4-2012

Abstracts of Technical Papers, Presented at the 104th Annual Meeting, National Shellfisheries Association, Seattle, Washington, March 24–29, 2012

National Shellfisheries Association

Journal of Shellfish Research, Vol. 31, No. 1, 231, 2012.

ABSTRACTS OF TECHNICAL PAPERS

Presented at the 104th Annual Meeting

NATIONAL SHELLFISHERIES ASSOCIATION

Seattle, Washington

March 24–29, 2012

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AN ANALYSIS OF TYPE IV PILI IN *VIBRIO PARAHAEMOLYTICUS* AND THEIR INVOLVEMENT IN PACIFIC OYSTER COLONIZATION.

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Bacteria, *Vibrio parahaemolyticus*, are natural inhabitants of coastal waters worldwide and cause gastroenteritis in humans, typically resulting from contaminated seafood, particularly raw oysters. Although several *V. parahaemolyticus* virulence factors for human disease have been identified, little is known about bacterial proteins that are important in the association of the bacteria with oysters. Type IV pili are often contributors to adhesion and biofilm formation in bacteria and might play a role in *V. parahaemolyticus* interactions with oysters. Genomic analyses of Type IV pili genes in *V. parahaemolyticus* revealed interesting sequence variations. In this study, the Type IV pili processing protein, PilD, and two Type IV pilins, MSHA and PilA from *V. parahaemolyticus* were assayed for their role in biofilm formation and oyster colonization. The *mshA*- and *pilA*- strains exhibited a decrease in biofilm formation consistent with a previous report, whereas the *pilD*-strain showed a drastic reduction in biofilm formation compared to the wild type. When tested in a Pacific oyster infection model, differences in initial colonization, as well as long-term depuration, were observed for some of the mutant strains. Establishing a role of Type IV pili in *V. parahaemolyticus* colonization of Pacific oysters could ultimately lead to novel intervention strategies.

MULTIPLE GENE SEGMENTS ISOLATED BY NEXT-GENERATION SEQUENCING INDICATE EXTREME DIVERGENCE OF *MIKROCYTOS MACKINI*.

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Mikrocytos mackini is a 'microcell' protistan parasite of Pacific oysters found on the Pacific coast of North America. Molecular diagnostics development is hindered by a lack of knowledge about its evolutionary history. The three other described microcell parasites (genus *Bonamia*) belong to the phylum Haplosporidia; however, several *M. mackini* features argue against a close relationship to these. SSU-rDNA does not resolve the phylogenetic position of *M. mackini*, so we aimed to build a multi-gene phylogeny to determine its position in the overall eukaryotic phylogeny. DNA was prepared from parasites concentrated from host material. Next-generation sequencing was used to generate a genome sequence survey and all potential oyster sequences were removed using the large mRNA sequence dataset from *Crassostrea* in Genbank. Surprisingly few remaining genes were identifiable: only seven protein-coding genes of *M. mackini* were unambiguously identified. After extending these sequences by 3' and 5' RACE, the *M. mackini* dataset included >6500bp of DNA sequence coding for >2000 amino acids. While this is a great improvement over the previous dataset for phylogenetics consisting only of SSU, phylogenetic analyses remain inconclusive as all *M. mackini* genes are uniquely divergent. Analyses suggest a relationship with Rhizaria, but this conclusion is not strongly supported.

A JOURNEY TOWARDS MOLECULAR DIAGNOSTICS FOR AN UNCLASSIFIABLE INTRACELLULAR MICROCELL PARASITE OF PACIFIC OYSTERS (*CRASSOSTREA GIGAS*), *MIKROCYTOS MACKINI*.

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Mikrocytos mackini is a microcell parasite of Pacific oysters and is a Reportable disease in Canada. Molecular assay development has been hampered by the paucity of information on its diversity, distribution, and phylogenetic position. Further, *M. mackini* is not culturable, is temperature-dependent, its life cycle is unknown, and it has no known relatives. We initiated multiple avenues of research to redress some of these knowledge gaps and support future molecular diagnostics development. We attempted to resolve the phylogenetic placement of *M. mackini*using data from multiple genes isolated from a genome sequence survey using next generation sequencing. Intriguingly, the phylogenetic placement of *M. mackini* remains unclear; it appears to be uniquely divergent from all other taxa. Concurrently, we isolated ITS1-5.8S-ITS2-rDNA of *M. mackini*, which evolves faster than SSU-rDNA and may therefore offer a more suitable target for PCRassays. We sequenced this region from over 70 *M. mackini* samples collected throughout its known geographic range to characterize extant diversity, and interpreted the complete absence of within-species sequence variation by two, alternative evolutionary hypothesis. We generated 1903 bp of rDNA sequence from *Mikrocytos* sp., and discovered regions of high divergence from *M. mackini* that will be useful for molecular diagnostics.

ACOUSTICALLY TAGGED FISH UTILIZATION OF AN ARTIFICIAL REEF CONSTRUCTED FOR NATIVE OLYMPIA OYSTER RESTORATION.

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Two artificial reefs constructed in San Francisco Bay as part of a native Olympia oyster (*Ostrea lurida*) restoration program were set up with Vemco acoustic receivers in order to determine if

acoustically tagged salmonids used the reefs. The results of three years of monitoring suggest the reefs in the north west side of San Francisco Bay were used extensively by acoustically tagged late fall run Chinook salmon (*Oncorhynchus tshawytscha*) and green sturgeon (*Acipenser medirostris*). The timing and duration of visitation are discussed in the context on reef age and environmental factors. A second reef constructed on the east side of San Francisco Bay was used extensively by striped bass. There were fewer detections of salmonids, green sturgeon and tagged sharks. The repeated visitation by some striped bass suggests utilization of the reef structures as a seasonal territory.

WHAT INDUCES *DREISSENA BUGENSIS* TO SPAWN UNDER LABORATORY CONDITIONS?

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One of the challenges in life history studies on *Dreissena bugensis* is to understand adult fecundity, larval recruitment and mortality, all of which are crucial for control of the species. There are few studies regarding the induction of spawning and egg production for *D. bugensis* under laboratory conditions. In this study, we attempted to induce spawning of *D. bugensis* through external application of serotonin, temperature shock, and gonad slurry. In separate trials, we tested field-collected mussels under each of these three conditions, recording the success or failure to produce gametes. The results suggested that temperature shock was the least effective of the three treatments, whereas serotonin was the most effective. Reproduction in response to temperature shock was slightly increased with the addition of gonad slurry, but still much lower than for serotonin. Our results show that serotonin exposure leads to consistent and dose-sensitive spawning of both male and female *D. bugensis*.

AN ECONOMIC DESCRIPTION OF THE FLORIDA SHELLFISH CULTURE INDUSTRY.

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The shellfish culture sector represents an important component of the commercial marine aquaculture industry in Florida. The industry is primarily composed of hard clam hatcheries, nurseries and grow-out operations. However, other candidate species are currently being examined as viable additions to the industry portfolio. Providing a current assessment of the industry, in terms of number of growers, acres under cultivation, seed sales, clams harvested, grower revenues, average prices, and other descriptors is problematic given the recent cessation of periodic statewide industry survey efforts. Such information is needed to provide an accurate estimation of the collective economic importance of shellfish culture on a national basis. Some current descriptive information and historical data are available to provide insight into the local/statewide economic presence and importance of the industry. The most recent secondary data will be presented that describe the shellfish culture industry in Florida. In addition, historical and recent economic impact assessments will be presented and changes over time, in terms of pricing, out of state sales, key market channels, and other factors, will be discussed.

ESTIMATING DISCARD RATES IN ESTUARINE RECREATIONAL CRAB FISHERIES USING SIMULATED FISHING.

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Most of Oregon's estuaries support year-round recreational crab fisheries targeting Dungeness and red rock crab. Fishery participants use rings and/or traps to harvest crabs from small boats or from public fishing piers. Non-target crabs are generally released back into the water for two reasons: regulatory (male Dungeness crabs below the legal size limit and all female Dungeness crab) or preference (soft-shelled legal-sized male Dungeness crab or unwanted red rock crab). Discarded crab could have elevated short-term mortality due to handling, and a better understanding of discard rates will be valuable for managing the fishery. Oregon Department Fish and Wildlife staff has conducted a multi-year simulated fishing study to estimate discard rates in the estuarine recreational crab fishery. The simulated fishing study mimics the gear and methods of the fishery and has provided data such as sex ratios, shell hardness, and size frequency to illustrate seasonal cycles and spatial variations found in the catch. In addition, the fishery is monitored by another multi-year independent survey that estimates total recreational fishery catch. By

combining data from these two projects, we have estimated the total number of crab retained by the fishery, as well as crab discarded for regulatory or preference reasons.

WATER CHEMISTRY, LARVAL OYSTERS, AND OCEAN ACIDIFICATION IN A COMPLEX, URBANIZED ESTUARY (PUGET SOUND, WASHINGTON).

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Recent observations have revealed subsurface marine waters in Puget Sound that are undersaturated with respect to aragonite throughout the year. Decreasing oyster populations and high mortality in larval stages throughout the Pacific Northwest have suggested that ocean acidification may be changing water chemistry and causing the observed shellfish declines. To examine linkages between water chemistry and oyster settlement, we collected water and biological samples during the growing seasons of 2009 and 2010 at two index stations in Puget Sound (Dabob Bay and Totten Inlet). During both sampling years, partial pressures of CO_2 (pCO_2) were higher at Totten Inlet, and saturation states and pH values substantially lower. In 2010, pCO_2 levels were much lower and pH and saturation states higher at both stations than in 2009. Comparison between the two index stations suggests that the dominant controls on water chemistry at Dabob Bay are physical (upwelling), whereas at Totten Inlet, biology dominates nearshore carbon chemistry. Decreased pH and saturation states at the end of summer or early fall appear to coincide with the end of larval oyster settlement and the transition of the dominant shell mineralogy in juvenile oysters from aragonite to calcite, the less soluble form of calcium carbonate.

OLYMPIA OYSTER STOCK AND HABITAT RESTORATION IN PUGET SOUND: A COMMUNITY-LEVEL EXAMINATION OF POPULATION AND HABITAT DYNAMICS FOR A RESTORATION AREA.

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A prime rationale for native oyster enhancement efforts Washington State is the development of nearshore habitat types that then benefit the estuary and provide refuge and forage space to native fishes and a rich invertebrate community. Here we examine the shift and succession of this community, including *Ostrea lurida*, as it develops within a large-scale, 6-year old ongoing habitat enhancement. Presented topics include observed recruitment dynamics for oysters, completion and predation, microhabitat conditions and ''age''effects of the emergent structured substrate. The enhanced habitat and community in the restoration area will be compared by similar measures collected in natural oyster beds; and the efficacy of habitat enhancement as a stock restoration tool will be discussed along with recommendations for appropriate siting.

WHAT CONSTITUTES NORMAL FOR TETRAPLOID *CRASSOSTREA VIRGINICA*?

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According to the latest survey, over 90% of growers in Virginia are choosing triploid *C. virginica* as their cultivar. Because the Aquaculture Genetics and Breeding Technology Center is the principal supplier of brood stock to make triploid oysters, we must have a reliable supply of tetraploids. Tetraploid x tetraploid spawns have been characterized by their wide variation. In 2010 we began to systematically look at individual spawns of tetraploids to try to determine what is normal. Twenty families of tetraploids were produced (5 individual families among 4 founder families) and 12 were deployed to the field for evaluation of survival, growth, and ploidy, specifically reversion. At 16 months, survival was not significantly different among families and between replicates. All ranged between 47 and 81% survival. For growth, there were significant differences among individual families and family groups for both length and weight. Average sizes among families ranged from 47-56mm and 16-24g body weight. Ploidy analysis had not been completed yet. The variance within and between family groups can be used to estimate broad sense heritabilities for these traits.

STATUS OF THE GEODUCK CLAM, *PANOPEA GLOBOSA* AND *P. GENEROSA* (BIVALVIA: HIATELLIDAE) FISHERIES, BIOLOGY AND ECOLOGY IN NORTHWEST MEXICO.

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There are two species of geoduck under exploitation in Northwest Mexico: *Panopea generosa* in the Pacific coast of the Baja California Peninsula and *P. globosa* in the Gulf of California. This study describes status of the geoduck clam fishery in Northwest Mexico. Recent surveys suggest a wide distribution of beds along both coasts of Baja California and Sonora. Production has increased from 49 t in 2002 to greater than 1,200 t annually during the period 2006-2011. The fishery has a current annual value around US$30 million. In Sonora, stock assessments estimate the potential yield to be approximately 1800 metric ton. There is an increasing interest of fishermen from the south of Sonora to enter into this fishery and therefore we have conducted surveys in 24 beds; in two beds no individuals were found. From February 2009 to October 2011 a total of 1,293,499 pieces have been authorized for extraction. Long life cycle, low recruitment rate, and high fishing rate threaten the viability of this resource unless immediate management actions are taken.

SUMMER MORTALITY OF *CRASSOSTREA GIGAS* AND PREVIOUS WINTER'S CLIMATIC CONDITIONS.

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Summer mortalities of the Pacific oyster in France started in late 1990s. While high temperature and other environmental stressors may be contributing factors, mortalities are mainly associated with high reproductive output. Consequently, triploid oysters are less susceptible than diploids. Samain *et al.* (2007) noticed that wet and warm winters are often followed by high summer mortalities. Runoff of land-based contaminants during a rainy winter has been proposed as one explanation. Another hypothesis can be suggested based on trophic considerations. Rainy winters provide good conditions for phytoplankton blooms during the oyster energy storage period. We simulated in mesocosm two winter conditions for high and low trophic levels. After this pre-conditioning, gonia proliferation was quantified with PCNA expression, and animals were then subjected to a second conditioning with high food supply at increasing temperatures as typically done in hatchery broodstock conditioning. Reproductive effort was evaluated by quantitative histology. Strong thermal shock was given to oysters following both treatments and any mortalities quantified. Relationship between trophic level, energy storage and utilization during gametogenesis have been demonstrated and regulated by an insulin signaling pathway. From analysis of the transcriptome, we have identified genes involved in this pathway and the expression supports the role in this physiological process in oysters.

SENSORY-MOTOR INTEGRATION OF GILL LATERAL CILIA IN THE BIVALVE MOLLUSC, *CRASSOSTREA VIRGINICA*.

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Lateral gill cilia of *Crassostrea virginica* are controlled by a serotonergic-dopaminergic innervation. The motor aspects have been well studied but the sensory side has not. We examined effects of sensory cues on beating of gill lateral cilia of *C. virginica*. Irritating the mantle caused a 70% drop in beating rates. Similarly, shining a light on the mantle rim decreased beating by 50%. Applying crab extract reduced rates to zero. Cutting the branchial nerve prevented the crab extract from having an effect. When the cerebrovisceral connective tissue was cut the basal cilia rate was lower than controls and the crab extract was still effective in slowing beating rates. The mantle rim is a major site of sensory cells in the animal. Excising the rim prevented the crab extract from affecting the cilia beating rates. The study demonstrates a sensory-motor integration of the beating rates of the lateral cilia that involves the sensory rim of the mantle and the visceral and cerebral ganglia. It appears that the animals may be interpreting the sensory cues as hostile. In their natural environment, oysters would then close their shells, reducing their water pumping rates with a corresponding drop in cilia beating rates.

THE STRUCTURE AND IMMUNE FUNCTION OF HEMOGLOBIN GENES FROM BLOOD CLAM *TEGILLARCA GRANOSA*.

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Hemoglobin (Hb) is the iron-containing oxygen transport metalloprotein found in red blood cells. Hb is rare in molluscs, where most species have copper-containing hemocyanin instead. Here, three unique and highly compartmentalized blood clam Hb globin genes (Tg-HbI, Tg-HbIIA and Tg-HbIIB) are cloned and characterized. All three proteins have the conserved characteristics of a functional globin fold. The three genes are highly similar in amino acid sequence and protein structure but considerably

different in DNA sequence. Divergence between molluscan Hbs and other Hbs is high and may reflect the complex evolution of Hb proteins and potential differences in their biological functions. A qRT-PCR assay was developed to measure the expression of globin genes in different clam tissues and their temporal change after clams challenged with *Vibrio parahaemolyticus*, lipopolysaccharide and peptidoglycan. As expected, high levels of expression were detected in hemocytes, with low expression in other tissues. Expression of all three genes significantly increased 6-12h after challenge, and decreased thereafter, although expression level and peak varied among different genes and pathogenic factors. These results indicated Hb is an acute-phase, inducible protein that plays an important role in the immune responses against gram-negative and gram-positive bacteria in the blood clam.

TREATY SHELLFISH MANAGEMENT ON PRIVATELY OWNED TIDELANDS.

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Federal District Court Judge Edward Rafeedie ruled the treaties' "in common" language meant that the tribes had reserved harvest rights to half of all shellfish from all of the usual and accustomed places, except those places "staked or cultivated" by citizens – or those that were specifically set aside for non- Indian shellfish cultivation purposes. His decision requires tribes planning to harvest shellfish from private beaches to follow many time, place, and manner restrictions on harvest. The Suquamish Tribe initiated harvest on privately owned tidelands in 2004 after substantial intertidal areas of Dyes Inlet in Central Puget Sound were re-classified by the Washington State Department of Health (WDOH). The Tribe has worked cooperatively with local agencies since 1998 to open beaches with good commercial shellfish harvest potential in the Suquamish exclusive Usual and Accustomed (U&A) Area. Since then the Tribe has surveyed and harvested natural beds of Manila and Littleneck clams on over 200 private beaches. Harvest management of the private tidelands' treaty share is based on a three to four year rotation cycle depending on recruitment. Success and challenges of private tideland treaty share management will be discussed.

THE PCSGA MONITORING PROGRAM: UTILIZING ENVIRONMENTAL MONITORING TO ENHANCE OYSTER SEED PRODUCTION IN COMMERCIALLY IMPORTANT BAYS OF THE PACIFIC NORTHWEST.

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Dramatic mortality events observed in commercial shellfish hatcheries since 2007 have led to a significant decrease in seed production, and pose an ongoing threat to the Pacific Northwest shellfish industry. Preliminary research indicates a strong correlation between these mortality events and the intrusion of upwelled, acidified seawater into historically productive bays along the Oregon and Washington coast. The Pacific Coast Shellfish Grower's Association (PCSGA) has developed a comprehensive monitoring program, extending from Northern Puget Sound to Central Oregon, with sites positioned at the exact locations of commercial hatcheries, and in areas of historically high natural recruitment. Continuous monitoring of high resolution pH, pCO_2, temperature, salinity, and dissolved oxygen at each site provides hatchery personnel the predictive capacity to maintain production in the face of challenging, and highly dynamic, seawater conditions, and the extensive larval performance data routinely collected at these sites offer an unprecedented opportunity to understand the effects of seawater chemistry on sensitive larval species in today's real ocean.

TOXIC EFFECTS OF *HETEROCAPSA CIRCULARISQUAMA* ON THE SHORT-NECK CLAM, *RUDITAPES PHILIPPINARUM*.

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The toxic dinoflagellate *Heterocapsa circularisquama* forms recurrent blooms in western and central Japan associated with bivalve mass mortalities, leading to serious hardship to shellfish industries. The underlying toxicity mechanism to bivalve is still poorly understood. To gain a better understanding of the toxicity of *H. circularisquama*, we investigated the physiological, pathological and defense responses of the short-neck clam, *Ruditapes phillipinarum*, when exposed to the toxic alga under laboratory conditions. The clearance rate, respiration rate, prevalence, and intensity of pathologies observed in the gills as well as the total circulating hemocytes and densities of mucocytes in clam gills were all affected, highlighting the occurrence of cytotoxicity and tissue repair failure. Inhibition of feeding and respiration as well as extensive necrosis in the gills, coupled with depression of the defense mechanisms resulting from depletion of hemocytes and mucocytes, ultimately resulted in the death of the clams.

INLAND AQUACULTURE OF *CERASTODERMA EDULE* – FATE AND IMPORTANCE OF DIETARY FATTY ACID.

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The cockle *Cerastoderma edule* is a valuable natural resource in Europe. This burrowing bivalve is commonly harvested from tidal flats, but there has been recent interest in using *C. edule* in an

integrated land-based multi-trophic aquaculture system (Zeeuwse Tong). The cockles will be fed live microalgae grown in nutrient rich water derived from flatfish ponds. However, information on the dietary requirements for this species and dietary effects on growth and survival are lacking. One of the most recently researched topics in shellfish nutrition is the fatty acid (FA) composition of the diet. It has been shown that eicosapentaenoic acid (20:5 *n*-3 EPA) and docosahexaenoic acid (22:6 *n*-3 DHA) are essential for shellfish, but whether the presence of one or both will provide better growth seems to be species specific. To evaluate the effect of dietary *n*-3 polyunsaturated fatty acids live microalgae diets were fed to cockles (6mm). The growth response, as well as survival and fatty acid composition of the neutral and polar lipids of the animals was determined. Present results provide a better understanding of the importance of dietary FA for growth, as well as their impact on FA composition of neutral and polar lipids of *C. edule*.

NOVEL LARVAL TRAPPING TECHNIQUE INDICATES IMPORTANCE OF RARE EVENTS DRIVING CONNECTIVITY OF GEODUCK CLAMS IN PUGET SOUND, WASHINGTON.

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Quantifying connectivity among populations of sessile bivalves has implications for key conservation and management questions. Traditional sampling techniques, such as nets and pumps, collect larvae during a discrete period. These approaches are further limited by the resource-intensive task of sorting and identifying larvae from the rest of the plankton, restricting the number of time points that can realistically be sampled. We use a novel approach, passive larval trapping, which takes a time-integrated sample, paired with Fluorescent In Situ Hybridization with Cell Sorting (FISH-CS), which automates the sorting and identification processes, to map larvae of geoduck clams (*Panopea generosa*) in Quartermaster Harbor, Puget Sound, WA. Our preliminary findings indicate that although there is a low level of small larvae in the Harbor throughout the season, a pulse of larger larvae was captured in early summer. In contrast, medium sized larvae were found in the slow-moving inner harbor. Our results imply that at least some of the larvae were retained in the harbor for their entire planktonic larval duration. Our results underscore the importance of time-integrated sampling of ecological parameters that are dominated by rare events rather than average conditions.

CAN AN ABALONE IN THE BAG SAVE TWO IN THE BUSH?

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An explicit consideration of pathogens into fisheries management can profoundly shift reference targets and criteria for sustainability. By driving host populations below thresholds for transmission, fishing supports a number of desirable outcomes, including the extirpation, or *fishing out*, of pathogens. Southern California abalone fisheries closed in 1996 due to a combination of heavy exploitation and a fatal infectious disease caused by a Rickettsiales-like pathogen. Although this disease remains enzootic in southern California, red abalone (*Haliotis rufescens*) populations have shown widespread signs of recovery, particularly at San Miguel Island. The re-opening of a small-scale, limited access fishery is currently proposed for this region, and in response we constructed an age-structured epidemiological model, parameterized by fisheries-independent demographic and epidemiological data, to test whether harvest could not only provide fishery benefits but enhance the recovery of red abalone at San Miguel Island as well. The results show that a limited effort fishery will enhance the recovery of red abalone, as long as size limits and effort are strictly maintained. These results will have widespread implications for epidemiological theory and the management of fisheries in the face of enzootic disease.

SIGNIFICANT WAVE HEIGHT, TIDAL LEVEL, AND DISTANCE BETWEEN NEIGHBORING INDIVIDUALS OF OPPOSITE SEX AFFECT PROBABILITY FOR FERTILIZATION IN SPAWNING BLACK ABALONE (*HALIOTIS CRACHERODII* LEACH, 1814).

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Black abalone (*Haliotis cracherodii* Leach, 1814) are listed as endangered in their U.S. range due to disease and overexploitation. Abalone are dioecious broadcast spawners, and as intertidal organisms exposed to oceanic surf, black abalone typically spawn in conditions of strong and turbulent water flow. To determine the minimum and maximum proximity between male and female

abalone for successful fertilization, we conducted field experiments releasing surrogate gamete-sized polyethylene particles, spherical in shape and slightly negatively buoyant, at varying separation distances. We collected water samples at predetermined post-release times and distances from release points to measure concentrations of surrogate egg and sperm particles. Optimal densities for successful fertilization are 10^4 to 10^6 sperm/mL with eggs present, based on literature. Densities less than 10^4sperm/mL have reduced probability of fertilization, and densities over 10^6 sperm/mL pose risks of polyspermy and consequent deformation of larvae. We ran experimental releases with variations in separation distance and significant wave heights, with results differing significantly in dispersal and mixing patterns. Initial results indicate that tidal stage and wave action significantly affect gamete dispersal and may influence the distance over which gametes may travel before fertilization.

THE EFFECTS OF MANGANESE AND COPPER ON MITOCHONDRIAL MEMBRANE POTENTIAL IN THE GILL OF *CRASSOSTREA VIRGINICA*.

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Accumulation of manganese or copper is characteristic of the neurodegenerative disorders Manganism and Wilson's Disease, respectively. The mitochondrion is a source and target of oxidative stress. Previously we found gill mitochondria from C*rassostrea virginica* treated with manganese or copper had impaired oxygen utilization. Here we used two fluorescent dyes, TMRM and JC-1, to determine effects of manganese and copper on mitochondrial membrane potential (MMP). For JC-1 we compared fluorescence intensities at 525 nm ex and 590 nm em of manganese treated mitochondria. Manganese treated showed dose dependent decreases in fluorescence of up to 70%. For TMRM we compared slopes of the 573/564 nm ex, 590 nm em fluorescence intensity ratio. Decreasing slope indicates loss of MMP. Treating mitochondria with copper or manganese caused dose-dependent reversal in slopes. Copper was more toxic than manganese and both fluorescent dyes were equally effective in demonstrating that short-term treatments with manganese or copper could de-energize gill MMP. This information correlates well with our previous findings on toxic effects of manganese and copper on mitochondrial respiration. Identifying the molecular and cellular mechanisms of metal-induced oxidative stress will provide a better understanding of the pathophysiological features of neurodegenerative disorders associated with metal toxicity.

PINTO ABALONE (*HALIOTIS KAMTSCHATKANA*) RECOVERY STRATEGIES IN WASHINGTON STATE: WHERE WE'VE BEEN AND WHERE WE'RE HEADED.

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Populations of Pinto abalone (*Haliotis kamtschatkana*) in Washington State have been declining since index surveys began in 1992. Nearly two decades after closure of the fishery in 1994, surveyed populations have decreased by over 80%; substantial recruitment has not been observed. Pinto abalone are a federal Species of Concern and a State Candidate Species. To effect restoration of this species, a collaborative recovery team was formed to test and implement recovery strategies including optimizing captive rearing methods, juvenile outplants, broodstock rotation and adult aggregation. Captive rearing began in 2003 using adult broodstock collected from the San Juan Archipelago (SJA), a region of historic abalone abundance. Larval and juvenile abalone are being produced in our hatcheries for research and restoration projects. Six restoration outplant sites in the SJA were seeded with 3500 juvenile abalone from multiple families in 2009 and 2011. Sites are being surveyed for survivorship and growth; monitoring will continue as the sites are overseeded with genetically diverse hatchery cohorts. Two SJA aggregation sites have been established using both reproductively isolated wild-collected adults and reintroduced hatchery broodstock; survival and spatial relationships are being monitored. A brief description will be given of present and future restoration strategies and associated research.

SURVEY RESULTS ON THE ACCEPTANCE OF TRIPLOID OYSTERS.

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Oysters are popular in Louisiana restaurants, commonly eaten raw on the half shell, or chargrilled. However, due to spawning in the summer, diploid oysters are often thin and watery and may not be as desirable to the consumer. As a substitute, triploid oysters are bred in a hatchery for reproductive sterility, due to an extra set of chromosomes, and are able to maintain a higher meat yield than diploid oysters in the summer. The objective of this survey was to determine if triploid oysters are an acceptable substitute for diploid oysters in the summer. Twenty-six visitors to the 2011 Louisiana Restaurant Association Expo were shown diploid and triploid oysters, both processed and unprocessed with High Pressure

Processing. The visitors were read a brief explanation about triploid oysters, and asked several questions. Overall, 69% of those surveyed rated triploid and diploid oysters differently based on the appearance of the shell, color and condition of the meat, and the size of the oyster; 92% said they would purchase triploid oysters, but only 63% said they would pay more for them during the summer; and, 61% deemed the term "triploid" unacceptable as a commercial product, and suggested a catchier, less scientific name.

TEMPERATURE THRESHOLD OF NORTHERN QUAHOG (=HARD CLAMS) AND EVALUATION OF BACKCROSSED F1 HYBRIDS (*MERCENARIA MERCENARIA* X *M. CAMPECHIENSIS*).

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Mercenaria mercenaria is an important aquaculture species in Florida. Florida water temperatures are at the upper temperature range of the northern hard clam and high mortalities in the summer months may be a result of the combined stress of high temperatures, extreme salinities, and low dissolved oxygen. Our group has been exploring a variety of breeding methods (triploidy, hybridization, backcrossing) to develop a hardier clam for the industry. The objectives of this research were 1) to determine the upper chronic temperature limit of *M. mercenaria* and 2) to evaluate the performance of backcrossed F_1 hybrids (*M. mercenaria* X *M. campechiensis*) in laboratory challenges. Challenges mimicked summer stressors in Florida: oxygen stress (<3ppm), high temperature (32°C), and various salinities (15, 25, 35ppt). The upper chronic temperature limit was 38°C and all *M. mercenaria* died within 28 hours of exposure after acclimation. In the first of two challenges, family and cross were not significant factors in mean survival time, however treatments (hypoxia, 15ppt) were significant. Understanding temperature limits in Florida strains of *M. mercenaria* will contribute to potential management of summer mortality events and further development of hardier clam strains for Florida, including the current examination of backcrosses.

INHIBITORY EFFECTS OF GABA ON SEROTONERGIC INNERVATION OF GILL LATERAL CELL CILIA IN BIVALVE MOLLUSCS.

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In most bivalves the gill lateral cell cilia are regulated by a neuro or endocrine mechanism. In several bivalves, including *Crassostrea virginica* and *Mytilus edulis*, the beating rate of gill lateral cilia is controlled by a serotonergic-dopaminergic innervation from their ganglia. GABA (gamma-aminobutyric acid) is a major inhibitory neurotransmitter in molluscs and other animals, but has not been well studied in bivalves. We examined effects of GABA on beating rates of gill lateral cell cilia in *C. virginica* and *M. edulis*. Beating was measured in whole animal preparations. In both animals, GABA had no direct effect on lateral cell cilia activity whether super-fused to the cerebral ganglia or applied directly to gill. However when GABA and serotonin were both applied to the cerebral ganglia, the presence of GABA, whether applied prior to or after serotonin, blocked the normal excitatory response of serotonin on the beating of the cilia. The GABA antagonist bicuculline methchloride blocked the inhibitory effects of GABA on the serotonergic system. The study demonstrates GABA is working centrally as an inhibitory ganglionic neurotransmitter in these bivalves to inhibit serotonin neurons that innervate the gill and speed up beating of the lateral gill cilia.

NEW TREATMENTS FOR BLISTER WORM, A POLYCHAETE THAT INFESTS EASTERN OYSTERS.

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The polychaete worm *Polydora websteri* burrows into the shell of the eastern oyster *Crassostrea virginica* where it feeds, grows, and reproduces. Muddy wastes excreted by the worm cause irritation to the oyster, which in turn produces a blister over the worm by secreting new shell material. In addition to the physiological stress this places on the oyster, the resulting "mud blisters" detract from the appearance of the oyster and reduce market value. A number of anecdotal reports suggest potential remedies for blister worm though few of these methods have proved consistently reliable. We report on recent work at the Center for Cooperative Aquaculture Research showing that cold storage of oysters for as little as 3 weeks can kill 100% of infesting *P. websteri* with minimal mortality to the oysters. Smaller scale experiments at the University of Maine have examined the osmotic stress tolerance of *P. websteri* and suggest that a combination of a low salinity bath with cold storage may reduce the treatment time to as few as 10 days. Future work will focus on scaling up and refining these treatments as well as the design site-specific pest management plans for the control of this pest.

RESTORING OYSTER REEFS IN HEMPSTEAD BAY, LONG ISLAND, NEW YORK

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The natural reef formations of the eastern oyster (*Crassostrea virginica*) have been lost from most of their range. The eastern oyster is an ecosystem engineer on which many other species depend. The loss of oysters, and the ecosystem services that they provide, continues to be an additional anthropogenic impact to our ecosystems that is difficult to gauge, but seems to include loss of salt marsh and several fish species. The Hempstead Department of Conservation and Waterways has embarked on a restoration program that is tailored to local conditions with the objective of self-sustaining reef formation. The goal is to test the efficacy of a raised bed that is composed of shell bound together in mesh for stability. Some loose oysters were housed in AquaPurses® at several points in the bay to estimate spatial trends in growth and mortality rates. In 2009, the installation of small raised beds was initiated, consisting of two tiers of bagged shell. Additional sections were added in 2010 and 2011 for a combined total of 187 m of reef containing roughly 2,000 adult oysters over 1 1/2 years old. The oysters on reef sections from 2009 and 2010 are showing growth in a vertical orientation and show signs of the initial formation of oyster rock. Future additions are planned and will be monitored for growth, survival, resettlement, and changes in the diversity of other species at these locations.

A MECHANISM FOR THE SENSITIVITY OF LARVAL OYSTER CALCIFICATION TO AMBIENT SEAWATER CARBONATE CHEMISTRY.

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The underlying mechanisms for deleterious impacts of acidification on larval shellfish have not been fully described, in part because early shell formation is not thoroughly understood. We measured elemental and isotopic composition of *Crassostrea gigas* larval cohorts from fertilization to settlement size in a production hatchery setting under upwelling and non-upwelling conditions along the Oregon Coast. During the first 5 days following fertilization, larvae completed a rapid transition from fertilized eggs to shelled larvae with significantly depleted energy reserves (lipids). Stable carbon isotope analyses found that initial shell material has proportionally more seawater DIC, and with increasing age a greater contribution of metabolic carbon was found in the larval shell. This transition coincides with an increasing reliance on exogenous food sources. Greater dependence on ambient carbonate ion availability and the energetic demands of early calcification processes are thus linked, and provide a clear mechanism for the susceptibility of early larval stages to ocean acidification. These data speak to the importance of larval life history and development, variable conditions in coastal ecosystems and suggest the presence of multiple bottlenecks in larval bivalve susceptibility to ocean acidification.

THE EFFECTS OF ACUTE HIGH pCO_2 EXPOSURE ON GROWTH AND SURVIVAL OF *CRASSOSTREA GIGAS* LARVAE.

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We determined if acute exposure (24 h) of developing Pacific oyster eggs to high pCO_2 (approx. 1400µatm) had a detrimental effect on subsequent development when cultured under ambient pCO_2 conditions. After exposure, high pCO_2 conditions resulted in a higher proportion of abnormal larvae and a smaller average shell width compared with eggs exposed to ambient pCO_2. However, both percentage and total number of normal larvae acutely exposed to high pCO_2 increased during a subsequent 11-day period under ambient pCO_2. Since density increases could cause bias, a second experiment stocked cultures with larvae from ambient and high pCO_2 exposures based on either the total number of normal D-larvae (same as first experiment), or the total number of normal and abnormal D-larvae. A significant difference ($P < 0.001$) in shell width was found on Day 7 in cultures stocked with the same concentrations of normal D-larvae, while only a marginally significant ($P = 0.049$) difference in shell width was observed in cultures stocked based on total D-larvae. These results suggest that abnormal larvae acutely exposed to high pCO_2 conditions can recover if subsequently raised in ambient seawater. Furthermore, the study emphasizes the need to correct for density effects when conducting pCO_2 exposure experiments with bivalve larvae.

GENOTYPE-ENVIRONMENT INTERACTION IN SELECTIVELY BRED DIPLOID AND TRIPLOID *CRASSOSTREA VIRGINICA* LINES.

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Whole live weight (WLW) and tissue weight (TW) of diploid and triploid oysters from four selectively bred lines of *Crassostrea virginica* were measured across three sites to determine genotype-environment interaction (GxE). Genotype x environment interaction is an additional source of variation in the classical $P = G + E$ paradigm. GxE was analyzed by an additive main effects and multiplicative interaction model (AMMI). For

WLW, there was no significant GxE for either the diploid or triploid lines. For diploids, E accounted for 95.0% and G – 5%. For triploids, E explained 94% and G – 4%. For TW, both diploid and triploid lines were significantly affected by genotype (G), environment (E), and GxE ($p<0.05$). GxE variance explained about 14% in diploids and 15% in triploids. The remaining variance for TW is apportioned differently in diploids and triploids. For diploids, E explains 78% and G – 8%. For triploids, E explains 42% and G – 42%. The impact of GxE, when it occurs, comes in the form of rank order changes that make breeding goals more difficult to achieve because like diploids, specific types of triploids may need to be selected for specific environmental conditions.

QUANTITATIVE TRAIT LOCI FOR REPRODUCTIVE INVESTMENT, GAMETE DEVELOPMENT, AND VIABILITY IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*).

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High fecundity of the Pacific oyster, *Crassostrea gigas*, contributes to summer mortality. Pedigree and phenotype-based selective breeding for survival is complicated by genotype-by-environment interactions and low genetic correlations across environments.

Marker assisted selection is an attractive alternative. As a first step, we identified quantitative trait loci (QTL) for reproductive investment, gamete development and viability using an F_2 family derived from non-inbred grandparents from highly differentiated families. We reared these progeny under conditions that promote gametogenesis, and quantified their gonad area in a histological cross section. We created a linkage map consisting of 52 microsatellite and 172 AFLP DNA markers and used MQM mapping to search for QTL for reproductive effort and gamete development. We identified three statistically significant QTLs for reproductive effort that account for a total of 31.9% of the total phenotypic variation, but no statistically significant QTLs for gamete developmental stage. Viability mapping based on segregation distortion detected nine viability QTLs, one of which was in the same chromosomal region as a significant QTL for reproductive effort. Within-family marker assisted selection to decrease reproductive effort could both improve product quality and increase survival, but further study is necessary to verify the effects and refine the positions of these QTL for more general use in selective breeding.

THE EFFECT OF SPATIAL HETEROGENEITY IN MUSSEL DENSITY ON THE PRODUCTIVITY OF A COMMERCIAL MUSSEL BED.

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Mortality and growth on commercial mussel beds are highly influenced by seeding density. The seeding process, however, induces seeding patterns with initially high spatial heterogeneity in mussel densities. This results in spatial patterns of density dependent processes such as growth and mortality and has an effect on the production at the scale of a commercial mussel bed. The question we address is: how does the heterogeneity in mussel density within a commercial mussel bed relate to its productivity? We hypothesize that spatial heterogeneity has an effect on the productivity of a commercial mussel bed, by an increase in competition at high local densities and a higher growth rate, but also a higher vulnerability towards hydrodynamic stress and predation at low local densities. To compare the effect of the spatial heterogeneity in mussel density a field experiment was conducted on an intertidal commercial mussel bed at the Oosterschelde estuary, The Netherlands. Mussels were seeded with a varying spatial heterogeneity; effects on growth and mortality were measured at an early period (3 months) after seeding. The results will be discussed.

TRACKING THE POPULATION DYNAMICS OF A LARGE SEA SCALLOP YEAR CLASS IN THE GULF OF MAINE.

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In 2009 we discovered a large abundance of juvenile sea scallops on the offshore banks in the Gulf of Maine. In August 2009, 2010, and 2011 we video surveyed 88 stations on Platts Bank, 48 stations on Cashes Ledge, 80 stations on Fippennies Ledge and 50 stations on Jeffreys Ledge using a centric systematic design with stations on a 1.0 km grid. In 2009 we estimated that these four areas contained 470 million scallops, equivalent to 10% of the total number of scallops on Georges Bank in only 1% of the area. The mean shell height of scallops was 41.6 mm (SD = 13.73 mm, n = 1,898) and size frequencies suggested they were mostly from a single year class. In examining changes in density, spatial distribution and size composition we observed growth and declines in abundance in both the 2010 and 2011 surveys. Cashes, Jeffreys and Fippennies ledges are closed to scallop fishing, while Platts Bank is exposed to low levels of fishing effort, presenting the unique opportunity to track a large year class of sea scallops through time and observe mortality in both fished and unfished areas.

OYSTER POPULATION CONNECTIVITY IN LOWER CHESAPEAKE BAY: POSSIBLE IMPACTS OF DISEASE SELECTION IN STRUCTURING POPULATIONS.

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Understanding the connectivity among oyster reefs is essential for designing effective conservation and restoration strategies, yet dispersal patterns are not necessarily obvious and easily determined. While physical modeling may indicate particular patterns of larval flow, the parasites *Perkinsus marinus* and *Haplosporidium nelsoni* could create such intense disease pressure that realized net gene flow bears little relation to what would be predicted based on net larval dispersal. In several Virginia tributaries of Chesapeake Bay, models indicate a strong downstream force suggesting that many larvae are originating from broodstock in upstream refugia from parasitism. Broodstock residing on reefs in disease-intense mesohaline waters may, however, be making the more substantial contributions to oyster populations in these systems. Virginia tributaries may represent a case study for the limitations to inferences of oyster population sources and sinks based simply on larval dispersal models. Genetic tools can be used to most accurately assess patterns of population connectivity and should incorporate both neutral markers and those linked to disease selection. We will synthesize existing data on the current genetic structure of Virginia oyster populations and present perspective on approaches to determine patterns of reef connectivity.

ARE MULTIPLE PREDATOR EFFECTS ON A SHARED PREY INFLUENCED BY HABITAT COMPLEXITY?

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It is becoming increasingly important to understand how multiple predators impact a shared prey resource, especially within the context of both invasive species and species experiencing poleward range expansions. Typically multiple predators will interact with each other, leading to either risk enhancement or risk reduction for shared prey. However, in multiple predator effects studies, habitat is often ignored, despite its importance in influencing predator-prey relationships. It is possible that increasing habitat complexity can either alleviate or exacerbate the effects of multiple predators. In New York estuaries, the long-established invasive green crab, *Carcinus maenas*, is an important consumer of bivalve prey. Additionally, populations of blue crabs, *Callinectes sapidus*, are increasing in New York estuaries, likely due to warmer winters associated with a changing climate. During mesocosm experiments, green and blue crabs were allowed to forage alone and together as both con- and hetero- specific pairs at 4 levels of habitat complexity. Rates of consumption when crabs foraged as pairs were compared to predicted values based on consumption rates when crabs foraged singly. Both the presence of multiple predators and habitat complexity had significant effects on prey consumption.

OYSTER HEALTH AND REPRODUCTION ONE YEAR AFTER THE DEEPWATER HORIZON OIL SPILL.

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This project is a continuation of a one-year project in which caged oysters and wild oysters across coastal Louisiana were sampled in May 2010, prior to the Deepwater Horizon oil spill event reaching the coast, and in October 2010 to determine acute responses to oil using a suite of biological effects measurements in relation to polyaromatic hydrocarbons (PAH) concentrations. Wild oysters and oysters deployed in cages at two oil-impacted (Grande Terre, Bay Batiste) and two reference sites (Grand Isle, Breton Sound) were sampled in August and October 2011, respectively. Hemocyte density, viability, phagocytosis and lysosomal stability were measured in individual oysters. Oysters were then shucked and fragments of gills and digestive gland tissues were used to measure Hsp70 stress proteins in gills and lipid peroxidation in digestive glands. Standard tissue sections were processed to assess histopathology. The bulk of the tissue from each oyster was then homogenized and processed to measure dermo (*Perkinsus marinus*) intensities, condition index and PAH concentrations. Additional oysters were collected from each site and their gamete fertilization success, larval development and survival are being measured to assess the impact that oil accumulation in brood stock gametes has on offspring larval stages.

INFLUENCE OF REEF DESIGN, WAVE ENERGY ENVIRONMENT, AND LOCATION ON OYSTER DENSITY, MORTALITY, AND SIZE DISTRIBUTION OF RESTORED REEFS.

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Along the northern Gulf of Mexico coast, reefs created by eastern oysters are increasingly targeted for restoration. Site selection and reef design are critical factors for the sustainability and functionality of these restored reefs. This project examined the effects of reef design (narrow and wide) and site, as defined by energy environment (low and medium), on reef sustainability in three locations (north, west, and south Caillou Lake, LA), with location used as a proxy for different water quality conditions (salinity, total suspended solids, and chlorophyll a) and proximity to the Gulf of Mexico. At each location, three reefs were created (low energy narrow, high energy narrow, high energy wide) in February 2009 using oyster shells. Oyster recruitment, density,

mortality, and size distribution were estimated seasonally through December 2011. While all reefs and locations experience recruitment every year, the southernmost site, located in closest proximity to the Gulf of Mexico had greatest recruitment, oyster density and more even size distribution. Significant differences in oyster density, mortality, and size distribution by energy environment or reef structure were not found, and water quality did not differ significantly between sites. Proximity to oyster beds and hydrological characteristics may have influenced reef performances between locations.

POPULATION AND MIDDEN ASSESSMENT OF QUEEN CONCH, *LOBATUS GIGAS*, IN SOUTHERN ELEUTHERA, THE BAHAMAS.

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Subject to subsistence and commercial harvest for decades, the queen conch, *Lobatus gigas* (formerly *Strombus gigas*), has been subject to additional pressure in the Bahamas as stocks have been depleted in other countries that were historically prominent exporters. With concerns about local stocks and interest in culture of this species in the Bahamas an analysis of conch populations in south Eleuthera was executed. A survey of middens in the region to assess recent and historical fishing efforts was also performed. An area surveyed in 2003 (Clark *et al.* 2005) and determined to be a conch nursery ground was surveyed again in 2011 bi-monthly from July through November 2011 to assess population dynamics at nine sites in southern Eleuthera. Preliminary results suggest a decrease in abundance. Middens in the area were also evaluated to quantify any change in size at harvest over time. Through observation, newer middens appear to contain a higher ratio of juveniles to adults than older middens. Statistical analyses are underway for both objectives. This information suggests that juvenile harvest has increased substantially throughout the years, raising concerns about the sustainability of this important fishery. The potential for aquaculture of queen conch to alleviate this pressure is discussed.

ADDRESSING BIOFOULING IN FLORIDA'S HARD CLAM *MERCENARIA MERCENARIA* AQUACULTURE INDUSTRY: PERFORMANCE OF TWO NET COATINGS.

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In Florida, northern quahog (=hard clams) *Mercenaria mercenaria* are typically cultured in polyester mesh bags on open-water leases in near shore coastal waters. During this phase of production (~1.5 years), bags are subjected to natural conditions, including biofouling. Biofouling can compete with clams for resources, hinder harvesting efforts, and intensify equipment upkeep. Some clam growers treat bags with an alkyd-based coating that stiffens the bag, thus reducing predation. However, no coating is currently being utilized to specifically reduce biofouling. In a preliminary evaluation, two foul release coatings—a photoactive release coating (A) and a silicone-based release coating (B)—were compared to the standard alkyd coating (C) and uncoated netting (D, control). Thirty-six treated netting pieces (30cm × 30cm) were attached to PVC racks and deployed for three or six months on two lease areas near Cedar Key, Florida. At three months, netting treated with Treatment B coating had significantly less coverage of biofouling than other treatments and significantly less wet weight of biofouling than other treatments except Treatment A. At six months, netting coated with Treatment B had significantly less wet weight of biofouling than other treatments. Implications for use of foul release coatings within hard clam aquaculture are discussed.

OCEAN LOW-OXYGEN ZONES AND OCEAN ACIDIFICATION: HOW ARE THEY CONNECTED AND WHAT DOES IT MEAN?

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In the Pacific Northwest, summer upwelling currents lead to the formation of low-oxygen zones in the coastal ocean. Such low-oxygen zones are also associated with greatly elevated concentrations of carbon dioxide. Using data collected from the Oregon coast, we explore the connections between these potential biological stressors. In particular, we examine the coupling between oxygen and carbon chemistry over the course of successive upwelling seasons from sensors deployed offshore over the mid continental shelf and nearshore close to the surf-zone. Using a decade-long record of dissolved oxygen, we further examine the factors that are responsible for driving inter-annual changes in the intensity of low-oxygen events and evaluate their implications for future ocean acidification changes.

GENETIC AND MEASUREMENT APPROACHES TO NOVEL AND COMMERCIALLY IMPORTANT TRAITS OF EDIBLE ECHINODERMS: A REVIEW.

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Commercially important traits play a central role in animal breeding programs. They are not only essential in classical breeding methods, including hybrid breeding and selective breeding, but also extremely important for molecular breeding approaches, for example quantitative trait loci (QTL) and genome-wide association study (GWAS). Compared to livestock and poultry, however, commercially important traits of marine organisms have only a poor understanding, imprecise measurement and preliminary genetic analysis. Here, we reviewed genetic analysis and measurement approaches to some novel and commercially important traits in the sea cucumber *Apostichopus japonicus* (traits of papillae) and sea

urchins *Strongylocentrotus intermedius* and *Glyptocidaris crenularis* (traits of gonad quality). In the present review, we also elaborated the 5W1H measurement system (what, when, where, who, why and how) to better understand commercially important traits in breeding programs of edible echinoderms. The endeavor on novel and commercially important traits increases our understanding on the genetics and breeding of edible echinoderms.

EFFECTS OF MICROALGAE ON THE IMMUNE RESPONSES AND DIGGING ABILITY OF HARD CLAM, MERETRIX LUSORIA.

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The hard clam, *Meretrix lusoria*, is the most commercially important cultured species of bivalve in Taiwan. Clams are filter-feeding animals. The phytoplankton which contained different compositions and levels of fatty acids were the major food sources of bivalve. Microalgae, *Chaetoceros muelleri*, *Tetraselmis chui*, and mixture of *C. muelleri* + *T. chui* were selected as feed to evaluate the immune responses and digging ability of hard clam, *M. lusoria* after 14 days feeding trial. The unfed treatments were also conducted as the control group. After 14 days feeding trial, the clearance efficiency against pathogen *V. alginolyticus* was significantly higher in clams fed *T. chui* or *C. muelleri* + *T. chui* than in clams fed *C. muelleri* or unfed treatment. No significant difference was observed in THC, PO activity and phagocytosis of clams fed different microalgae diet or unfed treatment. The digging ability of clams fed different microalgae diets was higher than the unfed treatment. There were 100%, 100%, 90% and 67% of clams fed *T. chui*, *C. muelleiri*, *C. muelleri* + *T. chui* and unfed treatments digging into the sand, after transfer to new culture tank for 10 hrs.

PRODUCTION AND ENVIRONMENTAL EFFECTS OF MANILA CLAM FARMING IN NORTH PUGET SOUND: COMPARISON OF YIELDS AND RESPONSES OF MACROFAUNA TO MECHANICAL AND HAND HARVEST.

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Currently up to 6,400 metric tons of Manila clams are harvested annually in Washington State, U.S., most from aquaculture facilities. This production accounts for all clams of this species harvested in the U.S. Most farm-reared Manila clams in Washington are now grown semi-intensively using open or net-protected culture systems, or net-bag culture for smaller scale production. Although these tactics allow Manila clam aquaculture to expand, production costs are generally increasing and overseas competitors are driving profits down. A new technology for Manila clam farming is now available that mitigates production costs, promising to enhance the economic stability and growth of the industry. This technology, which uses a combination of culture methods and tools more commonly associated with land-based farms, has been applied at several sites in north Puget Sound, Washington. Our research examines the environmental effects of this technology and explores methods improvement through on-farm experiments and observations, analyses of production data, and production capacity modeling. This is a report on aspects of the research to compare mechanical and traditional hand-harvest methods and yields, and examine site-specific effects on water quality, sediments, and macro fauna. This research effort received support from the NOAA Saltonstall-Kennedy Grant Program (FY10) grant number NA10NMF4270309.

AN OVERVIEW OF THE LAKE TAHOE AQUATIC INVASIVE SPECIES PREVENTION AND CONTROL PROGRAMS.

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The Lake Tahoe Aquatic Invasive Species (AIS) Program is a bi-state, local and federal multiagency partnership that is tasked with the implementation of the Lake Tahoe Region Aquatic Invasive Species Management Plan. It was approved in November 2009 by the federal Aquatic Nuisance Species Task Force and endorsed by the governors of Nevada and California. The Plan is implemented by various agencies that are coordinated into working groups that focus on the unique challenges and issues presented by individual species or programs (i.e. invasive weeds and motorized watercraft inspections). These working groups are in turn organized into a regional program by the Lake Tahoe AIS Coordination Committee. The Committee is co-chaired by the USFWS and the Tahoe Regional Planning Agency and is comprised of senior managers and senior technical staff from agencies involved in policy, implementation, and regulation of AIS projects and programs. Funding for the implementation of the Plan is provided by multiple federal, state and private sources. The prevention effort is comprised of the motorized watercraft inspection program, non-motorized inspection program, as well as risk assessment and regional coordination activities. The control effort is focused on the removal of AIS currently found in the Lake Tahoe Region.

CULTURE AND PHYSIOLOGICAL TOLERANCE OF THE HAWAIIAN OYSTER, *DENDOSTREA SANDVICENSIS*, IN HYPOXIC AND NORMAL CONDITIONS.

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The native Hawaiian oyster species, *Dendostrea sandvicensis*, is a small flat rock oyster that is both culturally important and potentially economically useful. Little previous research has been done on *D. sandvicensis*, though it has been identified throughout the Pacific Ocean. Given its potential for aquaculture, the Pacific Aquaculture and Coastal Resources Center (PACRC) at the University of Hawaii at Hilo has been conducting basic biological research and attempting to develop this species for aquaculture purposes for the last four years. In addition to conducting trials to determine the growth rates of larvae and spat, oysters were tested for hypoxic tolerance over a period of 20 days. Oysters were kept in sealed jars sparged with nitrogen gas to remove all dissolved oxygen. Mortality of the hypoxic oysters was around 90% between 8–10 days, while control jars had 0% mortality throughout the experiment. Control jars were aerated to maintain dissolved oxygen in the water; hypoxic jars were sealed throughout the experiment. These results have implications for determining environmental tolerances, shelf-life and resiliency to climate change impacts. Future research will include respiration analysis in different water conditions and survival tolerances.

THE EFFECT OF pH ON THE SURVIVAL OF DREISSENID MUSSELS.

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Dreissenid mussels are an environmental and economic nuisance across North America. When present in the source of raw cooling water, they become a serious problem for industrial facilities unless defensive steps are taken. The treatment of choice tends to be one of chemical control, as it has proven to be convenient and effective. Most chemicals are non-selective and therefore toxic to all forms of aquatic life. Dreissenid mussels have a relatively narrow range of pH tolerance, with the optimum range being pH 7.5 to 9.3. It was hypothesized that by manipulating this environmental variable, it may be possible to control the growth, settlement, and survival of dreissenid mussels in raw water systems. Single point of addition can adjust the pH level of a cooling water system or of a water conveyance. Several experiments have been conducted to verify this hypothesis. The field experiments were carried out using a custom built flow-through laboratory using water from three different sources. The experiments tested the ability of dreisseinid pediveligers to settle under different pH conditions as well as the long term survival of adult dreissenids under the same conditions. In addition, impact of very high and very low pH on adult dreissenids was also examined. This presentation will summarize the experimental results obtained.

THE PRESENCE OF GABA IN GANGLIA OF BIVALVE MOLLUSCS.

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The nervous systems of most studied bivalves contain serotonin and dopamine in their ganglia, serving as neurotransmitters regulating various physiological functions. GABA (gamma aminobutyric acid) is an inhibitory neurotransmitter found in the CNS of vertebrates and many invertebrates. Its presence and functions have not been well studied in bivalves. Here we used an HPLC method with pre-column derivatization and fluorescence detection to look for GABA in cerebral ganglia and visceral ganglia of the oyster *Crassostrea virginica*, the clam *Mercenaria mercenaria* and the mussel *Mytilus edulis*; and the pedal ganglia of *M. edulis* and *M. mercenaria*. GABA was detected in low nanogram amounts in each of the ganglia of each of the bivalves. This study coupled with other work in our lab demonstrates for the first time that GABA is present and has a neurophysiological role in ganglia of three orders of bivalves.

INTERACTIONS OF REEF STRUCTURE WITH LOCAL FLOW ENVIRONMENTS: IMPLICATIONS FOR RESTORATION.

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Biogenic reefs, like those formed by the eastern oyster, *Crassostrea virginica*, provide habitat and structural refuge to numerous species in estuarine systems. In addition to interactions with estuarine biota, reef structures interact with the local flow regime by altering flow fields in a manner that potentially benefits resident oyster populations. Historically, intertidal reefs in Chesapeake Bay were oriented perpendicular to the shore, facing into tidal flow. This orientation probably maximized flow speeds over the reef, enhancing food delivery, recruitment, elimination of waste products, and erosion of sediments from the reef surface. We examined the biological consequences of interactions between the local flow environment and reefs varying in orientation relative to tidal flow. Experimental reefs of three configurations (parallel to tidal flow, perpendicular to tidal flow and circular) were built in four locations of lower Chesapeake Bay. Perpendicular reefs were

intended to resemble the historical shoal-like structure. Oyster recruitment, growth and mortality were measured to determine if reef orientation resulted in differences in the local flow environment, and if those changes were reflected in the resident oyster population. Determining the reef structural characteristics that provide the greatest benefit for oyster recruitment and growth will help ensure persistence of restoration oyster reefs.

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

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Community Supported Agriculture (CSA) has become a popular business structure for producers to market their products directly to consumers. The CSA concept, now gaining appeal in the aquaculture industry, provides producers a competitive edge in the marketplace. An assessment of seafood consumers in Connecticut revealed an interest in having access to local shellfish and a desire to support the development of local "community" farms. Out of a total of 68 respondents, 90% said that they were willing to pay a premium for shellfish grown at or harvested from a Connecticut farm. 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 poll of municipal shellfish managers revealed that their demand for shellfish seed regularly exceeded supply. Seventy-five percent of the managers expressed interest in investing in a CSA as means to supply their shellfish demands, however, the balance between the level of investment and acceptable risk remains to be explored. As such, the Connecticut Sea Grant program is developing a business plan for the State's first Community Supported Aquaculture Project. This project is a partnership with the Noank Aquaculture Cooperative in Groton, Connecticut.

ONE HEALTH IMPLICATIONS OF SHELLFISH INVASIONS TO HUMAN AND ANIMAL DISEASES.

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Invasion of new geographical locations by exotic shellfish, both molluscan and crustacean, poses the potential for significant changes in biotic interactions among biota already inhabiting the invaded region. Among the most important of these changes is the potential for parasitic and other infectious diseases to be introduced, or to gain enhanced transmission potential, as a consequence of the invading shellfish acting as intermediate hosts or vectors of newly introduced or existing pathogens. In particular, the exacerbation of trematode diseases is a point of major concern. Adopting a One Health approach (i.e., integrating animal, human, and ecosystem health concerns), we have reviewed situations in which introduced shellfish have altered disease patterns among certain wildlife, fish, livestock, and human populations, involving both marine and freshwater systems. These range from long-past invasions such as that of the edible northeast Atlantic marine periwinkle snail, *Littorina littorea* invading northwest Atlantic nearly two centuries ago, to more recent invasions of North American freshwater systems by European dreissenid bivalves in the last few decades. We also present new data on parasites associated with introduced molluscs, as well as use of these invaders as sentinels to predict outbreaks of waterborne pathogens.

EFFECTS OF EXPOSURE TO THE WITHERING SYNDROME PATHOGEN ON THE GUT MICROBIOME OF BLACK ABALONE FROM SAN NICOLAS ISLAND AND CARMEL, CA.

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California abalone species are greatly threatened by the agent of withering syndrome, *Candidatus* Xenohaliotis californiensis. The Rickettsiales-like organism (RLO) infects abalone gastrointestinal epithelial cells and leads to starvation by interfering with food digestion and nutrient uptake. Black abalone from two California locations, San Nicolas Island (exposed to high withering disease selective pressure and thought to have a degree of RLO pathogen resistance) and Carmel (no prior withering disease selective pressure) were exposed to the RLO pathogen to investigate the roles disease and disease resistance have in shaping gut microbial communities of abalone. Microbial analyses of fecal samples collected during the course of infection were conducted using Automated Ribosomal Intergenic Spacer Analysis (ARISA). Gut microbial richness was found to be significantly greater for naïve Carmel abalone when compared to resistant San Nicolas Island abalone. Richness was driven more by origin site than by exposure to RLO. Microbial species composition, however, was found to be more dependent on exposure to RLO than origin site. Microbial richness and composition both varied significantly by date. Findings suggest disease and disease resistance have effects on the richness and composition of the gut microbiome and encourages further study of their interaction.

A SHELL-NEUTRAL SUSTAINABLE OYSTER HARVEST MODEL.

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We present a simulation algorithm for predicting the impact of fishing *Crassostrea virginica* from Louisiana's coast using no net shell loss as a basis for sustainability. The model accepts as input a set of n oyster size/count pairs, shell mass, and monthly fishing rates. It outputs expected remaining shell mass after m discrete fishing months. The model applies an inverse Von Bertalanffy equation to estimate each size group's age. An iterative $O(m \times n)$ loop is then entered that operates on each size group and the reef as a whole. Mortality rates are used to convert select oysters into shell mass credit. Parameterized fishing rates act on sack, seed, and shell independently, debiting each resource. Oysters that survive to the end of each month grow larger and add shell. Using heuristics, a combination of fishing rates can be selected that minimizes the net effect on the region's total shell.

AGE AND GROWTH OF *PANOPEA GLOBOSA* (BIVALVIA: HIATELLIDAE) OFF SONORA, MEXICO.

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This paper first describes the age and growth of the cortes geoduck *Panopea globosa* off coast of Sonora, Mexico. It was motivated because the limited knowledge of biological aspects of this species in Mexico. The objective of this study was to know the age and growth of *P. globosa*. Geoducks were collected from November 2008 through February 2010 in the central eastern coast of the Gulf of California (Bahia del Sol, Sonora, Mexico). Age was established using the "acetate peel" method. Von Bertalanffy growth parameter were estimated as = 122.86 mm, k = 0,33 and t_o = –0,2. Ages varied from 2 to 27 years old but the mode was 10 y. Average size was 109.8 ± 3 mm shell length. The conclusion is that according to our findings this stock is a recent established population in response to a favorable environmental event.

A TRANSCRIPTOMIC APPROACH IN SEARCH OF DISEASE RESISTANCE IN ENDANGERED BLACK ABALONE (*HALIOTIS CRACHERODII*).

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Withering Syndrome (WS) is a bacterial disease of abalone caused by a rickettsia-like organism (WS-RLO), resulting in up to 99% losses of black abalone in central and southern California, including San Nicolas Island (SNI). Beginning in 2002, abalone densities at SNI have trended upward which led us to hypothesize that disease pressure led to the development of WS resistance in selected populations. We tested this hypothesis in the lab using WS selected abalone from SNI and "naive" or non-selected abalone from Carmel, CA (CAR). Significantly lower mortality was observed in SNI abalone than CAR ($p < 0.05$), while no differences in survival were observed between control groups ($p > 0.05$). Microscopic examination suggests that resistance may be related to host response to initial infection. Using a transcriptomic approach, we recently began to discover and characterize genes actively expressed and specifically involved in black abalone innate immunity. By comparing the host response between SNI and CAR populations, we hope to better understand mechanisms associated with WS resistance.

CONSERVATION OF THE NATIVE EUROPEAN OYSTER *OSTREA EDULIS* THROUGH CONTROL OF ITS MAIN PATHOGEN *BONAMIA OSTREAE*.

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The native European oyster has been severely impacted on in recent decades by the effects of several pathogens and diseases that have decimated populations and resulted in European production being reduced to a fraction of what it was back in the 1960s.The most significant of these pathogens has been the haplosporidian parasite *Bonamia ostreae* that is present in most significant oyster growing regions throughout Europe and has threatened the viability of populations in some regions. In addition to the issues caused by pathogens and disease there is historical evidence that a number of regions have been overfished for the last century or more. A European Union funded study, OYSTERECOVER currently on-going, is looking at mechanisms to conserve this native oyster species through control of *Bonamia*. The study is looking at aspects of the life cycle, host parasite interactions, the genetic basis of varying susceptibility to infection and assessing habitats throughout a number of European countries to determine their suitability for oyster on-growing. The study is a good example of both researchers and industry coming together throughout the European Union to solve a common problem.

A STUDY OF THE IMPACTS OF *BONAMIA OSTREAE* ON A SIGNIFICANT OYSTER BED IN LOUGH FOYLE, IRELAND THROUGHOUT 2011.

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Lough Foyle on the North Coast of Ireland is the most recent area in Ireland where the haplosporidian *Bonamia ostreae* has been detected in the native European oyster *Ostrea edulis* - being first detected at a low prevalence in 2005. Since then prevalence of infection has been variable with some mortalities observed being attributed to the pathogen. Some control of fishing within the Lough takes place and in this study information on the status of the disease within one area of the Lough was assessed to inform fishing rights in a particular area. Oysters were sampled most months in 2011 commencing in January and prevalence and intensity of infection was determined. Environmental data was also assessed to determine what impacts other factors might be having on the evolution of the disease within the area. Results indicate that the pathogen is present – but not at a very high prevalence of infection as might be thought the case in a relatively naïve population. Prevalence of infection remained relatively stable throughout the year with higher intensities during the summer months indicating that the stressors present to exacerbate infection did not vary much throughout the year.

MIKROCYTOS LIKE PROTOZOANS AND THE SHELLFISH *DONAX TRUNCULUS* MORTALITY EVENTS IN FRANCE.

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In 2010, important mortalities were reported in wild beds of the shellfish, *Donax trunculus*, in France mainly during summer. Histological and transmission electronic microscopy analyses revealed the presence of a protozoan most certainly belonging to the genus *Mikrocytos*. Its prevalence was between 13 and 80% depending on the areas. PCR and *in situ* hybridization specific to *Mikrocytos* genus protozoans were performed and confirmed microscopic observations. DNA sequencing showed 79 to 80% of homology with *Mikrocytos mackini*, 79% with *Mikrocytos* sp. detected in *Crassostrea gigas* from China and 79% with *Mikrocytos* sp. detected in *Ostrea edulis* from Canada. These results strongly suggest that the detected protozoan belongs to the genus *Mikrocytos* but it seems different from the parasites previously reported in this genus. This parasite was also detected on archived material collected during a mortality event in 2008. In 2011, different monitorings were implemented in order to determine the best period to detect this protozoan and define accurately its involvement on *Donax trunculus* mortality. It was no longer detected in one specific wild bed and no mortality was notified. In two other beds, it was detected during and just after mortality outbreaks, which could suggest its role in *Donax* mortality.

HARD CLAM RELOCATIONS TO MITIGATE QPX DISEASE SEVERITY WITHIN AN ENZOOTIC ESTUARY.

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Monitoring of persistent QPX infections in wild clams of Raritan Bay shows certain areas of the estuary have remained without any significant disease prevalence. The field observations were concomitant to lab experiments showing an influence of environmental conditions on QPX disease development, including remission and healing, in northern quahog (=hard clams), *Mercenaria mercenaria*. A field study was conducted to investigate the potential to mitigate QPX disease by relocating clams to utilize favorable environmental conditions suggested to deter infection. All sites were located within the Raritan Bay complex to avoid risk of spreading infection to new populations. Clams were collected from a centralized bay location with consistent disease prevalence and brought to near shore habitats subject to lower salinities and higher summer temperatures. A treatment of reduced clam densities was included in the study to examine the common observation of high clam density in the most persistently infected locales. An additional treatment retained clams above the sediment since sediments are thought to represent a QPX reservoir. After four months, all treatments displayed less QPX disease than the control group. The most substantial reduction of QPX disease was for clams that were relocated to a tidal creek.

IN SITU PREDATION AND BEHAVIORAL PLASTICITY OF JUVENILE RED KING CRABS (*PARALITHODES CAMTSCHATICUS*).

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Juvenile predation may create a bottleneck for red king crab (*Paralithodes camtschaticus*) populations in Alaska, USA. Gut content analysis shows that groundfish consume juvenile red king crabs; however, observations from shallow nursery areas are limited. We tethered recently-settled red king crabs of different sizes (2.0–3.9 mm carapace width) during July and September 2011 to explore in situ predation rates and identify predators. Survival was similar between size classes and deployment month. Crabs

were consumed by a range of predators, including flatfish, ronquils, kelp greenling, and hermit crabs, yet some expected predators, including Pacific cod, demonstrated little interest in king crab juveniles. In laboratory experiments, we examined behavioral responses of recently-settled crabs by exposing them to fish predator cues, using video cameras to observe predator-prey interactions. Crabs increased cryptic behavior with higher survival after 48 h exposure to Pacific halibut suggesting crab predator avoidance behavior improves with experience. Our data suggest that a broad range of predators including demersal fishes and benthic invertebrates may be significant consumers of recently-settled red king crabs in nearshore habitats. More information is needed on predator avoidance behavior and predation in the field to evaluate if juvenile predation limits red king crab populations.

EVALUATING THE PRODUCTIVITY AND COST BENEFIT OF HIGH PRESSURE WATER TREATMENT REGIMES ON *CIONA INTESTINALIS* INFESTED MUSSEL SOCKS ON PRINCE EDWARD ISLAND.

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Successful management of aquatic invasive tunicates has become a critical component in the maintenance of mussel aquaculture. Over the past thirteen years, four exotic tunicate species (*Styela clava*, *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis*) have been detected in the waters surrounding Prince Edward Island. The species have rapidly become invasive and are now considered serious nuisance species by acting as fouling organisms. The mussel aquaculture industry has been the most significantly affected by *C. intestinalis*. Working with industry we conducted a clinical field trial to determine the optimal regime to treat mussel socks with high pressure water, the treatment of choice for mitigating *C. intestinalis* on Prince Edward Island. Biologically, the optimal regime results in the greatest reduction of *C.intestinalis* and the highest yield of mussels on the socks A cost-benefit analysis was conducted on each treatment regime to determine the most economical treatment regime for the mussel industry.

AQUACULTURE AND FISHERIES FOR GEODUCK CLAMS (*PANOPEA GENEROSA*) ON THE WEST COAST OF NORTH AMERICA.

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Geoducks are among the world's largest burrowing clams, they inhabit both intertidal and subtidal gravel, sand and mud sediments living to a depth of one meter or more, are extremely long lived and form extensive aggregations in western North America, Mexico, eastern South America and New Zealand among other locales. Aquaculture development for geoducks has increased rapidly over the last decade mainly in Washington State. Intertidal culture has been the focus in Washington where tidelands are either owned or leased. Geoduck clams possess characteristics that make it an exceptional candidate for aquaculture. Though 4–6 years are necessary to produce a harvestable clam, in excess of 300,000 kg are harvested annually with landings from farmed product increasing annually. Wholesale price for the clams has increased dramatically in recent years with current farm gate value in excess of $45.00 per kg for farmed product. Developments in hatchery rearing of larvae and seed include a better understanding of nutritional requirements and husbandry practices that focus on controlling clam density and pathogenic bacteria. An adequate seed supply to supply markets and permitting issues related to establishing new farms and expanding existing farms currently limit the growth of the farmed sector.

GROWTH AND MATURATION IN TRIPLOID GEODUCKS.

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Maturation control may preclude genetic interactions between cultured and wild geoduck. If sterility is conferred via triploidy, genetic risk from hatchery-produced geoduck clams to naturally occurring populations could be reduced. In addition, triploid geoduck (3N) may also exhibit better growth performance compared to diploids (2N). Our primary goal was to investigate whether 3Ns can function as biologically and commercially effective mitigation for potential genetic interactions. We planted 300 3Ns and 2Ns (1:1 ratio) in June 2005 and another 1656 3Ns and 2Ns from 14 separate families in June, 2011. We observed no differences between 3N and 2N survival, shell length, proportion ripe, or wet weight. However, we detected a highly significant difference in shell width between 2Ns and 3Ns ($p << 0.0001$), yielding a potentially valuable distinguishing shell morphometric. In the first group, we also found a significantly greater proportion of 2Ns exhibited evidence of gamete release ($p = 0.02$) and a preponderance of females among 3Ns ($p = 0.02$) but not among 2Ns ($p = 0.46$). As expected, egg diameters were significantly greater for 3Ns ($p << 0.0001$). Acini In 3N males primarily contained spermatocytes; we observed no evidence of spermiogenesis and no viable embryos derived from mated 3N females; these data suggest that 3Ns are effectively sterile.

EFFECT OF STOCKING DENSITY ON QUEEN CONCH, *LOBATUS GIGAS* (FORMERLY *STROMBUS GIGAS*), RAISED IN NEAR-SHORE SEA CAGES AND IMPLICATIONS FOR COMMERCIAL SCALE PRODUCTION.

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Queen conch, a staple within the Caribbean, is exported to increasing global markets. Rising fishing pressure has resulted in depletion of wild stocks; increasing interest in commercial aquaculture programs. Caicos Conch Farm (CCF) has developed commercial-scale hatchery and juvenile rearing techniques. This study, conducted at the CCF, sought to determine the optimum stocking density of conch in nearshore cages and how density affects profitability of grow-out farms. Hatchery-reared conch were stocked in nearshore cages at densities of 8.5, 14.2, and 21.2 conch/m^2 and measured seasonally for 27 months to determine growth rate and average length. Density became a significant factor after 12 months in cages. Upon harvest, whole tissue mass, meat mass, and developmental stage were recorded along with siphonal length and presence of a lip. Conch stocked at 8.5 conch/m^2 grew fastest, had a near even male:female ratio, and the most lipped shells. Although there was not a significant difference in mean tissue mass between conch held at 8.5 and 14.2 conch/m^2 the meat yield from those animals fell into different market categories thereby affecting the net profit of each density treatment. The results of this study can be directly applied to commercial-scale conch grow-out and hatchery broodstock development.

HYPOOSMOTIC CELLULAR VOLUME REGULATION IN BIVALVES: EFFECT OF THE CALCILYTIC DRUG, RONACALERET®.

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Exposure of the tissues of osmoconforming molluscs to a decrease in the osmotic concentration of the ambient medium results in osmotic gain of water. To regulate volume, the cells release amino acids (AA) to bring the cytoplasm into osmotic equilibrium with the medium. The mechanisms involved in the control of the release of AA are unknown. AA release is increased by removal of Ca^{++} from the medium and phorbol esters; La^{+++} inhibits release. In higher vertebrates, calcium-sensing receptor proteins (CaR) control the secretion of parathyroid hormone (PTH); a decrease in the ambient concentration of Ca^{++} stimulates PTH secretion. This regulatory pathway is potentiated by phorbol esters and blocked by La^{+++}. To investigate the role of CaR in volume regulation, we exposed the ventricles of *Crassostrea virginica* acclimated to 1000 mOsm seawater (SW) to either 500 mOsm SW or 500 mOsm SW containing 1 mg/ml of the CaR antagonist Ronacaleret. Ventricles in 500 mOsm SW released 6.1 ± 2.1 μmol/g dry wt AA; ventricles in 500 mOsm SW + Ronacaleret released 17.5 ± 9.6 μmol/g (n = 9). These results implicate CaR in the initiation and control of AA release during hypo-osmotic cellular volume regulation in bivalves. Ronacaleret was a generous gift of GlaxoSmithKline Inc.

IMPACT OF DIET ON DIGESTIVE ENZYME ACTIVITY IN LARVAL EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.

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The eastern oyster, *Crassostrea virginica*, is an ecologically and economically important species in the US. Increased understanding of the stage-specific digestive processes of this species could facilitate diet selection, enhance growth in aquaculture, and give further insight into the ecological role of these organisms. Previous studies have examined digestive enzymes in bivalves, yet few have focused on changes associated with development or dietary influence. The purpose of this study was to examine dietary impact on digestive enzyme activity over ontogeny in *C. virginica*. Larval *C. virginica* were exposed to one of three feeding treatments: 1) starved, 2) a combination of *Pavlova pinguis* and *Cheatoceros muelleri* (rich in chrysolaminarin), or 3) a diet of *Rhodomonas lens* (rich in starch) for a period of two weeks. Prior to conducting enzymatic assays, pH optimum was determined. For larval *C. virginica*, a pH of 5.5 was optimal in alpha amylase assays. The results from subsequent p-hydroxybenzoic acid hydrazide (PAHBAH) reducing sugar assays indicate that while alpha amylase activity (using starch as substrate) levels do increase around day six, there was not a significant difference between dietary treatments. In the future, trial period should be extended and other carbohydrases tested.

THE NORTHERN QUAHOG (=HARD CLAM), *MERCENARIA MERCENARIA* IN A CHANGING OCEAN: INTERACTIVE EFFECTS OF ELEVATED CO_2, TEMPERATURE AND SALINITY ON PHYSIOLOGY AND SHELL PROPERTIES.

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Ocean acidification has been shown to increase mortality, alter energy metabolism and respiration, and diminish shell mechani-

cal properties of marine calcifiers. In estuaries, fluctuations in environmental parameters such as salinity and temperature are common, which can affect ocean chemistry and modify or exacerbate the effects of ocean acidification on calcifying organisms. Here, we tested if elevated CO_2 levels combined, with either increased temperature or decreased salinity alters physiological and shell mechanical properties of the hard clam, *Mercenaria mercenaria*. The effect of temperature was tested in adult clams. Shell formed during exposure to increased P_{CO2}(800 ppm) at elevated temperature (27°) showed significantly reduced hardness, compared to shell formed during exposure to increased P_{CO2} or temperature alone. The effect of salinity was tested in juvenile clams. Increased P_{CO2} (800 & 2000 ppm) at reduced salinity (15 ppt) lead to profound differences in shell physical properties. After 8 weeks exposure, clams shells appeared chalky with considerable pitting and flaking. After 16 weeks, the hinge region had deteriorated in a large number of individuals, resulting in separation of shell valves. Such changes in shell properties may compromise survival of hard clams under future ocean conditions. Supported by NSF award IOS-0951079 to E.B. & I.M.S.

C-CAN: WORKING TOGETHER TO CHARACTERIZE OCEAN ACIDIFICATION.

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The West Coast shellfish industry has been observing shellfish recruitment failures and larval mortalities both in hatcheries and the wild for the past few years. One hypothesis is that these dramatic declines in productivity are related to increasing ocean levels of carbon dioxide found along the West Coast, and the corresponding decrease in the saturation state of the carbonate minerals that shellfish use to create their shells. The California Current Acidification Network (C-CAN) was formed as a collaborative effort between members of the West Coast shellfish industry and scientists to explore what is causing shellfish losses on the Pacific coast, what role ocean acidification and other factors might be playing in this problem, and how to adapt to these changes in order to sustain West coast shellfish resources. Our first goal has been to develop a roadmap for integrating ocean acidification observing activities on the U.S. West Coast that ensures balanced participation of academic, governmental, and commercial stakeholders. We have held two workshops to date (July 2011 & December 2011) discussing these issues, and a brief overview of these will be provided.

CHANGING OCEAN CHEMISTRY: THE EFFECTS OF OCEAN ACIDIFICATION.

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The seemingly unstoppable rise of carbon dioxide in the atmosphere has caused a corresponding change in ocean carbon dioxide levels: a process known as anthropogenic ocean acidification. The consequent changes in seawater composition causes a variety of problems for many marine organisms, and in particular for the early life stages of such organisms. Organisms whose shells (or other body parts) are made up of calcium carbonate are especially at risk and this risk is growing each year as is the potential to affect the marine food web. Nevertheless, such changes are not uniformly distributed around the world. The sensitivity of any particular seawater to changes depends upon its original composition, and that varies around the world. This presentation will outline clearly the chemical changes to seawater composition that underlie the phenomenon of ocean acidification, will discuss the likely magnitudes of such changes, and will illustrate why scientists believe that some geographical areas have the potential to be more sensitive to these changes than others. I will also indicate the additional complexities introduced by the ability of many animals to internally regulate carbon dioxide and pH levels, thus partially enabling such organisms to buffer themselves against changes in the external seawater composition.

EFFECTS OF TEMPERATURE, SALINITY, AND SUBSTRATE TYPE ON GROWTH AND SURVIVAL OF *OSTREA LURIDA* LARVAE.

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Despite being the only native oyster on the Pacific Northwest coast and its status as a "species of special concern" under the Canadian Species At Risk Act, basic life history knowledge of *Ostrea lurida* is limited. Olympia oysters are found in estuarine environments, therefore, it is expected that they are tolerant of a dynamic range of abiotic conditions. My research focuses on the influence of abiotic factors on the larval growth, settlement and survival of the Olympia oyster. Using field and laboratory experiments I examined the effects of temperature and salinity on larval growth and development. I will also determine their preferred settlement substrate. Preliminary results indicate a strong settlement preference for natural materials, such as shell and rock, over synthetic materials like ceramic and PVC, which are favoured for standardized population monitoring. Similar to other bivalve species, it is expected that *O. lurida* larvae will demonstrate increased growth rates with increased temperature and normal growth over a range of salinity conditions. Physical conditions in marine systems are changing and understanding how organisms and populations will be affected by these changes is imperative for

conservation, restoration and aquaculture efforts, particularly for at-risk species such as *O. lurida*.

MULTIVARIATE STATISTICS REVEAL SEASONAL PATTERNS IN PACIFIC GEODUCK (*PANOPEA GENEROSA*) DISEASE IN THE PACIFIC NORTHWEST.

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Pacific geoduck (*Panopea generosa*) aquaculture in Washington State exists within close proximity to native populations, but little information of disease patterns or epizootics specific to geoduck clams exist. To characterize pathogenic organisms, three *P. generosa* populations (Totten Inlet, Thorndyke Bay, Freshwater Bay) were sampled from 2008–2010, and multivariate statistical analyses were used to explore trends of parasite presence within geoduck populations and identify the environmental factors (locale, depth, and season) that influence the presence of parasite assemblages. Results from non-metric multidimensional scaling showed strong seasonal gradients in geoduck parasite assemblages. Analysis of similarity revealed significant parasite assemblage differences among collection depths and seasons, with the exception of summer and winter. Although further characterization of each parasite identified in this study is crucial to fully understand the factors that drive their presence, multivariate statistical analyses was successful in elucidating environmental drivers that influence the occurrence of prevalent parasites within Northwest geoduck populations. *Vibrio tubiashii* (Vt) is a causative agent of vibriosis in molluscan bivalves. Recent re-emergence of vibriosis in economically valuable shellfish, such as the Pacific oyster (*Crassostrea gigas*) in Washington State, has increased the urgency to understand the ecology of this pathogen. It is currently unknown how predicted environmental changes associated with ocean acidification, such as elevated surface seawater temperature, increased partial pressure of CO_2 (pCO_2), and Vt abundance, will impact marine organismal health and disease susceptibility. This study investigates how environmental cues predicted with ocean acidification influence physiological changes and pathogenicity in Vt. Using laboratory experiments manipulating temperature and pCO_2, we examined how these environmental factors influenced pathogen growth. Larval susceptibility to vibriosis was determined by exposing *C. gigas* larvae to a combination of elevated pCO_2 and Vt concentrations. These experiments provide insight into the environmental parameters that may drive pathogenicity or influence proliferation of the bacterium. Investigation of single and multivariate parameters such as temperature, pCO_2, and pathogen levels will help assess how predicted shifts in ocean conditions can impact shellfish survival and disease resistance.

EXAMINING THE NURSERY VALUE OF INTERTIDAL OYSTER AQUACULTURE, EELGRASS, AND UNSTRUCTURED MUDFLAT FOR FISH AND INVERTEBRATES IN WILLAPA BAY, WASHINGTON.

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Structured benthic habitats such as salt marshes, seagrass beds and oyster reefs are recognized as critical nurseries for fish and crustaceans in estuaries. Most U.S. West coast estuaries include substantial areas of unstructured habitat such as intertidal sand and mud flats as well as areas that are utilized for shellfish aquaculture where structure exists in the form of oysters or clams being grown on or under other structures. We quantified these areas for Willapa Bay, Washington where 20% of the intertidal area is utilized for shellfish aquaculture and present results of surveys and experiments conducted to examine use and function of these habitats for juvenile salmon, English sole, and Dungeness crab. Results from breeder trap and underwater video surveys suggest that English sole are more abundant in structured habitat whereas no difference was detected for Dungeness crab (mostly 1+ age class). Juvenile salmon were also captured in similar abundance over all three habitats using tow nets. Further experiments conducted by ourselves and others suggest the nursery value of structured habitat as refuge from predation and for benthic food production depends on species and juvenile size and mobility relative to the structure as well as location in the estuary.

CAN BURROWING SHRIMP POPULATION DECLINES IN U.S. WEST COAST ESTUARIES BE EXPLAINED BY FLUCTUATIONS IN RECRUITMENT?

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Two species of burrowing shrimp, *Neotrypaea californiensis* and *Upogebia pugettensis* are important members of intertidal mudflat communities in Pacific Northwest coastal estuaries, but cause problems for shellfish farmers. We have monitored populations of these shrimp in Willapa Bay, Washington for two decades and in several other estuaries since 2005. Densities of both shrimp species were either increasing or stable through the mid 1990's in Willapa Bay and then began to decline. *U. pugettensis* are now almost absent in Willapa Bay and many other estuaries along the U.S. West Coast due in large part to the introduced parasitic isopod, *Orthione griffenis* which compromises their reproduction. Recent surveys of *N. californiensis* populations however, suggest

that they too are declining. Since both shrimp have pelagic larval stages that develop in the coastal ocean, we asked whether interannual fluctuations in larval survival and estuary recruitment influence adult populations. We found a significant relationship between recruitment and the density of 1+ shrimp the following year. By tracking a substantial recruitment event for ghost shrimp to Yaquina Bay, Oregon in 2010 however, we demonstrate that the relationship may be more complex with broad recruitment, but high mortality during the first year and density dependent growth and survival thereafter.

THREE DISEASES OF SOFTSHELL CLAMS, *MYA ARENARIA* IN CHESAPEAKE BAY: 2000–2009.

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Trends are reported for the decade 2000-2009 on three prevalent diseases that affect benthic *Mya arenaria* softshell clams in Maryland waters of Chesapeake Bay, whose populations have been vastly and consistently diminished since 1990. *Perkinsus chesapeaki* infects members of at least six clam species in Chesapeake Bay. Infections occurred during 2000–2009 at variable mean summer-fall prevalences, which respectively ranged at 26–83% and 13–100% among commercially harvested *M. arenaria* and *T. plebeius* clam populations. In contrast to *M. arenaria* clams, *P. chesapeaki* prevalences remained high among *T. plebeius* razor clams during seasons of low water temperatures. An unnamed virus commonly infects nuclei of gill epithelial cells in *M. arenaria* clams, where it causes extreme, pathological nuclear hypertrophy. Gill epithelium nuclear hypertrophy (GENH) virus infections occurred during 2000–2009 at variable mean annual prevalences of 18–90%. Infection prevalences showed a consistent decreasing trend during 2000-2003, and a consistent increasing trend during 2003–2009. Lethal disseminated neoplasias (DN) occurred among *M. arenaria* clams during 2000–2009 at variable mean annual prevalences of 2–44%. Periodically or locally significant softshell clam mortalities from DN disease in Chesapeake Bay are likely, especially when DN disease compromises defensive capabilities of clams that are coincidentally infected by microbial pathogens.

WHAT MAKES OR BREAKS ALASKAN CRAB FISHERIES IN THE CONTEXT OF RESILIENCE AND RECOVERY?

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Resilience, the capacity of a system to respond to perturbation without loss of structure or function, is a critical feature in marine fishery systems and a feature for which we would ideally manage. Yet, our understanding of resilience in marine systems is limited, and resilience is currently not incorporated in single-species stock assessments, the process by which a large majority of living marine resources are managed in the US. When fisheries collapse and do not recover, the lack of resilience is easy to perceive, but the conditions which make the system vulnerable are often not clear. Historically, major crab fisheries in Alaska consisted of king crab (red king crab *Paralithodes camtschaticus*, blue king crab *Paralithodes platypus*, and golden (or brown) king crab *Lithodes aequispina*), Tanner crab (*Chionoecetes bairdi*), and Dungeness crab (*Cancer magister*). Crashes of many of these fisheries led to regional closures and depressed returns that by the mid 1990's were only 20% of their historical maximum. Incorporation of the concept of resilience is needed to create and implement conservation and management measures for rebuilding depleted stocks. I compare Alaskan crab fisheries collapses and recovery failures to better inform ecosystem-based fishery management approaches for rebuilding these stocks.

GROWTH OF HATCHERY REARED GREEN SEA URCHINS *STRONGYLOCENTROTUS DROEBACHIENSIS* UNDER VARIOUS CULTURE CONDITIONS.

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Growth rates for two hatchery cohorts of the green sea urchin (*Strongylocentrotus droebachiensis*) were monitored over the course of four years under various culture conditions and dietary regimes. Culture conditions included rearing in recirculating aquaculture systems (RAS), or release on-bottom and unconfined at aquaculture lease sites. In the RAS, growth rates were tracked for discrete groups of urchins, and at the lease sites growth of tagged released urchins was monitored with diver surveys. Dietary regimes in land-based culture included kelp and four formulated diets. Over 5,000 measurements were recorded for test diameter (TD) and weight, and these were compared with a relatively new method for estimating test diameter based on the cubed root of the weight. Urchins in the RAS grew significantly faster than urchins released on bottom, with the fastest growth in the RAS seen with a 20% protein diet, for urchins grown in V-shaped troughs. Based on projected growth, less than 30% of the RAS cohort will achieve the Maine legal minimum harvest size of 58 mm TD within three years of settlement. Improvements in hatchery protocols, diets, and aggressive size grading offer potential to improve the growth and economic viability for culture of this species.

THE FUNCTIONAL GENETICS OF OSMOREGULATION IN *CRASSOSTREA VIRGINICA* EXAMINED USING EXPERIMENTAL AND POPULATION GENETIC APPROACHES.

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When habitat quality varies spatially, high gene flow is expected to constrain local adaptation at habitat margins. Yet, along

estuarine salinity gradients, benthic invertebrates with sedentary adults and high fecundity may experience strong viability selection that causes functional genetic differentiation each generation. To test the hypothesis of functional population differentiation in osmoregulatory genes of *Crassostrea virginica* (eastern oysters), this study combined two complementary approaches. First, we experimentally measured the relative importance of genotype (population source) and broodstock conditioning on larval salinity tolerance. Adult oysters from low, intermediate and high salinities of the Delaware Bay were conditioned to both high and low salinities in a hatchery. Larvae were reared in high and low salinity for 10 days and survival was analyzed in a three-way ANOVA to test for a significant broodstock source by treatment interaction indicating functional genetic differentiation or persistent maternal effects. Second, in the context of a larger genomic study, DNA sequence comparisons were made for *C. virginica* within an estuary and to outgroups to test for signatures of selection in taurine transporter, a gene whose product regulates the influx and efflux of taurine, a critical osmolyte, during both hyper- and hypoosmotic stress.

MICROBIAL STUDY AND HEALTH OF NATURAL ASSEMBLAGES OF THE CARPET SHELL CLAM, *TAPES DECCUSATUS*, FROM EGYPTIAN COASTAL WATERS.

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Clam production and trade is often affected by clam and human pathogens, respectively. To initiate clam aquaculture in Egypt, the current status of carpet shell clam, *Tapes deccusataus*, health in Egyptian natural fisheries and possible risk associated with their consumption were investigated from December 2010 till November 2011. Potential clam pathogens (vibrios and *Aeromonas*) and human pathogens (*Escherichia coli*, *Staphyllococcus* and *Salmonella*) were counted monthly in clams from Alexandria, Ismailia, and Damietta. Severe contamination with vibrios was observed in Ismailia from April to June with counts within the range 1125–1750 CFU/ml, reflecting possible negative effect on clam production in hatcheries if broodstock clams are collected during vibrios outbreaks. *Aeromonas* and *Staphylococcus* were not detected during winter and spring, but the highest count in summer was within World Health Organization (WHO) permissible limits. Faecal indicators and potentially harmful human pathogens (*Salmonella* spp. and *E. coli*) were recorded at all sites with highest *Salmonella* spp. count in Alexandria in all months and in Damietta and Ismailia in July. Results showed that these sites are not suitable for clam aquaculture or natural harvest of clams for human consumption unless government regulations are enacted (e.g. depuration before use for human consumption) to protect public health.

DENMAN ISLAND DISEASE IN KUMAMOTO OYSTERS - IMPACT AND MANAGEMENT.

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Denman Island disease is caused by the protozoan parasite *Mikrocytos mackini*, first discovered in Pacific oysters (*Crassostrea gigas*) in British Columbia in the 1960s. The disease can cause visible pustules on the skin surface in Pacific oysters in colder water areas but the prevalence of such lesions in Pacific oyster growing areas further south is rare and considered incidental. *Mikrocytos mackini* was delisted by the World Animal Health Organization (OIE) as an internationally reportable disease in 2007. This is the first report of *M. mackini* infection in Kumamoto oysters (*C. sikamea*) and the first report of *M. mackini* in any species of oyster in California. We found the infection first by histology in brood stock oysters from Humboldt Bay, California in February 2011. By histology, prevalence was 7% in the first population examined. By evaluating pooled PCR (polymerase chain reaction) samples of additional different source groups of Kumamoto brood stock oysters, the prevalence was estimated to range from about 4% up to about 30%. Evaluation of brood and seed stock Pacific and seed stock Kumamoto oysters from Humboldt Bay by PCR revealed no additional positive samples. Identity with west coast *M. mackini* was further confirmed by ITS sequence comparison.

REPRODUCTIVE BIOLOGY OF THE CARPET SHELL CLAM, *TAPES DECCUSSATUS*, FROM EGYPTIAN COASTAL WATERS.

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The reproductive cycle of the commercially important carpet shell clam, *Tapes deccussatus* was studied as the clam is a potential candidate for aquaculture in Egypt. Clams from six sites in three main natural fisheries (Alexandria, Ismailia and Damietta) were examined over a twelve month period (December 2010–November 2011). Smear preparations and histological examination (n = 30/site/month) showed that minimum shell length at first maturation was 18.5 mm. Gametogenesis in females began in March with a

maximum percentage of 86% ripe females observed in April. Spawning started in April and continued to June (100% spawning/partially spent). In July, all examined females were at spent stage. In males, active gametogenesis was evident in almost all months. However, the highest percentage (100%) of ripe males was observed in May. All males were spawning/partially spent in June. Ripe males were re-observed in July, reflecting a possible occurrence of a second spawning later in the year. From August to November, most individuals were either spent or in a gonadal resting stage. Clam broodstock should be collected no later than February for final conditioning in the hatchery to avoid natural spawning in the field. Clam fishing should be avoided from April to June for clam fisheries management.

USING PAST, PRESENT AND FUTURE ESTIMATES OF WATER FILTRATION AS A TARGET FOR OYSTER RESTORATION.

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The well documented 200 year decline in US oyster reefs is increasingly discussed from the perspective of lost ecosystem services. Alongside lost fisheries, the reduction in water filtration and consequences for water quality has received considerable attention. Indeed this is regularly cited as part of the justification for restoration. As the result of a new assessment of historic and present-day oyster reef extent and biomass, together with an overview of in situ filtration capacity we revisit the role of oyster reefs in bay-scale water filtration. Historic maxima appear to show that oysters were capable of filtering quantities equivalent to the entire volume of many major bays within just a few days. In many cases such numbers represent the equivalent of filtering the entire water volume several times within the residence time for those bays. Generally such numbers have declined dramatically in recent decades. Restoration planners can utilize this approach to set ecologically meaningful targets and to make powerful arguments to stakeholders and funders. Future goals could be set based on some fraction of historic capacity; a desired increase in filtration relative to current; the rate of clearance relative to water volume or residence times; or indeed on some combination of these.

ASSESSING THE EFFECTS OF WATER TREATMENTS ON LARVAL OYSTER (*CRASSOSTREA GIGAS*) GROWTH AND SURVIVAL IN A PRODUCTION HATCHERY SETTING.

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In response to high mortality rates amongst larval oysters (*Crassostrea gigas*) at the Taylor Shellfish Hatchery in Quilcene, WA, a series of experiments were run to investigate a remediation strategy. It was hypothesized that deep water upwelling events were bringing low pH, high pCO_2 waters into the hatchery and adversely effecting larval health. With this in mind, a $NaCO_3$ injection system was established to both elevate pH and decrease pCO_2 in the deep water. Treatment success was determined by larval growth and survival. Preliminary results indicated that deep water was indeed capable of killing larvae at day 14, and that a carbonate injection system helped to counteract the lethal effect of deep water.

DEMONSTRATING THE VALUE OF SHELLFISH RESOURCES: PUBLIC EDUCATION AND OUTREACH THROUGH COMMUNITY-BASED OYSTER RESTORATION IN DELAWARE.

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Delaware's coastal lagoons, known locally as ''inland'' bays, exhibit the effects of chronic eutrophication and sediment erosion from several decades of sustained development and nutrient input from within the watershed. This cumulative impact has degraded water quality and has reduced diversity and abundance of various species of fishes, invertebrates and submerged aquatic vegetation. In 1994 the Delaware Legislature established the Delaware Center for the Inland Bays (CIB) to foster a program for stewardship of the estuary and its indigenous flora and fauna. The presentation reviews how a community-based oyster gardening program initiated in 2003 and associated field research and restoration activities have demonstrated the habitat value and related ecological benefits of oysters and bivalve filter feeders. Using different aquaculture methods, oysters in fixed and floating gear and the three dimensional off-bottom structure of rip-rap commonly used for shoreline stabilization and erosion control have facilitated enhancement of living shoreline habitat and natural recruitment. Field research and educational outreach validating the important contribution of oysters and shellfish resources to estuarine health have increased public awareness in local coastal communities. Shellfish ecosystem services and their intrinsic value are also increasingly recognized by state officials responsible for managing estuarine resources and public policy development.

THE ROLE OF CV1MMP IN HEMOCYTE MIGRATION AND ENCAPSULATION.

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In *Crassotrea virginica*, hemocytes, the blood cells, are responsible for digestion and nutrient transport, shell formation and repair, excretion, development, immunity, and wound healing. In order to perform most of these functions, hemocytes migrate through epithelial tissues. Although the mechanisms governing cellular diapedesis in bivalve molluscs are poorly understood, our collaborative efforts to explore encapsulation have resulted in the discovery of two important genes, a zinc-dependent matrix metalloproteinase (*Cv1MMP*) and a mucin-like phosphoprotein associated with the foliated shell layer (*Folian*), that both localize to the shell forming outer mantle epithelium. *Cv1MMP* is up-regulated in oyster hemocytes after infection and has been localized at the leading edge of migrating cells during feeding. Glass inserts placed into the oyster trigger an encapsulation response and are mineralized within 24 hours. *Cv1MMP* and *Folian* positive hemocytes are associated with those crystalline deposits. Within 2 weeks, these inserts are covered in mineral and periostracal material. Our findings suggest that *cv1MMP* and *Folian* may be functionally related by mediating hemocyte migration and encapsulation as an immune response in the eastern oyster.

EVALUATION OF VARIABILITY IN *PERKINSUS OLSENI* POPULATIONS USING ANALYSIS OF PROTEIN EXPRESSION BY 2D-PAGE AND MASS SPECTROMETRY.

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Perkinsus olseni is a protozoan parasite of marine molluscs in Asia, Australasia, Europe and South America. A proteomic approach was used to evaluate the variability of *P. olseni* in populations of the Spanish coast. *P. olseni* parasites were isolated from four clams *Ruditapes decussatus* from each of the following locations: Ría de Arousa and Ría de Pontevedra in Galicia (NW Spain), río Carreras (Huelva, SW Spain) and four clams *Ruditapes philippinarum* from Delta de l'Ebre (Catalonia, NE Spain). A *P. olseni* clonal culture was produced from each clam (four clonal cultures per geographic location). The protein expression both in parasite cells and in extacellular products released to the culture medium was compared between the four locations, by 2D-PAGE and applying PD Quest software. In the case of parasite cells, 35 spots exclusive of some location and 19 spots common to all locations were selected for further identification; with regard to extracellular products, 23 spots exclusive of some location and 11 common to all locations were selected. Just 38 proteins could be sequenced by mass spectrometry and 27 of them were identified. The low number of protozoan proteins in databases makes difficult protein identification.

EFFECTS OF CULTURE ENVIRONMENT ON THE GENE EXPRESSION OF DIFFERENT STRAINS OF QUAHOG PARASITE UNKNOWN (QPX).

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Quahog Parasite Unknown (QPX), a facultative parasite, has plagued populations of wild and cultured northern quahogs, *Mercenaria mercenaria*, along the Eastern U.S. This study is proposed to examine whether QPX gene expression differs based on culture environment. Different culture environments, as well as different strains, are expected to result in differences in QPX gene expression. Two strains of QPX obtained from different locations in Massachusetts (ATCC and 5-1) were cultured in three conditions: clam tissue and seawater, culture media, and sterile seawater. Using a Genefishing technique for differentially expressed genes, RNA extracted from cultured QPX cells was examined. Bands of interest were isolated and will be sent for sequencing and analyzed using NCBI Blast technology. Overall, seawater only cultures showed amplification with more primers than other environments, demonstrating differences in gene expression. Also, the ATCC strain produced more amplification in culture media and seawater and tissue cultures whereas the opposite was true for sterile seawater cultures. Sequencing of bands must still be completed. These results will potentially lead to a better understanding of the genes playing a role in the virulence of the QPX parasite. With this awareness, improved prevention and treatment means could be explored.

PRODUCTION AND ENVIRONMENTAL EFFECTS OF MANILA CLAM FARMING IN NORTH PUGET SOUND: APPLICATION OF THE FARM MODEL TO DETERMINE OPTIMAL CULTURE CONDITIONS AND SUSTAINABLE CARRYING CAPACITY.

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Described is a specific example of carrying capacity assessment, which includes both the ecological aspects, and the social dimension for a 6 acre Manila clam farm in North Puget Sound. Seventy percent of the farm area is under cultivation, and the harvest ranges

from 70,000 to 100,000 pounds per year. The farm and surrounding area have been monitored regularly with information gathered on water quality, the culture practice regime, and appropriate economic indicators. The research program includes the application of the Farm Aquaculture Resource Management (FARM) model, applied to this experimental site for analysis of shellfish production and environmental effects. FARM is able to match the production figures for the test farm, and provides important quantitative information on the mass balance of algae, detritus, and nutrients, biodeposits, and the role of fouling seaweeds over the culture cycle. The model is also used to classify the farm area with respect to its environmental footprint, by applying the well-known ASSETS eutrophication assessment model. Finally an analysis of culture practice is made, in order to evaluate optimal farming conditions with respect to profitability, environmental impact, and social aspects. This research receives support from the NOAA Saltonstall-Kennedy Grant Program (FY10) grant number NA10NMF4270309.

PRODUCTION AND ENVIRONMENTAL EFFECTS OF MANILA CLAM FARMING IN NORTH PUGET SOUND – APPLICATION OF THE FARM MODEL TO DETERMINE OPTIMAL CULTURE CONDITIONS AND SUSTAINABLE CARRYING CAPACITY.

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There is some discussion in the Puget Sound area concerning the use of intertidal zones for shellfish cultivation. In order to help inform that discussion, we present an assessment of the ecological carrying capacity of a 6 acre Manila clam farm in North Puget Sound. 70% of the farm area is under cultivation, and the harvest ranges from 70,000 to 100,000 pounds per year. The farm area has been monitored regularly, through funding from the NOAA Saltonstall-Kennedy Program; this has made available water quality data, culture practice, and economic indicators. The research program includes the application of the Farm Aquaculture Resource Management (FARM) model, applied to this experimental site for analysis of shellfish production and environmental effects. FARM is able to match the production figures for the test farm, and provides information on the mass balance of algae, detritus, and nutrients, biodeposits, and the role of fouling seaweeds over the culture cycle. The model is also used to classify the farm area with respect to its environmental footprint, by applying the well-known ASSETS eutrophication assessment model. Finally an analysis of culture practice is made, in order to evaluate optimal farming conditions with respect to profitability, environmental impact, and social aspects.

GROWTH AND FEEDING RATES EXHIBITED BY GEODUCK CLAM LARVAE (*PANOPEA GLOBOSA*) IN THE LABORATORY.

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Two sets of independent experiments were carried out in the laboratory at 22 °C in order to document the growth, clearance (CR) and ingestion rates (CR) of the geoduck clam, *Panopea globosa*, during larval development. Larvae were batch-cultured in 500 L conical tanks at 22 °C. Clearance rates and IR were evaluated in 20-ml containers using 50, 100, 200 y 300 cells μl^{-1} of *Isochrysis sp*. (clone T-ISO). The larval period of *P. globosa* lasted 13-14d, and metamorphosed at a shell size of 330 – 350 μm. Growth rate evaluated in 5 cohorts was 19.8 ± 1.0 μm d^{-1}, and survival varied between 20% and 50%. Larvae exhibited decreasing CR values with increasing algal concentrations, while IR steadily increased, except at the highest concentration (300 cells μl^{-1}) where the IR of all developmental stages reached asymptotic values or decreased. Both CR and IR scaled allometrically as a function of AFDW, with an exponent value between 0.59 and 1.55, depending on the cell concentration. The IR of 115 μm "D" larvae (75 – 85 cells h^{-1}) increased by 30× (50 cells μl^{-1}) or 75× (300 cells μl^{-1}) once they reached the premetamorphic stage.

EXPLORING THE USE OF HABITAT EQUIVALENCY ANALYSIS TO EVALUATE BENEFITS AND IMPACTS OF SHELLFISH FARMING.

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Habitat equivalency analysis (HEA) is a tool used by NOAA to evaluate natural resource damages from oil spills and other environmental contamination events, and to allocate economic liabilities for remediation and restoration costs. The model evaluates natural resource injuries, and calculates the damages over the time period they have occurred as 'discounted service acre years' (dSAYS). Remediation actions are evaluated as the time taken for habitat resources to achieve full functional equivalency, in consideration of the adversely affected habitat type(s) in the injured species 'service area'. More recently, we have used this model to evaluate habitat benefits from restoration in conservation banking instruments. Most recently, the model is being explored to evaluate how changes in habitat from shoreline development may affect ESA-listed salmonids, with the intent of providing a 'normalized currency' from which appropriate mitigation could be proposed. In

this presentation, the use of HEA is explored to evaluate the long-term ecosystem services provided by shellfish aquaculture relative to the pulse disturbances that occur with farming practices. Representative examples from oyster and geoduck culture will illustrate the model as a means to better quantify the ecosystem services and impacts associated with these types of shellfish farming in Washington State.

LABORATORY EXPOSURE OF JUVENILE *CRASSOSTREA VIRGINICA* TO CADMIUM OR MERCURY: ARE CHANGES IN PHYSIOLOGY DUE TO CELLULAR PARTITIONING OF METAL STORAGE?

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Oysters transplanted to highly degraded sites (i.e., inorganic pollutants, habitat removal, etc.) have been shown to accumulate metals, and have reduced physiological functions and altered energy budgets (Mass *et al*., 2011). In this study, laboratory exposures to Cd or Hg were used to understand the effects of toxic metals on oyster physiology. A 4-week dissolved exposure was designed with 3 treatments: Control, Low (0.014 mM Hg or 0.6 mM Cd), and High (0.056 mM Hg or 2.4 mM Cd). Following the exposure, oysters were analyzed to determine condition index and biochemistry, energy budgets, clearance rates, cellular biomarkers of stress, and subcellular binding of metals. Oysters exposed to High-Cd treatments had significantly decreased clearance rates (ANOVA, $p < 0.05$); however, there were no differences found in Hg- exposed oysters. Condition indices were reduced significantly in oysters with High-Cd or High-Hg exposures (ANOVA, $p < 0.05$) Cellular responses also showed significant differences with respect to Cd or Hg exposure; oysters exposed to High treatments had less energy storage (i.e., carbohydrates), but higher energy usage (ETS assay). Correlations suggest that accumulated metal burdens in different subcellular fractions (i.e., heat-denature proteins) may be related to the changes in physiology.

AN ASSESSMENT OF THE ONGOING IMPACT OF THE PATHOGEN *BONAMIA OSTREAE* IN THE EUROPEAN FLAT OYSTER *OSTREA EDULIS* AT TWO SITES WITH DIFFERENT HISTORIES OF EXPOSURE.

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The protozoan parasite *Bonamia ostreae* has had a significant impact on the European flat oyster, *Ostrea edulis*, with major losses occurring since the 1970's in Europe. Since the pathogens introduction into various sites some evolution of the host parasite relationship has occurred. *O. edulis* populations were studied at two sites along the Irish coast to investigate the current status of *B. ostreae* in Irish waters. Live oysters were collected every 3 months from Clew Bay on the west coast, where *B. ostreae* infection has occurred since 1988, and Lough Foyle on the north coast, recently infected in 2005. Prevalence of infection was determined through a combination of heart smear screening and conventional PCR with *B. ostreae* specific primers (Bo Boas). Lough Foyle was expected to display a significantly higher prevalence of infection compared to Clew Bay due to its recent exposure to *B. ostreae* and its large oyster population. This was not the case, with prevalence of infection in Lough Foyle being only slightly higher than Clew Bay for some periods, 9.8% compared to 6% in January, and lower at others, 5.5% compared to 10.7% in July. The influence of other factors such as temperature and density were also investigated.

ECONOMIC AND ENVIRONMENTAL EFFICIENCY: THE CASE FOR ALASKAN PACIFIC OYSTER (*CRASSOSTREA GIGAS*) INDUSTRY.

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Projections of financial performance of a small-scale Pacific oyster farm using lantern nets, with a maximum production of 605,625 oysters, was conducted to determine economic efficiency. Estimates of initial capital investment and annual operating costs were formulated, and an annual cash flow and enterprise budget were developed. Results show that the largest costs contributing to annual operating expenses are: labor including farm owner's opportunity cost (39.15%), freight for harvested oysters (21.98%), and seed cost (12.48%). Net returns over a 20-year farm horizon based on an 8% discount rate indicate a positive Net Present Value of $44,836.40, with a suboptimal Internal Rate of Return of 9.58%. In order to increase profitability for the Alaskan oyster industry, stakeholders are in the process of adopting business cluster development strategy to reduce regulatory costs and encourage resource sharing, development of cooperatives for bulk purchase of equipment and oyster seed and collaborative marketing, and adopting new production techniques to reduce labor costs. This presentation focuses on how in the course of achieving cost efficiencies for oyster operations, the environmental impact is reduced as the footprint size of the farms decrease with consolidation.

DESCRIPTION AND HISTORY OF SHELLFISH CO-MANAGEMENT WITH WESTERN WASHINGTON INDIAN TRIBES.

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Bivalve shellfish have provided sustenance to Pacific Northwest Tribes for thousands of years and figured prominently in tribal spiritual beliefs. Following an historic treaty ruling in 1974, known as the Boldt Decision, re-affirming the tribes' fishing rights in western Washington, the Northwest Indian Fisheries Commission (NWIFC) was formed to assist 20 treaty tribes in their new roles as natural resource co-managers and to establish a framework for joint tribal and state fisheries management. Tribal treaty rights subsequently were expanded in the mid-1990's in what is known as the Rafeedie Decision to include tribal shellfish harvests from public and private tidelands, except for shellfish cultured in created beds. Tribes now co-manage shellfish harvests divided 50-50 between the tribal and non-tribal fishers. The NWIFC and the tribes also play an increasingly important function in collaboration with commercial shellfish farmers and others in culturing shellfish, and in monitoring and maintaining water and habitat quality.

MANAGING WITH UNCERTAINTY IN THE KING AND TANNER CRAB FISHERIES OF THE EASTERN BERING SEA.

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Bering Sea and Aleutian Islands king and Tanner crabs are co-managed by the Alaska Department of Fish and Game and the National Marine Fisheries Service. Agency surveys provide most of the data on ecological and physical factors affecting crab distribution and abundance that support the North Pacific Fishery Management Council stock assessment and fishery evaluation of ten stocks. Survey, biological, economic, and modeling data are reviewed annually to recommend biological reference points associated with the status of stocks. Crab stocks fluctuated dramatically in the past 40 years with total mature male biomass of federally managed species fluctuating between 100,000 t and 500,000 t annually. Although biomass has stabilized around 200,000 t since 2005 and two stocks have been declared "rebuilt", two stocks remain "overfished". Uncertainty accounted for in the management of crab stocks includes survey uncertainty (stock biomass CVs range from 12–84%) and within assessment uncertainty for modeled stocks. Unaccounted uncertainty that has been identified includes assumptions made about survey catchability, natural mortality, and stock productivity. Although a number of agency and university studies have focused on the climate interactions with crab production, behavior, habitat use, and spatial distribution, the direct effects on management uncertainty and sustainability are largely unknown.

EFFECTS OF OCEAN ACIDIFICATION ON EMBRYO, LARVAE, AND JUVENILE SURVIVAL OF RED KING CRAB (*PARALITHODES CAMTSCHATICUS*) AND TANNER CRAB (*CHIONOECETES BAIRDI*).

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Shell building marine organisms in the North Pacific are particularly at risk to the effects of ocean acidification. To assess the potential effects on commercially important crab species, laboratory experiments were conducted to expose crab to increased CO_2 concentrations during critical and likely most sensitive life stages. Red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) ovigerous females, larvae, and juveniles were held in control (pH 8.0) and acidified (pH 7.8 and 7.5) treatments. Significant negative results of exposure to increased CO_2 levels were found during embryological, larval, and juvenile development of red king crab. Although embryos and larvae exposed to higher CO_2 levels were larger and had higher calcification, embryos took longer to hatch, embryo condition was reduced, and larval survival was significantly reduced. The morphology of juvenile red king crab and Tanner crab was unaffected by increased CO_2 exposure, however, growth and survival were significantly reduced in both species. Calcification was only significantly reduced in Tanner crab and only the energetic condition of red king crab was significantly reduced. These laboratory results indicate an immediate need to apply *in situ* measurements of temperature and CO_2 concentrations to laboratory studies to estimate population level effects of ocean acidification.

MODULATION OF PUMPING RATE BY TWO SPECIES OF MARINE BIVALVE MOLLUSCS IN RESPONSE TO NEUROTRANSMITTERS.

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This study investigated modulation of feeding rates in bivalves exposed to a variety of neurotransmitters, including serotonin (5-hydroxytryptamine), dopamine (3-hydroxytyramine) and the dopamine agonist apomorphine on isolated gill tissue and intact members of two bivalve species: blue mussel, *Mytilus edulis*; bay scallop *Argopecten irradians*. A three-pronged approach was taken to examine effects of drugs at the level of organelle, organ and whole animal. Four potential loci of control were examined: exhalant velocity (V), cross-sectional area of the exhalant aperture (Ax), clearance rate and ctenidial morphology. Volume flux (Q) was calculated as V*Ax. Results show differential effects of neuromodulators *in vivo* and *in vitro*; e.g., threshold inhibition of

apomorphine on excised scallop gill was 1×10^{-6}M, whereas intact scallops closed their shells at 1×10^{-7}M. Furthermore, *in vivo* responses of mussels were different than scallops when exposed to the same drugs. Mussels exhibited a significant decrease in Ax and Q ($p < 0.05$) at 1×10^{-6} M dopamine with no reduction in V, whereas increasing doses of dopamine resulted in reductions in V ($p < 0.05$) in scallops with no significant effect on Ax or Q. Results suggest both autonomous and physiological regulatory control mechanisms can be employed in species-specific manners.

ESTABLISHING THE RELATIONSHIPS BETWEEN LARVAL AND FIELD TRAITS IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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The focus of selective breeding programs for the eastern oyster hitherto has been targeted solely at improving post metamorphic, field traits and in particular, disease resistance. Whilst its success has played a central role in the development of oyster aquaculture in the Chesapeake Bay, much work is still to be done for the growing industry, especially in the hatchery. Improving larval performance through selection could reduce the burden on hatcheries to acquire and condition large numbers of broodstock, save production costs and ultimately make the venture more profitable. After observing genetic variation for larval traits, our aim was to establish the relationships between larval and field traits in order to determine the possible effect on improvements already realized in the field. Using data from 35 families, we examined the genetic and phenotypic relationships between larval setting order and subsequent field performance – spat growth and survival– over an 18 month period. We observed no difference in survival at the end of the trial between spat that metamorphosed in the first three days of the setting period, i.e. faster growing larvae, and those that metamorphosed later. These animals, however, were significantly larger. The difference in size was reduced as the trial progressed.

CONTINENTAL SHELF LOW pH AND OXYGEN CONDITIONS: IMPLICATIONS FOR *MYTILUS CALIFORNIANUS* AND *DORYTEUTHIS OPALESCENS* EARLY DEVELOPMENT.

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While ocean acidification may present a threat to ocean health, determining the implications of these threats for the coastal zone are confounded by key knowledge gaps related to present-day observations of carbon system dynamics. The coastal zone is a highly variable environment particularly in upwelling-driven ecosystems that are characterized by low oxygen as well as low pH intruding onto the shelf. With the use of physical and biogeochemical sensors we have characterized the pH and oxygen environment of nearshore habitats along San Diego. Results thus far reveal a strong relationship between oxygen and pH, with low oxygen and pH occurring on seasonal and event time-scales. We have simulated these *in situ* results in laboratory experiments (independently controlled carbon chemistry, oxygen, and temperature) to determine larval developmental responses to low oxygen and low pH for *Mytilus californianus* and *Doryteuthis opalescens*. At 11°C, *D. opalescens* embryos are developmentally delayed in low pH/oxygen conditions (7.6 and 80 μmol kg^{-1}, respectively) while at 11°C *M. californianus* is not inhibited by low pH/oxygen. These results suggest that upwelling coasts are exposed to a range of pH and oxygen conditions that have effects on the early life stages of some of its economically important species.

WHERE THERE'S A WILL, THERE'S A WAY–FARMING MUSSELS OFF THE COAST OF SANTA BARBARA.

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Santa Barbara Mariculture operates California's only offshore mussel and oyster farm and has been in business since 2003. All the necessary permits from government agencies have been obtained, and part of our success is due to the respect and acceptance we have earned from other users of the ocean and the people of Santa Barbara, and the integration of the farm and product into the community. For the past 3 years, production has leveled off at about 50,000 lbs. of mussels and 60,000 oysters due to a number of constraints: 1) farm management is limited by use of a single boat, 2) the rise of Amnesic Shellfish Poisoning in the last two years has dramatically decreased harvesting periods, 3) strong currents during storms have damaged gear which require time and capital to repair, and 4) no public or private scientific investment has been made. The strengths and weaknesses of Santa Barbara Mariculture's operations will be discussed with an emphasis on possible solutions. California is ready for more open ocean shellfish farms, and if there is a will, then there will be a way.

RESISTANCE TO BACTERIAL DISEASE IN ABALONES: ROLE OF HOST AND BACTERIAL HYPERPARASITE.

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Withering Syndrome, WS, is a fatal disease of abalone caused by a *Rickettsia*-like organism (WS-RLO). Susceptibility varies among species with up to 99% losses of black abalone in

lab and field studies. Since 2002, recruitment has augmented black abalone densities on San Nicolas Island CA (SNI) as compared to the 2001 minimum. In a lab trial, fewer RLO-exposed juvenile black abalone from the SNI (WS selection) died relative to those from Carmel (no WS selection) ($p < 0.05$). Microscopic examination suggests that resistance relates to the host response to initial infection rather than to the ability to resist infection. We observed a second RLO variant (RLOv), morphologically distinct from but infecting the same tissues as the WS-RLO. When SNI and Carmel black abalone were exposed to the WS-RLO alone, SNI abalone remained significantly more resistant to RLO infection than did Carmel animals ($p < 0.03$), but losses were higher and occurred much faster in both groups than when both RLOs were present. Molecular and electron microscopic examination suggests that the RLOv may be the WS-bacterium infected with a phage hyperparasite. These data illustrate disease resistance in the SNI populations but also suggest that phage infection may reduce pathogenicity and dampen losses from the WS-RLO.

EFFECTS OF OCEAN ACIDIFICATION ON THE HEALTH OF WASHINGTON STATE PINTO ABALONE.

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Ocean acidification may threaten nearshore marine molluscs, including members of the genus *Haliotis*. Seawater pH as low as 7.3 has been observed in some parts of Puget Sound and as low as 7.6 in Washington and Oregon coastal waters. Populations of pinto abalone, *Haliotis kamtschatkana*, have declined ~80% since 1992 and conservation efforts have focused on protection of remaining wild stocks combined with restoration aquaculture for supplementation. Survival of vulnerable larval abalone may be influenced by water quality, including pH, calcium carbonate saturation states, pathogens (e.g. bacteria) and predators (e.g. ciliates). We observed reduced survival of day 6 veliger larvae exposed to 750 µatm CO_2 relative to controls (400 µatm) after 48 hr ($p < 0.01$) and 72 hr ($p < 0.001$) exposures. The pathogenicity of *Vibrio tubiashii*, a pathogen of larval molluscs, did not appear to be influenced by 750 µatm CO_2 ($p > 0.05$). Ciliate population growth, however, was strongly inhibited by CO_2; we observed >20-fold fewer ciliates in 750 µatm CO_2 than in controls ($p < 0.001$). Ciliate susceptibility to elevated CO_2 revealed the same trend in a second trial conducted at 400, 750 and 2000 µatm CO_2 ($p < 0.001$). Species identification of the ciliates is in progress.

CONSTRUCTION OF A HIGH-DENSITY LINKAGE MAP AND QTL ANALYSIS OF GROWTH-RELATED TRAITS FOR ZHIKONG SCALLOP, *CHLAMYS FARRERI*.

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Scallops are a major group of bivalves which in turn constitute the second largest group of molluscs. In spite of their species abundance and diverse geographical distribution, very limited molecular data have been accumulated so far, which are far from enough to carry out any systematic biology studies. Fortunately, the recent advent of high-throughput sequencing technologies provides the turning point for scallop research, which can dramatically speed up genetic or genomic studies such as genome/transcriptome sequencing, high-resolution linkage and QTL mapping, population genomics and phylogenomics. Here we report the construction of a high-density linkage map from an F_1 cross of Zhikong scallop (*Chlamys farreri*) using a type IIB enzyme-based tag sequencing technique. More than 3,500 SNP markers have been included in the integrated map with the total map length of 1907 cM and the average marker interval of 0.55 cM. Growth related QTLs have been detected in five linkage groups, which are also verified by the genome-wide association analysis. This genetic map will serve as an important basis for further studies such as genome assembling, comparative genomics, physical mapping and population genomics.

MOLT CYCLES OF GREEN CRABS, *CARCINUS MAENAS*, IN NEW HAMPSHIRE.

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Green crabs, *Carcinus maenas* (Linnaeus, 1758) were sampled monthly from two New Hampshire estuaries (Hampton-Seabrook Estuary and Great Bay Estuary) over the course of a one year field season (Nov. 2009 – Oct. 2010) in order to determine the timing of molt cycles expressed by both populations. Molt timing was assessed by length of average intermolt period, which was estimated based on the color of the ventral side of the crab using the Munsell paint color charts, 40 hue ed. A total of 29,793 green crabs in both estuaries appeared to be on a simultaneous molting schedule centered around the month of June. Females experienced a population-wide molt beginning in June and completed by November. Males experienced population-wide molts in April and November that were completed in June. The bottom temperature profile of both areas sampled was quite disparate; therefore, photoperiod (day length) appears to be a more important environmental cue for green crab molting and reproduction.

QUANTIFYING THE FEEDING BEHAVIOR OF RIBBED MUSSELS IN LONG ISLAND SOUND FOR POTENTIAL NUTRIENT BIOEXTRACTION USE.

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The ribbed mussel (*Geukensia demissa*) is an intertidal bivalve that inhabits the salt marshes of the North American Coast. Although not harvested commercially, this is a keystone species in salt marsh biogeochemical processes, especially pelagic-benthic coupling. A current project is exploring the possible use of this species for nutrient bioextraction in the Bronx River, New York City. We studied the feeding behavior of ribbed mussels in two different sites in Long Island Sound (Bronx River and Milford Harbor) with very different seston characteristics using two portable, flow-through devices designed to quantify mussel feeding using the biodeposition method. Seston in the Bronx River had higher Total Particulate Matter (TPM) (8.75 ± 0.38 mg L^{-1}) than Milford Harbor (5.81 ± 0.21 mg L^{-1}), but seston organic content was consistently higher in Milford ($43.38 \pm 0.97\%$) than in the Bronx ($22.58 \pm 0.45\%$). These seston differences induced different physiological strategies by which mussels were able to maintain the same absorption efficiency (0.71 ± 0.01). In Milford there was a negative relationship between clearance rate and TPM ($R^2 = 0.79$); whereas, in the Bronx mussels had a positive relationship between gut transit time and TPM ($R^2 = 0.96$). This adaptability to different seston conditions is encouraging for the use of ribbed mussels in nutrient bioextraction.

KEY HABITAT FACTORS STRUCTURING CLAM COMMUNITIES IN TILLAMOOK BAY, OR USA.

Stacy Galleher, Anthony D'Andrea, Amy Hutmacher, Eva I. Riedlecker.

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The Shellfish and Estuarine Habitat Assessment of Coastal Oregon (SEACOR) project conducted a comprehensive study of bay clam populations using a stratified-random design on major tide flats in Tillamook Bay, OR. On each flat, we collected clam population data along with habitat characteristics (tide flat, tidal strata, sediment type, and eelgrass *Zostera marina* parameters) to identify patterns in bay clam species composition, abundance, biomass, size, and spatial distribution. Standard parametric and non-parametric statistical analyses combined with non-metric multidimensional scaling show that there are distinct differences in abundance and biomass between tide flats for the four most abundant species studied (*Clinocardium nuttallii*, *Tresus capax*, *Macoma nasuta*, *M. inquinata*). However, eelgrass parameters (e.g. % cover, shoot density, presence) were the key environmental factors affecting bay clam species composition, abundance, and biomass. Several species (*C. nuttallii*, *T. capax, M. nasuta*) had significantly higher densities and biomass in eelgrass beds relative to unvegetated areas of the tideflats. In addition, *M. inquinata* populations were almost exclusively found within eelgrass habitat. These relationships highlight the need to better understand the interactions between bivalve populations and eelgrass beds in Pacific Northwest (PNW) estuaries.

GROWTH AND BORROWING RATES OF EARLY GEODUCK CLAM SPAT (*PANOPEA* SPP.) UNDER LABORATORY CONDITIONS.

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Growth and size-dependent borrowing rates of two Mexican geoduck clam species (*Panopea globosa* and *P. generosa*) were assessed during their early spat development (6 to 7 months) using 30 L aquaria, whose bottom was covered with silt-clay sediment. Pre-burial response time, digging rates, and growth were recorded for organisms with an initial shell length of 8 mm. Once at the sediment, both species exhibited a lag in their burial response that was directly related to their size, with a rate of 21 min mm^{-1} (*P. globosa*) and 16 min min^{-1} (*P. generosa*). Digging rates were similar in both species (ca. 1.2 mm min^{-1}) over a size range of 7 to 36 mm), but were slower in 5-mm seed (0.4 mm min^{-1}). Shell growth rate of buried organisms increased linearly with time and was faster in *P. globosa* (157 μm d^{-1}) than *P. generosa* (58μm d^{-1}). These results suggest that short-term temporary enclosures may be useful for restocking natural populations of geoduck clams with laboratory-produced seed of a size as small as 7 mm.

CULTURING THE FLORIDA APPLE SNAIL FOR RESTORATION PURPOSES.

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The apple snail is an ecologically important animal to numerous endangered species throughout Florida wetland habitats. Stock enhancement of the snail is being explored as a potential management strategy for the conservation of the endangered snail kite. In 2007, Harbor Branch at FAU in collaboration with the SFWMD, began to research the techniques necessary to culture apple snails at a commercial scale. Developing methods that provide high growth and survival rates, reliable captive breeding, and survival post release are critical to ensure the feasibility of a restoration program. The ideal stocking density for juveniles and adults, the diet needed for growth and reproduction, and the parameters allowing for year-round egg production have all been determined. The Florida apple snail can now be reliably cultured

at the facility to address pressing restoration and management concerns such as helping to predict the uptake of copper in the snail kite or assessing the impacts of invasive apple snails on native populations. Highlights from this research program will be discussed and will include culture methods, restoration experiments, and invasive snail studies.

COPPER AND ZINC IN THE FLORIDA KEYS: IMPLICATIONS FOR QUEEN CONCH LARVAL RECRUITMENT.

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The queen conch, a keystone herbivore and once an important fisheries species of the Florida Keys, has not sufficiently recovered after a 25-year fishery closure. Research has shown high levels of copper and zinc in the gonads and digestive glands of adult conch found in the nearshore waters, yet metal concentrations in these areas has not been explicitly tested. Four sites relevant to queen conch larval recruitment were tested in 2010 for the presence of copper and zinc in the water, phytoplankton, substrate, and seagrass epiphytes over seven months. Surface water concentrations from the field were used to conduct acute and chronic toxicity laboratory tests on various ages of queen conch larvae and their microalgae food source. When field zinc concentrations (0–40 µg/L) were used, there was no significant impact on conch survival and development. However, field concentrations of copper (0–15 µg/L) often surpassed water quality standards and impacted the growth, survival, and development of the larvae. Chronic exposure to copper, through the water and food, disrupted the metamorphic success of competent larvae. Exposure to copper at later life stages decreased survival, suggesting that heavy metals have a negative effect on larval recruitment in localized areas of the Florida Keys.

THE EFFECTS OF A PREDATOR ON PHENOTYPIC PLASTICITY IN THE RIBBED MUSSEL, *GEUKENSIA DEMISSA*

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Elevational differences in the salt marsh create different habitats in terms of the physical environment. These differences translate into differences in biotic interactions for organisms, including the risk of predation. Four salt marsh habitats were studied: creek region, low marsh, middle marsh and high marsh. These four habitats differ in the risk of predation for ribbed mussels. Crab predation is greatest in lower elevation areas (low marsh and creek), which are also the areas where bivalve recruitment is highest. Laboratory experiments were used to assess whether the ribbed mussel could produce morphological defenses against predators. Juvenile ribbed mussels were exposed to cues from the crab predator, *Hemigrapsus sanguineus*, to assess whether chemical cues from crabs could induce defensive morphologies, or phenotypic plasticity (e.g., differences in shell mass or shape). The shells of mussels exposed to chemical cues from predators and control animals were not different in shell morphology, but those exposed to crabs grew less and had heavier shells for a given size than control mussels. Field-collected mussels from different regions in the marsh supported the laboratory results. Mussels from environments with higher predation risk had heavier shells than those from low risk environments.

EPIGENETIC MECHANISMS AS A SOURCE OF PHENOTYPIC PLASTICITY IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*.

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DNA methylation is an epigenetic mechanism with important regulatory functions in animals. Considering the importance of DNA methylation in gene regulation, its susceptibility to environmental influence, and its potential heritability, this mechanism is an ideal candidate for providing insights into how shellfish respond to their environment. Previously, we have applied in silico approaches to characterize DNA methylation in Pacific oysters (*Crassostrea gigas*). Our results suggest that DNA methylation is a common feature of the *C. gigas* genome and that functionally distinct categories of genes have significantly different levels of methylation. Specifically, ubiquitously expressed genes, such as those involved in DNA and RNA metabolism, are predicted to be hyper-methylated whereas genes associated with tissue-specific or inducible functions (e.g. response to stress) are predicted to be hypo-methylated. Currently, we are using both genome-wide (MBD-seq) and gene specific (bisulfite sequencing) approaches to experimentally evaluate DNA methylation in *C.gigas*. Results of these analyses are consistent with previous findings. Based on this evidence, we propose that the DNA methylation system in *C. gigas* may be facilitating random variation in environmental response genes that could provide a source of phenotypic plasticity.

ARE BAY SCALLOP POPULATIONS IN FLORIDA RESPONDING TO RESTORATION EFFORTS?

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Bay scallop subpopulations in Florida have been targeted for restoration efforts since the late 1990s. Some subpopulations have responded and appear to have stabilized, while others appear to be recovering. The initial effort included the historically fished waters around Crystal River and Homosassa. This local fishery was closed

to harvest for seven years and enhancement was conducted. As a result, the fishery reopened in 2002 and drives a vibrant local tourism industry. Others, such as Tampa Bay, suffer from repeated collapses and may need constant intervention or may never achieve sustainability. An important component of this recreational fishery involves public awareness. Recent restoration efforts have focused on involvement of local partners and an adaptation of the oyster gardening method. With the renewed interest in bay scallops in Florida, estimates of increasing harvest rates suggest caution. This year, several thousand boaters participated during the opening weekend as well as the July 4th holiday weekend, creating a derby-like atmosphere. Unfortunately, accurate estimates of harvest are challenging and expensive. Pre- and post-season surveys show that harvest did not always focus on most dense populations. Heavy harvest in depleted populations and poaching in closed areas may threaten previous and ongoing restoration efforts.

BASELINE ASSESSMENT OF OYSTER REEFS AND THEIR ASSOCIATED FAUNA IN MULTIPLE FLORIDA GULF ESTUARIES.

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Oysters (*Crassostrea virginica*) are an abundant natural resource of considerable importance throughout the Gulf of Mexico, producing extensive and variable habitats. Most sites in the Gulf of Mexico lack detailed quantitative data on population demographics and the biologically-diverse associated communities for this critical biogenic habitat. Available historical data also were collected using many different methodologies, often with little or no replication. Baseline data are critical to adequately assess potential impacts from discharges related to the Deepwater Horizon spill, related restoration and post-recovery status, as well as anticipated future stressors such as climate change and demographic shifts. The goal of this two-year oyster focused effort is to assess pre-spill conditions at replicate sites in the Florida Panhandle, Tampa Bay, SW Florida and South Florida using identical methodologies. We are assessing 60 natural oyster reefs for multiple metrics: abundance and size, resident reef communities, recruitment, growth and survival and PAH concentrations. We are also sampling tissues at the above sites and oyster reefs in areas likely to have been exposed to DWH oil for genetic diversity. We stress how important collecting baseline *C. virginica* data are to assessing the magnitude of any oil-related impacts and discuss preliminary results.

IMPROVING SHELLFISHERIES MANAGEMENT WITH INTERACTIVE MAPPING TECHNOLOGY.

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Connecticut's municipal resource managers are required to develop comprehensive management plans that identify appropriate sites for recreational and commercial shellfishing activity to avoid potential use conflicts and adverse environmental effects. The use of geospatial information is critical to this decision making process. The variety of information and range of technical skill required to utilize datasets of various sources and scales has been an impediment for managers. These challenges have resulted in confusion and delays in the decision making process. The Connecticut Sea Grant Program, the Connecticut Department of Agriculture, Bureau of Aquaculture and the University of Connecticut's Center for Land Use Education and Research collaborated to develop tools and training to better equip managers in their decision making processes. The result is an interactive online map viewer *The Connecticut Shellfisheries Mapping Atlas* http://seagrant.uconn.edu/whatwedo/aquaculture/shellmap.php. The Atlas integrates a multitude of shellfisheries related geospatial information. The site allows the user to view and overlay GIS datasets and create, share and print maps. The Atlas contains a dual viewer that displays Google and Bing imagery for selected sites. The online viewer is built using ESRI's ArcGIS API for Flex that allows for the creation of rich Internet applications on top of ArcGIS Server.

THE INFLUENCE OF BENTHIC SUSPENSION FEEDERS ON THE OCCURRENCE OF HARMFUL ALGAL BLOOMS IN SHALLOW ESTUARIES.

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The shallow and often poorly-flushed nature of many estuarine systems can make them susceptible to outbreaks of harmful algal blooms (HABs). Historically, many shallow estuaries had been dominated by benthic suspension feeders that were capable of rapidly filtering the water column and preventing the accumulation of algal biomass. In recent decades, overfishing, habitats loss, and hypoxia have diminished suspension feeder populations and may have, in turn, influenced the occurrence of some HABs. This presentation will focus on the key role suspension feeders can play in regulating phytoplankton blooms in shallow estuaries. Specific examples from NY estuaries will include the abilities of the

Northern Quahog, *Mercenaria mercenaria*, the dwarf surf clam, *Mulinia lateralis*, and the common Atlantic slippersnail, *Crepidula fornicata*, to feed on the brown tide alga, *Aureococcus anophagefferens*, and the red tide-forming dinoflagellate, *Cochlodinium polykrikoides*. Data indicate that the differential ability of each filter feeder to consume these HABs combined with the temporal and spatial dynamics of their populations has influenced the occurrence of blooms events in NY during the past 12 years. Finally, negative feedback loops that are established when estuarine productivity shifts from the benthos to the pelagic zone will be discussed.

CONSEQUENCES OF OCEAN ACIDIFICATION FOR NORTH ATLANTIC LARVAL BIVALVES.

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This talk will present the effects of elevated levels of CO_2 on larvae and early stage juveniles from three species of commercially and ecologically valuable bivalve shellfish native to the North Atlantic. *Mercenaria mercenaria* and *Argopecten irradians* larvae grown under low CO_2 concentrations (250 ppm) displayed significantly higher survival as well as faster growth and metamorphosis compared to individuals grown under higher CO_2 levels (>400 ppm). This finding suggests ocean acidification during the past two centuries may be inhibiting the survival of larvae from these bivalves. In contrast, *Crassostrea virginica* suffered significantly reduced survival under higher CO_2 levels (>700 ppm). Short term physiological effects of higher CO_2 included significantly decreased size, RNA:DNA ratios, calcification rates, and lipid content, all which would promote enhanced mortality in an ecosystem setting. Exposure of bivalve larvae to high CO_2 (750 ppm) for only four days was enough to significantly decrease survival compared to normal levels (400 ppm). Longer term experiments demonstrated that *A. irradians* reared under low CO_2 (250 ppm) as larvae were still significantly larger than those reared under higher levels (>400 ppm) after ten months of growth under ambient CO_2 levels. Collectively, these findings demonstrate that larval stage exposure to high CO_2 concentrations has profound implications for bivalve populations.

ECOLOGY OF CLAM BEDS WITH DIFFERENT HYDRAULIC DREDGING HISTORIES.

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Harvesting of clams with hydraulic dredges affects the benthos directly, but the rate of ecological recovery is related to the degree of disturbance and many biotic and abiotic factors. A field experiment was conducted in Long Island Sound on leased shellfish beds where hard clams, *Mercenaria mercenaria*, are routinely cultivated. From June through October 2011 we compared abundance and biodiversity of benthic fauna on plots that were hydraulically dredged 1-year, 2-years, and 5 years ago, as well as a site recently dredged (0-year). Benthic organisms (>1 mm) were identified and enumerated from Smith-McIntyre grab samples. Results indicate distinct assemblages of benthic biota across beds that reflect an ecological succession of recruitment following the dredging disturbance. Pioneer species, such as *Nucula*, *Yoldia*, and *Nephtys* rapidly recruited to the 0-year site, while climax species such as ampeliscid amphipods were most abundant on the 5-year site. The intermediate 1- and 2-year sites had relatively high numbers of individuals, species, and biodiversity. This study demonstrates that benthic communities on managed clam beds are robust and recover quickly after hydraulic dredging.

OYSTER REEFS IN PUMPKIN AND FAKA UNION BAYS, TEN THOUSAND ISLANDS, FLORIDA, AND THEIR PHYSIOLOGICAL AND ECOLOGICAL RESPONSES TO TERRESTRIAL DRIVERS.

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Alterations in freshwater inflow, resulting from watershed development and water management practices, have impacted salinity and water quality within southwest Florida estuaries thereby affecting responses of valued ecosystem components such as oysters. The Comprehensive Everglades Restoration Plan (CERP) Picayune Strand Restoration Project (PSRP) involves the restoration of natural sheet flow distribution across 85 square miles (220 square kilometers), which were drained in the early 1960s in anticipation of extensive residential development. The development's infrastructure changed the hydrology of the area and altered the salinity regimes and substrate of Faka Union and Pumpkin Bays. The refined project includes 83 miles of canal plugs, 227 miles of road removal, and the addition of pump stations (3) and spreader swales to aid in rehydration of the wetlands. It is anticipated that this will result in shifts in oyster reef distribution, community structure and viability of oysters. This study is focusing on spatial changes and correlating the responses to water flow and salinity conditions. This currently includes monitoring of: density of living oyster reefs, condition index of oysters, disease prevalence of the oyster parasite *Perkinsus marinus*, juvenile growth, spat recruitment and reproductive state of oysters.

AUTOMATED IDENTIFICATION OF BIVALVE LARVAE USING A POLARIZED IMAGE ANALYSIS TECHNIQUE.

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Our understanding of the distribution, abundance, and transport of bivalve larvae is limited due to their small size, the similarity between species, and lack of a low-cost automated approach for identification. The objective of this research is to investigate how physical-biological interactions influence spatial and temporal patterns in the distribution of bivalve larvae in the Choptank River, a tributary of Chesapeake Bay. A polarized image analysis technique (PIAT) will be used to identify bivalve larvae in a large number of samples (>1000) from the sub-estuary. With this technique, larvae are identified using 1) birefringent images of the larval shell produced with a polarizing microscope and 2) support vector machine software written in MATLAB. We will describe the methods for training the software with larvae spawned in the laboratory, as well as the results of a series of experiments used to estimate the accuracy and precision of PIAT.

CHEMICAL INDUCTION OF SETTLEMENT IN LARVAE OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN).

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Although numerous studies have been conducted to examine the effects of neuroactive compounds on bivalve larvae, few have identified chemicals capable of inducing settlement behavior in the eastern oyster *Crassostrea virginica*. In this study, we treated competent *C. virginica* larvae with select chemicals to identify those capable of inducing settlement behavior at an average salinity of 9.6 ppt (±0.1). The compounds y-aminobutyric acid and acetylcholine chloride, both at 10^{-4} M, did not significantly increase the percentage of larvae exhibiting settlement behavior. As compared with the control, a significant increase in settlement behavior was induced by treatment with 3-isobutyl-1-methylxanthine, 5-hydroxytryptamine, and L-3, 4-dihydroxyphenylalanine all at 10^{-4} M, as well as ammonia as a solution of 7.9 mM NH_4Cl (pH = 8.0). These findings differ somewhat from the results of similar studies involving other species in the *Crassostrea* genus and may be of value to hatchery personnel or researchers interested in the chemical induction of settlement behavior in the eastern oyster.

THE FEEDING PHYSIOLOGY OF OLYMPIA OYSTERS (*OSTREA LURIDA*).

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Despite numerous studies that have highlighted the benefits of filter-feeding bivalves in estuarine ecosystems, little attention has been paid to the Olympia oyster (*Ostrea lurida*). We have measured allometric relationships between filtration rates and body weights at several temperatures for Pacific and Olympia oysters fed on microalgae. In addition, the effects of suspended sediments on adult clearance rates were also examined. Finally, we have compared particle size preferences of these two species by feeding adults on a range of sizes of fluorescently-labeled wax beads and comparing size distributions in the suspended, fecal and pseudofecal fractions. Olympia and Pacific oysters differ in feeding strategies with Olympia oysters having lower weight-specific filtration rates and a wider particle size preference compared with Pacific oysters, under the same laboratory conditions. Additionally, the clearance rates of Pacific oysters were impacted proportionately more than Olympia oysters when exposed to highly turbid conditions. The potential implications of these laboratory findings will be discussed in terms of acquisition of nutrients and competition for food between these two oyster species, as well as clearance of suspended material in coastal estuaries and bays.

ASSESSING THE POTENTIAL FOR EXPANSION OF OYSTER (*CRASSOSTREA VIRGINICA*) AQUACULTURE IN NEW HAMPSHIRE'S COASTAL WATERS: A GIS-BASED APPROACH.

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This project was initiated in 2009 to ameliorate the economic effects from recurring harmful algal blooms (HABs) in New Hampshire's coastal waters by making shellfish farming more accessible to offshore mussel farmers who are typically more affected by HAB events, and/or others interested in developing or expanding shellfish culture operations in estuarine waters. In 2010, the aim of the study was expanded to also include an assessment of how oyster farms might be used in management of nutrient (mainly nitrogen) pollution to the Great Bay estuarine system. ArcGIS software was used to characterize spatial distributions of HAB toxins, other environmental factors, and social factors potentially affecting molluscan shellfish aquaculture in coastal and estuarine waters. A "map overlay" approach was used to identify those areas with high potential for shellfish (mainly oysters) aquaculture. To date, approximately 500 acres have been identified in the Great Bay estuarine system that may support expansion of oyster aquaculture. Initial experiments quantifying

the amount of nitrogen potentially extracted from the estuary by farmed oysters indicated that expansion of existing oyster farms (total: 15 acres) to 50 acres could be an important tool in nitrogen management in the estuary.

BIOLOGY AND MANAGEMENT OF THE PINK SHRIMP (*PANDALUS JORDANI)* FISHERY OF OREGON.

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Oregon's trawl fishery for pink shrimp is among the states most valuable and dependable. The management of this fishery has exhibited many years of success culminated in recent (2007) Marine Stewardship Council (MSC) sustainability certification. Success of this fishery has depended largely on 1) the favorable biology of the pink shrimp, 2) understanding of stock via continuous and focused research, 3) active communication leading to rapport with the fleet. Presented here is a description of the biology of *P. jordani*, pertinent results of long term and focused research, application of research to management, the methods we use to monitor this fishery cooperatively with the fleet, and the resultant management strategies employed.

GENETIC STRUCTURE OF EASTERN OYSTER POPULATIONS IN DELAWARE BAY AND SELECTION BY DISEASES.

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Oysters, because of their pelagic larval stage, can disperse over vast distances. It is questionable whether genetic structure can develop and be maintained in a well-mixed and flushed estuary such as Delaware Bay. However, oyster populations in the bay have been under strong selection by diseases for many decades. The selection pressure is uneven because diseases cause less mortality in low salinity areas of the bay, so it is conceivable that differential selection by diseases may cause genetic differentiation of oyster populations. We tested this hypothesis by analyzing genetic differentiation of oyster populations with microsatellite markers, some of which are closely linked to disease-resistance genes and some are apparently neutral. The resistance markers were identified by family-based association studies. We sampled oyster populations throughout the bay including lower salinity areas and tributaries. Our analysis shows that the main part of the bay is genetically homogenous while tributaries and the upper most part of the bay are genetically distinct. This structure is strong in disease-resistance markers but weak or absent in neutral markers. Our results indicate that selection by diseases may have caused some genetic differentiation in oyster populations in Delaware Bay which may be transient depending on disease pressure.

PRODUCTION AND BREEDING OF TETRAPLOID EASTERN OYSTER *CRASSOSTREA VIRGINICA*.

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Tetraploids are organisms with four sets of chromosomes. They can mate with normal diploids and produce 100% triploids that have three sets of chromosomes. Triploid oysters are valuable for aquaculture because of their sterility, superior growth, improved summer meat quality and increased survival under summer stress. Tetraploids were first produced in the Pacific oyster and subsequently proven to be highly effective in triploid production. Triploids produced from tetraploids are not only 100% pure and free of induction treatments, but also genetically superior to induced triploids. We produced populations of tetraploid eastern oysters in 2000 and have successively bred them for six generations. The first two generations of tetraploids suffered severe spring mortalities associated with early gametogenesis. Starting from the 4th generation, tetraploids showed significant improvement in growth and survival. Their juvenile growth was even faster than diploids. Their triploid progeny grew 22-74% faster and survived 25–56% better than diploids, leading up to 109% increase in yield. Despite some improvement in genome stability, some tetraploids of the 6th generation could still revert to triploids or mosaics. Gonadal development in tetraploids remained limited. Inbreeding might have caused some regression in performance. Further improvement of tetraploids especially in genome stability is clearly needed.

DEVELOPMENT OF GENOMIC RESOURCES AND TOOLS FOR HEALTH ASSESSMENTS OF MARINE MUSSELS (THE MYT-OME PROJECTS).

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Despite playing critical roles in the function of the marine environment, our coastal and estuarine ecosystems are being exposed to ever-increasing threats caused by human activities as well as through large-scale potential climate change impacts. Through

a Genome BC funded grant, we are using a combination of normalized and subtracted libraries generated by the *Myt*-OME project, along with a developed microarray and qPCR assays to study the responses of marine mussels (*Mytilus edulis* and *M. galloprovincialis*) to environmental and anthropogenic factors. To date approximately 35,000 ESTs have been generated from various tissues over timed exposures, following the application of environmental, biological and physical stressors. Bi-directional sequences were then trimmed and annotated where possible, before development of the 15K feature oligoarray (15,744). The final array composition includes *Myt*-OME sequences with informative annotation, *Myt*-OME sequences of unknown function, sequences from public sources and control features. Specific genes were then used for qPCR validations of gene expression data, such as *hsp*s. This presentation will provide a brief overview of the project and results obtained from microarray analysis of *M. galloprovincialis* subjected to temperature and physical stress, and will also include a discussion of the future application of these resources.

NOT JUST FOR VERTEBRATES ANYMORE: USING MICROCHIPS TO TAG ABALONE.

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Since 1969, global abalone landings have declined more than 50% (FAO, Fisheries and Aquaculture, 2008), with many species now recognized as endangered, threatened, or species of concern. As restoration efforts evolve to include population supplementation, a reliable tagging method is needed. Current abalone tagging methods are unsatisfactory due to tag loss, shell erosion, and encrustation. Observing tag numbers of cryptically positioned abalone can be difficult. To obviate these issues, we evaluated passive integrated transponders (PIT) as tags for Pinto abalone (*Haliotis kamtschatkana*). We applied PITs (8 mm) with cyanoacrylate glue to the dorsal exterior, ventral interior, and by injection into the foot muscle. We subsequently tracked growth, survival, and tag retention over nine months in captivity. Among treatments, differences in relative growth rate and survival were not significant (ANOVA, 3 df, $F = 1.8495$, $P = 0.160$; $X^2 = 6.061$, 3 df, $P = 0.109$, respectively). Shell PIT retention was significantly higher than injected ($X^2 = 18.373$, 2 df, $P < 0.001$; 90% and 10%, respectively). Applying PITs on the ventral interior is a promising method as abalone formed nacre over the tags, incorporating them into the shell. Trials are underway to characterize PIT retention in natural habitats, to determine tag longevity, and to use PITs to track adults re-introduced to aggregations.

DECOUPLING CO-VARYING CHEMICAL EFFECTS ON LARVAL SHELLFISH.

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Understanding responses of organisms to rising CO_2 is confounded by the co-variance of *p*H, pCO_2, mineral saturation and HCO_3^-, all of which have been considered as impactful to physiological processes including shell formation. The environmental processes of upwelling, downwelling, freshwater inputs, metabolism and gas exchange can lead to subtly divergent relationships between these chemical species, and this can complicate the interpretation of experimental or observational data. We present a discussion of observed and potential environmental trends and the first results of a new feedback-controlled chemical manipulation system that can fix one of these parameters while varying others. By precisely controlling alkalinity and total dissolved inorganic carbon, we have created conditions that span wide ranges of mineral saturation and pCO_2 at constant *p*H; widely varying *p*H and pCO_2 at constant mineral saturation state, and varying *p*H and mineral saturation at constant pCO_2. When subjecting organisms to these conditions in experimental work, the three-axis experimental manipulation will allow determination of the parameter to which organisms are most sensitive.

ASSEMBLING A PROGRAM TO MONITOR THE PREVALENCE OF PATHOGENS OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) IN THE NORTHERN LIMIT OF ITS RANGE.

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Global climate change is expected to raise the temperature of southern New England waters by up to 4.5°C in this century, to temperatures similar to the current mid-Atlantic region. One result is expected to be a northward expansion of blue crab (*Callinectes sapidus*) and other fishery species along with their associated pathogens. Using sensitive quantitative molecular methods, a coalition of academic, state and federal partners is beginning to assess the prevalence of two fatal blue crab pathogens (a reovirus and the protozoan parasite *Hematodinium* sp.) from Delaware Bay to the south shore of Massachusetts. After a successful first year,

we are planning to continue disease monitoring for one more year in order to establish a 2 year timeline and cultivate additional partnerships that will allow this survey to garner external funding and continue for an additional 3 years. This project, which also involves both graduate and undergraduate students, can serve as a template for long-term studies of the effects of climate change and latitude on the prevalence of diseases of blue crab or other fishery species in the Northeast.

CORRELATING ENVIRONMENTAL PARAMETERS TO TOTAL BACTERIA AND VIBRIONACEA POPULATION FLUCTUATIONS IN THE LEWES-REHOBOTH CANAL, DELAWARE.

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This study is looking to correlate environmental parameters with total bacteria and *Vibrio* population fluctuations in water and eastern oysters (*Crassostrea virginica)* in the Lewes-Rehoboth Canal, Delaware. Oysters were placed in enclosed trays and submerged in the canal at four sites. Physical and chemical water quality parameters were measured at the four sites, three of which are proximate to a municipal wastewater treatment plant discharge, and one control site away from the discharge. *Vibrio* was detected in canal water and in oysters via the COPP assay, which identifies bacterial colonies that exhibit strong peptidase activity such as *Vibrio*. *Vibrio* counts in the canal water were highest during August and September, while total bacteria counts were high virtually throughout the study period. *Vibrio* counts in oysters were erratic with a significant spike occurring the last week of August after Hurricane Irene however, total bacteria counts were more stable and numerous. Total bacteria and *Vibrio* overall were significantly more concentrated in oysters than in canal water. Oyster growth and mortality rates are also being examined to determine if the discharge has any effect on the growth and survival of the oysters.

RAISING THE BAR ON OUTREACH: MAKING YOUR RESEARCH PROPOSAL MORE COMPETITIVE.

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In a competitive funding environment it is critical that researchers make a compelling case for the impact and relevance of their proposed work to funding agencies and organizations. One way to make this case is to include a strong outreach element. An increasing number of funders now require such an element in their grant programs. The question then becomes; how to do this? Historically, many researchers have included outreach elements (presentations, workshops, factsheets, etc) that they, or more often their students, will produce or conduct. However, as reviewers become more sophisticated in their expectations for outreach it will likely be necessary to rethink this approach and solicit input from outreach professionals. One such group of outreach professionals can be found in Land Grant and Sea Grant Extension programs. Extension professionals have been conducting outreach for more than 100 years and can provide valuable insight into effective approaches that are appropriate to the research subject. Moreover, these professionals are more than ''hired guns'' to conduct outreach; they specialize in engaging with stakeholders and have a high degree of credibility locally and nationally. In this presentation we'll talk about Extension and how Extension professional can make research projects more competitive.

MECHANISMS LIMITING GENE FLOW AMONG EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS ALONG ATLANTIC FLORIDA.

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A third of the Atlantic seaboard consists of barrier island geomorphology with semi-isolated lagoons connected to continental shelf waters through tidal inlets. For many benthic invertebrate and fish species, connectivity among lagoon populations will depend on larval tolerances to marine salinities and hydrodynamic mechanisms facilitating exit, longshore transport and re-entry to lagoons through tidal inlets. We measured among-lagoon dispersal in eastern oysters (*Crassostrea virginica*) along Atlantic Florida and tested several mechanisms potentially constraining gene flow. Using Amplified Fragment Length Polymorphisms (AFLP) to assay genomic variation, subdivision was found between regions north and south of Cape Canaveral. This regional break is coincident with previously described genetic clines in *C. virginica*. Assignment tests provided no indication of inter-regional dispersal during the study period, yet genetic patterns in the south were consistent with historic admixture. The genetic homogeneity within each region, coupled with gene flow comparisons with co-distributed species, suggests that *C. virginica* experiences homogenizing gene flow among lagoons in each region. Between the north and south regions, however, genomically diverse patterns of substructure suggest that natural selection limits gene flow. Field experiments with reciprocally transplanted oysters north and south of Cape Canaveral provide support for regional adaptation and lower viability of migrants.

AN E-QTL APPROACH TO STUDY THE RESISTANCE TO BONAMIOSIS IN THE EUROPEAN FLAT OYSTER *OSTREA EDULIS*.

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Bonamiosis has widely contributed to the decline of the European flat oyster, *Ostrea edulis*, in the natural beds and in the aquacultural production in Europe. Since the middle 1980s, as no ecological solution has yet been found against this disease, efforts have been focused on the selection of naturally resistant animals against the intrahaemocytic parasite *Bonamia ostreae*. Five QTL regions involved in the resistance to *B. ostreae*have previously been identified in this oyster species, confirming the link between genotype of individuals and their resistance against the parasite. Here, we present new QTL regions to precise the link between genotype and phenotypes related to the response of individuals in the context of an experimental infection with *B. ostreae*. Genotypes were assessed with microsatellite markers and SNPs from direct sequencing and from New Generation Sequencing Technologies. Two different types of phenotypical parameters, previously identified as potential indicators of resistance to bonamiosis, have been measured: 1- differential expression of five candidate genes. 2- two haemocytic parameters. The present association between genetical and immunological parameters consists in a new approach to better understand relationships between a marine bivalve and one of his parasite, to enhance, *in fine*, the survival rate of marker-assisted selected animals (MAS).

ENVIRONMENTAL ADAPTATIONS IN BIVALVE SHELLFISH, EVIDENCED FROM STANDARDISED MEASURES DURING THE CALIBRATION OF SHELLSIM.

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ShellSIM (http://www.shellsim.com) is a mathematical model that simulates population and community dynamics, the User defining any combination of up to 14 species of bivalve shellfish, including associated spatial distributions (i.e. farm, raft or patch size) and culture practice (i.e. seeding, mortality and harvesting), whether suspended or on the bottom. Simulating in real time, forced by the minimal practical set of environmental drivers (i.e. temperature, salinity, dissolved oxygen, current speed and food availability), outputs help to quantify consequences for production, environmental effects and economic value. A common model structure simulates effectively in different species, including for the same species at contrasting sites. To calibrate that common model structure, standardised protocols have been used to measure dynamic responses in feeding and metabolism to environmental variations through Europe and Asia. Comparison of those responses has quantified impressive behavioural differences both within and between genera, establishing that there can be no typical mussel, oyster, scallop or clam. Those differences will be described here, including how they infer adaptation to natural habitats defined primarily in terms of food availability and/or temperature regimes.

SHELLFISH BIOLOGY IN THE GENOMIC AND POST-GENOMIC ERAS.

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Genomics provides powerful approaches to understanding the genetic and physiological bases of variation in survival, growth and reproduction. Indeed, one could argue that shellfish are model organisms for understanding metabolic adaptation and evolution (in contrast to an "evo-devo" focus on morphological and developmental evolution). The root of modern shellfish physiological genomics was 1990s research into the correlation of allozyme heterozygosity and growth, which ultimately stimulated the development of a systematic experimental breeding program, which, in turn, provided much of the rationale and the biological material for the genomic resources currently available for the Pacific oyster. The genomics era of shellfish biology has dawned with the imminent publication of a genome sequence for the Pacific oyster *Crassostrea gigas*. Much work remains to assemble scaffolds of genome sequence into an annotated genome, but this work is in progress and much of the transcriptome is already accessible. The interesting questions in the post-genomic era of shellfish biology will likely remain focused on mechanisms of metabolic adaptation and the evolutionary consequences of a life history featuring high fecund and high early mortality.

IMPACTS OF *ALEXANDRIUM OSTENFELDII* ON BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF MANILA CLAMS *RUDITAPES PHILIPPINARUM* NATURALLY INFECTED WITH THE PARASITE *PERKINSUS OLSENI*.

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This study assessed the impact of the toxic dinoflagellate *Alexandrium ostenfeldii*, producer of spirolids on behavioral and physiological responses of Manila clams *Ruditapes philippinarum* naturally infected with the parasite *Perkinsus olseni*. After a week of acclimation, clams were exposed for one week to the toxic algae, followed by a week of depuration. Few effects of *P. olseni* burden, evaluated at the end of this experiment, could be observed on clam physiology. The exposure to *A. ostenfeldii* caused a non significant decrease of *P. olseni* intensity within clams. Although spirolids

accumulated in clams exposed to *A. ostenfeldii*, neither burrowing capacity nor condition index nor digestive enzyme activities were significantly modified with algal exposure. Histological observations showed a significant impact of an exposure to *A. ostendeldii* on digestive gland with desquamation and hemocyte diapedesis of the stomach and intestine epithelia, and presence of *A. ostenfeldii* in the digestive tubules and the intestine lumen. Gills were also affected by the presence of the toxic dinoflagellates causing cell vacuolation and hemocyte infiltration, associated with an increase in concentration of circulating hemocytes. Both *P. olseni* and *A. ostenfeldii* increased the production of reactive oxygen species of hemocytes, which was enhanced when both factors were combined.

TESTING THE ACCURACY OF THE PATCH MODEL USED TO ESTIMATE DENSITY AND CAPTURE EFFICIENCY IN DEPLETION EXPERIMENTS FOR SESSILE INVERTEBRATES AND FISH.

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The Patch model is used to analyze depletion experiment data for sessile invertebrates and fish that do not randomize after sampling. It uses spatially explicit tow path information to form expectations of catch relative to the degree of overlap in successive tows. We tested the Patch model in simulation over a wide variety of possible scenarios. Simulations indicate that density and capture efficiency estimates were useful under realistic conditions for Atlantic surfclam (*Spissula solidissima*) and many other sessile demersal species. Density estimates were generally biased low by position data errors while efficiency estimates were relatively unbiased. A new "hit" matrix method improved accuracy of efficiency estimates, reduced variability for efficiency and density estimates and simplified assumptions about movement of organisms after sampling.

EXTENDED LARVAL CARRY-OVER EFFECTS: SYNERGISMS FROM A STRESSFUL BENTHIC EXISTENCE IN JUVENILE OLYMPIA OYSTERS.

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Although a growing body of evidence suggests that ocean acidification can negatively impact bivalves, few studies have examined how these effects are transferred across life stages in the natural environment. Our previous laboratory work demonstrated that planktonic exposure of oyster larvae to elevated pCO_2 had negative carry-over effects that persisted into the juvenile life stage. In this study, we tested whether the strength of these carry-over effects can be modified by environmental stress in the juvenile habitat. Juvenile Olympia oysters reared in laboratory cultures under ambient and elevated pCO_2 levels as larvae were outplanted to field sites in Tomales Bay, CA, at two intertidal heights that differed in emersion time and exposure to stress. We found that juvenile survival and growth were lowest for juveniles exposed to elevated pCO_2 during the larval stage, and outplanted to the higher, more stressful intertidal zone as juveniles. These effects persisted in juveniles halfway to reproductive age. Overall, our results indicate that negative effects of elevated pCO_2 experienced during the larval stage can lead to juveniles that do not survive or perform as well in a stressful juvenile habitat, suggesting potential demographic consequences of ocean acidification for oyster populations.

THE ENVIRONMENTAL COSTS OF FOOD PRODUCTION.

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All food production has environmental costs, whether measured as greenhouse gasses, habitat transformation, use of resources such as water, energy and chemicals, our outputs such as nitrogen. This talk will review the data that are now available on the environmental costs of aquaculture (including shellfish), livestock, dairy, crops and capture fisheries. While the data are not nearly as extensive as one might like, they indicate quite strongly that there are great differences in the environmental costs of different forms of food production, and that shellfish production has perhaps the lowest environmental impact of any form of food production. In particular a major concern of most food production is release of nitrogen and related compounds that lead to eutrophication of fresh water and coastal zones. The data available for shellfish production suggest that shellfish production actually absorbs chemicals that lead to eutrophication. Shellfish production is also among the most productive forms of food production measured in terms of tonnes of food produced per unit area.

ADULT MALE DUNGENESS CRAB (*CANCER MAGISTER*) MOVEMENTS NEAR REEDSPORT, OREGON FROM A FISHERIES COLLABORATIVE MARK-RECAPTURE STUDY.

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As the permitting process proceeded for Ocean Power Technologies (OPT) proposed wave energy development near Reedsport, Oregon, the fishing community expressed major concerns

about wave energy devices potential impacts on Dungeness crab movement, behavior and harvests. There was no data available to predict the potential impact of wave energy devices on the movement of adult Dungeness crab. Alongside baseline studies conducted by H.T. Harvey and Associates, the Oregon Wave Energy Trust (OWET), the Oregon Dungeness Crab Commission (ODCC) and Oregon Sea Grant initiated a tagging study to fill this knowledge gap. Legal sized male crabs were tagged at three locations near Reedsport Oregon in the fall of 2009. 2788 tags were deployed and crabs were recaptured in the commercial crab fishery. 952 crabs were recaptured and tags returned to Oregon Sea Grant. 626 tags were returned with location data of sufficient detail for analysis. Crabs travelled distances ranging from 0.27 km to 90.68 km. 65% of crabs traveled less than 20 km, 77.7% of crabs traveled less than 30km, and 95.5% of crabs traveled less than 50km. Crabs moved primarily in the alongshore direction, with minimal across shelf movement.

BLUE CRAB FISHERY STOCK DYNAMICS: MANAGING FOR RECRUITMENT LIMITATION.

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The Chesapeake blue crab (*Callinectes sapidus*) spawning stock declined by >80% from 1991–2000 and remained persistently at record low levels through 2007. Fishers blamed various causes from poor water quality to predation by predatory fish (striped bass). Fishery scientists developed stock models that showed the stock was overfished 9 of 11 years, and our experiments releasing cohorts of hatchery-reared juvenile crabs showed that the nursery areas of the upper Bay were recruitment limited. Many attempts by fishery managers in Maryland and Virginia to adjust regulations to reduced fishing pressure were unsuccessful for over a decade. Maryland attempt to reduce fishing pressure focused mainly on the recreational fishery and limiting commercial fishing during short seasonal windows; but this was never very successful, with 70% of females being caught before migrating to the spawning area. Virginia first created a large MPA that seasonally protected spawning females in summer, which was successful. However, protection of the spawning stock was removed in winter, when an intense dredge fishery impacted the females, such that females comprised 85% of the Virginia catch. In 2008 Virginia banned the winter dredge fishery, and the stock rebounded by 50% in two years. Males are now also limiting reproduction.

MONITORING SUCCESS OF LARGE-SCALE (*CRASSOSTREA VIRGINICA*) OYSTER RESTORATION AS MITIGATION IN A SOUTH CAROLINA INDUSTRIALIZED HARBOR.

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The SC State Ports Authority, as mitigation for seaport expansion, has contracted with South Carolina's Department of Natural Resources to construct 9 acres of intertidal oyster reefs within the Charleston Harbor watershed. This five-year project is structured to provide direct impact ecological services to aquatic resources and wetlands in areas adjacent to a newly constructed shipping terminal. In the first four years of this five-year restoration project, 116,374 bushels of oyster shells have been planted at 24 sites to create 9.45 acres of intertidal hard-substrate footprint. Newly constructed reefs are monitored for a minimum of three years to verify successful reef development and allow timely adaptive management. Success criteria for constructed reefs include oyster density and size, footprint retention, recruitment potential (larval supply) and shoreline stabilization. This paper decribes the success evaluation of reefs which were constructed in year one and evaluated at three years of age and discusses challenges associated with restoring habitat in an industrialized, high energy harbor.

THE SPREAD AND ABUNDANCE OF VELIGERS IN LAKES MEAD AND MOHAVE.

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Adult quagga mussels (*Dreissena rostriformis bugensis*) were found in Lake Mead in January 2007. The mussels are now thought to have been introduced between 2002 and 2004 and apparently spread slowly after the initial introduction. Within a few months after the discovery of the adult mussels, veligers were found throughout Boulder Basin, as well as in upper basins of the lake. Veligers were identified at all sampling stations throughout the lake by May 2008. Monthly veliger counts at 20 sampling stations throughout Lake Mead, including four stations with a focus on veliger detection, and an additional four sampling station in Lake Mohave downstream of Lake Mead, have identified seasonal patterns in distribution. Maximum observed veliger concentrations in Lakes Mead and Mohave are lower than those reported in the Great Lakes, but some veligers are found in every month of the year. Possible ecological implications of the observed veliger distribution and challenges with early detection of dreissenid mussels are discussed.

THE EFFECTS OF OCTOPAMINE ON BIVALVE HEART RATE.

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Octopamine is a biogenic amine first identified in octopus. It is well studied in arthropods and gastropods, serving as a neurotransmitter and hormone. Its presence and functions have rarely been reported in bivalves. We identified octopamine in ganglia and tissues of *Crassostrea virginica* and found octopamine a cardio-acceleratory agent. Here we examined octopamine on hearts of the clam *Mercenaria mercenaria* and the mussel *Mytilus edulis*. Heart preparations were prepared *in situ* and monitored with a Physiograph. Mussel basal heart rate averaged 12 beats/min. Superfusion of octopamine (10^{-6} – 10^{-3} M) increased it to 22 beats/minutes, an 83% increase. Clam basal heart rate averaged 8 beats/min. Superfusion of octopamine (10^{-6} – 10^{-3} M) slowed it to 4 beats/minutes, a decrease of 50%. The actions of octopamine were prevented by the antagonist phentolamine. The study shows octopamine affects heart rate of species in 3 orders of bivalves, Osteoida the oyster, Mytilorida the mussel and Veneroida the clam. The different result on clam with respect to oyster and mussel appears at first to be confusing, but Veneroida hearts are well known to respond differently to drugs and nervous stimulations compared to the other orders of bivalves.

DETECTION OF DREISSENID MUSSELS IN THE WESTERN UNITED STATES: LESSONS LEARNED.

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Detection of dreissenid species in water systems is critically important to maintaining structure and function of dam related structures. If mussels are detected early, facility operators may have three to five years to adjust systems before the population of mussels are large enough to restrict the flow of water, restrict water intakes, and clog pipes which affect cooling systems, and impede power generation. Reclamation received ARRA funding for a program to determine the extent of the dreissenid invasion in the 17 western states. Early detection methods for the detection of dreissenids in water has some inherent issues with reliability and variability in sampling which creates management decision dilemmas. Since current control methods of these mussels are very limited in regulated water systems, Reclamation is conducting intensive research. The testing methods that Reclamation has applied in the western state-wide program in conjunction with the data that has been generated is beginning to reveal more insights into dreissenid mussels and their spread. To improve detection in water systems, Reclamation has developed an analytical strategy that includes cross polarized light microscopy, scanning electron microscopy (SEM), flow cell cytometry, and polymerase chain reaction (PCR).

ECONOMIC IMPACT OF WEST COAST SHELLFISH AQUACULTURE.

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The Pacific Shellfish Institute (PSI) is surveying shellfish growers in Washington, Oregon and California in an attempt to fill critical socio-economic information gaps related to the shellfish industry. Survey data includes sales revenue, production volume by species and product, total payroll, number of employees, taxes, and detailed or grouped expenses. Key informants were selected in each state to provide detailed expenses for the 2010 calendar year. Expenses were sorted by vendor and assigned an IMPLAN® category based on the type of service or product purchased, then used to estimate a production function and build an input-output (I/O) model for shellfish aquaculture. An I/O model depicts inter-industry relations of a regional economy and shows how the output of one industry is an input to other industries. For Washington, the study team will develop an I/O model using the state as the study region and will calculate the economic impacts from the counties to the state using tidelands as a proxy for where expenses occur. The geographic unit for Oregon and California, if data allows, will be the state level. The purpose of this research is to quantify the economic impacts of commercial shellfish production and support sustainable and vibrant coastal communities.

ECONOMIC CONTRIBUTIONS OF VIRGINIA SHELLFISH AQUACULTURE.

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The shellfish aquaculture industry in Virginia consists of two species; the northern quahog (=hard clam), *Mercenaria mercenaria* and the eastern oyster, *Crassostrea virginica*. The Virginia Sea Grant Marine Extension Program has captured the trends of these fisheries by producing a crop reporting survey each year since 2004. The crop reporting tool evaluates the relative growth in inputs and farm output based on industry response. These annual assessments provide an accurate and consistent gauge of shellfish culture growth and its economic contribution. Shellfish culture in Virginia has grown significantly since the first survey in 2004 and added significant value to the State's seafood market place which in 2010 was estimated at $70 million in total economic impact. While Virginia continues to lead the nation in hard clam production a noteworthy transition to intensive aquaculture of native oysters is underway. The forecast for the future is positive with continued

growth in oyster plantings and eyed larvae production. Market and distribution channels for Virginia aquaculture products have been consistent over the years with the bulk sold in wholesale markets and out of state, increasing the value added to the Commonwealth.

USE OF INJECTABLE EUGENOL FOR EUTHANASIA AND ANESTHESIA OF AMERICAN LOBSTERS (*HOMARUS AMERICANUS*) AND SIMILAR SPECIES.

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Crustaceans are economically and ecologically significant, but current treatment and diagnostic protocols for crustacean health is very limited. According to standards given by the American Veterinary Medicine Association there are no current methods of euthanizing lobsters, also there are no effective quick release anesthetics. The objective of this project is to show that eugenol by direct injection is a safe, efficient and reliable method for euthanizing or anesthetizing crustaceans. Anesthetic levels were determined by behavior responses, death was determined by a lack of response to stimuli. Eugenol injected into the pericardial sac will work as a euthanizing agent for American lobsters (*Homarus americanus*) and rock crabs (*Cancer irroratus*) at a dose of 7 µl/g for lobsters and 10 µl/g for crabs. Crabs and lobsters will be anesthetized for 30 minutes (±4.6) by a dose of 0.15 µl/g of eugenol diluted with a solution of 70% ethanol and sterile sea water injected into the pericardial sac.

SCIENCE AND OFFSHORE MUSSEL CULTURE AT SAGRES, PORTUGAL.

John Icely.
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Installation of offshore longlines for mussel culture at Sagres, Portugal was started in 2011, based on the experience of oyster culture at the same site since 1990. The contribution of science to this operation is a series of short-term studies on the recruitment and growth of mussels; studies on biofouling; and identification of phytoplankton, particularly dinoflagellates, as potential sources of toxins. More recently, the validation of the Medium Resolution Image Spectrometer sensor for the European Space Agency has provided calibrated remote sensing images for interpreting fluctuations in primary production and linked to other remote sensing data for sea surface temperature, as well as wind velocity and direction. In the future, temperature, salinity and turbidity will be monitored continuously at the longlines and linked to regular samples for chlorophyll, nutrients, and phytoplankton. All data will be used to calibrate shellfish models to assist with monitoring and forecasting for the mussel culture.

PHYLOGENY OF LIVING SPECIES OF GEODUCK CLAM *PANOPEA* (BIVALVIA:HIATELLIDAE).

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Two species of geoduck clam occur in temperate and warm waters of Baja California and Gulf of California, Mexico. Although for a long time, a close genetic relationship was assumed between *Panopea generosa* and *P. globosa*, our phylogenetic survey using mtDNA (CO1) and nuclear DNA (28S and 18S) as molecular markers have shown otherwise. *Panopea generosa* and *P. globosa* belong to different lineage. In contrast, *P. generosa* it's closely related to *P. japonica*, even sharing one haplotype in the 18S gen. Using CO1 gene, both species (*P. generosa* and *P. japonica*) had more than 15% of divergence with *P. globosa*. Although *P. globosa* mtDNA marker showed a high percentage of divergence, nuclear genes allow for identifying phylogenetic relationships with *P. abbreviata* and *P. zelandica* (clams from the Southern Pacific).

According to the results, *P. zelandica* could be a relict species or even be the most similar to the ancient species within the *Panopea* genus that had a wide distribution during the Cenozoic. There is no genetic evidence to suggest that *P. globosa* diverged from *P. generosa*. *P. globosa* could be subject to a faster process of divergence, possibly because it is evolving into a new genus.

COST ASSESSMENT OF OYSTER SEED BEDDING IN LOUISIANA.

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Louisiana is the leading state in the nation for oyster production. Seed bedding is a means of oyster production where oyster farmers in Louisiana harvest seed oysters from public grounds and transport or "bed" them to their private leases. Natural seed production is unreliable; therefore other methods of oyster production are being investigated. It is important to document the cost of seed bedding to later compare against new technologies and ensure the change was economically efficient. A survey was conducted to document the costs of seed bedding, as well as measure the potential for other oyster production methods in Louisiana. It costs an average of $6.00 per barrel to bed seed. Responded oystermen show remote setting as a viable alternative to seed bedding for oyster production in Louisiana.

DISTRIBUTION AND VARIABILITY OF *BONAMIA EXITIOSA* IN FLAT OYSTER *OSTREA EDULIS* POPULATIONS IN EUROPE.

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Bonamia ostreae has been reported in flat oysters *Ostrea edulis* in several European countries since 1979. Routine diagnosis was generally based on histology and cytology which do not allow differentiation between parasites of the genus *Bonamia*. Following the detection of *B. exitiosa* in flat oysters from Spain in 2007 and from Italy in 2008, a working programme was proposed at the European level to find out the actual spread of this parasite previously considered exotic to EU. We present herein the sampling and diagnostic approaches followed in some European countries between 2008 and 2010 in the context of this working programme. *Bonamia exitiosa* has been detected in four Member States up to now, sometimes in association with mortality. Mixed-infection (with *B. ostreae*) at the same location and even in the same oyster was observed. The analysis of sequences including ITS-1 and ITS-2 reveals low polymorphism among European *B. exitiosa*. More studies are required to determine the accurate geographical distribution of *B. exitiosa* within Europe, to understand the presence and spread of this parasite and to evaluate its impact on flat oyster populations.

SURFCLAMS, SPATIAL SCALES AND REMARKABLY SERIOUS PROBLEMS IN AN OTHERWISE WELL MANAGED FISHERY.

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Environmental change may necessitate changes in stock definitions for broadly distributed species. Surfclams in federal waters between Cape Hatteras and Georges Bank are managed as one stock but the fishery is concentrated in the historically productive south. Georges Bank in the north is lightly fished due to PSP risk. During 1994 growth rates, recruitment, biomass and catch rates in the south began to decline while landings were stable and fishing effort increased. Biomass shifted north as growth and recruitment in the south declined. Poor southern conditions may be due to warming, low primary production, low regional spawning biomass, loss of dense beds possibly required for spawning, poor post settlement survival and by the single stock definition that hinders regional management attention. Smaller management units are not used because of: the rigid stock definition in the fishery management plan, negative experiences in the 1970s, a general desire to avoid meddling in the ITQ fishery, and the combination of high biomass and low fishing mortality for the stock as a whole. Management action is not technically required or anticipated despite declining biological and fishery performance in the south because the single stock definition effectively masks regional problems.

CAN THE RAGS TO RICHES TALE OF SPINY LOBSTER LIVE EXPORTS FROM NEW ZEALAND BE APPLIED TO THE KING CRAB FISHERY IN NORWAY?

Philip James, Sten Siikavuopio, Kjell Midling.

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The live export of spiny lobsters from New Zealand has been a remarkable tale of success. Twenty years ago 85% of the spiny lobsters fished in NZ were sold as a low value product that were frozen and exported to the USA. Today there has been an almost complete reversal with 97% of the lobsters landed in NZ being exported live to high value markets in Asia. Norway now finds itself in a similar position to the spiny lobster fishery in NZ twenty years ago. It has a developing king crab fishery based on the spread of this species from the Barents Sea where they were introduced in the 1960's. Currently less than 10% of the catch is exported live, the remainder being sold as frozen product. However, there is now a rapidly developing live king crab export industry based in Bugøynes in northern Norway which is modeling itself on the NZ lobster industry. This talk compares these two industries and looks at the similarities and lessons than can be learnt from each to enhance live export of crustacean species around the world.

OPTIMAL FEED STRATEGIES FOR THE CULTURE OF THE GREEN SEA URCHIN (*STRONGYLOCENTROTUS DROEBACHIENSIS*): CAN PERIODS OF STARVATION OPTIMIZE GROWTH?

Philip James, Sten Siikavuopio.

Nofima, Muninbakken 9-13, Tromsø, Troms, 9291, Norway.

The present study investigated the effects of feed frequency on somatic growth performance, feed intake and feed conversion efficiency of different size groups of sea urchins (*Strongylocentrotus*

droebachiensis) fed the Nofima manufactured dry feed. Three size classes of urchins were randomly allocated one of four feed regimes: 1) fed weekly, 2) fed one week/starved one week, 3) fed two weeks/starved two weeks and 4) fed four weeks/starved four weeks (n = 15 urchins per feed treatment). The animals were fed the Nofima sea urchin manufactured feed *ad libitum* and their feed consumption was monitored each week for a period of 54 weeks. There was no mortality throughout the experimental period. Growth was significantly affected by the feed frequency with sea urchins being fed on a weekly basis having the highest growth rates. However, when the FCR was taken into consideration it appears that the optimal feed frequency for medium and large sea urchins should be reduced to every second week to optimise feeding efficiency and reduce production costs. This was not the case for small sea urchins which required weekly feeds to maintain optimal growth. The results of the experiment are discussed in terms of the aquaculture of sea urchins.

A NATIONAL ASSESSMENT OF POST-SECONDARY AQUACULTURE-SUPPORTING EDUCATIONAL PROGRAMS IN THE UNITED STATES.

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The American Fisheries Society Fish Culture Section, National Shellfisheries Association, US Aquaculture Society, and USDA National Institute of Food and Agriculture assessed available training for a world-class workforce to support aquaculture-related sciences, educational programs, policies, and sustainable commercial development in the United States. Post-secondary institutions (>100) offering any level of aquaculture-related instruction, subject matter specialization, diplomas, certificates or degrees were identified. An on-line questionnaire to assess current status, future trends, and critical needs for a skilled workforce was finalized after review by survey development personnel, and approved by a university Institutional Review Board. Because 'aquaculture-related' is subjective, responders used their best judgment regarding how their programs relate to aquaculture. Data for 2000, 2010, and forecasted trends for 2015 are presented regarding training and degree programs, teaching faculty, enrollment trends, aquaculture-related courses, new programs, and job placement. The resulting national database provides an assessment of workforce preparedness for educators, practitioners, entrepreneurs and policymakers, an assessment of training issues and constraints; and recommended follow-up actions to support aquaculture education.

HEMATODINIUM SPP. IN THE PACIFIC NORTHWEST.

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Hematodinium perezi, a parasitic dinoflagellate of decapod crustaceans, was described in Europe in 1927 from the swimming crab (*Polybius depurator*). *Hematodinium* has since been identified in over 40 decapod hosts and is thought to be one of the most economically significant diseases of crustaceans. First diagnosed in the Pacific Northwest in 1985 in Alaskan Tanner crabs, it is fatal with prevalence exceeding 90% in some areas. *Hematodinium* has a complex life history with only the parasitic stages known. The final parasitic stage, the dinospore, is released before host death and may be a free-living, photosynthetic, resting, and/or infective stage. The means of infection are unknown, but may be associated with host molting. In the past 15 years, there has been renewed interest in *Hematodinium*, with investigations of occurrence, transport, life history, culturing, and molecular identification and characterization. Northern hemisphere *Hematodinium* is known to be a complex of at least two, and possibly four, ecotypes or species with probably two ecotypes in the Pacific Northwest. We have conducted a continuous monitoring program with associated research on *Hematodinium* in the eastern Bering Sea since 1987 and will present our findings on new hosts, *Hematodinium* distribution, life history and presumptive ecotypes.

OVERVIEW OF THE NOAA OCEAN ACIDIFICATION PROGRAM: FOCUSING ON SHELL FISHERIES.

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NOAA's Ocean and Great Lakes Acidification Research Plan (http://www.oar.noaa.gov/oceans/ocean-acidification/feel3500_without_budget_rfs.pdf) establishes a robust but targeted research approach which includes observing, experimental and outreach components. Particularly relevant to the National Shellfisheries Association is NOAA's growing portfolio of research focused on determining current and future impacts of changing ocean chemistry on commercial and recreational fishery species and their broader ecosystems, using realistic projections for pH, pCO2 and saturation state. I will give a brief overview of the extent (geographic location, target species, experimental systems, recent findings) of the fisheries impact work but more

importantly provide context for this research within the broader NOAA Ocean Acidification research agenda and those of other federal agencies. The NOAA OA research program, both present and future, includes opportunities for collaboration and partnership with academic and industry stakeholders.

GENETIC DIVERSITY ANALYSIS IN DIFFERENT STOCKS OF HEXAGRAMMOS OTAKII BASED ON MITOCHONDRIAL DNA CONTROL REGION PARTIAL SEQUENCE.

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Nucleotide sequences of mitochondrial DNA control region partial sequence from six wide stocks of *Hexagrammos otakii* were amplified using PCR techniques. A total of 148 variable sites were detected among the aligned sequences of 352 bp and the mutation rate was 42.05%. Twenty-two haplotypes were identified from 31 individuals according to the determined sequences, and the haplotype diversity was 0.963. The nucleotide diversity was 0.164, and the average number of nucleotide differences was 44.68. The value of Fu's Fs of neutrality tests was -0.14881 ($P < 0.01$). AMOVA analysis demonstrated that: Fst = 0.7398 ($P < 0.01$), 73.98% variances occurred among populations and 26.02% variances occurred within populations. The NJ and MP molecular phylogenetic trees constructed by the distances among different individuals were similar. The phylogenetic trees were all divided into two branches. The Qingdao population and one part of the Lvshun population made up one branch, the rest populations were clustered into the other branch. The results suggested that, the genetic diversity of the populations of *Hexagrammos otakii* was abundant, and there was a huge genetic differentiation among populations. It was proved that the gene sequence of mitochondrial DNA control region could be used to analyze the genetic diversity of *Hexagrammos otakii* within or among populations.

SIM DATABASE: A DATABASE FOR THE INTEGRATED MANAGEMENT OF SHELLFISH WILD, RESTORED, AND FARMED POPULATIONS.

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Multiple factors can severely impact shellfish populations, with serious biological, social, and economic consequences. Despite intensive efforts in the monitoring of shellfish populations, there are no consistent mechanisms for storing, managing, and analyzing the data, constraining the ability to reliably monitor restoration efforts and shellfish management on a local, regional, or national level. As a result, shellfish restoration practitioners have recognized a pressing need to build a strategy to better manage shellfish restoration efforts and monitoring, including coordinated data management between different agencies. In partnership with the Rhode Island Shellfish Technical Working Group, we have developed a relational database and graphic user interface for storing and managing oyster population data. The Shellfish Integrated Management Database has the capacity for systematic data capture, storage and management of shellfish monitoring data, disease surveys, and management of data pertaining to commercial aquaculture such as hatchery source and genetic broodstock. This effort provides a framework for better management of shellfish restoration efforts, including data storage and coordination, regional and habitat prioritization and goal setting, systematic ecological monitoring protocol of restoration sites, and provides a model for how the shellfish community can coordinate to proactively increase the success of oyster restoration.

RAPID COLONIZATION OF *CANCER IRRORATUS* IN ICELAND.

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The Atlantic rock crab (*Cancer irroratus*) was first recorded in Icelandic waters in 2006. In its new habitat competing decapods are scarce, as only two large crab species are commonly found there, *Hyas araneus* and *Carcinus maenas*. Since first discovered, rock crab has been found widely, both as adults and larvae, in the southwestern and western part of Iceland. During summer and fall of 2011 the first studies on its abundance was carried out. At two locations in the bay of Faxaflói, crabs were tagged for recapture and subsequently abundance was estimated on transects with scuba-divers. During the first experiment carried out in the beginning of June, few crabs were caught and consequently few individuals recaptured. Females with eggs were 10% of the catch in June. In September, the catch per trap was close to 3 kg and 1167 crabs were tagged and 38 recaptured. Using mark-recapture analysis the density was estimated to be around 0.07 crabs per square meter. Rock crab was the dominant species caught on both occasions and during the latter experiment it was in density which could be feasible for future harvest.

EUROPEAN TECHNOLOGY DEVELOPMENT FOR A RELIABLE SUPPLY OF HIGH QUALITY SEED IN BLUE MUSSEL FARMING.

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From 2005-2007 the EU project BLUE SEED was carried out. The objectives were to secure a reliable supply of blue mussel seed and to develop techniques allowing farmers to market blue mussels year round. A problem blue mussel producers face is the unpredictability seed supply. The amounts of wild seed available are extremely variable from year to year. A reliable supply of seed from hatchery sources will allow mussel farmers to overcome this. A second problem is that recently spawned mussels cannot be sold due to insufficient meat content. Producers will benefit greatly from a hatchery-based technique, such as triploid induction, that produces non-maturing mussels that can be marketed year round. In this project mussel farmers and sellers, a network for training and technology transfer, universities and research institutes collaborated. Attention was given to broodstock conditioning and larval rearing, production of triploid larvae and tetraploid broodstock, spat settlement and on rearing of diploid and triploid spat to seed size. In addition, the allowed costs of hatchery produced seed with conventional wild-caught seed is compared. Considering the normal 2 to 3 year production cycle for blue mussel in Europe, the focus of this 2-year project was on spat and seed production.

MARTHA'S VINEYARD SHELL RECOVERY PARTNERSHIP – 2011 PILOT PROJECT.

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Shell is a necessary component of oyster habitat providing hard substrate for setting oyster spat; and of increasing importance as a buffering agent for acidified waters which can inhibit shell formation in bivalves and other marine organisms. Each year hundreds of cubic yards of shell are brought to the island and planted in the ponds in an effort to replace shell that is removed during the harvest of bivalve shellfish. At the same time, dumpsters outside restaurants are filled with discarded shells destined for removal to off-island landfills. During the summer of 2011 with funding from the local Edey Foundation, the Martha's Vineyard Shell Recovery Partnership was initiated to recover and recycle shell from the waste stream; thereby, reducing bulk waste and providing a local source of shell to protect and maintain shellfish habitat. Between June and August, over 1,700 gallons of shell were recovered from seven restaurants. Efforts are underway to expand the program and make it economically sustainable.

EVALUATION OF A *BACILLUS* SP. PROBIOTIC CANDIDATE UPON SURVIVAL OF OYSTER LARVAE (*CRASSOSTREA VIRGINICA*) DURING PILOT-SCALE TRIALS AND ITS EFFECTS UPON OYSTER HEMOCYTE IMMUNE FUNCTIONS.

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Rising concerns over antibiotic residues found in food, the environment, and the emergence of antibiotic-resistant bacterial pathogens has resulted in the need for environmentally-friendly methods for controlling microbial pathogenesis. In aquaculture, probiotic bacteria are becoming increasingly preferred over antibiotics. Research at the Milford Laboratory has identified a *Vibrio* sp. bacterium (OY15), isolated from oysters, that significantly improved survival ($p < 0.014$) of oyster larvae (*Crassostrea virginica*) when challenged with a *Vibrio* sp. shellfish-larval pathogen (B183). Possible mechanisms of this probiotic effect were revealed in a study showing that OY15 stimulated immune functions of adult oyster hemocytes *in vitro*. This study investigated the potential probiotic effects of a *Bacillus* sp. bacterium (S1), isolated from bay scallops (*Argopecten irradians irradians)*, in larval oysters. This strain inhibited pathogen B183 in Kirby-Bauer disk-diffusion testing, showing promise as a probiotic candidate for shellfish larviculture. During pilot-scale trials, however, survival of 2-day-old oyster larvae challenged with B183 was not improved significantly with S1 supplementation compared to larvae challenged with B183 alone. In addition, significant immuno-suppression of oxidative burst upon adult oyster hemoytes, suggested ineffective probiotic effects. Future work will investigate potential probiotic effects of S1 on bay scallop larvae in pilot-scale trials. Probiotic bacteria may be species-specific.

ROLE OF ANTIBIOTIC PRODUCTION ON THE PROBIOTIC ACTIVITY OF *PHAEOBACTER* SP. S4 AGAINST BACTERIAL PATHOGENS OF OYSTERS.

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Bacterial infections are considered a major problem for the shellfish aquaculture industry, causing mass mortality events during larval and juvenile stages. Although probiotics can be used to manage these diseases, there is little known about the actual

mechanisms of action of probiotics. We investigated the role of antibiotic production on the probiotic activity of *Phaeobacter* sp. S4 against bacterial pathogens of oysters. *Pheobacter* sp. S4 is known to produce the antibiotic tropodithietic acid (Tda) and protects larval oysters against challenge with the bacterial pathogen *Vibrio tubiashii* (Relative Percent Survival of 66.5). Purified Tda showed antibiotic activity against *V. tubiashii*, with an MIC of 6.25 μg/mL. We created several mutants of S4 that does not produce Tda. Pretreatment of larval oysters with sublethal concentrations of purified Tda (1.56 μg/mL) and several *Phaeobacter* sp. S4 mutants demonstrated partial protection against challenge with *V. tubiashii*, suggesting that mechanisms other than antibiotic activity may be involved in protection of oyster larvae against bacterial infection. This study will help in understanding specifically mechanisms of protection in probiotics thus helping in the future development of improved methods for probiotic screening.

OCEAN ACIDIFICATION AS INTERSECTION OF SCIENCE, LAW, AND POLICY.

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Ocean acidification is a strikingly interdisciplinary problem, one that requires us to recalibrate the communication among scientists, industry representatives, lawyers, and government officials. In particular, there is a powerful interaction between emerging data and the legal tools we might use to mitigate acidification: as we learn more about the changing chemistry of the oceans, more and different legal options become relevant. This, in turn, gives industry (and many others) new policy directions to pursue. Recent data indicate that nutrient runoff and other non-atmospheric inputs strongly influence the carbonate chemistry of coastal oceans, making coastal land-management and water quality laws increasingly important for mitigating these auxiliary acidifying factors. While atmospheric CO_2– the fundamental driver of ocean acidification – remains an elusive regulatory target, existing legal tools offer a means of buying time by addressing these other drivers. In doing so, we may mitigate the most economically important effects of ocean acidification, and accomplish clean water goals in the bargain.

RESPONSES OF THE BENTHIC REEF COMMUNITY TO THE PHYSICAL AND BIOTIC COMPONENTS OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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Benthic organisms can utilize the complex oyster reef structure as refuge, habitat, foraging and nursery grounds. The ecological value of the oyster's physical structure has been well studied, but the effect of the biotic input of the oyster, in the form of biodeposits, on the reef community is unclear. This study investigated contributions of the oyster's physical structure and biodeposits 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 from July 2009 till July 2010. Fauna that colonized the reefs were enumerated, identified, and are currently being biomassed. MANOVA of abundance data by feeding guild showed no differences between live oyster and oyster shell reefs. In addition there was no difference in number of species between the treatments. However, ANOVA of species level abundance showed that *Apocorophium lacustre* was present in greater numbers ($p = 0.03$) and comprised a greater proportion of the community ($p = 0.05$) on live oysters. While species level analysis suggested that biodeposits may have benefited amphipods, the overall analysis demonstrated the important, perhaps dominant, role physical structure may play in reef community colonization and development.

EFFECTS OF HYPOXIA ON ANTIOXIDANT STATUS, MICROBIAL COMMUNITY, TISSUE METAL ACCUMULATION AND TISSUE DAMAGE IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.

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Aquatic ecosystems are exposed to multiple stressors like hypoxia, pH shifts and metal contamination simultaneously. Hypoxia and pH shifts are important environmental variables in estuarine ecosystems that may contribute to oxidative stress and tissue injury, as well as affecting microbial flora and bioavailability of metals. Therefore metal exposures can cause oxidative damage that may be exacerbated by hypoxia and pH. The overall purpose of our study is to investigate the effects of hypoxia on oxidative damage and antioxidant status, and consider the potential impacts on microbial flora and metal bioavailability in eastern oysters, *Crassostrea virginica*. Laboratory studies were conducted wherein the effects of hypoxic regimes on oxidative stress (antioxidant levels and lipid peroxidation) were measured, and the effects on metal bioaccumulation were also determined. The microbial flora of the oysters was also characterized using genomic techniques. Changes in oxidative damage and bioaccumulation of metals were observed in hypoxic regimes, and pH was found to be an important factor in determining oxidative damage. Changes in microbial flora were also related to hypoxia stress. This study suggests that the interactions between these environmental stressors are important determinants of the health of bivalve populations in estuarine ecosystems.

SOFT TUNIC SYNDROME OF THE ASCIDIAN *HALOCYNTHIA RORETZI* CULTURED IN THE SOUTH COAST OF KOREA IS POSSIBLY CAUSED BY BI-FLAGELLATED PROTISTS.

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The edible ascidian *Halocynthia roretzi* is a commercially important fisheries resource in Korea. However, for the last several years, outbreaks of mass mortalities of the species have been occurring along the south and east coasts of Korea. The episode is known as tunic-softness syndrome because the tunic of the diseased ascidian becomes softened and finally ruptured. To determine the agent causing tunic-softness syndrome, healthy and diseased ascidians were collected in March 2011 from Tongyeong, on the south coast of Korea, and were used for pathological investigations. The results showed that diseased ascidians exhibited bi-flagellated protozoans (10–15 μm length and 3–5 μm width) were observed specifically in the tissue imprints and tunic cultures of diseased ascidians at an occurrence rate of 97.5%. Healthy ascidians which inoculated with purified bi-flagellates showed 40% of mortality while control group was only 6.6% and the typical symptom of soft tunic syndrome was observed. Our study clearly shows that bi-flagellated protists are present only in the softened ascidians, suggesting that the flagellates are partly or entirely associated with soft tunic syndrome. Accordingly, further investigations to verify the effects of the flagellates found in the present study on soft tunic syndrome should be conducted.

MYTILUS GALLOPROVINCIALIS STARVATION ON A COMMERCIAL SUSPENSION CULTURE FARM IN PUGET SOUND DURING THE SUMMER OF 2010.

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During the fall of 2010 a significant mortality of *M. galloprovincialis* was observed on a commercial suspension culture in farm in Totten Inlet, Puget Sound. Further sampling showed in excess of 25% mortality had occurred on mature rafts of mussels. Reference to samples of broodstock sent three weeks previously for routine pathology showed a very low digestive gland indicating the animals had not been feeding. Further data collected by Pacific Shellfish Institute showed a period of low ph followed by a bloom of the *Akashiwo sanguinea* a dinoflagellate had developed in early summer and persisted in high concentrations through October in this area.

THE EVOLVING WORLD MARKET FOR GEODUCK.

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The market for Pacific Geoduck has changed dramatically over the past 20 years. A species whose highest economic value used to be as clam chunks in Ivar's chowder served to chilly Washingtonians boarding a ferry is now a premium product that can be harvested in the Pacific Northwest and sold live in Mainland China less than two days later. With this strong increase in per-pound value came the development of a burgeoning private aquaculture industry for geoduck and difficult management decisions for the State of Washington. In 2004, the State of Washington commissioned a study on the potential growth of the private industry over the next decade and the wisdom of allowing private production on public lands. Geoduck culture requires a long lead time—estimated at 5 – 8 years from seeding to harvest. Would private seeding of public lands overwhelm geoduck markets? Could existing private investment meet rapidly increasing demand? Now, one geoduck production cycle later this presentation looks back on how those early predictions regarding the growth of private industry matched with reality and how the world geoduck market has evolved.

DEVELOPMENT OF MUSSEL FARMING IN NEW ZEALAND: PAST, PRESENT, AND FUTURE.

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The New Zealand Greenshell™ mussel industry has grown from a small wild-catch fishery in the 1970s to a US$150M aquaculture industry today. While some of this success can be attributed to the intrinsic advantages of New Zealand's natural environment, including a wild spat supply and excellent growing conditions, the role of innovation has been critical to the industry's success. Prior to the late 1990s, development of efficient farming systems and establishment of an effective value chain through to market drove strong industry growth. Regulatory constraints on the development of new farm space slowed this growth at the end of the 1990s. The next generation of innovation focuses on opportunities available through techniques including hatchery spat production, selective breeding, and farming new environments such as the open ocean. These techniques offer the potential for both production and value gains, and mitigate the risks of reliance on wild spat. Initial results have shown that selective breeding for yield related traits is effective. Current research focuses on identifying new traits with potential for industry benefit. Strong partnerships between researchers and industry are helping align research strategy with industry objectives, and enable research findings to be tested at scale in a production context.

FIELD EVALUATION OF DIPLOID AND TRIPLOID EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) IN SOUTH CAROLINA: AN INDUSTRY COLLABORATION TO INVESTIGATE THE VALUE OF FUTURE INVESTMENT IN POLYPLOID OYSTERS.

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The development of an oyster mariculture industry in South Carolina has been hindered by high levels of natural oyster recruitment which often result in severe 'overset'. The production of oysters in a short timeframe that avoids the intense spatfall season would allow this industry to generate a highly valued single oyster. Triploid oysters have been shown to be of commercial value due to their concomitant sterility that allows greater nutritional resources to be made available for growth. While the performance advantages of triploid oysters have been evaluated in other regions of the United States, they have not been previously studied in South Carolina. In collaboration with the Virginia Institute of Marine Sciences' Aquaculture, Genetics and Breeding Technology Center and 4Cs Breeding Technologies, Inc. to address patent and pathological considerations, and with funding from the South Carolina Sea Grant Consortium, triploid and diploid single eastern oysters (*Crassostrea virginica*) were deployed at industry sites across the coastal region of South Carolina in the fall of 2011. Comparative survival and growth data will be presented here, along with some commentary on the interest, obstacles and future potential for oyster aquaculture in South Carolina.

EFFECT OF PRIOR SALINITY ACCLIMATION ON SALINITY TOLERANCE FOR THE SUNRAY VENUS CLAM *MACROCALLISTA NIMBOSA*.

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The sunray venus clam (SRV), *Macrocallista nimbosa*, is showing promise as a potential aquaculture candidate for Florida shellfish growers. Salinity tolerance of this species has been examined using step-change challenges and found to range from 16–40, but prior salinity acclimation was not taken into account. This study examined the effect of prior salinity acclimation on salinity tolerance. SRV clams (n = 3–4, ~36 mm length) acclimated to salinities of 16, 24, 32 and 40 from a prior experiment were subjected for 18 days to different salinity treatments in duplicate as follows: 16 to 12, 14 and 16; 24 to 12, 24 and 40; 32 to 12, 16 and 32; and 40 to 16 and 24. SRV clams acclimated to 16 ppt were found to survive rather well (83 and 88%) when exposed to lower salinities (12 and 14). In general, SRV clams experiencing a salinity change of ≥12 had no survival regardless of initial salinity acclimation. Hemolymph osmolality of surviving clams followed water osmolality (R^2 = 0.9991). Large juvenile SRV clams in growout may be more resilient to lower salinities (12–16) when acclimated to lower salinities and if the change is gradual versus abrupt. This work was supported by NOAA/FL Sea Grant.

EFFECTS OF NORTHERN QUAHOG (=HARD CLAM, *MERCENARIA MERCENARIA*) FARMING ON LOCAL BIOTA: RESULTS FROM NEW JERSEY AND VIRGINIA.

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Since the mid 1970's northern quahog (=hard clam) (*Mercenaria mercenaria*) aquaculture has become the most intensive form of mollusc production on the US east coast. High clam density, predator exclusion nets and harvest techniques may affect biotic assemblages on or near the farm. Little is known about these potential effects. We sampled the macrobiota of intertidal/shallow subtidal clam culture areas in spring, summer and fall on two commercial sites (New Jersey and Virginia) that have been used for at least a decade. Samples were obtained from control (not farmed) plots, recently harvested plots, and plots planted for over a year. Motile fauna were sampled with fyke nets, haul seines and minnow pots. Suction dredge samples estimated surface/infauna biota, and in the fall infauna were sampled with cores. We present the preliminary analysis of these data showing that despite differences in epibiota and infaunal composition and abundance between culture sites and controls, few differences were observed in the utilization of these sites by motile fish and decapods. The data suggest that many species may be moving between habitats within the landscape.

VARIABILITY IN OYSTER FOOD QUANTITY AND QUALITY IN THE DELAWARE ESTUARY.

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Oysters filter vast quantities of seston to balance their nutritional demands, which vary with age and season. Since the amount and type of seston can also vary in time and space, natural diet

composition might rarely match demands, hence constraining productivity. To examine how oyster food availability and quality varies in a large and complex system, seston was collected at eighteen stations over ten months in each of three years, 2009–2011, in the Delaware Estuary. Seston was analyzed for total particulate matter (PM), particulate organic matter (POM), organic content, and biochemical composition (protein, lipid, carbohydrate). Seston quantity and quality varied widely throughout the year and among locations. Seston quantity (PM and POM) was greater in spring and fall and also was more abundant in the upper estuary. Seston organic content was inversely related to PM and POM concentrations, being greater downbay. Particulate protein, lipid and carbohydrate declined as the years progressed, as bio-unavailable POM increased. Oyster condition in the fall correlated best with summer concentrations of seston protein (and to a lesser extent carbohydrate) and was inversely correlated with food quantity. Bottom-up limitation of oyster production is governed more by food quality than food quantity in the Delaware Estuary.

HALIOTIS RUFESCENS X *H. DISCUS HANNAI*: A NEW HYBRID TO IMPROVE THE CHILEAN ABALONE AQUACULTURE?

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Two introduced abalone species are currently produced in Chile, red abalone *Haliotis rufescens* and Japanese abalone *Haliotis discus hannai*. However, red abalone accounts for 99% of total production, while the Japanese abalone has not adapted well to Chilean contidions. This study reports the hatching and growth performance of interspecific hybrids produced between red (R) and Japanese (J) abalone. Additionally, genetics studies like chromosome analysis, microsatellite variability and gene expression are also reported. Our results shows that egg age and sperm concentration were critical factors to produce hybrids. Further, larval development stages were similar in RR, JJ and RJ hybrid abalones. Among the experimental trials, settlement rate varied from 12.3 to 18.6% and final survival from 20.1 to 31.7%; being the RJ hybrid rates intermediate between parental species. The final shell lengths were similar between RR and RJ hybrids, but significantly higher in JJ abalones. Regarding to genetic studies, hybrid abalones show a conservative chromosome number of $2n = 36$, but with slightly differences in chromosome composition. Microsatellite variation showed that the hybrids are comparatively more similar to *H. rufescens* than *H. discus hannai*. Furthermore, hybrid status was confirmed by the presence of specie-specific bands for each parental species of microsatellite locus *Hco97*. Finally, thermal tolerance was ascertained through *HSP70* gene expression, showing a highest thermal tolerance in hybrids in comparison with their parental species. This study reports new knowledge of abalone hybrids and gives new possibilities to improve the Chilean abalone production.

DEVELOPMENT OF EST-SSR MOLECULAR MARKERS FOR RED ABALONE *HALIOTIS RUFESCENS*

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The red abalone *Haliotis rufescens* is among the 15 most valuable abalone species. Despite its ecological and economical importance, genetic studies had only been carried out using heterologous DNA markers, due to a lack of specific molecular markers for this specie. Herein, the increasing of genomic information in public EST-database allows us to discover new DNA markers, such as microsatellite or Simple Sequence Repeat (SSR). These markers have been successfully used to characterize either aquaculture or wild populations, and also for genomic mapping, DNA fingerprinting, and marker-assisted selection. The advantage of ESTSSR is that these markers could be associated to candidate genes involved in biological process as growth and reproduction. The main goal of our research was to mine and develop EST-SSR markers for red abalone from an EST-database generated by pyrosequencing 454. Thus, mRNAs was sequenced from gonad and digestive gland of ten individuals through a 454 GS-FLX platform. We obtained 213,967 reads with an average length of 384 bp. After *De Novo* assembly, contigs and singletons were screened using QDD2 software. Therefore, 2,860 and 3,858 EST-SSR were obtained respectively, and a total of 627 EST-SSR from both groups that had flanking sequences suitable for PCR primer design were identified. Furthermore, ESTSSR markers were selected considering their annotation relevance after a BLAST analysis. The present study reports the first specific DNA markers for *H. rufescens*, and gives new genomic resources from Next Generation Sequence technology.

AN ONTOGENETIC COMPARISON OF EGG QUALITY OF FEMALE *CRASSOSTREA VIRGINICA* FROM THE NORTHERN CHESAPEAKE BAY.

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In order to investigate potential relationships between oyster age and egg quality, young (3–4 y) and old (9–11 y) female *Crassostrea virginica* oysters from both the Magothy and Choptank rivers were collected during 2010 and 2011 and spawned at Horn Point Oyster Hatchery (HPOH). The fatty acid composition of each female's eggs was used as a measure of egg quality. In 2010, oysters were spawned within two months of being collected from their sites of origin, and significant differences were found in the fatty acid composition of eggs by site (global R = 0.368), with polyunsaturated fatty acids being the most influential in separating the fatty acid signatures. In 2011, oysters were kept in a small boat basin connected to the Choptank River for 9 months prior to spawning, in order to test whether overwintering location caused the site differences detected in 2010. The overwintering of animals in the same location between 2010 and 2011 eliminated differences in the fatty acid composition of eggs detected in 2010. These findings call into question the idea that reproductive outputs have singular formulas within a species and bring to light the physiological effects of the environment on not only the animals, but also on their offspring.

THE MOLLUSCAN BROODSTOCK PROGRAM – PAST, PRESENT AND FUTURE.

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The USDA/NIFA-funded Molluscan Broodstock Program (MBP) was established on the West Coast, US, in 1996. The program has focused on selective breeding to improve yields of Pacific oysters, the most valuable aquaculture species on the West Coast. Over the last 15 years, we have developed a land-based hatchery and nursery system to a) exclude specific pathogens, b) improve water quality during periods of upwelling of aragonite-corrosive seawater and c) reduce common-environment effects. Family-based mating designs and statistical analyses are being applied to identify genetically superior broodstock. Average yields of progeny from selected broodstock are significantly greater than those of control families derived from non-selected broodstock. Selected broodstock has been amplified to produce large quantities for commercial production. We have developed working relationships with industry members to host test sites and manage a commercial broodstock repository. MBP is currently developing strategies to transfer the breeding program to industry so that future improvements can be made without dependency on Federal and State funding sources.

DIFFERENCES IN TIMING AND DURATION OF PROLONGED FRESHWATER EXPOSURE ON EASTERN OYSTERS IN BRETON SOUND, LOUISIANA.

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The re-engineering of many of the world's large rivers results in altered flow regimes, marked especially by changed frequencies and magnitudes of extreme events. Downstream estuaries and their biological resources, such as the economically important eastern oyster are particularly affected by changes in physicochemical attributes (i.e., salinity). We monitored oyster recruitment, growth, mortality and dermo disease along a salinity gradient in Breton Sound, LA through 2010 and 2011. During both years, there was limited recruitment throughout the estuary until late fall, and low dermo intensities. In contrast, mortality was significantly higher, and growth lower in 2010 as compared to 2011 at the three higher salinity sites examined, likely due to differences in the timing and extent of freshwater influence into the estuary. In 2010, significant freshwater was diverted into Breton Sound resulting in salinity of less than 3 at all sites for more than 115 days between April and August; in contrast, despite a record Mississippi flood in 2011, freshwater input only resulted in lowered salinity at all sites in spring (April–June). The extended freshwater input and its timing through the hottest months of the year in 2010 likely contributed to the difference in impacts between the two years.

TOXIC EFFECTS OF CRUDE OIL AND THE COREXIT 9500 DISPERSANT ON CONCH (*STROMBUS GIGAS*), OYSTER (*CRASSOSTREA VIRGINICA*) AND SHRIMP (*PENAEUS DUORARUM*) LARVAE.

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Static acute toxicity tests were conducted on ecological and economically important invertebrate larvae with artificially weathered crude oil, dispersant and dispersed oil (1:10 ratio) to determine LC_{50} values following short term exposure. Larvae exposed included conch veligers (2, 4 and 6 lobed stages), oyster veligers (D and "eyed" stages), shrimp larvae (nauplii, zoea 1, mysis 1 and PL6 stages). Dispersed oil was more toxic than crude oil for larvae at all stages of development and as toxic as the dispersant alone at 48 hours for most larvae. The dispersant was equally toxic to all invertebrate larvae at all life stages. LC_{50} levels for the dispersant ranged from a high of 36 ppm (2 lobed conch veligers) at 24 hours to <1.25 ppm at 72 hours (shrimp zoea 1). Survival decreased with an increase in exposure time and younger stages were generally more sensitive than older stages. Shrimp were more sensitive to oil and dispersed oil than conch or oysters. Zoea 1 shrimp were the most sensitive to the contaminants (81 ppm oil, 15 ppm mix, 4 ppm dispersant; 24 hours). Eyed oyster larvae were the most resistant (>1200 ppm oil, 97 ppm mix, 23 ppm dispersant; 24 hours).

CONTINUAL MONITORING FOR THE PARASITE *BONAMIA* IN OYSTERS AND ASSOCIATED BIVALVES IN FLORIDA'S INDIAN RIVER LAGOON.

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PCR analysis revealed *Bonamia* positive bivalves at 8/10 sites in the southern Indian River Lagoon (IRL) in summer 2010. Two sites (WCC, RB) were revisited in summer 2011 along with new sites in the southern (TC, BP, SLE) and middle IRL (SR, WA, OCA). Bivalves were tested for the presence of *Bonamia sp.* (BoF/BoaR). Positive samples were re-tested using species-specific primers for *B. exitiosa* (CaBon146F/471R) and *B. perspora* (OEBon154F/472R). Preserved *C. virginica* tissues collected fall 2010 from the middle (SR, WA) and southern (WCC, TC, SLE, FPI) IRL were also assayed. All sites surveyed in summer 2011 were positive for *Bonamia* (5–62% prevalence) and 5/6 sites surveyed in fall 2010 were positive (5–48% prevalence). No clearly defined seasonal prevalence patterns were evident at sites visited both summer and fall: decreased fall prevalence (WA, TC), increased prevalence (SLE), no change (SR). With the exception of *O. equestris* no *Bonamia* species specific associations were evident, however site specific associations were noted at locations sampled multiple times. This data indicates that a wider geographic range for *Bonamia* exists than has been previously reported in the USA and underscores the need for continual monitoring of *Bonamia* in Florida waters.

IMPACTS OF *ALEXANDRIUM OSTENFELDII* EXPOSURE UPON ANTIOXIDANT SYSTEM OF THE MANILA CLAM *RUDITAPES PHILIPPINARUM* WITH VARYING PARASITE *PERKINSUS OLSENI* BURDEN.

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Manila clams *Ruditapes* (= *Venerupis*) *philippinarum* in Arcachon Bay, France, have decreased in standing stock since 2003. Consequently, studies have been conducted to determine the causes of this decrease. Arcachon Bay clams, naturally infected with the parasite *Perkinsus olseni*, are exposed recurrently to blooms of the toxic dinoflagellate *Alexandrium ostenfeldii*, and accumulate spirolides. This study evaluated effects of both *A. ostenfedlii* and *P. olseni* upon antioxidant gene expression and SOD activity in gills, hemocyte variables and histopathology. Manila clams with variable levels of *P. olseni* infection were exposed for seven days to T-*Iso* (control) or a mix of T-*Iso* and *A. ostenfeldii*, followed by seven days of depuration with only T-*Iso*. Clams with high *P. olseni*burden had high SOD Cu/Zn gene expression. Exposure to *A. ostenfeldii* decreased expression of this gene, as well as hemocyte mitochondrial membrane potential, indicating metabolic depression, and induced vacuolation of cells in gill filaments. These effects may be attributable to allelopathic substances excreted by *A. ostenfeldii* when directly in contact with the gills. No interactions of *A. ostenfeldii* and *P. olseni* were significant, but trends observed in SOD Cu/Zn gene expression suggested that *P. olseni* burden and *A. ostenfeldii* exposure may have synergistic effects.

JUVENILE OYSTERS *CRASSOSTREA GIGAS* EXPOSED TO INFECTIOUS AGENTS MODIFY THEIR HEMOCYTE RESPONSES TO TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA*.

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Many studies conducted to determine factors responsible for high mortality rates in juvenile oysters *Crassostrea gigas* in France, have implicated an infectious agent, herpes virus OsHV-1 (or

variants), as the principle suspect, possibly coupled with other factors, including blooms of toxic algae. In this study, 4cm-oysters were exposed for 9 days to *Alexandrium catenella*, which blooms recurrently in the south of France, and compared to control oysters fed non-toxic *Heterocapsa triquetra*. In an additional experimental treatment, oysters previously held in Bay of Brest (presumably contaminated by herpes virus) were added to half of the tanks containing each algal treatment, to infect experimental oysters. Hemocyte responses, after 4 and 9 days, showed specific effects of either exposure to *A. catenella*, either to infectious agents, on oyster physiology. Exposure to *A. catenella* and to infectious agents simultaneously led to additive effects on several parameters; oysters exposed to both factors showed the highest hemocyte complexity, size and reactive oxygen species production. But some synergistic effects were also observed, as shown by a large increase in hemocyte mortality in oysters exposed to both *A. catenella* and infectious agents, highlighting the relevance of considering combined effects.

SAN FRANCISCO BAY SUBTIDAL HABITAT GOALS REPORT: SETTING 50 YEAR CONSERVATION GOALS FOR OLYMPIA OYSTER RESEARCH, RESTORATION, AND MANAGEMENT IN THE SAN FRANCISCO BAY.

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The San Francisco Bay Subtidal Habitat Goals Report was released in January 2011 and is the first time that comprehensive information about submerged areas in the bay has been compiled. The 50-Year conservation plan is non-regulatory and presents a bold, comprehensive and long-term vision for research, restoration and protection of intertidal and subtidal habitats of the San Francisco Bay. The Project is a collaborative interagency effort between the California Coastal Conservancy, Bay Conservation and Development Commission, National Oceanic and Atmospheric Administration, and the San Francisco Estuary Partnership. The report presents the regional GIS maps of these submerged areas, and a specific adaptively phased approach to learn more about Olympia oyster ecosystem services, functions, and interactions with other habitat types. The presentation will cover actions that have occurred since the recommendations were released and next steps in regional oyster restoration. Implementation of the goals will occur through a number of avenues, and depends on voluntary participation from local governments, resource agencies, researchers, consultants, and non-profits. I will discuss new projects focused on oyster climate change research and living shorelines approaches just getting started in this estuary. The report, appendices, and interactive maps are all available online at www.sfbaysubtidal.org.

A COMPARISON OF TOTAL MERCURY CONTENT BETWEEN FARMED AND WILD SHELLFISH COLLECTED ALONG THE EASTERN COAST OF THE U.S.

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As a result of national health warnings concerning elevated mercury levels in fish, the consuming public is wary of all seafood as potential sources of mercury exposure. While there is a reasonable amount of information on mercury levels in wild shellfish, few data have been reported on levels in farmed shellfish. We investigated total mercury in shellfish from farms located along the eastern seaboard of the U.S. and compared them with mercury levels determined for wild specimens. Oysters (*Crassostrea virginica*) and quahogs (*Mercenaria mercenaria*) representing two size classes were collected from farms and wild sites in selected states along the Atlantic Coast. Total mercury content of the soft tissue was measured using a DMA-80 Total Mercury Analyzer. The level of total mercury in the edible tissue of oysters and clams was not significantly different between farmed and wild samples. On average, the total mercury content of farmed oyster wet soft tissue was 20.6 (SE +0.9) μg/kg (ppb) and for quahog wet soft tissue was 10.8 (+1.5) μg/kg; while, for their wild counterparts, levels were 27.5 (+2.8) μg/kg and 9.0 (+0.5) μg/kg respectively. These values are similar to those previously reported, e.g. FDA - oyster: 25.3 μg/kg; quahog: 17.5 μg/kg.

SHIFTING BASELINES OF A ROCKY REEF FOODWEB: PLACE AND TIME MATTER TO NORTHERN ABALONE CONSERVATION.

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Shifting baselines can elicit vexing conservation challenges when society comes to value attributes of altered ecosystems. Such is the case with northern abalone on temperate rocky reefs in BC where sea otters, important abalone predators, were extirpated by the industrial fur trade a century ago. In the absence of otters, the abundance and size of their benthic macroinvertebrate prey increased dramatically, facilitating development of shellfisheries. Poor fisheries management led to precipitous declines in northern abalone abundance and their listing as threatened, with recent uplisting to endangered. Re-introduction and range expansion of sea otters is again shifting abundance and distribution of macroinvertebrates and kelp. Setting conservation targets and management objectives for abalone and sea otters becomes complex because both are species at risk in Canada, and the hyperabundance of abalone following otter extirpation currently informs reference points for recovery. We combine historical knowledge and observational data across gradients of sea otter occupation in two regions of BC to understand how recovery of sea otters is

impacting benthic rocky reef foodwebs and recovery of northern abalone. While poaching remains a threat, assessment of abalone recovery in an ecosystem context challenges us to re-evaluate the status of northern abalone and targets for conservation.

A NOVEL BIOMARKER FOR CHRONIC DOMOIC ACID EXPOSURE IDENTIFIED IN A ZEBRAFISH MODEL AND VALIDATED IN SEA LIONS.

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There is a critical knowledge gap regarding the impacts of chronic low-level exposure to common environmental toxins such as the neurotoxic amino acid domoic acid (DA), a phycotoxin naturally produced during harmful algal blooms (HABs). Accumulation of DA in the food web poses significant health threats to humans and wildlife and is responsible for a neurotoxic illness known as amnesic shellfish poisoning (ASP). Regular monitoring of DA levels in edible shellfish tissues has been effective in protecting human consumers from acute DA exposure. However, there is no protection from chronic low-level DA exposure, which may pose significant human health risks, particularly in coastal and tribal communities that subsistence harvest shellfish known to contain low levels of the toxin. Using a zebrafish chronic exposure model, we have identified a biomarker specific for chronic low-level domoic acid exposure and that chronic exposure increases toxin susceptibility in subsequent exposures. We have further confirmed the presence of the biomarker in naturally exposed California sea lions. Our next steps are to translate the diagnostic tools developed in these sentinel species to platforms for human health diagnostics.

SHELLFISH RESTORATION BEST PRACTICES: CO-OPERATIVE STRATEGIES FOR SUCCESSFUL PROJECTS.

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Project objectives were to establish protocols and safeguards and educational programs to ensure that shellfish grown in unapproved areas do not reach the market. The project, recommended by the Restoration Committee of the Interstate Shellfish Sanitation Conference (ISSC,) was designed around seven regional workshops, bringing together diverse stakeholders to identify critical issues and solutions. The focus was on identifying methods to restore critical shellfish areas while protecting public health. The resulting BMPs were grouped under 5 headings: protect public health while restoring the environment; define goals and objectives of restoration projects; expand communication and education; expand community-wide restoration and, use of noncommercial species. Participants suggested forming partnerships among vested parties to promote restoration of native shellfish and ecosystem services, and to conserve and restore coastal water quality. Most regulators preferred restoration activities sited only in approved waters, but if in unapproved waters, restoration would require additional plans for the biosecurity and an education component (biology, growing methods, pests competitors, diseases and public health) for restoration programs using volunteers.

A PRELIMINARY EVALUATION OF HATCHERY PRODUCED SPAT CHIPS ON LOUISIANA OYSTER REEFS.

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The current method of harvesting oysters in Louisiana, seed bedding, relies on the production of wild oyster seed to be transplanted to private leases for grow-out. However, such production can vary due to environmental and anthropogenic influences. A more reliable method of producing seed may entail using larvae from a hatchery, where outside influences that affect larval survival can be minimized. The success of using hatchery-produced oyster spat on Louisiana seed beds is being evaluated as an alternative to wild seed. Competent pediveliger larvae were set on different cultch materials to produce ''spat chips'' at the Louisiana Sea Grant Bivalve Hatchery on Grand Isle, LA for planting on Louisiana oyster reefs. From this evaluation, proper site selection, handling techniques and monitoring methods for measuring spat survival are being determined. The information gathered from this preliminary evaluation is useful for future research and implementation of hatchery technology on public or private oyster leases in Louisiana.

TECHNIQUES IN HABITAT MODELLING FOR STOCK ASSESSMENT IN CANADA'S PACIFIC RED SEA URCHIN COMMERCIAL FISHERY.

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With the move towards ecosystem based management, the Dept. of Fisheries and Oceans Canada (DFO) is currently exploring ways to use ShoreZone data as an input for habitat modelling in order to identify potential habitat in the shallow subtidal 'white strip' for stock assessment of commercial dive fisheries. The ShoreZone data set is a georeferenced biophysical

characterization of British Columbia's shoreline based on aerial video and photography taken near zero tide. ShoreZone data were originally applied to the TRIM shoreline (Terrain Resource Information Management), whereas DFO uses a Canadian Hydrographic Services (CHS) defined shoreline. The ShoreZone have been successfully transferred to the CHS low water shoreline via a Thiessen polygon method. Integration of multiple data sets (fishing events, industry knowledge and dive surveys) from DFO with the ShoreZone data has allowed predictions of previously undefined sections of shoreline as red sea urchin habitat, or non-habitat. Urchin habitat predictions in data-limited areas were made with varying degrees of confidence using the Biomod package in the statistics program 'R'. Truthing of predictions is underway.

PROTEIN PROFILES OF PARASITE INFECTED MANILA CLAMS *RUDITAPES PHILIPPINARUM* EXPOSED TO THE HARMFUL ALGA *KARENIA SELLIFORMIS* AS REVEALED BY PROTEINCHIP® AND SELDI-TOF-MS PROTEOMIC TECHNOLOGY.

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Manila clams *Ruditapes philippinarum* with high or low intensity of infection with the protistan parasite, *Perkinsus olseni* were exposed to the toxic alga, *Karenia selliformis*, or to a control alga, *Chaetoceros neogracile*. Protein expression in hemocytes was studied using ProteinChip and Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-TOF-MS) technology. Previous studies demonstrated that *K. selliformis* had more-severe effects on hemocytes than *P. olseni*, and clam cellular immune responses to *K. selliformis* were modified by *P. olseni*. Aims of current research were to compare the protein profiles of hemocytes from clams from the different treatments and to explore the relationship between protein expressions and immunological response. Presence of *P. olseni* caused the up-regulation of 5 proteins and down-regulation of 15 proteins. Similarly, exposure to *K. selliformis* also caused up-regulation of 9 proteins and down-regulation of 17 proteins. Certain proteins were selectively up- or down-regulated in clams highly infected by the parasite and exposed to the HAB, suggesting a potential synergistic effect of these two stressors. The profiles of HAB-exposed clams had the highest degree of departure from the control (no HAB, low parasite) clams, indicating a tight relationship between protein expressions and immunological responses.

OFFSHORE MUSSEL FARMING IN SOUTHERN NEW ENGLAND: RESEARCH PLANS FOR OPTIMIZING ECONOMIC YIELD.

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The development of domestic offshore mussel farms offers promising options for fishermen and shellfish farmers to create jobs and product for local markets. After successfully testing growing methods for longline mussels *(Mytilus edulis)* in offshore waters and harvesting them in the fall of 2010, fishermen in Rhode Island and Massachusetts have doubled their investment, expanded their leases and installed eight 150-meter subsurface longlines in waters averaging 30 meter deep. With complementary support from NRAC and RI Sea Grant our current investigations plot a path to more sustainably economic operations. Paramount among these is sourcing sustainable and steady supplies of mussel seed via wild collection and hatchery production to supply year round sales. A series of experiments are proposed to test the efficacy of eradicating tunicates that may be found on wild-collected seed. Secondly, we will compare different types of mussel socks and stocking densities for developing optimal marketable yield for our region. Details of our plans and results so far will be discussed.

HAA AANÍ, LLC AND MARICULTURE: SOUTHEAST ALASKA RURAL ECONOMIC DEVELOPMENT.

Anthony Lindoff.

Haa Aaní, LLC: Economic Development Subsidiary of Sealaska, One Sealaska Plaza, Suite 400, Juneau, AK, 99801, USA.

A primary goal of Haa Aaní is to actively support and facilitate initiatives that will improve economic and employment opportunities throughout Southeast Alaska. Haa Aaní's main goals are to:

- create employment opportunities in rural SE communities that are lifestyle compatible
- create economic expansion and sustainable economies across SE enabling Sealaska Corporation shareholders to work and reside in their respective communities (i.e. curb the current outmigration)
- remove impediments and bottlenecks to economic stimulation

Haa Aaní firmly believes that the nascent shellfish mariculture industry in Alaska can offer a viable entrepreneurial opportunity for its shareholders. While Haa Aaní isn't interested in owning/operating shellfish farms, it has been steadfastly attacking and removing impediments and other barriers to entry so as to insure its efforts and those of its shareholders are truly sustainable. Through

Haa Aaní's partnerships, collaborative efforts, its oyster seed business, and by creating more favorable public policy, the Alaska mariculture industry is on the verge of unprecedented production and growth; sustaining that growth and building upon our success is Haa Aaní's mission.

ALTERNATIVE STABLE STATES IN NATIVE OYSTER POPULATIONS.

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Alternative stable states (AltSS), the occurrence of different community states under similar environmental conditions, have been documented or suggested for various ecosystems including coral reefs, lakes, grasslands, hard- and soft-bottom marine communities, and kelp forests. Despite the likelihood of AltSS in marine species, there have been few comprehensive studies that concurrently document the patterns, causal mechanisms, and ecological consequences of AltSS at the population level. We present modeling and empirical studies that demonstrate the likelihood of AltSS in native oyster populations in Chesapeake Bay. The approach is multi-disciplinary, and integrates field sampling, manipulative field experiments, hydrodynamic modeling, and demographic modeling. Specifically, the project provides empirical evidence and the theoretical foundation for the hypothesis that a positive feedback mechanism in oyster populations is disrupted by an interaction between environmental disturbance and connectivity. This process produces a source-sink metapopulation composed of persistent and degraded AltSS. The project findings advance the theory and empirical evidence for AltSS in exploited marine species such as the oyster, and concurrently augment the scientific foundation needed for effective restoration of populations in decline.

RECOVERY OF THE BLUE CRAB IN CHESAPEAKE BAY.

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Over the past decades, marine species have experienced population declines worldwide due to overfishing and habitat degradation. In contrast to the widespread examples of population declines, there are few examples of successful management actions that both prevented population collapse and catalyzed recovery to historical abundance levels. In Chesapeake Bay overfished populations of various species, such as Atlantic sturgeon, striped bass, and the native oyster, have sequentially collapsed. In the early 1990s, the blue crab population experienced a decline of 80% that persisted for nearly two decades. In 2008, forceful restoration measures, based on robust ecological and fishery data and aimed at the spawning stock, catalyzed a dramatic recovery of the population and fishery. The spawning stock recovered to historically high levels, followed by enhanced recruitment and a continuing robust spawning stock. The resurgence thus serves as a definitive example of the means by which overfished marine species can be restored and their ecosystems placed on a path to recovery.

TOTAL LIPID CONTENT AND FATTY ACID PROFILES IN THE NOBLE SCALLOPS *CHLAMYS NOBILIS* RELATED TO SHELL COLORS.

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Total lipid content (TLC) and fatty acids composition among three kinds of tissues including adductor, mantle and gonad sampled from male and female noble scallop *Chlamys nobilis* with orange and brown shell colors were compared. TLC ranged from 3.80 to 19.11 ($mg \cdot g^{-1}$dry mass), which was significantly affected by shell color, tissue, and gender ($P < 0.05$). There were significant differences in SFA, MUFA and PUFA among three tissues. In adductor muscle, both SFA and PUFA were significantly affected by shell color and gender. In mantle and gonad, SFA, MUFA and PUFA were all affected significantly by shell color and gender. Moreover, absolute content of DHA and EPA was significantly affected by shell color, tissue, and gender ($P < 0.05$). The content of DHA and EPA was the highest in gonad among three tissues, which was ranged from 1.99 $\mu g \cdot g^{-1}$ to 3.59 $\mu g \cdot g^{-1}$ and from 3.44 $\mu g \cdot g^{-1}$ to 4.52 $\mu g \cdot g^{-1}$, respectively. The present results showed that the fatty acids content was related to shell color in the noble scallop.

DEVELOPING SPATIALLY-EXPLICIT ASSESSMENT TOOLS FOR EASTERN OYSTER IN CHESAPEAKE BAY.

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Understanding spatial and temporal recruitment variability is important for managing fisheries for sessile species, especially when determining placement of marine protected areas and spatial fishery regulations. The eastern oyster *Crassostrea virginica* is

of current concern because of its declining abundance and its ecological, economical, and cultural importance. An overall declining trend in recruitment has been observed in Chesapeake Bay, but spatial and temporal dynamics as well as environmental factors affecting recruitment have been relatively unexplored. We evaluated how spatial patterns in autocorrelation of recruitment and adult relative density varied over time by fitting semivariogram models to survey data for each year. We used these patterns to determine appropriate scales to develop a stage-based model for the lower Potomac River using data from the Maryland Department of Natural Resources fall dredge survey and the Potomac River Fisheries Commission. Oyster bars in close proximity to one another had similar dynamics and recruitment in lower salinities was more variable than recruitment in higher salinities, suggesting salinity is important factor in oyster recruitment variability. This information can be used to determine appropriate scales for stock assessment models and can help guide spatial management of eastern oysters in Chesapeake Bay.

HELPING THE FLORIDA CLAM INDUSTRY SURVIVE THE SUMMER.

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Florida is at the southern-most limit of the northern hard clam *Mercenaria mercenaria*, where subtropical temperatures allow for a longer growing season and shorter production time. However, increased summer temperatures have led to mortalities of market size clams, impacting the industry. In a previous study examining heat-tolerance and triploidy, it was found that heat shock proteins (Hsp) may be a suitable biomarker for development of a heat-tolerant clam. In the current study, individual variation and potential inheritance of Hsp70 is being examined. A survey of cultured and wild groups of clams (total n = 540) found that hemolymph Hsp70 levels varied within and between groups; size or age may be a factor. Clams classified with high- and low-expressing Hsp levels were spawned individually to produce three putative high- and three low-Hsp families. Families were reared as similarly as possible and planted recently in the field. If Hsp levels in progeny are correlated to parental Hsp levels and if high-Hsp families exhibit higher survival in the field and under laboratory challenges, Hsp may be considered a biomarker for selective breeding of heat-tolerant hard clams that would assist the Florida cultured clam industry. Support by USDA and Sea Grant (R/LR-A-47).

HEMOLYMPH pH AFTER CLAMPED EMERSION AND HYPOXIC GAPING RESPONSE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, AND THE ASIAN OYSTER, *CRASSOSTREA ARIAKENSIS*.

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The eastern oyster, *Crassostrea virginica*, is known for its ability to withstand low oxygen conditions. However, the taxonomically and morphologically similar Asian oyster, *Crassostrea ariakensis*, dies significantly earlier than *C. virginica* during hypoxic exposure. Our study was aimed at understanding the physiological basis for this difference in tolerance. We exposed the oysters to water with oxygen concentration below 0.5 mgL^{-1}, and assessed the gaping response and hemolymph pH at specific time intervals for 24 hours. We found that *C. ariakensis* gaped more frequently and wider than *C. virginica*, and that gaping was associated with acidification of the surrounding water during hypoxic exposure. We also found that when gaping was inhibited by clamping, the time an oyster was clamped had a significant effect on the hemolymph pH for each species and the hemolymph exhibited a more acidic shift in *C. ariakensis* than *C. virginica*. Further, we investigated if hypoxic-induced gaping was a behavioral or physiological response. This research has applications for conservation and restoration decisions as well as comparative physiology by explaining fundamental differences between two closely related species and how they respond to environmental stress. Specifically, these comparisons may provide insight into the mechanisms behind the well-documented hypoxic tolerance of *C. virginica*.

MEET ME AT THE FOOD COURT: IMPLEMENTING A BIOPHYSICAL MODEL TO TEST THE EFFECTS OF FOOD ABUNDANCE ON THE SUCCESS OF OYSTER LARVAE RECRUITMENT IN DELAWARE BAY.

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Recent advances in the application of biophysical models have provided valuable insights into larval dispersal and recruitment. In the present study, we coupled an Individual-Based Model with a regional hydrodynamic model to simulate larval dispersal of the eastern oyster, *Crassostrea virginica*, in Delaware Bay. We incorporate a new parameter, food abundance, to study its effects on larval recruitment. Food values were obtained from seston samples collected once every month at 18 stations along Delaware Bay and River during 2009–2010. Total food available was estimated as the

sum of the protein, lipid, and carbohydrate values (mg/L) in each station and data were interpolated throughout the Bay to create a 3D food field. Particles were released from populations along the Bay every five days from June–September and tracked until they reached a competent settlement size (330 um). Recruitment patterns were similar to those observed in an 'unlimited food' scenario. However, low food values (spatial and temporal) contributed to increase larval development times, resulting in higher mortality. While survival is limited by low salinity levels in the upper bay areas, our results suggest that food may be the limiting factor in the mid-to-lower bay areas.

MUSSELS AT WORK: DEVELOPING METHODS TO INTEGRATE RIBBED MUSSELS INTO LIVING SHORELINES AS FOUNDATION REINFORCERS.

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Habitat restoration is a fundamental component of the overall strategy to improve ecological conditions throughout the Delaware Estuary. Our previous work has established that natural, biodegradable coir fiber logs can stabilize salt marsh shorelines, and that ribbed mussels (*Geukensia demissa)* and grasses can be successfully planted into these structures to form the basis of a living shoreline. Because collecting ribbed mussels from natural populations is destructive to the marsh habitats that these living shorelines are trying to protect, our next goal is to develop methods to spawn, rear, set and grow ribbed mussels. Our first objective was to determine the ribbed mussel reproductive cycle in Delaware Bay. Ten mussels from three sites spanning upper, mid and lower regions of Delaware Bay were collected bimonthly from April to November 2011. All three sites behaved similarly. Gonad development was underway in April and peak maturation occurred in July. Spawning was first apparent in late July and continued to late October. Sex ratio averaged 1:1 throughout the season. Initial spawning attempts via thermal, physical or chemical stimulation as well as strip spawning were unsuccessful. Efforts are underway to condition mussels for spawning trials at the Rutgers Cape Shore Shellfish Hatchery in early 2012.

SENTINELS ON DUTY: CAN RIBBED MUSSELS (*GEUKENSIA DEMISSA*) RELIABLY MONITOR *PERKINSUS* SPP. ABUNDANCE IN DELAWARE BAY?

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Mussels have been widely used as indicators of long-term ecosystem health. The purpose of this study was to evaluate the potential use of ribbed mussels, *Geukensia demissa*, as sentinels for monitoring *Perkinsus* spp., the protist responsible for Dermo disease in the eastern oysters (*Crassostrea virginica*), in Delaware Bay. Three locations (Money Island, Maurice River, and Reeds Beach) were targeted to be representative of upper, middle, and lower areas of the Bay, respectively. Mussel gill samples were collected every other week from June to November 2011 and incubated in RFTM for at least a week before *Perkinsus* spp. cells were counted. Subsamples were preserved for species-level identification using PCR. Two peaks in *Perkinsus* spp. cell counts were evident in the Maurice River mussel gill data. The first peak, in late August, occurred during peak summer temperatures, and was consistent with observations from previous studies. The second and highest peak in abundance occurred at all sites, and coincided with peak oyster mortality in the fall. The results indicate the potential to use ribbed mussels as 'natural collectors' as opposed to more logistically or economically restrictive methods.

DEVELOPMENT OF A Q-PCR ASSAY TO DETECT *MIKROCYTOS MACKINI* AND ASSESSMENT OF OPTIMUM TISSUE FOR DIAGNOSTIC TESTING.

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Mikrocytos mackini is a microcell parasite of Pacific oysters (*Crassostrea gigas*) found on the Pacific coast of North America. To date, detection methods have included histopathology, which lacks sensitivity, or a conventional PCR assay, which cross-reacts with *Mikrocytos* sp. Our aim was to redress the limitations of these techniques by developing a qPCR assay. Recent characterization of ITS-rDNA of *M. mackini* and related *Mikrocytos* sp. enabled development of a *M. mackini*-specific TaqMan qPCR assay targeting ITS2-28S. It detects $\leq$65 copies of plasmid containing the target sequence in a matrix of host tissue. Assay specificity was confirmed by the absence of amplification in *Mikrocytos* sp. and a broad range of other shellfish parasites and commercially important shellfish hosts. Due to the focal nature of *M. mackini* infections, qPCR sensitivity was evaluated across several tissue types selected based on historical observations of the distribution of infections in the host. The following tissue types were sampled from 62 Pacific oysters and tested by qPCR: mantle; palps; adductor muscle; a mid-body cross-section including gill, digestive gland, mantle, connective tissue, and gonad; and hemolymph. Statistical analyses showed that the mid-body slice was the optimal tissue to sample for detection of *M. mackini* by qPCR.

LUMPY BUMPY THE SEA STAR: REVISITING AN INTERNAL MOLLUSCAN PARASITE OF SEA STARS.

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During annual NOAA Fisheries stock assessment surveys, the Fisheries Resources Pathobiology team monitors diseases of fish and shellfish in the Bering Sea, Alaska. As a routine practice, we record rare cases and new anomalies. In summer 2010, a grossly misshapen sea star of the species *Leptasterias polaris* was encountered with large swollen regions and will be presented as a case study. The sea star arms were parasitized by multiple individuals of a gastropod in the family Eulimidae, known to be the only gastropod group parasitizing echinoderms. The body plan of the endoparasite, likely a species of *Asterophila*, is modified from most gastropods. Within the arm of the sea star, females deposit and brood an egg mass, leading to the physical distortions of the host as eggs develop into veliger larvae. Parasitism of sea stars by *Asterophila japonica* in the Chukchi Sea, Alaska was examined in 1980, but currently the range of this parasite in the Bering Sea is unknown. We will present molecular and morphological data on this case.

HETEROSIS ANALYSIS ON INTERSPCIFIC HYBRIDS BETWEEN *HALIOTIS DISCUS HANNAI* AND *H. GIGANTEA*.

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Haliotis discus hannai is the most important species of gastropods in aquaculture for China, however many abalone farms have been particularly affected by germplasm degradation of *H. discus hannai*. *H. gigantea*, which possesses excellent disease resistance, is a valued commercial species along the coast of Japan. This species was introduced from Japan to China for mariculture in 2003. A 2×2 complete diallele cross was conducted between *H. discus hannai* (D) and *H. gigantea* (G) to produce hybrid abalones. Growth performance and survival of the reciprocal hybrids of *H. discus hannai* and *H. gigantea* were investigated. Results showed that the fertilization and hatching rate of the reciprocal hybrids were significantly lower than those of the parental species ($P < 0.05$). At grow-out stage, both reciprocal hybrids grew at similar rates ($P > 0.05$), but significantly faster than thir parents ($P < 0.05$). Moreover, survival was similar in reciprocal hybrids and one of conspecific group *H. gigantea*, while a significantly higher mortality occurred in the other conspecific group *H. discus hannai* ($P < 0.05$). The superiority of growth and survival of the reciprocal hybrids over their parental controls indicates hybrid vigor and has significant implications for the improvement of abalone aquaculture in China.

DIFFERING SUSCEPTIBILITY TO COMMENSALS AND POTENTIAL PATHOGENS COULD GIVE *MYTILUS GALLOPROVINCIALIS* AN ADVANTAGE OVER *MYTILUS EDULIS*.

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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. In Wales, it is believed that only *M. edulis* is present. This study investigated the health status of these mussels. Samples of wild and cultured *Mytilus* spp. were collected from twenty-four sites encompassing all coasts of Ireland and the Welsh Coast, at different times of the year over several years. In total, eight hundred and forty-one *Mytilus* spp. were examined. Habitat description and the environmental factors influencing the study sites were recorded. All *Mytilus* spp. samples were screened using histology to determine health status and the presence of any potential pathogens/parasites. At certain study sites a cPCR was carried out to differentiate which mytilid species were being screened. Copepods, ciliates, trematode spp. prokaryote inclusion bodies and *Nematopsis* spp. were detected in the mussels. The prevalence of infection and/ pathological changes varied among the sites and were observed in both mytilid species and hybrids.

AN UPDATE ON SURVEY FINDINGS AND RESEARCH EFFORTS ON OLYMPIA OYSTERS (*OSTREA LURIDA*) IN CANADA.

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Olympia oysters, *Ostrea lurida* Carpenter, 1864, were first assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2000 and were listed under the Species At Risk Act in 2003 as a species of Special Concern. Fisheries and Oceans Canada posted a five-year management plan in response to the listing. The goal of the plan is to maintain stable populations of Olympia oysters in BC. The indicator used to measure success in attaining that goal is to measure the relative abundance of Olympia oysters at index sites over the six year period 2008–2013. Thirteen index sites were identified, based on a combination of pre-determined criteria (previous data, accessibility, collaborative interest and representative of potential threats) and random selection. All 13 sites have had baseline surveys completed. Surveys have also been conducted to verify historical records; beaches with historical records were targeted and adjacent sites were added opportunistically. A total of 196 beaches were surveyed between

2009 and 2011. The presence of Olympia oysters was confirmed at 120 of these beaches (61%). Restoration research in collaboration with Vancouver Island University has focused on broodstock collection and rearing techniques with plans to identify preferred substrate suitability and optimum settling parameters.

LINE-SPECIFIC SUSCEPTIBILITY TO ROD IN EASTERN OYSTERS.

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In northeastern U. S., Roseovarius oyster disease (ROD) has had a significant impact on eastern oyster (*Crassostrea virginica*) culture operations causing upwards of 90% mortality in juvenile farmed oysters. Genetically improved stocks and husbandry strategies have reduced losses from this disease, but outbreaks continue to impact culture throughout the region. Presently, it is unknown whether increased survival among improved lines is due to tolerance or resistance to ROD. Further, there is a lack of sufficient understanding of the conditions and host-parasite interactions that affect the pathogenicity of the etiological agent of the disease, *Roseovarius crassostreae*. To address these gaps in knowledge of the dynamics of ROD, we deployed oyster seed from 6 genetically improved lines at an oyster farm in Maine where ROD is endemic and are monitoring line-specific disease prevalence and disease-associated mortality through an ROD outbreak. Concurrently, we are using molecular biological approaches to examine seasonal variation in *R. crassostreae* abundance in environmental samples taken at the same oyster farm and to identify potential virulence factor(s) associated with *R. crassostreae* pathogenicity. We anticipate these combined approaches will help in the development of integrated methods for minimizing the impact of ROD on oyster culture in the northeastern U.S.

WELL-FED BUT METABOLICALLY STARVING: IMPLICATIONS OF GENOTYPE FOR ENERGY ALLOCATION IN LARVAE OF *CRASSOSTREA GIGAS*.

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For decades, well-fed Pacific oyster larvae have grown optimally at temperatures of 25°C or higher. Recently, many facilities worldwide are reporting high mortality under these long-established and routine culturing conditions. These ''failures'' have been attributed to various environmental causes (e.g., pathogens, ocean acidification, warming ocean temperatures). We tested the hypothesis that there is a genetic basis for variation in metabolic efficiency. This would have important consequences for avoiding stress-related mortality by understanding the energetic scope required to mount physiological responses to environmental change. The cost of protein synthesis was measured in larval families to define the biochemical allocation of available energy. Surprisingly, for certain larval families reared at 25°C, over 90% of available metabolic energy was allocated to the single process of protein synthesis. This ~90% allocation was substantially reduced to ~50% when larvae of the same full-sibling larval family were reared at 20°C. These findings suggest optimal temperatures that maximize growth might in fact ''starve'' larvae, in terms of their capacity to allocate additional energy to respond to environmental stress. Understanding the genetic basis of metabolism has implications for optimizing both growth and survival of larvae under changing ocean conditions.

SO WHAT SHOULD A NATURAL MORTALITY CURVE LOOK LIKE FOR OYSTERS?

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Natural mortality rate (M) of a population describes the interaction of recruitment, growth and loss to environmental factors (both physical and biological). It reflects species life history traits, a product of selection over evolutionary time scales. Fishing mortality (F) describes loss to exploitation. Estimates of natural mortality in extant oyster populations arguably diverge from these evolved norms because of cumulative impacts of environmental degradation, age truncation by disease, and fishing. Pre-1900 literature describes very large oysters that, extrapolating from truncated modern growth curves, are suggested to have terminal ages in the 15–20 year ranges. The lengths of these oysters can be used with Hoenig plots to estimate natural mortality in pre-exploitation, pre-disease situations. A Hoenig plot inherently suggests a constant mortality rate with increasing age, but is this correct for oysters? We describe a length frequency distribution for an unexploited population of *Crassostrea gigas*, currently invading the Oosterschelde in the Netherlands, that includes representation of all size classes up to 200mm in length – an analog of a pre-exploitation, pre-disease population. From this demographic we suggest a probabilistic age structure and estimate age specific mortality for a long-lived, undisturbed oyster population.

A COMPARISON OF TWO METHODS FOR *IN SITU* HYBRIDIZATION USING PARRAFIN TISSUE SECTIONS.

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Two methods for colorimetric *in situ* DNA probe hybridization (CISH) assays on paraffin tissue sections were compared. Both procedures were tested on Davidson's and formalin-fixed, paraffin-embedded histological tissue samples harboring the mollusc pathogens *Perkinsus chesapeaki* or *Haplosporidium nelsoni*, or the

fish pathogen *Myxobolus cerebralis*. One method is labor intensive and is highly prone to human error; while the other is less labor intensive, less expensive, and can be run in a shorter period of time. Both methods yielded similar results. Use of complex and expensive pre-hybridization buffers did not improve the performances of the tested CISH assays. Pre-hybridization heat-denaturation of DNAs in assayed samples increased both assay duration and loss of samples, but did not improve hybridization signals.

FIVE DECADES OF OYSTER FISHERY ENHANCEMENT STRATEGIES IN UPPER CHESAPEAKE BAY: EFFECTS ON LANDINGS AND RELATIVE ABUNDANCE.

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Three key strategies have been used over the past fifty years in an attempt to rehabilitate the oyster (*Crassostrea virginica*) fishery in the Maryland portion of the Chesapeake Bay: the planting of oyster bars with shell, wild seed, and more recently, hatchery seed. The goal of these methods was not only to enhance oyster habitat and oyster populations in the bay, but to achieve a widely distributed (>150 km) productive fishery. We compiled three primary but disparate datasets: seed and shell planting history, harvest records (landings), and relative oyster abundance to determine the effects of the enhancement strategies on the oyster fishery. Records of specific seed and substrate plantings were available from 1960-present. Landing reports required from seafood dealers and relative oyster abundance data from the annual fall survey conducted by the Maryland Department of Natural Resources were available from 1980-present. We were able to reconstruct the enhancement histories of 292 individual oyster bars and, utilizing multiple regression and time-series techniques, compare strategies over large spatio-temporal scales. Future enhancement efforts can be based on where and when these strategies were most successful in the past.

USING NOVEL GROWTH MEDIA TO CONTROL BIOFOULING AND SHELL DEFORMITIES IN SUSPENDED CULTURE OF BIVALVES IN BRITISH COLUMBIA, CANADA.

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Biofouling and shell deformities are problems commonly associated with suspended culture of bivalve shellfish and can reduce bivalve condition and final product marketability. Labour-intensive mechanical treatments and/or intertidal culture are often used to address these problems. As an alternative to these treatments, juvenile Pacific oysters (*Crassostrea gigas*) and Manila clams (*Venerupis philippinarum*) were tray-reared in two varieties of culture media: expanded clay aggregates and lava rock. The hypothesis was that substrates lower the incidence of shell deformities (by providing structural support), brush fouling off with wave action, and act as a tumbling agent that strengthens oyster shells. Both species were grown in suspended trays (55 × 55 cm) containing 0, 1, 2, and 3 l of substrate. Stocking densities were 200, 600, and 1000 ind/tray for oysters and 800, 2400, and 4000 ind/tray for clams. Discussed are the effects of substrate type, substrate volume, and bivalve stocking density on biofouling coverage, growth, survival, shell shape, shell density and meat content of bivalves.

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

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Flow-through (high density) larval culture boasts the capability of rearing larvae at stocking densities higher than those of static larval culture. Full-scale implementation of flow-through culture for the eastern oyster, *Crassostrea virginica*, has yet to occur. Information on the consistency and predictability of flow-through larval culture performance is lacking. The objective of this experiment was to determine the variation in larval performance among highly replicated flow-through cultures, thereby establishing expectations for commercial hatchery success. Flow-through tanks were stocked with 10 larvae/mL and reared at two flow rates: 1.4 and 2.8 L/min. Expected survival for the 1.4 L/min flow rate is 0.59 (±0.19) and 0.46 (±0.13) for 2.8 L/min. Larval growth at 1.4 L/min was significantly faster than at 2.8 L/min. Overall cell consumption was significantly higher for larvae reared at 2.8 L/min. Eyed larvae harvests for cultures reared at 1.4 L/min were inconsistent, with two flow-through cultures experiencing earlier harvests than the others. Larval cultures grown at 2.8 L/min had more uniform harvests. Two million eyed larvae were harvested from the 1.4 L/min flow rate, and 1 million from the 2.8 L/min flow rate. The 1.4 L/min flow rate results in better overall performance of eastern oyster larvae.

INTERACTIVE EFFECTS OF ELEVATED CO_2 AND TEMPERATURE ON PHYSIOLOGY OF TWO BIVALVE SPECIES.

Omera Matoo.

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Estuarine bivalves are susceptible to environmental stressors such as ocean acidification and elevated temperature which can interactively affect their performance and survival. We tested the

interactive effects of elevated CO_2 and temperature on energy metabolism and oxidative stress in two common bivalves, *Crassostrea virginica* (eastern oyster) and *Mercenaria mercenaria* (hard shell clam). Organisms were exposed for 2 and 15 weeks to a combination of two temperatures (22°C and 27°C) and two P_{CO2} levels – 380 and 800 ppm representative of the present-day conditions and a moderate IPCC scenario for the year 2100, respectively. Respiration rate was significantly increased in clams but not oysters exposed to hypercapnia and elevated temperature. In clams, levels of oxidative stress biomarkers were higher and the total antioxidant capacity (TAOC) – lower after 15 weeks of exposure at the elevated temperature. In oysters, higher levels of oxidative damage and increased TAOC were detected after 15 weeks at elevated P_{CO2}. Our results indicate that elevated temperature and P_{CO2} compromise the long term survival of these bivalves with negative effects more pronounced in clams than oysters under future ocean conditions. Supported by NSF award IOS-0951079 to E.B. & I.M.S.

NOROVIRUS MONITORING SURVEY IN JAPANESE SHELLFISH GROWING AREAS.

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Norovirus (NoV) causes acute gastroenteritis worldwide, and has contaminated oyster cultivation cites in coastal waters over the last three decades, including Japan. The aim of this study was to monitor fluctuation of noroviruses in an oyster cultivation area of Hiroshima Bay, the most important oyster growing area in Japan, to elucidate the contamination pathway of norovirus from rivers to the oyster cultivation area. We have developed a rapid and sensitive direct detection method for norovirus using RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification), coupled with simple ultrafiltration. This method successfully detects norovirus from small volumes (0.5 to 1 L) of seawater within 3 hours. Biweekly field monitoring in seawater revealed that norovirus was highly distributed in the fresh water area, and they intrude into oyster cultivation areas through surface layer. Further, our results showed that almost all NoVs in seawater exist in the < 0.2 μm fraction, suggesting that NoV do not bind with any suspended materials such as phytoplankton and detritus in coastal waters.

BYCATCH REDUCTION DEVICES IN BLUE CRAB TRAPS AFFECT CATCH WHILE PROMOTING DIAMONDBACK TERRAPIN CONSERVATION.

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Bycatch mortality in commercial fishing gear often reduces abundance of non-target species. In Chesapeake Bay, a species of ecological concern is the diamondback terrapin, *Malaclemys terrapin*, which often enters and drowns in blue crab pots. Although BRDs in commercial fishing gear have been shown to reduce bycatch mortality, previous studies have mostly been conducted at small spatial scales. Here we examined BRD effects on blue crab catch and terrapin bycatch over the whole of lower Chesapeake Bay. At 22 locations on the eastern and western shores of the lower bay, we used 4–20 standard pots and 4–20 BRD pots, which were baited and fished for 10 days each. Catch per pot, sex, and sub-legal catch differed between standard and BRD pots, whereas no terrapins were captured in BRD pots, which demonstrates the utility and the tradeoffs of using BRDs in blue crab pots for conservation.

CRABS IN HOT WATER: ASSESSING THE VULNERABILITY OF DUNGENESS CRAB (*CANCER MAGISTER*) FISHERIES IN CALIFORNIA, OREGON, AND WASHINGTON TO CLIMATE CHANGE.

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Assessing Dungeness crab (*Cancer magister*) fisheries' vulnerability to climate change is essential to develop management responses that will promote future sustainability and focus research. In our vulnerability assessment framework, three components shape response to climate change: 1) the degree to which the stock and fishery experience climate change (i.e., exposure); 2) the degree to which the stock and fishery are affected (i.e., sensitivity); and 3) ability of the stock and fishery to adjust to changing conditions and related impacts (i.e., adaptive capacity). In May 2011, NOAA, Sea Grant, and other partners convened a workshop to evaluate the effects of climate change on selected northeastern Pacific fisheries, including Dungeness crab. A working group consisting of scientists, managers, and industry representatives evaluated the exposure, sensitivity, and adaptive capacity of the Dungeness crab stock and fishery along the contiguous US West Coast. Here we highlight the results of the workshop and present details of the vulnerability assessment. Climate change is anticipated to affect every Dungeness crab life history phase. Despite likely impacts to the fishery, fishing communities are expected to adapt to

anticipated changes. The approach and results are useful to managers and others charged with planning for the inevitable consequences of climate change.

NEKTON, NETS, AND TUBES: MACROFAUNA RESPONSE TO INTERTIDAL GEODUCK AQUACULTURE OPERATIONS IN PUGET SOUND, WASHINGTON, USA.

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Aquaculture of geoducks (*Panopea generosa*) involves large-scale out-planting of juvenile clams to beach habitats and installation of PVC tubes and netting to exclude predators thereby increasing early survival. Here we examine whether structures associated with this culture method affect patterns of use by nekton through observations made during regular SCUBA and shore surveys of aquaculture areas and reference beaches at three sites (Fisher, Rogers, and Stratford). The data support previous observations that habitat complexity associated with geoduck aquaculture may attract some structure-associated species observed infrequently on reference beaches, while displacing other species that typically occur in areas lacking epibenthic structure. A pronounced seasonal response is evident as well, with most species less abundant in winter. A multivariate approach incorporating Analysis of Similarity (ANOSIM) and nonmetric Multidimensional Scaling (nMDS) was used to reveal shifts in the community composition of fish and macroinvertebrates between aquaculture and reference plots at the three sites. Preliminary analyses of shore survey data have not indicated differences in use of habitats by juvenile salmonids, although these data are limited by low sample sizes. These results have implications for management of current and future aquaculture operations in southern Puget Sound.

NETWORK AND EXPRESSION ANALYSIS OF THE TRANSCRIPTOME OF EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, JUVENILES IN RESPONSE TO THE BACTERIAL PATHOGEN *ROSEOVARIUS CRASSOSTREAE*.

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Eastern oysters farmed in the Northeast US can suffer severe mortality due to *Roseovarius* Oyster Disease (ROD), caused by the bacterium *Roseovarius crassostreae*. We sequenced the transcriptome of oysters in response to challenge with *R. crassostreae*. In a family resistant to ROD, highly upregulated contigs at early time points after challenge included thioester-containing proteins, IMAP family members, and jagged-like proteins, while downregulated contigs included heat shock proteins and ADAM-like metalloproteinases. In a family susceptible to JOD, highly upregulated contigs included the signaling molecule MAPKKK, while highly downregulated contigs included multiple EGF-like proteins. Most genes whose expression varied significantly between the resistant and susceptible families were related to metabolism, peptidases in particular. We identified families of related proteins using gene network analysis. The largest families included tripartite motif-containing proteins, EGF domain-containing proteins, HSP70, and scavenger receptor cysteine-rich (SRCR) proteins. A new tool for visualizing and mining gene network and expression data was developed to help select genes for further characterization, including genes previously characterized as important in immunity in other organisms, like SRCR, and genes minimally characterized in their relation to immunity, like ankyrin-containing proteins. Furthermore, function will be putatively ascribed to some of the ~35,000 novel transcripts in the assembly.

ECOREGION VARIATION IN CARBON CHEMISTRY: IMPLICATIONS FOR SHELLFISH OCEAN ACIDIFICATION EXPERIMENTS.

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For the majority of shellfish ocean acidification species-response experiments, treatments are exposure of animals to seawater equilibrated to global average atmospheric CO_2 concentrations at the current time (~387 ppm) and some projected future scenario (e.g., 750 ppm by 2100). A challenge with interpreting these studies is that few shellfish experience global atmospheric CO_2 concentrations for extended periods of time because pH/pCO_2 varies in time and space. In order to understand the ecological context for interpreting ocean acidification species-response studies, we conducted a global ecoregion-scale evaluation of the observed and modeled mean values, ranges and temporal patterns of local marine carbon chemistry patterns. This analysis revealed substantial variation in carbon chemistry, particularly in costal areas relevant to shellfish. Using information on species spatial distributions (including depth) we compared treatment conditions used in published shellfish ocean acidification experiments to the carbon environment the species would likely experience in the field. The analysis indicates some miss-match between experiments and conditions in the field. It is important to consider spatial and temporal variation in marine carbon chemistry for the design and interpretation of ocean acidification experiments.

GROWTH AND GAMETOGENESIS OF THE INVASIVE GREEN MUSSEL, *PERNA VIRIDIS*, IN ESTERO BAY, FLORIDA.

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Growth and gametogenesis are important factors to aid in predicting the spread of invasive bivalves such as *Perna viridis*, a recent marine invader to the coastal regions of southwest Florida. Previous studies have suggested that green mussels may impact native oyster reefs. In an attempt to understand the influence of seasonal and environmental factors in SW Florida, growth, reproductive and immune parameters were measured in green mussels from Estero Bay, SW Florida. Individual green mussels were tagged and monthly growth measurements were obtained. Reproductive activity was assessed using standard histological techniques while immune parameters (hemocyte viability, production of reactive oxygen species, mitochondrial membrane potential, lysosome activity, and phagocytosis) were assessed using flowcytometry. Thus far *P. viridis* has exhibited high growth rates (6 – 10 mm/month) that are comparable to those from their native range (Indo-Pacific). In addition, green mussels also have an extended spawning period in Estero Bay compared to those reported from Tampa Bay, Florida. Given the warmer waters prevailing in SW Florida, it is anticipated that while growth rate may decrease during winter, reproductive activity will continue with little lag (1–2 months). Seasonal variation in growth, reproduction and immune parameters will be discussed in relation to water quality parameters.

STUDYING ABALONE REPRODUCTIVE BIOLOGY TO SUPPORT A CAPTIVE BREEDING PROGRAM OF THE ENDANGERED WHITE ABALONE, *HALIOTIS SORENSENI*.

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In 2001, the white abalone, *Haliotis sorenseni*, became the first marine invertebrate to be federally designated as an endangered species. As part of the recovery and restoration plan for the species, there is an effort underway at the UC Davis Bodega Marine Laboratory to successfully breed the white abalone in captivity. There is limited information about white abalone biology and reproduction since the species was rapidly overfished and currently exists mainly in deep-water refugia. In order to refine and maximize efficiency of future captive breeding efforts, we are developing endocrine biomarkers of reproductive condition using the red abalone, *H. rufescens*, as a proxy for the endangered white abalone. Our research is focusing on using non-lethal sampling throughout the reproductive cycle to detect the presence and titers of peptide and steroid hormones in hemolymph and other tissues. In addition, we are attempting to condition adult white abalone for a possible spring spawn using information about environmental conditions in their historical, native geographic and depth ranges as well as methods used in other threatened abalone species.

WHAT'S NEW WITH OLYMPIA OYSTERS? AN INTRODUCTION TO THE SESSION.

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During the last decade there has been increased interest in the restoration of, and research on, the Olympia oyster, *Ostrea lurida* (Carpenter 1864), the only native oyster on the west coast of the US and Canada. From California to British Columbia, Canada, restoration practitioners, shellfish growers, and scientists have been working together to better understand the biological and physical requirements of the Olympia oyster in order to propagate and restore it. Continued research and monitoring are important in this effort and are providing insights into, e.g., better restoration techniques, anthropogenic threats to native oyster populations, and ecosystem services provided by Olympia oyster beds. Restoration of the Olympia oyster has also been highlighted in the recently released Washington State Shellfish initiative, a joint commitment between Washington State and the National Oceanic and Atmospheric Administration's (NOAA) National Shellfish Initiative. This session includes results of a wide range of restoration and research topics on Olympia oysters on the US west coast and British Columbia, as well as some perspectives on an emerging public awareness of the role of oysters and other shellfish in marine ecosystems.

A MONITORING AND EARLY DETECTION SYSTEM FOR ZEBRA MUSSEL INVASION OF NORTHEASTERN TEXAS WATER BODIES.

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Zebra mussels (*Dreissena polymorpha*) (ZM) infest Lake Texoma (LT) on the Texas/Oklahoma border. Boater traffic/water transfers from LT are potential vectors for ZM spread to other Texas lakes. A ZM detection system was tested consisting of plankton tows for veliger larvae during spawning periods examined both in the field and laboratory along with deployment at 1 m depth of a nylon scouring pad mussel settlement monitor and temperature data logger. The monitoring system was applied to 14 northeastern Texas lakes including LT during the early summer and fall of 2011. Veligers occurred only in LT from early summer to late fall, with settlement only during early summer. Settlement-competent pediveligers were present in plankton tows only when settlement occurred suggesting that high water temperatures may have prevented veligers from developing to the settlement stage during the late summer and fall. Average summer surface water

temperatures ≥32°C and/or calcium concentrations ≤12 mg L^{-1} indicated that four of the 14 lakes were unlikely to support ZM while pH and dissolved O_2 were suitable at all lakes. Thus, risk assessments based on water temperature and calcium concentration could allow management actions to be focused on Texas lakes most at risk of ZM invasion.

EFFECTS OF GEODUCK AQUACULTURE ON THE GROWTH AND STABLE ISOTOPE SIGNATURES OF PACIFIC STAGHORN SCULPIN.

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Aquaculture operations are a frequent and prominent cause of anthropogenic disturbance to marine and estuarine communities. In Puget Sound, Washington aquaculture of the Pacific geoduck clam (*Panopea generosa*) is on the rise, however little is currently known about impacts of the industry on ecological communities. Our study took place during the initial phase of geoduck aquaculture that utilizes nets and PVC tubes to protect immature geoducks from predators. We examined the site fidelity, growth and stable isotope signatures of a local ubiquitous predator, Pacific staghorn sculpin (*Leptocottus armatus*) at geoduck aquaculture sites and nearby reference areas. A pilot mark-recapture study indicated that staghorn sculpin show fidelity to their site of initial capture and grow at different rates in cultured and reference sites. Prior research on sculpin diet showed that types of prey consumed differed by site type. Preliminary results from carbon and nitrogen stable isotopes suggested that sculpin consumption was chemically similar at cultured and reference areas. Future research efforts will focus on elucidating such predator-prey relationships at geoduck aquaculture sites.

IMPACT OF OCEAN ACIDIFICATION ON SPECIES ADAPTATION AND ABUNDANCE ACROSS 11° OF LATITUDE.

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Upwelled coastal waters in the California Current system are naturally acidified, and already experience low pH levels not expected in the open ocean for another 50-100 years. An NSF-supported team of researchers (Ocean Margin Ecosystem Group for Acidification Studies or OMEGAS) spread over 7 institutions and spanning 1600 km of coast is conducting oceanographic, ecological, physiological, and genetic studies to track responses of sea urchins and mussels to spatial and temporal variation in ocean acidification (OA). We ask: How does OA vary along the coast? Do growth and survival of key species respond to changing carbonate chemistry? How do larvae respond to present pH regimes and how will they respond to future changes? Can species adapt and evolve as OA, and consequent reduced abilities to calcify, increases with rising CO_2 emissions? How do abundances of calcifying species vary along an upwelling gradient? The first-ever time series of pH and pCO_2 at 7 sites from the central Oregon coast to Santa Barbara show that, as predicted, OA varies (1) latitudinally, (2) between sites within region, and (3) within sites on hourly to daily to weekly time scales. Drivers of this variation include large-and regional-scale upwelling-driven currents, and regional- to local-scale biological processes. Laboratory mesocosm studies have found that sea urchin larvae are smaller, and grow less skeletal material at future-scenario pH levels compared to present levels; these effects were most pronounced in southern urchins. Mussel larvae were even more strongly affected by future-scenario conditions, growing thinner, smaller, and more fragile shells. The first genome-wide survey of evolutionary response to acidification shows that hundreds of sea urchin genes shift allele frequencies when cultured under elevated pCO_2 and that geographic patterns in important physiological genes seem to be directed by these selective changes. Analyses of community surveys indicate that contrary to expectation from carbonate chemistry, high magnesium-calcite (coralline algae, echinoderms, crabs) and aragonite (chitons, abalone, scallops, some whelks) calcifiers are more abundant toward the southern CCLME, where upwelling is stronger, temperatures are lower and planktonic food (phytoplankton) is lower. Consistent with expectations, low magnesium calcifiers (mussels, barnacles, limpets, snails) show opposite patterns, with higher abundances under less intense upwelling conditions. Thus, as expected, OA in this highly dynamic and productive upwelling ecosystem varies naturally in concert with periodic intrusions of deep, high CO_2 water, with the intensity of how these intrusions vary alongshore, and with variation in algal photosynthesis and respiration. Although larvae of key calcifiers show some resistance to this variability at present, they do poorly under future-scenario CO_2 levels. Adult abundance patterns are more difficult to interpret, because (1) current conditions may not be severe enough to influence abundances, and (2) other factors are likely to interact with OA, possibly enhancing or negating the expected effects of high CO_2 on calcification.

EXTENSION, OUTREACH AND PUBLIC RELATIONS: LESSONS LEARNED FROM TALKING OYSTERS.

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Extension professionals often create programs that address identified community issues. These frequently involve controversial subjects that arise between interest groups. Distrust, poorly founded opinions and disregard of science impedes progress and perpetuates conflict resulting in lack of progress. For program success, common ground must be found between groups. The

Maryland oyster industry provides an example of how a resource can generate conflict as struggle between groups continued over decades to determine whether public harvest or private cultivation would prevail as the principal production method. Oyster harvesters, scientists, environmental groups, politicians, and management agencies all contributed to the problem throughout this period as disease became the principal cause of the nine-five percent (95%) reduction in harvest. The University of Maryland's Oyster Hatchery at the Horn Point Laboratory has been incorporated into an oyster restoration program to re-habilitate shellfish populations in Chesapeake Bay for both ecological and economic value. Extension programs developed to include multiple user groups, which led to changes in attitude by those working together to solve common problems. This helped minimize conflict when aquaculture laws were recently changed and led to many former commercial harvesters participating in programs designed to provide them with the skills to succeed as growers.

IMPACTS OF ELEVATED pCO_2 CONDITIONS ON THE *RUDITAPES PHILIPPINARUM* LARVAL TRANSCRIPTOME.

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Changing ocean conditions as a result of anthropogenic carbon dioxide emissions is a concern for the health of shellfish populations. Evaluating the molecular process altered by changes in dissolved carbon dioxide levels can provide insight in to the mechanisms affected by ocean acidification and potentially reveal processes vital for adaptation and survival. A major hurdle in evaluating these processes is limited genomic resource for shellfish, however developments in sequencing technologies and analyses are facilitating such studies. In this study, the transcriptome of the commercially important bivalve species *Ruditapes philippinarum*, is characterized in two different pCO_2 environments. Data from this study provides valuable information about the molecular processes in shellfish larvae that are affected by ocean acidification, as well as providing a foundation for future transcriptomic analysis of shellfish larvae.

GENOTYPING AND EXPRESSION PROFILING ON A BUDGET IN NON-MODEL SPECIES.

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High-throughput sequencing (HTS) technologies are now widely used to profile genetic variation and gene expression in model systems. To illustrate the general suitability of these methods for non-model organisms like shellfish, we describe pilot studies evaluating the feasibility and costs of HTS for genotyping and gene expression analysis in *Crassostrea gigas*. Genetic variation was profiled by sequencing short DNA tags associated with type IIb restriction sites (2b-RAD). Both parents and pooled offspring from a cross were genotyped, allowing us to measure changes in allele frequencies in larvae challenged with a pathogen (*Vibrio tubiashii*). Gene expression was profiled in other *C. gigas* families resistant or susceptible to the pathogen using a tag-based RNA-Seq approach. Comparison with existing EST resources for this species made it possible to profile a large fraction of the transcriptome and analyze differential expression. These constitutive differences in gene expression between susceptible and resistant families highlight candidate genes for variation in pathogen resistance. The low-budget strategies described here will make it possible to profile gene expression and genetic variation at the high throughput needed for well replicated experiments in *C. gigas* and other shellfish species.

100 DAYS IN HOT WATER, TALES FROM A SIXTH INSTAR DUNGENESS CRAB (*CANCER MAGISTER*), OCEAN ACIDIFICATION IMPACTS ON EARLY JUVENILE STAGES.

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Dungeness crab (*Cancer magister*) is a commercially, culturally and ecologically important marine organism inhabiting coastal and estuarine waters of the Pacific Northwest. This study explores the potential impacts of ocean acidification on early life stages for this species. Dungeness crab megalopae collected from Skagit Bay, North Puget Sound, WA were exposed to either control (400 micro atmospheres CO_2; n = 84) or elevated CO_2 (1000 micro atmospheres; n = 83) conditions. Survival, mortality, time to molt, carapace width and length (from molted carapaces), and physical characteristics were recorded for 110 days when all surviving crabs had molted to 4th, 5th and 6th instar. Water quality (spectrophotometric pH, TA and DIC) was measured weekly to bi-weekly. By day 106, 27 crabs had survived under control conditions, while 21 crabs survived the high CO_2 treatment. Studies to assess changes in crab behavior under acidified conditions are in progress. Surviving crabs will take part in a behavioral y-maze pilot study exploring any preference to high or low pH levels as well as ability to detect food.

NEARSHORE ACIDIFICATION: WHY COASTAL OCEAN ECOSYSTEMS ARE DIFFERENT.

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Because of their relative shallowness and often reduced salinity and typically reduced alkalinity, coastal marine habitats and estuaries are inherently less buffered to changes in pH than is the

open ocean, making them prone to CO_2-induced changes in pH. Despite their natural variability in pH and pCO_2, an overall increase in atmospheric CO_2 will likely create a shifting baseline for this environmental variability, much more complex than is expected in the open ocean. How individuals, populations, and ecological communities will respond to such changes will be more complicated than current ocean acidification models predict. Relying on the prevailing air/sea equilibrium assumptions of ocean acidification will surely result in unsound ecological prediction in coastal habitats.

STAKEHOLDER-DRIVEN ONE HEALTH RESEARCH.

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A 'One Health' approach to problem solving and disease investigations includes consideration of how human, animal, and environmental factors interact. One Health efforts are usually transdisciplinary and may involve scientists, stakeholders, and students working together on issues of common interest. An example of 'One Health' research in California is a previous Sea Grant project where shellfish growers, dairy farmers, and university researchers came together to investigate fecal pathogen pollution and water quality on a watershed scale, and showed that selected Best Management Practices (BMPs) helped to reduce the transport of fecal pathogens in storm runoff and thereby improved water quality downstream where shellfish are grown. The results suggested that BMPs such as vegetated buffer strips and straw mulch application, especially when placed near calf areas, will reduce environmental loading of fecal pathogens and improve stormwater quality. In contrast to other areas along the California coast, the fecal pathogen *Cryptosporidium* was not detected in water or shellfish in the receiving waters of the study watershed. The study findings are assisting working dairies in their efforts to improve farm and ecosystem health along the California coast.

EARLY DEVELOPMENTAL EVENTS IN ZEBRA AND QUAGGA MUSSELS AND POSSIBLE TARGETS FOR CONTROL.

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Dreissenid mussels (*D. polymorpha and D. bugensis*) are unusual among North American freshwater bivalves in their reproductive strategy. More similar to marine mussels, dreissenid mussels broadcast spawn their gametes with fertilization and larval development occurring in the water column. This planktonic development is one factor leading to their success as an invasive species. Here we present a comparison of fertilization and early development between the two North American invasive dreissenids, the zebra and quagga mussels. There are conserved mechanisms between these species including serotonin induced spawning, general egg morphology, and sperm incorporation events. However, there are also marked differences including gamete morphology, timing of events, and biochemical characteristics. Our understanding of the basic mechanisms of early development can help identify lifecycle stages that may be ideal for potential control mechanisms. We will discuss the use and effects of ultraviolet radiation and gamma radiation on both adults and gametes with regards to early developmental stages of dreissenid reproduction.

RECRUITMENT, GROWTH, MATURATION, AND HEALTH OF OLYMPIA OYSTERS *OSTREA LURIDA* NATURALLY SETTLED ON CULTCH DEPLOYED IN SAN FRANCISCO BAY, CALIFORNIA, USA.

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A series of mounds of *Crassostrea gigas* cultch in bags was deployed in San Francisco Bay in June 2007 to attract native Olympia oysters. We sampled the cultch approximately monthly for one year, recording oyster densities and size. Sixty of the largest individuals in each sample were examined histologically to determine reproductive status and to record the presence of symbionts. Oyster density increased through February 2008 with a peak population estimate of 549,000 (mean 17 spat per shell) before gradual decline. At day 56, 12% of the sampled oysters had male gonad and 4% were hermaphrodites that were predominantly male. By day 92, all oysters showed reproductive development with 44 % being hermaphrodite/predominantly male, 24 % male, 23 % hermaphrodite/predominantly female and 11% hermaphrodites with equal proportions of male and female gonad. Among all reproductive oysters in the study, 80 % were hermaphrodites while 18 % were male and only 2 % were female. Apparently-mature sperm balls were observed in male gonad in day 56 oysters and the first fully mature female gonad tissue was seen at day 92. The oysters appeared to be minimally affected by infectious disease, harboring low numbers of commonly described symbionts.

COMPARISON OF THE HEALTH STATUS OF THE COMMON COCKLE *CERASTODERMA EDULE* AT TWO SITES IN SOUTHERN IRELAND.

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Significant mortalities of the cockle *Cerastoderma edule* have been reported at a number of European sites over the past few years with complex and varied causes. In Ireland surfacing and subsequent mortality events are observed particularly over the summer months leading to an investigation of cockle health at two sites on the Irish Sea, southern Ireland. Over a 16 month period, March 2010–June 2011, 30 surfaced and 30 buried cockles were taken monthly from Flaxfort Strand, Co. Cork and Bannow Bay in Co. Wexford. Length, weight, age and sex were determined; tissues sections were screened for the prevalence of parasites or any pathogens. Cockles were larger in Bannow Bay, although the difference in mean age between sites was negligible. At both sites the prevalence of neoplasia was greater in surfaced rather than buried cockles; the advanced stages of the disease were more common in surfaced cockles. Trematode sporocysts and metacercriae showed seasonality, but peaks in their prevalences differed between sites. Prevalence of trematode metacercariae was higher at both sites in moribund cockles, being more pronounced at Bannow Bay. A range of other pathogens were identified including gregarines and copepods however unidentified haplosporidians were seen exclusively in Bannow Bay.

HOW DO MARINE PROTECTED AREA STRATEGIES INFLUENCE METAPOPULATION GENETIC CONNECTIVITY? A MODELING STUDY WITH OYSTERS.

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Effective management of marine resources like oysters relies on understanding how populations are connected. Application of a modeling framework including distinct populations connected via larval transport is effective for examining complex metapopulation dynamics and how anthropogenic activities influence them. Here, our goal was to assess how various marine protected area (MPA) strategies alter genetic connectivity. An individual-based metapopulation model integrating population dynamics, dispersal, and genetics was used to examine mechanisms and dynamics of metapopulation genetic connectivity. The model was parameterized to simulate four eastern oyster (*Crassostrea virginica*) populations from Delaware Bay for two periods (1970's and 2000's). In our simulations, no fishing was allowed within MPA populations. Simulations included all possible combinations of MPA location (which population was protected) and fishing mortality rates for non-MPA populations (this included low (4%), medium (8%) and high (30%)). Results showed (i) MPAs can enhance genotypes originating within the protected area when surrounding fishing rates are relatively high (30%), and (ii) a strong temporal difference in the influence of MPA strategies on metapopulation genetic connectivity between the two time periods (1970 vs. 2000). Generally, these results suggest that MPA location, exploitation rates and regimes play a role in metapopulation genetic connectivity.

PREDICTING THE INFLUENCE OF SEED AND COMMERCIAL OYSTER FISHERIES ON METAPOPULATION GENETIC CONNECTIVITY USING MODEL SIMULATIONS.

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Previous research has demonstrated that changes in population demographics (mortality and abundance) can alter genetic connectivity among populations in an oyster metapopulation. Through increases in mortality rates and creation of mortality gradients in space, fisheries could potentially influence metapopulation genetic connectivity and source-sink dynamics. Our goal was to assess how various oyster seed and commercial fisheries alter metapopulation genetic connectivity. An individual-based metapopulation model integrating population dynamics, dispersal, and genetics was used to examine mechanisms and dynamics of metapopulation genetic connectivity. The model was parameterized to simulate four eastern oyster (*Crassostrea virginica*) populations from Delaware Bay for two periods (1970's and 2000's). Simulations included a range of fishing and seed fishing scenarios using rates currently observed in commercial oyster fisheries from Chesapeake and Delaware Bays and connectivity was tracked using population-specific neutral alleles. Results show (i) high rate (30%) seed fisheries can lead to loss of neutral alleles from exploited areas, (ii) relatively low rate fisheries (4%) affect minimal change in genetic connectivity, and (iii) a strong temporal difference between the two periods (1970 vs. 2000). These results suggest that fishery management, including size restrictions, and exploitation rate, can influence metapopulation genetic connectivity.

THE CAPE COD RESEARCH FARM NETWORK: A TOOL FOR APPLIED RESEARCH AND EXTENSION.

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This program, initiated in 2005, seeks to improve the production of regional shellfish aquaculture by conducting applied research into issues relevant to regional shellfish farmers, specifically, through the establishment of a network of collaborating commercial shellfish farms across southeastern Massachusetts. At each site, identical research questions have been selected to provide both regional and site-specific information on questions of importance to the industry (e.g., differences in seed from different hatcheries, test of diploid vs. triploid oyster seed, differences in gear, etc.). Identical shellfish, gear, and methods are supplied to participants and shellfish are grown under the permit of the license holder who acts as site foreman, following the proscribed methods, keeping accurate written records, and allows access to research personnel. Through the application of these field-based studies members of the aquaculture industry actively participate in the scientific process of gathering data to support or refute anecdotal evidence or data to scientifically describe their observations. These experiments are replicated at all sites to ensure relevant and significant comparisons between sites and across the region. A summary of results to date will be presented, and the value of this approach as an extension tool will be discussed.

QUANTIFICATION OF INFLAMMATION IN THE MANILA CLAM *RUDITAPES PHILIPPINARUM* BY ASSAYING FOR CYCLOOXYGENASE ACTIVITY ASSAY (COX).

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The Manila clam *Ruditapes philippinarum* is a commercially important marine bivalve species that is currently undergoing mass mortality in Korea. One of the agents responsible for this mass mortality is the protozoan parasite *Perkinsus olseni*. Many studies have shown that this pathogen elicits a severe inflammatory response in the Manila clam. This observation suggests that inflammation in the Manila clam is an important sign of infection by this parasite. The present study was designed to develop a technique for the quantification of inflammation in the Manila clam by assaying for cyclooxygenase (COX) activity. For the experiment, hemocytes of the clam were incubated with oxytetracycline dihydrate (a proinflammatory reagent) or diclofenac (a nonsteroidal anti-inflammatory drug [NSAID]) for 18 hours. The hemocytes treated with oxytetracycline showed a marked increase in COX activity, while those treated with diclofenac showed a significant decrease in COX activity. Our study suggests that the inflammatory response in the Manila clam is regulated by inflammatory reagents or NSAIDs, and this response can be measured by assaying for COX activity. Therefore, a COX assay can be used to quantify the inflammatory response to *P. olseni* infection in the Manila clam.

ADENYLYL CYCLASE INHIBITORS REVERSE THE NEUROTOXIC EFFECTS OF MANGANESE ON POST-SYNAPTIC DOPAMINE D2 RECEPTORS.

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Manganese, a neurotoxin causing Manganism, a Parkinsons-like disease, disrupts dopaminergic neurotransmission, but the mechanism is not fully understood. Gill lateral cell cilia of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations. Dopamine causes cilio-inhibition, serotonin cilio-excitation. The post-synaptic dopamine receptors in the cells are D2 type G protein-coupled metabotrophic receptors. We showed manganese blocks the cilio-inhibitory effects of dopamine by blocking dopamine post-synaptic receptors. Here we observed membrane potentials of lateral cells of *C. virginica* with a voltage sensitive fluorescent dye while measuring cilia beating rates. Serotonin caused membrane depolarization and increased beating, dopamine repolarized the membrane and decreased beating. Manganese prevented the cilio-inhibition and repolarization. ATP and forskolin, an adenylyl cyclase activator, increased beating without changing membrane potentials in control or manganese treated gill. MDL and SQ, adenylyl cyclase inhibitors, decreased beating without affecting membrane potential in controls or manganese treated gill. The study shows correlation between membrane potential and cilia beating rates, that the actions initiated by activation of D2 post-synaptic receptors can be differentiated into effects on adenylyl cyclase and membrane channel conductance, and the neurotoxic effects of manganese can be overcome by application of adenylyl cyclase inhibitors. It helps elucidate the neurotoxic mechanism of action of manganese.

SHELL-GIS – A NEW GIS TOOL FOR OYSTER FARM SITE SELECTION, OYSTER GROWTH SIMULATION AND PRODUCTION CARRYING CAPACITY.

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NRAC funding of the project "Assessment of Environmental Impacts of Oyster Aquaculture in New England Waters" was used to develop a shellfish GIS software which was successful in incorporating the spatial and temporal presentation of site hydrodynamics (on a 50 m grid scale), environmental forcing functions including temperature, salinity and food availability, and growth of the eastern oyster, *Crassostrea virginica* in bottom culture using ShellSIM at a test site in Maine in 2010 and 2011, and validated in Connecticut in 2011. Shell-GIS provides a convenient tool for bay scale oyster aquaculture management, and marine spatial planning.

THE EFFECTS OF HYDRODYNAMICS ON THE FOOD SUPPLY AND DEMAND OF MUSSEL RAFTS.

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The objectives of this study were to characterize the hydrodynamic conditions within and around mussel rafts, and relate them to the rates of filtration or Chl-*a* consumption by the mussels within those rafts. The raft hydrodynamics, which are a function of site specific ambient conditions (i.e. tidal current velocities, directions and wave climate), may also be characterized with respect to optimum flow conditions related to feeding (i.e. 2-8 cm s^{-1}), and sufficient particle flux such that depletion of Chl-*a* in the center if the raft is minimized. While physical oceanographers have used finite difference and finite element models to simulate large scale currents in the ocean and coastal zone, the application to individual aquaculture structures such as mussel rafts have been limited. In this paper, we use FLOW-3D to understand detailed mussel raft hydrodynamics at Maine sites and the effects of various mussel raft parameters on the food supply and demand of these marine bivalve suspension culture systems.

NANOOS CONTRIBUTIONS TO UNDERSTANDING OCEAN ACIDIFICATION.

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The Northwest Association of Networked Ocean Observing Systems (NANOOS) is contributing to our emerging understanding of ocean acidification in the Pacific Northwest. Primarily studied in oceanic waters, little is known regarding its status in estuaries. Anthropogenically acidified coastal waters upwelling along the western North American continental margin can enter Puget Sound through the Strait of Juan de Fuca. Our results showed deep marine waters in Puget Sound are undersaturated with respect to the biomineral aragonite (Feely et al., 2010). Ocean acidification accounted for 24–49% of the pH decrease compared to estimated pre-industrial values. The remaining change in pH results from remineralization of organic matter due to natural or anthropogenically stimulated respiration processes within Puget Sound. To further observe ocean acidification status, autonomous buoys, part of NANOOS, have been outfitted with sensors for pCO_2 and pH. The scale of variation in pCO_2 in the atmosphere and surface waters is different but both records reflect dynamic processes. The pattern of variation in seawater appears to correlate with processes such as mixing and primary production that can vary on short timescales. Assembling a timeseries is leading to a better understanding of range of variation and the mechanisms involved with ocean acidification locally.

IDENTIFICATION OF POTENTIAL MARKERS OF DISEASE IN EASTERN OYSTERS THROUGH ANALYSIS OF GENE EXPRESSION PATTERNS DURING DISEASE CHALLENGE EXPERIMENTS.

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Many diseases can severely impact oyster populations in the US. Markers indicative of physiological stress and disease could provide a general idea on the health status of an oyster population

without having to diagnose for individual diseases. Candidate novel (arginine kinase, matrix metalloproteinase 1, a Toll like receptor, IkB, NFkB) and previously annotated genes (serine protease inhibitor 1, heat shock protein 70, histone H4, lysozyme 1, and defensin) were identified through screening of transcriptomic data of resistant and susceptible oysters exposed to the bacterial pathogen *Roseovarius crassostreae*. Temporal patterns of expression of these candidate genes in response to challenge with the protozoan parasite *Perkinsus marinus* were evaluated using quantitative real time PCR and related to temporal patterns of variation in levels of *P. marinus*. Oysters from the two genetic lines show different patterns of gene expression in response to challenge with *P. marinus*. Our results confirm previous research on the role of serine protease inhibitor-1 in immunity in oysters and strengthen the evidence that SP1 could be a potential marker of disease resistance. Elucidation of the molecular mechanisms controlling these differences in expression could led to the identification of genetic markers for disease resistance or disease status in *C. virginica*.

PRELIMINARY OBSERVATIONS OF GONAD STRUCTURE AND GAMETOGENIC TIMING IN A RECOVERING POPULATION OF *OSTREA LURIDA*.

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In the Pacific Northwest, Olympia oyster, *Ostrea lurida*, stocks were dramatically reduced by overharvesting and habitat loss. Restoration efforts of the native oyster require information on the timing of gametogenesis and spawning. Using routine histological methods on monthly samples from Coos Bay, we confirmed early work describing the *O. lurida* gonad. Gonadal structure of *O. lurida* is similar to that of other *Ostrea* species; Gonads extend through the connective tissue between the digestive gland and epithelium. The genital sinuses become engorged with gametes during breeding then shrink considerably after periods of spawning. *Ostrea lurida* are protandric, sequential hermaphrodites, alternating release of male and female gametes throughout their life cycle. Follicles containing partially spawned, mature gametes are frequently found in a state of transition alongside reproductive cells of the opposite sex in various stages of proliferation. Gonad samples collected from July–August 2010 and September–October 2011 revealed a reproductive peak in early to mid August, followed by a reduction in follicle volume and gamete density in September and October. Accounting for estimated brood time and planktonic larval duration, these results (suggesting spawning in early August), are in agreement with previous Coos Bay research suggesting a peak in settlement around mid-October.

SAN FRANCISCO BAY NATIVE OYSTER RESTORATION: LESSONS LEARNED AND NEXT STEPS.

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San Francisco Bay subtidal areas are a challenging habitat for restoration. Small-scale native Olympia oyster restoration efforts have been on-going in San Francisco Bay for nearly a decade. Several habitat enhancement techniques have been tested on a pilot scale, mainly Pacific oyster shell cultch and Reefballs®. Using the success criteria of increased oyster population, increased species richness of both fish and invertebrates, increased utilization by commercial and T&E fish species, the reefs are largely successful. The success of the reefs goes beyond a single species but encompasses ecosystem-wide benefits and the creation of EFH. The restoration techniques are successful, but not self-sustainable. The main problems are scour and sedimentation that reduce the functional life of a created reef between 3–10 years and requires maintenance. In spite of the physical problems, the biological successes are reason enough to justify expanding the restoration effort. Plans to increase the size of Bay reefs in conjunction with eelgrass planting to determine if there are synergistic effects are moving ahead through a program being lead by the California Coastal Conservancy. These larger reef structures may and help alleviate the problems associated with global warming and sea level rise by wave attenuation, reducing shoreline erosion.

A CURRENT UNDERSTANDING OF CORRELATIONS BETWEEN FIB AND PATHOGENS IN COASTAL WATER.

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Fecal indicator bacteria (FIB) such as total coliform, fecal coliform, *Escherichia coli* and members of the genus *Enterococcus* (the enterococci) have been widely used to assess fecal contamination and microbial quality in drinking water, recreational water and shellfish farming areas. The underlying assumptions are that (1) the presence of pathogens correlates well with the presence of FIB, (2) FIB are present mostly in human and warm-blooded animals' intestines and cannot survive for a long time outside their hosts, and (3) FIB are inactivated at rates similar to those of pathogens in natural environments and engineered treatment systems. In addition, FIB measurements are relatively easy, and hence FIB are suitable for monitoring on a regular basis. Many studies, however, have pointed out violations of these assumptions and have raised questions regarding the use of FIB in estimating public health risks. Furthermore, a statistical analysis of literatures published in these 40 years suggested that there is no single

microbial indicator identified as the most correlated with pathogens (Wu, et al., 2011). In this presentation, a current understanding of correlations between FIB and pathogens will be reviewed to elucidate the limitations of FIB with the emphasis on coastal water managements.

DELAWARE VOLUNTEER OYSTER GARDENERS: STEWARDS OF THE BAY!

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Delaware is the only coastal state along the eastern seaboard which has no commercial aquaculture. However in 2003, Delaware's Volunteer Oyster Gardening Program was created to help and promote oyster restoration within the Delaware Inland Bays (DIB). Today, these volunteers do much more than wash and care for oysters in floating aquaculture gear. These waterfront homeowners are allowed the opportunity to help collect useable data to help determine ideal oyster growing locations. For the past several years, oyster growth and survival data has been collected by some of our volunteers. Digital calipers, datasheets and instructions were distributed at training meetings. Some volunteers assist by collecting physical water quality data through the use of YSI 556 Multiprobes, which generates many more data points that our research team can collect. Several oyster gardeners are also members of The Inland Bays Citizen Monitoring Program, which helps monitor water quality throughout the DIB. Additionally, some volunteers allowed use of their property as research bases, allowing our research team use of their kayaks, water supply, room for storing field equipment, etc. Without many of these generous volunteers and their contribution for the oyster restoration efforts, logistical operation at these field sites would be near impossible.

RECENT DISCOVERIES OF RARE FRESHWATER MUSSELS IN THE URBAN CORRIDOR OF THE DELAWARE ESTUARY.

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Freshwater mussels are the most imperiled animals in the Delaware River watershed and across North America. They naturally form dense beds in streams and rivers, and declines in mussel populations contribute to degraded water and habitat quality. Up until 2009, we believed many of the 12+ native mussel species were lost from the area. During 2009–2011, extant populations of at least seven species were discovered in the tidal freshwater portion of the upper Delaware Estuary: pond mussel, *Ligumia nasuta;* tidewater mucket, *Leptodea ochracea; a*lewife floater, *Anodonta implicata;* creeper, *Strophitus undulatus;* eastern floater, *Pyganodon cataracta; y*ellow lampmussel, *Lampsilis cariosa*, and the elliptio, *Elliptio complanata*. At least four are critically imperiled and two were classified as extirpated. The mussels formed robust, mixed species beds in shallow subtidal areas where they might help sustain water quality. Their survival in tidal freshwater areas likely results from a lack of dams interfering with passage of fish hosts, essential for mussel reproduction. Since they live in the urban corridor, their protection is paramount for sustaining future mussel restoration efforts in support of broad water quality, habitat and living resource goals across the watersheds of the Delaware Estuary and possibly also Chesapeake Bay.

VARIATION IN THE IMPACTS OF THE HARMFUL BROWN TIDE ALGA ON THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*.

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Blooms of *Aureococcus anophagefferens* have been suggested to prevent the recovery of *Mercenaria mercenaria* populations. In years with brown tide blooms, peak spawning condition in Great South Bay (GSB), Long Island, was 10–15% lower than expected based on fall and spring condition. In Shinnecock Bay (SB), however, no deleterious effects of brown tides were observed, despite more frequent and severe brown tides than in GSB. Brown tides have reoccurred annually since 2007, yet clams have reconditioned in spring and fall, and have maintained high condition and gonad ripeness. We propose three hypotheses for differences in clam performance between GSB and SB in the presence of brown tides: 1) The toxicity of brown tide differs between the two bays. Laboratory cultures of *Aureococcus anophagefferens* can lose toxicity; cell toxicity may also vary in nature. 2) Other environmental and/or physiological factors mediate the effects of brown tide, and these factors differ between the two bays. 3) Brown tide had no effect on clams in either bay, and the low condition of clams in GSB was due to other factors. Other factors may play a more important role in regulating clam condition, and clams can fail to recondition in years without brown tides.

MORPHOLOGICAL CONSTRAINTS, LARVAL DIET AND POST-METAMORPHIC SURVIVORSHIP AND FEEDING IN THE ATLANTIC SLIPPERSNAIL, *CREPIDULA FORNICATA*.

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The adaptability of individuals in the face of environmental change is of fundamental important for assessing the resilience of populations and the robustness of performance. Limits on early life stages can have lasting impacts on later life stages of organisms. Throughout ontogeny organisms can experience significant changes in their biotic and abiotic environments, and can respond through changes in morphology, physiological responses and behavior, or through phenotypic plasticity. All organisms undergo changes in size during ontogeny, but performance of morphologies, physiologies, and behavior may not scale simply with size. Morphological and physiological systems often have size-dependent functions, i.e., all features of organisms cannot be expected to function similarly as individuals change size through ontogeny, which creates challenges for organisms with respect to metabolism and food acquisition among other functions. Suspension-feeding molluscs are ecologically important, provide important ecosystem functions and are important fisheries and aquiculture species. Their ability to suspension feed effectively, especially at a small size has been questioned due to differences between metabolic demands and energy acquisition. Performance of small individuals, especially those just past metamorphosis, thus may pose an important bottleneck for molluscan suspension feeders.

ISOLATION AND APPLICATION OF SUCCINYL THIOKINASE.

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In our earlier studies, the succinate concentration in clam body fluid was used as an anoxia-stress indicator. In order to assess the vitality of hard clams, developing an enzymatic-coupling reaction to determine the succinate concentration in their body fluid is required. Succinyl thiokinase (STK) is the key enzyme in the enzymatic-coupling reaction and is commercially unavailable. Therefore, the aim of this study is to isolate STK from pig heart. STK from pig heart was purified over 850-fold to apparent homogeneity. It has a dimeric structure with a relative molecular mass of 69,200, and there are two types of subunits, a and b, with respective apparent molecular weights of 38,000 and 47,000. Among the isolation steps, the purification factor (14.6) of a CHT ceramic hydroxyapatite column was the highest. STK is heat-labile, and the addition of 2.4 M ammonium sulfate made it more heat-stable than that in 20% glycerol at 60°C. No STK activity was found in the absence of $MgCl_2$. Fifty percent of the activity of STK was inhibited by 8 mM $CaCl_2$ in the presence of 20 mM $MgCl_2$. The stability of STK stored in glycerol was greater than that stored in ammonium sulfate at 4 °C.

AFLP TECHNIQUE WAS USED TO ANALYZE THE GENETIC DIVERSITY AND DIFFERENTIATION OF FIVE NATURAL POPULATIONS OF *HEMIFUSUS TUBA* DISTRIBUTED ALONG THE CHINESE COAST IN ORDER TO UNDERSTAND GENETIC VARIATION.

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Hemifusus tuba in China coastal waters has been facing considerable fishing pressure, and stocks of this species have been exhausted in recent years. To understand genetic variation in its populations, five natural populations of *H. tuba* distributed along the Chinese coast were collected. Amplified Fragment Length Polymorphism (AFLP) technique was used to analyze the genetic diversity and differentiation of these populations. A total of 310 loci were generated from 150 individuals using two primer combinations, of which 277 were polymorphic. The five populations had the same high level of genetic diversity. The expected heterozygosity *ranged* from 0.3824 to 0.4766. Most of the genetic variation was found among individuals within populations. F_{st} value and inter-population genetic distance showed no significant differentiation among populations, and the UPGMA tree of the populations also showed the close relationship among them. Dominant gene frequency in the five populations was similar. Thus, all wild populations had high intra-population genetic diversities and low inter-population differentiation. This information on population structure should be useful for stock management and conservation as well as for genetic improvement of this species.

MONITORING OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS AT RESTORATION SITES IN THE ST. LUCIE ESTUARY.

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In June 2009, NOAA awarded Martin County, Florida more than $4 million to construct approximately 20 acres of reef in the St. Lucie estuary (SLE). The main objective of this project was to monitor background oyster populations in concert with oysters populating restored reefs in the estuary. FWRI monitored settled oyster density, reproductive development, physiological condition, juvenile recruitment, and prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo) at four restoration stations.

Eleven natural reef stations were also monitored serving as reference populations against which to compare the success of the restored reefs. Monitoring at restoration stations commenced at two sites in late 2009 and at another two sites in early 2010 as reef construction was completed. Live oysters were present on each restored reef by the end of the study. Two stations had oysters present at densities near background abundances, oysters that were reproductively active, and disease and mortality rates comparable to natural levels. Oysters colonized the reef quickly at the other two stations, but experienced a significant mortality event during the winter of 2010/11 with only a limited rebound during the 2011 recruitment season. The most likely factor for mortality at those sites was sedimentation and burial.

IMPACTS OF INVASIVE JAPANESE EELGRASS (*ZOSTERA JAPONICA*) AND ITS MANAGEMENT ON MANILA CLAM PRODUCTION IN WILLAPA BAY, WA.

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Thick infestations of Japanese eelgrass *(Zostera japonica)* over thousands of hectares of commercial clam beds in Willapa Bay, Washington have become problematic for the shellfish industry. They claim *Z. japonica* results in significant reduction in production, increased harvest cost, and major and deleterious modifications in estuarine processes. To investigate these claims, numerous replicated research trials with and without Japanese eelgrass were established on commercial clam beds at several locations in Willapa Bay. Plot sizes ranged from 20 to 2000 m^2. *Z. japonica* was removed using the herbicide imazamox. Duration of treatment comparison ranged from 0.5 to 3 years. Data were collected on manila clam recruitment, growth of seeded clams, condition index, total commercial production, and net revenue. Additional data were collected on sedimentation and elevation changes over time, and treatment interactions with netting or gravelling, used for predator protection. Clam growth, production, recruitment and net revenue were usually reduced by *Z. japonica*, but treatment significance was highly site-dependent. The net dollar returns on sites where *Z. japonica*was removed increased by an average of $16,000/ha. Differences in sedimentation rates and elevation were site-dependent, but lower sedimentation rates and site elevations were usually found in sites where *Z. japonica* was removed.

PATENT TONG SURVEYS OF TWO OYSTER SANCTUARIES IN MARYLAND.

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Two intensive patent tong surveys were undertaken in Fall 2011 in the newly declared Harris Creek and Little Choptank oyster sanctuaries in Maryland, both located in the mesohaline portion of Chesapeake Bay near the mouth of the Choptank River. Three strata were created based on bottom classification data of acoustic surveys provided by the NOAA Chesapeake Bay Office and the Maryland Geological Survey. One stratum was mud or soft bottom, another represented sand or mud mixed with oyster shell, and the third was classified as mostly shell. A fourth stratum was added that represented those areas within which some restoration activity had taken place. In each sanctuary and within each stratum, 150 grabs at random locations were taken. For each of the resultant 1200 grabs, a shell score (amount of oyster shell present in tongs), substrate type, oyster number and the sizes of all live and dead oysters were recorded. The data will be used not only to describe oyster populations within each sanctuary but also to assess the accuracy of acoustic bottom classification and refine the survey protocols based on statistical analyses of strata variances.

INFLUENCE OF MICROALGAL SPECIES AND DIETARY RATION ON LARVAL DEVELOPMENT AND SURVIVAL OF THE PURPLE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*.

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This study evaluated the effect of diet and ration on the growth and survival of larvae of the purple sea urchin, *Strongylocentrotus purpuratus*. In the first experiment, seven algal diets [*Dunaliella tertiolecta*, *Chaetoceros muelleri*, *Isochrysis* sp. (Tahitian strain), and all possible binary and tertiary combinations] were assessed, along with a control treatment of no food. Larvae reared on the bi-algal diet of *D. tertiolecta* and *Isochrysis* sp. and the uni-algal diet of *D. tertiolecta* had significantly better growth than those in all other treatments. In the second experiment, a bi-algal diet (*D. tertiolecta* and *Isochrysis* sp. at equal bio-volumes) was evaluated using five rations: (1) low ration: 1.25×10^3 cells ml^{-1}; (2) normal ration: 2.5×10^3 cells ml^{-1}; (3) standardized ration: 2.5×10^3 to 10.0×10^3 cells ml^{-1}, with increasing ration according to developmental stage; (4) medium ration: 5.0×10^3 cells ml^{-1}; and (5) high ration: 10.0×10^3 cells ml^{-1}. Larvae reared on the standardized ration had significantly better growth than those in all other ration treatments. Overall survival (from prism stage to metamorphic competency) for the best treatments in both experiments was 44.4 ± 2.9 and 53.3 ± 1.9% (mean ±SE) for the binary diet of *D. tertiolecta* and *Isochrysis* sp. and standardized ration treatments, respectively.

COMPARISON OF REPRODUCTIVE CYCLE OF THE GEODUCK CLAM, *PANOPEA GLOBOSA* AND *P. GENEROSA* (BIVALVIA: HIATELLIDAE) IN NORTHWEST MEXICO.

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The recent exploitation of geoduck (*Panopea generosa*) in Northwest Mexico on the Pacific coast of the Baja California and *P. globosa* in the Gulf of California shows the lack and need of general biological knowledge on this species. This study describes timing of gametogenesis, development, and spawning for geoducks in Northwest Mexico. Clams were collected monthly for one year (March 2008–March 2009) in San Quintin Bay (Pacific coast), and San Felipe, Baja California and Guaymas, Sonora (Gulf of California). Monthly samples of approximately 30 clams (size range 110–190 mm shell length) were collected subtidally between 10 and 25 m depth. Standard histological analyses and measurements of oocyte diameters were used to describe the timing of gametogenic development and spawning. In the Gulf of California, upper and central gulf, the maturity period is restricted from November to January (winter season), but in the Pacific coast, the reproductive period is year round. The reproductive timing seems to be correlated with sea temperature. In the Gulf of California the results demonstrated that reproductive activity was triggered by a steep decrease in temperature 4 months prior to the peak of productivity, while the Pacific coast experiences no abrupt temperature changes.

SEASONAL ABUNDANCE AND TIDAL-TIMED MIGRATION OF OLYMPIA OYSTER LARVAE IN COOS BAY, OREGON.

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Olympia oysters (*Ostrea lurida*) are estuarine-dependent, and tidal cycles have an important influence on larval export and retention. Simple larval behaviors, such as vertically migrating to the bottom during falling tides, have been observed in several species as a potential mechanism of limiting export out of estuaries. We examined the seasonal pattern of larval abundance and tidal-timed migration for *O. lurida* in Coos Bay, Oregon during the summer of 2010. Weekly zooplankton tows and CTD casts were conducted from June to October, during a series of alternating rising and falling tides. No *O. lurida* larvae were observed in the water column until the end of July and greatest abundance was observed during the dry season (August–September). Dry season was characterized by elevated water temperatures (>16°C), high salinity (>25), and low stratification of the water column. Although we observed a trend toward lower larval abundance in surface waters during falling tides, no significant differences occurred with regard to tidal phase. Preliminary results suggest that differences in vertical abundance of *O. lurida* larvae may be related to larval supply from different parts of the bay and determined by the interaction between current velocities and tidal cycles.

DREISSENID PREVENTION ACTIVITIES IN THE WESTERN UNITED STATES—THE 100TH MERIDIAN INITIATIVE.

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Zebra mussels and quagga mussels have been one of the most costly aquatic invaders in U.S. history as millions of dollars are spent each year in managing zebra mussel infestations in the Great Lakes, Mississippi and now Colorado River drainages. The introduction of zebra and quagga mussels into the Columbia River Basin could not only threaten native species, but also industrial, agricultural, recreational, navigation, and subsistence use of the infested waters. Dreissenid mussel transfer between basins in the western United States is most likely to occur through the movement of trailered watercraft. Government agencies and organizations in the western US have implemented watercraft interception programs designed to prevent contaminated watercraft from being launched in unaffected waterways since 2007. Helping to coordinate this effort has been the 100th Meridian Initiative, a cooperative effort between state, provincial, and federal agencies to prevent the westward spread of zebra mussels and other aquatic nuisance species in North America. Current 100MI projects include rapid response planning, support of coordinated monitoring, protocols for decontaminating watercraft, and educational products such as the video 'Don't Move a Mussel'.

TRANS-SPLICING ELEMENTS IN *PERKINSUS MARINUS*.

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Perkinsus marinus, a protistan parasite that causes "dermo" disease in oysters, possess a spliced leader (SL) within their mRNA. Multiple SLs have been identified in *P. marinus*. Comparison of four different SLs of 22 nucleotides (nt) in length determined the consensus sequence to be (**NY**CGUAGCCAU**Y**UUGGCUCAAG). A truncated 21 nt SL, with a (U) deletion at nucleotide 13, is also present. Our group and others have identified SLRNAs for both the 22nt (SL-1) and 21 nt (SL-2) variants, ranging in size from 80–83 nt. We present here preliminary evidence for a SLRNA variant of only 53 nt in length, with a secondary structure predicted to be appreciably different from that of SL-1 and SL-2. Sequence

analysis of several cDNAs suggests a condensed 3 nt consensus (YAG) splice acceptor motif although a definable upstream polypyrimidine tract, essential in most systems for splice-site recognition, is absent. In summary, *P. marinus* contains multiple SLRNAs, each of which may contain SLs of varying sequences and length. Variability at positions 1 and 2 suggests variability of cap structure between the different SLs. Overall these data suggest a complex gene regulatory system both at the level of mRNA generation and of translational control.

HIGH GENOTYPE-DEPENDANT MORTALITY AT METAMORPHOSIS IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*.

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Settlement/metamorphosis is a critical period in the life cycle of marine bivalves, during which larvae undergo substantial morphogenetic, sensory, and ecological changes. High mortality during this transition is well documented for many marine bivalves and is generally interpreted as occurring post-settlement and being environmentally derived; little is known, however, about whether mortality occurs during the process of metamorphosis itself, and what role endogenous genetic variation may play. Using QTL mapping methods, we examine the stage-specific expression of deleterious loci in the Pacific oyster, Crassostrea gigas, which carries a large load of deleterious recessive mutations that cause massive, zygotic marker-segregation distortion and genotype-dependent mortality in F_2 families. We found that half of the loci causing genotype-dependent mortality (5–7 mutations within a family) act during metamorphosis. Further fine-scale dissection of mortality during metamorphosis revealed a mutation causing selection during metamorphosis, possibly affecting the morphogenetic pathway, while another mutation caused a delay in, or inhibition of, metamorphosis, suggestive of a defect in the competence pathway. Overall, selection during the larval-juvenile transition appears to be confined to the induction of metamorphosis and metamorphosis itself, which highlights the importance of understanding the developmental-genetic pathways associated with this critical transition.

UTILIZING GEOSPATIAL TECHNOLOGY IN THE MANAGEMENT OF SHELLFISH GROWING AREAS IN NORTH CAROLINA.

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The Shellfish Sanitation Program in North Carolina collects a significant amount of water quality and pollution source data within shellfish growing areas in order to properly manage the harvest of molluscan shellfish for the protection of public health. In the past, much of this data has been stored in a tabular format or represented on hand-drawn paper maps. However, in recent years, a geographic information systems (GIS) database has been developed and become the basis for data collection and analysis within the program. As a result, field data collection efforts have been improved and standardized through the use of handheld GPS devices. The geospatial component of the data enhances analysis by allowing better visualization of bacteriological sampling results and observed shoreline pollution sources in relation to the affected shellfish harvesting waters. GIS software tools enable a more accurate determination of closure boundaries and growing area classifications. In addition to making data access and analysis more efficient internally, these technologies also make it easier to distribute data to stakeholders in a format that is readily accessible, understandable, and interactive.

PATCHINESS OF DERMO (*PERKINSUS MARINUS*) DISEASE FOCI IN THE ARANSAS-COPANO, TEXAS ESTUARINE SYSTEM.

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The intensity of Dermo (*Perkinsus marinus*) disease has been monitored in the Aransas-Copano estuarine system for several years. For these data see www.oystersentinel.org. A recent study involving both hatchery-reared and natural oysters (*Crassostrea virginica*), indicate that some areas are more supportive of Dermo disease development in oysters than others. Water temperature and salinity obtained at the time of sample collection indicated that this estuarine system was well mixed during an extreme drought period. The water salinities at all seven collection sites ranged between 36 and 38 ppt. We believe that this information will be useful in siting future oyster restoration projects.

OYSTER FOOD SUPPLY: ITS ESTIMATION IN DELAWARE BAY FROM A HYDRODYNAMIC MODEL AND THE INTERACTION WITH THE OYSTER POPULATION.

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To evaluate oyster food supply, water samples were collected at fifteen sites in the Delaware Bay near-monthly in 2009/2010. Food was estimated as the sum of protein, carbohydrate, and lipid.

Five variables each based on temperature were significant contributors to a multiple regression. Cluster analysis on residuals identified two large groups of sites, one comprising most sites on the eastern side of the bay including all of the New Jersey oyster bed sites downestuary of the uppermost beds and one including most of the sites along the central channel and waters west. Food values over the New Jersey oyster beds were often depressed by 50% relative to the bay-wide mean. Food values did not follow an upestuary-downestuary trend anticipated from the salinity gradient. Rather, the differential was cross-bay and was distinctive throughout the estuarine salinity gradient, thus explaining the lack of significance of any salinity-related variable in the multiple regression. The oyster reefs of Delaware Bay are dominantly sited on the New Jersy side, suggesting that oysters can influence food values on the New Jersey side of the bay at present biomass and this would explain the cross-bay gradient in food values as an outcome of oyster feeding.

INTERACTIONS BETWEEN *OSTREA EDULIS* GALECTIN (OE-GAL) AND THE PROTOZOAN PARASITE *BONAMIA OSTREAE*.

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Bonamia ostreae is a protozoan parasite affecting the flat oyster *Ostrea edulis*. This parasite targets haemocytes, cells notably involved in oyster defence mechanisms. The open reading frame of a gene encoding a galectin (OeGal) was completely sequenced allowing the obtaining of a recombinant protein and antibodies anti-OeGal. Interactions beween OeGal and *B. ostreae* were first investigated *in vitro* by measuring internalization of parasites in haemocytes previously submitted to different treatments. These treatments consisted in incubating haemocytes with galectin inhibitors including glucose, galactose, Beta-lactose and anti-OeGal at different concentrations. These different contact experiments generaly yielded to a decrease of parasite internalization in haemocytes. On the contrary, an incubation of parasites with recombinant galectin prior to contact with hameocytes increased the number of infected haemocytes. OeGal appears involved in the internalization of *B. ostreae* in haemocytes *in vitro*. This study was completed by testing flat oysters originating from a *B. ostreae* endemic area. Parasite load and Oe-Gal expression were determined for six organs. A positive correlation was observed between parasite load and OeGal expression in gills. These results contribute to better understand how the parasite installs and survives within haemocytes.

EFFECTS OF HARVEST ACTIVITY ON INFAUNAL COMMUNITIES IN GEODUCK CLAM AQUACULTURE PLOTS IN SOUTHERN PUGET SOUND, WASHINGTON, USA.

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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 unplanted reference plot of equal size. For several months prior to, during, and after harvest, we collected data on benthic community abundance and composition. Each site presented a slightly different benthic community structure and therefore responded differently to harvest practices. Data analysis indicated that variance in infaunal data was attributable to time of year (seasonality) and plot status (cultured versus uncultured). There was little evidence to indicate that activities associated with geoduck harvest caused significant long-term damage or disruption to the benthic ecosystems on the intertidal sand flats of southern Puget Sound.

HISTOPATHOLOGICAL EVALUATION OF THE CARPET SHELL CLAM, *TAPES DECCUSATUS*, FROM EGYPTIAN COASTAL WATERS.

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A histopathological evaluation of the commercially important carpet shell clam, *Tapes deccussatus* from six sites in three main Egyptian clam fisheries (Alexandria, Ismailia and Damietta) was conducted over a twelve months period (December 2010–November 2011). Smear preparations and histopathological examination (n = 30 and 10/site/month, respectively) showed infection of market size clams (shell length >20 mm) with digenean larvae and redia of cercaria that started to appear in March–April. Maximum prevalence (% clams infected) was observed in May samples at the two Ismailia sites (81% & 92%), followed by El-Max, Alexandria (25%) and no infection at the other Alexandria or Damietta sites. Maximum infection intensity (% area of longitudinal tissue section occupied by parasites, n = 12 slides examined per site) was observed in the May samples of Ismailia, (78%), followed by El-Max, Alexandria (45%). Digenean larvae

caused a wide range of damage to clam tissues and organs with gonad degeneration noted most, which could lead to reduced reproductive success and eventual castration. Infection intensity increased with clam size (predominantly found in clams ≥31.9 mm shell length with ripe gonads). Histopathological abnormalities (e.g., lesions, infiltration, malformation of epithelial cells, degeneration) not caused by parasites will also be discussed.

RESEARCH AND DEVELOPMENT OF SHELLFISH AQUACULTURE ON THE ANNETTE ISLAND RESERVE, ALASKA.

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For twenty years the Alaska Sea Grant Marine Advisory Program and the Department of Fisheries of the Annette Island Reserve have conducted collaborative research and development projects on shellfish aquaculture to provide local employment opportunities and to assist the Alaska industry by providing necessary and timely applied research. The collaborations have been productive with projects completed on alternate methods for Pacific oyster (*Crassostrea gigas*) culture, cadmium testing of oysters, marine biotoxin monitoring, intertidal geoduck clam (*Panopea generosa*) aquaculture, oyster quality assessment, and evaluation of alternative rapid test kit for paralytic shellfish poison and domoic acid. As the only Alaska Native Reserve, a major advantage of collaborating with Annette Island is the ability to conduct rapid response research because the reserve is not required to obtain authorization permits from the State of Alaska. The reserve currently has three oyster farms and is planning a major intertidal geoduck farming venture using results of a seven year research project that provides production data for farm planning. Future activities being considered are shellfish nursery culture, paralytic shellfish poison research, additional studies on alternative Pacific oyster growout methods, and growout trials of Molluscan Broodstock Program high performance Pacific oysters.

EXPANDING KNOWLEDGE OF OCCURRENCE AND DISTRIBUTION OF PARALYTIC SHELLFISH POISON AND DOMOIC ACID TOXINS ALONG THE COAST OF ALASKA.

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Since 2007, a flurry of research and monitoring projects dealing with paralytic shellfish and domoic acid toxins have revealed new and significant information on their occurrence and distribution in Alaska marine shellfish. Five outbreaks of illness occurred in 2010–11 that included two fatalities attributed to consuming toxic shellfish. Two very localized and unusual HAB events illustrated the complexity of HAB events in Alaska and challenge conventional means used to forecast HAB occurrences and toxicity in shellfish. Alerted to the marine toxin problem, biologists are investigation and finding toxins in blood and urine of stranded marine mammals. HAB events have impacted every major marine sector that includes commercial fisheries, recreational harvest, subsistence use, tourism, and shellfish aquaculture. Work continues with expansion of monitoring efforts ranging from the Bering Sea southeast to the Alaska/Canada border, participation in field evaluation of ELISA based toxin testing kits, and improvements of rapid response efforts associating with human illness. An expanded interagency education and outreach program is now underway to improve reporting and media notifications of toxic events and expand monitoring to assure safe shellfish harvest from popular recreational and subsistence harvest beaches.

EXTENSION AS A CENTRAL FOCUS OF RESEARCH: SUCCESSFUL METHODS TO SPREAD THE WORD.

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A primary goal of research is to provide reliable information necessary for decision making and fostering behavioral change. Extension is the accepted mechanism to distribute research information utilizing methods that effective access and impact society. Linking these two endeavors would appear to be of vital importance, but extension is often relegated to a subservient role where results are often extended to stakeholders after the research is completed. In the new age of communication, websites, media productions, and publications are often employed to meet extension requirements of research projects. Although well-produced mass communication is useful in disseminating information to a large diverse audience, evaluation of effectiveness and assessment of impacts of the research, as is more increasingly demanded by funding sources, is challenging. In support of expanding the role of extension, this presentation provides detailed examples of successful methods utilized in Alaska that incorporated extension throughout research projects from planning to completion, enhanced engagement and participation with stakeholders, improved the prospects for acceptance and application of research results, and utilized methods of measuring social and economic impacts.

INVESTIGATING USE OF INTERTIDAL BAG CULTURE OF PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) FOR ALASKAN FARMERS: AN ALASKA SEA GRANT AND FARMER RESEARCH COLLABORATION.

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Farming oysters in the cold northern waters of Alaska is physically and financially challenging. At conclusion of a financial management project in 2009, Alaska shellfish farmers for the first

time were exposed to accurate costs associated with oyster farming that lead to a research project directed toward reducing growout time and labor costs to achieve profitability. The research experimented on floating and intertidal bag culture techniques and compared the results to traditional raft and tray culture as the control. Five farmer/collaborators participated in the project, deploying growout gear on their farm sites, collecting environmental and operational data, and provided oyster samples at regular intervals for assessment of growth and condition. Results show significant reduction in labor cost during the first growout year and lower cost of gear construction compared to raft and tray culture. Oyster condition was excellent. Final analysis shows that a combination of intertidal bag growout during the first year then transferring the oysters to raft and tray culture during the second year is likely the most cost effective method for oyster farming in Alaska. Several oyster farmers are in the process of converting to the new growout method.

PERKINSUS SPP. IN CLAMS *RUDITAPES DECUSSATUS*, *RUDITAPES PHILIPPINARUM*, *VENERUPIS SENEGALENSIS* AND *TAPES RHOMBOIDES* IN GALICIA (NW SPAIN): FIRST DETECTION OF *PERKINSUS CHESAPEAKI* IN SPAIN BY DGGE.

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The genus *Perkinsus* includes parasites of molluscs world wide and includes seven valid species. *P. olseni* and *P. mediterraneus* are the species of this genus known to occur along the Spanish coast, although the number of molecular studies to identify the species responsible for perkinsosis in Spain is very low. A survey to identify parasites of this genus occurring in four commercial clam (*Ruditapes decussatus*, *Ruditapes philippinarum*, *Venerupis senegalensis* and *Tapes rhomboides*) species in Galicia (NW Spain) was carried out. The clams were collected from 16 beds scattered along the Galician coast. The prevalence of parasites of this genus in samples of 30 clams was determined by PCR using genus specific primers. The highest prevalence of perkinsosis corresponded to *R. decussatus* and *V. senegalensis*, while lower values were detected in *R. philippinarum* and *T. rhomboides*. Identification of the *Perkinsus* species involved in each positive case was performed by denaturing gradient gel electrophoretic (DGGE) assay. *P. olseni* was detected in every clam species. Additionally, *Perkinsus chesapeaki* was detected in clams *R. phillippinarum* from a single location. Identification of *P. chesapeaki* was further confirmed by sequencing the ITS rDNA region.

SPATIAL DISTRIBUTION OF THE OYSTER *OSTREA EDULIS* PARASITES *BONAMIA OSTREAE* AND *BONAMIA EXITIOSA* IN GALICIA (NW SPAIN) AND INFECTION DYNAMICS THROUGH OYSTER ON GROWING.

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Bonamiosis affects various oyster species world round causing high mortalities. *Bonamia ostreae* has been responsible for this disease in Europe since late 1970s, but the recent detection of *Bonamia exitiosa* infecting *Ostrea edulis* in the Galician coast (NW Spain) and, subsequently, in other European countries has raised the question of the impact of *B. exitiosa* on the European oyster industry. Seven oyster beds and 3 farming areas scattered through Galician Bays were sampled in autumn 2009 and spring and autumn 2010. *B. ostreae* was detected in every location and *B. exitiosa* in all locations except in one natural bed. Additionally, four oyster spat cohorts produced in a hatchery, deriving from different brood-stock batches, were transferred to a culture raft in the Ría de Arousa in summer 2009. Monthly sampling was performed to evaluate growth, survival and health status through on-growing up to June 2011. PCR assays confirmed the infection with both *Bonamia* spp. early in the spat with increasing prevalence through on-growing. Histology showed cases of heavy intensity for both *Bonamia* spp. Individual oysters co-infected with both *Bonamia* spp. were detected. Results confirmed that *B. exitiosa* is well adapted to infect *O. edulis* in the Galician marine environment.

NEW PCR-BASED SPECIES SPECIFIC PROCEDURES TO DIAGNOSE *BONAMIA EXITIOSA* AND *BONAMIA OSTREAE*, PROTOZOAN PARASITES OF OYSTERS.

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Bonamiosis is responsible for mass mortalities of various oyster species around the world. Two species of the genus *Bonamia*, *B. ostreae* and *B. exitiosa*, are highly pathogenic and widely spread in the Northern and Southern Hemisphere, respectively. Furthermore, *B. exitiosa* has been recently detected in Europe and northern Africa. The World Organisation of Animal Health has included both species in the list of notifiable diseases. However, official methods for diagnosis of *B. ostreae* and *B. exitiosa* have certain limitations: histological methods do not allow a clear discrimination between both species and the recommended PCR procedure is

genus specific, which requires further DNA sequencing or RFLP assay for species specific diagnosis. New conventional PCR and real time PCR species-specific diagnosis procedures for *B. exitiosa* and *B. ostreae* have been developed, as well as an *in situ* hybridization assay specific for *B. exitiosa*. The new PCR-based procedures showed higher sensitivity than the OIE recommended ones.

DUNGENESS CRAB PRODUCTION: AN ECOSYSTEM SERVICE PROVIDED BY *OSTREA LURIDA* AND *CRASSOSTREA GIGAS* IN WILLAPA BAY, WASHINGTON.

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Reef forming shellfish provide substantial habitat for other fish and invertebrates in estuaries. This ecosystem service, once provided by the native oyster, *Ostrea lurida* in many US West Coast estuaries has been substituted with that provided by actively cultured Pacific oysters, *Crassostrea gigas*. We examined the present service provided by Pacific oysters as habitat for juvenile Dungeness crab (*Metacarcinus magister*), and estimated that provided historically by native oysters in Willapa Bay, Washington. Pacific oysters are now cultured and cover approximately 20% of the intertidal area of this estuary while native oysters are virtually absent, but were estimated to have covered 12% of the low intertidal area. Reef morphology and location of these two oysters in the estuary however differ substantially. An experiment where shell bags were placed at four locations and four tide heights was conducted in Yaquina Bay, Oregon to examine how these factors affected crab settlement. We also surveyed crabs in oyster aquaculture, remnant and restored populations of native oysters, eelgrass, and open mud habitat in several estuaries to estimate tradeoffs in crab production which should be considered when defining goals for both aquaculture and native oyster restoration in Willapa Bay and other estuaries along this coast.

MUSSEL MARICULTURE IN QUARTERMASTER HARBOR: ENGAGING COMMUNITIES AND IMPROVING WATER QUALITY THROUGH NUTRIENT BIOEXTRACTION.

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Poor circulation and nutrient inputs have resulted in critically low dissolved oxygen levels in portions of Quartermaster Harbor (QMH) on Vashon Island, particularly in late summer and early fall. The negative impact on overall estuary health and function prompted King County to initiate the 2009–2012 QMH Nitrogen Loading Study. In concert with this study, Pacific Shellfish Institute, Puget Sound Restoration Fund and an assortment of industry and tribal partners worked together to culture native mussels (*Mytilus trossulus*) on an experimental field station in QMH as an alternative form of nitrogen removal. Project objectives included: 1) working with the community of Vashon to build a creative strategy that mitigates for chronic nutrient inputs in QMH, 2) engaging residents in the recovery of healthy marine resources, and 3) developing market-based mechanisms for cleaning Puget Sound. The study quantifies the nitrogen removal services of mussels grown in QHM, evaluates mussel production and market potential of products grown in the Harbor, and fosters public engagement and community-based solutions to chronic wastewater issues.

TEXAS' 2011 BUMPER OYSTER SET JEOPARDIZED BY PROLONGED DROUGHT.

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Unusually high water salinities enjoyed in the spring of 2011 resulted in a very high oyster set in all Texas Bays. Unfortunatly, the severe drought, which began in October 2010, has continued through 2011. High water salinities have promoted significant Dermo (*Perkinsus marinus*) disease in spat and juvenile oysters. Spat as small as 15 millimeters have become moderately infected with Dermo. In mid-fall 2011 salinities in the high twenties and low thirties ppt existed near river mouths. The current drought is predicted to last 18 months or more. An oyster-killing flood in each bay system will be required to effect recovery. The levels of Dermo disease in adult and juvenile oysters of major Texas Bays will be presented.

SHELLFISH REEF RESTORERS: ADJUST GOALS TO CURRENT, NOT BY-GONE CONDITIONS.

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Most Shellfish reef restoration projects begin with this premise: declining shellfish populations are due primarily to over-harvesting and pollution. There are other major causes such as diseases and predation as well as reduced fresh water inflow and increased salt water intrusion brought on by navigation, flood control and other projects that have resulted in vast changes in the physical nature of estuarine systems. Such changes are often ignored when

planning projects to return shellfish populations to "levels of the good-old-days." Such lofty restoration goals are often doomed to failure along with wasted time, substrate and money. Repeated failures will result in loss of confidence by the supporting public. A more prudent approach appears to be that of using "aquaculture" as employed in Virginia and other states, as well as using all factors (not just the easy ones to blame) in planning restorations. The "aquaculture" approach may result in "limited success" as enjoyed in Virginia and the Northwest USA, rather than the "sanctuary" approach that has led to repeated failures such as experienced by the state of Maryland in their efforts to restore Chesapeak Bay oyster populations. Perhaps "improving" the shellfish populations is a more achievable goal than "restoring." Examples of proposed restoration projects in Texas and Maryland will be discussed.

OUTPLANTING DESIGN IMPROVEMENT FOR JUVENILE ABALONE (*HALIOTIS KAMTSCHATKANA*): THE ADDITION OF COMPLEX SUBSTRATE INCREASES SURVIVAL.

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We assessed the effectiveness of complex substrate in reducing predation on outplanted northern abalone (*H. kamtschatkana*) by supplementing fenced 1 m^2 outplanting plots with layers of cobbles or boulders. We released 30 juvenile abalone (mean SL = 51.5 mm) into each plot. We found that adding boulders or cobble significantly increased the survival rate of outplanted large juveniles over the first six days. However, we detected no difference between plots with added cobbles and those with added boulders and no difference between plots with 30 cm of added substrate and those with 90 cm of added substrate. Our surveys suggest that the abalone emigrated from the substrate-addition plots at a lower rate than from the control plots. The addition of complex substrate may also have provided crypsis from predators, however the substrate-addition plots actually contained significantly higher densities of abalone predators than the control plots. In a separate study we genotyped abalone from three outplanting sites for six microsatellite loci and used three different programs to assign them to either wild or hatchery origin. Our data suggest outplanting hatchery-reared northern abalone at the competent larval stage may be more effective than outplanting them as large juveniles.

DELAWARE'S ANTHROPOGENIC ROCKY SHORELINE: A LOCATION FOR OYSTER RESTORATION?

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Efforts to restore the once abundant eastern oyster population, *Crassostrea virginica*, in the northwest Atlantic Ocean have had limited success in the past 160 years. The Delaware Inland Bays are void of any natural hard bottom substrate or preexisting oyster reefs, but riprap provides locations for potential larval recruitment. Two year old oysters, grown in Delaware Volunteer Oyster Gardening Program, have been stocked in riprap over the past several years for restoration purposes, as previous work showed that oysters survived poorly on an artificially created oyster reef. Survival of these planted bivalves is our number one goal, while propagation of a new generation of naturally setting oysters is our second aspiration in this study. This study aims to discover if riprap may hold a future in oyster restoration, especially in Delaware. Several sites containing usable ripraps were stocked with 75 measured oysters in summers of 2009 and 2010. Results varied per site based upon survival and re-location ability. To calculate more precise oyster growth data in the future would require a more consistent re-location effort of oysters, or to follow the fate of individual oysters for an extended period of time.

INVESTIGATING BIOLOGICAL DIVERSITY FROM STOCKING RIPRAP WITH OYSTERS (*CRASSOSTREA VIRGINICA*) IN JEFFERSON CREEK, SOUTH BETHANY, DELAWARE.

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Coastal shorelines are often modified around the world into artificial structures (i.e. bulkhead and riprap) in hope of shoreline strengthening and stabilization. Impacts on the local aquatic communities are of high concern in such modified areas. Few studies have been performed in estuaries along riprapped shorelines to assess how aquatic organisms may be utilizing shoreline habitats differently. Fish species assemblages along rocky shorelines are sampled differently depending upon location, since some habitats are easier to study than others. Riprap, a 3-dimensional complex habitat comprised of randomly placed rocks, has the ability to be stocked with abundant bushels of oysters. In this study in a tidally flushed creek in South Bethany, Delaware, we attempted to compare transient and resident fish population diversity and richness along the three different shoreline habitats: riprap stocked with oysters, riprap with no oysters, and a natural shoreline as a reference site. Un-baited minnow traps were deployed every two weeks to collect specimens from the three different shorelines in triplicate. Seine nets, modified lift nets and cast nets proved ineffective during this study. Additionally, physical and chemical water quality parameters (nitrogen and phosphorous) along the different shorelines were studied to ensure overall bay health for oyster restoration.

EAST COAST SHELLFISH AQUACULTURE STATUS AND TRENDS.

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The east coast shellfish aquaculture industry harvests approximately $100 million in clams, oysters and mussels. Half of that production comes from just two of the fourteen east coast states; Virginia and Connecticut. Clams (*Mercenaria mercenaria*) comprise 67% of this value, and oysters (*Crassostrea virginica*) are responsible for the bulk of the remainder. The industry supports over a thousand farms; mostly small-scale owner-operator farms with less than ten employees. The east coast industry also supports 55 hatcheries. The industry provides direct full-time employment for thousands of individuals and part-time direct employment for hundreds more. The industry is maturing and this presentation will examine industry trends and predict some of the opportunities and threats that face the industry. The application of economic multipliers allows the projection of the economic impact of the industry on local communities. A preliminary economic valuation of certain ecosystem services will also be presented.

EFFECT OF SORTING SEED BY SIZE AND TUMBLING ON THE GROW-OUT OF FARMED OYSTERS, *CRASSOSTREA VIRGINICA*, IN THE GULF OF MEXICO.

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Oyster farmers suggest like size seed perform better than unsorted seed and often assume small seed are "runts". This study analyzes the effect of size sorting seed and tumbling of sorted seed on oyster growth. Oysters seed spawned in Spring 2011 were nurseried in 12 mesh bags at a commercial farm. In August 2011, six bags were sorted into three sizes: 1) the smallest 12.1%, 2) the middle 76.5%, and 3) the largest 11.4%. Six replicate longline baskets were stocked (75/basket) per group. Half of the baskets from each size group were tumbled monthly, the other half were not handled. For comparison, 3 replicate baskets were stocked (75/basket) from the remaining unsorted bags, half were tumbled monthly, the other half were not handled. Stocked baskets were maintained on an adjustable long-line system in Grand Bay, Alabama. Shell metrics and condition were mesured for 5 oysters from each basket at quarterly intervals. Initial shell heights for size groups were significantly different and small and large size groups were significantly different from unsorted seed. By November, large, medium and unsorted seed maintained a size advantage over small seed but there was no significant difference in growth rate among all the groups.

TESTING FACTORS DETERMINING SETTLEMENT OF THE OLYMPIA OYSTER IN COOS BAY, OREGON.

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The Olympia oyster, or *Ostrea lurida*, population of Coos Bay is more dense in some locations than in others. Understanding the factors contributing to this population structure may help us understand larval settlement requirements. I will track temperature, salinity, and chlorophyll concentration over time at several locations where *O. lurida* is present in the intertidal and in the subtidal, as well as locations where it is not present. I will also test the survivorship and growth of newly settled individuals outplanted at these sites at times of year when the population is reproductive and when it is not reproductive. Lastly, I will examine larval settlement success under different conditions of salinity, temperature, and substrate availability in the lab. Determining larval settlement requirements could help better inform restoration projects for this native species.

EPIGENETIC REGULATION OF OYSTER HOX ORTHOLOGUES BY DNA METHYLATION PLAYS A CRITICAL ROLE IN THE EARLY DEVELOPMENT OF *CRASSOSTREA GIGAS*.

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A proper temporal and spatial pattern of gene expression is mandatory for a normal embryogenesis. An appropriate DNA methylation is a critical epigenetic feature in the development of vertebrate embryos. However, the situation in Lophotrochozoans remains poorly described. Indeed, despite oyster genomic DNA being methylated, the role of DNA methylation in *Crassostrea gigas* development remains unknown. We examined oyster genomic DNA and found it differentially methylated during early development. Consistently, RT-qPCR indicated stage-specific mRNA levels of DNA-methyltransferases (DNMTs) and methyl-binding domain (MBD) proteins. In addition, *in vivo* 5-aza-cytidine treatment induced alterations in the quantity and localization of methylated DNA, a severe early development delay, and was lethal after zygotic genome reinitiation. Furthermore, *Hox* gene orthologues, which exhibit specific temporal expression patterns, were modulated by 5-aza-cytidine treatment. Accordingly, methyl-DNA-Immunoprecipitation (MeDIP)-qPCR indicated for most of them an inverse correlation between their specific DNA methylation and transcription level. We demonstrated that DNA

methylation influences gene expression and is critical for oyster embryonic development by specifically controlling *Hox* genes transcription. These findings assess the importance of epigenetic regulation of development in Lophotrochozoans and bring new insights into the early growth processes of oysters.

COMPARING IMMUNE RESPONSES IN SHELLFISH USING SHORT-READ SEQUENCING TECHNOLOGY.

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Advances in sequencing technology provide new opportunities to explore functional genomics in commercially important molluscs, especially those with limited sequence information. In order to better understand immune function and disease tolerance in these shellfish, short-read sequencing technologies (i.e. SOLiD, Solexa) were used to examine differential gene expression in discreet populations of oysters, abalone, and clams. A suite of sequencing efforts have been completed that include the characterization of: 1) disease-tolerant, wild populations of hard clams and black abalone challenged with pathogens, 2) manila clams subjected to altered water chemistry, and 3) Pacific oysters grown in environments with varying levels of anthropogenic impacts. These combined data reveal gene expression patterns that are correlated with an effective immune response and provide insight into functional genetic variation that could be incorporated into aquaculture practices to improve production. Results from these sequencing efforts that utilize different technologies and different experimental designs will be presented. Furthermore, advantages and challenges associated with using short-read sequencing for functional genomic analyses in aquacultured shellfish will be discussed.

GENETIC AND MORPHOLOGICAL VARIATION OF *PANOPEA* CLAMS IN THE NORTHEAST PACIFIC.

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Geoduck clams have recently become the most profitable emerging fishery resource in Northwest Mexico. The fishery targets two species—*Panopea globosa* and *P. generosa* — but is managed indistinctively. Despite its growing importance, scientific research on the basic biology of the Mexican stocks is limited. Major gaps in knowledge are the interspecific distinction in structural and functional biological attributes and the levels of population genetic diversity. Consequently, we used genetic (nuclear and mitochondrial) and morphological data to characterize *Panopea* populations in the northeast Pacific to understand the patterns of genetic structure and connectivity. We found evidence of morphometric differentiation between *P. generosa* and *P. globosa*. Genetic data from the nuclear ribosomal DNA revealed very large genetic differentiation between species. This was found to be useful for molecular species identification, which was used to corroborate the presence of *P. globosa* in the Pacific coast of Baja California (Bahía Madgalena), thereby extending its distribution outside of the Gulf of California. Preliminary analyses of mtDNA sequences from structural genes (COXI and COXIII) showed limited polymorphism and differentiation among samples of *P. generosa* from the U.S. (Washington) and Mexico (Baja California). We discuss our findings in the light of their evolutionary and management implications.

HARMFUL ALGAL BLOOM IMPACTS ABALONE AND SEA URCHINS POPULATIONS IN NORTHERN CALIFORNIA.

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A Harmful Algal Bloom (HAB) coincided with a die off of marine invertebrates along the northern California coast in August 2011 impacting the red abalone, *Haliotis rufescens* fishery. This is the first reported abalone HAB related die off in the region, which supports a major recreational red abalone fishery landing 1,125,000 pounds (500 mt) per year. Subtidal surveys at four established fishery sites revealed that 19–35% of the abalone and 30-45% of the red sea urchins *Strongylocentrotus franciscanus* died. Transects revealed that mortalities were most severe in shallow water (<10 m) including wave swept headlands. Sea stars and large chiton also died in large numbers however no dead fish were

observed. Phytoplankton samples indicated that at the time of the mortality event the dinoflagellate, *Gonyaulax spinifera*, was the dominant species. Tissue samples revealed trace levels of yessotoxin in the gut and digestive gland but not the foot muscle of the abalone. Histological examination revealed normal gill tissue structure. Managers recommended an emergency closure in Sonoma County, which accounts for 50% of the fishery, prematurely ending the season. More work is currently underway, including population assessments, microscopic examination of the phytoplankton as well as mRNA screening of abalone before and after the die off.

MANAGEMENT PROCEDURES, BIOLOGICAL REFERENCE POINTS AND DECISION RULES FOR MANAGING AND RECOVERING ABALONE POPULATIONS IN CALIFORNIA.

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The recreational red abalone fishery in northern California is the largest wild abalone fishery in the world landing in excess of 1.1M pounds (500 mt) and is currently managed using management procedures (MPs). The decision rules, which are the foundation of the MPs, are outlined in California's Abalone Recovery and Management Plan. The plan requires periodic Scuba surveys to assess local densities of red abalone at eight sites throughout the fishery. These density data are then compared with densities from previous surveys. Built into the decision rules are pre-agreed on Biological Reference Points based on density, which trigger adjustments in the Total Allowable Catch (TAC). Current density levels at Sonoma County sites have fallen below these triggers but the average of all the fishery sites is hovering at the 0.5 red abalone/m^2 trigger. At the most heavily fished site, Fort Ross, which produces roughly 180 mt per year, current densities are at 0.33/m^2 which is nearing the trigger for site closure of 0.25/m^2. The challenge for management and restoration of abalone in California will be to set TACs to levels that ensure population sustainability as well as inform restoration of depleted populations such as the endangered white abalone.

IMPACTS OF THE RED TIDE ORGANISM *KARENIA BREVIS* ON THE EARLY LIFE STAGES OF OYSTERS *CRASSOSTREA VIRGINICA* AND NORTHERN QUAHOGS (= HARD CLAMS) *MERCENARIA MERCENARIA*.

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The brevetoxin-producing dinoflagellate, *Karenia brevis*, impacts many commercially and ecologically important shellfish species in the Gulf of Mexico. In Florida, the northern quahog (= hard clam; *Mercenaria mercenaria*) and eastern oyster (*Crassostrea virginica*) contribute markedly to the local economy and ecology. It is thus important to determine the effects of *K. brevis* during early development of these species. In this study, embryos of *M. mercenaria* and *C. virginica* were exposed at the 2–4 cell embryonic stage to both whole and lysed *K. brevis* isolated from Manasota Key, Florida at simulated bloom concentrations (500, 1500, 3000 cells ml^{-1}) for 96 h. Growth, percent abnormalities and mortality were measured throughout the experiment and overall survival (%) was measured at 96 h. Effects of *K. brevis* on larval growth were dose-dependent and observed from 24 h to 96 h exposure in both species. *K. brevis* had a significant negative effect on mortality and abnormality rates of both species following only 6 h of exposure. Overall, lysed cells of *K. brevis* had a more pronounced effect on clam and oyster survival and percent abnormalities than whole cells.

BIOFOULING TUNICATES ON AQUACULTURE GEAR AS POTENTIAL VECTORS OF HARMFUL ALGAL INTRODUCTIONS.

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Biofouling tunicates are ubiquitous in coastal ecosystems and are among the main overgrowers of aquaculture gear. Our study tests the hypothesis that the transport, removal, and transfer by aquaculturists of fouling tunicate species provide mechanisms for concentration and distribution of harmful-algal cells to new areas.

Wild-caught specimens of common, biofouling ascidians species (*Styela clava, Ciona intestinalis, Molgula manhattensis, Bottryloides violaceus, Didemnum sp.*, and *Botryllus schlosseri*) were exposed individually to cultured strains of co-occuring harmful algae (*Prorocentrum minimum, Alexandrium fundyense, Alexandrium monilatum, Karenia mikimotoi, Aureococcus anophagefferens*, or *Heterosigma akashiwo*) at simulated bloom cell densities of each HAB. After feeding, ascidians were transferred to ultrafiltered seawater. Immediately after exposure, and after 24 and 48 h in ultrafiltered seawater, biodeposits were collected and observed microscopically for the presence of intact, possibly viable cells. Subsamples of biodeposits were transferred into ultrafiltered seawater and monitored for algal growth. Thus far, cells of all HAB species have been found to pass intact through the ascidian digestive system and remain viable, re-establishing a population 48 h post-ingestion. Potential mitigation strategies to prevent transport of harmful-algal species through movement of fouled shellfish-aquaculture gear and disposal of biofouling material are being investigated.

THE ROLE OF PARTICLE SURFACE CHARACTERISTICS IN PARTICLE CAPTURE IN BIVALVE MOLLUSCS.

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Recent studies suggest that non-specific physicochemical interactions play a role in mediating a passive selection mechanism in *C. virginica* and *M. edulis*. Combined with findings on the role of lectins in particle selection, this suggests an expanded role of mucus in the feeding process (e.g., selection, capture and transport). Several studies have found that live cells may adhere to mucus preferentially over other surfaces, attributable to the specific qualities of the mucus. These findings raise the question of whether or not the surface properties of a given particle can affect its interactions with mucus, and if some properties are more likely to make a particle adhere than others. This study seeks to examine that question with the goal of further elucidating the mechanistic aspects of particle selection. Mucus isolated from the labial palps and ctenidia of mussels and oysters will be used in adhesion assays, in which the mucus extracts are fixed to a slide and immersed in a solution containing a particle of interest for 1 h. Adhesion of particles with different surface properties is quantified using image analysis. Multivariate statistics will be used to assess the relative importance of different particle properties to adhesion.

BYCATCH MITIGATION THROUGH FISHING GEAR BASED APPROACHES: EVALUATION OF THE IMPACTS OF THE COONAMESSETT FARM TURTLE DEFLECTOR DREDGE ON THE SCALLOP RESOURCE AND FINFISH BYCATCH.

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The focus of recent management measures for the sea scallop, *Placopecten magellanicus* fishery has increasingly been dictated by issues relating to bycatch. In an effort to mitigate the impact of the scallop fishery on threatened and endangered sea turtles, a modified dredge frame was designed and tested. While the primary goal of these modifications were focused on sea turtles, the performance of the Coonamessett Farm Turtle Deflector Dredge (CFTDD) with respect to the sea scallops and finfish bycatch is critically important if the modifications are to be considered for the fishery. A series of paired-tow comparative gear experiments were conducted to assess the relative performance characteristics of the CFTDD in relation to the standard New Bedford style scallop dredge. Of critical interest is the magnitude of any differences in the overall catch rates and size selectivity for both scallops and the major finfish bycatch species. Results indicate that the two dredge designs performed equivalently with respect to sea scallops and most species of finfish, supporting the implementation of the CFTDD as an acceptable sea turtle bycatch mitigation approach. These results will be discussed within the context of a suite of complimentary bycatch mitigation strategies employed by sea scallop fishery managers.

HUMAN PERCEPTIONS AND ATTITUDES REGARDING GEODUCK AQUACULTURE IN PUGET SOUND, WASHINGTON: A Q-METHOD APPROACH.

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Recent growth and planned future expansion of geoduck (*Panopea generosa*) aquaculture is causing disagreement and conflict among some coastal stakeholders. The resulting dispute has been fueled by rhetoric and a lack of peer reviewed data focused on impacts of geoduck culture methods in nearshore ecosystems. While work is underway to understand environmental impacts, work has not been done that can provide structure to the social dimensions of this controversy. This research explores the subjective perceptions and attitudes of six sub-groups of coastal stakeholders involved in geoduck aquaculture, including; state/local regulators, scientists, shellfish growers, tribes, landowners and non-governmental organizations. The goal is to elicit prevailing social constructions and to see how these differ across

stakeholder groups and research sites. To give structure to those constructions, procedures are being implemented collectively known as Q-methodology.

TEMPERATURE AND PH AS DRIVERS OF OYSTER LARVAL SURVIVAL OVER 50 YEARS OF FIELD DATA.

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Pacific oysters (*Crassostrea gigas*), introduced a century ago, dominate cultured oyster production on the US west coast. The distribution of established populations is well-known to hinge on sufficiently warm temperatures for reproduction. At the same time, within established populations, settlement can vary by orders of magnitude interannually, and this variability has been suggested to arise from a suite of abiotic and biotic factors that influence larval performance. Here we focus on mean water temperature, variability in water temperature, and pH as environmental factors with physiological importance, tracked in the field over 50 years, and predicted to change due to anthropogenic activities. Compared to data from the 1960s, recent water temperatures are warmer and pH values lower in river-influenced regions of Willapa Bay, Washington, USA, whereas no trend was evident closer to the ocean. Over this time period, larval mortality rates varied from –0.12 to –0.34 d^{-1} with no clear trend. Nevertheless, high mortality rates were often associated with temperature variability, pointing to the role of weather events, not only climate trends, in near-term forecasts relevant to bivalve aquaculture.

DETECTION OF pH SHIFTS IN THE SOUTH SLOUGH ESTUARY (OREGON, USA): POTENTIAL RELATIONSHIPS BETWEEN RESPIRATION, NET ECOSYSTEM METABOLISM, AND CHANGING CARBONATE CHEMISTRY.

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Time-series analysis reveals a long-term directional shift in pH measurements within the tidal waters of the South Slough estuary (Oregon, USA). Dataloggers operated by the South Slough NERR documented a shift toward increased medium pH values over the period of 2002–2010, and the shift in pH values was consistent at four locations located along the estuarine gradient. Median pH values shifted from 7.9 in 2002 to 8.1 in 2010 within the marine-dominated region, from 7.4 to 8.0 within the mesohaline region, and from 7.2 to 7.4 in the riverine region. Substantial diel variability in estuarine pH values occurs with fluctuations over 0.4 pH units in a single day, and estuary pH values are directly correlated with concentrations of dissolved oxygen. An adjacent eelgrass bed exhibited slight increases in density and spatial cover, but we did not observe concurrent increases in water-column Chl-*a* concentrations over the period of 2004–2010. These observations indicate that net ecosystem metabolism of the estuary is currently dominated by production, and increased metabolism may buffer the local effects of acidification. The trend toward increased alkalinity in the estuary is influenced by many factors including freshwater inputs, tides, photosynthesis/respiration, salinity, carbon cycling, nutrient availability, denitrification, and ocean acidification.

POPULATION RECOVERY, HABITAT ENHANCEMENT, AND REPRODUCTIVE ECOLOGY OF OLYMPIA OYSTERS (*OSTREA LURIDA*) IN COOS BAY, OREGON.

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Olympia oysters (*Ostrea lurida*) became locally extinct in Coos Bay (OR) prior to written history in response to a combination of coastal fires, sedimentation, burial, and perhaps a large-scale tsunami. Individuals of *O. lurida* were returned to Coos Bay in the 1950's when they were inadvertently re-introduced as hitchhikers during commercial mariculture of Pacific oysters (*Crassostrea gigas*), and the population was re-established by the late 1980s. However, it is likely that basin-wide re-colonization of *O. lurida* has been hampered by several limiting factors including habitat loss and alteration, dredging, decreased availability of shell substrata, diminished recruitment, predation, competition, and ecological interactions with native and non-native species. Recent efforts have been initiated to re-establish and enhance populations at several sites in Coos Bay, and new research is underway to investigate the reproductive biology and ecology of local Olympia oysters as factors that regulate population recovery. Enhancement actions have included deployment of larval collector bags, import of Olympia oyster spat, placement of *C. gigas* shell-and-rock rubble, and experimental development of modular Oly-ROCS (cement paving stones with *O. lurida* shell fragments). Pedi-veliger larvae of *O. lurida* also frequently settle on the shells of living Pacific oysters which are cultured extensively throughout the tideflats.

SETTLEMENT PREFERENCE AND THE TIMING OF SETTLEMENT OF THE OLYMPIA OYSTER, *OSTREA LURIDA*, IN COOS BAY.

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Populations of the native Olympia oyster (*Ostrea lurida*) were decimated by overharvesting. Full-scale recovery appears to be limited by a lack of available hard substrata for larval settlement

and Pacific oyster shell is often added. I investigated whether providing live oysters or conspecific shell could enhance settlement. Settlement was monitored on four substrata: live *O. lurida*, *O. lurida* shell, live *C. gigas* and *C. gigas* shell. Settlement occurred from August-December with a peak in October. I found no significant difference between conspecific shell and Pacific oyster shell nor between live oysters and dead oyster shell. There was significantly higher settlement on bottoms of horizontal substrata compared to the top. Larval choice experiments in the laboratory showed no trends in preference among hard substrata. The lack of settlement preference has positive implications for restoration projects since Pacific oyster shell is much easier to obtain and seems to be no less beneficial.

CORRELATING WATER CHEMISTRY PARAMETERS TO LARVAL OYSTER (*CRASSOSTREA GIGAS*) GROWTH AND SURVIVAL IN A PRODUCTION HATCHERY SETTING.

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A monitoring network was established at the Taylor Shellfish Hatchery in Quilcene, WA to track fluctuating water chemistry in and around the hatchery, as well as oyster (*Crassostrea gigas*) growth and survival data. The data collected through this network was used to both to correlate changing water conditions to varying oyster performance, and to characterize water chemistry at the hatchery. Preliminary analysis has shown consistent water conditions as well as consistent larval performance. While this made it difficult to correlate these data sets, what will be key in going forward are both the monitoring network that was established, and the process that was developed to analyze and characterize the collected data. When changes do arise, the Taylor Shellfish Hatchery will be well situated to characterize these changing waters and determine their effects on *C. gigas* growth and survival.

MICROBIAL SOURCE TRACKING IN COASTAL WATERS.

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Fecal contamination is the main source for waterborne pathogens in surface waters. Consequently, water quality objectives were formulated with regards to the occurrence of gastrointestinal bacteria as indicators. Due to high release concentrations these bacteria were thought to be an easy detectable representation of the many possible waterborne pathogens that pose a direct or indirect risk to human health. However, research has shown that these traditional fecal indicator bacteria, namely total coliforms, *Escherichia. coli*, fecal coliforms, and *Enterococci* are not only able to survive outside of their native environment but can even reproduce under certain conditions. Thus, *Bacteroidales* based microbial source tracking (MST) assays represent a viable alternative to detect recent fecal contamination. Rapid molecular methods targeting these strictly anaerobic bacteria have the additional advantage to be selective for various hosts and can thus indicate for example human or bovine sources. The method can be used as a valuable management tool in various situations along watersheds and coastal areas. The results of *Bacteroidales* based MST application for coastal waters will be presented.

TRIBAL MANAGEMENT OF WILD SHELLFISH ON THE WASHINGTON COAST: DUNGENESS CRAB AND PACIFIC RAZOR CLAM MANAGEMENT CHALLENGES.

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Tribes with treaty rights on the coast of Washington State co-manage fisheries resources within their treaty ocean areas and adjacent shorelines. Two shellfish species of great cultural and economic importance to the Quinault Indian Nation are Dungeness crab, *Cancer magister*, and the Pacific Razor Clam, *Siliqua patula*. Both species are seasonally managed as wild stocks with no population enhancements. Some current challenges to conducting and maintaining these fisheries include accurate population estimation, annual recruitment and survival of clam and crab juveniles, harmful algal bloom toxins, ocean acidification, hypoxia events and anthropogenic inputs. The importance of proper management of these two species is critical as a loss of access to either would be a devastating blow to the Quinault Nation.

POPULATION DECLINE AND RESTORATION OF SOFT-SHELL CLAMS IN CHESAPEAKE BAY: ROLE OF PREDATION, HABITAT, DISEASE, AND ENVIRONMENTAL FACTORS.

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We examine the causes of the decline in soft-shell clam, *Mya arenaria*, populations in Chesapeake Bay. The population of *M. arenaria* in Chesapeake Bay has been declining for several years with a severe decline in the 1990s to record low levels that have been sustained to the present. The population decline is likely due to multiple factors including: recruitment, predation, habitat loss, disease, and overfishing. We address influential factors sequentially, focusing on predation by crabs and cownose rays, and subsequently on habitat (structured vs. unstructured), disease, and environmental factors. We know that recruitment of *M. arenaria* in certain systems within Chesapeake Bay has continued to be high,

yet survival is low in all but those habitats with sufficient structure to protect clams from predation (e.g., seagrass). We know that disease may also be an important factor in population dynamics, particularly in recent years, as *Perkinsus chesapeakei* has reached epidemic levels in *M. arenaria*. Thus far, broad-scale sampling has revealed several species of bivalves, with variable *M. arenaria* densities and the presence of disease in upper-Bay locations. Long-term predator densities are negatively correlated with *M. arenaria* densities, suggesting that predation influences *M. arenaria* densities over the long term.

CAUSES OF VARIATION IN THE ABUNDANCE OF RETURNING *CANCER MAGISTER* MEGALOPAE AND THE SIZE OF THE COMMERCIAL CATCH.

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Annual return of *Cancer magister* megalopae has been followed for 11 years (1997–2001, 2006–2011). Megalopae were caught daily with a light trap fished in Coos Bay, Oregon. Trapping begins in early spring and continues into fall. During the first 6 years the number of returning megalopal varied with the date of the spring transition (early transitions = more megalopae) and megalopal abundance was strongly correlated with commercial catch. In 2007 through 2009 megalopal abundance jumped by >20 times. This step change in megalopal abundance appears to be due to a shift in the Pacific Decadel Oscillation (PDO); high catches have occurred during years with negative PDOs. In 2010 and 2011 catches were much lower than expected. In these years, shortly after the spring transition upwelling stopped and did not resume for weeks. The number of returning megalopae appears to be related to three factors, the PDO, the spring transition date, and the amount of upwelling in spring/early summer. Through 2006, the abundance of megalopae was linearly related to the commercial catch, but with the recent spike in megalopal abundance this relationship has become non-linear; density dependent mortality following settlement has become more important.

PATHOGEN AGGREGATION: UNDERSTANDING WHEN, WHERE, AND WHY SEAFOOD CONTAMINATION OCCURS.

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Contamination of seafood with pathogens depends not only on their presence in harvesting waters, but also on the physical state of harmful microorganisms in the water column. Specifically, whether or not pathogens are present as individual particles or attached to aggregates can determine their transport to, and eventual fate within shellfish. Macroaggregates (also known as ''marine snow'') are ubiquitous in aquatic environments, and their significant role in biogeochemical processes has been recognized for decades. Yet, only recently studies have begun to explore the potential role of aggregates in disease transmission. Unlike freely suspended particles in the water column, pathogens that are attached to aggregates are likely to experience enhanced settling velocity rates, and entrained within organically rich matter they are more readily ingested by invertebrates such as shellfish. Initial laboratory experiments by our research team demonstrate that protozoan pathogens, bacteria, and viruses are likely to aggregate in estuarine and marine waters more readily than in freshwaters. Ongoing studies will provide further insight on the attachment of pathogens to aggregates in environmental waters, with a future goal of providing science-based recommendations for water quality monitoring strategies and shellfish growing practices that target seafood safety.

EFFECTS OF TEMPERATURE ON FEED INTAKE, GROWTH AND OXYGEN CONSUMPTION OF THE RED KING CRAB *PARALITHODES CAMTSCHATICUS* HELD IN CAPTIVITY AND FED MANUFACTURED DIETS.

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The red king crab (*Paralithodes camtschaticus*) was introduced into Norway in the 1960's and a commercial fishery has now been established with a TACC of 1200t of male crabs. A live export industry for king crabs is being developed in Norway which will rely on the ability to hold king crabs in landbased facilities for extended periods whilst maintaining, or increasing, their meat content and quality. The current study investigates whether it is possible to increase the meat content of captive king crab (average = 2,2 kg) by feeding manufactured diets at different temperatures (4, 8 and 12°C). A 120 days trial was undertaken with groups of king crabs held in 12 land-based holding tanks. Feed intake increases with increasing temperature from 1 gram per kg animal at 4°C to 2.8 gram feed per kg animal at 12°C. The meat content increased in all temperature groups and there were no significant differences between temperature groups at the end of the experiment. Oxygen consumption was significant affected by the temperature and increased with increasing temperature.

EFFECTS OF SIZE AND TEMPERATURE ON GROWTH, FEED INTAKE AND FEED CONVERSION OF JUVENILE SEA URCHINS (*STRONGYLOCENTROTUS DROEBACHIENSIS*).

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Juvenile green sea urchin from 0.5 g to 30 g were held in individual compartments at four constant temperature regimes (6, 10, 12 and 14°C) ($n = 54$ per temperature treatment) for a period of 100 days. There was no mortality throughout the experimental period. Temperature group 6 had the significant lowest specific growth rate (SGR) (% body growth per day). There were no significant differences in specific growth rate (SGR) between temperature groups 10, 12 and 14°C. Temperature had a significant effect on feed intake (FI) (g feed per animal pr week) in relation to body weight and temperature. Feed conversion (FCR) (grams of feed used to increase the body wet weight by one gram) was significantly affected by temperature with sea urchins having significantly better FCR values at 10°C (2.2), followed by temperature group 6°C (3.2), 12°C (7.02) and 14°C that had the highest FCR of 8.8. Inside the temperature groups, FCR was size depends, with decreasing FCR with increasing size. In conclusion, the present study showed that temperature has significant effects on growth, FI and FCR of juvenile green sea urchin. A rearing temperature of 10°C appears to be the optimal temperature when taking into account both feed conversion and growth.

A PLAN TO GROW THE MUSSEL FARMING INDUSTRY IN THE NORTH EAST UNITED STATES.

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In 1975 a Sea Grant helped establish Abandoned Farm of Maine as the first mussel farm in North America. In 2010 farmed mussel production in New England was less than 450 metric tons and imports from Atlantic Canada were 12,690 metric tons. The market for live mussels in the U.S. is growing from 10% to 20% per year. The recently formed Mussel Marketing Council of North America will further drive demand. Near shore production in the U.S. is constrained by regulation and user conflicts. Recent research by Rich Langan at the University of New Hampshire and Scott Lindell at the Marine Biological Laboratory in Woods Hole, MA has demonstrated that an offshore mussel farming industry is technically possible. There is a regulatory abyss that confronts the development of this industry. It will take creative thinking, bold action and undaunting courage from both the public and private sector to make offshore mussel farming a reality. The upside is jobs, a raw material to support vibrant working waterfronts, a green industry providing positive ecological services, food security, an offset to the seafood trade deficit, and a wholesome ''locally'' produced food for the American consumer. A five year, 3500 metric ton plan is proposed.

STATUS OF *PERKINSUS* SPP. IN OYSTERS *CRASSOSTREA RHIZOPHORAE* AND *C. BRASILIANA* FROM BRAZIL: FIRST REPORT OF *P. MARINUS*.

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Perkinsiosis is a major constraint for wild and cultured bivalves worldwide. We provide a recent update of the status of the genus *Perkinsus* in Brazil, with data on prevalences and intensities, pathologies, *in vitro* proliferation, and molecular analyses. Until 2008, nothing was known about the occurrence of *Perkinsus* spp. infecting bivalves from Brazil. The first result showed a *Perkinsus* sp. infection in oysters from the estuary of Pacoti River (Ceará State). One year later (2009), *Perkinsus* sp. was detected in cultured and wild oyster populations from the estuary of São Francisco River (Sergipe State, SE). During 2010, oysters were studied for pathologies in estuaries of the Maraú and Graciosa Rivers of the southern Bahia State (BA), and were shown to harbor *Perkinsus* sp. In 2011, *Perkinsus* sp. was detected in two estuaries (Paraíba, and Mamanguape) of Paraiba State. Infected oyster species were genetically identified as *C. rhizophorae* and *C. gasar*. Prevalences were high for most locations (35%–90%), except Ceará (5%). *In vitro* cultures were established. Analyses of rDNA ITS and LSU sequences suggest a diverse group of *Perkinsus* spp. infecting Brazilian oysters, including *P. beihaiensis*-like, *P. olseni*-like, an unidentified *Perkinsus* sp., and *Perkinsus marinus*.

SIGNS OF EXTRAORDINARY MORTALITY OF GEODUCK CLAMS (*PANOPEA GENEROSA*) AT SITES IN PUGET SOUND, WASHINGTON.

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The wild geoduck clam fishery in Washington State began in 1970 and commercial landings and ex-vessel values are now at an all-time high (2150 mt and $US 36.1M). Recent state auction prices for geoduck clams have exceeded $17.60 per pound, and the high value is an incentive to illegally harvest geoduck clams. Scuba surveys are conducted on established discrete commercial harvest "tracts", which provide for site-specific time series estimates of changes in abundance. Six tracts in southern Puget Sound have been surveyed multiple times following commercial harvest, and the rates of recovery of density to pre-fishing levels range from –0.035% to 5.19% per year. Rates of recovery have changed in the last decade, and the abundance on 3 of the 6 surveyed tracts in this study has recently declined. During the same time frame, the abundance at two geoduck index stations has been stable or has increased. In southern Hood Canal a decrease in abundance on certain unfished tracts indicates extraordinary mortality that may be attributed to low dissolved oxygen events or other environmental factors. Management recommendations including evaluation of sustainable harvest rates are discussed.

PRODUCTION CAPACITY OF ESTUARINE ECOSYSTEMS AND FILTER FEEDER STOCK SIZE: INDICES FOR UNDER- OR OVERGRAZING.

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For extensive shellfish culture, the trophic capacity of the culture system is a major production factor and this is determined by the primary production, the flux of food to the culture sites and the role of competing filter feeders. Various control mechanisms play a role in these processes: bottom-up control of primary production may shift to top-down control at increasing filter feeder stocks. Nutrient limitation, hence bottom-up control, may decrease through enhanced nutrient regeneration, while increased grazing pressure will reduce phytoplankton biomass to the benefit of picoplankton and macro-algae. To evaluate the production capacity of a given ecosystem, as well as the potential and the impact of expanding shellfish culture, there is a need for simple parameters, in addition to the development of more sophisticated simulation models. Efforts have been made to develop indices for shellfish culture capacity evaluation, such as the ratios of clearance time, residence time, primary production time and nitrogen turnover time, and the ratio between pico- en microphytoplankton. These indices are now further tested in a project in Dutch coastal waters to evaluate the impact of increasing the mussel biomass in conjunction with a massive invasion of exotic oysters and razor clams; the outcomes will be discussed.

HOW TO BENEFIT FROM THE SERVICES OF SHELLFISH TO THE ECOSYSTEM?

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Shellfish communities are nowadays recognized as a key factor in processes and structures of coastal ecosystems. Due to their large abundance, high filtration capacity, reef building capacities and their link between lower and higher trophic levels, they provide various goods and services to the ecosystem. However, an integrated approach for coastal zone management that fully benefits from these services is still a challenge. So far, shellfish functions have been in use for seafood production, nature conservation, ecosystem restoration, water quality remediation or coastal protection. In various cases, functions like exploitation and conservation are considered as antagonistic. Yet, sustainable exploitation can be defined as shellfish culture that also contributes to other societal goals, and for shellfish this is not difficult to imagine. An analysis of the goods and services they deliver is a helpful tool to illustrate this point. Moreover, there is synergy to be achieved by an integrated approach. This is particularly relevant for coastal protection in combination with shellfish eco-engineering and sustainable exploitation. It is evident that global costs for coastal protection of low-lying areas against sealevel rise will be tremendous. The options to combine these costs with direct economic use need to be sorted out. Shellfish beds have the capacity to both contribute to coastal infrastructure, to improve water quality, to serve as food for birds as well as to be exploited by man. Various ways to improve the benefits of these features will be discussed.

CULTURE AND GROWTH COMPARISONS BETWEEN MULTIPLE STRAINS OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* IN HEEIA AND KEAWANUI FISHPONDS, HAWAII.

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Growout trials in 2009-2011 demonstrated that traditional Hawaiian fishponds are ideal sites for *Crassostrea gigas* culture. Now that Hawaii is poised the brink of beginning an oyster culture industry, determining whether particular oyster strains perform better is key to starting the industry off on the right foot. Five strains, some obtained from the OSU MBP were tested: $Adam_2$ w/ $Adam_2$ ($A_2 \times A_2$), $Adam_1$ w/ Eve_1 ($A_1 \times E_1$), Eve_2 w/ Eve_1 ($E_2 \times E_1$), naturalized oysters from Oahu (O), and Midoris

(M), from Japan. The results of these trials showed that larvae (24 hrs post-hatch) from all strains averaged the same size immediately after spawning, reaching between 50 to 85 microns (m) on the first day. At four months of age, all four strains have shown successful growth reaching minimum lengths (DVM) of 1.5 cm; widths of 1.0 cm; and heights of 0.5 cm. Growout trials will continue for one year and data on the condition index will also be collected.

A RETROSPECTIVE ANALYSIS OF SUSTAINABLE OYSTER HARVEST FROM THE LOUISIANA STATE PRIMARY SEED GROUNDS, 1999-2010: A SHELL-NEUTRAL MODELING APPROACH.

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The Louisiana Department of Wildlife and Fisheries manages nearly 1.7 million acres of public water bottoms for the cultivation of the eastern oyster, *Crassostrea virginica*. The Department sets seasons, monitors harvest, and plants cultch. Currently, annual stock assessments, combined with best professional judgment, are used to both inform management and to predict the success of the upcoming oyster season. While these data provide crucial information for tracking oyster stock on an annual basis, present management has no established biological reference point and consequently no criterion by which sustainable harvest can be estimated. A numerical model is presented which defines a sustainability criterion as no net loss of shell, and calculates a sustainable harvest of seed and sack oysters. Stock assessments of the Primary State Seed Grounds conducted east of the Mississippi from 1999–2010 show a trend toward decreasing abundance of sack and seed oysters. Retrospective simulations provide estimates of annual sustainable harvests. Comparisons of simulated sustainable harvests to actual harvests show a trend toward unsustainable harvests toward the end of the time series. Stock assessments combined with shell-neutral models can be used to estimate sustainable harvest and manage cultch through shell planting when actual harvest exceeds sustainable harvest.

CHEMICAL AND PHYSIOLOGICAL MEASURES ON EASTERN OYSTERS FROM OIL-EXPOSED SITES IN LOUISIANA.

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On April 20th, 2010, an explosion on the Deepwater Horizon oil well, located 80 km off the coast of Louisiana, triggered a human, economic, and environmental disaster. When the well was capped on July 15, 2010, nearly 5 million barrels of oil had been released into the offshore waters. Oil laden waters advected into the estuaries and demonstrated the risk of offshore oil activities to inshore oyster populations. Potential lethal and sub-lethal effects of oil from the Deepwater Horizon spill to oysters (*Crassostrea virginica*) in Louisiana east of the Mississippi River were examined along a biophysical gradient of oil pollution, salinity, and disease. Approximately 6 months after the capping of the Deepwater Horizon wellhead, no polycyclic aromatic hydrocarbons were detected in oysters from oil-exposed sites. Variations in oyster condition and reproductive state, and infection with the oyster parasite *Perkinsus marinus* are consistent with natural differences along the salinity gradient and not with impacts of polycyclic aromatic hydrocarbon contamination. Although no impact was observed in this study, we caution the over application of the results of this spatially and temporally limited study to other areas and other times where impacts from the Deepwater Horizon spill may have occurred.

ROTATIONAL HARVEST IN THE RAPPAHANNOCK RIVER, VIRGINIA: A REVIEW OF PROGRESS TO DATE.

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Ransom Myers once described rotational harvest as a ''fisheries management strategy robust to ignorance.'' (Can. J. Fish. Aquat. Sci. Vol. 57:2357-2362, 2000). We present a history of rotational harvest management of oysters in the lower Rappahannock River, in the Virginia portion of the Chesapeake Bay and pose the question: does it work? In 2007 a collaboration between industry, academia, non governmental organizations, and regulators agreed to adopt a three year rotational harvest protocol using six geographic areas in the lower river, with two of the six areas open each year, and an area lying ''fallow'' for two years after a harvest period. We review the success of this program in terms of the relationship of annual assessments to eventual production, and options to enhance production in specific time frames and areas through shell substrate and/or spat on shell addition.

MAXIMIZING ALGAE GROWTH FOR LARVAE AND SEED PRODUCTION.

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Aquaculture provides more fish for human consumption than wild harvested fish, and in the last 20 years, the yield of fish meal has been essentially constant. [Barrows, USDA Agricultural Research Service, ABS, 2011] This makes the need for efficient growth of algae for larvae and seed production essential. Taylor Shellfish and BioVantage have experimented with the growing of high-density algae in 200 L and 600 L photobioreactors and raceways. This algae is then used for further growth in either batch (tank) or continuous (bag) aquaculture systems. Data will be shared on the optimum growth of the algae, represented as millions of cells per day of algae grown. Variables experimented with include various light wavelengths, nutrient inputs and variability, CO_2 and pH values. In addition, data on algae growth efficiency based on initial inoculum densities, such as from bubble columns, will be shared. Data from species that are key in the aquaculture industry, such as *Isochrysis* will be shared, as well as reproducibility data.

IDENTIFICATION AND MAPPING OF GROWTH-RELATED QTL USING MICROSATELLITE AND AFLP MARKERS FOR THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*.

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Traditional quantitative trait locus (QTL) mapping strategies use crosses among inbred lines to create segregating populations. Unfortunately, even low levels of inbreeding can depress economically important quantitative traits in the Pacific oyster, potentially complicating subsequent QTL analyses. To circumvent this problem, we constructed an integrated linkage map, consisting of 65 microsatellite (18 of which were previously unmapped) and 212 AFLP markers using a full-sib cross between phenotypically differentiated outbred families. We identified 10 linkage groups spanning 710.48 cM, with an average genomic coverage of 91.39% and average distance between markers of 2.62 cM. We identified 12 QTLs and 5 potential QTLs in the F1 outcross population of 236 full-sib Pacific oysters for four growth-related morphometric measures (individual wet live weight, shell length, shell width and shell depth measured) at four post-fertilization time points (plant-out, first year interim, second year interim and harvest). Mapped QTLs accounted for an average of 11.2% of the total phenotypic variation, ranging between 2.1 and 33.1%, and were not randomly distributed across the genome. We conclude that alleles accounting for a significant proportion of the total phenotypic variation for traits that influence harvest yield remain segregating within broodstock of West Coast Pacific oyster selective breeding programs.

THE SEARCH FOR NORTH PUGET SOUND OLYMPIA OYSTER BROODSTOCK: A SURVEY OF THREE NEWLY REPORTED EXTANT POPULATIONS AND THE RESTORATION EFFORT LOCATED IN FIDALGO BAY.

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The Olympia oyster, *Ostrea lurida*, is the only oyster species native to the Pacific Northwest. Ecological benefits provided by oysters as well as the species' historical significance has motivated numerous restoration efforts. Research has recently provided evidence of substantial genetic population structure among extant Olympia oyster populations. As a result, restoration projects should consider using genetics to monitor and evaluate the success of their efforts. We used microsatellite markers to survey 508 Olympia oysters collected from two North Hood Canal (Bywater and Dabob Bay) and one Strait of Juan de Fuca (Discovery Bay) extant populations and one North Puget Sound restoration effort (Fidalgo Bay) with the goal of identifying suitable broodstock for augmenting existing restoration-based projects. Fidalgo Bay samples included natural set in addition to original hatchery-produced plant-out oysters. We observed no significant genetic differentiation between Hood Canal and Discovery Bay sites. As expected, significant genetic differentiation between all extant populations and the original Fidalgo Bay plant-out were found. We did not, however, find any evidence that the Fidalgo Bay natural set originated from the original hatchery-produced plant-out. Although the new Fidalgo Bay recruitment appears to originate from an unidentified population, it is a valuable, naturally occurring broodstock source.

CONSTRUCT–CREATING OYSTER NICHE STRUCTURES THROUGH RESTORATION USING CRAB TRAPS.

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Abandoned crab traps are a major source of marine debris in coastal South Carolina waters, causing "ghost fishing" mortality of both target and non-target species, visual pollution, and damage to

sensitive habitats. Previous work at the SCDNR has shown that crab traps are an effective alternative substrate for establishing restored and enhanced oyster reef habitat, particularly in "pluff mud" environments where more traditional approaches (e.g., planting loose shell) are less effective. The primary goal of the CONSTRUCT project is to establish a crab trap revitalization program that converts marine debris with negative ecological and aesthetic impacts into structurally complex habitat that supports shellfish and finfish populations. To date, 365 crab traps that would otherwise have been destined for the landfill have been collected through the combination of direct retrievals from the marine environment and donations from commercial crabbers and the general public. In the spring of 2011, with the help of volunteers, a total of 216 cement coated crab traps were deployed at 8 sites along the South Carolina coast. Preliminary data will be presented here on reef "footprints" collected quarterly using photographic methods to estimate oyster coverage on the reefs and to assess the relative success of different project sites.

MICROSATELLITE PARENTAGE ASSIGNMENT INDICATES HIGH VARIANCE IN REPRODUCTIVE SUCCESS AND DECREASED GENETIC DIVERSITY IN CULTURED GEODUCK, *PANOPEA GENEROSA*.

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Aquaculture for the Pacific geoduck (*Panopea generosa*) is a small but expanding industry in Washington State, USA, where geoducks are native and genetic interactions between wild and cultured geoducks are likely. To examine the potential genetic implications of geoduck aquaculture, we used five microsatellite loci to compare genetic diversity in wild geoducks and cultured geoducks produced in two hatcheries with different culture protocols. We estimated effective number of breeders (N_b) using two demographic and three genetic methods. Cultured populations were characterized by reduced heterozygosity, allelic richness, and N_b and increased mean pairwise genetic relatedness. Parentage assignment revealed that genetic diversity and N_b was affected by large variance in reproductive success; though many parents contributed to most seed cohorts, single full-sib families comprised from 11% to 94% of the offspring. Although both hatcheries produced geoduck seed with reduced genetic diversity compared to wild geoducks, this difference was far more pronounced in one of the hatcheries. Our results demonstrate that hatchery practices affect genetic diversity in their progeny and may aid in developing geoduck breeding practices that reduce risk to wild populations.

SHELLFISH SAFETY: DEVELOPING AN EARLY WARNING SYSTEM FOR BIOTOXINS AND PATHOGENS.

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Public health concerns and the economic impacts of harvest closures from marine biotoxins and pathogens significantly affect the West Coast shellfish industry. One major impediment to safe shellfish is the limited availability of predictive tools to forecast toxic harmful algal blooms (HABs) and the presence of marine bacterial pathogens such as *Vibrio parahaemolyticus*. NOAA's Northwest Fisheries Science Center is using a multidisciplinary approach to develop an integrated early warning system for HABs and *V. parahaemolyticus*. Such a system will utilize weather forecasts and climate modeling, measurements of environmental parameters such as temperature and salinity, an existing monitoring network for HABs in Puget Sound (SoundToxins), pathogen genetics, and a novel biosensor instrument called the Environmental Sample Processor. It is envisioned that such a system will help shellfish growers and public health managers to better forecast potential HAB and pathogen presence, and allow proactive decisions for harvest closures prior to any illness from shellfish consumption.

EXTENSION'S ROLE IN THE DEVELOPMENT AND SUSTAINABILITY OF A SMALL-SCALE HARD CLAM AQUACULTURE INDUSTRY ON FLORIDA'S GULF OF MEXICO COAST.

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The commercial hard clam *Mercenaria mercenaria* culture industry on Florida's Gulf of Mexico coast traces its roots back to job retraining programs for underemployed fishermen during the mid-1990's. Resulting efforts by Harbor Branch Oceanographic Institution, the University of Florida, and Florida Sea Grant helped in the successful development of a viable industry. Educational efforts in these programs focused on production techniques for the hatchery, nursery and growout phases of the culture process. Early extension efforts were directed toward specific hurdles found in the path of the developing industry, such as seed production, product quality, industry organization, and risk management. This presentation focuses on current integrated research and extension activities addressing the sustainable development of hard clam aquaculture by increasing yield, farm efficiency and profitability. Specific programmatic areas are advancement of management practices, genetic stock improvement, and species diversification. Examples of successful industry-driven projects, such as access to "real-time" water quality and weather information at lease areas and evaluation of the culture and market potential of the sunray venus clam *Macrocallista nimbosa*, will be provided. Further, annual workshops allow

faculty to present findings and progress on these projects as well as obtain feedback and direction from the industry.

IMPROVING HARD CLAM PRODUCTION IN FLORIDA THROUGH CULTURE OF BACKCROSSED HYBRIDS (*MERCENARIA MERCENARIA*, *M. CAMPECHIENSIS*).

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The need for a hardier clam strain has become evident as shellfish growers in Florida report below average survivals or total losses during prolonged hot summers. The local southern quahog *Mercenaria campechiensis* may have suitable production characteristics for Florida environments and readily hybridizes with the northern hard clam *M. mercenaria*, but gapes during refrigerated storage. We previously reported on parental species and their hybrid crosses. Differences in hybrid performance indicated that backcrossing of hybrids to the northern hard clam may improve some measures. Five families of backcrossed hard clams were produced using multi-parent crosses from hybrid and parental stocks. No differences were noted between stocks during larval and nursery culture in the hatchery. In field nursery, survival of backcross stocks (71–82%) was greater than hard clam controls (65%). At harvest, 66% of backcross stocks yielded higher survival (81–91%) and production (34–38 kg/bag), compared to hard clams (79%, 31 kg/bag). After 10 days in refrigerated storage, survival of backcross stocks (97–99%) was similar to hard clams (100%). Although gaping was higher in the backcross stocks (4–17%) versus hard clams (3%), these results are commercially acceptable. This breeding approach can increase summer survival and productivity of cultured hard clams while maintaining product quality standards.

GROWTH AND SURVIVAL OF THE EXOTIC HARD CLAM (*MERCENARIA MERCENARIA*) IN TOKYO BAY, JAPAN BASED ON SCLEROCHRONOLOGICAL ANALYSIS.

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The hard clam (*Mercenaria mercenaria*) was unintentionally introduced to Tokyo Bay, central Japan during 1990's. This species inhabits abundantly in the inner eutrophied environment of the bay. Recently, this species has been attracted by researchers' attention not only for fishery resource, but also its impact to native ecosystems. In order to obtain fundamentals for growth character in an introduced eutrophied tidal flat, we analyzed the growth pattern of the population at the intertidal flat in the innermost part of the bay during July 2010 to June 2011 by mark-recapture experiment. We also monitored seawater temperature and salinity by a logger and measured gonad index of monthly collected specimens by histological method. After the experiment, sclerochronological analysis was made for the lunar-day incremental sequence in the outer shell layer of each specimen. The result of our analysis revealed that in the clams smaller than 60 mm in shell length the daily growth rate was highest in September and thereafter decreased daily growth rate during late October to next March. Daily shell growth decreased when the gonad index was high. During the rainy season (June–July), clams sometimes stopped shell growth. We revealed that the hard clam could survive under a low salinity and high temperature environment at the Tokyo Bay.

WATER QUALITY MONITORING AT WASHINGTON STATE SHELLFISH HATCHERIES AND SETTING SITES.

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This presentation is a brief look at current and past water quality monitoring efforts at the Lummi Shellfish Hatchery and at remote setting sites in Willapa Bay, Washington. Real time data stations have been installed and upgraded at these sites to provide accurate and cost effective water quality data that is pertinent to growers, researchers and regulators. This data is accessible to anyone with internet access through the NANOOS data portal. Data from each site shows unique differences in when preferred water quality conditions (increased pH, oxygen and salinity) occur. Diel and seasonal fluctuations along with upwelling events that impact these areas also add clarity to yearly and daily trends. The back bone of these efforts, carbon sampling and analysis, give a solid base to further understand these estuarine systems. With this information and tools, acidified waters can be avoided to help grow shellfish in these areas while increasing our understanding of daily and longer-term water quality trends.

LOUISIANA OYSTER PRODUCTION: A TALE OF TWO VIEW POINTS.

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Since the early 1960's Louisiana's oyster landings has been dominated by private lease production, with over 71% during 2010, with fluctuations relative to seed abundance on the public

oyster grounds. The long term average (1960–2010) for private landings is 8.012 million pounds of meat versus 3.049 million pounds for public landings, at a value of approximately $35 million dockside and a total economic impact of about $300 million. Louisiana relies heavily on wild oyster seed. Seed planting onto private oyster grounds was estimated at approximately 22,000 sacks during the 2010–2011 season. Cultch planting by the state onto public oyster reefs during 2011 equaled just over 40,000 cubic yards at a cost of approximately $3.2 million. Natural oyster production on Louisiana's primary seed grounds has waned since 2004. This seed shortage is expounded by an average return of only 35% of the seed planted on private leases, based on a 2010 industry survey. In response, oyster aquaculture programming has focused on promoting private oyster nursery production utilizing hatchery-raised oyster larvae as a supplemental seed source, coupled with cage culture to improve production efficiency.

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

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A previous study has shown that triploidy improves yield and condition in the bay scallop *Argopecten irradians*. This technology could be what the industry needs to 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. From 2007 to 2010, triploid F_1 groups were produced each year with chemical induction. In the spring of 2008, 2009, 2010 and 2011, the F_1 triploid populations were tested to isolate a triploid broodstock. Approximately 5% of the triploid group showed some signs of sexual development. In 2008, 2009, 2010 and 2011 F_1 triploid broodstock were spawned producing variable amounts of eggs. The triploid eggs were fertilizedwith diploid sperm and treated to induce tetraploidy (F_2). Only the eggs from the 2008 spawn developed into larvae and survived passed the setting stage. Ploidy testing of this F_2 population at 1-2 mm revealed the presence of 5% of tetraploids in the F_2 group. This population did not survive overwintering. Attempts to replicate the 2008 results were unsuccessful.

EXPLORING RECOVERY TOOLS FOR PINK (*HALIOTIS CORRUGATA*) AND GREEN (*HALITOIS FULGENS*) ABALONES IN SOUTHERN CALIFORNIA.

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Populations of pink abalone (*Haliotis corrugata*) and green abalone (*Haliotis fulgens*) in Southern California, U.S.A. were over harvested up until the fishery closure in 1995. Since 2005, the recovery of these species and all other abalones has been guided by California's Abalone Recovery and Management Plan. Recovery to sustainable fishery levels will take many decades relying mostly upon natural recovery. Human interaction in the form of translocation, moving abalone together to form dense aggregations, may be used to increase the probabilities of spawning success in depleted areas, thus enhancing recovery. Green and pink abalone were tagged, translocated, and monitored on a small-scale level to determine their survival, persistence, and movement over two years. Abalone survival was high after the translocation and tagging process with mortalities of three percent for pink and two percent for green abalone. A higher percentage of translocated pink abalone remained on the sites compared to green abalone for the duration of the study. Although both species of abalone had high survival after the translocation and tagging processes, pink abalone exhibited higher rates of persistence and less movement than green abalone; therefore, pink abalone would be a stronger candidate for translocation as a recovery tool.

EFFECT OF CHLORAMPHENICOL AND GENTAMICIN ON THE LARVAL DEVELOPMENT OF THE GEODUCK CLAM, *PANOPEA GENEROSA*.

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Two independent experiments were carried out in the laboratory in order to test the effect of two antibiotics, chloramphenicol and gentamicin, on the growth and survival of larvae of the geoduck clam *Panopea generosa*. Straight-hinge through pediveliger larvae were grown in 6-L triplicate plastic containers at 17°C at four antibiotic concentrations: 0, 3, 6, and 9 mg L^{-1}. They were fed the microalgae *Isochrysis sp*. (clone T-ISO). There was observed a significant effect of chloramphenicol on the length of the larval period. Metamorphosis took place on the 25th day at 6 and 9 mg l^{-1}, while control larvae did not attain the metamorphic stage after 34 days. Growth rate of larvae treated with antibiotics (12 to 14 μm d^{-1}), and percent survival (83 to 93%) doubled that of the controls. Growth rate was not significantly different among gentamicin and control treatments, yet survival was highest in the treatment of 9 mg l^{-1} (69 ± 2%), followed by 6 and 9 mg l^{-1}. No significant difference was found between the survival of the control and the lowest

gentamicin concentration. It is concluded that chloramphenicol may be a potentially important antibiotic for improving the growth and survival of *P. generosa* larvae.

EFFECT OF CHLORAMPHENICOL AND GENTAMICIN ON THE GROWTH AND SURVIVAL OF GEODUCK CLAM (*PANOPEA GENEROSA*) LARVAE.

Sandra Tapia-Morales, Zaul Garcia-Esquivel.
Universidad Autonoma de Baja California, km 103 carretera Tij-Ensenada, Ensenada, BC, 22860, Mexico.

Two independent experiments were carried out in the laboratory in order to test the effect of two antibiotics, chloramphenicol and gentamicin, on the larval growth and survival of geoduck clams, *Panopea generosa*. Straight-hinge through pediveliger larvae were grown in 6-L triplicate plastic containers at 17°C and four antibiotic concentrations: 0, 3, 6, and 9 mg L^{-1}, and fed the microalgae *Isochrysis* sp. (clone T-ISO). A significant effect of chloramphenicol was observed on the length of the larval period. Metamorphosis took place on the 25th day at 6 and 9 mg l^{-1}, while control larvae were unable to metamorphose after 34 days. Growth rate (12 to 14 μm d^{-1}) and percent survival (83 to 93%) of larvae treated with chloramphenicol doubled that of the controls. Growth rate was not significantly different among larvae from the gentamicin and control treatments, yet survival was highest in the treatment of 9 mg l^{-1} (69 ± 2%), followed by 6 and 9 mg l^{-1}. No significant difference was found between the survival of control larvae and those from the lowest gentamicin concentration. It is concluded that chloramphenicol may be a potentially important antibiotic for improving the growth and survival of *P. generosa* larvae.

HARD CLAM WALKING: ACTIVE LOCOMOTION OF ADULT *MERCENARIA MERCENARIA* (LINNE, 1758) AT THE SEDIMENT SURFACE.

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Crawling of recently settled Venerid clams at the sediment surface is well known, but the literature suggests that for *Mercenaria mercenaria* >17 mm this type of movement is minor and inconsequential. Here, we document active locomotion of adult *Mercenaria mercenaria* (55–113 mm SL) at the sediment surface (here referred to as 'walking'), over significant distances, in a shallow water population at East Marion, New York. Walking clams were observed at this site in June-July of two consecutive years, as well as in October – at which times clams were aggregated. However, in August, when no walking clams were observed, clams were more randomly distributed. Rates of movement were documented via videotaping; mechanics of movement were the same in all clams: leading with the anterior end, clams rocked forward, with the umbo as much as 6 cm above the sediment surface. Gender ratio of walking clams was ~1:1; parasite loads of sampled clams were minimal. There was no consistent directionality of clam movement. We suggest, as has been done previously for several species of Unionids, that walking behavior serves to aggregate reproductively mature individuals in preparation for spawning – presumably so that the probability of fertilization success is increased.

USING MICRO-RAMAN SPECTROSCOPY TO ASSESS FUNDAMENTAL QUESTIONS ABOUT BIVALVE LARVAL SHELL FORMATION.

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Micro-Raman spectroscopy has been used in bivalve shell studies to investigate organic and inorganic shell components, yet most work has focused on adults. It is known that the organic matrix in larvae contains polyphenol compounds important for shell pigment, but less is known about the source of these molecules. We took Raman spectra of hatchery-reared larvae to assess how the inorganic:organic material ratio changes along a dorsoventral line. In laboratory experiments, we reared two species of bivalves in waters with different organic signatures to test if larvae incorporated compounds from source waters into their shells. Our preliminary results suggest that there are differences between the amount of pigment, but not the types of pigments, present along the larval shell. Pigment intensity was often strongest in growth bands. We could not resolve differences in spectra of individuals of the same species reared in different waters, but there were significant differences in spectra between different species. Organic material present in larval shells may be species-specific and not a function of environment, although further work is needed to verify this. With this information, it may be possible to identify unknown species in the wild, which could aid management and restoration efforts.

INDENTIFYING SPAWNING EVENTS OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, ON GEORGES BANK.

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The regional spawning patterns of the sea scallop, *Placopecten magellanicus*, on Georges Bank are unknown despite this aggregation being the largest wild scallop resource in the world. Current

scallop management employs a rotational system, which depends on recruitment and growth rates and assumes annual autumn spawning. However, semiannual spawning may have important implications for growth rate. This project will identify spawning events in scallop management areas Closed Area I (CAI) and Closed Area II (CAII) on Georges Bank. We hypothesize that spring spawning occurs in addition to autumn spawning. Scallops are collected during a monthly dredge survey in CAI and CAII, which began in March 2011 and will continue through March 2012. Gonads from frozen samples will be freeze-dried and dry gonad weight will be analyzed to identify significant differences between months. Preserved gonads will be examined using histological techniques to verify reproductive stage. Spawning events will be determined by a significant decrease in gonad weight between months and confirmed by histological examination. Bottom temperature will also be measured and analyzed to determine differences between the two areas. This study will contribute to an understanding of spawning events on a regional scale, which is important to effectively implement rotational management.

FINDING THE PHYSIOLOGICAL LIMIT OF EXPOSURE TO OCEAN ACIDIFICATION AND HEAT STRESS IN THE PACIFIC OYSTER.

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Wide-scale climatic changes are projected for the world's oceans over the next century, fundamentally changing the habitat of aquatic organisms. Among the projected changes for the coming century, ocean acidification and warming are expected to be two of the more dramatic alterations. Many invertebrates have physiological mechanisms to respond to ocean acidification, but the response is metabolically costly. Environmental stressors rarely occur in isolation, and exposure to multiple stressors can have synergistic effects. Juvenile Pacific oysters, *Crassostrea gigas*, were exposed to a range of pCO_2 (400–1400 µatm) for either 1 week or 1 month. After exposure to ocean acidification, the ability of the oysters to acclimate to a heat stress was tested. Differences in survival and changes in molecular physiology were compared across treatments. The results from this experiment shed light on how exposure to a primary stressor (ocean acidification) can affect an organism's ability to launch an effective physiological response to a secondary stressor (heat shock). Ocean acidification and other environmental shifts associated with climate change will create an aquatic environment characterized by novel stresses to marine invertebrates.

DIARRHETIC SHELLFISH TOXINS IN WASHINGTON STATE: A NEW THREAT TO THE SHELLFISH INDUSTRY.

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On June 25, 2011, three people became ill with suspected diarrhetic shellfish poisoning (DSP) after eating mussels from Sequim Bay, Washington State. To the north on Salt Spring Island, Canada, there were an estimated 60 illnesses from DSP in August 2011. This illness is caused through ingestion of shellfish that have accumulated toxins from the harmful algal bloom organism, *Dinophysis*, which produces a suite of toxins including dinophysistoxins and okadaic acid (OA). Approximately 2000 lbs of shellfish were recalled due to these poisoning events. A collaboration between the Washington State Department of Health and the SoundToxins partnership, which monitors phytoplankton and environmental factors in Puget Sound, resulted in a rapid response to increased abundances of *Dinophysis* at locations throughout Puget Sound to ensure the safety of shellfish to consumers. Analysis of data from Sequim Bay showed that *Dinophysis* cell abundances initially increased to 40,000 cells/L on June 6, reaching a maximum abundance of 84,000 cells/L on July 15. The dominant species was *Dinophysis acuminata*. Liquid chromatography mass spectrometry analysis of mussels collected on in June and July 2011 showed toxin concentrations above the FDA guidance level of 16 µg/100g shellfish with dinophysistoxin-1 as the primary toxin isoform.

STATUS OF THE COMMERCIAL RED SEA URCHIN (*STRONGYLOCENTROTUS FRANCISCANUS*) FISHERY IN WASHINGTON, USA.

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Washington's commercial red sea urchin fishery began in 1971. Fishery regulations limit harvest to diver hand-pick only and employ minimum and maximum size limits. Peak landings occurred in 1988, when annual statewide landings reached 3,658 mt, prompting the first emergency closure of the fishery. More active management followed with restricted seasons, limited entry, and utilization of a size-structured yield model to recommend harvest rates. Areal quotas are based on an array of

fishery-dependent and –independent estimates of biomass available from various sources. The fishery is considered to have been overexploited during the late 1980's and early 1990's and a progression of precautionary management steps have been implemented to promote stock recovery, including areal harvest closures. Results from recent surveys suggest that stock recruitment and abundance may be responding positively to lower harvest rates, but that recovery is punctuated, area-specific, and may be complicated by unaccounted for environmental factors. Since 2000, the market for Washington red sea urchins has diminished significantly; large in-state processing facilities have closed and annual harvest quotas are underutilized.

DISEASE-INDUCED FLUCTUATIONS IN BLACK ABALONE (*HALIOTIS CRACHERODII* LEACH, 1814) POPULATIONS OVER A 32-YEAR TIME SPAN AT SAN NICOLAS ISLAND, CALIFORNIA, WITH IMPLICATIONS FOR REPRODUCTIVE POTENTIAL.

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Black abalones (*Haliotis cracherodii* Leach, 1814) dwell in exposed rocky intertidal habitats between northern California, USA and northern Baja California, Mexico. US populations were listed as "endangered" in 2009, largely due to mortalities from the bacterial disease, withering syndrome (WS). Twenty-two surveys for black abalones were completed between 1981 and 2012 in nine permanent study locations on San Nicolas Island, California (SNI). WS-induced mortalitiesfirst occurred at SNI in 1992, causing a 99.2% reduction in numbers by 2001, with catastrophic mortalities observed at all study sites. Since 2002 black abalone numbers have increased at four study sites, producing a six-fold increase in Island-wide counts between by 2012. Numbers at the five remaining study sites have remained low since the late 1990s. Sites with positive trends in black abalone density show genetically-based resistance to WS and have potential in development of restoration strategies. Densities and nearest-neighbor distances indicate stability of aggregations even when populations were at minimal levels in 2001 and 2002, with two-thirds of abalones in close proximity or in direct contact with nearest neighbors when overall densities were depressed by WS effects. Aggregation behavior and patchy habitat attributes appear to facilitate substantial population reproductive potential despite disease-induced mortalities.

EFFECTS OF PREDATOR EXCLUSION STRUCTURES AS AGENTS OF ECOLOGICAL DISTURBANCE TO INFAUNAL COMMUNITIES IN GEODUCK CLAM AQUACULTURE PLOTS IN SOUTHERN PUGET SOUND, WASHINGTON, USA.

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Geoduck clam (*Panopea generosa* Gould, 1850) aquaculture is an expanding industry on intertidal beaches of southern Puget Sound. Culture operations involve several major phases, each constituting potential disturbance to resident infauna. Here we consider effects of placement of predator exclusion structures on infaunal communities by comparing assemblage dynamics in cultured plots and adjacent uncultured reference plots. Outplanted clams are protected by placement in PVC tubing covered with netting. Predator exclusion structures may disturb resident infauna by altering flow, modifying rates of sedimentation, altering detrital carbon concentrations in sediments, increasing hard substrata for use by sessile epibiota, reducing sunlight levels at the sediment surface, or altering activities of mobile macrofauna. We assessed effects of predator exclusion structures at three aquaculture sites over three years using infaunal dynamics as response variables. Infaunal data were obtained from sediment coring before, during, and after the predator exclusion phase, in cultured and reference plots. Preliminary results to date suggest significant but temporary alterations of infaunal patterns by structural presence, with differences in patterns of disturbances among study sites.

CASCADING ECOSYSTEM IMPACTS OF DREISSENID MUSSELS IN THE GREAT LAKES.

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During the early years of the dreissenid invasion of the Great Lakes, ecosystem impacts were often associated with nearshore areas or bays with some impacts seen in the offshore region because of shunting of C and P into nearshore communities. As the quagga mussel replaced the zebra mussel and moved onto soft sediments in deep water, the nearshore constraint was removed and whole basins or whole lakes such as Lake Michigan were impacted from

not just the nearshore but also from the deeper regions of the lake. This expansion has re-engineered nutrient and carbon flows, greatly reduced the phytoplankton food base during all seasons and dramatically increased light intensity in offshore regions leading to cascading, spatially complex interactions at all trophic levels. We believe increased light was a master variable for changing spatial coupling in the food web and altering the balance between vertebrate and invertebrate predation, especially that of the visually feeding, invasive predatory cladoceran *Bythotrephes* relative to planktivorous fishes. It is also possible the offshore expansion of quagga mussels onto softer sediments in the more eutrophic lakes will lead to greater promotion of harmful algal blooms over greater areas.

SIZE MATTERS: DIFFERENTIAL SETTING OF HATCHERY-REARED EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) LARVAE.

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At Horn Point Laboratory Oyster Hatchery in Maryland, we have informally observed differences in mean setting efficiency between larvae retained on different sized grading sieves. To verify this trend in a controlled environment, I placed *Crassostrea virginica* larvae retained on 200, 212, and 224 micron sieves in small scale setting vessels with conditioned tiles. I removed the tiles 24, 48, 72, 96, and 120 hours after larval introduction. I counted the number of spat to calculate the setting efficiency. Time and larval size were significant main effects, and there was no significant interaction. Overall, larvae retained on larger grading sieves had higher setting efficiencies; however, in general, within size treatments setting efficiencies did not significantly differ across time. These data suggest that the size of eyed larvae is important in spat production, even when larvae exhibit behavioral competency.

TEMPERATURE, SALINITY AND DESSICCATION TOLERANCE OF THE GREEN MUSSEL *PERNA VIRIDIS* IN SOUTHWEST FLORIDA.

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Temperature, salinity and desiccation tolerances of the invasive green mussel were investigated under environmentally realistic conditions in southwest Florida to assess whether green mussels compete with native oysters for food and space / substrate. Acute and gradual salinity change (5–35 ppt), temperature (10–45°C), and desiccation tolerance (0–8 hrs) of green mussels were assessed. While gradual salinity changes (3 ppt/2 days) resulted in excellent survival of mussels at salinities ≥9 ppt (>97%), acute salinity change resulted in low survival (0%) below 10 ppt. Green mussels showed poor survival at temperatures below 10°C or above 35°C. While oyster survival was high (>95%) under direct sunlight, green mussels encountered heavy mortality (~85%) within 3 hours. Results suggest that while green mussels are less tolerant of lower salinities (<10 ppt), with gradual salinity changes they may invade estuarine portions and could compete with oysters for resources such as food and substrate. Results also suggest that green mussels may not be able to tolerate low (<10 ppt) and high salinities (>40 ppt) prevailing in the estuaries, and given their poor desiccation tolerance, may not be able to settle on and overtake intertidal oyster reefs.

EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) AS AN INDICATOR FOR THE RESTORATION OF EVERGLADES ECOSYSTEMS.

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One component of the Comprehensive Everglades Restoration Plan (CERP) is an attempt to restore hydrology in south Florida estuaries. The eastern oyster *Crassostrea virginica* is a dominant feature of those estuaries. We have developed a communication tool (Stoplight Report Card system) based on CERP performance measures for oyster biological responses. The communication tool involves a suitability index score for each organism metric as well as a trend score (– decreasing trend, ± no change in trend, and + increasing trend). Based on these two measures, a component score (e.g., living density) is calculated by averaging the suitability index score plus the trend score. The final eastern oyster index score is obtained by taking the geometric score of each oyster component and translating it into a stoplight color for success (green), caution (yellow), or failure (red). Based on the oyster responses in select estuaries in south Florida, the system is at stages failure - caution. This communication tool instantly conveys the status of the indicator, and the suitability and trend curves provide information on progress towards reaching a target. The tool also has the advantage of being able to be applied regionally, by species or other ecological characteristics, and collectively system-wide.

USE OF TRIPLOID OYSTERS, *CRASSOSTREA VIRGINICA*, IN AQUACULTURE: EFFECT OF GROW-OUT GEAR ON FIELD PERFORMANCE.

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Though prior studies have indicated the importance of site selection in the performance of triploid oysters in aquaculture production, we sought to conduct a test of the importance of grow-out gear on the performance of triploid oysters, *Crassostrea virginica*, relative to half-sibling diploid oysters at a single site (Grand Bay, Alabama). In 2010, two batch spawns were conducted simultaneously at the Auburn University Shellfish Laboratory, producing two lines sharing female parentage (3 yr old broodstock, F1 Cedar Point); one line was produced with males collected from the same site (the diploid line), while the other line was produced with tetraploid males provided by Louisiana State University (the triploid line). Seed were raised side-by-side under identical conditions, until deployment into four different gear types in May 2011: an adjustable long-line system, floating bags, OysterGro cages and LowPro cages. Seed and gear were regularly tended, with oysters sampled in August and November 2011. Oysters were measured for survival, shell metrics, dry shell weight, dry tissue weight and condition index. There was a strong interaction between ploidy and grow-out gear type. Results of ongoing analyses will be presented.

MICROSATELLITE GENETIC VARIATION BETWEEN AND WITHIN WILD AND IMPORTED AQUACULTURE *BABYLONIA AREOLATA* POPULATIONS.

Aimin Wang.

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The spotted babylon *Babylonia areolata* is an important mariculture gastropod in China and Thailand. The individuals from Thailand have greater growth vigor than native ones in Hainan Island, so most hatchery populations in Hainan come from Thailand lines. Genetic diversity between two native wild (from Beihai and Sanya, respectively) and three farmed populations (one native, one originally imported from Thailand -08T and its second generation population -10T) of spotted babylon were analyzed using 8 microsatellite markers. High levels of polymorphism were observed over all populations with the average number of alleles and average heterozygosity at 14.8 and 0.68, respectively. The farmed (the native and 10T) babylon showed less allele number than the wild and 08T. Four of the eight loci deviated from Hardy–Weinberg equilibrium ($P < 0.05$ after Bonferroni correction) in at least 3 populations, probably because of the presence of null alleles and/or non-random mating, since significant heterozygote deficiency was found at three of them. Significant genetic differentiation was revealed by pairwise and globe *F*st between native and imported populations, especially for original population 08T. The significant genetic differentiation between the native and imported populations suggested that the diverse genetic resources could be applied to develop particular strains for the benefit of aquaculture.

CHARACTERIZATION OF PEARL OYSTER, *PINCTADA MARTENSII* MANTLE TRANSCRIPTOME USING 454 PYROSEQUENCING.

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The pearl oyster, *Pinctada martensii*, is a marine bivalve widely distributed in tropic and subtropic marine. Mantle is the special tissue of *P. martensii* that secretes biomineralizational proteins inducing shell deposition, as well as iridescent nacre both in the inner shell and artifial nucleus. Thus, *P. martensii* is quite famous for artificial pearl production, and an ideal candidate for studies into the processes of biomineralization. However, deficiency transcriptome information limits the insight to biomineralizational mechanism and pearl formation. In the study, we firstly present the *Pinctada martensii* mantle transcriptome using massively parallel pyrosequencing. From a total of 220824 reads, 24747 putative unique transcripts were assembled, annotated, and classified. 45 unique transcripts may involve in biomineralization, such as calcineurin-binding protein, amorphous calcium carbonate binding protein 1, calmodulin, calponin, carbonic anhydrase 1, Glycine-rich shell matrix protein, Lysine-rich matrix protein, mantle gene, nacrein, pearlin, PIF, regucalcin and shematrin. The sequence data contained 10285 potential single nucleotide polymorphism (SNP) loci and 7836 prabable INDELS, providing a resource for mollcular biomarker, population genetics and also gene function. These sequence data will notably advance biomineralization and transcript-wide study in *Pinctada martensii* and other *Pinctada* species.

BUILDING A ROADMAP TO RECOVERY FOR THE ENDANGERED BLACK ABALONE (HALIOTIS CRACHERODII).

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Due to recent population declines associated with disease and historical overfishing, the black abalone (*Haliotis cracherodii*) was listed as endangered under the federal Endangered Species Act

(ESA) in 2009. As a result, several regulations were established to protect the species and its critical habitat. While these regulations provide protection from further harm, a more comprehensive plan is needed to delineate, prioritize, and guide the steps forward to achieve species recovery. The National Marine Fisheries Service is charged with developing that roadmap, in the form of a recovery plan. A recovery plan defines the vision of what species recovery looks like and provides a strategy for recovery, serving as a guide for sound decision-making and effective, targeted actions to achieve the recovery vision. We will provide an overview of ESA regulations currently in place for black abalone and outline the recovery planning process, focusing on what decisions need to be made, how they are made, and progress to date. By providing a clear understanding of the ESA regulations and recovery planning process, we hope to promote effective participation by stakeholders, a key component of successful recovery planning and implementation.

"PUTTING HEADLIGHTS ON THE CAR:" WILL MONITORING FOR "CORROSIVE" SEAWATER BECOME STANDARD PRACTICE FOR SHELLFISH HATCHERIES?

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Documented increases in average surface-ocean CO_2 concentration and associated changes in seawater chemistry have generated widespread concern about possible impacts on shellfish production. Many aquaculture systems and wild harvest shellfish fisheries require stable water chemistry (e.g. pH, pCO_2, aragonite Ω, DO, etc) in order to maintain recruitment and growth rates. Monitoring methods that have enabled Pacific Northwest oyster hatcheries to avoid some impacts of corrosive, upwelled water are now being embraced by hatcheries in other regions. Plans are under development to expand use of monitoring significantly. This presentation reviews current and evolving practices for monitoring seawater chemistry and managing consequences of increasing acidification on shellfish production in estuaries and marine waters.

THE OYSTER RESTORATON RESEARCH PARTNERSHIP 2010-2011: INITIATION OF A LONG-TERM PROGRAM TO RESTORE OYSTER POPULATIONS IN THE NEW YORK HARBOR REGION.

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The Hudson River Foundation, New York/New Jersey Baykeepers, U.S. Army Corps of Engineers, University of New Hampshire, and other partners initiated in 2010 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 major focus of the program is on ecosystem services such as water filtration and habitat provision provided of the oyster reefs rather than human harvest and consumption. Five small experimental reefs consisting of a rock base overlain with surf clam shell then 'seeded' with juvenile oyster spat on shell (SOS) were constructed in fall 2010, and monitored for oyster survival and growth during 2010 and 2011. Two of the five reefs had good SOS survival and growth, and three showed newly recruited spat from natural oyster populations in the region. These data are being used to design Phase 2 of the ORRP, which will likely include construction of larger reefs at one or two of the existing sites.

REPRODUCTIVE ECOLOGY OF COMMERCIALLY IMPORTANT LITHODID CRABS.

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Six species of Lithodid crabs, *Paralithodes camtschaticus*, *Paralithodes platypus*, *Paralithodes brevipes*, *Lithodes aequispinus*, *Lithodes santolla*, and *Paralomis granulosa* annually account for >99% of king crab landings worldwide. Fisheries for many of these species have collapsed with mixed recovery success. Differences in reproductive potential among these species are related to divergent modes of larval development (planktotrophy versus lecithotrophy). Cumulative egg production, an index of female reproductive potential, was estimated to range among species from $\sim 10^6$ for *P. camtschaticus* to $\sim 10^4$ for *P. granulosa*. Reproductive potential was also three to four times greater for large versus small females within each species. Female *P. camtschaticus* had the highest estimated cumulative and annual reproductive potential after normalization for differences in egg size. Decreased female reproductive potential among species was associated with decreased frequency of reproduction, increased egg size, and decreased range of mature female body size. High levels of interspecific variation in female reproductive potential may be associated with variability in male reproductive potential but there is a paucity of information available to evaluate these differences and likely linkages with mating dynamics, sexual selection, female reproductive success, and resilience to large-male-only harvest.

EXTENSION, OUTREACH AND PUBLIC RELATIONS: THE DIFFERENCE IS IN THE OUTCOME.

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Research organizations often emphasize the distribution of results of funded projects as a way to generate further public and private support. Funding agencies are more often including mandates for outreach or extension activities in requests for proposals. Researchers frequently find these to be bothersome or confusing to include in projects and give little thought or effort to properly designed programs. But well-designed outreach and extension components can aid in disseminating results to provide documented positive outcomes. There are distinct differences in the aims and results of these methods of disseminating information. We discuss the principal objectives of extension, outreach and public relations programs and show how they differ from each other. Examples of each are provided to highlight their uses and show how they may apply in shellfish aquaculture. An overview of the extension system in the United States is provided to demonstrate the application of programs. Steps from identifying needs through program design and delivery to documenting outputs and outcomes are followed to show the benefits of program implementation. Finally, discussion of integrated programs conceived by the US Department of Agriculture is provided to show how components are combined to effectively use research generated information for positive outcomes.

SEASONAL CHANGES IN THE DIET OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) IN THE RHODE RIVER, MARYLAND.

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Oysters were placed in cages suspended from a dock in the Rhode River, Maryland. Water column, gut contents, and feces were sampled monthly for a year and analyzed for algal composition. The water column was dominated by diatoms in fall and spring, dinoflagellates in the winter, and Cyanobacteria in the summer. Vanderploeg and Scavia's electivity index, which accounts for the abundance of other food types present, indicates preference for chlorophytes and diatoms in early fall, flagellates and dinoglagellates in late fall; diatoms in winter; flagellates, chlorophytes, and dinoflagellates in spring; and diatoms and chlorophytes in summer. Cyanobacteria were avoided throughout the year. Diatoms and dinoflagelletes often comprised a larger proportion of the gut contents than feces, suggesting these taxa were preferentially digested. Flagellates comprised a larger proportion of the feces than gut contents except in fall, suggesting these taxa were excreted without digestion most of the year. Relative composition of cyanobacteria in gut contents and feces varied monthly, indicating temporal differences in digestion. Chlorophytes comprised equal proportions of gut contents and feces except in August, when they were preferentially digested.

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

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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. The focus of this talk will be on the ecosystem service valuation of shellfish production especially the economic benefits of nitrogen removal as related to water quality enhancements. Results provide guidance to managers on the best options for integrated nutrient management including the policy decision as to whether to include shellfish growers in the current Connecticut Water Quality Trading Program.

THE PRESENCE OF OCTOPAMINE IN GANGLIA AND TISSUES OF DIFFERENT CLASSES OF BIVALVE MOLLUSCS.

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Octopamine is a biogenic amine first identified in the octopus. It is well studied in arthropods and a few gastropods where it serves as a neurotransmitter and hormone. The presence of octopamine

has rarely been reported in bivalves. We show that it is present in ganglia and tissues of *Crassostrea virginica*, a member of the order Osteoida, where it is a cardio-excitatory agent. The present study sought to examine if octopamine is present in other species of bivalves. We tested tissues of *Mytilus edulis* and *Mercenaria mercenaria* utilizing HPLC with fluorescence detection to identify and measure octopamine in cerebral ganglia, visceral ganglia, pedal ganglia, gill, palps, heart and foot. The results show that octopamine is present in ng amounts in gill, palps, cerebral ganglia, visceral ganglia, pedal ganglia, heart and foot along with serotonin, dopamine and norepinephrine. The study now identifies octopamine in the nervous system and innervated organs of bivalves in two additional orders, the order Mytilorida (*M. edulis*) and Veneroida (*M. mercenaria)*.

APPLYING A SOILS-BASED APPROACH TO CLAM AQUACULTURE IN FLORIDA.

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Relationships between terrestrial agriculture and soils have been thoroughly investigated, yet links between shellfish aquaculture and subaqueous soils have only recently been examined. As infaunal bivalves spend a majority of their life buried, traditional soil characterization is being applied in Florida to the production of the northern hard clam *Mercenaria mercenaria* and a potential new aquaculture species, the sunray venus clam *Macrocallista nimbosa*. Results will be discussed for ongoing studies determining effects of hard clam farming intensity and varying fallow times after harvest on soil properties (bulk density, organic matter and particle size distribution). Soil type preference for the sunray venus clam was preliminarily determined from a six-month *in situ* mesocosm study that revealed variations in production characteristics. Sunray venus clams grew larger (11.0 g) and had fewer deformities (2.2%) in sandier soils (>95% content) than clams (9.6 g, 18.7% deformities) in soils that contained more silt (>4%). In addition, soil properties at commercial leases were sampled and characterized to evaluate suitable for sunray venus culture. Industry-acceptable production (survival, 55–71%; weight, 11.3–23.0g) occurred at leases that contained higher sand (>87%) and lower silt (<2.5%) content. Understanding these soil relationships is critical to increasing productivity, directing management practices, and selecting lease sites.

EFFECTS OF ELEVATED $_pCO_2$ ON ATLANTIC SURFCLAM, *SPISULA SOLIDISSMA*, LARVAE.

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Atmospheric CO_2 emissions continue to increase and diffuse into our ocean waters, leading to ocean acidification. Increased CO_2 affects seawater chemistry, leading to hypercapnia and decreasing saturation states of aragonite and calcite. These global changes could potentially affect the surfclam fishery on the Atlantic coast of the United States, worth 25.9 million US dollars in 2010 (NMFS). Surfclams form their shell in the first 48 hours and the initial shell is made of aragonite, a very soluble form of calcium carbonate. Surfclam larvae were spawned by thermal stimulus and eggs and sperm were combined in ambient seawater (380 ppm). Embryos were added to seawater at a density of 15 embryos/ml pre-equilibrated to three different pCO_2 levels (380, 1200 and 2200 ppm). Larvae were reared in triplicate at 20°C in 15L buckets. After 3 days, mean shell lengths were 84, 81 and 79 μm and after 15 days mean shell lengths were 145, 131 and 134 μm at pCO_2 levels of 380, 1200 and 2200 ppm, respectively. Elevated pCO_2 levels did not affect the shell length of surfclam larvae during the first 15 days of development; however, after only three days surfclams experienced a 40% decrease in individual weights at 2200 ppm vs. ambient.

QUAGGA MUSSEL VELIGERS AT DIFFERENT DEPTHS OF LAKE MEAD: ABUNDANCE, DEVELOPMENTAL STAGES, AND MORPHOLOGY.

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We collected veliger samples weekly at depths of 5, 10, 20, 30, 40, 50, and 60 meters in the open Boulder Basin of Lake Mead, NV, from June 30, 2010 until June 16, 2011. Compared to other months of the year, the abundance of veligers was always higher from August to September 2010 with the highest recorded on August 18, 2010 (41 veligers/L). The highest number of veligers was found at the sampling depth of 20 meters from June 30, 2010 until March 19, 2011, as well as from June 9, 2011 to June 16, 2011. Between these two periods, the abundance of veligers was the highest at a depth of 10 meters. Dynamics of different developmental stages of quagga mussels at different depths of the lake were also quantified during this annual survey. The morphology of veligers at different depths did not differ significantly. The present study provides information about life history of invasive quagga mussels in Lake

Mead and is valuable for understanding mussel population dynamics and ecosystem impacts that is critical for enhancing resource management.

CHROMOSOMAL SET MANIPULATION OF THE CHINESE SHRIMP, *FENNEROPENAEUS CHINENSIS*.

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Using temperature shock (hot or cold) and /or chemical shock (CB, or some new chemical with lower toxicity and high efficiency) to induce triploidy Chinese shrimp, *Fenneropenaeus chinensis*, which is an important commercial species in China, in some cases 100% triploidy could be obtained. We discovered the female/male ratio was 4:1 in the triploidy population instead of the ratio 1:1 in normal population. Also, comparative studies on meiosis behavior of spermatocytes between diploid and triploid males were carried out by synaptonemal complex (SC) analysis. To identify key molecular changes involved in ovary of triploid shrimp, two subtracted cDNA libraries between triploid and diploid shrimp were constructed using suppression subtractive hybridization (SSH) technique. To further dissect exact gene functions for gonad development of shrimp, three significantly differentially expressed genes between diploid and triploid ovary, PCNA (proliferating cell nuclear antigen), CAS/CSE1 (cellular apoptosis susceptibility protein/chromosome segregation and SSRF (spermatogonial stem-cell renewal factor) were characterized by full-length cloning, qRT-PCR, in situ hybridization or prokaryotic expression.

IDENTIFICATION OF A NOVEL METAL BINDING PROTEIN IN OYSTER PLASMA.

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The second most abundant protein of eastern oyster plasma was purified and characterized. The 40 kDa protein as determined by SDS-PAGE under reducing conditions made up about 17% of plasma proteins and was in extrapallial fluid. RACE reactions with primers designed from an EST sequence identified by BLAST search in GenBank using the N-terminal amino acid sequence obtained by Edman degradation of the purified protein, predicted a 997 bp complete cDNA that encoded 277 amino acids including a 16-residue signal peptide at the N-terminus. The deduced mature protein was composed of 261 amino acids with a calculated molecular mass of 30,484 Da, lower than the molecular mass of the purified protein measured by MALDI. The protein mRNA was detected in hemocytes by in situ hybridization. Immunohistochemistry revealed the protein was most abundant in tissues with high densities of blood sinuses like the gills and dorsally along the base of the mantle. ICP metal analysis of purified protein indicated highest association with zinc, calcium and iron. Results of N-terminal and internal peptide sequencing of SDS-PAGE separated plasma proteins from Pacific, Asian and European flat oysters indicated that this second most abundant plasma protein is conserved. Its function is currently being investigated.

THE QUANTIFICATION OF THE POTENTIAL SPAWNING CONTRIBUTION FROM THE RESTORED OYSTER (*CRASSOSTREA VIRGINICA*): A COMPARATIVE STUDY AMONG RESTORATION SITES AND SUBSTRATES IN COASTAL ALABAMA.

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Numerous ecosystem services of oyster reef restoration have been documented. But the service that the oyster reef putting in an increasing larvae has not been previously quantified. In this study, we selected 4 restoration sites in coastal Alabama (Billy Goat Hole, Alabama Port, Coffee Island & Little River Bay areas) with 6 different substrates (Reef Balls, Reef Blocks, bagged oyster shell, loose oyster shell and 2 types of WADs). In 2011, we sampled the oysters monthly during the presumed reproductive season (June to November), dividing the oysters into 2 size classes, with up to 30 oysters collected per size class per sampling. Oysters were sexed by microscopic examination of the gonad, and the number of eggs per female was estimated using the method of Cox & Mann (1992). There were clear differences among the sites across the season. Differences among substrates are currently being analyzed and will be presented. The annual potential spawning contribution of each of the restoration sites will be estimated, compared and discussed.

ELECTROCHEMICAL CHARACTERIZATION OF THE SHELL OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.

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The valves of the eastern oyster (*Crassostrea virginica*) are composed of multiple mineralized layers. Valve closure forms an effective barrier to incursion by surrounding water. The material

properties of shell were investigated to determine the effects of shell thickness, valve orientation, and shell layer composition on the electrochemical behavior using electrochemical impedance spectroscopy (EIS), potentiodynamic polarization, and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS). Microscopic analysis confirmed that the multilayered shell material is composed of calcium carbonate ($CaCO_3$) in all layers (prismatic, foliated, chalky) and that material density and elemental composition varied between foliated and chalky layers. EIS measurements in 3.5 wt% NaCl indicated that the impedance of the whole oyster shell in the low frequency region exhibited high impedance values (high resistance). Electrical current transit through the shell valve increased when the outer layers of the shell were sequentially removed by grinding (decreased shell thickness) while impedance (resistance) values were shown to decrease with decreasing shell thickness. These findings suggest that the presence of prismatic (outermost) shell layer in combination with the organic matrix between all shell layers may influence the electrochemical parameters and ionic conductivity through the oyster shell.

PERFORMANCE AND GENETIC VARIATIONS FOR BACKCROSS PROGENIES OF SMALL ABALONE.

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Haliotis diversicolor is a commercially important species cultured along the coast of southern China. ''Dongyou No.1'' small abalone, which was confered for ''new species certificate'' by Ministry of Agriculture, has been proved for excellent disease-resistant ability and was large-scale produced in China. It was created by crossbreeding between Japan (as male) and Taiwan (as female) population of small abalone. In this research, ''Dongyou No.1'' was backcrossed with Japan and Taiwan population, respectively. But growth and survival for reciprocal backcross progenies were significantly different. Reciprocal backcrossing with Taiwan population, survival rate for the exprimental groups were decreasing significantly, while production performance was improved when backcrossing with Japan population. Japan population was then selected as recurrent parents for grading cross with Taiwan population. Survival rate of F_3 (83.4%) progenies were higher than that of F_2 (77.6%) and F_1 (71.4%) at grow-out stage. Genetic variation for backcross progenies from different populations were also studied in this study by microsatellite markers.

ECODYNAMIC SOLUTIONS FOR THE PROTECTION OF INTERTIDAL HABITATS: THE USE OF OYSTER REEFS.

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Ecosystem engineering processes are relevant to many environmental problems and management concerns. Within the program ''Building with Nature'' (www.ecoshape.nl) we investigate the use of bivalve reefs as ecodynamic measures to protect tidal flats against erosion, which poses a serious problem in the Oosterschelde estuary (Netherlands). Bivalve reefs are ecosystem engineers that influence tidal flow and wave action and therefore modify sediment transport patterns. The development of such infrastructural solutions that aim for an integration with the natural environment can only be achieved through experimentation and learning-by-doing. Artificial oyster reefs were constructed in different erosional intertidal environments in the Oosterschelde. Reefs consist of gabions filled with oyster shells (*Crassostrea gigas*), offering a stable substrate that allows for the settlement of oysters, while minimizing shell loss. Monitoring results indicate that artificial reefs can develop into self-maintaining, living oyster reefs which stabilize tidal flats. Site-specific effects in reef development (e.g. recruitment of oyster larvae) and in sediment dynamics were observed. Knowledge about local hydromorphological conditions and a thorough understanding of the ecosystem engineering properties and habitat requirements of *C. gigas* are needed to implement this concept in management practices. We present the concept, monitoring and modeling results and derive design rules from these.

TRANSCRIPTOMIC ANALYSIS OF *CRASSOSTREA HONGKONGENSIS* REVEALS MOLECULAR TOLERANCE MECHANISMS TO WIDE RANGE OF SALINITY FLUCTUATION.

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Crassostrea hongkongensis is an economically important aquaculture species inhabiting in estuary where salinity fluctuates significantly with tide cycle and variable freshwater input. Physiological tolerance to acute salinity variation guarantees the oyster's

successful survival in its habitats. To explore its molecular mechanism of euryhaline adaptation in this non-model species, *de novo* transcriptome and transcriptional response to an acute salinity variation were analyzed. The RNA from various tissues and early developmental stages were pooled and normalized after reverse transcription. Through a full 454 GS-FLX titanium sequencing, approximately 1.6 million reads were generated with an average length of 387 bp, which resulted in assembly of 35,207 contigs, with 1,271bp of N50. Using Blast searches of NCBI non-redundant protein database, around 16,000 transcripts with conserved protein domains were identified and approximately 10,000 transcripts were assigned with Gene Ontology terms. Digital gene expression (DGE) analysis showed a significant up- or down-regulation of salinity-responsive transcripts involved in osmotic effector proteins, osmotic stress specific transcription factors, osmo-sensory signal molecules, osmo-regulatory hormone and their receptors, suggesting a high complexity and universality of osmo-regulation in oysters. These results provided global insight into the fundament of physiological adaptability to acute salinity fluctuation at genomic level.

BIOACOUSTIC EVALUATION OF RESTORED OYSTER REEFS – SPATIAL, TEMPORAL AND SPECIES SPECIFIC VARIATION IN BIOLOGICAL SOUND PRODUCTION.

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Passive acoustics uses naturally occurring sounds produced by marine organisms to study their behavior, biology and location. The aim of this research was to use passive acoustic technology to measure the progress (reef use and colonization) of a large restoration oyster reef project, where more than 24 acres of oyster reefs were restored. Three sites along the Saint Lucie Estuary, Florida were acoustically monitored. Oyster toadfish, naked goby, mud crabs, and snapping shrimps inhabit oyster reefs, and they are known to produce sounds. This study focused on the sound production of snapping shrimp because they are one of the most abundant decapod crustacean species in oyster reefs, and they are well known for their sound production. Total power and number of snapping shrimp snaps were calculated for each field recording. Results indicated that total power and number of snaps can be useful in detecting differences between natural and restored reefs, seasons, river regions, and day periods. In addition, number of snaps can be useful for estimating number of species present in an oyster reef. As snapping shrimp are common in various ecosystems, this methodology could be extrapolated to monitor number of snapping shrimp and number of species in other ecosystems.

COUPLED PHYSICAL AND BIOLOGICAL MODELING OF ATLANTIC SURFCLAM LARVAL TRANSPORT AND SUB-POPULATION CONNECTIVITY IN THE MIDDLE ATLANTIC BIGHT AND GEORGES BANK.

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The Atlantic Surfclam (*Spisula solidissima)* is one of the most commercially important shellfish species along the Northeast U.S. coast. In the past, systematic variations in the surfclam sub-populations in this region, thought to be associated with progressive environmental change, have been reported. The larval dispersal stage of the surfclam plays a key role in total recruitment rate and sub-population connectivity. With this in mind, we couple a physical circulation model, based upon the Regional Ocean Modeling System, and a surfclam individual-based larval model to simulate surfclam larval transport and sub-population connectivity throughoutthe Middle Atlantic Bight (MAB) and Georges Bank (GBK) regions. Preliminary results for the period 2006–2009 show the connection direction among the surfclam sub-populations inside the MAB and GBK to be downstream, from the northeast to the southwest. Typically, only two adjacent regions are closely connected. As expected from its retentive circulation, the GBK surfclam population is relatively isolated. The coupled simulations also confirm large inter-annual variation in surfclam sub-population connectivity patterns.

CLONING AND EXPRESSION OF VITELLOGENIN GENE IN NOBLE SCALLOP *CHLAMYS NOBILIS* (BIVALVE: PECTINIDAE) WITH BROWN AND ORANGE SHELL COLORS.

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In this study, the full-length cDNA encoding vitellogenin (Vg) in noble scallop *Chlamys nobilis* was cloned and expressed at different tissues and different gonadal development stages using male and female individuals with orange and brown shell. The complete Vg cDNA consists of 7760 nucleotides with an open reading frame encoding 2289 amino acid residues. According to the phylogenetic analysis of Vg gene, *Ch. nobilis* was clustered together firstly with its sister species *Ch. farreri* and another scallop *M. yessoensis*, next with other molluscs such as oyster and abalone, and then other invertebrates, finally with vertebrates. In common with molluscs Vgs, the Vg gene was expressed only in the ovary. Both orange and brown shell color scallops show the same trend that the amount of Vg mRNA expression significantly increased at

the growing stage, then dramatically decreased at the spawning stage, and finally resumed to higher level at the post-spawning stage. Moreover, the orange individuals had a higher Vg expression level at all the maturity stages, suggesting that there maybe a positive relationship between Vg expression level and total carotenoids content in the noble scallop *C. nobilis*, because total carotenoids content in orange individuals is more than that in brown ones.

HYBRID OYSTER (*CRASSOSTREA HONGKONGENSIS*♀ × *CRASSOSTREA ARIAKENSIS* ♂) AS AN AQUACULTURE CANDIDATE FOR NORTHERN CHINA.

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Crassostrea hongkongensis is now a popular aquaculture species in southern China because of its high quality adductor; however, this species cannot survive during the winter in northern China. This study evaluates the aquaculture potential of hybrids between *Crassostrea hongkongensi s*and *Crassostrea ariakensis* reared in the north of China. The results showed an asymmetry in fertilization; *C. hongkongensis* eggs can be fertilized by *C.ariakensis* sperm, with 36% of eggs reaching D-larvae. The growth of hybrid progeny was intermediate between pure *C. hongkongensis* (HH), and pure *C.ariakensis* (AA) *a*t both larval and juvenile stages. Positive hybrid vigor ingrowth rate was observed during over-winter and grow-out stages (156%, and 65%, respectively). The survival of HH juveniles was 0%, while the HA and AA showed higher survival rates of 72% and 75%, respectively, during the over-winter phase. Morphological and molecular genetics analysis revealed that hybrid progeny were true hybrids. Furthermore, the adductor of the adult hybrid was similar to that of *C. hongkongensis*. F_2 hybrids could be produced, but died before metamorphosis. The results suggest that the hybrid between *C. Hongkongensis* and *C. ariakensis* may be an aquaculture candidate as a substitute for *C. hongkongensis* reared in northern China.