is related to the demographic (shell length, age, and biomass). Oysters were collected from June through August 2008 at 11 sites in the James, Rappahannock and Great Wicomico Rivers, Virginia. Sex ratios (fraction female), age-length, and biomass-length relationships were determined for each population. The fraction of oysters examined that were female, irrespective of hermaphrodites or indeterminate individuals, generally increased within increasing shell length, age, and biomass at all sites. Simultaneous hermaphrodites were rarely observed at most sites in any size or age class. Sex ratios by shell length and age were strongly biased towards males while the sex ratio by biomass was strongly biased towards females across sites. We will discuss the application of Gompertz models to the observed sex ratios with regard to population dynamics observed across sites and years.

GIGASNP: INTEGRATING GENETIC, PHYSICAL AND CYTOGENETIC MAPS OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS*.

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The Pacific oyster *Crassostrea gigas* is among the highest producing farmed aquatic organisms in the world. Since 2004, an international consortium of shellfish scientists has advocated for sequencing the Pacific oyster genome. In late 2008, whole-genome shotgun sequencing began in China, using DNA from an inbred oyster developed with support from USDA and Taylor Shellfish Farms. To aid assembly of a draft sequence for this highly polymorphic genome, we launched GIGASNP, a USDA-funded project integrating genetic, physical, and cytogenetic maps, using single nucleotide polymorphisms (SNPs). By mapping Sanger and Illumina RNA-Seq reads to a transcriptome reference sequence, for which exon boundaries were annotated by mapping to genome scaffolds, we identify SNP candidates in coding sequences. A SNP-discovery pipeline automates this process and creates FASTA files suitable for SNP-assay design. Taking exon boundaries into account, PCR amplification from gDNA is 95% successful. We discard SNP candidates that have insufficient flanking sequence, lie too close to an intron, or are located in SNP-dense regions; we verify SNP polymorphism in parents of mapping families by high-resolution melt assays. The goal is to genotype >3000 candidate SNP markers by multiplex assays in mapping families and in oysters from diverse stocks and closely related species.

DESICCATION AND TEMPERATURE TOLERANCES OF THE INVASIVE GREEN MUSSEL, *PERNA VIRIDIS*, IN ESTERO BAY, FLORIDA.

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The green mussel, *Perna viridis*, is non-indigenous to southwest Florida. The mussels were discovered in Tampa Bay in 1999 and have spread south, their range now including Estero Bay. *P. viridis* grows on bridge pilings in the passes of the bay and offshore structures, but has not been found on intertidal oyster reefs. To better predict the range of potential spreading of *P. viridis*, experiments were conducted to determine its tolerance to temperature (10–35°C) and desiccation (2–12 h). Results indicate a high tolerance to desiccation alone, but a much lower tolerance when desiccation occurs at higher temperatures. Daily exposure to air temperatures reaching above 30°C for more than 2 hours resulted in 100% mortality after only two days, while all mussels exposed to lower air temperatures (<30°C) showed strong survivability over 21 days of exposure. Mussel survival was high at water temperatures between 11 and 30°C, the typical annual range for Estero Bay. The results of these experiments suggest that direct sunlight and resulting extreme high air and inside the shell temperatures in intertidal areas throughout most the year would inhibit the survival of green mussels in intertidal areas, while surviving in shaded areas under mangroves.

MULTIPLE PATERNITY IN THE BLUE CRAB (*CALLINECTES SAPIDUS*) ASSESSED WITH MICROSATELLITE MARKERS.

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In the blue crab, *Callinectes sapidus*, double ejaculations have been observed, however, it is not known if these multiple inseminations are the products of different males or if they lead to effective multiple paternity. In this study seventy-five mature females and their spermathecae were genotyped for five polymorphic loci. The incidence of multiple mating was analyzed by comparing the genotypes of either spermathecae or individual ejaculates with the corresponding mother, and the male contribution was then deduced. Of these females 5.3% contained double ejaculates, which genotyping confirmed as the products of different

males. In addition, a routine screening of sixty-eight crabs from a single hatchery brood at two loci indicated more than two parents. Mitochondrial analysis confirmed that the brood shares the same mother; therefore, the extra alleles must be due to different fathers. Our findings indicate that female blue crabs are not only capable of mating with more than one male but that multiple inseminations can lead to multiple paternity within a brood.

FIELD ASSESSMENT OF THE FEASIBILITY OF ENHANCING THE CHESAPEAKE BLUE CRAB STOCK.

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The Chesapeake blue crab population declined from 1991 to 2007 to an unprecedented, sustained low level, with 84% drop in spawning stock and 70% drop in fishery. The population was recruitment limited. We present the results of a multi-year (2002–2010) study of stock enhancement linked to fishery management and marine protected areas, and designed to (1) assess potential for enhancing local populations of blue crabs with hatchery-reared juveniles and (2) identify factors influencing survival of hatchery-reared 20 mm juveniles in the wild. We tagged and released 57 cohorts of 2,000–25,000 tagged hatchery-reared juveniles (378,000 crabs total) into nursery habitats of upper Chesapeake Bay. Average enhancement (~300%), survival (~15%), and production (~300 adults ha^{-1}) of released crabs varied significantly among sites and years. Hatchery crabs behaved and survived comparably to wild crabs, grew rapidly to maturity, mate, migrate to spawning areas in the lower Bay within their first year. Survivorship of hatchery-reared juveniles was inversely density-dependent, and emigration was low. Experiments indicated that survivorship and growth were favored in early season releases at low densities in shallow habitats. Stock enhancement and spatial management could be coordinated effectively to restrict fishing along migration corridors linking nurseries to a spawning sanctuary.

DELAWARE BAY ECOLOGY OF INFECTIOUS DISEASE PROJECT OVERVIEW AND RESULTS.

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As part of the NSF Ecology of Infectious Diseases initiative, a program was developed to understand how oysters and disease interact in Delaware Bay, and how these interactions might be modified by climate change. This program investigated the timeline of natural selection to establish disease resistance, the role of disease refugia in the genetic structure of a population, the relationship between range contraction of a species and disease resistance in preventing local extinction of oysters, and the effects of a warming climate on oyster lifespan, oyster reproduction and parasite transmission. Laboratory and field studies identified genes related to MSX and Dermo disease resistance, potential disease refugia and the mechanisms that allow them to exist, the differences among oysters from suspected refugia and high-disease areas, and the effect of space and time on the size of spawning populations. Integration of experimental results with numerical models of explicit genetic structure, disease processes, and post-settlement oyster-population changes, and a Delaware Bay circulation allowed studies of disease transmission, larval transport, and current and future climate condition effects on oyster populations. These results are providing insights into changes in Delaware Bay oyster populations that may occur in response to climate, environmental, and biological variability.

COMMUNICATING SCIENCE: VIDEO AS A MEDIUM FOR HAB AWARENESS.

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Communicating science to the public in a clear and effective manner is a challenge. Consider how difficult it is then to attempt to communicate a complex scientific topic that is *also* a potential threat to human and animal health and rife with controversy, and political and economic implications. That scenario describes the intrinsic difficulty in harmful algal bloom (HAB) outreach. As part of two separate grant-funded research projects into HABs, two outreach videos were created. The videos illustrate various considerations that go into the creation of outreach videos. We briefly describe the video production process, discuss lessons learned, and provide information on inexpensive, often free, resources for video content and dissemination. The lessons learned from the production of the videos may be helpful to scientists, resource managers, and industry as they consider multimedia products to communicate their products. These ''products'' caninclude research findings, event responses, marketing new or different products, and food safety messaging.

EFFECT OF STARVATION ON THE ENERGY BUDGET OF TWO ASIAN HORSESHOE CRAB SPECIES: *TACHYPLEUS TRIDENTATUS* AND *CARCINOSCORPIUS ROTUNDICAUDA* (CHELICERATA: XIPHOSURA).

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Energy budget is one of the best known and most studied parameters in aquatic animals under environmental stresses. In this study, respiration rate, ammonia excretion rate, O:N ratio and scope for growth (SfG) in two Asian horseshoe crab species, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, were investigated, so as to understand how prolonged starvation affected their energy budget. Significant interactive effects between feeding regimes (fed or starved) and time courses on these physiological parameters were evident in the two species during the seven-week experiment. No significant difference was observed in absorption efficiency in either fed horseshoe crab species. In both species, respiration and excretion rates of the starved groups significantly decreased, while their O:N ratio significantly increased, as compared to the fed groups during the experiment. *C. rotundicauda* showed a greater decrease in SfG under starvation than *T. tridentatus*, suggesting that they may have a more competitive life history strategy for adapting to different nutritional conditions.

MANGANESE IMPAIRS THE ACTIVITY OF MITOCHONDRIAL ACONITASE IN THE GILL OF THE BIVALVE *CRASSOSTREA VIRGINICA*.

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Manganese is an essential trace metal but excessive exposure leads to manganese accumulations and toxicity. Excess manganese is neurotoxic and causes Manganism, a Parkinson's-like disorder. The mechanism of manganese neurotoxicity is unknown. It is hypothesized excess manganese causes mitochondrial dysfunction. Manganese accumulates in mitochondria and can raise levels of reactive oxygen species or participate in catalyzing unwanted redox reactions. We showed manganese accumulates in tissues of *Crassostrea virginica* and disrupt the dopaminergic system controlling lateral cilia in gill. We also showed it impairs gill mitochondrial respiration. We examined effects of manganese on the Kreb Cycle enzyme aconitase in gill. Mitochondrial suspensions were exposed to manganese minutes on ice, repelleted, resuspended, then sonicated to free aconitase from the mitochondrial matrix. Aconitase activity was determined spectrophotometrically. The reactions were monitored by measuring the increase in absorbance at 340 nm associated with the formation of NADPH. Results show short-term exposure to manganese (1–50 mM) caused up to a 90% loss in aconitase activity. The results corroborate our previous findings that manganese disrupts mitochondrial respiration in oyster gill and further demonstrates a mechanism by which manganese can disrupt energy homeostasis by impairing aconitase, an oxidative stress-sensitive enzyme of the Kreb Cycle.

THE TOXIC EFFECTS OF METALS ON MITOCHONDRIAL CYTOCHROME C OXIDASE ACTIVITY IN THE GILL OF THE BIVALVE *CRASSOSTREA VIRGINICA*.

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Many metals are believed to exert their toxic effects by raising cellular levels of reactive oxygen species (ROS). We showed metals accumulate in gill and other tissues of *Crassostrea virginica*, and some, particularly copper and manganese, impair mitochondrial respiration. In this study we treated gill mitochondria with copper, manganese, lead or cadmium to determine effects on cytochrome c oxidase (COX). COX is the principle terminal oxidase of high affinity O_2 in aerobic respiration. Agents that inhibit the function of respiratory complexes not only disrupt energy homeostasis but also increase production of ROS. Gill mitochondria were prepared from *C. virginica* and exposed to Cu, Mn, Pb or Cd, re-pelleted then resuspended in fresh media. COX activity was determined spectrophotometrically. Compared to controls results indicate mitochondrial COX activity decreased in response to Cu, Pb and Cd, but remained unaffected by Mn. Mitochondrial COX was most affected by Cu. We also showed the loss of COX activity due to copper treatments could be prevented if mitochondria were pre-incubated with glutathione suggesting the toxic effects of Cu on COX is due to increased oxidative stress. Studying the effects of metals on cellular processes will provide insight into the mechanisms that underlie metal toxicities.

HAS SHELLFISH MODELLING ANY RELEVANCE TO THE BUSY, FINANCIALLY STRAPPED FARMER?

John Icely.
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Many examples of shellfish models have been developed in recent years with a range of objectives from modeling: shellfish growth; environmental impacts on shellfish; impacts of shellfish on the environment; carrying capacity of the habitat for shellfish; socio-economic impacts etc. The objective of this presentation is to look at these modeling activities at the scale of the individual shellfish farm and ask do these activities have any relevance to a busy, financially strapped farmer! This is not mere academic indulgence; the presenter is currently a partner in a newly-funded aquaculture enterprise for offshore culture of bivalves on "long-lines" off the SW coast of the Portugal.

MODELING AND FORECASTING THE DISTRIBUTION OF *VIBRIO* SPP. IN CHESAPEAKE BAY.

John Jacobs [1], **Chris Brown** [1], **Rita Colwell** [2], **David Green** [1], **Raleigh Hood** [2], **Wen Long** [2], **Guillaume Constantin De Magny** [2], **Bala Krishna Prasad Mathukumalli** [2], **Raghuram Murtugudde** [2], **Matt Rhodes** [1], **Robert Wood** [1].

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The estuarine bacteria *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* are capable of causing severe and occasionally life threatening infections in humans. While 50–60 cases are reported annually in the Chesapeake region, few efforts have focused on understanding the distribution of these opportunistic pathogens on a scale relevant to regional management. To address this concern, a partnership was established to enhance monitoring capabilities, model the distribution of these species, and develop ecological forecasts.Through collaboration with Maryland and Virginia water quality monitoring programs, *Vibrio* spp. are enumerated using quantitative PCR and linked directly to the associated water quality data. ChesROMS, a regional adaptation of the Rutgers Ocean Modeling System, is used to force empirical models derived from these large data sets. Nowcasts, 3-day, 14-day, and seasonal forecasts are provided through restricted access to state and county health officials for use in education and decision making. Models are also being used to evaluate other scenarios, such as regional climate change. Finally, monitoring efforts are continuous allowing for validation of empirical models, evaluation of forecast model skill, and tuning over time.

GENE EXPRESSIONS THROUGH THE BROWN RING DISEASE IN HEMOCYTES AND MANTLE OF MANILA CLAMS, *RUDITAPES PHILIPPINARUM*.

Fanny Jeffroy, **Franck Brulle**, **Christine Paillard**.

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The Brown Ring Disease (BRD) touching the manila clam production in Brittany has been settled since the 80's. The disease is caused by the bacteria *Vibrio tapetis* which adhere on the periostracal lamina. This *Vibrio* involves hemocyte parameters modifications (increase hemocytes number, rounding of cells or decrease of the phagocytic activity). Moreover, BRD is characterized by the presence of a brown deposit of conchiolin coating the bacteria. Some clams are able to resist to *V. tapetis* by recovering the conchyolin deposit with a new calcified layer. In order to highlight genes implicated in response against *V. tapetis* and BRD development, we performed Suppressive Soustrative Hybridations (SSH): 1- *in vitro* experiment with hemocytes in contact with *V. tapetis* during the cells rounding; 2- *in vivo* experiment with *V.tapetis* inoculation in the extrapalleal cavity of clams. We obtained more than 900 EST from both SSH. Then, the expression level *in vitro* and *in vivo* of selected genes has been analyzed to identify specificities of genes expressed in hemocytes in contact with *V.tapetis* or in the BRD development in clams.

COMPLEMENTARITIES BETWEEN MICROSATELLITES AND TWO PHENOTYPES, SHELL COLOR AND ORNAMENTATION, TO IDENTIFY FAMILIES OF *RUDITAPES PHILIPPINARUM* : A TOOL FOR RESEARCH SURVEY AND AQUACULTURE MANAGEMENT

Fanny Jeffroy, **Christine Paillard**.

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Microsatellites are generally employed to analyze populations genetic in diversity, migration and policy to find criminals. Another application allows recognizing of the paternity or the genealogy. Microsatellites have been identified in most of species. In the manila clam, *Ruditapes philippinarum*, more than twenty microsatellites have been published in revues by Asian's research. A few studies have been carried out, but only concerning the Asian populations of this specie. Moreover, no data was acquired for French *R. philippinarum* populations. In our study, we have first seen differences in microsatellite allelic diversity and microsatellites frequencies, in a few culture populations in several North French areas. We have secondly shown that distinguishable shell color/ornamentation heredity results principally from females visible characters. The parenthood and the haplotypes were associated with the families. So the crossing between genitors with different shell color or ornamentation could be a tool for aquaculture production or for lab experiments.

EVIDENCE FOR LARGE-SCALE RECRUITMENT LIMITATION OF BLUE CRABS IN UPPER CHESAPEAKE BAY.

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The recent decline in the Chesapeake Bay blue crab population appears to have been largely driven by declines in the spawning stock and subsequent reductions in juvenile recruitment. Herein, we summarize evidence from (*i*) existing fishery-independent data sets, (*ii*) large-scale field surveys and experiments within shallow blue crab nurseries throughout Chesapeake Bay, and (*iii*) small-scale field releases of hatchery-reared juvenile blue crabs in upper Chesapeake Bay, that provide strong indications that many areas of Chesapeake Bay suffer from recruitment limitation. Population declines are evident in landings data, fishery-independent surveys, and in independent measures of spawning stock abundance in the

lower Bay spawning sanctuary. Field surveys within shallow nursery habitats of Chesapeake Bay clearly show that juvenile blue crab density declines with distance from the Bay mouth. Conversely, survival of blue crab juveniles is highest in up-estuary sites with abundant prey at all sites. Field releases of hatchery juveniles directly augmented local wild stocks and demonstrated that release sites were not limited by prey availability and are below carrying capacity. Overall, our results indicate that important blue crab nursery habitats are recruitment-limited and hatchery releases can successfully enhance local crab populations.

THE OCCURRENCE, ABUNDANCE, PHYLOGENY AND VIRULENCE POTENTIAL OF PATHOGENIC *VIBRIO* SPECIES IN NEW HAMPSHIRE SHELLFISH WATERS

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Vibrio parahaemolyticus (Vp), *Vibrio cholerae* (Vc) and *Vibrio vulnificus* (Vv) are potentially pathogenic bacteria that can cause infections in humans who consume raw or undercooked shellfish. To provide consumers with safe raw oysters, it is essential to understand their incidence, persistence and potential to cause disease. There are historical reports on the incidence of Vp and Vv, and for Vc in 2010, yet disease incidence in New Hampshire is rare. From 2007–10 we collected oysters, water and sediment from two sites in the Great Bay Estuary from May to December. Culture-based and qPCR MPN methods were used to isolate and determine concentrations of each species and strains with clinical markers. The abundance of the Vibrios peaked in warm summer months and was significantly related to water temperature, salinity, dissolved oxygen and/or large rainfall events. No clinical gene markers were detected except Vp tdh/trh using qPCR in temperature-abused oysters. The diversity and degree of recombination that is evident with Vp and Vc suggests a potential for lateral transfer of virulence genes. The public health threat is low at present, but the potential for evolution of endemic Vibrio populations in New Hampshire and other northern temperate shellfish waters is a concern.

MARYLANDERS GROW OYSTERS PROGRAM: RESULTS OF CITIZEN BASED EFFORTS TO ENHANCE OYSTER SANCTUARIES IN CHESAPEAKE BAY

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Citizen involvement in oyster restoration occurs in many coastal regions. Gardeners, or growers, tend young oysters which are eventually planted on non-harvest sanctuary sites. Such outreach/educational programs motivate citizens regarding estuarine issues and create oysters to enhance sanctuary sites. Marylanders Grow Oysters is a citizen based oyster growing (gardening) program initiated in 2008 in the Tred Avon River, Maryland. Famously popular, it expanded in both 2009 and 2010 and is now located in 18 tributaries. Mail-box sized wire cages and spat are distributed at no charge to waterfront property owners, who tend the oysters for one year, after which the oysters are collected and planted in a sanctuary to provide ecological services and broodstock. The program consists of over 1,500 growers and about 8,000 cages. Approximately 1.8 million oysters were planted in 2010. The program is conducted by the Maryland Department of Natural Resources, with numerous partners, including the prison system which makes the wire cages. Results of the Marylanders Grow Oysters Program will be presented covering organization, logistics, costs per cage and per oyster, spat survival rates, planting numbers, survival on the sanctuary sites, as well as results from the viewpoint of the motivated growers.

INTERACTIONS OF A PROBIOTIC BACTERIUM AND A SHELLFISH LARVAE PATHOGEN DURING HATCHERY LARVICULTURE OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*).

Diane Kapareiko [1], **Gary H. Wikfors** [1], **Jennifer H. Alix** [1], **Eric Schott** [2], **Harold J. Schreier** [3], **Dennis McIntosh** [4], **Jessica M. Rash** [4], **Oluchi Ukaegbu** [3].

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Use of probiotic bacteria as an "environmentally-friendly" method for controlling microbial pathogenesis in aquaculture has advantages overuse of antibiotics, which can leave residues in farmed food fish, and lead to emergence of antibiotic-resistant bacteria. Recent research at the Milford Laboratory has identified a *Vibrio* sp. bacterium (OY15), isolated from the digestive glands of oysters, that significantly improved survival ($p < 0.0141$) of oyster veligers (*Crassostrea virginica*) when challenged with a shellfish larval pathogen, B183 (*Vibrio* sp). Mechanisms of this probiotic effect were elucidated in an *in vitro* study suggesting that probiotic candidate OY15 stimulated the immune functions of adult oyster hemocytes. Molecular analysis (bacterial16S rRNA amplification) of larvae (both challenged and unchallenged with pathogen B183) and culture water, revealed diverse but different bacterial communities. Addition of OY15 did not significantly change the diversity of the bacterial community associated with oyster larvae, suggesting no selective retention or exclusion of different bacteria within larvae. This study investigated the interaction of OY15 and B183 within the oyster larval host and associated microbial environment during larviculture, and tracked these two isolates at various times post-exposure using qPCR and species-specific primers.

ELUCIDATING THE MECHANISMS OF PROBIOTIC ACTIVITY IN PROTECTING LARVAL OYSTERS FROM BACTERIAL INFECTION.

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Roseovarius crassostreae and *Vibrio* species are major disease causative agents in Eastern oysters *Crassostrea virginica*. Although probiotics can be used to manage these diseases, the protective mechanisms of probiotic activity are not clearly understood. We investigated the role of antibiotic production on probiotic activity against bacterial pathogens of oysters. Probiotic isolate RI0695, which has antibiotic activity against *R. crassostreae* but not *V. tubiashii*, was able to protect larval oysters against challenge with these two bacterial pathogens. SWAT 3 is a *Vibrio* spp. that produces the antibiotic andrimid. Mutant isolates SWAT 3-111 and SWAT 3–4 show no antibiotic activity against bacterial pathogens of oysters. Pretreatment of larval oysters with SWAT3 or the mutant isolates showed protection against mortality due to challenge with *V. tubiashii*. Oysters were treated with 10^4 cfu/ml of candidate probiotics 24 to 48 hours prior to exposure of 10^5 cfu/ml of the pathogen. SWAT isolates producing the Green Fluorescent Protein were seen colonizing the mantel and gut of larval oysters under confocal microscope. Our results indicate that andrimid production is not responsible for the probiotic activity of SWAT3. The protection conferred by these particular candidates may be due to mechanisms other than antibiotic activity.

STRATEGIES TO RESTORE OYSTER POPULATIONS IN TWO SALT PONDS ON MARTHA'S VINEYARD.

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The wild oysters on Martha's Vineyard occur as relatively isolated populations in salt ponds along the southern coast. In 1996 Edgartown Great Pond oysters were first positively diagnosed with Dermo. Despite regular, sometimes heavy, natural sets, the majority of the oysters recruited in the pond succumbed to Dermo. By the early 2000's, the pond's oyster population was slowly recovering despite the continued presence of Dermo. Suspicions that the unique breeding isolation of the pond's oysters under constant selection pressure of the disease was resulting in a Dermo-resistant oyster strain were supported by a study in which hatchery-spawned offspring of survivor oysters survived 17–19% better than a non-resistant control.

Tisbury Great Pond oysters first tested positive for Dermo in 1999. By 2001, 95% perished. Like Edgartown, a remnant population survives and oysters are slowly returning.

In 2008, restoration efforts using local Dermo-resistant broodstock were expanded. The program includes conventional methods of planting cultch, deployment of shell bag collectors, and seeding of hatchery-spawned single oysters, and remote-set spat on shell. New strategies include the deployment of broodstock in floating "spawning sanctuaries;" hanging shell bags from the "spawning sanctuary" floats, and outlawing the harvest of larger oysters that survived exposures to Dermo.

NITROGEN REMOVAL AND SEQUESTRATION CAPACITY OF A RESTORED OYSTER REEF.

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Oyster reefs provide habitat for an abundant and diverse reef-associated community. Both oysters and reef-associated organisms sequester nitrogen in the form of animal tissue. In addition, oysters modify biogeochemical cycles by filtering large quantities of organic matter from the water column. The majority of this material is either used directly for growth and maintenance or deposited on the sediment surface as feces and pseudofeces. These biodeposits are a food source for high abundances of organisms capable of enhancing denitrification rates. In Maryland, the majority of oyster reef restoration projects have been implemented in shallow-water, aerobic environments (4–6 m) with limited light levels. These conditions are conducive to efficient removal of nitrogen by microbially-mediated denitrification.

Our work compares denitrification rates and nutrient sequestration capacities of a restored reef (Choptank River, MD; oyster age: 3–7 yr; oyster density ~100 m^{-2}) to an adjacent non-restored site. Denitrification rates were assessed by bringing 0.13-m^2 sections of a restored oyster reef and their associated macrofauna into the laboratory and directly measuring net fluxes of dinitrogen. Once laboratory incubations were complete, macrofauna retained on a 0.5-mm sieve were identified and counted. Total biomass and nitrogen content of major faunal groups was assessed.

ASSESSING THE IMPACT OF THE PHYSICAL AND BIOTIC COMPONENTS OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, ON THE BENTHIC REEF COMMUNITY.

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Complex oyster reef structure provides benthic organisms with refuge, habitat, and food sources. While the ecological value of the physical structure of the oyster has been well studied, it is not clear

what effect the additional biotic input of the oyster, in the form of biodeposits, has on the development of the reef community. This study investigated the contributions of oyster's biodeposits and physical structure to the development of the reef community. Small reef structures were created with live oyster clumps and with oyster shell clumps. Ten trays of each treatment were placed in the Patuxent River, MD on June 30th and July 3rd 2009. Three trays of each treatment were removed in October 2009 to perform a preliminary assessment, and the remaining fourteen trays were removed in July 2010. Fauna on the small reefs are being enumerated, identified, and biomassed. Analysis of benthic fauna data from the October 2009 collection showed no differences in faunal abundance, biomass or diversity. In addition, initial analyses of fish abundance and biomass from the 2010 collection showed no differences between live and shell treatments. This demonstrates the important, perhaps dominant, role complex physical structure plays in reef community colonization and development.

EFFECTS OF CYCLIC AND CONTINUOUS HYPOXIA ON EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* AND ATLANTIC RIBBED MUSSELS, *GEUKENSIA DEMISSA*.

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Dissolved oxygen (DO) and pH of seawater play critical roles in structuring estuarine ecosystems. In shallow-water coastal environments, DO levels can fluctuate diurnally or during low tide exposures between hypoxia and normoxia. The overall purpose of these studies was to determine antioxidant levels and impacts on microbial flora under cyclic and continuous hypoxia in oysters, *Crassostrea virginica* and mussels, *Geukensia demissa*. Bivalves were exposed to different oxygen regimes using CO_2 and N_2 gas, and minisondes were used to record water quality parameters semi-continuously over the course of the exposures (4 and 8 days). Gill and hepatopancreas tissues were dissected and used to measure antioxidants (glutathione and catalase) and tissue damage (lipid peroxidation).The bacterial concentrations (total bacteria and *Vibrio* sp.) were determined, and bacterial genomic techniques (ARISA, Automated Ribosomal Intergenic Spacer Analysis) were used to characterize microbial diversity of hepatopancreas tissues. Differences in antioxidant and tissue damage levels between cyclic and continuous hypoxia treatments were observed, and the results indicated that reduced pH along with low DO was more damaging than hypoxia alone. These studies also suggested that low DO and acidic conditions can affect microbial concentrations and diversity, and may increase susceptibility to bacterial pathogens in marine bivalves.

PERSISTENCE OF NOROVIRUS AND OTHER FOOD-BORNE VIRUSES IN BIVALVE SHELLFISH.

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Viruses have a unique ability to persist within bivalve shellfish tissues, avoiding biologic processes that should purge or inactivate these viruses. USDA ARS research indicates that circulating phagocytic cells of the oyster (hemocytes) play an important role for the retention of virus particles within bivalves. We find that persistence of hepatitis A virus (HAV) within oyster hemocytes correlates with the presence of virus within whole oysters. If viruses are phagocytized by hemocytes, viruses should be localized within low pH endo-lysosomal vesicles within these cells. Supporting this idea,we find that the ability of different enteric viruses to resist acidic inactivation correlates with virus persistence time within shellfish. We have recently utilized these hemocyte findings to demonstrate that purifying HAV or murine norovirus RNA directly from hemocytes is an efficient and rapid method for testing shellfish. This test simply involves removing or draining hemocytes from oyster tissues, pelleting, and lysing the pellet and purifying virus RNA using commercial RNA purification kits. We anticipate that this newly developed method will lead to a practical method for routine testing of viruses in shellfish.

UPDATES ON THE STATUS OF INVASIVE AND NON-INDIGENOUS SPECIES WITH IMPACTS FOR SHELLFISH COMMUNITIES IN SOUTH CAROLINA, USA.

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In recent years, considerable attention has been directed towards the increasing prevalence and ecological and economic impacts of invasive species on coastal marine and estuarine communities. Intensive commercial shipping traffic and recreational activities along the South Carolina coastline make this region susceptible to introductions of invasive, non-native species through ballast water exchange, transport on ship and barge hulls, and overland transport. As in other areas, the aquarium trade also provides a known vector for the introduction of invasive, non-native species through accidental and wanton releases. This presentation will provide updates on the status of selected coastal marine, estuarine and freshwater invasive species, specifically those with implications for shellfish communities. Such species include the Asian tiger shrimp, *Penaeus monodon*, the green porcelain crab, *Petrolisthes armatus*, the isopod *Synidotea laevidorsalis*, the red lionfish *Pterois volitans*, and two species of non-native callinectid crabs (*Callinectes exasperatus* and *C. bocourti*). Where available, trends in the occurrences of these species will be reported, along with the implications and contributions of climate change to the current and future distributions of these species.

FLORIDA NSP EXPERIENCE: COMMERCIALLY AND RECREATIONALLY HARVESTED SHELLFISH.

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Neurotoxic shellfish poisoning (NSP) is caused by the consumption of bivalves contaminated with the toxins from *Karenia brevis*. To satisfy the National Shellfish Sanitation Program (NSSP) guidelines, water and shellfish meat samples are collected by Florida Division of Aquaculture for testing. Determination of red tide cell counts and brevetoxin toxicity are completed by Florida Fish and Wildlife Research Institute personnel. A live count method is used to determine the number of *Karenia brevis* cells per liter of seawater. Counts of 5,000 *K. brevis* cells per liter or greater are criteria to close shellfish harvesting areas. A shellfish harvesting area is not reopened until cell counts drop below 5,000 *K. brevis* cells per liter and bioassay tests confirm the shellfish are not toxic. Due to this aggressive monitoring protocol, there have been no cases of NSP reported from commercially purchased shellfish. However, in recent years, there have been cases of NSP reported from recreationally harvested shellfish. In response, a collaborative effort of three Florida agencies identified the area where the toxic shellfish were harvested and an aggressive outreach campaign in the identified county was launched to prevent any further poisonings.

A COMPARATIVE ANALYSIS OF ABALONE HARVEST RECORDS BETWEEN JEJU, KOREA AND CALIFORNIA, USA, USING THE COMMON PROPERTY FRAMEWORK.

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Shellfish stocks are sensitive to collapse, due in part to open-access to these marine living resources. To evaluate the effectiveness of open-access vs. common-property management practices on shellfish stocks, we compared the harvest trends and managerial policies for abalone in Jeju, Korea, and in California (USA), for the period 1960–2009. On Jeju Island, there exists the historically and anthropologically well-established women divers communities (called '*Haenyo*'), who have maintained their village common-property fishing grounds for much of the past 400 years. In contrast, open access to abalone has been a major managerial problem in California, resulting in a 1997 commercial harvesting moratorium, despite having implemented science-based approaches such as size and/or numerical limits, restricted harvest seasons, and modifying harvesting techniques. We found that the common property-based management policies in place on Jeju Island has reduced fishing pressure and decreased the monitoring cost as opposed to the open-access policies in place in pre-1997 California. Based on the experience of the Jeju Island Haenyo, we believe that the new NMFS common property-based management program, known as catch and share being tried for the New England groundfish fishery would be the most applicable for establishing a sustainable commercial harvest of abalone in California.

CLIMATE CHANGE AND THE DELAWARE BAY OYSTER POPULATION: AN EXPLORATORY LOOK.

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Climate change could affect estuaries through a number of interacting mechanisms such as changes in temperature, pH, the timing and/or intensity of rainfall, and sea-level rise. Three of these effects on estuaries with easily retrieved historical records are sea-level rise, river flow and temperature. Effects of other variables are more difficult to document. Because of the natural geomorphology and channel deepening sea level rise will increase the salinity of the Delaware estuary even with expected rainfall increases. In addition, shoreline changes due to inundation may alter circulation patterns. The *Crassostrea virginica* population within the system exists along an environmental gradient based primarily on salinity. This gradient and the temperature also control the oyster diseases dermo, *Perkinsus marinus* and MSX, *Haplosporidium nelsoni*. The interactions between the diseases, salinity and temperature currently control oyster population dynamics, and are expected to continue to do so. We use a 60+ year data base to explore the sensitivity of oyster recruitment and mortality to river flow and temperature. The resulting information highlights the sensitivity of various sectors of the oyster population. When more precise estimates of future condition are available these data could be used to describe change to the oyster population.

ANNUAL, SEASONAL AND SIZE-DEPENDENT PATTERNS OF JUVENILE BLUE CRAB, *CALLINECTES SAPIDUS*, MORTALITY IN UPPER CHESAPEAKE BAY.

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We assessed annual, seasonal and size-dependent patterns of blue crab mortality using long-term (1989–2010) field tethering experiments with juvenile blue crabs conducted during June-August

within the Rhode River, a small mesohaline subestuary of upper Chesapeake Bay. Annual mortality rates of juvenile blue crabs declined significantly during 1989–2008, but increased in 2009–2010. Mortality appears to be primarily driven by annual changes in the abundance of key predators, primarily larger blue crabs. The majority of mortality was directly attributed to cannibalism based on the characteristics of tethered crab remains that were indicative of predation by adult blue crabs. Thus, declines in the mortality rates of juvenile blue crabs in our study may have resulted from declines in adult blue crab predators in Chesapeake Bay, which are reflected in multiple fishery-independent indices of blue crab abundance over this period. Relative mortality rates of tethered juvenile crabs varied seasonally and were lowest in early spring, peaked in summer, then declined through the fall. Relative mortality decreased with size up to 50 mm CW, suggesting that juvenile crabs may attain a partial refuge from predation at this size. Our results highlight the potential importance of density-dependence mediated by cannibalism to population dynamics for this species.

CLIMATE CHANGE AND BIVALVES: WINNERS, LOSERS AND ADAPTATION IN THE DELAWARE ESTUARY.

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Shellfish biologists were shown downscaled climate predictions for the Delaware River Basin and then polled using a risk assessment approach to build consensus on top vulnerabilities for bivalve molluscs in the watershed. Increased temperature, sea level, salinity, storms, and altered seasonal precipitation are expected to interact with ongoing landscape change, perturbing many ecological relationships and affecting bivalve molluscs. In non-tidal streams, environmental tolerance limits for several of 12 species of native freshwater unionids are likely to be breached. Since dams and other impediments block dispersal of freshwater mussel larvae carried by fish hosts, newly open niches vacated by extirpated native species are unlikely to be filled with southern species without assisted migration or other tactics. In the tidal estuary, a predicted loss of >25% wetland habitat by 2100 threatens populations of ribbed mussels (*Geukensia demissa*). Interactions between disease and increased salinity and temperature may threaten commercial populations of oysters (*Crassostrea virginica*). Although some species may eventually benefit from a longer growing season or shifting habitat types, we predict net effects on the collective assemblage of 60+ species of bivalves, and their associated ecosystem services, to be substantially negative unless proactive "adaptation" measures are adopted to stem losses.

WEST COAST UPWELLING IMPACTS SEED AVAILABILITY FOR PACIFIC OYSTER FARMERS

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Pacific (*Crassostrea gigas*) oysters are the main shellfish species cultured on the West Coast, USA, with annual sales valued at about $83 million. About 2000 farmers earn a living by rearing and harvesting Pacific oysters, often in rural coastal communities. Farmers are dependent on either collection of seed ("eyed" larvae) from natural spawns in a few localities—the main source being Willapa Bay, WA, or purchase of seed from commercial hatcheries. Unfortunately, since 2005 there have been no commercial quantities of natural seed produced in Willapa Bay. In addition, since 2007 two of the four commercial hatcheries have reported severe problems rearing oyster larvae. Periods of poor larval production at the Whiskey Creek Hatchery, Netarts Bay, OR are strongly correlated with upwelling events. Oceanographers have reported that upwelled deep water on the West Coast is characterized by low aragonite saturation states. Aragonite is the main form of calcium carbonate in shells of larval oysters. At this time, it is not clear if low aragonite saturation states, high concentrations of dissolved carbon dioxide or some other characteristic of upwelled water is detrimental to oyster larvae.

MARINE FISHERIES ENHANCEMENT: AN IDEA BEFORE ITS TIME

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In science, new ideas are formulated into hypotheses, tested and evaluated. Some ideas survive experimentation and the test of time and become theory; others are tossed aside or recycled and reevaluated. Marine Fisheries Enhancement is a management idea that was implemented a century ago in a complete void of science. Millions of cod, haddock, pollock, and flounder larvae were hatched and stocked into the sea for over half a century, before the hatcheries at Woods Hole and Gloucester, MA, were closed for lack of effectiveness. With no science as a base, this idea crumbled to the ground as the way to manage fisheries, replaced by more effective fishery management ideas about controlling fish catch. But some ideas die hard, and the attractiveness of stocking continued to invite funding from politicians. By the time the Blue Crab Advanced Research Consortium (BCARC) was funded, scientific study of fisheries enhancement had finally begun in earnest the previous decade. A prescription for a 'responsible approach' emerged and BCARC seized it as a working model for how to proceed. In the backdrop of a rapid increase in scientific study of marine fisheries enhancement, I consider here what BCARC's contribution to this new science has been.

DETERMINATION OF SEX AND GONADAL DEVELOPMENT OF EASTERN OYSTERS *CRASSOSTREA VIRGINICA* GMELIN THROUGH PROTEIN PROFILES OF HEMOLYMPH BY SELDI-TOF-MS TECHNOLOGY.

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In hatchery practices, the readiness of broodstock oysters for spawning is important. The conventional method of determining sex and gonadal developmental stages in Eastern oysters involves sacrificing individuals and making gross-anatomical observations. In this study, we reported a novel, non-lethal method of determining oyster sex and developmental stages through protein profiling of extracted hemolymph using a relatively new proteomic technique, Proteinchip® and SELDI-TOF-MS. Among hundreds of peptides/proteins detected from oyster hemolymph, 62 appeared to be involved in reproductive activities. Using the protein-profile information and multivariate statistical analysis, individual broodstock oysters were categorized successfully into one of five groups: undifferentiated, female developing, female ripe, male developing and male ripe. The accuracy of categorization, confirmed by traditional histological methods, was 98.8% ($p < 0.05$). Wide application of this method is still limited by cost; however, results of this research open doors for further study to develop more-affordable and portable methods based upon detection of specific hemolymph peptides and proteins.

SHORT TIME RESPONSES OF *CRASSOSTREA GIGAS*, SELECTED FOR RESISTANCE OR SUSCEPTIBILITY TO SUMMER MORTALITY, TO A BACTERIAL CHALLENGE PERFORMED DURING ACTIVE GAMETOGENESIS.

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Resistant (R) and susceptible (S) *Crassostrea gigas* to summer mortality were exposed to a bacterial challenge (*Vibrio splendidus* + *V. aestuarianus*) during active gametogenesis. Three samplings were performed at 12, 24 and 48 hours post challenge. As a result, higher mortalities were observed on S oysters as compared to R ones (38% vs. 25%). The bacterial burden increased in the hemolymph of challenged oysters after 12 hours but was cleared quickly (24 hours). Data on hemocyte parameters showed few and transient changes after 12 hours challenge and more differences between R and S oyster lines rather than between the challenged and non-challenged oysters: oxidative activity was significantly higher on susceptible (S) oysters compared to R ones. Analysis of expression of a peroxiredoxin 5 homologous gene (known as a hydrogen peroxide scavenger) in gills showed a higher mRNA level in R oysters. In hemocytes, analysis of expression by qPCR of genes known to be involved in oyster immune response showed no differences according to bacterial challenge but higher mRNA levels in S oysters likely leading to higher energy expenditure. These results seem to reinforce the evidences of a potential role of oxidative stress to explain *C. gigas* mortality events during gametogenesis.

HAVING AN EGG-CEPTIONAL TIME: MODELING OVERALL FECUNDITY IN *CRASSOSTREA VIRGINICA* FEMALES FROM THE CHESAPEAKE BAY USING BOTH EGG QUANTITY, LIPID CONTENT AND FATTY ACID COMPOSITION.

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Reports on oyster (*Crassostrea virginica*) reproduction tend to be based on animals that are approximately market size (75 mm) due to both the commercial importance of data on animals of market size and the relative paucity of animals larger than 75 mm. Oysters have been known to survive longer than ten years and therefore comprehensive data on oyster reproduction of older and larger oysters may be important to understand the ecology of long-lived populations. The ages of some oysters on restored oyster bars in Maryland is known due to a seeding program by the Oyster Recovery Partnership. We took advantage of this scenario and measured relative fecundity (eggs spawned) and egg quality (egg total lipid content (ETLC) and egg fatty acid composition (EFAC)) for oyster populations from the Maryland portion of the Chesapeake Bay (Choptank (3 and 9 y) and Magothy Rivers (4 and 11 y)). The egg quantity and quality data were incorporated into a model to describe the correlation between size/age and maximum energy-rich reproductive output. Although these data point to differences in overall fecundity in oysters, several more years of data will be necessary to confirm the trends observed during the first year of sampling.

IMPROVEMENT IN YIELDS OF PACIFIC OYSTERS *CRASSOSTREA GIGAS* ON THE WEST COAST, USA, IN A HETEROGENEOUS AND CHANGING ENVIRONMENT.

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The Molluscan Broodstock Program (MBP) is a selective breeding program designed to increase yields of Pacific oysters *Crassostrea gigas* by improving growth and survival. Five lines were originally created that were derived from broodstock collected from wild populations in Washington State and Pipestem Inlet, British Columbia. Each line consisted of a series of cohorts selected since MBP was established in 1995. After three selection cycles, cohorts with genetic contributions from Pipestem Inlet founders have performed best, with average yields per cohort up to 36% greater than those of control families derived from non-selected broodstock, resulting in realized heritabilities for yield, survival and final individual harvest weight (growth) of up to 0.57, 0.49 and 0.19, respectively. Improvements in yield and realized heritabilities varied greatly among test sites. Environmental heterogeneity of test grow-out conditions, over different scales of space and time, is a difficult challenge for oyster breeding programs. Adverse effects of long-term changes in ocean conditions, linked to global warming and increased ocean acidification, will need to be addressed by breeding programs in order to reduce impacts on oyster farmers.

INTERVENTION STRATEGIES TO CONTROL FOODBORNE VIRUSES DURING PROCESSNG AND HANDLING.

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According to the United States Centers for Disease Control and Prevention, human enteric viruses are estimated to cause two-thirds of the foodborne illness in the U.S. each year, with the majority attributed to norovirus (NoV). Enteric viruses including NoV, hepatitis A and E viruses can enter shellfish through contaminated seawater or by contamination during handling and processing, resulting in outbreaks ranging from small isolated ones to epidemic. A number of strategies, on-farm through to retail, have been used to mitigate the risk viruses pose to our food supply. The effects of food processing technologies such as high pressure processing can result in more than 3.5-$\log_{10}$ $TCID_{50}$ ml^{-1} reduction of hepatitis A virus and feline calicivirus, a surrogate for NoV. Similarly handling of contaminated shellfish during the preparation of foods, either at home or food service kitchen, can result in cross contamination of other foods and spread NoV. Behavioral changes in food preparation such as proper hand washing and food handling can prevent or reduce virus transfer from hands during food preparation. Such strategies could be incorporated into a quantitative risk assessment model which may be used to determine the risk management strategies that will determine appropriate process criteria on reducing the contamination of shellfish.

TO DEVELOP A VISUAL ASSAY KIT FOR MONITORING HYPOXIC STRESS OF HARD CLAMS *MERETRIX LUSORIA*.

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The dissolved oxygen concentration that induces the onset of anaerobic metabolism in hard clams was found to be 1.11 mg O_2/L, at which time, the concentration of succinate in the body fluid was increased to be 4.4 μmol/ml. Therefore, this level of succinate concentration could be considered as an indicator for the hypoxic stress of hard clam. The concentration of succinate could be determined by measuring the amount of NADH oxidation in the coupling reaction of succinyl thiokinase (STK), pyruvate kinase and lactate dehydrogenase. However, NADH is not visual. In order to visualize the concentration of NADH, tetrazolium salts and phenzaine methosulphate are included in the reaction. NADH can reduce tetrazolium salts to form formazan whose color is dark blue. Therefore, the amount of formazan produced is proportional to the concentration of NADH in the reaction. The visual assay kit for succinate is constructed to contain 10 mM NADH. A color of light yellow or light blue will respectively be shown in the kit when the concentration of succinate in the body fluid is more than 10, or less than 5 mmol/ml. Therefore, this kit can be used to monitor the hypoxic stress of hard clam.

CONTROL OF HUMAN VIRUS CONTAMINATION IN MOLLUSCAN SHELLFISH: WHERE WE ARE AND WHERE WE NEED TO BE.

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The problem of human virus contamination (particularly noroviruses and hepatitis A virus) is well documented. Unfortunately,controlling contamination remains problematic. The presence or numbers of the traditional fecal indicators (fecal coliforms and *E. coli*) is poorly correlated with virus contamination in molluscan shellfish and their harvest waters, and sources of contamination (e.g., untreated human sewage overflow because of catastrophic weather events, illegal boat discharges, and septic tank leakage) are unpredictable and appear to be relatively rare events. As a consequence, there remains substantial interest in development of novel pre- and post-harvest controls. The purpose of this presentation is to describe the current state knowledge and

technology regarding the control of human virus contamination of molluscan shellfish. Specifically, we will cover (i) the epidemiology of shellfish-associated viral food borne disease; (ii) emerging pre-harvest control measures (e.g., alternative microbiological indicators, novel microbial source tracking methods, and virus testing strategies); and (iii) novel post-harvest control measures (e.g., high pressure and combination approaches). While advancements are being made, there is much yet to do and the scientific community needs to work together to solve this persistent public health problem.

THE ECOLOGICAL AND COMMERCIAL FUTURE OF "SHELLFISH" IN AN ACIDIFIED OCEAN.

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There is little doubt at this point in time that global climate change, and its effects on biological systems, is occurring. Much of the concern surrounds the well-documented accumulation of carbon dioxide in the atmosphere and the corresponding coupling of that phenomenon to increasing levels of carbon dioxide in the world's oceans. While much of the attention regarding global climate change effects have centered on coral reef ecosystems many important effects have been documented from Polar and temperate ecosystems. A number of physiological studies have now documented significant effects not only on calcifying organisms such as corals, but many other marine organisms of ecological and commercial importance such as bivalve molluscs. Increasingly, the larvae of many of these organisms have been identified as a critical life history stage that is significantly affected by ocean acidification and likely represents a bottleneck to successful recruitment in natural settings, and production of larvae in commercial settings. In this overview I present an integrated description of what we know at this point in time and identify critical areas of study that should be undertaken in order to respond successfully to what many believe is the inevitable changes coming in the future for our oceans.

POTENTIAL FOR OYSTER REMOVAL OF NITROGEN: 15 BILLION CAN'T DO THE JOB ALONE IN A NY BAY.

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The eastern oyster *Crassostrea virginica* grew more rapidly in Jamaica Bay than in any other of 9 widespread sites in the New York – New Jersey region. Most Jamaica Bay chlorophyll is in the > 5 um size fraction and therefore could possibly be filtered by oysters, if not partially converted to oyster body mass. If suitable habitats were covered with oysters, could they be harvested in order to remove a significant fraction of the nitrogen? We measured oyster soft tissue growth, mortality, and nitrogen content across a water quality gradient and calculated potential nitrogen removal in an aquaculture setting using a GIS-based analysis of oyster habitat. Assuming oysters occupy grow-out bags in cages, covering 50% of suitable habitat, our data at several sites suggest that the hypothetical 14.9 x 10^9 oysters would remove 1.96 x 10^6 kg of nitrogen over the maximum growing season, which compares to 5.76 x 10^6 kg of total annual nitrogen input into Jamaica Bay. Of course this is an overly generous coverage of oysters and the GIS estimate is an overgenerous habitat assessment. Our measurements and calculations allow the design of an approach that might use oysters as part of a larger nitrogen removal scheme.

OFFSHORE MUSSEL FARMING IN SOUTHERN NEW ENGLAND; RESULTS AND CONCLUSIONS FROM COMMERCIAL GROW-OUT.

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Approximately 90% of mussels consumed in the U.S. are imported. The development of domestic offshore mussel farms offers promising options for fishermen and shellfish farmers to create jobs and product for local markets. In the fall of 2009 fishermen installed and maintained four 150 meter subsurface longlines for suspended mussel culture in 30 meter deep waters in Rhode Island and Massachusetts. These pilot commercial-scale lines grew mussels to market in less than 12 months. Growth rates averaged 3 to 3.5 mm per month until harvest in August 2010. No pea crabs were found in 2 of the 3 sites harvested. The net yield of processed and marketable mussels from the gross weight of the harvested socks was 75%. The yield of live mussels was lower than expected at <3 kg/m of rope. The steamed meat yield of the mussels dropped from 30% in early July to 20% in late August. An overset of mussel seed on the harvested lines could have provided up to half of the seed necessary to restock the line. An economic break-even analysis using various production and market scenarios will be presented.

ASSESSING POTENTIAL BENTHIC IMPACTS OF SUBTIDAL GEODUCK CLAM HARVESTING.

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The geoduck clam, *Panopea generosa*, is the largest burrowing clam in the world and adults of this species live a meter or more below the sediment surface. In order to extract these clams, harvesters use high-volume water hoses to liquefy the surrounding sediment. Disturbing the sediment to a depth of a meter or more could have profound effects on the local benthic environment, but little research has focused on this issue. We examined the potential benthic effects of harvesting a 60 x 100-m subtidal area that had been planted with clams 8 years prior to harvest. We took replicate sediment samples within the harvest zone and at varying distances from the area of impact (5, 10, 25, 50, 75 m) at various time points ranging from 12 months prior to harvest through to 12 months post-harvest. We examined various sediment qualities (grain size, percent organics, total organic carbon, total nitrogen, sulphide concentration, redox), infaunal diversity and numbers, and suspended sediment levels. In addition, we examined potential effects on a nearby eelgrass bed (examining shoot length, biomass, and shoot density). Sample processing and data analysis are currently underway, but preliminary evidence suggests that impacts appear to be relatively limited in time and space.

EFFECT OF HYPOXIA AND ACIDOSIS ON ADDUCTOR MUSCLE FUNCTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, AND THE ASIAN OYSTER, *CRASSOSTREA ARIAKENSIS*.

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Hypoxic environments disproportionally affect sessile benthic organisms as they are permanently affixed furthest from atmospheric sources of oxygen. Therefore, some members of this community, including the Eastern oyster, *Crassostrea virginica*, have evolved metabolic adaptations enabling survival of hypoxic episodes. Interestingly, the Asian oyster, *Crassostrea ariakensis*, does not exhibit strong hypoxic tolerance. Previous studies showed that when exposed to hypoxia, *C. ariakensis* underwent more significant hemolymph acidosis and died earlier than the closely related *C. virginica*. Additionally, these species exhibited different gaping responses when exposed to low oxygen environments. To determine if the difference in gaping response in *C. virginica* and *C. ariakensis* in low oxygen environments is a result of physiologically-induced changes in adductor muscle function, we assessed the effects of low oxygen and acidosis on adductor muscle function in *C. ariakensis* and *C. virginica*. This study sheds light on physiological differences between two closely related species, while helping explain mechanisms for the well-documented hypoxic tolerance of *C. virginica*.

THE DERIVATION OF A SAFE LEVEL FOR COPPER BASED UPON AN IMPROVED UNDERSTANDING OF ITS CHEMISTRY IN THE MARINE ENVIRONMENT, AND AN ASSESSMENT OF THE RISK OF USING COPPER IN THE PREVENTION OF BIOFOULING.

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The last 5 years have seen significant developments in the approaches used to derive safe environmental levels for metals. In particular, an understanding of bioavailability has provided new tools for risk assessment which have allowed the derivation of safe levels for metals based upon new data, and allowing the improved use of existing data. The approach described shows how these tools and data have been used within the EU to derive a safe level for copper in the marine environment and, in addition, shows how the derived safe level was tested experimentally. The conclusions of the research are extrapolated into the context of a risk assessment on the use of copper in the prevention of biofouling, challenging historical conclusions regarding the adverse impacts from the use of copper in the marine environment.

SCALLOP RESTORATION IN THE VIRGINIA COASTAL BAYS: A PROGRAM FOR RESTORING A POPULATION 75 YEARS AFTER LOCAL EXTINCTION.

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During the early 20th Century the coastal bays along Virginia's outer coast supported an extensive commercial fishery for the bay scallop, *Argopecten irradians*. Following the nearly complete loss of eelgrass, *Zostera marina*, from the region in 1933, the scallop population collapsed and has been effectively extinct from Virginia waters since that time. Over the past decade efforts to restore eelgrass to the coastal bays have been remarkably successful and grass beds are now expanding throughout much of the system. Restoration of bay scallops to this region faces several unique challenges, including the lack of regionally available brood stock, the likelihood that this region was formerly a zone of overlap between two sub-species and the sheer scale of the bays. We used F1 generation animals, produced from quarantined broodstocks (to avoid introduction of hitchhiker species), to test grow-out methods, determined spawning times and evaluate growth rates in the region. We have subsequently deployed over 75,000 caged scallops to serve as spawning stock within the grass bed. Though this represent only the first step in a multi-year effort, qualitative observations within the grass bed have revealed the presence of juvenile scallops and suggest that spawning and successful recruitment has occurred.

ZEBRA MUSSELS IN IRELAND—FOURTEEN YEARS OF INVASIVE EXPERIENCE.

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When the zebra mussel (*Dreissena polymorpha*) was discovered in Ireland, in 1997, there was quick recognition of the potential negative impacts to both ecosystems and the human aquatic resource. The national response was to promote research, education and outreach with the aim to develop effective policy and legislation.

The initial invasion was in the Shannon, the major Irish navigational waterway (257km). Colonization developed upstream; the passage of recreational boats acted as the main vector. Biofouling of the native unionid duck mussel *Anodonta* quickly followed and by the year 2000, no living specimens were observed. A three year study on Lough Key (9 km^2) (2001–2003) estimated the population at 34 billion (34×10^9), with a total biomass of 4.4×10^6 kg, capable of lake filtration in a 10-day period. The geology of a river basin (pH and calcium) is one of the main limiting factors for invasion of a lake. Water abstraction facilities are impacted by biofouling. Education and outreach have not been effective in preventing the spread of zebra mussels. New invasive species legislation will shortly be enacted—fourteen years later. On a positive note, zebra mussels are used as effective monitors for human waterborne pathogens, e.g. *Cryptosporidium*, in Irish waters.

INVESTIGATION INTO THE PREVALENCE AND DISTRIBUTION OF PATHOGENS IN CULTURED AND WILD POPULATIONS OF THE MUSSEL *MYTILUS* SPP. IN IRELAND.

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In Ireland, both wild and cultured *Mytilus* spp. are widely distributed on all coasts. *Mytilus edulis*, *Mytilus galloprovinicialis* and hybrids of both species are found on the west and south coast of Ireland while *M. edulis* is found on the east coast. Although high mortalities caused by pathogens and parasites have not yet been encountered in *Mytilus* spp., several pathogens may be potentially harmful.Prior to this study, very little was known about the health status of *Mytilus* spp. in Ireland. The monitoring of shellfish populations and their pathogens is important in the decision making involving shellfish movements. Samples of *Mytilus* spp. were collected from fourteen sites encompassing all coasts of Ireland at different times of the year. Habitat description and the environmental factors influencing the study sites were recorded. *Mytilus* spp. samples were screened using histology and conventional polymerase chain reaction. At certain study sites a cPCR was carried out to differentiate which mytilid species was being screened. Overall almost a third of mussels examined in this study were hosts to parasites. Four types of symbionts were detected including ciliates, trematode spp. prokaryote inclusion bodies and *Nematopsis* spp.

THE INCIDENCE OF HAPLOSPORIDIANS, *HAPLOSPORIDIUM NELSONI* AND *HAPLOSPORIDIUM AMORICANUM*, IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*, AND THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS*, IN IRELAND.

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Haplosporidium nelsoni is the causative agent of the disease MSX (multi-nucleated unknown). *H. nelsoni* has been observed in the Pacific oyster *Crassostrea gigas*, in California (USA), Korea, Japan, France and Canada.Multinucleate plasmodia are the typical life stage and the disease is usually systemic with *H. nelsoni* spreading to all tissue via hemolymph sinuses.During heart smear screening of *C. gigas* in Ireland multinucleate plasmodia were detected. Conventional polymerase chain reaction (cPCR) was carried out using generic haplosporidian primers and *H. nelsoni*-specific primers on both the tissue and shell cavity fluid of *C. gigas*. Products were amplified at the expected product size; the DNA was isolated and purified for sequencing.Sequencing results deemed the DNA sequences to be *H. nelsoni*. This is the first reporting of *H. nelsoni* in oysters in Ireland. *Haplosporidium amoricanum* was detected in the heart smear of a single *O. edulis*. cPCR was carried out on the corresponding DNA of that individual and sequencing confirmed it to be *H. amoricanum*. The impact *H. nelsoni* and *H. amoricanum* will have on *C. gigas* and *O. edulis* stocks in Ireland is unclear at this time.

INVESTIGATION INTO THE INVOLVEMENT OF OSTREID HERPES VIRUS 1 (OSHV-1) IN SUMMER MORTALITIES OF PACIFIC OYSTER, *CRASSOSTREA GIGAS*, SPAT AND MARKET SIZED ADULTS IN IRELAND.

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Significant summer mortalities of the Pacific oyster, *Crassostrea gigas*, have been reported worldwide since the 1950's to the present day. Many studies have indicated that a complex aetiology is involved in these mortalities but one consistent pattern is the presence of herpes virus (OsHV-1) in moribund oysters. A single cohort of *C. gigas* spat sourced from a hatchery was monitored every two weeks at two cultivation sites in Ireland from July to November in 2003. Samples were screened for ostreid herpes virus 1 (OsHV-1) using conventional polymerase chain reaction (cPCR) and histology. OsHV-1 was detected by cPCR in the juvenile *C. gigas* gill tissue. The hemolymph of a sample of market sized adults from one of the sites was also screened for OsHV-1 as this group of oysters was experiencing significant mortalities. OsHV-1 was detected in the hemolymph cPCR. ''Abnormal cells'', which are normally associated with OsHV-1 infections, were observed in the histology of most *C. gigas* spat deemed positive in the cPCR.

Prevalence of OsHV-1 infection in the oyster spat ranged from 2% to 71% over the four month study period and prevalence of infection in the adult *C. gigas* was 90%.

HUGE DECLINE IN LANDINGS OF WILD COMMERCIAL BIVALVE MOLLUSCS IN THE NORTHEAST UNITED STATES, 1980 TO 2010.

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Over the past 30 years, 1980–2010, the wild-caught bivalve fisheries in the northeastern United States declined sharply in landings and harvesters. From Maine to Virginia, landings fell in all four of the major shellfisheries: oysters (90%), hardshell clams (22%), softshell clams (67%), and bay scallops (93%). The collapses of wild-caught harvests were acute most everywhere. Maryland, once a major supplier of oysters and softshell clams, lost over 95% of its production, and Virginia landings of oysters and hardshell clams plunged 85% to 90%. In some cases, there were regional shifts in the relative share of production (e.g. the majority of hardshell clam landings in 1980 were from New York, but Connecticut was the leading producer by 2004). Concomitant with these plummeting catches was the departure of harvesters from the shellfisheries. The number of full-time oyster harvesters in Maryland fell from 2,246 in 1980 to 244 in 2009, and Virginia licenses for both oyster and hardshell clam harvesting declined 73%. Similarly, the number of hardshell clam diggers dropped by 75% and softshell clam diggers by 100% in Raritan Bay, New Jersey. These huge declines in landings have only been slightly compensated for by aquaculture production, especially hardshell clam harvests, but not the other species.

EFFECT OF *IN-VITRO* PESTICIDE EXPOSURE ON OXIDATIVE BURST POTENTIAL OF SYDNEY ROCK OYSTER HEMOCYTES.

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Production of Sydney rock oysters (*Saccostrea glomerata*) has declined in Australia due to outbreaks of QX-disease (*Marteilia sydneyi*). The disease occurs in estuaries where the catchment has been modified by agriculture or urban activities. Scientific studies have shown that rock oysters are immuno-compromised prior to *M. sydneyi* infection and rainfall reduces the activity of oxidative enzymes involved in resistance to QX disease. Pesticides found in agriculture rainwater runoff have been suggested as a potential risk factor. The current study investigated sub-lethal concentrations of agricultural pesticides on the oxidative burst potential of *S. glomerata* hemocytes. Production of reactive oxygen species (ROS) by hemocytes in response to agricultural pesticides and a model immunogen (zymosan) was measured using an *in-vitro* chemiluminescence assay. Atrazine, diuron, chlorpyrifos and carbendazin at concentrations of $5 - 500\ \mu g.mL^{-1}$ had no effect on ROS production of *S. glomerata* hemocytes ($p > 0.05$). However, exposure of hemocytes to low levels of all pesticides ($5\ \mu g.mL^{-1}$) and zymosan induced a significant increase in ROS production compared to zymosan controls ($p < 0.05$). These results suggest that when rock oysters are exposed simultaneously to low concentrations of pesticides and pathogens, hemocytes overstimulate ROS production and oxidative damage to host cells could occur.

THE EFFECTS OF DISEASE ON THE SPATIAL DISTRIBUTION OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, ALONG AN INTERTIDAL STRESS GRADIENT.

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Spatial distributions of species can be shaped by factors such as mortality, growth, and disease—all of which can be influenced by physiological and environmental stress. In habitats such as the intertidal zone, various durations of air-exposure that occur during low tide create defined stress gradients that shape the spatial distributions of benthic marine organisms. The eastern oyster, *Crassostrea virginica*, an economically and ecologically important species in Chesapeake Bay, lives in both the intertidal and subtidal in Virginia, but only in the subtidal in Maryland. We used field experiments and sampling from Maine to North Carolina to determine whether disease (Dermo), mortality, and growth of oysters vary along an intertidal-subtidal stress gradient during summer in the Maryland and Virginia regions of Chesapeake Bay. Results indicated that Dermo prevalence and mortality decreased and growth increased from the peak (high intertidal) to the bottom (subtidal) of the intertidal gradient. Dermo prevalence was higher in habitats with longer durations of air-exposure but progression of the disease did not differ consistently along the intertidal gradient. Patterns in summer mortality, growth, and disease in combination with recruitment, winter mortality, and predation likely contribute to the variation in spatial distributions of oysters within Chesapeake Bay.

GROWING THE *CHAETOCEROS* PHYTOPLANKTON SPECIES IN THE BRITE-BOX, AN ALGAL PHOTO-BIOREACTOR.

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The Brite-Box system is a closed photo-bioreactor designed to maximize algal production with minimal energy input and reduced labor costs It has been used to sustain the complete hatchery

production cycle of the bay scallop, from the conditioning of the brood-stock to the rearing of 3-mm juvenile bay scallops. The Brite-Box has control systems capable of delivering stable temperature and pH conditions and the ability to alter light levels and spectra. These system attributes are important while researching the optimal culture conditions for different phytoplankton species. Repeated sustained harvesting of *Chaetoceros mulleri* and *Chaetoceros calcitrans* has proved to be challenging for hatchery operators equipped with continuous algal production systems. The practical solution has often been to use continuous culture systems for flagellates species and rely on batch culture systems for the diatom species.We will report on some of the culture conditions which appear to be necessary for the sustained harvesting of *Chaetoceros* species from the Brite-Box photo-bioreactor. Results will be presented in terms of growth profiles (fluorescence and cell counts), production rates (dry weight per day), and cost of production per unit dry weight.

DNA-BASED DIETARY ANALYSIS OF WILD MARINE BIVALVE LARVAE.

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Dietary analysis of planktonic organisms and understanding competitive interactions between species has proven difficult because of the small size of predators and their microscopic prey. We present a PCR-based approach using universal eukaryotic primers for the dietary analysis of individual bivalve larvae. To reduce amplification of larval DNA and enhance PCR sensitivity to DNA of ingested organisms, a bivalve specific restriction enzyme and blocking primer were evaluated. Using the blocking primer alone, 80% of recovered sequences originated from gut contents. This increased to 100% in combination with a restriction enzyme. When applied to the dietary analysis of naturally feeding larvae of *Mysella vitrea* and *Ostrea edulis*, 75% of the recovered sequences originated from centric and pennate diatoms belonging to at least 4 different family groups, 16% originated from Fungi of the phyla Ascomycota and Basidiomycota, and almost 5% were flowering plant sequences, presumably originating from pollen grains. At the level of taxonomic resolution attained (genus or family), there was no significant difference between the larval diets of *O. edulis* and *M. vitrea*, suggesting that there was direct competition for food resources. Detailed characterization of the diets of wild larvae should aid in the development of hatchery diets for emerging species.

DNA-BASED DIETARY ANALYSIS OF MARINE BIVALVES AND ITS CONCURRENCE WITH STABLE ISOTOPES.

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The cryptic nature and wide diversity of dietary organisms ingested by marine suspension feeding bivalves presents distinct challenges when trying to determine trophic and competitive interactions between species. Using a polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) approach targeting eukaryotic organisms, we have obtained dietary profiles from five species of suspension feeding bivalves. In all cases the dietary profiles were significantly different between species and distinct from that of the water column. These results suggest that different bivalve species actively reduce feeding competition through selective feeding. In addition, the spatial variation in dietary profiles from *Mytilus edulis* was evaluated along a strong environmental gradient and compared with corresponding fractionation values of isotopic carbon and nitrogen. While the similarity of stable isotope measurements were significantly correlated with those based on dietary profiles, the DNA-base data more closely reflected the underlying environmental gradient. Such variation likely results from the time lag inherent between DNA-base ingestion data and assimilation data as measured by stable isotopes. On-going attempts to merge these two techniques are focused on using the increased taxonomic resolution of DNA studies to refine the mixing models used in stable isotope analyses.

ENVIRONMENTAL AND PHYSIOLOGICAL GENOMICS OF MARINE LARVAL FORMS.

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Larval forms are dominant in the complex life history strategies of most marine animals. From enhancing aquaculture production to modeling recruitment ecology under natural conditions, a lack of understanding of the biology of larval forms often limits predictive power. In general, marine larvae are "hard to work with"—many species are difficult to culture under controlled laboratory conditions, and their small and variable sizes pose challenges for quantitative and integrative ("genes to whole organism") biological analysis. Additionally, the lack of defined genetic lines has often hampered the interpretation of comparative physiological studies of most marine larval species. Recent studies of sea urchin and bivalve larvae are opening up new approaches to

the study of marine larval forms. Genetic analysis and functional genomic approaches are now providing new insights into the biochemical and physiological bases of environment-genotype interactions in early developmental stages. Such analyses offer the potential of improving predictions through a mechanistic understanding of growth and survival of contrasting phenotypes in the changing environmental conditions of the ocean.

THE CHESAPEAKE BAY EXECUTIVE ORDER, OYSTER RESTORATION GOALS AND DOLLARS - DID ANYONE THINK ABOUT THE COSTS?

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There is often a disconnect between the lofty goals of restoration programs and the reality of on the ground success and associated costs—both initial and generally unappreciated continuing costs. Executive Order 13508, entitled a "Strategy for Protecting and Restoring the Chesapeake Bay Watershed" proffers the goal of restoring 20 estuaries within the Chesapeake Bay to self sustaining oyster populations by 2025. I present a series of calculations that broadly interpret this goal with associated needs in terms of shell to build habitat, shell to maintain habitat (assuming that adequate shell is available for both, and this is not assured at this time), and dollar estimates to support the enterprise. Depending on the restoration scenario chosen, the cost estimates vary between $71 million and $2.8 billion dollars for a ten year period, and that is without any assurance that the "restored" systems will be self sustaining thereafter. So did anyone think about the costs?

MANAGING SHELL BUDGETS THROUGH ROTATIONAL HARVEST IN SEED PRODUCTION AREAS.

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Recent publications have underscored the importance of habitat (shell) budgets to maintain oyster populations, and the challenge this presents in managing oyster fisheries where shell is applied as a substrate to encourage recruitment and removed by harvesting. Under a traditional "annual put and take" approach to shell planting and seed harvest it is not possible to sustain a positive or even equilibrium shell budget, that is bushels of seed produced will be less than the bushels of shell applied. We describe a 10-year record of recruitment, growth and mortality for oyster populations in the Piankatank River, VA, a river traditionally used for seed oyster production. The mean recruit:stock ratio over the study period approaches 4.0. Growth and age specific mortality rates are known for this population. We present a series of calculations that examine an alternate seed harvest strategy with harvest every other year in combination with a quota based on fall stock assessment. The results illustrate how the shell budget can be markedly changed and even approach an equilibrium situation with rotational harvest alone as a management tool. The implications for long term stability and cost efficiency are obvious. We encourage examination of this option in other seed fisheries nationwide.

THE EFFECTS OF CHRONIC HABITAT DEGRADATION ON THE PHYSIOLOGY AND METAL STORAGE OF EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.

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Prior to the urbanization of the Hudson River Estuary (HRE), Eastern oysters (*Crassostrea virginica*) were significant contributors to the ecological and economic health of the region. However, due to overharvesting, pollution, and declining water quality, this ecosystem engineer has become 'functionally extinct' in the lower HRE. Recent restoration efforts have yielded mixed results, mainly due to the unique suite of pollutants seen in the HRE. Changes in physiology (i.e., energy reserves, mitochondrial energetics) and metal storage (i.e., storage in subcellular fractions) may lead to changes in the overall health of the oyster, and affect growth, reproduction, and survival. A field-based study examining juvenile *C. virginica* at a variety of sites within the urbanized HRE was conducted in the summer of 2010. Juvenile oysters were placed along a contaminant gradient, and subsampled over time to determine changes in physiology and metal storage. Site specific differences in condition index and biochemistry ($p < 0.05$), and changes in metal storage were seen across sites and time. These results indicate that any potential future restoration of *C. virginica* to the urbanized HRE must take into account the differences in energy budgets and condition of juveniles. Future studies with adult oysters will complement this research.

DIFFERENTIAL GENE EXPRESSION IN EASTERN OYSTER SEED (*CRASSOSTREA VIRGINICA*) IN RESPONSE TO THE BACTERIAL PATHOGEN ROSEOVARIUS CRASSOSTREAE.

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In culture facilities, the eastern oyster, *Crassostrea virginica*, has suffered severe mortality due to Juvenile Oyster Disease (JOD), caused by the bacterial pathogen *Roseovarius crassostreae*. The

immune defense response of *C. virginica* remains poorly characterized. To that end, seed oysters (less than 25 mm in shell height) from two families (GX09 and F3L, Rutgers University) were challenged by bath exposure to *R. crassostreae* (7.5×10^6 cfu/L). Among challenged groups, GX09 had a significantly higher survival rate (70%) than F3L (20%) 86 days after challenge. Survival in control, non-challenged tanks ranged between 66–70%. RNA was isolated from whole oysters at 1, 5, 15, and 30 days after challenge. At 30 days, differential expression of several immune and stress-related proteins was observed between oysters from the high and low survival families. We are also analyzing approximately 46 Mbp of 108-bp reads of cDNA, sequenced by Illumina GAII, from challenged and control oysters in order to identify immune-related transcripts, both previously-annotated and novel. High-throughput transcriptome analysis may prove a powerful tool in deeply characterizing the immune response of oysters in response to bacterial infection and in identifying single nucleotide polymorphisms useful as genetic markers in breeding programs.

INFLUENCE OF ACUTE CHANGE SALINITY ON OSMOLALITY OF HEMOLYMPH IN THE GREEN MUSSEL, *PERNA VIRIDIS*, AND THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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The green mussel *Perna viridis* is a recent invasive species to southwest Florida and while it is currently only found in high salinity areas it is of concern that they may threaten estuarine oyster reefs. This study contrasted the osmoregulatory capacity and salinity tolerance of, *P. viridis* with the native species - eastern oyster, *Crassostrea virginica*. Both species of shellfish that were at an ambient salinity of 30 ppt were exposed to acute salinity change (5, 10, 15, 20, 25 30 and 35 ppt) under laboratory conditions and hemolymph osmolality examined over a period of 1 week. Oysters at 5 and 10 ppt were able to adjust their hemolymph osmolality to ambient conditions within 24–96 hrs, while green mussels at similar salinities were unable to adjust even after 1 week. Both species at 15 ppt and above were able to adjust their hemolymph osmolality to ambient conditions within 24 hrs. Green mussels may not be able to adapt to acute salinity changes, especially at salinities <15 ppt.

THE GROWTH, REPRODUCTIVE AND NUTRITIONAL DYNAMICS OF QUAGGA MUSSELS, *DREISSENA ROSTRIFORMIS BUGENSIS*, IN LAKE MEAD, NV/AZ.

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The quagga mussel (*Dreissena rostriformis bugensis*) was sampled bimonthly from 07/2009-11/ 2010 at a depth of 1.5 m in Las Vega Boat Harbor, Lake Mead. The shell lengths (SL) of all sampled individuals were measured (nearest 0.1 mm) and dry tissue weight and gonad condition determined for 100 individuals. Sample size distributions indicated a maximum age of four years at ≤25 mm SL. Adults became gravid in spring and in fall. Fall juvenile settlement began on 10/06/2009 when water temperature fell below 23°C, newly settled juveniles making up 85.1% of the population by 12/21/2009. Mussels again became gravid on 03/15/2010 leading to a second smaller spring settlement initiated on 05/03/2010 after water temperatures fell below 18°C, newly settling individuals making up 14.5% of the population. Shell growth did not occur during summer, the majority of shell growth occurred during fall-spring at 15°–25°C. Mussels lost dry tissue mass during summer months at water temperature >25°C. Tissue dry mass increased from fall through winter, peaking on 03/15/2010 prior to spring reproduction. The data indicate that quagga mussels were in negative energy balance above ≈25°C and that rapidly rising spring water temperatures may have inhibited spring juvenile settlement.

EVIDENCE FOR DENSITY-DEPENDENT SURVIVAL OF *CRASSOSTREA VIRGINICA* SPAT ON RESTORED OYSTER BARS IN THE NORTHERN CHESAPEAKE BAY.

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Oyster (spat-on-shell) plantings of *Crassostrea virginica* have been conducted throughout the Maryland portion of the Chesapeake Bay in an effort to restore oyster reefs. Since 2008, we have conducted post-planting monitoring to examine spat survival and growth. It is unclear what factors contribute to post-planting spat mortality, although environmental conditions, sediment, and predators are thought to play a role. We suspect that environmental conditions and sediment loads may contribute to density-independent mortality while predation rates may be affected by prey density. In this study, we investigated the relationship between initial spat density (spat/m^2) and the post-planting spat survival rate (four to eight weeks post-planting). 2010 spat survival data from eight restored reefs showed a significant positive density-dependent effect ($P = 0.0002$); higher spat/m^2 densities were correlated with higher spat survival. Although similar data are not available for previous years, this is interesting relative to 2009 data that indicated higher spat per shell densities were associated with lower spat survival. The presence of density-dependent effects may indicate predator influences and could have important management implications.

DIETARY PHYTOSTEROLS AND POSTLARVAL SCALLOPS: NOT ALL STEROLS ARE TREATED EQUALLY.

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Considerable work has examined the role of fatty acids in bivalve nutrition, yet less attention has focused on that of sterols. As bivalves are limited in their ability to synthesize sterols, they are largely reliant on their diets to provide these compounds. Feeding trials were conducted with bay and sea scallops (*Argopecten irradians* and *Placopecten magellanicus*, respectively) to examine the role and importance of individual dietary sterols. Scallops were fed various diets, all of which consisted of either *Chaetoceros muelleri* or one of two *Pavlova* spp., for 3–4 weeks, and sterol composition was examined in algal diets and scallop tissues. Across scallop species, stigmasterol and cholesterol were the dominant tissue sterols, yielding a combined 37–68% of all identified sterols. High cholesterol was attributed to *C. muelleri* (38–48%), whereas stigmasterol was the dominant sterol in *Pavlova* spp. (28–43%). While diet influenced tissue composition, this relationship was not necessarily linear and a comparison between sterols in the tissues versus those provided by the diet showed relative enrichment of some sterols (campesterol and cholesterol) and depletion of others (4α-methyl-24-ethyl-5α-cholest-22E-en-3β-ol). Determination of the preferred sterols out of the wide variety produced by microalgae could help develop diets capable of enhancing growth of scallop postlarvae.

NOAA FISHERIES OFFICE OF HABITAT CONSERVATION – YOUR PARTNER IN HABITAT PROTECTION AND RESTORATION.

Patricia A. Montanio.
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Our coastal, marine, and riverine habitats are among some of the most biologically rich and economically valuable areas on Earth and provide a range of benefits to our nation. The NOAA Fisheries Office of Habitat Conservation (OHC) protects, restores, and promotes stewardship of coastal and marine habitat to support our nation's fisheries and preserve our coastal communities for future generations. We believe that healthy habitat is essential to the reproduction, growth, and diversity of fish and shellfish, and our programs directly support NOAA's priority to end overfishing and rebuild our nation's fisheries and fish habitat. Oyster restoration is one of our areas of expertise. This presentation will highlight the activities and contributions of NOAA Fisheries in oyster restoration.

The NOAA Restoration Center, located within the OHC, has as its primary mission the restoration of the nation's coastal ecosystems and preservation of diverse and abundant marine life. The Restoration Center is celebrating its 20th anniversary this month, which will emphasize the growing interest in restoration by community groups and organizations nationwide. Through several programs, the Restoration Center provides funding for projects, including oyster reef restoration. Oyster reefs continue to be a significant focus of NOAA Fisheries with more than 200 oyster restoration projects completed in the last 20 years on all coasts, including a few that incorporate shell recycling as a basic component of their projects. Since there is a scarcity of shell resources in many coastal areas, shell recycling will become an increasingly important issue as we continue to restore oyster reef habitat on all coasts. For example, in response to President Obama's Executive Order regarding cleaning up the Chesapeake Bay, NOAA will pursue restoring and creating oyster reefs in 20 tributaries of the bay during the next 10 years. This will require vast amounts of shell or other suitable materials, with the goal of restoring the native oyster population to levels that will once again provide significant ecological and economic benefits. Likewise, there will be a need for more shell along the Gulf Coast to rebuild reefs that were damaged as a result of the *Deepwater Horizon* BP oil spill. On the West Coast, NOAA Fisheries has been a major partner with organizations and community groups trying to restore the Olympia oyster, which was once plentiful from California to Washington. NOAA's major accomplishments include sponsoring workshops for scientists and restoration practitioners to share research and restoration results of projects involving the Olympia oyster, and publishing proceedings of the meetings. We also contributed to a peer reviewed journal containing important research on this species. The NOAA Office of Habitat Conservation and its Restoration Center are committed to continuing restoration of coastal and marine habitats to ensure their health and sustainability, as well as their ability to adapt to climate change. In this talk, attendees will learn more about the NOAA Restoration Center and the important part it is playing in the restoration of oyster reefs nationwide.

INVESTIGATING THE ROLE OF RIBBED MUSSELS (*GEUKENSIA DEMISSA*) IN STABILIZING SALT MARSH SHORELINES.

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Marsh erosion is a major concern for estuaries as increasing storm severity, boat wakes, and sea-level rise threaten shorelines. The ribbed mussel *Geukensia demissa* is a prominent component of estuarine ecosystems typically associated with the lower edge of marshlands in close association with the marsh grass *Spartina*

alterniflora. Nutrient rich mussel feces enhance *S. alterniflora* production creating levees along the marsh edge by trapping sediments. Shoreline erosion threatens the ability of the marsh to create these natural levees. In addition to enhancing marsh grass production, dense aggregations of ribbed mussels may stabilize the marsh edge, but evidence is lacking. To test the effects of mussel aggregations on salt marsh shoreline erosion, mussel demographics, mass transport rate and annual lateral shoreline movement were quantified along marsh shorelines in four tributaries of the Delaware Estuary (n = 12 sites). Relationships between these factors will help determine the potential role of marsh mussels as a living fortification to reduce erosion along salt marsh shorelines.

ASPECTS OF THE BIOLOGY AND PATHOLOGY OF THE COCKLE *CERASTODERMA EDULE* IN THE IRISH SEA.

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The cockle *Cerastoderma edule* is being studied at two sites on the Irish coast to investigate the biology and health status of the populations and to investigate inter-site variation. Factors contributing to surfacing and subsequent mortality of cockles are being studied. Tissue sections are being screened to assess the health status and gametogenesis of the cockles. The influence of environmental parameters such as temperature, oxygen levels plus salinity is being investigated. Differences are apparent between sites in both biotic and abiotic factors. Pathogens such as trematodes are present at both sites as is hemic neoplasia. Condition indices vary between sites. The sex ratio was more biased towards males at one site and females at the other. Cockles are known to overwinter in spent condition and at the start of the study in March 2010 some had not initiated gametogenesis while others were already classed as late developing. Ripe individuals were observed from April at both sites, cockles were seen in spent condition from May onwards in tandem with other cockles that were starting development. Higher temperatures and a greater salinity range were recorded at one site, but proliferation of algal mats was a greater issue at the second site.

VOLUNTEER CITIZEN SCIENTISTS ASSIST SHELLFISH INDUSTRY TO MONITOR HARMFUL ALGAL BLOOMS.

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The Phytoplankton Monitoring Network (PMN) is a NOAA research-based program utilizing volunteers to monitor harmful algal blooms (HABs) and phytoplankton composition throughout the United States. The network is currently composed of over 150 sites located in 16 coastal states. Since the inception of the program in 2001, 7 toxic events and more than 150 algal blooms have been observed by volunteers. Data generated by volunteers are utilized to alert state resource managers on the extent and distribution of HABs. Three examples of how volunteer observations impacted the shellfish industry will be addressed in this presentation. The PMN offers an ideal pedagogic vehicle to explore the interrelationships between humans, the coastal environment and economically valuable resources, such as shellfish, while at the same time providing volunteers with meaningful opportunities for hands-on engagement in science.

INSIGHT INTO THE MOLECULAR DIVERSITY OF THE CARIBBEAN SPINY LOBSTER VIRUS, *PANULIRUS ARGUS* VIRUS 1.

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Panulirus argus virus 1 (PaV1) is the first pathogenic virus described in lobsters (Shields and Behringer, 2004).Prevalence of the virus can range from 5–8% in juveniles and the virus has a size-specific pathogenicity, with the smallest size classes being most susceptible (prevalence as high as 50% in some "hot spots"). During validation of a PCR assay for PaV1, sequence analysis showed significant variation indicating the presence of several viral genotypes or strains. A small-scale study was undertaken to study the molecular diversity of PaV1 in the Florida Keys. Results of that study were two fold; sequencing analysis suggested tremendous genetic diversity within PaV1; more importantly, PaV1 was described and sequenced from pueruli, non-feeding postlarvae. Previous work has shown that the Florida Keys receives larvae from many regions throughout the Caribbean Sea.Viral variation could either reflect local strain endemicity in nursery habitats or viral hypermutation. Through infection trials, we can demonstrate that observed strain variation is not due to hypermutation, but more likely is reflective of strains endemic to different geographic locations (a hypothesis we are now exploring further). We intend to use the molecular diversity of PaV1 to study disease connectivity throughout the Caribbean Sea.

BIOLOGICAL AND PHYSICAL INFLUENCES ON THE SHELL GROWTH OF THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS.*

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Shell growth of sea scallops is influenced by localized characteristics such as; density, food availability, shear stress, temperature, depth and substratum type. Therefore, scallop growth varies

throughout Georges Bank and Mid-Atlantic. We examined each of these variables and their interactions to highlight possible causes of growth rate differences. Tag-recapture experiments in each area provided shell growth increments over time and were used to calculate instantaneous growth rates. Scallop population densities were estimated using underwater video quadrat counts at each location during the tagging experiments and on a larger spatial scale from the yearly SMAST video survey. Substrate type for each study area was also classified from the video surveys. Remote sensing (SeaWiFS) of Chlorophyll-a levels were used as a proxy for available phytoplankton, while shear stress, bottom temperature, and depth data were derived from the Finite-Volume Coastal Ocean Model (FVCOM). This work aims to advance scallop ecology by explaining the relationships between scallop growth, density and environmental conditions, which will improve rotational management.

THE PROMISE OF FOULING DETERRENCE AS A NATURAL MARINE ANTIFOULING STRATEGY

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Marine biofouling is the unwanted accumulation of bacteria, algae, plants and marine animals on submerged structures including ships. Unfortunately, man's attempts to develop effective antifouling coatings have had deleterious effects on marine life and a less toxic deterrent to cuprous oxide based paints is needed. Larval marine invertebrates have highly developed sensory organs which investigate surfaces prior to settlement, attachment and metamorphosis. We investigated this tactile chemical sense as a potential natural antifouling strategy by covalently linking the neuroendocrine hormone noradrenaline (NA) to poly-hydroxyethylmethacrylate and to poly-methacrylic acid polymer surfaces. NA was selected since it is well established that the soluble form it inhibits larval settlement in molluscs, barnacles, bryozoans and annelid tube worms, all of which are major macrofoulers. The NA conjugate polymer surfaces induced oyster cellular apoptosis when compared to negative controls and also deter the settlement of barnacle and oyster larvae. Fouling deterrence is a promising strategy in that only treated surfaces would deter biofouling thus eliminating the need to release of any toxic substances into the world's oceans.

THE CELLULAR MODEL OF EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) SHELL FORMATION

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Nanocrystalline ceramics evolved from refractive (REF) cells over a billion years ago. We first discovered oyster REF cells in 2004. These cells produce calcite crystals by intracellular means and deliver them to the mineralization front. Subsequently, we have found that oyster cells enable simultaneous production and self-organization of organic and mineralized phases resulting in the extraordinarily strong nanocrystalline ceramic that forms the multi-lamellar shell. Cellular driven mineralization events include; mantle forms primary membrane for adherent hemocytes these cells adhere, aggregate, and organize the membrane by secreting a paracellular macromolecular complex (PMC); crystalline assemblies are formed either within cell masses or by individual cells; the mantle also organizes prismatic layer through organic wall secretions, vesicular bound calcium to augment mineralization is cellularly supplied and finally, a capping membrane also of vesicular origin terminates mineralization. The *transformative aspects* of this research are: a new understanding of molluscan shell formation from a cellular biology perspective which will further our understanding of the impact of ocean acidification on molluscs that inhabit estuarine, littoral and oceanic zones of the world ocean.

LONGTERM TRENDS IN PACIFIC OYSTER (*CRASSOSTREA GIGAS*) LARVAL ABUNDANCE AND RECRUITMENT IN PENDRELL SOUND, BRITISH COLUMBIA, CANADA.

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Pendrell Sound is an important area for the British Columbia shellfish industry; its unique geography and oceanography enables successful annual breeding of Pacific oysters (*Crassostrea gigas*) which can be utilized for seeding oyster farms. In 1959, the DFO

(Department of Fisheries and Oceans Canada) recognized the need for assessing this resource to enable optimal uptake of seed by the industry, and commenced a larval monitoring program. This long-term monitoring program was comprehensive, detailing larval abundances, settlement, recruitment success and environmental variables. By assembling these historical data and continuing to collect data from the Pendrell Sound region we are using this valuable long-term ecological dataset to examine changes in oyster larval density and recruitment in response to changing coastal ecosystems. Early results from this research show that average water temperature is positively correlated with both larval density and recruitment density from 1950 through 1975. As this research continues, we aim to collect larval and recruitment density from 2010 through 2015 that can be compared to historical patterns to examine processes that govern the dynamics of recruitment success of *C. gigas* in British Columbia, and generate a better understanding of how potential climate changes may affect successful settlement and recruitment.

A METAPOPULATION MODEL TO EXAMINE GENE TRANSFER AND POPULATION DYNAMICS IN SHELLFISH.

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Shellfish metapopulations are a complex network of interacting populations connected by larval dispersal. Studying metapopulation dynamics with model simulations requires a modeling framework that includes dynamics of individual populations and connectivity (immigration and emigration) between populations via larval transport. A metapopulation model in which gene flow, metapopulation dynamics, population abundances and diseases dynamics are explicitly represented has been implemented for Delaware Bay oyster populations. This model simulates the large-scale dynamics of abundance, mortality and gene transfer that have been observed for Delaware Bay oysters (*Crassostrea virginica*) from 1990–2010, a period that includes a regime shift. Simulation results demonstrating the influence of spatial relationships of population genetics on overall metapopulation genetics and the role of mortality and larval dispersal in maintaining abundance and gene transfer will be presented.

Preliminary simulation results show that metapopulation allele frequencies are influenced interactively by larval dispersal, mortality and relative population abundance. Additionally, juvenile mortality via predation may exert a notable constraint on transfer of neutral alleles among populations.

A MODELLING APPROACH TO UNDERSTANDING SURF CLAM (*SPISULA SOLIDISSIMA*) MORTALITY PATTERNS AND POPULATION DISTRIBUTION RELATIVE TO CLIMATE CHANGE.

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Increased mortality has been observed since 1997 for southern and inshore populations of Atlantic surf clams (*Spisula solidissima*) from Virginia through New Jersey. Mortality has been co-incident with unusually warm bottom water temperatures and has resulted in changes in metapopulation distribution that have major implications for the clam fishery within the region. In an effort to clarify the processes that have and will further change surf clam population distribution, a model was developed that includes a coupled larval-hydrodynamic model and an individual-based post-settlement population dynamics model. The post-settlement population dynamics model includes phenotypic variability in metabolic energetics such that simulated populations containing multiple cohorts include a range of individual adaptation to environmental conditions. Preliminary simulations of surf clam populations have replicated previously observed mortality events. High temperatures result in decreased ingestion leading to stunted growth, reproductive failure and eventual starvation and death. The model will be used to examine changes in population range and demographics due to altered circulation and thermal regimes that may arise from future climate forcing. These results will feed into socio-economic analyses intended to provide guidance to fishery managers and facilitate a proactive approach to potential changes in the surf clam fishery that may result from climate change.

A PRELIMINARY STUDY INVESTIGATING THE INFLUENCE OF ADULT *CRASSOSTREA VIRGINICA* ON LARVAL SETTLEMENT.

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Agreat deal effort has been devoted to restoring oyster populations in much of its natural range, and understanding the mechanisms that influence oyster larval settlement may improve spat collection success in restoration programs. Observations on Martha's Vineyard showingincreased recruitment on spat collectors adjacent to adult oysters, suggest an improvement to oyster spat collection for enhancement programs. A preliminary study was designed to investigate the hypothesis that augmented oyster larvae settlement occurs in close proximity to adult oysters. Thirty

shell bag spat collectors were hung from floating cages and seventy adult oysters were placed in one cage, while the other was left empty. They were deployed in Edgartown Great Pond on Martha's Vineyard, MA in June of 2010. After approximately 6 weeks the cages were retrieved and oyster spat were counted and recorded. There was a significant difference among treatments; a greater number of spat recruited to shells associated with the adult oysters than to those on the empty cage. Further development of analogous spat collection techniques should be pursued to include spatial and density factors as well as interannual variability. Currently, we continue to encourage the use of this method of spat collection to local shellfish resource managers.

POPULATION TRENDS, LIFE-HABIT TRAITS, AND ECOLOGICAL IMPACTS OF THE QUAGGA MUSSEL IN THE GREAT LAKES REGION.

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Although the quagga mussel became established in the Great Lakes at about the same time as the zebra mussel, it spread through the lakes at a slower rate. Once established, however, it attained densities that exceeded zebra mussels in many nearshore regions, and colonized deep, offshore regions where zebra mussels were never found. As a result, ecological impacts of the quagga mussel are more extensive and far-reaching compared to those of the zebra mussel, particularly in Lakes Michigan, Huron, and Ontario. Long term monitoring activities in Lake Michigan have documented recent food web changes corresponding to the expansion of quagga mussels at depths >50 m. Mussel density and biomass at these deeper depths began to increase in 2004 and have increased steadily since. Laboratory experiments have shown that quagga mussels can readily filter-feed even at the continually cold temperatures found at these depths. Observed lower food-web changes have been the loss of the spring diatom bloom, large declines in phytoplankton productivity, a decline in populations of the opossum shrimp *Mysis*, and the continued disappearance of the amphipod *Diporeia*. The latter two species are glacial relicts heavily utilized by fish populations in offshore waters.

RELATIONSHIPS BETWEEN SMALL-SCALE SPATIAL DISTRIBUTIONS OF NEW SETTLERS, ASARI CLAM *RUDITAPES PHILIPPINARUM*, AND PHYSICAL/TOPOLOGICAL ENVIRONMENTS ON THE MATSUNASE TIDAL FLAT, CENTRAL JAPAN.

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Much data on spatial distributions as related to *Ruditapes philippinarum* adults (Asari clam) on tidal flats have accumulated to date, but few studies dealt with spatial distributions of the new settlers in relation to physical/topographic conditions on the flat. In order to identify factors by which characteristics of spatial distributions of the new settlers on the flat may be generated and maintained, we examined small-scaled spatial distributions of the new settlers on the Matsunase tidal flat, central Japan, during the ebb of spring tide for successive two days, simultaneously to monitor shear stresses due to the wave-current flows on the flat. Using generalized linear models (GLM), physical/topographic characteristics responsible for affecting the new settler density were identified so that "crest" sites always had a negative influence on the density for both sampling days. Based on the above shear stress at a "crest" site on the flat, it was deduced for the new settlers, as well as sediment particles, to be easily displaced at a "crest" site on the flat. Small-scale spatial distributions of Asari new settlers on the flat may alter with the advanced benthic stages depending mainly on the wave shear stress.

EFFECTS OF LARVAL DISPERSION ON THE MOVEMENT OF DISEASE RESISTANT GENES BETWEEN OYSTER POPULATIONS.

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In oyster populations, the exchange of individuals, and therefore genetic material, occurs during the larval phase where biophysical processes determine the fate of the larva. A hydrodynamic model developed for Delaware Bay coupled with a larval model is used to represent these processes and estimate exchange rates between low and high disease-resistant populations. This information is used in an individual-based genetics model in which each individual oyster has 10 chromosome pairs with 4 genes per chromosome. An initial population with a random geneticstructure was established and a varying number of individuals (estimated from the larval-hydrodynamics model) with a neutral marker genewere added to the population. Simulations are run

for 100 generations and the frequency of the marker allele determined. For low resistant populations that receive immigrants from highresistant populations, the disease-resistant gene will become common in 4 to 12 years and dominant in about 10 to 30 years. Depending on the exchange rates and the size of the populations, the gene transfer might take more than 100 years. For immigrants going from low to high resistant populations, the gene transfer occurs between 20 and 40 years. These simulations are illuminating for fisheries management decisions and oyster restoration efforts.

THE NATIONAL FISH HABITAT ACTION PLAN (NFHAP): SCIENCE SUPPORTING CONSERVATION FROM SUMMIT TO SEA

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The National Fish Habitat Action Plan (NFHAP) was launched in 2006 with the mission to protect, restore, and enhance the nation's fish and aquatic communities – freshwater, estuarine, and marine—through partnerships that foster fish habitat conservation. NFHAP recently completed a national-scale assessment of fish habitat quality based on existing information to assist conservation planning and ultimately help track the progress of the plan's habitat conservation and restoration efforts. The inland component of the assessment applies a hierarchical hydrologic framework to incorporate landscape-level disturbance data and provide results across regions from small river reaches to large watersheds. The coastal component of the assessment uses a modified version of NOAA's Coastal Assessment Framework to organize environmental indicator data sets pertaining to fish habitat for estuaries, coastal watersheds, and nearshore marine waters. Indicator parameters include pollution sources, water quality and eutrophication, changes in land use in coastal watersheds, and alteration of tributary river flows. Future work will incorporate information on the status of fish populations and specific biotic habitat types such as tidal wetlands, submerged aquatic vegetation, shellfish beds, and corals. NFHAP partnerships which feature coastal habitat restoration projects include the Atlantic Coastal Fish Habitat Partnership (ACFHP), and the Southeast Aquatic Resources Partnership (SARP). SARP has oyster reef restoration projects in progress in coastal North Carolina, South Carolina, Georgia, and Florida. These restoration projects involve local communities through direct participation (volunteers), education, and monitoring.

ARE THE NEUROTOXIC EFFECTS OF MANGANESE DUE TO BLOCKAGE OF POST SYNAPTIC DOPAMINE RECEPTORS.

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Manganese causes Manganism by disrupting dopaminergic neurotransmission, but the mechanism is unclear. p-Aminosalicylic acid (PAS) is reported an effective treatment but its mechanism is unclear. Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic and dopaminergic nerves. Dopamine produces cilio-inhibition, serotonin cilio-excitation. We showed manganese blocks cilio-inhibition of dopamine and PAS prevents that. We studied if manganese blocks dopamine post-synaptic receptor binding and if PAS prevents this. We observed membrane potentials of lateral ciliated cells using a voltage sensitive dye while measuring beating rates. Applying serotonin or 5 Hz electrical stimulation (ES) to the branchial nerve caused prolonged membrane depolarization and increased beating rates. Applying dopamine or 20 Hz ES after exciting cilia repolarized the cells and decreased beating rates. Manganese prevented the cilio-inhibition and repolarization. PAS prevented manganese from doing that. Adding ATP to gill increased beating rates without changing membrane potential. Applying MDL, an adenylcyclase inhibitor, after manganese decreased beating rates without affecting membrane potential. The study shows a correlation between membrane potential and beating rates. It helps elucidate neurotoxic mechanisms of action of manganese by showing the site of action is after post-synaptic dopamine receptors. This is helpful to understand causes and treatments of Manganism.

A COMPREHENSIVE HABITAT MAPPING APPROACH FOR SITING OF SUBTIDAL AQUACULTURE DEVELOPMENT AREAS.

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Northeast USA shellfish farmers face a multi-tiered regulatory review and permitting process for use of subtidal lands and the need to select suitable sites within ecologically diverse coastal habitats. This project provides technical support to two Cape Cod towns (Massachusetts, USA) in order to identify areas suitable for

community aquaculture development areas (ADAs) large enough to contain multiple grow-out sites for use by individual farmers. Mapping efforts incorporate publicly available habitat type data and management boundaries, as well as local knowledge of human use and shellfish abundance. Existing data are complemented by a habitat classification approach using acoustic backscatter and bathymetric data collected with an interferometric sonar system. Combined with sustainable aquaculture site selection criteria and the biological requirements of potential species to be grown, these data are used to identify suitable sites for subtidal ADAs in town waters. Potential ADAs are surveyed to ground-truth the mapping approach and assess existing shellfish abundance. Diver transects are conducted to identify bottom type, sediment characteristics, and vegetation. Benthic quadrat sampling is used to estimate shellfish density (organisms/m^2). These data are incorporated into the site selection process, which includes ongoing discussion with stakeholders regarding implications for ADA permitting and use.

IN SITU MONITORING OF LONGFIN INSHORE SQUID EMBRYONIC DEVELOPMENT.

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Embryonic development within longfin inshore squid (*Loligo pealeii*) egg masses was monitored at a commercial fish weir in northeastern Nantucket Sound (Massachusetts, USA) in June-July 2009. Two freshly discovered egg masses were collected and placed in a mesh enclosure on the bottom immediately adjacent to the weir to facilitate semiweekly sampling. Seawater temperature was recorded using a data logger affixed to the weir. Minimum development times for embryos within the two egg masses were 22 days (collected on 02 June) and 15–17 days (collected on 30 June). Daily mean seawater temperature increased from a minimum of 15.6° C on 01 June to a maximum of 21.6° C on 14 July. Mean temperatures during development of the embryos in the first and second collections were 16.6° and 19.6° C respectively. Embryos developed ~1.5 times faster at warmer temperatures, consistent with laboratory studies and *in situ* observations of the embryonic development of other loliginid squids. Understanding the relationships between temperature and development time may be useful for predicting hatch timing and mitigating impact of fishing and other activities on squid eggs.

ANTIOXIDANT STATUS OF *CRASSOSTREA VIRGINICA* UNDER MODERATE HYPOXIC CONDITIONS.

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A variety of environmental conditions in estuaries may affect the antioxidant status of organisms. When the production of free radicals (e.g. OH^-, H_20_2) exceeds the antioxidant potential, cellular and tissue damage may occur. Therefore, environmental stressors such as hypoxia that reduce oysters' antioxidant levels may lead to an increase in oxidative stress and increased susceptibility to other stressors. The purpose of these studies was to evaluate how hypoxia affects the levels of important antioxidants (glutathione, catalase, superoxide dismutase), and oxidative stress (lipid peroxidation) in oysters, *Crassostrea virginica*. Oysters were exposed to hypoxia for 4 and 8 days with two methods used to induce hypoxic conditions: nitrogen gas; and hypercapnia, which simulates the excess CO_2 accumulation that accompanies hypoxia in the environment. An important difference between these two regimes is the concomitant drop in pH during hypercapnia-hypoxia that does not occur with nitrogen. The results indicate that antioxidant levels were lower and lipid peroxidation levels were higher in oyster tissues (gill and hepatopancreas) exposed to hypoxic conditions. These effects were more pronounced in the hypercapnic regimes. These results support the hypothesis that oysters exposed to hypoxia, particularly when accompanied by hypercapnia, have reduced antioxidant capacity and are more susceptible to damage.

SYMBIOTIC AND FREE-LIVING CILIATES ASSOCIATED WITH *CALLINECTES SAPIDUS* AND OTHER DECAPODS.

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Ciliophorans play important harmful and helpful roles in aquaculture and natural habitats of *Callinectes sapidus*. Ciliates examined from the crab and water in Mississippi were compared with those found in the representatives *Farfantepenaeus aztectus* from Mississippi, *Litopenaeus setiferus* from South Carolina, and *Pandalus borealis* from Maine. Cultured and examined ciliates produced a total of 27 species in 6 of the 10 major orders. The crab hosted 7 of the 12 symbiotic species and 12 of the 15 free-living ones. Of the 3 shared symbionts, 2 apostomes are problematic in the decapods and 1 of the predatory suctorians indicates a healthy microbial community. Twelve of the 15 free-living species occurred in water or sediment associated with the crab, and most occurred with 1 or more of the other decapods, including *P. borealis*. They, like some of the symbionts, included suspension feeders and species that feed on relatively large animals and algae, on small organisms and detritus, and on bacteria. At least 2 can enter wounds and produce pathological alternations and death in the crab or shrimp hosts, and others are indicators of excess bacteria and organic

material in the systems. Funding from NOAA Subaward NA17FU2841 and NSF Grant No. 0529684.

DELAWARE BAY STOCK ASSESSMENT OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) IN DELAWARE BEDS.

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In the Mid-Atlantic States, there is a long history of using oyster dredges as a sampling tool for stock assessments. On both sides of the Delaware Bay, management officials have used oyster dredges in their annual surveys. We used similar survey methods to the dredge calibration to assess Delaware natural oyster grounds during 2007 and 2008 field seasons. After the state agents sorted and processed the samples for their annual survey, samples were returned to DSU and sorted into a number of categories depending on its makeup. Our objective was to determine abundance of live oysters in individual size classes, spat (smaller than 20 mm), submarket size oysters (between 20 mm–74.93 mm) and market size oysters (greater than 74.93 mm), and oyster recruitment on these beds. Both number of boxes and live oysters collected per square meter was analyzed for each of the three size classes at each bed. The results of these two surveys show that DSU survey underestimated total number of boxes and small oysters while the survey by the state agency underestimated the abundance of spat and market sized oysters. The findings presented here should allow for more successful restoration and protection of the natural oyster stocks in the Delaware Bay.

SPECIFICITY OF HOST-PATHOGEN INTERACTIONSIN MARINE ENVIRONMENT: COMPARISON OF MOLLUSC VIBRIOSIS

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The development of vibriosis in shellfish depends on the ability of vibrios to colonize epithelia, and (or) external secretions, to penetrate and multiply inside the tissues, and at each step to neutralize host defenses. The nature and specificity of these interactions determine the pathogenesis of the disease.

Our research aims at characterizing the specific molecular mechanisms governing the shellfish-vibrios interactions in the marine environment by comparing three models: *Vibrio tapetis-Ruditapes philippinarum*, *V. aesturianus-Crassostrea gigas* and *V. harveyi-Haliotis tuberculata*.

The complete genomes of the three pathogenic vibrios have been recently sequenced, allowing the identification of genomic islands and some specific virulence factors. Their modes of actions have been compared by cellular and molecular approaches, underscoring totally different mechanisms of evading the host immune systems.

Faced with the vibrio infection, the host triggers immune responses specifically adapted. Their knowledge would allow improving the prevention and helping selection of resistant mollusc.

SPREAD, POPULATION DYNAMICS, AND ECOSYSTEM IMPACTS OF ZEBRA MUSSELS VERSUS QUAGGA MUSSELS: WHAT WE KNOW AND WHAT WE DO NOT.

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The zebra mussel, *Dreissena polymorpha*, and the quagga mussel, *Dreissena rostriformis bugensis*, are both important invaders in freshwaters of the Northern Hemisphere. These two invaders have similar life habits and similar life history characteristics, and alter aquatic communities and habitats they invade. While *D. polymorpha* is among the best studied freshwater invertebrates worldwide, comparable information on the biology, population dynamics and impacts of *D. r. bugensis* are less well known, limiting our ability to predict the spread and ecological impacts of this important freshwater invader. Here we contrast what is known about these two invaders and highlight information that is needed, especially for the quagga mussel if we are to accurately predict the future spread of this species as well is its population dynamics and likely impacts on habitats and communities it invades.

PREDATION RATES ON *MERCENARIA MERCENARIA* BY CHANNELED AND KNOBBED WHELK.

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Predation by two species of whelk, *Busycotypus canaliculatus* and *Busycon carica*, is a major source of mortality for adult clams in Great South Bay, Long Island, and may threaten the success of restoration efforts for *Mercenaria mercenaria*. We conducted experiments to determine the vulnerability of *Mercenaria mercenaria* to predation by these two species with in-field mesocosms.

We contrasted predation on large (chowder) and medium (cherry stone) clams, and medium versus small (littleneck) clams. We also tested whether the accumulation of shell (common when clam densities were high) affected predation risk. Overall we found that whelk predation rates were lower than predicted from laboratory-based studies, and channeled whelk ate more clams than knobbed whelks. The presence of shell in the sediment did not have an effect on consumption rates. Both the channeled and the knobbed whelks consumed similar sized clams, and tended to consume smaller clams, but not the smallest size clams available. In some cases medium sized clams were eaten, but the large clams were immune from predation. In all cases, within a treatment, whelk did not appear to be size-selective. Rather, their feeding success was greater when offered smaller sized clams.

THE ROLE OF ALTERNATE HOSTS IN THE ECOLOGY AND LIFE HISTORY OF *HEMATODINIUM* SP., A PARASITIC DINOFLAGELLATE OF THE BLUE CRAB (*CALLINECTES SAPIDUS*).

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Hematodinium infections occur in populations of *Callinectes sapidus* in the western Atlantic Ocean. We examined several species of crustaceans to determine if the same species of *Hematodinium* that infects *C. sapidus* is found in other hosts. A total of 1829 crustaceans were collected from the Delmarva Peninsula, Virginia, and examined for infections over a two-year period. A portion of the first internal transcribed spacer (ITS1) region of the ribosomal RNA (rRNA) gene complex from *Hematodinium* was amplified and sequences were compared among 35 individual crustaceans putatively infected with the parasite by microscopic examination and 4 individuals putatively infected after PCR analysis. Of the 18 crustacean species examined, five were infected with *Hematodinium* after microscopic examination and PCR analysis, including three new hosts, and an additional species was positive via PCR analysis. The similarity among the ITS1 sequences and similarities in the histopathology of infected hosts is evidence that the same *Hematodinium* sp. found in *C. sapidus* infects other crustaceans along the Delmarva Peninsula. Our data indicate that this *Hematodinium* sp. is a host generalist, capable of infecting hosts in different families within the Order Decapoda, with some evidence suggesting it may be capable of infecting crustaceans within the Order Amphipoda.

STRAIN VARIATION IN *HEMATODINIUM* SP., A PARASITIC DINOFLAGELLATE FROM THE BLUE CRAB *CALLINECTES SAPIDUS*, ALONG THE DELMARVA PENINSULA, VA.

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We designed 12 microsatellite markers (8 polymorphic and 4 monomorphic) from an enriched microsatellite library constructed from a non-clonal culture of *Hematodinium* obtained from an infected blue crab (*Callinectes sapidus*). With these markers, several strains of *Hematodinium* were genotyped from blue crabs from six sites along the Delmarva Peninsula. Data suggest that the parasite cells in the host are primarily haploid. When allele frequencies were compared among sites, some alleles were seen at all sites, indicating a high degree of mixing between sites. We also found evidence for simultaneous infections with multiple genetic strains in blue crabs in this system, with evidence of up to seven different strains infecting the same blue crab host. After excluding multiple infections, multi-locus genotypes (MLG) were compiled and 104 different MLGs were found, of which 81 (78%) were unique. This high level of genotypic variation is comparable to that reported in free-living dinoflagellates. In addition, this level of variation suggests that a sexually reproducing stage may be present in the life cycle of this parasite. Ongoing work is comparing these results to microsatellite data obtained from alternate hosts from the same locations and blue crab hosts from other geographic areas.

DEVELOPMENT AND VALIDATION OF PREDICTIVE MODEL FOR THE GROWTH AND SURVIVAL OF *VIBRIO VULNIFICUS* IN POST-HARVEST SHELLSTOCK OYSTERS FROM TWO ESTUARIES AND TWO OYSTER SPECIES.

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Post-harvest growth of *Vibrio vulnificus (Vv)* in oyster increases risk of human infection but knowledge is limited about viability patterns in oysters over a wide range of storage temperature, in oysters harvested from different estuaries and in different oyster

species. In this study, we developed a predictive model for *Vv* viability in American oysters (AMO) (*Crassostrea virginica*) harvested from Chesapeake Bay (CB), Maryland over a temperature range of 5–30°C, and then validated the model against *Vv* viability rates in AMO and Asian oysters (ASO) (*C. ariakensis*) harvested from Mobile Bay (MB), Alabama and CB. *Vv* was slowly inactivated at 5 and 10°C at −0.0043 and −0.0042 log MPN/h. The estimated growth rates (GR) at 15, 20, 25 and 30°C were 0.022, 0.042, 0.087 and 0.125 log MPN/h. GR estimates for CB and MB oysters stored at 25 and 30°C were lower than FAO/WHO *Vv* Quantitative Risk Assessment model predictions. The *Vv* growth model developed in CB oysters appears to be applicable to AMO and to the MB harvest region. The lower GR estimates in this study than in previous risk assessments are cause to re-examine other model assumptions such as time to refrigeration and cool down times.

OYSTER RESTORATION IN THE MARYLAND PORTION OF THE CHESAPEAKE BAY: MEASURES OF SUCCESS AND FAILURE.

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The goals of ecological restoration are necessarily subjective. The Society for Ecological Restoration defines ecological restoration as returning an ecosystem to its former **trajectory**, recognizing that all ecosystems exist in a state of flux and that the ultimate end product of ecological restoration is a historical trajectory that requires no more external intervention. However, many restoration efforts may never become completely self-sustaining and may require management activities to maintain the target trajectory. Oyster restoration in Maryland is faced with such a challenge. Currently, our poor understanding of broodstock abundance and location as well as hydrodynamics of the Bay prevents informed actions that might lead to a self-sustaining population. However, many studies continue to contribute to a growing knowledge base of the ecology within and around restored oyster reefs including oyster sex ratios, fertilization rates, benthic and nektonic abundance and diversity, and benthic biogeochemistry.These studies may offer a greater understanding of the ecological trajectories of oyster reefs. Ecologically productive reefs created by the hatchery-based oyster restoration program in Maryland may resemble their historic trajectories in many ways. Thus, restored Maryland oyster reefs may represent a measure of success without immediate or short-term sustainability of any given population.

IMPROVING CONSERVATION OF NEW ENGLAND WHELKS *BUSYCOTYPUS CANALICULATUS*: SIZE AT MATURITY.

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The New England whelk fishery has expanded rapidly from a small amount of bycatch to a directed fishery. As lobster fisheries have declined in Southern New England, much of that effort has been redirected to channeled whelks (*B. canaliculatus*), increasing the utilization of horseshoe crabs (for bait) which are already fully exploited in New England. Whelks are slow growing, mature late, and have low fecundity, characteristics which make them extremely susceptible to overfishing. Virtually nothing is known about growth, age, or sexual maturity of whelks in New England, so current management is based solely on size, season, and effort restrictions. In August 2010, we collected, marked, and released 4050 whelks in Buzzards Bay, MA. We hope that some of these will be recaptured and returned to us by fishermen, in order to obtain information on growth. We also dissected 224 specimens to determine sexual maturity. Although histological examination has not yet been completed, males seemed to dominate the 110-150 mm (total length) range while females dominated above 160 mm. This suggests that males may have a lower maximum size and/or slower growth than females. Any proposed adjustments to size limits will need to consider this information and its impact on the population reproductive potential.

DEVELOPMENT OF NEW FISHERIES IN THE NORTHERN GULF OF MEXICO USING HATCHERY-REARED BLUE CRABS

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The Gulf Coast Research Laboratory in Ocean Springs, MS has successfully operated a hatchery for blue crabs (*Callinectes sapidus*) for over five years. The ability to reliably produce ''seed'' crabs has great potential for expanded development of soft crab fisheries in the Gulf of Mexico and for the development of a bait crab for use by recreational anglers. Hatchery-reared blue crabs are stocked as juveniles into non-vegetated one-quarter acre ponds for soft crabs and into one-quarter acre vegetated ponds for grow-out to bait crabs. Crabs are fed a diet of manufactured feed and scrap fish. Pre-molt crabs (40–50 mm carapace width) are harvested from non-vegetated ponds using bushlines constructed of wax myrtle. Bushlines are placed across the pond in close proximity to provide shelter for pre-molt and molting crabs. Peeler crabs collected in the bushlines are harvested daily and maintained in recirculating shedding systems until they molt. Harvesting techniques for bait crabs include use of minnow traps, seines, and dipnets. Continuing market demand, profitability, and regional familiarity with pond

cultured products all suggest that blue crab aquaculture for softshell and bait production would be an economically viable enterprise. Use of hatchery-reared blue crabs would allow for fishery development independent of wild stocks.

"OILY" DROPLETS ASSOCIATED WITH BLUE CRAB MEGALOPAE COLLECTED IN THE NORTHERN GULF OF MEXICO

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The blue crab, *Callinectes sapidus*, has planktotrophic larval development with seven zoeal molts and the metamorphic molt to the megalopal (postlarval) stage occurring in offshore waters. The *Deepwater Horizon* oil spill coincided with the spawning of blue crabs in the northern Gulf of Mexico and movement of megalopae from offshore waters to inshore estuarine environments. Blue crab megalopae, along with early life history stages of other invertebrates, were sampled on passive collectors positioned along the coastline. Megalopae collected in May in Louisiana waters contained unusual "oily-appearing" droplets wedged between the exoskeleton of the cephalothorax and the inner integument. These droplets were not observed in blue crab megalopae during eleven years of extensive monitoring of blue crab early life history stages by personnel of the Gulf Coast Research Laboratory. Additional collections of blue crab megalopae from Florida to Louisiana and larvae/postlarvae of other brachyuran crabs, polychaetes, and barnacles also contained similar droplets. Analyses are being conducted to determine the precise composition and origin of the droplets using an open-beam mass spectrometer (JEOL AccuTOF™ DART® Direct Analysis in Real Time with Time-of-Flight Mass Spectrometer). Results of these analyses will be discussed.

OYSTER RESPONSES TO THE *DEEPWATER HORIZON* OIL SPILL ACROSS COASTAL LOUISIANA: EXAMINING OYSTER HEALTH AND HYDROCARBON BIO-ACCUMULATION.

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The eastern oyster (*Crassostrea virginica*) is a major component of the Gulf Coast seafood industry, especially in Louisiana, and is also a vital constituent of estuarine environments. Polynuclear aromatic hydrocarbons (PAHs) present in oil pose a risk to filter-feeding bivalves such as oysters, which can effectively take up and accumulate these toxic substances. PAHs can have a wide range of deleterious effects on oysters from sub-lethal effects that include reductions in growth, impaired immunocompetence, increased susceptibility to dermo disease caused by the oyster pathogen *Perkinsus marinus*, decreased reproductive capability, to death. To assess the consequences of the *Deepwater Horizon* oil spill on oyster populations in Louisiana, the overall health and PAH concentrations of caged and wild oysters sampled pre-spill and at 3 and 9 months after oil exposure are being assessed. Specifically, using a number of biomarkers we are conducting a spatial and seasonal investigation of the oysters' health, nutritional, reproductive and immunological status in relation to their body total hydrocarbon and PAHs concentrations in oiled and non-oiled sites. This work is funded by the Louisiana Sea Grant College Program.

MATRIX METALLOPROTEINASES AND HEMOCYTE MIGRATION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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The mechanisms governing hemocyte migration in invertebrate defense are poorly understood. In mammals, migration is mediated by a family of zinc-dependent proteases called matrix metalloproteinases (MMPs). These proteases break down components of the extracellular matrix allowing cells to migrate through tissues. One MMP, Cv1MMP, has been identified to be upregulated in hemocytes of the Eastern oyster and is localized at the leading edge of migration in cells migrating through digestive tissues during feeding. These findings suggest that Cv1MMP may play a role in hemocyte migration. The effect of the metalloproteinase inhibitor GM6001 on hemocyte migration in response to immune stimuli like zymosan A from *Saccharomyces cerevisiae* and heat-killed and live *Vibrio tubiashii* isolate RE22 was tested using a 96-well chemotaxis chamber. Data showed there were a differential response in the migration of hyalinocytes and granulocytes to zymosan A and RE22, with hyalinocytes preferentially migrating in response to zymosan A and granulocytes preferentially responding to bacterial products. The migration of hemocytes pre-treated with GM6001 was reduced compared to controls. These results suggest that matrix metalloproteinases are involved in hemocyte migration in oysters. This research provides valuable insight into oyster immunity that could aid in developing strategies to manage disease.

GENOME-WIDE ANALYSIS OF INBREEDING DEPRESSION IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*, USING A QTL MAPPING APPROACH.

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Discovery of a large number of deleterious recessive mutations (genetic load) in the Pacific oyster explains distorted segregation ratios, seen widely in bivalves, and supports the dominance theory

of heterosis and inbreeding depression. Up to this point however, analysis of molecular data on segregation data in F_2 families has been constrained by single or two-locus models which lack whole genome information. We report advances in our analyses of these data using a 'viability- selection model' that applies quantitative trait locus (QTL) mapping techniques to the identification of deleterious mutations (viability loci) across the genome. With this approach we can better address a number of complicated questions in inbred F_2 families such as the effect of environment (algal diet) and the developmental timing of viability loci. We find a large number of viability loci (14–15), half of which are expressed during metamorphosis. We find that selection increases at viability loci in more harsh (nutrient poor) diets, but also that dominance is increased, exposing more of the population to selection than expected from recessive mutations alone. Outbred families also exhibit substantial viability selection.Genetic load may be responsible for much of the high early mortality (Type-III survivorship) in Pacific oysters.

GENOTYPE BY ENVIRONMENT INTERACTION AFFECTS GENETIC LOAD IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*.

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Inbreeding depression is a major concern in the management and conservation of threatened shellfish populations. Inbreeding generally reduces fitness, but its magnitude and effects are highly variable because they depend on the genetic constitution of a population and on interaction with the environment. In general, harsher environments significantly increase inbreeding depression. However, most studies have examined the interaction of environment and inbreeding depression at the population level; less is understood about underlying genetic mechanisms. Using QTL methods, we performed a genome scan for deleterious (viability) loci in two inbred F_2 families of the Pacific oyster, *Crassostrea gigas*, reared in two environments; nutrient-poor (single-algal) and nutrient-rich (mixed-algal) diets. We found 14 viability loci, causing 98 % mortality up to the juvenile stage. There were four types of environmental interaction with viability loci: 1) selection against the affected allele increased in the harsher environment, 2) dominance increased exposing heterozygotes to more selection (harsher environment) 3) viability loci were lethal in both environments (no interaction), and 4) viability loci were only present in the harsher environment. These findings suggest that individual viability loci respond differently to the environment and have implications for small or inbred populations coping with climatic shifts and habitat destruction.

CAN OYSTERS DEVELOP RESISTANCE TO DERMO DISEASE: EVALUATION USING A GENE-BASED POPULATION DYNAMICS MODEL.

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Oysters are commonly limited by disease mortality. Resistance to MSX disease has developed in some cases, but little development of resistance to Dermo disease mortality has occurred despite frequent epizootics. We utilize a gene-based population dynamics model to simulate the development of disease resistance in *Crassostrea virginica* using mortality as the agent of selection. Simulated populations were exposed to four levels of mortality covering the range in mortality observed in Delaware Bay in the 1990s. Dermo resistance increased in each over time proportional to the increase in mortality rate imposed by the disease. However, the simulations show that the population responds on multi-decadal to half-century time scales. As the mortality rate declines with increasing disease resistance, the rate of further improvement in disease resistance likewise declines. Thus, a mortality rate of 25% per year, yielding a rate of selection profoundly slow, still produces significant decrements in oyster abundance in Mid-Atlantic estuaries. Evidence from fisheries retrospectives suggests that oysters cannot withstand a constant removal at this scale without compromising population integrity noticeably. So, a mortality rate that grievously limits the development of disease resistance still sorely strains the species' ability to maintain a vibrant population necessary to its long-term survival.

BAD YEAR(S): RECENT AND CONTINUED CHALLENGES TO OYSTER REEF RESTORATION IN THE NORTHCENTRAL GULF OF MEXICO

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To put it mildly 2010 was a bad year for oysters and oyster fishermen in the northern Gulf of Mexico: the largest oil spill in U.S. history, the largest fisheries closure in U.S. history, unprecedented freshwater diversions in Louisiana and a resumption of drought conditions in Alabama, Mississippi, and the panhandle of Florida. Unfortunately, 2010 is not the only recent bad year for oyster reefs and the fishery they support, several years of drought conditions have resulted in low survival (presumably as a result of oyster disease or drills) in many areas. The socioeconomic and ecological systems of the region are still recovering from the 2005 and 2006 hurricanes seasons, which combined to produce the costliest storms in U.S.

history. We review the past and current natural and anthropogenic impacts to oyster reef resources and discuss the many challenges that must be addressed in restoring oyster reefs and revitalizing the oyster fishery. Two of the largest issues facing oyster reefs remain regional (1) climatic conditions, which influence salinity regimes and may be influenced by larger climatic oscillations and global climate change, and (2) large scale landscape changes brought about by wetland restoration (i.e. freshwater and sediment diversions), hurricane activity, and continued development of coastal areas.

BENTHIC COMMUNITY STRUCTURE AND RESPONSE TO HARVEST EVENTS AT GEODUCK (*PANOPEA GENEROSA*) AQUACULTURE SITES IN SOUTHERN PUGET SOUND, WASHINGTON.

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Geoduck (*Panopea generosa)* aquaculture has become a lucrative and widespread practice on intertidal beaches in South Puget Sound, WA. The techniques used to plant, grow, and harvest these clams have come under scrutiny by various public and private agencies and individuals.

In June of 2008 we began a long term investigation to assess the effects of the harvest process at three geoduck aquaculture sites using changes in benthic invertebrate assemblages to evaluate disturbance. At each site a treatment plot of mature planted geoduck was paired with an adjacent reference plot of equal size. For several months prior to and for six months after harvest we collected benthic cores screened though 0.5 mm square mesh.

Each site presents a slightly different benthic community structure and therefore may respond to harvest practices differently. Data analysis completed to date indicate that variance in infaunal data is attributable to time of year, plot status (cultured versus uncultured), and harvest timeline (pre- versus post-harvest). As of yet we have seen little evidence to indicate that activities associated with geoduck aquaculture cause significant long term damage or disruption to benthic ecosystems on the intertidal sand flats of southern Puget Sound.

PHARMACOLOGICAL STUDY OF THE EFFECTS OF OCTOPAMINE ON HEART RATE OF *CRASSOSTREA VIGINICA*.

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Octopamine, a biogenic amine first identified in the octopus, has been well studied in arthropods and a few gastropods, serving as a neurotransmitter and hormone. Its presence and possible functions have rarely been reported in bivalves. We identified octopamine in cerebral ganglia, visceral ganglia, gill, palps and hemolymph of *Crassostrea virginica*. We also found octopamine was a cardio-acceleratory agent and postulated that it may have a neuroendocrine role in *C. virginica*. The present pharmacological study examined octopamine agonist and antagonists to determine if octopamine is activating octopamine receptors in the oyster heart *C. virginica* heart preparations were prepared *in situ*. Mechanical and EKG rates were monitored. Basal heart rates averaged 4.2 beats/min. Superfusion of octopamine (10^{-6}–10^{-2} M) more than doubled heart rates in a dose dependent manner. The octopamine agonists, synephrine and phenylephrine were slightly more potent that octopamine in increasing heart rates. The antagonists, phentolamine, metaclopramide and yohimbine blocked the cardio-acceleratory effects of octopamine; however, they did not block the cardio-acceleratory effects of dopamine. The study provides further evidence that octopamine, which is present in the nervous system, innervated organs and hemolymph of *C. virginica*, may have a neuroendocrine role as a cardio-regulatory hormone.

EXPLOITATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS* BY ILLUMINA TRANSCRIPTOME SEQUENCING.

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Here we reported the work on SNPs discovery based on population transcriptome data. Total mRNAs from twenty wild Pacific oysters collected from Qingdao, China were mixed in equal amount and submitted to transcriptome sequencing according to Illumina's recommended protocol. A total of 49,344,041 reads (accounting for 83% of all reads) with length of 90 nt were aligned to the Pacific oyster genome (Oys_Scaffold_V4.1 from OGP) by TopHat software. There are 4,174,329,225 (78.0%) bases mapped, and at the depth of more than 5 the length of all mapped regions is 53,957,669 nt. The length of mapped exon regions is 23,118,168 nt, which is 59.5% of the length of de novo predicted exon regions in the genome. One mutation site where the consensus quality and SNP quality are both more than 20 (Phred-scaled likelihood, corresponding to an error rate of 0.01) and the depth of minor allele was more than 5 was identified as one high reliable SNP locus. Totally 878,022 SNPs were discovered, including 199,817 of homozygote and 678,205 of heterozygote. There are 532,078 transition sites (including 29.3% of C/T and 29.1% of A/G) and 345,944 transversion sites (including 9.7% of A/C, 9.5% of G/T, 13.1% of A/T and 5.7% of C/G).

EVALUATING THE EFFICACY OF A NATURALLY DERIVED INVASIVE MUSSEL CONTROL TECHNOLOGY, ZEQUANOX™.

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In 2008, Marrone Bio Innovations and New York State Museum entered into an agreement to turn the effective invasive mussel control discovery, *Pseudomonas fluorescences* CL145A (*Pf* CL145A), into a commercially viable product. Dr. Dan Molloy of New York State Museum discovered this specific strain of *Pseudomonas fluorescences* and demonstrated the products effectiveness in killing adult invasive quagga and zebra mussels in laboratory and pilot studies. Since 2008, MBI has been conducting extensive research to develop this discovery into a commercial product, Zequanox™. Because the effectiveness of *Pf* CL145 is highly selective to dreissenid species and does not significantly impact other tested non-target organisms, the product has great potential for use under a wide range of applications. In addition to bringing a more environmentally friendly invasive mussel control product into the market for use by industrial facilities, MBI is conducting evaluations to develop management strategies for environmental and recreation restoration. Zequanox shows potential for being used by fish hatcheries to prevent the spread of the planktonic life stage, veligers, during fish stocking.

This presentation will provide an overview and current status of commercialization of Zequanox. The focus will be on describing demonstration trials for different applications and available results.

A COMPARISON OF GROWTH AND OVER-WINTER MORTALITY OF HATCHERY-REARED *CRASSOSTREA VIRGINICA* SPAT SET IN TANKS AND IN A FIELD ENCLOSURE.

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In areas with limited natural spat set, restoration sites have been seeded with spat-on-shell, made by setting hatchery-reared eyed larvae onto shells in tanks. However, shell transport proves both expensive and logistically challenging, suggesting the need for an additional restoration tool that requires less materials handling. Previous work in Blackwalnut Creek (Annapolis, Maryland USA) has suggested the feasibility of setting larvae *in situ* by surrounding a newly-constructed oyster reef with a temporary flexible boom to prevent larval loss from the target site. Preliminary results from three replicate trials in August 2010 showed comparable spat-on-shell densities among tank and boom treatments when data were pooled among experimental trials. To follow up this work, spat from the trials of *in-situ* and traditional tank setting were combined by treatment (boom-set and tank-set), and moved to bottom cages in College Creek (Annapolis, MD USA). Variability in oyster heights, lengths, and over-winter mortality between the boom and tank-set oysters was analyzed to assess long-term effects of setting method on the oysters, and provide more information of the potential for using *in-situ* setting of hatchery-reared larvae as a restoration tool.

DIFFERENCES IN CHEMICAL DYNAMICS BETWEEN CHEMOAUTOTROPHIC AND THREE DIFFERENT HETEROTROPHIC BIOFLOC-BASED SHRIMP (*LITOPENAEUS VANNAMEI*) CULTURE SYSTEMS.

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Biofloc-based shrimp aquaculture can be an ecologically friendly alternative to traditional shrimp culture. A dense microbial community forms in these systems and is responsible for cycling otherwise toxic nitrogen compounds. System managers can rely on chemoautotrophic nitrifying bacteria or can add various exogenous carbon sources to promote heterotrophic bacterial ammonium assimilation. An experiment was conducted using 16, 500-L tanks. The tanks received water that contained an established nitrifying bacterial community. Four treatments were developed, each with four randomly assigned replicate tanks. A nitrifying treatment (T-N) had no additional carbon added, and three heterotrophic treatments had different carbon sources added: sucrose (T-HS), molasses (T-HM), and glycerol (T-HG). Shrimp were stocked at 300 m^{-3}, and cultured for eight weeks at 16 ppt. salinity. Inorganic nitrogen species remained low in the heterotrophic treatments; there was a spike of nitrite-nitrogen in the T-N treatment and subsequent increase in nitrate-nitrogen. Biochemical oxygen demand was highest in the T-HS treatment and lowest in the T-N treatment. Total suspended solids concentrations were higher in heterotrophic treatments than the T-N treatment, requiring more biofloc concentration management. These, and further results from this project illustrate differences in system performance based on management of microbial function in biofloc systems.

ELUCIDATING HOST AND GEOGRAPHIC RANGES FOR *PERKINSUS* SPECIES.

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Perkinsus species are destructive parasites of molluscan species worldwide and have serious impacts on many fishery and aquaculture production industries. Molecular detection tools and

genetic information have been used during the past decade to facilitate identification of these protozoan parasites and to determine their host and geographic distributions. Polymerase chain reaction (PCR) assays, *in situ* hybridization (ISH) using DNA probes to target parasite cells in infected host tissues, DNA sequence-based phylogenetic analyses, as well as field and laboratory studies were used to identify and discriminate among *Perkinsus* species from five continents. Unique *Perkinsus* species were detected by screening Asian oysters proposed for introduction to Chesapeake Bay, Australian abalone that had suffered mass mortality, and clams being imported into the USA from Vietnam for the aquarium trade. Recently, a new strain or species of *Perkinsus* was found in *Saccostrea culcullata* oysters from India. Genetic data indicate that this new species is closely related to *P. beihaiensis* that was described from southern Chinese oysters and a *Perkinsus* sp. recently found in Brazilian *Crassostrea gasar* oysters. These discoveries emphasize the need for accurate identifications to properly assess risks of unintentional introductions of new *Perkinsus* species, and to develop informed management strategies.

HAB RISKS IN FLORIDA: DEVELOPING APPROPRIATE PUBLIC HEALTH RESPONSE STRATEGIES

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Florida Department of Health's (FDOH) Aquatic Toxins Program (ATP) addresses public health implications of four important harmful algal blooms (HABs) impacting the state including : Florida red tide (*Karenia brevis*), blue-green algae (cyanobacteria), and those associated with ciguatera and saxitoxin toxins. During many of these events, there are limited planned response procedures at local health departments, making it difficult to implement consistent and appropriate measures. Additionally, there is no "early warning system" for notification of potential health impacts for appropriate state and local entities.

FDOH is facilitating the development of county-specific HAB public health response plans that draw on local experience and expertise. In each of its 67 county health departments, collaborations between the FDOH Aquatic Toxins Program and the appropriate local program (Environmental Health, Epidemiology, Public Health Nursing, Public Information Officer, and Preparedness Coordinator) are being fostered. Local HAB plans are developed to represent local capacity for response (such as environmental health field staff available for sample collection; epidemiology expertise, primary care facilities, etc). Elements of the finalized response plans include county specific: process maps; surveillance assessments; water body inventory; environmental data sources; and partner contacts and activities (including emergency response personnel, media contacts, and environmental scientists).

EVALUATING THE USE OF FLOW-THROUGH LARVAL CULTURE FOR THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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The flow-through, or high density, larval culture has its origins on the west coast of the US, but its recent introduction to the east coast has made the eastern oyster, *Crassostrea virginica*, a candidate for this system. However, high density larval culture is relatively untested for this species. Successful adoption requires empirical trials varying density, flow rates, starting sizes, feeding rates, etc. For this work, a commercial hatchery provided space and food for six 400L flow-through tanks. Larval stocking densities of 10, 20, and 50/mL were tested with two water flow rates, first at 1.4 L/min, then at 2.8 L/min. Larval survival, growth, eyed larvae size, and duration of the eyed larvae stage were determined. At a flow rate 1.4 L/min, stocking density had a significant effect on survival and growth, but not on the eyed larvae duration. At 2.8 L/min, stocking density had a significant effect on survival, but not on growth or eyed larvae duration. Current recommendations for using flow-through to rear *C. virginica* larvae are to stock tanks at 20 larvae/mL and use a flow rate of 1.4 L/min. Stocking tanks at a higher density and using a faster flow rate only impaired larval growth and survival.

ARE ECOSYSTEM SERVICES RENDERED BY SHELLFISH AQUACULTURE SIMILAR TO NATURAL AND RESTORED BEDS?

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There is broad recognition of the ecosystem services rendered by natural and restored shellfish populations. These include mitigation of excess nutrients, improvements to water quality and clarity, and increases in habitat richness and diversity associated with increased vertical structure and niche complexity. From a functional ecology standpoint the ecosystem services provided by cultured shellfish are essentially indistinguishable from those provided by natural reefs, however there are several important distinctions.

Cultured shellfish are typically subjected to pulse disturbances related to planting, tending and harvest. The impact of these disturbances varies greatly between culture methods, but the timescale of recovery is brief, the spatial scale is restricted. The significance of these disturbances will be examined.

Another distinction between cultured and restored shellfish pertains to the nutrient mitigation associated with shellfish. Nitrogen and phosphate in tissue and shell is removed from the system at harvest. Additional nutrients are removed or sequestered via increased benthic-pelagic coupling and denitrification. Restored

shellfish are increasingly being protected from harvest to preserve their ecological value.

INACCURACIES IN PREDICTING NOROVIRUS INACTIVATION USING SURROGATE VIRUSES.

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Human norovirus (NoV) cannot be propagated in cell culture, so virus inactivation studies, including processing interventions, are generally performed on virus surrogates that may be readily quantified in the laboratory. However, there are fundamental differences in many closely related viruses, differences which limit their roles as surrogates. For example, feline calicivirus (FCV) and murine norovirus (MNV) are commonly used surrogates for human NoV, but their susceptibility to temperatures, pH, and environmental conditions have been shown to be dramatically different when directly compared with each other. Our laboratory showed that for high pressure processing, pressures of 250 mega-Pascals (MPa) for 5 min was sufficient to inactivate 7-$\log_{10}$ of FCV; however, 400 MPa for 5 min was required to reduce MNV by 4 $\log_{10}$, and using human volunteers, pressures over 400 MPa and up to 600 MPa for 5 min were required to reduce 4 $\log_{10}$ of human NoV in oysters. Data obtained from surrogate viruses must be carefully scrutinized and treated as presumptive evidence of how the pathogen may respond to a particular treatment. The true measure of the utility of NoV surrogates can only be determined when surrogate studies are performed in tandem with volunteer studies using the human pathogens themselves.

THE VARIED RESPONSES OF MARINE CALCIFIERS TO CO_2-INDUCED OCEAN ACIDIFICATION: HOW AND WHY?

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Anthropogenic elevation of atmospheric carbon dioxide (pCO_2) is making the oceans more acidic, thereby reducing their degree of saturation with respect to calcium carbonate ($CaCO_3$). There is mounting concern over the impact that future CO_2-induced reductions in the $CaCO_3$ saturation of seawater will have on marine organisms that construct their shells and skeletons from this mineral. I shall present the results of 60-day laboratory experiments in which the effects of CO_2-induced ocean acidification on calcification were investigated for 18 benthic marine organisms. Species were selected to represent a broad taxonomic range (crustacea, cnidaria, echinoidea, rhodophyta, chlorophyta, gastropoda, bivalvia, annelida) and included organisms producing aragonite, low-Mg calcite and high-Mg calcite forms of $CaCO_3$. The experiments show that 10 of the 18 species studied exhibited reduced rates of net calcification and, in some cases, net dissolution under elevated pCO_2. However, in seven species, net calcification increased under the intermediate and/or highest levels of pCO_2, and one species showed no response at all. Here, I will present these varied calcification responses to CO_2-induced ocean acidification and investigate potential reasons for such variation—including differential pH-regulation at the site of calcification, the role of protective organic coverings, differences in shell polymorph solubility, and direct utilization of CO_2 via photosynthesis. I will also present the results of a recent pH microelectrode study of the calcifying fluid of a coral to explore the role that proton-regulation at the site of calcification plays in determining an organism's specific response to CO_2-induced ocean acidification.

BIOLOGICAL GLUES AS ORGANIZERS OF MARINE COMMUNITIES.

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In our conceptual model, barnacle glue curing is similar to blood clotting. Try sin-like serine proteases activate structural proteins. Transglutaminases and potentially other enzymes cross link structural proteins and are curing agents. As in blood clotting, structural proteins and enzymes are activated from storage forms. Then structural proteins rearrange and present motifs to surfaces. Finally the polymers are cured by cross linking. The benefit of the model is in identifying paths for research by providing an evolutionary context and testable hypotheses. For example, the model enables the hypothesis that other marine glues cure by a similar mechanism. Pheromones, kairomones and other signal molecules arose from the peptide hydrolysis products generated during curing and degradation of glues. Chemical and biological evidence supports this hypothesis Thus, in a broad sense, the process of curing and breakdown of a variety of evolutionarily related macropolymers loosely categorized as glues can function to literally and figuratively cement and organize marine communities.

NEW GENERATION OF ENVIRONMENTALLY PREFERRED MARINE ANTIFOULANTS

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Biofouling has direct impact on fish health asit reduces water exchange through fishnets by up to 40% so:

- it reduces oxygen and feed supply

- it provide suitable media for fish/shellfish pathogens
- frequent changes of nets (up to every 20 days) stress the fish

While copper has been historically usedin fish nets due to its efficacy, it is not perfect thus the addition of organic co-biocides can improve its performance. Performance, environmental fate and environmental modeling of Sea-Nine 211N™ antifoulant (4,5 **di**chloro-2*n*-**o**ctyl-4**iso**thiazolin-3-one) proved to be highly effective when used as a copper co-biocide.

Sea-Nine antifoulant degrades rapidly in the environment by microorganisms. Bioaccumulation studies in fish showed essentially no bioaccumulation of the Sea-Nine biocides. It does not show chronic or reproductive toxicity to marine species. It is safe with low risk to human health.

Lastly, the new controlled release / microencapsulation technology will be introduced. Designed to increase formulation flexibility, it will prolong the service lifetime of thenet coatings due to more active retention and the delivery system thus more cost savings to fish farmers.

Sea-Nine 211N received the prestigious Presidential Green Chemistry Award in the US and most recently, has been given the recommendation for inclusion in the Annex 1 of the BPD for PT 21 (antifouling products) by the Rapporteur Member States: Norway.

POPULATION AND FISHERY DYNAMICS OF BLUE CRABS (*CALLINECTES SAPIDUS*) IN A SUBESTUARY OF CHESAPEAKE BAY.

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The blue crab spawning stock in Chesapeake Bay (CB) has experienced a persistent decline during the last 25 years, prompting an investigation of the feasibility of hatchery-based restoration for this stock. Experimental field releases have demonstrated that hatchery-reared juveniles can successfully enhance local populations at small spatial scales. However, to estimate levels of production and enhancement at larger spatial scales, an understanding of population and fishery dynamics at comparable scales is required. We conducted a mark-recapture experiment in the Rhode River, a small mesohaline subestuary of CB, during 2005–2008 to estimate: (1) population size, (2) exploitation rate, (3) tag-reporting rate, and (4) fishery composition. To date, over 40% of tags have been reported. Exploitation rates were high, but comparable to current bay-wide estimates. Tag-reporting rates were high for all fishery sectors, indicating a high level of cooperation with most fishers. Recreational catch was a considerable component of overall harvest (~20–35% per year), much larger than current bay-wide estimates. Population size estimates were generally consistent among years and increased in precision over the study period. These data provide an understanding of crab populations at the scale of a subestuary and the key factors that influence changes in population size.

THE ROLE OF PARTICLE SURFACE PROPERTIES ON FEEDING SELECTIVITY BY EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* AND BLUE MUSSELS, *MYTILUS EDULIS*.

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The mechanisms by which bivalve molluscs differentiate particles are not clearly understood, and the surface properties of a particle have been proposed as factors that contribute to particle selection. The effects of surface properties upon selection by suspension-feeding bivalves were examined. The charge and wettability of several different types of 10-μm spheres were determined. Microspheres with different surface characteristics were fed to oysters (*C. virginica*) and mussels (*M. edulis*). Microsphere proportions rejected as pseudofeces and egested as feces were determined using flow cytometry. Results suggest that, when given a choice, both mussels and oysters reject some types of microspheres (e.g., aluminum oxide), over other types (e.g. polystyrene). When bivalves were fed different microspheres with similar properties, no selection occurred. Both bivalve species had the same pattern of selectivity, suggesting a generalized mechanism of selection. Both wettable and non-wettable microspheres were rejected in some assays, indicating that wettability is not a sole qualifier for selection. Microspheres within a mid-range of charges were ingested preferentially, and positive or highly-negative particles often were rejected. The discrimination between microspheres based upon surface properties indicates that surface properties may play a role in the mechanism(s) underlying selection.

OVERFISHING AND DECADAL TRANSIENTS IN OCEAN PRODUCTIVITY

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''Overfishing'' is a central theme in modern fishery management—whether or not a stock is overfished and whether overfishing is taking place. These concepts are also key measures for appraising the performance of fishery management. However, determinations of overfishing are often equivocal for several

reasons. This is because there are many different definitions of overfishing, data often do not fit the mathematical models used to indicate whether or not a stock is overfished, and influences of the ocean environment and associated species are generally not taken into account, theoretically or analytically, in assessing reasons for stock declines. Data exist that allow contemporaneous analysis of time series of variations in ocean environments and fish stock abundances. These examples suggest that the dynamics of fish stocks can be decoupled from measures of fishing mortality. Thus some currently held overfishing scenarios might be interpreted as being driven by strong environmental changes rather than overharvesting. Implications of the findings are discussed.

VIRULENCE-RELATED GENES IN QPX, THE THRAUSTOCHYTRID PARASITE OF THE HARD CLAM *MERCENARIA MERCENARIA*.

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Natural and cultured populations of the clam *Mercenaria mercenaria* have been affected by a protistan parasite commonly referred to as Quahog Parasite Unknown (QPX). QPX belongs to the thraustochytrid group within Labyrinthulomycetes and was successfully isolated from infected clams in different locations from Canada to Virginia. Previous studies demonstrated that QPX produces and releases yet unknown factors that are cytotoxic to *M. mercenaria* hemocytes. Moreover, variability of pathogenicity was observed among different QPX isolates. To investigate molecular mechanisms involved in QPX virulence, we generated an expression library from a QPX organism isolated from an infected clam from Raritan Bay, New York. Sequence information was generated on a total of about 18,000 distinct ESTs among which several candidate genes potentially involved in QPX virulence were identified. These included genes encoding for cysteine and serine proteases which could facilitate host tissue invasion and degradation by the parasite. Full length sequence transcripts of selected candidates were obtained using RACE and their transcript levels were investigated. This work generated important molecular information on the understudied QPX parasite and provided valuable insight on potential mechanisms associated to its pathogenicity.

CALIBRATING INDUSTRY VESSELS TO THE NMFS SEA SCALLOP TIME SERIES.

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In anticipation of the retirement of the R/V *Albatross IV*, the NOAA vessel that had conducted the annual synoptic sea scallop survey virtually uninterrupted since the 1970's, a series of paired tow calibration experiments were conducted to estimate fishing power correction factors. The objective of these experiments was to facilitate the transition of the NMFS sea scallop dredge survey time series from the R/V *Albatross IV* to a future survey platform. Due to some uncertainty in the subsequent survey platform, this information would facilitate the use of the calibrated vessel to either conduct the survey, or at least form a link from the R/V *Albatross IV* to any future survey platform. Ultimately, two calibration experiments were conducted in 2007 and 2009 with the calibration process being conducted in a stepwise fashion. We used a Generalized Linear Mixed Model (GLMM) to analyze the paired catch data to test for differences in both the pooled over length catch data as well as differences in the length composition of the catch. Results indicate that scallop catch appears to be robust to the effect of vessel and based on our results, no adjustments to the NMFS sea scallop time series were supported.

A SIMULATION STUDY TO EVALUATE SAMPLING DESIGNS FOR HIGHLY AUTOCORRELATED POPULATIONS: WITH AN APPLICATION TO SEA SCALLOP CLOSED AREAS.

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The accurate estimation of sea scallop, *Placopecten magellanicus*, biomass represents a critical component of the spatially explicit management strategy currently in use. Uncertainty surrounding biomass estimates can stem, in part, from the positively autocorrelated nature of scallop distribution. In an attempt to improve these estimates, we evaluated candidate sampling designs via conditional simulation based on empirical data obtained from survey catch data. We considered a simple random design, a stratified random design (stratified by depth) and a systematic design at three levels of sample size. Comparisons were made with respect to the relative performance of the estimates of mean abundance as well as the uncertainty around those estimates. Results indicated that all estimates of mean abundance were unbiased, with levels of relative precision ranked: systematic > stratified > simple random. The performance of the estimators of the variance of the sample mean were less clear with the design based estimators proposed for systematic sampling observed to be both biased and imprecise. Conditional simulations represent a simple, yet powerful tool for the evaluation of sampling designs. Implementation of these results can serve to improve the estimates of biomass in support of sea scallop rotational area management.

THE INFLUENCE OF LAND – OCEAN EXCHANGE ON COASTAL CARBONATE MINERAL CHEMISTRY.

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River discharge mixes with and is broadly distributed in the surface waters of the continental shelf. Dissolved and particulate constituents in river discharge vary depending on regional climate, geology, land use, residence time, andatmospheric deposition over land. These regional differences can have considerable influence on the status of the carbonate mineral saturation (omega) over broad regions of the continental shelves. We discuss regional land-to-ocean flux variability in calcium, borate and non-carbonate alkalinity and their potential to confound estimates of omega in coastal waters. We also address the impact of land fluxes on net community production and coastal eutrophication, and the effect these processes can have on perturbing omega in coastal waters.

HATCHERY AND FIELD-NURSERY CULTURE OF BACK-CROSSED HYBRID (*MERCENARIA MERCENARIA*, *M. CAMPECHIENSIS*) HARD CLAMS.

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Nothern hard clam *Mercenaria mercenaria* production in Florida has seen various mortality events resulting from hurricanes, low salinities, and high water temperatures. The local southern hard clam (*M. campechiensis*) hybridizes readily with *M. mercenaria*. An examination of the parental species and their reciprocal crosses under commercial conditions has been reported. Differences in performance of hybrids indicated that backcrossing of hybrids to the northern hard clam may improve upon some performance measures. Four individual families of backcrossed hard clams were produced using single-parent crosses from our cultured hybrid and parental families. Spawning was induced by thermal stimulation with clams maintained in separate containers. Larvae were cultured separately utilizing standard hatchery practices of daily water change and batch feeding of microalgae. Larvae were transferred to downwelling systems for metamorphosis after approximately 7 days culture. No noticeable differences were observed during hatchery culture. Clams were cultured further in a land-based flow through system before being placed into nylon mesh bags for field-nursery culture near Cedar Key (Gulf of Mexico) and Sebastian (Indian River Lagoon). Preliminary analysis of growth and condition index data for nursery clams reared to grow-out seed size at Sebastian indicated no differences. Results for Cedar Key will also be presented. This work was supported by USDA-CSREES.

DEVELOPMENT OF NEW MARKERS FOR GENETIC CHARACTERIZATION OF THE PROTOZOAN PARASITES IN THE GENERA *PERKINSUS* AND *HEMATODINIUM*.

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Phylogenetic studies indicate that organisms in the phylum *Perkinsea*, which includes the *Perkinsus* species, are evolutionarily closest to the dinoflagellates, particularly the basal parasitic dinoflagellates such as those in the genus *Hematodinium*. Molecular markers that have been used to date for phylogenetic studies do not always provide the level of sequence variation required for confidently assigning new taxa as either new species or strains of currently described species. For example, within the genus *Perkinsus, P. beihaiensis*-like organisms have been found in Brazil and India with unique sequences that do not demonstrate the between species genetic distances observed among other *Perkinsus* species. Similarly, although molecular data are helping to clarify relationships among *Hematodinium* and *Hematodium*-like species, additional markers will facilitate species and strain-level discriminations in this genus. Toward this end with are obtaining sequence data for several new loci including the heat shock protein 90 (HSP90), cytochrome b (*cob*) and cytochrome c oxidase subunit 1 (*cox*1) genes from available *Perkinsus* and *Hematodinium* samples. These data will be used to do more extensive phylogenetic analyses among species within each genera, as well as to examine overall relationships among members of the Alveolata, which includes the dinoflagellates, apicomplexans, ciliates and the *Perkinsea*.

MODELING SUSTAINABLE CRAB HARVEST IN THE CHESAPEAKE BAY.

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The Blue Crab (*Callinectes sapidus*) is an important commercial and recreational fishing resource for the Chesapeake Bay. This study uses modeling techniques to assess the effects of two types of policies that can be applied to regulate crab fishing in the Chesapeake Bay area. One type of policy is spatially-based, and involves partitioning the bay into distinct parcels in which fishing is allowed for some parcels but banned for others for a period of years. This allows the crab population to recover over time. The second type of policy is to allow unrestricted fishing throughout the bay, but to impose a catch-tax that provides a financial penalty for fishermen who overharvest. This policy does not have a spatial component, but uses a social policy component instead.

The study uses a classical sustainable fisheries mathematical model as a basis for rough-order of magnitude calculations and initial validation metrics. The study uses an abstract spatially explicit model of the Chesapeake Bay, in which crab populations and fishermen are modeled as agents, and the bay is modeled as the

landscape. The model is used to assess the impacts of fish harvesting policies on the crab population dynamics and harvest rates.

THRESHOLD RESOURCE DENSITY FOR CRAB DISTRIBUTION IN NURSERY HABITATS.

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Shallow, marsh-fringed coves in the York River, Virginia, have abundant infaunal resources and are below carrying capacity for blue crabs; however, densities of ambient resident crabs could diminish survival of juveniles released for enhancement. In this study, we investigated whether there is a certain habitat suitability whereby benthic infaunal resources are ideal. Though infaunal densities are typically dominated by polychaetes and amphipods, infaunal biomass is dominated by bivalves, and crabs appear to respond principally to clams. Crab distributions established before June and is correlated with clam density and biomass and is highly correlated with juvenile clams, which make up 84–99% of the clam population. The relationship of ambient crabs to clams is sigmoidal, with a threshold at about 1000 clams m^{-2}, after which crab densities are elevated. Habitats are most suitable for crab enhancement in spring when juvenile clam densities are above a low-density threshold of 50 m^{-2} but below ~600 m^{-2}. In such habitats, food for juvenile crabs is plentiful but the density of cannibalistic conspecifics is relatively low. Based on resource availability and cannibal density, we predict that crab survival will be optimal in shallow coves where clam densities are sufficiently high yet predation on juvenile crabs is low.

PRELIMINARY ASSESSMENT OF SEASONAL TRENDS IN *VIBRIO VULNIFICUS* AND *V. PARAHAEMOLYTICUS* CONCENTRATIONS FROM SOUTH CAROLINA OYSTERS

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Recognizing the importance of oyster as critical habitat and a fisheries resource, the South Carolina Department of Natural Resources (SCDNR) initiated a comprehensive statewide oyster resource assessment in 1980. In 1999, SCDNR initiated another major cooperative monitoring program with South Carolina Department of Health and Environmental Control (SCDHEC) to evaluate the condition of estuaries with respect to general environmental quality and biotic condition. The South Carolina Estuarine and Coastal Assessment Program (SCECAP), provides an unbiased, state-wide assessment of estuarine habitat quality including an overall assessment of metal concentrations in sediments. SCECAP measures biological condition through an assessment of phytoplankton composition, a benthic index of biotic integrity, and an evaluation of demersal finfish and crustacean community composition. During the 2005 and 2006 surveys, SCECAP expanded the biological assessment to include measures of oyster condition in relation to general environmental quality including seasonal bacterial concentrations in oyster tissue. For this presentation, we quantified and compared seasonal and spatial concentrations of *Vibrio vulnificus* and *V. parahaemolyticus* across the two-year sampling period for coastal South Carolina sites selected using a probability-based, random tessellation, stratified sampling design. We also examined relationships between *Vibrios* and other bacterial concentrations in oysters including *Enterococcus* and fecal coliforms. Lastly we compared mean concentrations of both *Vibrio* species among sites classified as Approved, Restricted, and Prohibited for shellfish harvesting. *Vibrio vulnificus* concentrations differed significantly between the seasons, with a significant interaction between the two independent factors ($p = 0.038$ for season effect, $p = 0.005$ season versus site classification). Concentrations did not differ significantly among the three harvesting designations ($p = 0.086$). *Vibrio parahaemolyticus* concentrations were significantly higher in summer samples than winter samples ($p < 0.001$) and concentrations from Restricted sites were significantly higher than those from Approved sites ($p = 0.039$ for site class effect; Bonferroni post hoc $p = 0.035$). No other significant differences occurred for this species.

THE WEIRD AND THE WACKY: *ORCHITOPHYRA STELLARUM* INFECTIONS IN *CALLINECTES SAPIDUS*.

Hamish J. Small, **Terrence L. Miller**, **Anna H. Coffey**, **David Gibbs**, **Kelly Delaney**, **Jeffrey D. Shields**.
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Blue crabs, *Callinectes sapidus*, are known to harbor a number of parasites including parasitic protozoa, bacteria, viruses, and helminthes. A ciliate parasite, *Mesanophrys chesapeakensis*, has previously been documented in crabs from Chesapeake Bay, Delaware Bay, and a coastal bay of Maryland. In winter 2007 and 2009, systemic ciliate infections were discovered associated with mortalities of blue crabs held in laboratory bioassays at the Virginia Institute of Marine Science (VIMS). Initial analysis of hemolymph smears and histological tissue sections indicated that infective ciliate was *M. chesapeakensis*. However, subsequent sequence analysis of the ITS rDNA region revealed

that the infective ciliate was actually *Orchitophyra stellarum*, a known parasite of sea stars with a similar morphology to *M. chesapeakensis*. We report on the discovery of this unusual pathogen, its in-vitro growth, and the nature of its relationship with blue crabs. We also examined experimental infections in fiddler crabs, *Uca minax* to model infections in other decapod hosts. This study highlights the need for the combined use of morphological and molecular data when identifying pathogens of marine origin.

UNDERSTANDING AND MITIGATING THE IMPACT OF LOGGERHEAD (*CARETTA CARETTA*) TURTLE INTERACTIONS IN THE USA SEA SCALLOP FISHERY.

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Interactions between loggerhead turtles (*Caretta caretta*) and the Atlantic sea scallop (*Placopecten magellanicus*) fishery became a recognized problem in the past decade. Loggerhead injuries resulting from interactions with scallop dredges are being mitigated through shifts in fishing effort and modifications to the gear. The research effort, primarily funded by the sea scallop industry, has and continues to involve thousands of comparison tows of different dredge designs and modifications,studying loggerhead behaviorwith remotely operated vehicles (ROVs), and usingsatellite tracking tags torecord and analyze loggerhead diving behavior and movements. The result of this research has been a significant decline in the impact of the sea scallop fishery on loggerhead turtles. The dredge design work has also resulted in modifications that reduce fish bycatch inscallop dredges. This presentation will review the research that has taken place and summarize the results.

PREDATION OF GREEN MUSSELS (*PERNA VIRIDIS*) AND OYSTERS (*CRASOSSTREA VIRGINICA*) BY STONE CRABS (*MENIPPE MERCENARIA*).

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Green mussels (*Perna viridis*) are invasive bivalve species in the coastal areas of SW Florida. To examine the potential limitations on the population growth of mussels, this project examined the relative predation of native stone crabs (*Menippe mercenaria*) on native oysters (*Crassostrea virginica*) and green mussels. Individual crabs were offered either 4 oysters, 4 green mussels or 2 oysters and 2 green mussels (N = 5 replicates / treatment) under laboratory conditions and predation of shellfish enumerated every day. Shellfish were replenished daily. While stone crabs consumed green mussels in low numbers, they seemed to prefer to consume oysters. When green mussels were the only choice of prey species, crabs consumed 0.3 (±0.34) mussels/crab/day compared to 1.7 (±0.49) oysters/crab/day when oysters were offered as the only prey species. When offered both green mussels and oysters as prey species, crabs consumed 0.68 (±0.24) oysters/crab/day compared to 0.18 (±0.21) mussels/crab/day. The size of the shellfish also influenced the crabs' daily consumption rate. Future experiments will examine the predation preference of other crustacean species (e.g. blue crabs, *Callinectes sapidus*, and mud crabs, *Scylla serrata)* on these two shellfish species.

FDA'S RISK PROFILE ON TRANSMISSION OF NOROVIRUS VIA READY-TO-EAT FOOD.

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The FDA's Center for Food Safety and Applied Nutrition developed a risk profile in response to the increasing incidence of norovirus (NoV) foodborne illness outbreaks to help improve the Agency's understanding of the potential risks posed by NoV to the nation's food supply.

The specific objectives of the risk profile were 3-fold:

- Provide a comprehensive review of the available science on NoV and its mode of transmission, particularly related to ready-to-eat (RTE) food.
- Provide information on potential options to interrupt the NoV transmission pathway, with a special emphasis on those pathways leading to RTE foods.
- Identify knowledge gaps for the purposes of research planning.

Norovirus gastroenteritis can result from foodborne (including shellfish), waterborne, person-to-person, or environment-to-person means of transmission.The focus of this risk profile is the prevention of foodborne NoV gastroenteritis from RTE food in

the United States by developing a better understanding of all the NoV transmission pathways. Effective control options for NoV should address all possible modes of NoV transmission, including foodborne, environmental, waterborne, and person-to-person spread. Several data gaps are also identified in this document.

IS HARVEST FROM THE LOUISIANA STATE PRIMARY SEED GROUNDS SUSTAINABLE? WHAT NUMERICAL MODELING MIGHT RESOLVE.

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The Louisiana Department of Wildlife and Fisheries manages 1,650,000 acres of public water bottoms for the cultivation of eastern oysters (*Crassostrea virginica*). Stock estimate for these Public Grounds in 2010 were 694,670 barrels of seed oysters (25 to <75 mm) and 529,707 barrels of sack (market) oysters (≥75 mm). Traditionally, the Louisiana oyster industry uses the public grounds as a source of seed for transplant to private leases, where they grow to market size and are subsequently harvested. The abundance of suitable water bottoms and this public-private partnership make Louisiana a leading producer of oysters. Despite the abundance of the stock and success of the industry, managers are concerned about the long-term sustainability of the resource. Direct marketing of sack oysters is of special concern since the natural renewal of a reef requires that small oysters grow to be large ones and that sufficient numbers of large oysters remain unharvested and die in place. What this sufficient number is, remains unknown, in part because there is no sustainability criterion. A numerical model is presented which defines a sustainability criterion as no net loss of shell, and calculates the sustainable harvest of seed and sack oysters.

INFLUENCES OF TEMPERATURE, FOOD AND PHYSIOLOGICAL ON RESERVE AND MEMBRANE LIPIDS OF OYSTERS CRASSOSTREA GIGAS DURING AN ANNUAL CYCLE.

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The ability to synthesize or bioconvert sterols and polyunsaturated fatty acids *de novo* is generally low or absent, and implies that a dietary supply is necessary gametogenesis, embryogenesis and larval development in bivalves. The objective of this study was to investigate fatty acid and sterol localization and transfer during the natural reproductive cycle of the Pacific oyster collected every 2 weeks in Marennes Oleron Bay. Membrane and reserve lipids (fatty acids and sterols) were examined in muscle, digestive gland and gonad.

Multivariate analyses of fatty acids and sterols from reserve lipids of digestive gland revealed that oyster natural diet was dominated by flagellates from January to April and by diatoms from May to August. Analyses of muscle membrane lipids allowed identifying fatty acids and sterols positively (20:1n-9, 18:1n-9, 20:1n-7 and dehydrocholesterol) and negatively (20:1n-11, 22:6n-3 and desmosterol) correlated to temperature. During gametogenesis, cholesterol was preferentially accumulated in gonad steryl esters. There was also a specific accumulation of 20:5n-3 in gonad polar lipids. Oyster larvae obtained from these oysters consumed the 20:5n-3 and cholesterol enriched steryl esters during embryogenesis. This study allowed discriminating the respective influences of food composition, temperature and reproductive activity on oyster membrane and reserve lipid compositions.

BIODEPOSITION RATES IN SHALLOW WATER, INTENSIVE OYSTER FARMS.

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The Chesapeake Bay offers enormous opportunity for the development of intensive, on bottom oyster farms of considerable size, but unlike many locations worldwide the majority of farm development sites are in shallow water with limited tidal excursion and/or flushing. The challenge to the farmer is development of a best management practice that optimizes growth but limits the impact of biodeposit accumulation beneath the deployed trays. We present a growth and biodeposit budget for a working oyster farm over the period of culture of a single cohort of oysters from seed to

harvest. A cohort of approximately 900,000 seed oysters were grown to market size in a 18-24 month period using a system of 1.8 m × 1.2 m × 15 cm cages supported above the bottom on 15 cm legs. Individual oyster biomass increased 38-fold over the culture period. Associated total biodeposition is between 12 and 15 metric tonnes for the entire cohort. While this is substantial, farmers are proactively working to manage their lease bottom and the prospects for continuing high productivity is good.

FEDERAL SURVEILLANCE OF MOLLUSCAN DISEASES ON CANADA'S EAST COAST.

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As a partner in the National Aquatic Animal Health Program (NAAHP), Fisheries and Oceans Canada (DFO) has recently established the National Aquatic Animal Health Laboratory System, a group of four Reference Labs providing technical and scientific expertise on aquatic animal disease. The laboratory in Moncton, New Brunswick has a dedicated surveillance program for monitoring health of oysters on Canada's East Coast Since the detection of MSX in 2002 within the Bras d'Or Lakes, disease zonation has limited it's spread to the outer coast of Cape Breton. However, the threat of incursion of *Haplosporidian nelsoni* into the MSX disease free area of the lucrative Gulf of St. Lawrence is an industry concern. Surveillance for the maintenance of the MSX disease free status is ongoing and includes an area of increased surveillance based on oceanographic proximity and industry activities. Disease screening using histopathology and real time polymerase chain reaction (qPCR) allows for detection of both *H. nelsoni* and changes in the status of other endemic diseases, most notably Malpeque Disease. Disease advice is provided to other sectors within DFO for the implementation of restrictions under the Fisheries Act and the data also provides key baseline information for the development of the NAAHP.

DID 8.5 BILLION JUVENILE SEA SCALLOPS DIE IN THE MID-ATLANTIC DUE TO INCIDENTAL FISHING MORTALITY?

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A single large year class in the Mid-Atlantic consisting of 1.1×10^{10} sea scallops was observed in 2003. This year class was 1.0 to 1.4 times greater than the entire scallop stock in any year between 2004 and 2009. Over half of these scallops died between 2003 and 2004. This mortality did not appear to be caused by a shift in environmental conditions or from biological factors. However, the majority of fishing effort was focused in the Mid-Atlantic. During fishing operations these scallops were brought to the surface through water temperatures greater than their lethal limit and exposed to high air temperatures before being returned to the sea floor several hours later. Therefore, this mass mortality was likely the result of incidental fishing mortality. The recruitment event was reported to managers on 2 July 2003. The Elephant Trunk Closed Area, established 13 months later, has provided substantial landings for the past 5 years; the potential of the 2003 recruitment event was only partially realized. To achieve the full benefit of extreme recruitment events requires a closer examination of the target species ecology, real-time abundance and size distribution data and a management system that can respond rapidly.

WHAT DO CLAMS EAT? A PICTORIAL GUIDE TO MARINE PHYTOPLANKTON QUANTITY AND QUALITY FOR CLAM FARMERS.

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Hard clam, *Mercenaria mercenaria*, aquaculture supports many small businesses in Florida with over 150 million clams produced annually. Farms are located on submerged land leases in inshore coastal waters. Florida's warm climate supports year-round productivity and optimal growing conditions for hard clams. Knowledge of what clams eat and when those food items are most available can guide farmers in deciding when to plant seed and harvest clams. In addition, information on patterns of occurrence of harmful algal blooms can help farmers avoid or anticipate losses in clam sales or stocks. A web-based, pictorial guide assists farmers in identifying potential food sources for hard clams and their spatial and seasonal distribution. Measures of phytoplankton quantity (chlorophyll *a* patterns) and quality (biomass patterns), as well as species data, from two important clam aquaculture regions are presented in this guide. Biographical sketch pages describe over 50 common algal species with information about where and how often the species was found in samples from University of Florida phytoplankton studies, and whether the food is good (nutritious) or bad (noxious or harmful) for clams. The educational tool *What Do Clams Eat?* is accessible at http://shellfish.ifas.ufl.edu and available as a CD-ROM.

FLORIDA CLAM AQUACULTURE INDUSTRYS RESPONSE TO THE *DEEPWATER HORIZON* OIL SPILL.

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In response to the threat of the *Deepwater Horizon* oil spill, the clam farming industry in Florida, located primarily along the Gulf of Mexico coast, prepared for potential impacts. From Day 14, members of the state task force met weekly with agency and

university representatives via conference call to discuss plans and concerns. Clam farmers participated in area contingency planning sessions with Unified Command. Aquaculture leases were identified as environmentally sensitive areas and given a top tier designation for protection. Alternative countermeasures were identified with the U.S. Coast Guard to protect open-water leases in lieu of traditional booming strategies. The state shellfish control authority collected pre-impact samples for baseline characteristics and developed an emergency regulatory response should hydrocarbons from the oil spill reach Florida estuaries. As of Day 153 when the well was permanently sealed, none of the shellfish harvesting areas in Florida were closed due to the presence of oil products. The industry's biggest challenge was ensuring buyers and consumers that shellfish were safe. In response, a *Florida Gulf Safe* promotional campaign was initiated, which featured a seafood hotline with daily reports. Indirect impacts to and lessons learned by the clam culture industry during this disaster will be presented.

CHALLENGES TO CREATING A TETRAPLOID BROODSTOCK FOR THE BAY SCALLOP *ARGOPECTEN IRRADIANS*.

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Tetraploid broodstock have already been created for the Pacific oyster *Crassostrea gigas* and the American oyster *Crassostrea virginica* to allow production of natural triploid crops suitable for market in the US. A previous study has shown that triploidy improves yield and condition not just in oysters, but also the bay scallop *Argopecten irradians*. This technology might be what the industry needs to finally overcome the bottlenecks stalling bay scallop aquaculture. However, because bay scallops are hermaphrodites and more difficult to grow than oysters, challenges have risen while attempting to create a tetraploid broodstock. In 2007, 2008, 2009 and 2010, triploid F1 groups were produced with chemicals. In the springs of 2008, 2009 and 2010, the F1 triploid populations were tested to isolate a triploid broodstock. Approximately 5% of the triploid group showed some signs of sexual development. In 2008 and 2009, F1 triploids were spawned successfully. The triploid eggs were fertilized with diploid sperm and treated to induce tetraploidy (F2). Only the 2008 F2 individuals survived passed the setting stage. Testing revealed the presence of 5% of tetraploids in the F2 group. This population did not survive overwintering. Attempts to replicate these results are underway.

OXYGEN FLUCTUATION AND OXIDATIVE STRESS IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*.

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Oxygenation in the marine environment can be very variable due to several parameters as temperature, salinity, tides and irradiation. The oyster *Crassostrea gigas* is a marine intertidal oxyconformer mollusc that can experience important variations in oxygen levels during emersion/immersion tidal cycles and during eutrophication phenomena. Molecular, cellular and physiological responses upon short-term oxygen variations (hypoxia and reoxygenation) were measured through transcriptional antioxidant gene expression, hemocyte oxidative metabolism and mitochondrial functional modifications. Results revealed that oysters adapt fast to oxygen variations during hypoxia and re-oxygenation, and that they seem to own performing strategies in order to adjust metabolic needs, energy production, and antioxidant defense as well. During hypoxia, aerobic metabolism was reduced through a drastic fall down of the respiratory chain activity (40% of normoxic activity) and ATP production (20% of normoxic production). Upon re-oxygenation, antioxidant defense was up regulated not only through the activation of the "classical" antioxidant defenses (up regulation of Glutathione transferase and reductase genes) but also through an alternative pathway of electron transfer. This system, known as alternative oxidase, is presumed to be implicated in the observed modifications of oxygen consumption, oxidative phosphorylation, mitochondrial membrane potential and ATP production capacities of mitochondria in *C. gigas*.

IDENTIFICATION OF AGE LIMITATIONS IN HEMIC NEOPLASIA DEVELOPMENT IN THE SOFT SHELL CLAM *MYA ARENARIA*.

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Hemic neoplasia is one of the six most destructive diseases among bivalve mollusc populations, specifically *Mya arenaria*. The disease is characterized by development of mitotic, rounded hemocytes. In *Mya arenaria* a correlation between age and neoplasia development was suggested based on size limitations of neoplastic animals in natural populations. In this study we injected clams from seven different size classes (5-90mm) with 0.2 micron filtered lysed neoplastic hemocytes. Prior to injection all potential experimental animals were biopsied to ensure absence of neoplasia and separated based on their shell size. Animals were divided into two treatment groups, a control injected clam hemocyte medium and experimental treatment injected 0.2 micron filtered neoplastic hemocytes. Animals from control and treatment groups were injected 2.5% of total blood volume for each size class. All

experimental animals were biopsied weekly for 7 weeks to examine neoplasia development. *Mya arenaria* between 40-80mm were the most susceptible, while animals below 20mm were not susceptible to developing neoplasia. Using a mathematical model for age determination, animal sizes correlated to age groups of 0.5-2 years for neoplasia development. (Funding through WCUPA CAS student research award to NGT)

EXPRESSION OF ECDYSTEROID RECEPTOR COMPLEX (ECR-RXR) AND MOLT-INHIBITING HORMONE (MIH) IN A MOLT CYCLE OF THE FEMALE BLUE CRAB, *CALLINECTES SAPIDUS*.

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Molting (ecdysis) is the necessary process for somatic growth and reproduction in animals belonging to the phylum Arthropoda. The molt cycle is under positive and negative controls of ecdysteroids (Ecd) and molt-inhibiting hormone, respectively. Ecd exerts its physiological and molecular actions through a heterodimer complex of nuclear receptors named ecdysone receptor (EcR) and its partner, retinoid X receptor (RXR). We have identified a complex Ecd profile in the hemolymph from the last two molts. Also, we isolated the full-length cDNAs of *CsEcR and CsRXR* and analyzed 5' and 3' untranslated regions for their translational controls *in silico*. We report that *C. sapidus* express two isoforms of *CsEcR*, while it has only one *CsRXR*. We also found tempo-spatial distributions of *CsEcR-CsRXR* in eyestalk ganglia and Y-organ.

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RESURGENCE OF BAY SCALLOP, *ARGOPECTEN IRRADIANS IRRADIANS*, POPULATIONS AND FISHERIES AFTER LARGE-SCALE RESTORATION EFFORTS IN EASTERN LONG ISLAND, NEW YORK.

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Bay scallop (*Argopecten irradians irradians*) populations in the Peconic Bays of eastern Long Island, New York were decimated by a series of 'brown tide' algal blooms from 1985–1995. After the last of these events, commercial fishery landings remained at 1–2% of historical levels for 12 years. Bay scallop stocks probably did not recover on their own because densities and total population sizes of scallops in local embayments were too low to permit high rates of successful fertilization during spawning, thus limiting recruitment. Our present restoration efforts, begun in 2006, have focused on jump-starting populations by planting >4 million hatchery-reared scallops at high densities, in lantern nets or directly to the bottom, to ensure a high probability of fertilization of spawned eggs. In the 4 years following restoration, compared to the 2 years prior, we have documented increases in larval recruitment and benthic populations of >100× and 60×, respectively, in the three areas of the Peconic Bays where we have planted scallops. Unplanted areas did not exhibit population increases for 1–3 years following initiation of restoration efforts, but population rebuilding is now widespread. Commercial bay scallop landings are now ~10–30x those of mean annual harvests recorded from 1996–2007.

POST-HARVEST QUALITY OF SELECTED PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) CULTURED IN KACHEMAK BAY, ALASKA, AND PUGET SOUND, WASHINGTON.

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To date studies on quality characteristics of the Pacific oyster (*Crassostrea gigas*) have compared animals within and between locations, and the effects of different culture types. However, research has only been made upon animals of mixed genetic background. Selective breeding programs for oysters have been shown to improve yields and survival, and allow oyster growers to choose specific traits desirable for optimal growth and marketable characteristics. This study used the seven highest yielding families of oysters among a cohort developed and planted in April 2006 by the Molluscan Broodstock Program (MBP), which were grown in suspended culture at a site in Kachemak Bay, Alaska. The same cohort was planted in tandem and grown intertidally at Thorndyke, Bay in Washington. Animals from the top seven yielding families and control families were sample in October 2009 and again in June 2010. Biometric, chemical, and fatty acid data were collected. Reproductive condition and shell morphological and color characteristics were also recorded. Comparisons were made between families of selected and non-selected families of MBP oysters; within and between sites and across seasons. This presentation presents the results, the usefulness to the industry, future applications of the breeding program within Alaska, and the Pacific states.

CHARACTERIZING AN OVARIAN VTG GENE OF THE BLUE CRAB, *CALLINECTES SAPIDUS*: LOCALIZATION AND ITS EXPRESSION DURING OVARIAN DEVELOPMENT.

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Ovarian development in crustaceans requires two main processes: (1) oocyte recruitment and (2) yolk protein synthesis and accumulation. Vitellin (VT), a lipo-glyco-carotenoprotein, is the major yolk protein that is derived from the precursor vitellogenin (VtG). Depending on the species of decapod crustaceans, *VtG* expression is found in hepatopancreas or/and ovary. It is reported in *Callinectes sapidus* that *VtG* is mainly expressed in the hepatopancreas of adult female *C. sapidus* and three subunits of VT are derived from VtG. We isolated a second *VtG* gene from the ovary of *C. sapidus* using overlapping sequencing of PCR cloning and 5' and 3' RACE. The cDNA ovarian *VtG* (7881bp) contains an open reading frame encoding the putative 2564 amino acids of VtG that is identical to the *VtG* found in hepatopancreas (7833bp). However, we found some differences in 5' and 3' untranslated region of *VtG* between ovary and hepatopancreas. We are in the process of determining the localization by using *in situ* hybridization and the expression levels via quantitative PCR analysis of ovarian *VtG* during ovarian development of *C. sapidus*.

Acknowledgement: The work is supported by a Binational Agricultural Research and Development (BARD) UMBI-Israel program MB-8714-08, and Royal Thai Government Scholarship.

IS A GENETIC VARIATION RESPONSIBLE FOR THE DIFFERENTIAL GROWTH RATE OF ANIMALS OBTAINED FROM A SINGLE BROOD OF *CALLINECTES SAPIDUS*?

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Crustaceans do exhibit vast difference in the growth rate. In *Callinectes sapidus*, animals derived from a single brood and exposed to the same culture condition display great size variations. Considering the somatic growth in crustaceans only occur through the molt process at a 20–50% of molt increment/molt, we hypothesized that molt frequency may be responsible for such a size variation. It is known in crustacean endocrinology that molt is regulated by an endocrine axis of neuropeptides [crustacean hyperglycemic hormone (CHH) and molt-inhibiting hormone (MIH)] produced in eyestalk; ecdysteroids synthesized; and released by Y-organ and gut CHH. Interestingly, cDNA of MIHs found in several decapod crustaceans contain microsatellite sequences in the 3'untranslated region (UTR), despite the fact that the exact functional significance of these sequences in translation of *MIH* is unknown. We aimed to understand 1) the roles of CHH and MIH in the growth rate and size variation of *C. sapidus* and 2) a potential significance of the length of microsatellite sequence of *MIH* and *MIH* expression. This paper discusses a potential implication of genetic variation via the different lengths of microsatellite located in 3' UTR of MIH in *MIH* expression and translation in the growth rate of *C. sapidus*.

GENETIC VARIABILITY OF THE MEDITERRANEAN ISRAELI COAST POPULATION OF THE INVADING BLUE CRAB *CALLINECTES SAPIDUS*.

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Though the natural range of the American Bay blue crab (*Callinectes sapidus)* is the western Atlantic Ocean, from Nova Scotia to Argentina, it has also been successfully introduced into the Mediterranean Sea. The blue crab is found in the Israeli shores of the Mediterranean for the past 40 years. We have sampled 25 crabs in locations 50 kilometers apart and looked into their genetic diversity by sequencing five mitochondrial DNA genes (12S, 16S, ND4, ND2 and COI). We have found three different haplotypes without a geographic linkage. The three haplotypes were more similar to different Atlantic coast crabs than to each other. This clearly indicates a polymorphism that was introduced into the young Israeli population and a founder effect

FINDING AND FORECASTING HARMFUL ALGAL BLOOMS.

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Obtaining locations and forecasting positions are critical for managing Harmful Algal Bloom (HAB) impacts. As HABs pose a potential health risk to both human and animal health, the ability to provide early warning as to when a toxic bloom might occur is critical. NOAA has developed a nowcast/forecast capability in several regions of the U.S. by combining several data types and models to locate blooms and forecast where the impacts might be seen over the next few days. A suite of algorithms were developed for detecting HABs in coastal areas and toxic cyanobacteria in several freshwater systems. Through a combination of observations and anecdotal information, the extent of a bloom is estimated. In some regions, operational hydrodynamic models are available and applied to produce a nowcast when imagery is several days old, followed by forecasts approximately 3 days ahead. The result is an analysis of the risk of a bloom in a particular location. The value and challenges of merging observations and models will be

discussed. Improvements in the quality of the forecasts require validation through observations of bloom location and intensity. Continued improvement in the analysis can lead to data sets that will allow seasonal and climatic forecasts.

THE ROLE OF *CRASSOSTREA VIRGINICA* HEMOCYTES IN SHELL FORMATION: *EX VIVO* MINERAL DEPOSITION BY CULTURED HEMOCYTES.

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The Eastern oyster, *Crassostrea virginica*, produces a composite ceramic shell composed of the calcite polymorph of calcium carbonate and various proteinaceous organic constituents. There are two models for shell biosynthesis: the matrix-mediated model which states that mineral nucleation occurs on secreted organic matrix components, and the hemocyte-mediated model which states that mineral nucleation occurs intracellularly and nuclei are deposited at the shell formation front. In this study we examine the role of circulating refractive hemocytes in shell formation with the goal of further elucidating the mechanism of calcite nucleation and growth. Oyster hemocytes were collected from notched adult oysters and cultured *ex vivo* for up to 96 hours with resultant mineral formation on glass substrates. Hemocytes were also cultured on biomedical implant substrates (316L stainless steel and titanium alloy Ti6Al4V) with similar results. X-ray diffraction studies of isolated hemocytes reveal the presence of the calcite polymorph in *C. virginica* hemocytes. The *ex vivo* culture of mineral-bearing hemocytes opens up the possibility for site-directed mineral deposition on a variety of substrates of economic and biomedical interest.

EXPANDING POST-DISEASE RECOVERY IN POPULATIONS OF THE ENDANGERED BLACK ABALONE (*HALIOTIS CRACHERODII* LEACH 1814) AT SAN NICOLAS ISLAND, CALIFORNIA

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Black abalones (*Haliotis cracherodii* Leach, 1814) are found in rocky intertidal habitats from northern California, USA, into northern Baja California, Mexico. US populations were listed as "endangered" in 2009, largely due to mass mortalities associated with a bacterial disease known as withering syndrome (WS). Twenty population-scale surveys for black abalones were completed between 1981 and 2010 in nine permanent study locations distributed around the periphery of San Nicolas Island, California (SNI). WS was first seen at SNI in spring 1992, causing a 99% reduction in Island-wide density by 2001, with catastrophic mortalities observed at all nine study sites. Since 2002 black abalone numbers have increased at four study sites, three on the south side of SNI and one (site 3) on the north side. The increase was twelve-fold at sites 5 and 7 and threefold at site 8. Numbers at site 3 have shown high inter-annual variance but have increased twenty-four fold since 2001. Numbers at the five remaining study sites have remained low and constant or have continued to decline. Patches of black abalones with recent positive trends in density show resistance to WS and have obvious potential for contributions to the development of restoration strategies for depleted populations.

THE USE OF LOSS AND SETTING EFFICIENCY TO MAXIMIZE SPAT PRODUCTION OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) AND ITS APPLICATION TO A PRODUCTION HATCHERY.

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One measure of success in an oyster hatchery is setting efficiency. Increasing efficiency can involve keeping larvae in tanks as long as possible, which can result in loss of larvae to the sides of the tanks. This study used loss and setting efficiency to determine when introduction of larvae into setting tanks would maximize spat production. *Crassostrea virginica* larvae were grown under normal hatchery conditions. Once retained on a 200 micron sieve, they were placed in a rearing cone. The cone was drained, larvae were counted, a portion was placed in a setting vessel, and the remaining larvae were returned to the rearing cone. This generated daily loss and setting efficiency values, and occurred every day until 10% of larvae remained. These data were used in a hypothetical scenario to determine when spat production is maximized. Both the highest setting efficiency and lowest spat production occurred on day five. The greatest spat production occurred on day two. These data suggest that spat production may be increased by introducing larvae into setting tanks two days after they are retained on a 200 micron sieve, or by increasing the time larvae spend in setting tanks.

THE CAUSES OF ACIDIFICATION IN CHESAPEAKE BAY AND CONSEQUENCES TO OYSTER SHELL GROWTH AND DISSOLUTION.

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Ocean acidification, due to increasing release of fossil fuel emissions, has become recognized as a threat to shell forming animals in the world's oceans. However many important marine

resources, and in particular shellfish, reside in coastal embayments and estuaries where myriad processes affect the water's acidity. Analyses of Chesapeake Bay water quality monitoring data indicate that acidity is changing over the last two decades; acidity decreased in mesohaline habitats and increased in polyhaline habitats at higher than expected rates. These changes have significant potential to affect the sustainability and restoration of current oyster populations in Chesapeake Bay. Experimental results measuring biocalcification in juvenile oysters highlight the importance of temperature and salinity in moderating acidification impacts on shell deposition. Furthermore, dissolution rates of intact oyster shells indicate moderate changes in water acidity can have significant effects on the persistence of shell material, which is necessary habitat for new oyster recruits. Interestingly, the legacy of shell material affected its susceptibility to dissolution, with dredged shell showing increased tolerance to dissolution, relative to either fresh or weathered shell. In total, these results illustrate the importance of further study and understanding of the factors controlling estuarine acidification and the complex effects on oyster populations.

COMPOSITION, MORPHOLOGY, AND FORMATION OF THE CALCAREOUS SHELL OF THE SERPULID *HYDROIDES DIANTHUS*.

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The calcareous tubes of serpulid marine worms were studied in a biomineralization context. The structure and composition of the tube shell and adhesive cement of the marine tubeworm *Hydroides dianthus* were studied using a variety of characterization techniques, including powder XRD, FTIR, SEM, EDX, and AFM. The tube and cement were determined to be inorganic–organic composite materials, consisting of inorganic aragonite ($CaCO_3$) and Mg-calcite ($(Ca_{0.8}Mg_{0.2})CO_3$) crystals, and both soluble and insoluble organic matrices (SOM and IOM). SEM imaging revealed a variety of crystal morphologies. AFM nanoindentation of the inorganic components yielded Young's moduli of ca 20 GPa in the wet state, and ca 50 GPa in the dry state. Amino acid analysis of the SOM indicated substantial amounts of acidic and non-polar neutral amino acids. Part of the insoluble organic tube lining was identified as being composed of collagen-containing fibers aligned in a criss-crossed structure. The SOM and organic tube lining were found to contain carboxylated and sulphated polysaccharides. In an artificial seawater solution, the SOM and the organic tube lining mediated $CaCO_3$ mineralization *in vitro*.

NANOSTRUCTURES TO INFLUENCE MARINE BIOFOULING SETTLEMENT.

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We report recent results on the use of nanostructured block copolymers to inhibit the settlement of algal spores. Methods to make the films, to enhance durability, will also be discussed. The adhesion responses of other organisms, to these and related polymeric materials will be placed in the context of fouling inhibition and release.

TAXONOMIC STATUS OF A COMMON OYSTER FROM SOUTHERN CHINA.

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One kind of cupped oyster is common and abundant along the coast of southern China, but its taxonomic status is still unresolved. We collected and analyzed a large number of oysters along the southern coast of China. As controls, we included different populations of *C. gigas* from the northern coast of China, and samples from Korea, Japan, Portugal, the Netherland, Australia and the US, *C. angulata* from Portugal, *C. virginica* from Delaware Bay and Louisiana, and *C. rhizophorae* from Florida. DNA sequences from mitochondrial 16S rRNA and cytochrome oxidase I (COI) both indicate that the common oyster from southern China is the same as *C. angulata* from Portugal. While *C. angulata* and *C. gigas* are genetically distinct, sequence divergence between them is higher than that between the most divergent populations of *C. virginica*, but considerably lower than that observed between any sister species of *Crassostrea*. This finding and the fact that the two oysters have slightly different biological characters but can hybridize without difficulties suggest that *C. angulata* should be considered as a sub-species of *C. gigas*.

EFFECTS OF TEMPERATURE AND QPX (QUAHOG PARASITE UNKNOWN) INFECTION ON THE TRANSCRIPTION OF DEFENSE-RELATED GENES IN THE HARD CLAM *MERCENARIA MERCENARIA*.

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Quahog Parasite Unknown (QPX) is a protistan parasite infecting hard clams, *Mercenaria mercenaria*, along the Northeastern coasts of the United States and Maritime Canada. Our

previous studies have demonstrated the effect of temperature on QPX disease prevalence and intensity, although the mechanisms involved in disease modulation remain obscure. This study investigated the effects of temperature and QPX challenge on the transcription levels of an array of stress and defense related genes in clams. Naturally and experimentally infected clams were maintained at 13, 21 and 27°C and transcription levels of selected genes were assessed in different clam tissues after 2 and 4 months by real-time quantitative PCR. Results demonstrated a major modulatory effect of temperature and QPX challenge on both constitutive and QPX-induced transcription levels of several candidate genes, including lysozyme, lectin, hemocyte defensin and big defensin. Associated with previous results showing high disease development at low temperature (13°C) and enhanced healing processes in clams maintained at warmer temperatures (21 and 27°C), these findings have the potential for helping the identification of molecular mechanisms associated to the resistance of clams to QPX disease by mean of successful immune response to the parasite.

CONSTRUCTION OF A CYTOGENETIC MAP FOR THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*).

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Cytogenetic mapping of chromosomes is important for the characterization of genomes as chromosomes are the most basic and obvious units of genome organization. We are developing the first cytogenetic map for the Pacific oyster for the integration of genetic, physical and sequence maps. We mapped bacterial artificial chromosomes (BACs) with fluorescence *in situ* hybridization (FISH) for chromosome identification and map integration. Our strategy is to select microsatellites from the genetic map and use them to the BAC library. BAC clones that are positive for mapped microsatellites are selected for chromosomal assignment with FISH. FISH in oysters and other molluscs is a challenge due to the lack of cell lines needed for the preparation of high quality metaphases. We developed a protocol for FISH with metaphases obtained from early embryos. So far 31 clones were successfully mapped to 10 oyster chromosomes. Each BAC was mapped to a unique chromosomal location except one that was mapped to two chromosomes, probably due to locus duplication. Our results show that BACs can be reliably mapped to oyster chromosomes with FISH using embryonic material. More BACs are being selected and mapped for the development of the cytogenetic map and for integration with other genomic maps.

THE DNA REPLICATION AND REPAIR PATHWAY OF PACIFIC OYSTER, *CRASSOSTREA GIGAS*.

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The reasons for the high genetic diversity of pacific oyster remain unclear, one of which is the weak ability of DNA replication and repair in this species. The homologs of genes in eukaryotic DNA replication and repair reference pathway were verified in the genomes of pacific oyster, sea hare, limpet, human, chicken, zebrafish, fruit flying and nematode (e-value < 10^{-5}). Obvious difference was found in DNA replication pathway, which is one type of DNA replication and repair pathway. Five genes in eukaryotic DNA replication and repair reference pathway not found in pacific oyster genome were also not found in sea hare and limpet genome. In addition, another gene was found in oyster genome, but not in sea hare and limpet genome. Six genes were not found in nematode genome, 4 of which were the same as those that not found in above molluscs. More differences were found between invertebrate and vertebrate DNA replication genes. These results showed that the unconservative genes among species were inclined to be identical in the same evolutionary clade, which partly supported the accuracy of genome assembly. The genes involved in molluscan DNA replication and repair, no doubt an interesting issue, need further study.

CHARACTERIZATION OF THE SUBPOPULATIONS OF HEMOCYTES AND THEIR IMMUNE-RELATED PARAMETERS IN THE GREEN-LIPPED MUSSEL *PERNA VIRIDIS*.

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The *Perna viridis* is a bivalve species distributed widely in the Indo-Pacific region. Commercial production of *P. viridis* sometimes is restricted due to mass mortality in summer. However, limited information is available in the physiological and immunological nature of hemolymph; the understanding of which is important in managing the problem. Immunological activities and morphology of the hemocytes were investigated using flow cytometry, electron microscopy (TEM and SEM) and light microscopy. Three types of hemocytes were identified, including granulocyte, semi-granulocyte and hyalinocyte. Granulocytes, the most abundant one, were of intermediate size containing numerous granules in the cytoplasm. Semi-granulocytes, the largest but less abundant than granulocytes, contained some granules. Hyalinocytes contained few granules and were the smallest and least abundant. Flow cytometry revealed that the granulocytes were the most active in cell phagocytosis and lysosomal content, but had similar esterase and peroxidase activities and reactive oxygen

species (ROS) generation as semi-granulocytes. In contrast, the hyalinocytes showed the lowest values in phagocytosis, enzymic activities and ROS, but a higher mortality as compared with other hemocytes. The immune functions assessed by flow cytometry in this study indicated that the granulocytes and semi-granulocytes are the main hemocytes involved in the cellular defense in *P. viridis*.

INTER-ANNUAL VARIABILITY IN CIRCULATION AND WATER PROPERTIES IN DELAWARE BAY AND THEIR RELATIONSHIP TO DISEASE PREVALENCE.

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As part of the U.S. National Science Foundation Ecology of Infectious Diseases (EID) program, we have developed a high-resolution hydrodynamic model for the Delaware Bay. Validation studies conducted with the model confirm its ability to reproduce observed fields of water level, circulation and tracer (temperature and salinity) properties. Here, we apply the model to ask: can inter-annual variability in these fields account for the observed variations in disease prevalence in Delaware Bay? To address this question, model simulations are performed for several three-year periods which collectively span a variety of physical conditions (e.g., freshwater inputs) and disease occurrence. The three periods include 1974–1976, 1979–1981, 1984–1986, 1990–1992 and 2006–2008. The first two intervals correspond to periods of low observed prevalence of (e.g.) MSX in the upper Bay, whereas the latter periods were observed to have elevated disease levels. The presentation will summarize the results from these multi-year simulations and assess the ability of the present-generation circulation model to provide an explanation for observed patterns of disease prevalence.

WORKING WITH NEW HAMPSHIRE RESIDENTS TO RESTORE OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS TO THE GREAT BAY ESTUARY.

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Oysters have declined dramatically in the estuary since the 1800's, with high losses in recent years due to disease, pollution, and over-harvest. Our laboratory has involved concerned volunteers living throughout the Great Bay system in a variety of restoration projects. An Oyster Shell Recycling Program which provides a "drop-off" trailer located near an oyster harvesting area. After donation, oyster shell is stored and quarantined at UNH's Kingman Farm. In 2010 the Coastal Conservation Association greatly increased shell recycling using volunteers who provide shell "pick up" at local restaurants and markets. An Oyster Conservationist (OC) Program involving 30+ volunteers with access to a suitable dock who are trained to care for young oysters (spat) and raise them to the size needed for placement at the restoration sites in Great Bay. This program was started in 2006 by UNH and carried forward by The Nature Conservancy. In 2010, three Oyster Conservationists participated in the construction of oyster "mini-reefs". This new program expands OC outreach by engaging volunteers in the actual restoration and monitoring process. Together, these three programs have been highly successful in educating and involving the local community with oyster restoration efforts throughout Great Bay.

SETTING RESTORATION GOALS FOR MARYLAND'S OYSTER SANCTUARIES.

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Maryland recently expanded its oyster sanctuary network to include 24% of the oyster habitat remaining in the state's portion of Chesapeake Bay. While some sanctuary reefs are in good condition, others need restoration. We are attempting to set concrete, measurable restoration goals for the sanctuaries. These goals include the establishment of self-sustaining oyster populations, the accretion of shell, the supply of larvae to areas outside the sanctuaries, and the provision of ecosystem services such as water filtration and provision of habitat. Self-sustaining populations will require a balanced sex ratio, an oyster density sufficient to ensure a high fertilization rate, and reduced mortality in the presence of disease. Shell accretion must exceed loss to sedimentation and dissolution. Ecosystem services should follow from the establishment of viable oyster populations. Target population sizes may be based on the level of ecosystem service desired. In areas where establishment of a self-sustaining population is unlikely due to low salinity, ecosystem service goals may be achieved by regular seeding of reefs with hatchery-reared spat. The time frame to achieve the goals over this massive spatial scale is expected to be on the order of years to decades.

A HISTOPATHOLOGICAL EXAMINATION OF *HEMATODINIUM* SP. INFECTIONS IN BLUE CRABS (*CALLINECTES SAPIDUS*) FROM THE EASTERN SHORE OF VIRGINIA.

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Epizootics of the parasitic dinoflagellate, *Hematodinium* sp., have been reported in blue crabs in the seaside bays of the Delmarva Peninsula. The parasite infects the hemolymph of crabs

where it proliferates and ultimately kills its host. We examined the pathology caused by the parasite and documented histopathological alterations to the tissues of their blue crab hosts. Pressure necrosis was evident in the soft connective tissues of the hepatopancreas, the hemolymph sinuses within most organs, and soft connective tissues in the eyestalks. In heavy infections little remained of the soft connective tissues around the hepatopancreas; however, the reserve inclusion cells appeared relatively intact. The typical structure of most organs was altered by the proliferation of the parasite and subsequent edema and loss of spongy connective tissues. Damage to the gills varied, but in some cases it was severe involving an apparent thinning of the cuticular layers and loss of host epithelial cells. Affected lamellae exhibited varying degrees of hypertrophy with the loss of trabecular cells and swelling along the distal margins. In acute infections, blue crabs presumably die from tissue disruption because the reserve inclusions appear relatively intact, not metabolic wasting as is the case in more chronic types of infection.

LARVAL BAY SCALLOPS, *ARGOPECTEN IRRADIANS IRRADIANS*, GROW AND SURVIVE WELL WHEN FED LOW CONCENTRATIONS OF T-ISO, *ISOCHRYSIS* SP.

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Bay scallop, *Argopecten irradians irradians*, larvae were grown for six days and fed T-ISO, *Isochrysis* sp. concentrations ranging from 2,333–50,000 cells/ml. Phytoplankton were maintained at prescribed concentrations throughout the experiments utilizing an automated demand feeding system. In each experiment, daily water changes were made to maintain optimal water quality for larval growth. Larvae were stocked at 150,000 per15L of one micron filtered seawater held at 25 ± 1°C. Bay scallop larvae grew at similar rates independently of phytoplankton concentration. Even when larvae were fed on a once a day ration at a high cell concentration of 56,000 cells/ml we observed no significant difference in survival and shell length. There was a decrease in shell length variation of scallops grown at the single feeding concentration of 56,000 cells/ml. The decreased size variation observed could have major ecological and aquaculture implications. From an ecological viewpoint, when algal blooms are present there may be a narrower window available for settlement. Our recommendation would be to utilize low phytoplankton concentrations to feed larval bay scallops to conserve resources, increase profitability and maintain an efficient hatchery.

ECOLOGICAL, EVOLUTIONARY, AND PHYSIOLOGICAL PERSPECTIVES ON OCEAN ACIDIFICATION AND MOLLUSCAN SHELLFISH.

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Irrefutable evidence shows that the concentration of carbon dioxide in the earth's atmosphere is increasing as a consequence of human fossil-fuel combustion and that dissolution of a portion of this carbon dioxide into the oceans and other large water masses is causing changes in carbonate chemistry. Projected decreases in ocean pH and aragonite and calcite saturation states over the next century are raising concerns about the future abilities of calcifying invertebrates to produce skeletal structures, including shell formation in molluscs. Amidst a blizzard of experimental studies designed to anticipate the consequences of this climate-change component to molluscan species world-wide, relatively less attention is being paid to environmental conditions through which extant bivalve species have evolved and survived. The legacy of evolutionary responses of organisms to changing ecological conditions in the past can be found in the physiological processes and tolerances exhibited by species inhabiting a range of ecosystems in the present. I will describe and attempt to reconcile evolutionary, ecological, and physiological information about coastal bivalve molluscs in hopes of providing a context within which current, experimental studies can be evaluated.

COMPARISON OF GENETIC DIVERSITY IN DIFFERENT LIFE STAGES OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) WITH MITOCHONDRIAL AND MICROSATELLITE MARKERS.

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The blue crab *Callinectes sapidus* has been shown to have relatively high genetic diversity compared to vertebrates and also other invertebrates. This makes genetic comparisons between populations difficult to perform and results can often be unintelligible or even misleading. One major factor contributing to this difficulty is a lack of understanding the process of genetic inheritance in these populations for what is a highly migratory and possibly genetically unstable animal. The life stages of an individual blue crab can inhabit many different environments each with its own selective pressures. In order to begin to understand how the genetics of a blue crab population can change with each recruitment, individual megalopae, juveniles, and adults from different estuaries and years were genotyped for both a mitochondrial marker and four microsatellite loci previously shown to have high diversity in wild populations. Although computed diversity indices were similar for juvenile and adult populations, the megalopae had the same or higher levels of diversity in all cases.

Thus, a genetic expansion and contraction may be occurring with every recruitment cycle. Implications for genetic comparisons of populations as well as possible biological and environmental causes for these observed genetic differences are discussed.

INVASION BY QUAGGA MUSSELS (*DREISSENA ROSTRIFORMIS BUGENSIS* ANDRUSOV 1897) INTO LAKE MEAD, NEVADA-ARIZONA: THE FIRST OCCURRENCE OF DREISSENID SPECIES IN THE WESTERN UNITED STATES.

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On January 6, 2007, adult quagga mussels (*Dreissena bugensis*) were found in Lake Mead, the largest reservoir by volume in USA. Adult and juvenile mussels were first detected at a concession operated marina in Boulder Basin, the lower basin of the lake. In 2007, the average mussel density was 505 mussels/m^2. There were more mussels on rocks than silty areas. Size frequency of mussels collected from different locations in 2007 demonstrated that there were three to four cohorts with shell length from less than 1 mm to 25 mm. Quagga mussel veligers were present year round. Average veliger numbers from March 2007 to March 2008 were 6.6/L in Boulder Basin, 0.7/L in the Overton Arm, 0.3/L at Temple Bar area, and 0.04/L in Gregg Basin, the upper basin of Lake Mead. During 2007, veligers peaked in July and October in Boulder Basin, while in the middle basins of this reservoir only the October peak was found, although at much lower numbers than in Boulder Basin. The results of the veliger counts demonstrate that adult mussels first established in Boulder Basin before reaching upper basins of the lake. This matches the result of abundance and distribution of adults and juveniles.

ENTERIC VIRUSES IN SHELLFISH: RESEARCH PROSPECTIVE AND OUTBREAK ANALYSIS.

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A significant portion of infectious foodborne illnesses in the US is attributed to human enteric viruses and norovirus is the most common etiological agent identified in viral gastrointestinal illnesses. Bivalve molluscan shellfish can act as vehicles for transmission of enteric viruses. Viruses accumulate in shellfish digestive diverticula and can infect humans upon ingestion of raw or undercooked shellfish meat. The use of bacterial indicators as a predictor of fecal contamination in shellfish growing areas reduced bacterial gastrointestinal infections but this practice is believed to have limited predictive value for enteric viral pathogen contamination. The Food and Drug Administration Gulf Coast Seafood Laboratory has developed methods for concentration and detection of enteric viruses from molluscan shellfish. These methods have been utilized in field and retail studies and for analysis of shellfish implicated in outbreaks. Validation and inclusion of these methods in the Bacteriological Analytical Manual will provide regulatory analysts the tools necessary to conduct field and outbreak investigations of food concerning enteric virus contamination.

DEVELOPMENT OF TOOLS TO FACILITATE THE PROTEOMIC ANALYSIS OF OYSTER PLASMA AND EXTRAPALLIAL FLUID.

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Proteomics targets an entire set of proteins involved in a life process and forms a necessary complement to transcriptomic approaches in functional genomic research. Oyster plasma proteome contains proteins produced by cells in the entire body. Analysis of changes in this proteome in response to different stressors using proteomic approaches will efficiently generate information about physiological and pathological processes in oysters. Moreover, the generation of substantial amount of oyster genomic sequence data during the last few years is greatly facilitating proteomic research. However, oyster plasma proteome has an extremely broad range of protein concentration, which is far beyond the detection power of proteomic technology whether it is two-dimensional electrophoresis or mass spectrometry-based. Dominin, for example, constitutes nearly 50% of the total oyster plasma proteins. In contrast, cvSI-1, a serine protease inhibitor, is 4 to 5 $\log_{10}$ orders lower in concentration than dominin. Interestingly, dominin also represents the most abundant protein of extrapallial fluid. Thus development of tools to pre-fractionate proteins will permit the accurate measurement of proteins with different abundance and facilitate the full proteomic analysis of oyster plasma and extrapallial fluid.

CV-LYSOZYME-1 RECOMBINANT EXPRESSION AND 13C/15N-LABELING FOR NMR STRUCTURAL DETERMINATION USING *PICHIA PASTORIS* YEAST PROTEIN EXPRESSION SYSTEM.

Qinggang Xue, **Yanli Li**, **Richard Cooper**, **Jerome La Peyre**.

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Cv-lysozyme-1 purified from the eastern oyster (*Crassostrea virginica*) plasma is biochemically unique and likely has potentials for commercial uses as an antimicrobial. Structural study of

cv-lysozyme-1 will help understand the unique characteristics of this oyster lysozyme. However, cv-lysozyme 1 purification from oyster plasma is time-consuming and the purified protein cannot be labeled easily with heavy isotopes for structure determination using nuclear magnetic resonance spectroscopy (NMR). Therefore, a recombinant expression system using the *Pichia pastoris* yeast protein expression system was developed to produce cv-lysozyme-1. We constructed two recombinant vectors using the native oyster lysozyme signal sequence or the α-factor signal sequence to transform yeast cells. The results indicated that the α-factor signal sequence worked well for the secreted expression of cv-lysozyme-1 whereas the oyster native signal sequence did not. A transformed yeast cell of methanol utilization slow (Mut^s) phenotype was eventually selected, which produced about10 mg of cv-lysozyme-1 in 1 liter of culture supernatant. The expressed recombinant cv-lysozyme-1 was identical to that purified from oyster plasma in molecular size as detected by SDS-PAGE and in enzyme kinetic characteristics. The recombinant expression system is now being used for $^{13}C/^{15}N$-labeling of cv-lysozyme 1 so that the protein structure can be determined using NMR.

IMPROVED TOOLS FOR INVESTIGATING NATURAL SELECTION IN BLUE CRAB POPULATIONS.

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While most surveys of genetic variation in the blue crab (*Callinectes sapidus*) have found widespread genetic uniformity among locations, one early allozyme study uncovered significant temporal and spatial genetic differentiation on a regional scale. An increasing number of studies have found similar patterns for other meroplanktonic species, which could be explained by natural selection acting on dispersing larvae. Further evidence of selection has followed recent developments in the detection of the effects of natural selection on genetic variation. Single nucleotide polymorphisms (SNPs) are particularly useful subjects for tests of natural selection when they occur in genes that are likely to be the targets of selection. The current research is focused on novel SNP markers that are located in genes associated with environmental adaptation and detoxification to investigate selection in blue crab populations in Louisiana and throughout the species' North American range. Preliminary results identify significant differentiation among some locations and SNPs that are candidates of selection. We will discuss the implications of these findings in the context of conservation and management and review the advantages of using SNPs for population genetic studies.

ASSOCIATION BETWEEN THE SERINE PROTEASE INHIBITOR GENE AND DISEASE RESISTANCE IN THE EASTERN OYSTER.

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A serine protease inhibitor from the eastern oyster, cvSI-1, has been shown to inhibit the proliferation of the Dermo pathogen *Perkinsus marinus* in vitro. In this study, we identified polymorphism in *cvSI-1* and studied its association with disease resistance in the eastern oyster. Full-cDNA sequence of *cvSI-1* was sequenced in a diverse panel of oysters, revealing 12 single nucleotide polymorphisms (SNPs) in the 273 bp coding region: five were synonymous and seven non-synonymous. The Dn/Ds ratio, 1.4 SNPs, suggests that *cvSI-1* is under positive selection. Selected SNPs were genotyped in families before and after disease-caused mortalities, disease-resistant and susceptible strains as well as wild populations with different disease exposure histories. At SNP198, the C allele consistently increased in frequency after Dermo-caused mortalities. Its frequency in the disease-resistant strain is significantly higher than that in the susceptible strains and the base population. In wild populations, frequency of the C allele was positively correlated with their disease history. Furthermore, within the same estuary, areas with high prevalence of diseases had more C alleles than that with low disease pressure. These results indicate that polymorphism at *cvSI-1* is associated with Dermo resistance in the eastern oyster.

FRIENDS OR FOES? ECOLOGICAL INTERACTIONS BETWEEN NATIVE OYSTERS (*CRASSOSTREA VIRGINICA*) AND A NEWLY INTRODUCED SPECIES.

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In recent years, the number of introduced species has greatly increased in the marine environment, largely due to hull fouling and releases of ballast water. One such species that has been found along coastlines of the southeastern United States is the pink acorn barnacle (*Megabalanus coccopoma*). More specifically, this species has been found to cohabitate with the eastern oyster (*Crassostrea virginica*), an ecosystem engineer and keystone species, in Mosquito Lagoon, along the east coast of central Florida. Very little research has been done to understand how this anthropogenically introduced species interacts with the native eastern oyster in the novel range. We hypothesized that the pink acorn barnacle would limit the survival and growth of native oysters and potentially out-compete them. We tested the competitive interactions of survival and growth between this introduced species and native oyster spat. Thus far, our data indicate that oyster spat survival was significantly reduced in the

presence of the pink barnacle (contingency table analysis: $p < 0.005$). However, oyster spat growth was not significantly affected at the end of six week trial (ANOVA: $p = 0.5245$). These results can be applied in predicting the potential displacement of native structure-forming fauna.

RESTORATION OF OLYMPIA OYSTERS: OYSTER SETTLEMENT, SURVIVAL, GROWTH, AND COMMUNITY BIODIVERSITY ON CONSTRUCTED OYSTER BEDS.

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The Olympia oyster has experienced substantial population declines throughout its range (from Sitka, AK to Baja California, Mexico) since the early 1900's. We are testing the effectiveness of different restoration techniques for this species in southern California. We hypothesized that Olympia oyster larvae would settle, survive more and grow faster, and that epifaunal community diversity would be higher, and infaunal diversity would be lower on thick, consolidated oyster beds compared to thin, unconsolidated beds or compared to control mudflat plots. To test our hypotheses, we augmented intertidal mudflat with dead *Crassostrea gigas* shell into replicate ($n = 5$) 2 m $\times$ 2 m plots of two thicknesses, 4 cm or 12 cm, and two types, bagged/consolidated shell or loose shell (plus 5 control plots = 25 plots). Reefs were constructed in June 2010. We compared larval settlement among treatment and control plots using settlement arrays deployed onto each plot for two-week periods over summer and monthly periods in fall 2010 and winter. We are also measuring oyster survival and growth rates, bed degradation, and are monitoring community biodiversity before and after (at 0, 6, 12, 18, and 24 months) construction of experimental plots. Results will contribute to the design of future restoration efforts in southern California.

VARIABILITY IN EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, FEEDING BEHAVIOR IN A EUTROPHIC ESTUARY

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Bivalve suspension feeding results in the removal of water column particulates and promotes the transfer nutrients to the benthos. This ecological service (i.e. benthic-pelagic coupling) is often cited as an argument for oyster restoration. Management actions in the Hudson-Raritan estuary (NY/NJ) include a restoration plan that creates oyster reefs to improve ecosystem functioning. We predict that seston characteristics, influenced by nutrients and seasonal change, will drive oyster feeding and waste composition and thus benthic-pelagic coupling. Oysters were deployed in May 2010 at four sites in Jamaica Bay, NY along a nutrient gradient. We quantified oyster filtration, ingestion, absorption, selection and absorption efficiency using the *in situ* biodeposition method. Growth and condition index was also measured. Oyster clearance rates were negatively correlated with the high total particulate loads typical of high nutrient sites. In addition, feeding rates and efficiencies were low during spawning events and when food quality was poor. Our results show that feeding behaviors considered desirable from a management perspective will only be maximized under conditions dictated by controls from exogenous (i.e., particle load and food quality) and endogenous (i.e., spawning events) factors.

PASSIVE ACOUSTICS AS A MONITORING TOOL FOR EVALUATING OYSTER REEF RESTORATION.

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Passive acoustics uses naturally occurring sounds produced by marine organisms to study their behavior, biology, and location. Ambient marine sounds are known to vary from place to place, and these sounds can be used to detect differences in habitats. Oyster toadfish, naked goby, mud crabs, and snapping shrimps inhabit oyster reefs, and they are known to produce sounds. In an oyster reef, the combination of sounds produced by organisms' communication, feeding, or moving, may produce a unique acoustic signature. Therefore, individual acoustic signatures of oyster reefs may convey information about the habitat quality and the organisms that inhabit them. Three sites along the Saint Lucie Estuary, Florida were acoustically monitored. Restored and natural reefs were recorded for 5-minutes using a hydrophone. Acoustic signatures were compared using spectra (frequency (Hz) vs. intensity (dB)) overlays. Preliminary results showed that shortly after restoration, acoustic signatures from the natural and restored reefs differed. As time progresses, the acoustic signature of a fully restored reef may resemble that of the natural reef, representing a convergence of restored and natural habitats. Passive acoustics has the potential to provide a new methodology to rapidly monitor oyster reefs and other ecosystems, such as coral reefs and rocky reefs.

THE OYSTER GENOME PROJECT: AN UPDATE ON ASSEMBLY AND ANNOTATION.

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A relatively small genome size and economical and ecological importance make the Pacific oyster *Crassostrea gigas* a good candidate for genome sequencing. This project was initiated to sequence the whole genome of the Pacific oyster, using a combination of traditional Sanger and next-generation sequencing technologies: Illumina for deep coverage and Sanger sequencing of fosmid ends for connectivity. To date, we have obtained more than 200 × deep shotgun sequences with Illumina and end-sequences of 450,000 fosmid clones with Sanger. To overcome problems resulting from the extremely high polymorphism of the oyster genome, about 3,000 pools of 160,000 fosmid clones were sequenced. Sequences from fosmid pools were integrated with fosmid-end and shotgun sequences. Transcriptomes from seven tissues including hemolymph, digestive gland, gonad, gill, mantle, labial palp, adductor muscle, and one mixture of all eight tissues were analyzed by RNA-Seq. Currently, around 32000 genes were predicted with the combination of *de novo* and homolog annotation. Gene expression levels were estimated and alternative splicing was identified in different tissues. Genomic basis for nacre formation, stress and immune response, bivalve evolution, reproduction, high polymorphism and sex determination are being analyzed. Latest results from assembly and annotation analyses will be presented at the meeting.

THE GENOMIC UNDERPINNINGS OF APOPTOSIS IN *CRASSOSTREA GIGAS*.

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Lophotrochozoa is considered to be the basal Bilateria, however the apoptosis gene repertoire of this clade is still elusive. The survey of apoptosis-related genes in the recent sequencing pacific oyster genome (version 4) and its developmental expression profiling data reveal some interesting novelties. First, CED-4/Apaf-1 had paralogs in the pacific oyster genome, but with special domain organization. Second, the pacific oyster has unusually large number of proteins containing anti-apoptotic domain, which suggests the complexity of apoptotic regulatory network in the oysters. Third, the complexity of caspase protein families appears greater than that found in protostome model systems. Fourth, the large number of death receptor homologues suggesting significant gene loss in nematodes and insects. Fifth, the expression level of four selected typical apoptosis-related genes in the hemolymph increased dramatically in the presence of the bacteria, *Vibrio anguillarum*, indicating their role in bacterial defense. Finally, expression profiling data reveals that apoptosis-related genes present a gradually increase expression pattern during development, but fluctuates in certain period. Our results suggest the complexity of the oyster apoptosis system, which cannot be represented by model invertebrates. Moreover, their unique features may relate to specific requirements imposed by immunological defense and/or life cycle complexity of this organism.

THREE NEW RECORD SPECIES OF SMALL SHELLFISH IN SWAN LAKE IN RONGCHENG BAY, SHANDONG, CHINA.

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The small-gastropods are abundant on algae in intertidal and subtidal zones, but research on small-shellfish in China is still in blank. In a recent study, the author carried out biological investigation to the intertidal zone of the Swan Lake in Rongcheng Bay of China, and collected some small shellfish on the root parts and leaves of *Zostera marina* Linn growing in the lake, and the length of the shell is between 0.4 mm and 2 mm; and by sorting out these small shellfish, the author found out three new record small-gastropods species in China, belonging to two families and three genera, which are respectively *Alvania concinna* (A. Adams, 1861) and *Lucidestea cf. matusimana* (Nomura, 1940) in Rissoidae, and *Barleeia angustata* (Pilsbry, 1901) in Barleeidae.

THE INFLUENCING FACTORS OF TUBE BUILDING BEHAVIOR OF *POLYDORA CILIATE* JOHNSTON, 1838 (ANNELIDA, POLYCHAETA): A SPIONID PLOYCHAETE INFESTS SCALLOPS *PATINOPECTEN YESSOENSIS* IN CHINA.

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The scallop *Patinopecten yessoensis* is suffering a great economical loss by infestation of a spionid ploychaete, *Polydora ciliate* in China. Our study show that the larvae built mud tubes on shells

when they metamorphosis. During our field investigation, we found that tubes were composed of mucus and fine sand particles. The tube, which connected to the burrow and was about 1cm in height above the shell, could enlarge polydorids' movement scope. Thus, tubes were very important for the polydorids during their life history. However, it was found that they could use the bryozoan colonies (calcium carbonate) instead of mud tubes when there was little silt. A research was also made about relationship between the sediment grain size and the average number of *P. ciliate* on one scallop by using curvilinear regression analysis.

HEMATODINIUM SP. INFECTIONS IN THE BLUE CRAB *CALLINECTES SAPIDUS* FROM THE NORTHERN GULF OF MEXICO

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Hematodinium sp. is a complex of parasitic dinoflagellates that infect different decapod crustaceans throughout the world, including the blue crab *Callinectes sapidus* in the United States. A survey was conducted from 2004–2008 to identify and monitor the prevalence of *Hematodinium* sp. in wild caught blue crabs in the Northern Gulf of Mexico by PCR and histology. The parasite displayed a cyclical pattern of occurrence with a significant downward trend over the 4 yr of the survey. Annual prevalence of the parasite was greatest during the season comprising the months of November through February. Logistic regression analysis revealed temperature, carapace width, and season as significant risk factors associated with infection. Salinity was not a significant risk factor. Infected blue crabs occurred at all survey sites regardless of salinity, and reached a prevalence of 90% at low salinities (3 ppt). Laboratory transmission to crabs was effected by injection as well as by cannibalism. Median log lethal injection dose exposure was 6.3 organisms (95 % CI = 6.2 – 6.5). No exposed crab survived. Mean survival time was 39.9 d (95 % CI = 31.1 – 44.7 d).

DEVELOPMENT OF HATCHERY TECHNOLOGY FOR THE PRODUCTION OF BLUE CRAB (*CALLINECTES SAPIDUS*) JUVENILES FOR BASIC SCIENCE AND APPLIED STUDIES

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Responding to the rapidly declining abundance and harvests of the blue crab in the Chesapeake Bay, a multidisciplinary program was developed to (1) study the basic biology and life cycle of the blue crab, (2) develop hatchery and nursery technologies for mass production of crab juveniles, and (3) assess the potential of using hatchery juveniles to conduct ongoing studies toward a more comprehensive understanding of the fishery and improved management.

Understanding the environmental regulation of the reproductive cycle resulted in full photo-thermal control of the timing of ovulation and hatching of wild-caught inseminated females. As a result, consortium scientists were able to obtain crab larvae year-round. Mass production of crab juveniles was accomplished via intensive larval rearing using high nutritional value foods, resulting in up to 80% survival from hatch to Z8 larvae. Z8 larvae were collected, restocked at lower density and reared to C3-C4 stage (~8–12 mm CW), resulting in up to 35% survival. Post-sorting, juveniles were stocked in ponds at low densities and harvested as releasable size crabs (C6, ~20 mm CW). Survival rates at this point ranged from 50–90%, depending on multiple factors. During 2002–2008, approximately 800,000 hatchery-reared juveniles were released into Bay waters for consortium studies.