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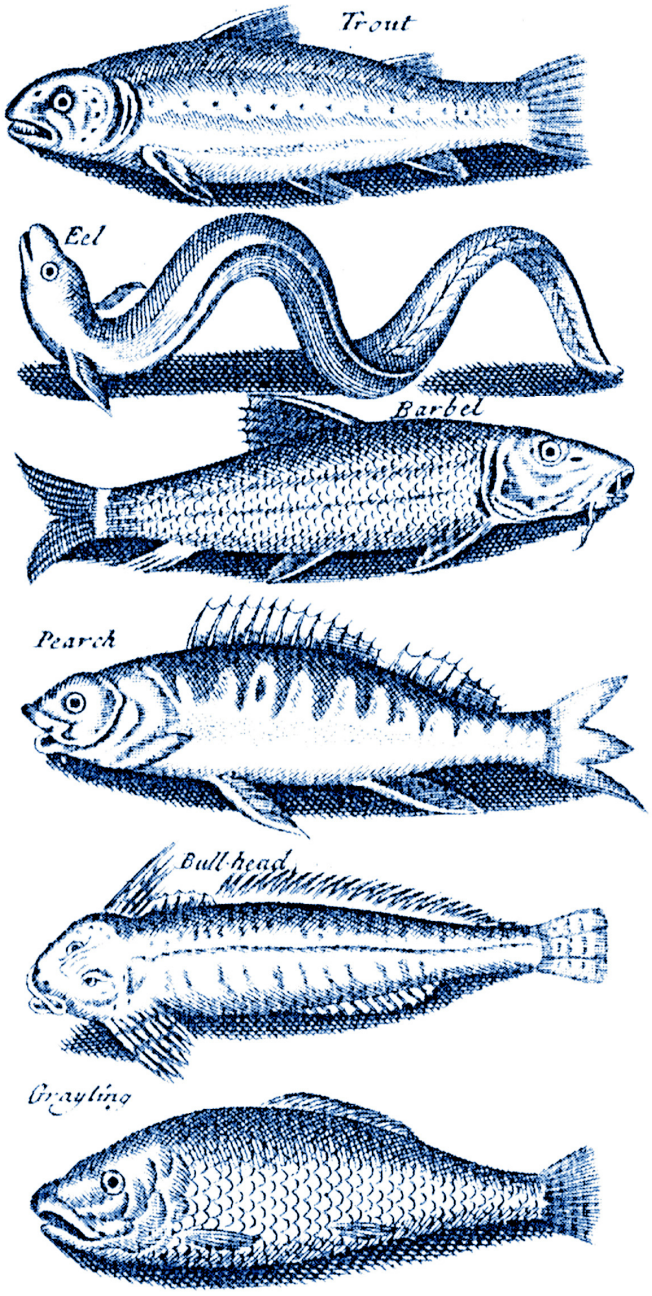

ABSTRACTS

AN INVESTIGATION OF THE SPONGE *MYRMEKIODERMA* SP. FOR NEW ANTIMALARIAL DRUG LEADS.

LaTasha D.J Amisial[1], Hilaire V. Kemami Wangun[2], Debopam Chakrabarti[3] and Amy E. Wright[1,2]

[1]Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC
[2]Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL
[3]University of Central Florida, Orlando, FL

Malaria is a devastating disease caused by infection with a parasitic protozoan of the genus, *Plasmodium*. The two most common forms of *Plasmodium* that infect humans are *Plasmodium vivax* and *Plasmodium falciparum,* with *P. falciparum* infection being the most dangerous. Transmission of the *Plasmodium* parasite is through the mosquito. Currently, forty-one percent of the world's population lives in areas where malaria is endemic, and each year 350-500 million clinical episodes of malaria occur worldwide. Over one million people die from malaria every year, and in regions with extremely high transmission of malaria, two people die per minute. There is emerging drug-resistance in both *P. vivax* and *P. falciparum,* and the discovery of novel chemotypes that inhibit the drug-resistant strains may lead to the development of new, critically-needed medicines. Marine organisms have been the source of many natural products with therapeutic properties. As part of a program to discover novel anti-malarial agents from marine organisms, extracts from the marine sponge *Myrmekioderma* sp. have tested positive for antiplasmodial activity. Presented here is a natural products investigation into the chemistry of the sponge *Myrmekioderma* with the goal of identifying the antiplasmodial natural products.

Funding: Gertrude E Skelly Charitable Foundation and HBOI at FAU.

Post-transcriptional regulation of the Cell Cycle in the Florida Red Tide dinoflagellate, *Karenia brevis*

Stephanie A. Brunelle[1,2] and Frances M. Van Dolah[1,2]

[1]Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC
[2]Center for Coastal Environmental Health and Biomolecular Research, National Oceanic and Atmospheric Administration, Charleston, SC

The red tide dinoflagellate, *Karenia brevis*, is responsible for harmful algal blooms in the Gulf of Mexico that cause extensive fish fills, marine mammal mortalities, and human illness on a nearly annual basis. The molecular mechanisms controlling the cell cycle in this dinoflagellate are important because vegetative cell division plays a central role in bloom development. In dinoflagellates, cell division is characterized by novel adaptations including the presence of permanently condensed chromatin throughout the cell cycle, a lack of nucleosomes to aid in chromatin packaging, a permanently intact nuclear envelope, and an extranuclear mitotic spindle that interacts with chromosomes via cytoplasmic channels. Microarray and qPCR studies further suggest that, unlike typical eukaryotes, dinoflagellate cell cycle genes are not regulated at the transcriptional level (Van Dolah et al., 2007). In the current project, we investigated the expression of proliferating cell nuclear antigen (PCNA), an S-phase gene whose expression is typically activated by the E2F transcription factor at the restriction point, which regulates the entry into S-phase. Immunolocalization with anti-*K .brevis* PCNA showed that although PCNA transcripts are constitutively present, PCNA protein is expressed only in cells actively traversing the cell cycle. However, PCNA is present in the nucleus well before S-phase is apparent by flow cytometry. These results suggest that PCNA protein expression is activated by translation initiation at some point prior to S-phase entry. We are currently using immunolocalization of pre-initiation complex MCM proteins to identify precisely when the restriction point occurs, in order to investigate

how *K. brevis* regulates S-phase entry in the absence of transcriptional control of S-phase genes.

Funding Source: Center for Coastal Environmental Health and Biomolecular Research, National Oceanic and Atmospheric Administration, Charleston, SC

Selenium and mercury concentrations in liver of stranded pygmy sperm whales (Kogia breviceps) affected by cardiomyopathy

Colleen E. Bryan[1, 2], Wayne E. McFee[3], Gregory D. Bossart[4], W. Clay Davis[2], Steven J. Christopher[2]
(1)Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC
(2) National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC
(3) NOAA National Ocean Service, CCEHBR, Charleston, SC, USA
(4) Division of Marine Mammal Research and Conservation, HBOI, FAU, Fort Pierce, FL, USA

Pygmy sperm whales are the second most frequently stranded toothed whale along the U.S. Atlantic and Gulf coasts. More than half of documented cases exhibit signs of cardiomyopathy (CMP). Many factors may contribute to the development of idiopathic CMP in *K. breviceps*, including genetics, infectious agents, contaminants, biotoxins, and dietary intake (vitamins, selenium, mercury, and pro-oxidants). Nutritional deficiencies of selenium (Se) have been shown in mouse and bovine models to contribute to CMP. This study assesses trace elements in *K. breviceps* (n = 62) exhibiting or lacking signs of CMP using liver samples collected from individuals that stranded along the coasts of MA, VA, NC, SC, GA, and FL between 1993-2007. Total Se was measured by inductively coupled plasma mass spectrometry (ICP-MS), and total mercury (Hg) was measured by pyrolysis atomic absorption (AA) to examine if the Se/Hg detoxification pathway inhibits the bioavailability of Se. Due to the important role Se can play in antioxidant biochemistry and protein formation, Se species were examined in addition to total Se by size exclusion chromatography coupled to UV visible spectrophotometry and ICP-MS (SEC/UV/ICP-MS). Mean total Se and Hg concentrations (wet mass fraction, ± SD) were 9.57 ± 4.33 μg/g and 11.5 ± 10.6 μg/g, respectively. Se concentrations ranged from 2.01-21.6 μg/g and Hg concentrations ranged from 0.385-56.9 μg/g. A strong positive correlation exists between total Se and Hg concentrations in liver ($p < 0.001$, r = 0.770). Data collected on trace elements and metalloproteins will be evaluated in the context of animal life history, disease state markers, and other complementary histological information to gain insight into the biochemical pathways contributing to the development of CMP in *K. breviceps*.

Funding for this study is provided by the National Institute of Standards and Technology and through interagency agreements established with the National Oceanographic and Atmospheric Administration (NOAA) National Marine Fisheries Service. This research is being performed in the context of a larger study on Kogia species in partnership with NOAA's Center for Coastal Environmental Health and Biomolecular Research and HBOI Division of Marine Mammal Research and Conservation.

The influence of methylmercury on vitamin D3-induced transcriptomic effects within skin of the Atlantic bottlenose dolphin (*Tursiops truncatus*)

Blake C. Ellis[1,2], Mark S. Kindy[1,2,3], Annalaura Mancia[1], Gregory W. Warr[1,4], and Sebastiano Gattoni-Celli[1,3,5]

(1) Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC
(2) Department of Neurosciences and Neuroscience Institute, Medical University of South Carolina, Charleston, SC
(3) Ralph H. Johnson VA Medical Center, Charleston, SC
(4) Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC
(5) Department of Radiation Oncology, Medical University of South Carolina, Charleston, SC

The Atlantic bottlenose dolphin has attracted attention as a potential sentinel for human health. Greater knowledge of how the dolphin responds to environmental stress is needed, but such studies are limited by its status as a protected species. As a consequence, we established cell strains and an SV40-transformed cell line to be used as *in vitro* tools for measuring the molecular-level effect of the environment on this marine mammal. Specifically, we are investigating the vitamin D3 pathway within these cells. Vitamin D is of interest because of its acknowledged chemopreventative and immunomodulatory properties within terrestrial animals. Whether aquatic mammals also possess this pathway and gain the same immune benefits from vitamin D3 as terrestrial mammals is unknown. Our question is whether the pathway exists in dolphin skin and whether environmental stressors interfere with the downstream effects of vitamin D, proposing a potential mechanism for the detrimental impact of environmental fluctuations on marine mammal health. The bioactive and hormonal form of vitamin D3, 1,25-dihydroxyvitamin D3 (1,25D3), interacts with the vitamin D receptor (VDR) which is a potent regulator of gene transcription. We have previously detected within dolphin skin a 1,25D3-induced upregulation of VDR levels and expression of several genes, as identified by cDNA microarray analysis. One stressor of interest relevant to the dolphin's environment is methylmercury, which has been detected at levels considered toxic within various tissues of the animal, including skin. We show here that sublethal concentrations of methylmercury compromise 1,25D3's ability to upregulate both VDR and the expression of those target genes identified by microarray analysis. This suggests that stressors such as mercury interfere with the downstream transcriptional responses induced by vitamin D3. Such findings may help elucidate the role of vitamin D on innate immunity in dolphin skin and the role that stressors play at the molecular level.

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IT'S NOT ONLY PFOS – OTHER PERFLUORINATED COMPOUNDS ARE PRESENT IN NORTHERN FUR SEALS

Jocelyn R. Flanary [1,2]; John R. Kucklick [2]; Paul R. Becker [2]

[1]*Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC*
[2]*National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC*

Perfluorinated compounds are an emerging class of anthropogenic contaminants. Most organic contaminants are deposited in fatty tissues because of their lipophilic nature; however, perfluorinated contaminants have an overall polarity which prevents their accumulation in fatty tissues. Instead, these compounds circulate in the blood and accumulate primarily in the liver. Perfluorinated compounds have a worldwide distribution and exhibit toxicological effects in laboratory animals, therefore they may pose a risk of adverse effects in marine mammals. Perfluorooctane sulfonate (PFOS) is the only compound that has been analyzed in northern fur seals (*Callorhinus ursinus*). In this preliminary study we measured thirteen perfluorinated compounds in 45 plasma samples from northern fur seals collected in 2006 from St. Paul Island (in the Bering Sea), Alaska. Liquid chromatography/tandem mass spectroscopy (LC-MS/MS) was used to perform the analytical measurements. Perfluoroundecanoic acid (PFUnA) was the most abundant compound (5.2 ng/g), followed by perfluorononanoic acid (PFNA) at 3.0 ng/g and PFOS at 2.0 ng/g. The higher concentrations of PFUnA and PFNA over PFOS have rarely been seen in wildlife studies of perfluorinated compounds. However, it is not surprising because the atmospheric oxidation of 8:2 and 10:2 fluorotelomer alcohols (FTOH) produce PFNA and PFUnA, respectively, allowing for increased exposure to these compounds. It is also possible that PFUnA and PFNA exhibit larger bioaccumulation factors in northern fur seals than PFOS, causing higher concentrations of these carboxylate compounds. The results reported here demonstrate that all thirteen perfluorinated compounds analyzed are at measurable quantities in northern fur seals. At this time it is unclear whether the concentrations reported here lead to adverse toxicological effects in northern fur seals.

Funding Source: National Institute of Standards and Technology internal funds

A Novel Hollow Fiber Model System and the Study of Oxygen Sensitive Genes in the Alveolar Epithelium of Marine Mammals

Christina L. Grek[1], Danforth A. Newton[1], John E. Baatz[1,2], Xuejun Wen[3], Demetri Spyropoulos[4]

[1] Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC
[2] Department of Pediatrics, Medical University of South Carolina, Charleston, SC,
[3] Bioengineering Program, Medical University of South Carolina & Clemson University, Charleston, SC
[4] Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC

Pulmonary surfactant, a complex mixture of phospholipids and proteins, is critical for lowering surface tension levels in the alveoli and preventing lung collapse. The molecular and cellular mechanisms that regulate surfactant secretion are numerous and complex. During fetal lung development, *in utero* surfactant production is essential for successful lung inflation and function upon birth. In fact, abnormal surfactant is the major problem associated with premature births and the development of respiratory distress syndrome (RDS). When compared to the airway of the newborn, the fetal lung develops under conditions of lower oxygen tension. Similarly, during deep diving activities of marine mammals, the lung is collapsed, resulting in a state similar to that of the neonate *in utero,* rapidly becoming hypoxic and further complicated by increasing hyperbaric pressure due to diving depths of 90 to 2,000 meters. Pulmonary surfactant is crucial in allowing for proper reinflation and restoration of lung oxygen tension upon surfacing. Studies from our laboratory and others have indicated specific oxygen-sensitive genes may have roles in the regulation of surfactant production, especially during times of low oxygen tension. Due to the complex nature of lung architecture that prevents easy access, studies of the alveolar epithelium are extremely difficult. Through the establishment of a novel lung model system that more accurately represents *in vivo* conditions by incorporating the three-dimensional nature and air-liquid interface of the native lung epithelium (both marine and terrestrial), this study provides the opportunity to elucidate the roles of specific oxygen-sensitive genes in the regulation of surfactant production. Our data indicate a direct relationship between hypoxia-inducible factor and the expression of hemoglobin and surfactant proteins and suggest a role for hemoglobin in ATII cells in the oxygen-sensing pathway in alveolar epithelial cells. The information gathered will facilitate the elucidation of particular genes that may be useful in the development of new therapeutics to treat and prevent airway disease associated with disruption of surfactant production.

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Influence of Coral Health on Composition and Structure of Bacterial Communities Associated with the Surface Mucopolysaccharide Layer and Tissues of *Montastrea Faveolata*.

Wesley Johnson[1,2], Reney A. Henderson[3], Garriet W. Smith[4], Ernesto Weil[5], Karen E. Nelson[6], and Pamela J. Morris[1,2,6,7]

[1]*Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC*
[2]*Hollings Marine Laboratory, Charleston, SC*
[3]*Department of Biology, Howard University, Washington, D.C.*
[4]*The J. Craig Venter Institute, Rockville, MD*
[5]*University of South Carolina Aiken, Aiken, SC*
[6]*Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC*
[7]*Center for Coastal Environmental Health and Biomolecular Research, NOAA National Ocean Service, Charleston, SC*

Corals naturally form associations with complex assemblages of microorganisms that are thought to play vital roles in coral ecology. However, the composition of these communities and potential structural changes related to disease are poorly understood. Our objectives in this work were to 1) assess the composition of the bacterial communities associated with the coral, *Montastrea faveolata*, 2) Compare the communities of healthy and diseased colonies of *M. faveolata*, and 3) Compare the assemblages from the surface mucopolysaccharide layer (SML) to those of the coral tissues. Samples were collected from La Parguera, Puerto Rico in March 2006. SML from three healthy and three diseased colonies was collected by aspirating with a syringe. Tissues from the same colonies were collected by chiseling a small fragment from each colony. Community DNA was isolated and clone libraries of the16S rDNA genes were constructed and sequenced. Comparisons of community structure were also performed using denaturing gradient gel electrophoresis (DGGE). Results from clone libraries showed tissues to be dominated by sphingobacteria, while SML communities were composed mostly of α-proteobacteria. Diseased tissues had fewer Clostridium sequences than did healthy tissues. SML samples also showed differences between healthy and diseased colonies, with healthy colonies containing numerous sequences of *Lactococcus lactis*, which is completely absent from diseased samples. DGGE showed differences between SML communities of healthy and diseased colonies that were not observed between healthy and diseased tissues. These data indicate shifts in the structure of *M. faveolata* bacterial assemblages related to host health. Furthermore, these results indicate that SML communities may be less stable than those associated with tissues, and that the occurrence of disease is linked to a shift from a stable, low-diversity community to a species-rich assemblage of opportunists.

This work was funded by the National Science Foundation Biodiversity Surveys and Inventories Program, award number DEB0516347.

THE FUNCTIONAL POTENTIAL OF CORAL-ASSOCIATED MICROBIAL COMMUNITIES IN *MONTASTREA FAVEOLATA* FROM LA PARGUERA, PUERTO RICO

Nikole E. Kimes[1,2], Joy D. Van Nostrand[3], Jizhong Z. Zhou[3], Ernesto Weil[4] and Pamela J. Morris[1,2,5,6]

[1]*Marine Biomedicine and Environmental Sciences Center, Hollings Marine Laboratory, Charleston, SC*
[2]*Medical University of South Carolina, College of Graduate Studies, Charleston, SC*
[3]*Department of Botany and Microbiology, University of Oklahoma, Norman, OK*
[4]*Department of Marine Sciences, University of Puerto Rico, Mayaguez, PR*
[5]*Center for Coastal Environmental Health and Biomolecular Research, NOAA National Ocean Service, Charleston, SC*
[6]*Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC*

Coral-associated microbial communities are increasingly recognized as important components of the coral holobiont that influence coral health and disease. Energy and nutrient cycling is one of the more common mechanisms suggested for their contributions. Few studies, however, directly address the functional role of these communities. In the present study, we examine the functional potential of the coral-associated microbial community found in the surface mucopolysaccharide layer (SML) and tissue of a common Caribbean coral, *Monastrea faveolata*. The samples were collected from both visually healthy and yellow band infected colonies in March of 2007 off the coast of La Parguera, Puerto Rico. DNA was extracted and amplified for use in a functional gene array, the GeoChip II, which targets 10,000 functional genes involved in biogeochemical processes. We identified over 6500 functional genes present in the microbial communities associated with *M. faveolata*. Our preliminary analysis reveals a consistency across all of the samples in the relative percentage of genes found in each biogeochemical process surveyed. These processes include carbon degradation/fixation (16.5%, +/- 1.04), dissimilatory sulfite reduction (7.2%, +/- 0.73), metal homeostasis (21.15%, +/- 1.33), methane generation and oxidation (3.45%, +/- 0.37), nitrogen processing (17.57%, +/- 0.97), and organic chemical degradation (31.75%, +/- 1.16). Interestingly, however, comparisons of the specific genes indentified within a given functional category display less than 50% overlap between healthy and diseased samples. Our data suggests that the microbial communities associated with healthy and diseased *M. faveolata* use different mechanisms to fulfill similar functional niches.

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Anti-viral immunity in the shrimp *Litopenaeus vannamei*: dissecting the role of RNA interference

Yannick Labreuche[1], Javier Robalino[1], Enrique de la Vega[1], Nuala O'Leary[1], Jessica Shockey[1], Craig Browdy[2], Paul Gross[1] & Greg Warr[1]

[1]Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Charleston, SC
[2]South Carolina Department of Natural Resources, Charleston, SC

RNA interference (RNAi) is a form of posttranscriptional gene silencing whereby double-stranded RNA (dsRNA) molecules trigger the sequence-specific degradation of cognate mRNA by an RNA-induced silencing complex (RISC). The biological importance of RNAi is underscored by its wide conservation throughout metazoans and also by emerging evidence indicating that RNAi and related pathways function in many fundamental biological processes, including antiviral defense, development or maintenance of genomic stability.

In the Pacific white shrimp *Litopenaeus vannamei*, our recent work has established, for the first time in a marine invertebrate, that the injection of dsRNA induces an innate antiviral immunity acting in a sequence-independent manner, but also that virus-specific dsRNA evokes a powerful and specific immune response. These results imply the existence in the shrimp of an intact RNAi machinery, which could act as a natural mechanism of antiviral defense.

Using large scale EST collections from the Pacific white shrimp and a candidate gene approach (degenerate primers), we have identified several genes that correspond, based on their sequence homology, to different components of the RNAi pathway in vertebrates and invertebrates. We therefore aimed at investigating their *in vivo* implication in this mechanism by developing a reverse genetic approach (using dsRNA) to knock down their expression, a method also called RNAi of RNAi.

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Nucleic acid adducts of brevetoxins

Tod Leighfield[1,2] and John Ramsdell[1,2]

[1]Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC
[2]NOAA National Ocean Service, CCEHBR, Charleston, SC

Brevetoxins are potent secondary metabolites produced during harmful algal blooms by the dinoflagellate, *Karenia brevis*. Brevetoxins have been implicated in the morbidity and mortality of diverse organisms, from invertebrates to humans. Metabolic activation after exposure may result in the formation of reactive brevetoxin intermediates, which can create conditions favorable for binding to nucleic acids. This study aims to characterize the adduction of brevetoxins with nucleotides and to determine their role and significance in the induction of epigenetic modifications. Further studies will investigate the role of these unique metabolites as tools for biomonitoring.

Funding: NOAA National Ocean Service

PUTATIVE METACASPASE ACTIVATION IN AGING *KARENIA BREVIS* CULTURES: PRELIMINARY INSIGHT INTO CELLULAR MECHANISMS REGULATING BLOOM TERMINATION

Jillian G. Lynch[1,2] and Frances M. Van Dolah[1,2]

[1] *Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC 29412*
[2] *Marine Biotoxins Program, NOAA Center for Coastal and Environmental Health and Biomolecular Research, Charleston, SC 29412*

Karenia brevis, a toxic dinoflagellate, is responsible for near annual harmful algal blooms (HABs) off the west coast of Florida. These blooms cause extensive ecological and economic losses due to massive fish kills, marine mammal mortalities, and human illness caused by neurotoxic shellfish poisoning and respiratory irritation. The development of successful management strategies for *K. brevis* blooms is contingent upon understanding the molecular mechanisms that govern bloom initiation, propagation, and termination. Molecular mechanisms regulating *K. brevis* bloom demise, in particular, have remained largely uninvestigated, although recent studies have discovered programmed cell death (PCD) pathways in several other bloom-forming phytoplankton species. We have identified in *K. brevis* putative metacaspases, known central mediators of PCD in plants, fungi, and protists, containing a well-conserved caspase catalytic diad domain. Western blot analysis of *K. brevis* protein extracts collected from an early stationary phase culture revealed immunohybridization of a distinct protein band to a polyclonal antibody raised against a recombinant *Emiliania huxleyi* metacaspase protein. Caspase-specific activity in aging cultures was examined by measuring specific cleavage of fluorogenic canonical caspase tetrapeptide substrates. Preliminary results from these studies indicate an increase in caspase-specific activity in stationary phase cultures when compared to early and mid-logarithmic cultures. Identification of putative metacaspases from our EST libraries, cross reactivity with an *E. huxleyi* metacaspase polyclonal antibody, as well as caspase-specific activities in aging cultures provide preliminary evidence that *Karenia brevis* contains PCD machinery and may utilize an apoptosis-like pathway during cell death. Further characterization of the involvement of metacaspases in cell death may lead to the identification of molecular biomarkers for bloom termination.

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Salinity Effects on DMSP Concentrations in *Fragilariopsis cylindrus*

Barbara R. Lyon[1], Peter A. Lee[2], Michael G. Janech[3] and Giacomo R. DiTullio[1,2]

[1] Marine Biomedicine and Environmental Science, Medical University of South Carolina
[2] Hollings Marine Laboratory, College of Charleston
[3] Dept. of Medicine, Division of Nephrology, Medical University of South Carolina

Sea-ice diatoms encounter extreme salinity gradients during seasonal environmental cycles within their natural habitat. Winter sea-ice brine channel salinities have been measured in excess of 200ppt, while salinities in summer melt ponds drop to 10ppt. Dimethylsulfoniopropionate (DMSP) is a compatible solute produced by certain phytoplankton species that may serve osmoregulatory functions during salinity acclimation. DMSP also plays important roles in climate and biogeochemical cycles and therefore the physio-biological parameters controlling its production are of critical interest. The current experiment investigates intracellular and extracellular DMSP levels in the polar sea-ice diatom *Fragilariopsis cylindrus* in response to shifts in salinity. Log phase cultures initially grown at 35ppt were manipulated over a 24 hour period with media of varying salinities to achieve final salinities of 10ppt, 20ppt, 35ppt, 50ppt, and 70ppt. Cell counts, chlorophyll a (chl *a*), photosynthetic efficiency (F_V/ F_M), and total and dissolved DMSP were quantified at various time points during the three week experiment. Osmolality, carbonate alkalinity, and pH were also monitored during the course of the experiment. We hypothesize intracellular DMSP will increase in response to increasing external salinities, and decrease in response to decreasing external salinities coinciding with increases in extracellular (dissolved) DMSP. These results will be used to refine conditions for a follow-up proteomics experiment aimed at elucidating proteins associated with salinity stress and DMSP metabolism.

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QUANTITATIVE IMAGING AND GENE EXPRESSION ANALYSIS OF A DOLPHIN LUNG ENDOTHELIAL CELL LINE: BASELINE DATA TO ASSESS MARINE MAMMAL HEALTH.

Annalaura Mancia[1,2,3], John T. Elliott[3], Michael Halter[3], Alessandro Tona[3], Gregory W. Warr[1], Anne L. Plant[3].

(1) Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Hollings Marine Laboratory, 331 Ft. Johnson Road, Charleston, SC, 29412, USA.
(2) Department of Experimental Evolutionary Biology, University of Bologna, Via Selmi 3, Bologna, 40126, Italy.
(3) Biochemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD, 20899, USA.

Development of stable dolphin cell lines is critical for studying biological differences between land-based and sea-based mammals and for understanding the molecular basis of dolphin health. Almost no baseline data for dolphin cell lines exists in the literature, and very few studies have characterized both the phenotypic and gene expression profile of a specific dolphin cell line. The goal of this project is to provide baseline data that describes the phenotypic properties and gene expression levels of a cultured primary dolphin lung endothelial cell line. We began with a comprehensive characterization of the growth and morphological characteristics of the cell line during *in vitro* culture. Interestingly, in some cases, we observed a systematic decrease in the distribution of cell volumes and cell spread area during extended passages of the cell line. This appeared to be a result of spontaneous conversion of the original symmetrical morphology of endothelial cells to a spindly, more migratory and more rapidly proliferating cell type. This phenotypic change is similar to changes that have been observed in endothelial cells from land mammals and may represent a "trans-differentiation" event. Expression of endothelial protein biomarkers and RNA transcripts are being monitored during culture to establish the endothelial nature of this cell line and develop baseline transcriptome information. These studies will likely be of significance to endothelial cell biology of many species, in addition to dolphin. In the future, analysis of the dolphin cells under different conditions and challenging with specific treatment (e.g. biotoxin) will open new insights on the marine mammal biology-physiology-health status and their relationship with human-health.

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ANALYSIS OF MOUSE LUNG INFLAMMATION FOLLOWING ASPIRATION EXPOSURE TO BREVETOXIN-2.

Kelli M. Sas[1] and John E. Baatz[1,2]

[1]*Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC*
[2]*Department of Pediatrics, Medical University of South Carolina, Charleston, SC*

Brevetoxins are potent marine algal toxins produced by *Karenia brevis*, the organism responsible for Florida red tide. Brevetoxin exposure through ingestion or inhalation has been associated with a variety of adverse effects including human illness, marine mammal and seabird deaths, and massive fish kills. Humans and manatees have been identified as the most at-risk groups for inhalation exposure, and previous reports of natural exposures in both humans and manatees have suggested that brevetoxin inhalation may result in an inflammatory response in the lung. In this study, our goal was to test the hypothesis that brevetoxin exposure results in pathological changes in the mouse lung, characterized by immune cell infiltration and tissue inflammation. C57BL/6 mice were exposed to 5 ug/kg brevetoxin-2 or sham control through aspiration exposure for 1, 4, 8, 16, or 24 hrs. Histological analysis revealed immune cell infiltration in both the airways and surrounding tissue at 16- and 24 hr. post-exposure with the infiltrate consisting primarily of neutrophils and monocytes, often resulting in multinucleated cell obstructions of airways. Additionally, areas of bronchiolar epithelium disturbances were seen at 24 hr. post-exposure. These results indicate that brevetoxin inhalation does trigger an immune response in the mouse lung, and further analysis of bronchoalveolar lavage fluid at 16- and 24 hr. will determine alterations in cytokine levels following brevetoxin inhalation.

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Polyketide synthases in *Karenia brevis*: characterization and expression of proteins involved in toxin biosynthesis in the Florida red tide dinoflagellate

Emily A. Monroe[1,2], Zhihong Wang[2], Richard K. Pierce[3], and Frances M. Van Dolah[1,2]

[1]Marine Biomedicine and Environmental Sciences Program, Medical University of South Carolina, Charleston, SC
[2]Marine Biotoxins Program, NOAA, Center for Coastal and Environmental Health and Biomolecular Research, Charleston, SC
[3]Mote Marine Laboratory, Sarasota, FL

Karenia brevis is the causative organism of Florida red tides that are responsible for detrimental effects on the environment and on human health through the production of brevetoxins. Brevetoxin biosynthesis is proposed to be mediated by polyketide synthase enzymes (PKS). We have recently demonstrated novel and unexpected type I PKS transcript structures in *K. brevis* that encode for single catalytic domains. The absence of differential expression of PKS's in microarray analyses and the presence of a spliced leader sequence led us to hypothesize that these transcripts are controlled post-transcriptionally. In this study, we employed peptide polyclonal antibodies to *K. brevis* PKS sequences to analyze protein abundance in a low toxin-producing *K. brevis* (Wilson) sub-strain compared to *K. brevis* (Wilson) cultures that produce 8 pg/cell intracellular brevetoxins. During log phase of growth, RNA, protein, and brevetoxins were isolated from the low toxin-producing cultures and control cultures. Brevetoxins were not detected in any of the low toxin-producing cultures by LC/MS (detection limit <0.1 pg/cell). Using our *K. brevis* microarray and qPCR to examine PKS transcript levels, PKS transcript expression in the low toxin-producing cultures was not significantly different from control cultures. However, abundance of certain PKS proteins was depressed 40-60% in the low toxin-producing cultures, suggesting these proteins may be involved in toxin biosynthesis. This is the first evidence relating expression of a gene or protein to toxin biosynthesis in a dinoflagellate. Identification of the molecular basis of toxin biosynthesis is a critical step in understanding regulation of toxin biosynthesis.

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Investigating the Role of Membrane Vesicles in Zn-Microbe Interactions

Benjamin A. Neely[1,2], Venetia D. Lyles[3], Paul M. Bertsch[1,4], Pamela J. Morris[1,2,5]

[1] *Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, South Carolina*

[2] *Hollings Marine Laboratory and Center for Coastal Environmental Health and Biomolecular Research, U.S. National Oceanic and Atmospheric Administration, Charleston, South Carolina*

[3] *South Carolina State University, Orangeburg, South Carolina*

[4] *Department of Plant and Soil Science, University of Kentucky, Lexington, Kentucky*

[5] *Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, South Carolina*

Membrane vesicles (MV) are extracellular formations derived from the outer-membrane of Gram-negative bacteria, ranging from 50-200 nm in size. Their biological roles include transport of virulence factors, protein and DNA exchange, cell-cell communication, and biofilm formation. In addition, MVs are considered a key vehicle in host-pathogen interactions. Also, because of their high surface area to volume, in the presence of high concentrations of metal, MVs may also play an integral role in modulating metal ion binding to the cell surface by increasing the number of potential metal binding sites. To address additional roles of MVs, we used the Gram-negative bacterium *Burkholderia vietnamiensis* $PR1_{301}$ (PR1) which in humans is involved in co-infecting cystic fibrosis patients. From an environmental perspective, PR1 has been used in bioremediation applications and has previously been studied by our laboratory for its resistance to divalent metals including Zn^{2+}. We have shown that PR1 possesses an uncharacterized pH-dependent mechanism of Zn^{2+}-resistance, and is 10-fold more resistant to Zn^{2+} at pH 5 versus pH 7. Interestingly, we have found evidence that MVs may be playing a role in pH-dependent Zn toxicity. When PR1 is grown in the presence of 100 mg/L Zn^{2+} at pH 7, using SEM/EDX, it was shown that Zn is localized to MVs but is not associated with cells. Additionally, at pH 5 versus 7 in the absence of Zn^{2+}, PR1 produces approximately half as much MVs (2.1 versus 4.1 mg MV protein/L, respectively). A MV purification scheme was then developed using centrifugation, filtration, and ammonium sulfate precipitation followed by density gradient centrifugation. Ongoing studies will characterize purified MVs produced at pH 5 and 7 using shotgun proteomics. These data will provide valuable insight into how the protein composition of MVs at different pH could be involved in Zn^{2+}-microbe interactions as well as provide the first study of the influence of pH on MV protein composition, which may be relevant to studying the host-pathogen relationship.

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Localization and Binding Capabilities of Crustins in the Pacific Whiteleg Shrimp, *Litopenaeus vannamei*

Jessica E. Shockey[1,2], P.S. Gross[1,2], John E. Baatz[1,3]

[1]Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, 221 Fort Johnson Road, Charleston, SC 29412, USA

[2]Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, USA

[3]Department of Pediatrics, Medical University of South Carolina, Charleston, SC 29425, USA

Crustins are a family of antimicrobial peptides originally discovered in the shore crab, and to date includes several types found in many crustacean species, including *Litopenaeus vannamei*. Studies suggest crustins have antibacterial action primarily against Gram positive bacteria, with only one report of its *in vitro* activity against a Gram negative species. The crustin peptides are constitutively expressed, and are present in large quantities even in unchallenged animals. In this study, the localization and bacteria binding properties of crustins *in vivo* are being assessed in "healthy" non-immune challenged shrimp. For native peptide localization, hemocyte smears and tissue sections were subjected to immunohistochemistry using a crustin-specific (C-terminal polyclonal) antibody, and crustins were detected within a population of hemocytes and within different tissue samples, most notably the gills and lymphoid organ. In order to test crustins' ability to act as indirect antimicrobial agents, hemolymph samples were exposed to Gram negative *Vibrio penaeicida*, and crustin was determined to bind these shrimp pathogens both by a bacteria bead binding assay and by immunohistochemistry. The results suggest that crustins are providing antibacterial activity by binding to bacteria.

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Vibrio coralliilyticus: Comparison of Anti-Microbial and Antibiotic Resistance with Sister Phylotypes Isolated from Puerto Rico

Maria I. Vizcaino[1,2,3], Gregory S. Matthews[2,4], Nikole E. Kimes[1,2], Katherine Williams[1,2], Peter D.R. Moeller[1,2,4], and Pamela J. Morris[1,2,5,6]

[1]Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC
[2]Hollings Marine Laboratory, Charleston, SC
[3]Toxin Chemistry, NOAA National Ocean Services, Charleston, SC
[4]Grice Marine Laboratory, College of Charleston, Charleston, SC
[5]Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC
[6]Center for Coastal Environmental Health and Biomolecular Research, NOAA National Ocean Service, Charleston, SC

Vibrio coralliilyticus ATCC BAA-450, a temperature-dependent coral pathogen first isolated from the Indian Ocean, induces bleaching in *Pocillopora damicornis* at temperatures greater than 24.5°C. Currently, we are comparing the anti-microbial and antibiotic resistance and susceptibility profiles of this *V. coralliilyticus* strain and possible sister phylotypes isolated from the southern coast of Puerto Rico. We hypothesize that *V. coralliilyticus* ATCC BAA-450 and the sister phylotypes will display similar anti-microbial and antibiotic resistance. We have several lines of evidence to support this hypothesis. First, five bacterial isolates, obtained from the surface mucopolysaccharide layer of visually-diseased *Pseudopterogorgia americana*, showed 97-99% 16S rDNA homology to *V. coralliilyticus*. Genomic profiling through repetitive element PCR (REP-PCR) demonstrated that one isolate had a similar profile to ATCC BAA-450, while the other four were different (but similar to one another). Second, we examined whether bacteria isolated from healthy and diseased *P. americana* colonies were able to inhibit *V. coralliilyticus* grown at 24 and 27°C. We observed that twelve (12/140) isolates inhibited *V. coralliilyticus* at 24°C, while only five strains inhibited growth at 27°C. Third, four of the homologous strains were screened against a subset of the coral isolates that exhibited bioactivity in anti-microbial tests. Three of the homologous strains responded similarly to the ATCC strain and showed a high level of resistance to the anti-microbial compounds produced by the coral isolates. Lastly, *V. coralliilyticus* ATCC BAA-450 and the three sister phylotypes were screened against 26 known antibiotics; they all exhibited similar resistance and susceptibility profiles (resistant to 10-14 antibiotics). To summarize, both *V. coralliilyticus* ATCC BAA-450 and three of the Caribbean phylotypes showed similar levels of resistance in our anti-microbial (temperature-dependent) and antibiotic assays. This study will further contribute to our understanding of the pathogenicity of *V. coralliilyticus* and the similarity among sister phylotypes in a broader ecological context.

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Organohalogen contaminant exposure in wild bottlenose dolphins: Combined influences of bioaccumulation, life history and tissue distribution.

Yordy, J. [1, 2], Wells, R.[3], McLellan, W.A.[4], Pabst, D.A.[4,] Guichard, A[2], Balmer, B.[3], Schwacke, L.[5], Rowles, T.[6], Kucklick, J.[2]

[1] *Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC*
[2] *National Institute of Standards and Technology, Charleston, SC*
[3] *Chicago Zoological Society, c/o Mote Marine Laboratory, Sarasota, FL*
[4]*University of North Carolina Wilmington, Wilmington, NC*
[5]*Cooperative Center for Marine Animal Health, NOAA, Charleston, SC*
[6]*Marine Mammal Health and Stranding Response Program, NMFS, Silver Spring, MD*

Bottlenose dolphins (BNDs) are long-lived, piscivorous marine mammals which represent apex predators for many coastal ecosystems. As a result, they are vulnerable to accumulating heavy burdens of persistent organohalogen contaminants (POCs). Total concentrations of POCs in marine mammals are strongly influenced by diet, sex, body condition, and life history traits (i.e., age and reproductive state); however, less is known about how these parameters impact compound-specific patterns and contaminant mixtures. To evaluate the impact of each of these factors on organohalogen mixtures in BNDs, tissue samples (blubber, n= 107; blood, n=68) and dietary items (milk, n= 20; fish, 3 species, n=45) were collected from Sarasota Bay, FL and analyzed by GC/MS for 68 polychlorinated biphenyl congeners, 12 organochlorine pesticides and 5 polybrominated diphenyl ether congeners. Principal components analysis (PCA) was used to investigate mixture differences between dietary sources and three dolphin life history groups (juveniles, adult males and females). Contaminant mixtures of the two dietary items in Sarasota Bay differed, with milk containing a greater proportion of the lower chlorinated PCBs and fish containing higher proportions of the organochlorine pesticides. Contaminant mixtures in bottlenose dolphins also varied between life history stages. Mixtures in juveniles were not purely reflective of a milk or fish-based diet, but were affected by both diet and metabolism. Patterns in adult males were influenced by biotransformation and shift to contain higher proportions of non-metabolizable congeners with age. Alternatively, contaminant patterns in adult females appeared to be strongly influenced by the selective offloading of lower halogenated compounds through milk upon reaching reproductive maturity. Body condition was also investigated as a potential factor in blood and internal tissue contaminant concentrations and mixtures. Supportive data from live animals and necropsied animals will be presented.

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