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# Towards Biomimetic Ceramic Coatings: Cellular Aspects of Oyster Shell Biomineralization

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blue crabs with hatchery-reared juveniles and to identify key factors that maximize survival of hatchery-reared juveniles following release. During 2002-2008, we released 53 cohorts of 1,000-25,000 hatchery-reared juveniles into nursery habitats of the upper Chesapeake Bay. Survival of released crabs varied among release sites, seasons, years and stocking densities. Overall survival was high (15%), but was highest in early spring and late fall releases when predation was lowest. Cohorts released in spring grew to maturity within the season of release; whereas cohorts released in summer and fall over-wintered and matured in their second year. Key next steps in our ongoing assessment are to assess fishery impacts on restocking success and to evaluate the cost-effectiveness of restocking relative to alternative strategies. Overall, our results continue to indicate that the potential for restocking with this species is encouraging.

**TOWARDS BIOMIMETIC CERAMIC COATINGS: CELLULAR ASPECTS OF OYSTER SHELL BIOMINERALIZATION.** Mary Beth Johnstone<sup>1</sup>, Karolyn M. Hansen<sup>2</sup>, Neeraj V. Gohad<sup>1</sup>, Douglas C. Hansen<sup>2</sup>, and Andrew S. Mount<sup>1</sup>. <sup>1</sup>Clemson University, Clemson, SC, 29634, USA; <sup>2</sup>University of Dayton Research Institute, Dayton, OH, 45469, USA.

The molluscan shell formation process is a promising model for development of bio-inspired ceramics for a wide variety of applications in fields as varied as nanotechnology and nanofabrication to name a few. Using a method similar to flat pearl formation, metal alloy disks placed *in vivo* between the mantle, a shell promoted the deposition of successive uniform layers of folia onto implant surfaces. The resulting coating mimics folia formed in shell which is comprised of laterally flattened crystal laths that fuse to form highly ordered folia sheets. These layers form on the surface of a substantial substrate membrane and are capped with a similar membrane which acts as a substrate for the next layer of folia deposition. We observed that cells initiate folia formation by depositing membrane bound nanoparticles containing crystals onto the substrate. Early folia formation commences with the formation of unorganized mineral patches over the entire membrane surface. The capping membrane originates from plasma membrane bearing vesicles which originate from hemocytes at the mineralization front.

**A BIOINFORMATICS APPROACH FOR DISCOVERING SHELL MATRIX PROTEINS.** Mary Beth Johnstone, Margaret E. Staton, Chris Sasaki, and Andrew S. Mount. Clemson University, Clemson, SC, 29634, USA.

The acidic proteins derived from molluscan shell are among the most unusual proteins known. They are heavily processed post-translationally and post-secretory, attributes which have made standard biochemical characterization problematic.

We recently identified folian, an acidic family of phosphoproteins in oyster shell primarily comprised of Asp, Gly and phosphorylated serine, at roughly 30 mol% each. Discrete protein identification using standard protein characterization methods has been difficult due to the high weight percent phosphate which contributes to the folian family heterogeneity. Using a bioinformatics approach, a virtual "perlscript" probe was constructed to a 20 amino acid N-terminal sequence previously determined for a 48 kDa folian phosphoprotein and was used to search against available EST databanks for *Crassostrea* oyster species. The identified EST sequence, when translated, contained the N-terminal probe sequence and substantial Asp-Ser rich domains. The full transcript was determined by screening a mixed *Crassostrea virginica*/*Crassostrea gigas* BAC library with 10X coverage; Southern analysis revealed that two copies of the protein exist in the genome. The protein has an estimated molecular weight of 34 kDa and contains 41% Asp, 31% Ser, 8% Glu with a theoretical pI of 2.37. This study demonstrates that bioinformatics is a powerful tool for identifying novel proteins.

**PREHISTORIC OVER-EXPLOITATION OF *MERCENARIA* SPP. IN THE SOUTHEASTERN USA.** Douglas S. Jones, and Irvy R. Quitmyer. University of Florida, P.O. Box 117800, Gainesville, FL, 32611, USA.

Human-induced changes in natural or pristine ecosystems of the Americas are thought to have occurred after European contact and colonization. However, age class determination of *Mercenaria* spp. shells using annual shell growth increments provides evidence for the over-exploitation of this resource at six pre-Hispanic archaeological sites from Florida, Georgia, and South Carolina. The data show that hard clams were collected at such a high intensity that the average age of their populations was significantly reduced. This occurs where there is evidence for hard clam harvest during the peak period of reproduction. Evidence of human sedentism and intensive use of hard clams may have also contributed to the decline in population age class structure. Further, modern age class data of three managed hard clam populations show a positive effect in those populations. These data span over 2,000 years of hard clam exploitation along the southeastern coast of North America.

**THE SEXUAL PREFERENCE OF *PERKINSUS MARINUS*.** Joshua Kauffman, Doug Zemeckis, and David Bushek. Rutgers University, 6959 Miller Avenue, Port Norris, NJ, 08349, USA.

In the Eastern oyster *Crassostrea virginica*, Dermo disease, caused by *Perkinsus marinus*, intensifies with age and therefore tends to remove larger, older animals from the population. Because oysters are protandric, populations shift from predominantly male to predominantly female as a cohort ages and the oysters grow. Therefore, all else being equal, Dermo disease should have a

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