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Electrochemical Characterization of the Shell of the Eastern Oyster, Crassostrea Virginica

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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