Generation and utility of hepatocyte-like cells from pygmy sperm whale (*kogia breviceps*) induced pluripotent stem cells.

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The pygmy sperm whale (PSW; *Kogia breviceps)* is the second most commonly stranded cetacean in the Southeastern United States. The PSW is a deep diving mammal (2km) that spends limited time at the surface thus restricting access for biological studies. For example, it is possible that their extreme lifestyle requires a resilient physiology that makes them more susceptible to environmental insult. However, sample availability would be required to test this possibility.

We have focused on efforts to utilize rare samples made available by PSW strandings to study normal cell biology and response to stressors. Unfortunately, these samples produce low numbers of primary cells with limited expansion capacity. To better understand PSW biology and test hypotheses regarding their strandings we have established PSW induced pluripotent stem (iPS) cells that will be differentiated to primary cell types for studies of normal physiology and responsiveness to stressors.

Heavy metal exposure has been argued to be a cause of PSW stranding/lethality. To test this hypothesis, we are developing iPS-derived PSW hepatocytes for toxicity to determine if PSW cells are more sensitive than their human counterparts. In humans, hepatocyte-like cells generated from embryonic stem (ES) cells and from iPS cells have been generated and used for various studies and even considered for transplant therapies. We have generated PSW iPS cells from fibroblasts derived from lungs from stranded/deceased individuals through the activation of 4 stem cell factors. These iPS cells are being cultured to generate hepatocyte-like cells to test the efficacy of current protocols developed for humans.

Successful creation of PSW hepatocyte-like cells will provide us with a reagent to assess heavy metal toxicity in marine mammals, which has potential to aid the prediction, mitigation, and prevention of stranding events.

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The Distribution of *Pseudo-nitzschia spp.* Along the Southeastern U.S. Coastline

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Phytoplankton is the base of the ocean's food web and they play a vital role in supporting life. However, under certain conditions phytoplankton populations can grow unchecked forming algal blooms which can deplete oxygen as blooms senesce. Some species of phytoplankton are known to produce toxins and when these species bloom they can produce far reaching negative effects. Domoic Acid (DA) is the neurotoxin produced by *Pseudo-nitzschia* a type of diatom. DA has long been a documented issue on the west coast of the United States. Over the last three years, a number of strandings of pygmy sperm whales and dolphins along the southeastern U.S. coast have been linked to DA, suggesting that *Pseudo-nitzschia* may be an issue in this area. Since little work has been done looking at *Pseudo-nitzschia* distribution in this area a collaborative study was implemented to assess this species distribution. NOAA Phytoplankton Monitoring network in conjunction with the South Carolina Maritime Foundation and South Carolina Department of Natural Resources set out to collect phytoplankton samples from Oregon Inlet, NC to Cape Canaveral, FL. The SCDNR program SEAMAP and the SC Maritime Foundation boat The Spirit of South Carolina collected samples from along the coast, while NOAA Phytoplankton Monitoring staff collected samples from within three different estuaries in South Carolina. These samples were screened using an inverted light microscope to assess what species of phytoplankton were present. When *Pseudo-nitzschia* was present, samples were settled on a Sedgwick Rafter counting chamber to determine the abundance present. A scanning electron microscope was then used to identify what species of *Pseudo-nitzschia* was present. *Pseudo-nitzschia* was found off the southeastern U.S. coast at nearly all stations sampled, as well as within, the South Carolina estuaries. Further research is necessary to identify any trends that may govern their distribution.

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Characterization of the toxic principles from field collected and laboratory-cultured samples of the harmful alga, *Prymnesium parvum*

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The golden alga, *Prymnesium parvum*, has been implicated in fish and aquatic animal kills in the United States since 1985. This alga is most often associated with blooms in estuarine and marine waters, but has been observed to affect high conductivity inland waters and aquaculture facilities. In addition to widespread ecological impacts through the loss of entire fish populations within lakes, an economic burden is also felt by state and local agencies due to year class losses of fish for stocking lakes as well as loss of fishing and recreational use of the affected body of water. Two ichthyotoxic and hemolytic compounds have been described and structurally characterized from *P. parvum*, prymnesin -1 and -2. Data from both the Moeller lab and others suggest that undescribed and more ecologically relevant toxins are present in *P. parvum*. To identify novel toxins *P. parvum* was first cultured and field unialgal blooms were also collected; these were extracted with ethyl acetate followed by methanol. Filtrate from *P. parvum* cultures was similarly extracted. These crude extracts were tested for cytotoxicity against mammalian cell lines (N2A and GH4) with activity observed in both the methanol and ethyl acetate fraction from cultured cell mass and in the methanol fraction from *P. parvum* filtrate. Bioassay-guided fractionation using high pressure liquid chromatography (HPLC) and mass spectrometry (MS) identified multiple cytotoxic fractions. Further characterization of these fractions with nuclear magnetic resonance spectroscopy (NMR) indicates that lipids dominate the cytotoxicity observed in cultured cell mass, while free fatty acids, specifically oleic acid and elaidic acid dominate the cytotoxicity observed from filtrate samples. A cytotoxic non fatty acid sample from the filtrate with a nominal molecular ion at m/z 540 is currently being characterized by high resolution NMR and accurate mass spectrometry. Isolating and characterizing the important toxin(s) from *P. parvum* will allow relevant experimentation with field and culture populations to assess cues for toxin accumulation, lead to improved detection methods and further research avenues including toxin neutralization, characterization of toxin bioactivity, and possible anticancer properties.

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Post-transcriptional Regulation of the Cell Cycle in the Red Tide Dinoflagellate, *Karenia brevis*

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The dinoflagellate, *Karenia brevis*, produces harmful algal blooms in the Gulf of Mexico that cause extensive marine animal mortalities and human illness nearly annually. The molecular mechanisms controlling cell cycle entry in this dinoflagellate are important because bloom development occurs through vegetative cell division. Microarray and qPCR studies have demonstrated that, unlike typical eukaryotes, dinoflagellate cell cycle genes are not regulated at the transcriptional level, including genes that code for replication fork proteins, typically activated by the E2F transcription factor at the G1/S transition. Post-transcriptional control of these genes is also suggested by the presence of a trans-spliced leader sequence on their transcripts. Sequence analysis and protein modeling were used to develop custom antibodies for Western blotting to investigate the abundance of replication fork proteins over the cell cycle and whether they are regulated at the translational or post-translational level. The *K. brevis* replication fork proteins, PCNA, RFC, RPA and RnR2 were shown to change over the cell cycle with highest expression at S-phase, suggesting translational control. PCNA also appears to be modified post-translationally, either by ubiquitin or SUMO concurrent with S-phase. Immunolocalization of PCNA showed that it is present in the nucleus throughout the cell cycle in cells actively traversing the cell cycle. However, PCNA showed a pattern of nuclear location that changes between a chromatin bound form and a pool that is peripheral. These results lead us to propose a novel mechanism of translational control of cell cycle entry as opposed to transcriptional control which is seen in most eukaryotes.

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Etiologic Studies of Skin Disease in Wild Bottlenose Dolphins (*Tursiops truncatus*) and their Relevance to Human Health

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Skin disease in free-ranging bottlenose dolphins is geographically widespread and highly prevalent in many populations near coastal areas. Reports of skin disease in wild bottlenose dolphins have been documented from waters surrounding South America, portions of Europe and the United Kingdom, New Zealand, and U.S. coastal waters, with evidence of skin disease affecting over 50% of the individuals in several of these populations. Underlying causes of skin disease vary and while some disease, such as poxvirus, is usually transient and non-life threatening, other infectious cutaneous disease such as erysipelas may be severely debilitating and/or fatal. The implication of the observed disease for overall ecosystem health and/or human health depends on the etiology and potential pathogen involved.

Skin diseases in free-ranging dolphins have been studied using a variety of research methods including photographic identification (photo-id) surveys, capture-release health assessment projects, and stranding investigations. In many cases, however, the etiology of lesions cannot be determined, thereby impeding estimates of disease occurrence and endemicity, as well as predictions of disease effects on population dynamics. The objective of this study is to utilize histological, microbiological, and genetic analyses of skin lesions occurring on stranded bottlenose dolphin carcasses to assist in the determination of lesion etiology for free-ranging animals. Members of the Southeast U.S. Marine Mammal Stranding Network have been asked to photo-document and biopsy lesions from any dead bottlenose dolphin with evidence of skin disease. To date, we have received 32 stranding samples, representing eleven etiologies, some of which are zoonotic or similar to pathogens that cause disease in humans. Obtaining a better understanding of the various etiologies underlying skin disease in bottlenose dolphins provides a means for improving our estimates of disease prevalence among wild populations, as well as an opportunity to enhance the surveillance of marine pathogens that are potentially harmful to humans.

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Measurements of Perfluorinated Compounds in Plasma of Northern Fur Seals (*Callorhinus ursinus*) and a Preliminary Assessment of their Relationship to Peroxisome Proliferation and Blood Chemistry Parameters

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Perfluorinated compounds (PFCs) are known to exhibit toxicological effects in laboratory animals and may pose a risk of adverse effects in marine mammals. With some of the highest concentrations of PFCs measured in marine mammals from the Arctic, it is important to understand their distribution in Arctic species. In this study we report concentrations of thirteen perfluorinated compounds measured in northern fur seal (*Callorhinus ursinus*) plasma from animals harvested on St. Paul Island, Alaska in 2006 and 2007. Perfluoroundecanoic acid (PFUnA) was the most abundant compound with a median concentration of 5.5 ng/g ranging from 1.2 to 16.8 ng/g, followed by perfluorononanoic acid (PFNA) at 3.3 ng/g (1.3 to 9.6 ng/g) and perfluorooctane sulfonate (PFOS) at 3.0 ng/g (0.9 to 18.6 ng/g). Interestingly, PFOS is not the most abundant compound as it is in most environmental studies, suggesting a different source or preferential metabolism of the C11 and C9 carboxylic acid compounds. The results reported here demonstrate that several perfluorinated compounds are at measurable quantities in the northern fur seal, with some PFCs being measured for the first time in this species. We also determined that peroxisome counts and blood chemistry parameters were not significantly correlated to concentrations of perfluorinated compounds measured in the plasma. This suggests the levels of PFCs in this population are too low to induce peroxisome proliferation or to affect other blood chemistry markers.

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The interactive effect of hypercapnic hypoxia and bacterial infection on oxygen consumption and protein synthesis in the Pacific whiteleg shrimp, *Litopenaeus vannamei*.

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Estuarine species frequently encounter areas of low oxygen (hypoxia) and high carbon dioxide (hypercapnia). Exposure to hypoxia and hypercapnia triggers a broad assortment of behavioral, physiological, biochemical and genetic responses in marine organisms. In combination, hypercapnic hypoxia (HH) is also known to impact disease resistance in crustaceans causing, for example, an increase in the rate of lethal infection from bacterial pathogens in shrimp. Inhibited immune function is particularly problematic for estuarine species, as anthropogenically altered coastal systems frequently exhibit higher than normal pathogen loads. With high concentrations of environmental bacteria, decreased immune resistance and the ability to travel over large distances, crustaceans become ideal vectors for disease transmission (via consumption or physical interaction), thereby posing a serious risk to human health. Moreover, both hypoxia and bacterial infection independently cause a reduction in the rate of oxygen consumption in the Pacific whiteleg shrimp, *Litopenaeus vannamei,* and Atlantic blue crab, *Callinectes sapidus.* This metabolic depression could have serious consequences to the animal by reducing growth rates, reproductive efforts, and swimming and/or migratory behaviors. The interactive effects of hypoxia and infection, which are largely unknown, could be even more severe, potentially leading to widespread mortality events. Here we examine the singular and combined effects of HH and bacterial infection (*Vibrio campbellii*) on whole-animal oxygen consumption rates (MO_2) and tissue-specific (muscle, hepatopancreas, gill) protein synthesis rates of juvenile *L. vannamei*. We use closed-system respirometry to determine resting MO_2, whereas protein synthesis rates are measured by calculating the rate of tissue incorporation of a radiolabelled amino acid ([^{3}H] phenylalanine). We hypothesize that MO_2 and protein synthesis rates (particularly in muscle) will be lowest in shrimp exposed mutually to HH and *V. campbellii*. Our forthcoming results may better enable us to stop the spread of human disease by marine organisms and prevent decreased growth rates and survival in many economically important fisheries.

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Temperature-dependent Virulence Factors in the Marine Pathogen *Vibrio coralliilyticus*: A Proteomic Analysis

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Over the past century, a correlation has been observed between increasing temperature and the increased incidence and/or severity of certain infectious diseases. An important example of this observation occurs within the Genus *Vibrio*, where human and coral *Vibrio* outbreaks often occur during the warm summer months. *Vibrio coralliilyticus* is a globally-distributed bacterium that infects corals and their endosymbionts at temperatures above 24°C. Evidence shows that this temperature-dependent virulence is multifactoral, however, the mechanisms underlying pathogenicity have not been fully elucidated. In this study, we use two-dimensional liquid chromatography coupled with tandem mass spectrometry (2D-LC-MS/MS) to detect proteins produced by *V. coralliilyticus* ATCC BAA 450 at a non-pathogenic (24°C) and pathogenic (27°C) temperature. Utilizing the newly sequenced genome of *V. coralliilyticus* ATCC BAA-450 (GenBank:ACZN00000000) in conjunction with TurboSEQUEST and Scaffold, we compare virulence factors produced by *V. coralliilyticus* at the two temperatures in order to identify potential mechanisms of temperature-associated pathogenicity. Our results reveal a significant increase in the number and expression level of virulence factors produced by *V. coralliilyticus* cultivated at 27°C. Proteins associated with quorum sensing, flagellar-mediated motility, secretion systems, host degradation, and antibiotic resistance were increased at 27°C, indicating they may contribute to the increased virulence of *V. coralliilyticus* at the higher temperature. This study's significance is enhanced by climate change predictions indicating that surface seawater temperatures will soon reach above 27°C for the majority of the year throughout the tropics.

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Distribution and elimination of brevetoxin metabolites

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Brevetoxins are potent neurotoxins produced by dinoflagellates during harmful algal blooms. Accumulation and metabolism of these toxins are risks to human health resulting from the consumption of contaminated shellfish and the inhalation of toxic aerosols. After exposure to brevetoxins, metabolic transformation results in the production of brevetoxin intermediates of varying potencies and concentrations. *In vivo* disposition studies of brevetoxin metabolites have been hampered by the lack of sufficient quantities of purified brevetoxin metabolites. Here we describe findings from an *in vivo* exposure study and measure brevetoxin metabolite distribution and elimination using radiolabeled analogues of three shellfish metabolites synthesized specifically for this study. Differences in the elimination rates and distribution of these metabolites suggest that observed differences in cellular toxicities of each metabolite may be augmented by the retention and distribution of the toxin in the body. This research will provide a better understanding into the adverse effects of exposure to brevetoxin and its metabolites in marine animals and humans.

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Global analysis of growth phase-associated transcriptomes in the toxic dinoflagellate, *Karenia brevis*

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Molecular mechanisms regulating senescence and cell death in phytoplankton have received increased attention within the last decade due to their prospective roles in population structuring, species succession, biodiversity/selection, bloom termination, and biogeochemical cycling (Bidle and Falkowski, 2004; Franklin, 2006). To gain insight into gene expression indicative of aging and/or cell death in *Karenia brevis*, the toxic dinoflagellate responsible for the near annual harmful algal blooms in the Gulf of Mexico, oligonucleotide microarrays were employed to monitor transcriptomic changes over a complete growth curve. Mid-logarithmic phase (day 6) was used as the point of comparison to the transition into stationary phase (day 10), mid-stationary phase (day 14), and late-stationary (day 18). The data were subjected to three stringency filters to encompass a wide array of statistical and technical requirements to identify the full breadth of future research directions. Of the 10263 genes assessed using the most stringent filtering scheme, 29% (2959) of the features on the array were significantly changing, suggesting a drastic reorganization of the *K. brevis* transcriptome at the transition from logarithmic to stationary phase growth. Analysis of the distribution of genes with increased and decreased expression in stationary phase with respect to functional classes (Gene Ontology enrichment) for each stringency filter (GO Slim, Modified Fisher's Exact, FDR < 0.05) identified coordinated expression of transcripts involved in energy production, calcium ion homeostasis, regulation of gene expression, and response to stress at the transition from logarithmic to stationary phase growth. While further research is needed to discern the functions of the *K. brevis* transcripts responsive to the transition between growth and maintenance phases, this work may serve as a diagnostic for determining the growth status of field populations.

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Proteome Changes associated with Salinity Stress and DMSP Accumulation in *Fragilariopsis cylindrus*

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Sea-ice diatoms comprise the greatest portion of biomass in fast and pack ice environments and have been identified as important contributors to global biogeochemical and climate cycles, in part through their production of dimethylsulfoniopropionate (DMSP). DMSP can be cleaved to form volatile dimethylsulfide (DMS), which affects both atmospheric chemistry and the earth's radiation budget. Therefore, understanding the impact of environmental parameters on the cellular biology associated with DMSP production is of critical importance to predicting interactions and effects of climate change and sea-ice loss. Ice-diatoms encounter extreme salinity gradients during seasonal environmental cycles. DMSP is a compatible solute that may serve osmoregulatory functions and act as an antioxidant during salinity acclimation. A previous experiment with *F. cylindrus* confirmed the hypotheses: (1) hypoosmotic conditions decrease intracellular DMSP with concomitant increase in the dissolved fraction (2) hyperosmotic conditions increase intracellular DMSP. The current experiment investigated global proteome changes associated with increases in intracellular DMSP under high salinity with the hypothesis that a subset of significantly up or down regulated proteins will be associated with osmotic stress and increased DMSP concentrations. Axenic log-phase cultures initially grown at salinity of 35 were gradually shifted over 24 hours to a treatment salinity of 70 or maintained at 35 control salinity (5 biological replicates per group). Cell density, DMSP, photosynthetic efficiency, pigment and protein changes were assessed at 48 hours. Two-dimensional gel electrophoresis was used to identify protein spots significantly increased or decreased in abundance (student's t-test, $p \leq 0.02$). These spots were selected for identification by tandem mass spectrometry. Results included decreases in light harvesting complex proteins, along with increases in general stress response, compatible solute synthesis, and S-adenosyl methionine (SAM) active methyl cycle proteins. This later group of proteins indicates the activated methyl cycle could be an important part of DMSP synthesis.

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Development of induced pluripotent stem cells from cryopreserved lung tissue of pygmy sperm whale (*kogia breviceps*).

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Concerns about marine mammal health and environmental threats have risen in the last decade due to increases in mortality, unusual mortality events, and previously unseen pathologies. Because opportunities for *in vitro* studies on pelagic organisms, such as marine mammals, are limited, it is necessary to establish systems whereby cultures of cells and reconstitution of functional tissues from these organisms can be used as surrogates for the animal in the wild. Isolated primary cells are, in fact, common models for the study of protein functions, cellular mechanisms, organ-specific functions, and responses to environmental parameters. Because there is a limited availability of marine mammalian samples and primary cell types we first developed a method to cryopreserve tissues and thereby generate a tissue bank (or biorepository). Starting from fragments of long-term cryopreserved tissues (several months after the stranding event), we were able to establish cultures of viable primary cultures of different lung cell types. To further circumvent the limited availability of samples, we then used one of these primary cultures (a primary culture of lung fibroblasts) to generate induced pluripotent stem (iPS) cells. iPS cells are a potential source for most primary cell types in large numbers, and therefore a useful approach to circumvent the limited availability of pygmy sperm whale samples. In a field with a lack of basic knowledge due to the limited availability of tools and samples and to the protected status of the animals, the development and establishment of primary cultures from 1) cryopreserved tissues and 2) reprogramming of pluripotent stem cells, has the potential to create the reagents necessary to revolutionize our understanding of the basic biology of marine mammals and understand the impact of stressors on marine and land animal health.

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The effects of increased temperature on *Karenia brevis* and *Symbiodinium microadriaticum*

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As global temperatures increase, so do the potential impacts on phytoplankton communities. To understand the potential effects of climate change on harmful algal blooms (HAB) and coral bleaching, heat stress will be applied to two model species; *Karenia brevis*, a toxic HAB species, and *Symbiodinium microadriaticum*, the coral symbiont. We will investigate the potential role of heat stress in oxidative stress and programmed cell death (PCD). *K. brevis* and *Symbiodinium* are both dinoflagellates and previous studies have documented that *Symbiodinium* experiences an increase in ROS levels in response to heat stress. Therefore, we hypothesize that *K. brevis* and *Symbiodinium* will both exhibit increased levels of reactive oxygen species (ROS) and metacaspase protein expression during heat stress when compared to control conditions. Since ROS production and metacaspase proteins are hypothesized to be involved in the heat stress response, we will examine ROS levels and metacaspase protein expression levels in *K. brevis* and *Symbiodinium* using flow cytometry and western blot methods. This experiment will allow us to determine if heat stress does in fact cause an elevation of ROS and metacaspase protein expression levels suggesting an induction of oxidative stress and PCD. This project will provide insight into how climate change may shape coastal phytoplankton species.

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The Effects of Nutrient Limitation and Light Levels on Symbiodinium

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Coral reefs are among the most diverse and ecologically productive ecosystems on Earth. Important to their survival are the endosymbiont flagellate, *Symbiodinium spp*. *Symbiodinium spp.* are responsible for creating a compound called DMSP which functions are hypothesized to include osmoregulation, anti-predation, antibiotic, and methyl donating properties. DMSP's production has been shown to be up regulated in times of nutrient limitation and oxidative stress. DMSP is cleaved by an enzyme called DMSP lyase, resulting in the creation of DMS. DMS is released into the surrounding environment where it becomes oxidized into sulfate aerosols condensation nuclei which are responsible for regulating solar reflectance and radiation. Because of this DMS, has a potential global impact. The goal of this experiment is to observe how nutrient limitation and light affect DMSP and other osmolytes levels, as well as the physiology of *Symbiodinium spp.* In this experiment, 2.5L *Symbiodinium spp.* cultures were grown in filtered seawater and K+Cu media in the following 6 treatments: control (200µE, N+,P+), nitrogen limited (200µE, N-P+), phosphorus limited(200µE, N+, P-), low light (20µE N+,P-) nitrogen limited/low light (20 µE, N-,P+), phosphorus/ low light (20µE, N+,P-). . These were monitored each day with cell counts and F_V/F_M (monitors the activity of photosystems). After the seventh day the cultures were harvested for chlorophyll, DMSP, and phospholipid concentrations. According to the published literature, it is predicted that DMSP functions as an antioxidant, and may be substituted for other osmolytes in times of nutrient limitation. In my experiment, DMSP levels should be the highest in the nitrogen limited culture in the high light due to lack of nitrogen and increased oxidative stress. The phospholipid concentration should be the lowest in the cell membrane of the phosphorus limited treatment because in times of phosphorus starvation some other phytoplankton species have been shown to preferentially utilize non- phosphorus containing lipids in their membrane and use the limited amounts of available phosphorus for construction of nucleic acids.

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The Use of NMR Based Metabolomics to Determine Biomarkers for Environmental Stressors

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Metabolomics is the study of small molecule metabolite profiles produced by specific cellular processes in an organism in response to normal physiological events or to an external stressful event. It is considered a complimentary 'omics' approach with genomics and proteomics. Nuclear Magnetic Resonance (NMR) based metabolomics can be used to rapidly assess the metabolic status of an organism and provide insight into the effects of environmental stressors. Through the use of NMR, metabolites in a biological sample may be measured in an unbiased manner with a targeted or non-targeted approach. Recent results of an international intercomparison exercise for NMR-based environmental metabolomics show that NMR metabolome analysis yields robust results with consistent trends in metabolite-based biomarker identification among laboratories (Viant et al. 2009). This type of demonstrated comparability is necessary as the technique is considered for regulatory environmental studies.

We propose to measure the metabolomic response of marine organisms to relevant stressors in lab experiments as well as field experiments. We hypothesize that particular metabolites in the organism of interest will provide information about the pathophysiological response of the organism. In turn, these measurements will be related to the overall health of the environment. To begin this study, the metabolic profile (in the form of a 1D NMR spectrum) of laboratory-grown *Spartina*, a sea grass native to the Atlantic coasts and prevalent in the Charleston area, has been analyzed. Exploratory polar and non-polar metabolite extractions were performed, and 1D and 2D NMR data collected using 700 MHz and 800 MHz instruments. Spectral annotations indicate the presence of sulphur cycle compounds as well as a host of others including amino acids, sugars, and others typical of the plant metabolome. These types of studies are key to understanding the effects of anthropogenic and natural stressors in coastal regions.

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The Potential for the Marine Bacterium *Pseudovibrio denitrificans* to Produce Novel Antibiotics

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Antibiotics produced by microorganisms are an important defense against infectious pathogens, and the search for novel antibiotics is of increasing importance due to the emergence of antibiotic-resistant bacteria. Antibiotics are also natural compounds that play a key ecological role within an environment, often creating a unique 'chemical' signature that exerts selection pressure on community members. We have focused our studies on the chemical ecology associated with the diverse microbial community found in the coral surface mucopolysaccharide layer (SML) due to the recent unprecedented global degradation of coral reef ecosystems. In our studies, we characterized the chemical ecology of the microbial community in the gorgonian octocoral, *Pseudopterogorgia americana.* We isolated three unique Gram-negative bacteria with 99% 16S rRNA gene sequence similarity (1200 b.p.) to *Pseudovibrio denitrificans* capable of inhibiting Gram-positive and Gram-negative bacteria, as well as a known coral and human fungal pathogen. We hypothesize that the species *P. denitrificans* may play a role in protecting the coral against potential pathogens due to the production of antimicrobial compounds. To characterize the antibiotic compounds from one of the three *P. denitrificans* strains, the cell-free supernatant was extracted using dichloromethane, acetone, and methanol, and each resulting fraction was screened for its antimicrobial activity. The acetone extract inhibited Gram-positive *Kocuria rhizophila*, and the methanol extract inhibited Gram-positive *Bacillus subtilis* and Gram-negative *Vibrio harveyi* and *V. coralliilyticus*. A bioassay-guided fractionation of the MeOH extract using high-performance liquid chromatography (HPLC) suggests the presence of at least two antibiotics in this extract, one inhibiting the Gram-negative and one inhibiting the Gram-positive bacteria. Studies are on-going to isolate, purify, and structurally characterize the three chemically distinct antibiotics using HPLC, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Pseudovibrio* species have only been recently isolated from marine environments and little is known regarding their production of antimicrobial compounds. These results highlight the potential role of *P. denitrificans* in protecting corals against invading pathogens, and as a source for novel bioactive natural compounds.

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Polyketide synthases (PKS) in dinoflagellates: New Insights into Their Cellular Localization and Functionality

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Polyketides are a large family of secondary metabolites that are synthesized from acyl- CoA precursors by polyketide synthase enzymes (PKSs). These enzymes are multi-domain complexes that structurally and functionally resemble the fatty acid synthases (FASs). To date, approximately 25 species of dinoflagellates have been found to produce polyketides. Recently, several putative PKS genes encoding ketosynthase (KS), ketoreductase (KR), and both acyl carrier protein (ACP) and KS domains were identified from *K. brevis* (Monroe and Van Dolah 2007). Their structure is unique in that their sequence is most similar to Type 1 PKS, but separate catalytic domains reside on separate polypeptides, like Type II. Their altered expression in a non-toxic isolate of *K. brevis* suggested their involvement in brevetoxin biosynthesis (Monroe et al., 2010); however, their chloroplast localization resembles FAS. Since no information exists on PKS proteins of other toxic dinoflagellates, we used antibodies developed against *K. brevis* PKS proteins to probe for the expression and intracellular localization of PKS domains in three harmful dinoflagellates (*Karenia brevis, Ostreopsis ovata*, *Coolia monotis*), one non-toxic species (*Karenia mikimotoi*) and a raphidophyte (*Fibrocapsa japonica*) which is known to produce high concentrations of free fatty acids (FFA). All species, including the raphidophyte expressed proteins cross-reactive with one or more *K. brevis* antibodies. These results lead us hypothesize that either (1) these proteins are FAS or (2) single PKS units could be cobbled together to form complexes that synthesize different polyketide compounds and/or fatty acids in different species.

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