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Abstracts

The isolation of two potentially new natural products from the deep water sponge *Scleritoderma*

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Pancreatic cancer is one of the deadliest forms of cancer. It has a five year survival rate of 1 to 2%. Natural products have proven to be an excellent source of new therapeutic agents with over 50% of all drugs approved since 1981 originating from natural products. Currently there has been a focus on the marine environment as a potential source of new natural products. The investigation of natural products from deep water marine invertebrates and microbes has increased the diversity of new natural products with therapeutic potential (e.g. the antitumor agent discodermolide). By applying a traditional bioassay guided fractionation method using a standard cytotoxicity assay (the MTT assay), the isolation of two potentially new natural products from the deep water sponge *Scleritoderma* are presented here. These two compounds have shown strong cytotoxic effects against two pancereatic cancer cell lines: PANC-1 and AsPC-1

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MONITORING THE HEALTH STATUS OF THE CALIFORNIA SEA LION (*Zalophus californianus*) USING A CANINE MICROARRAY.

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California sea lions (*Zalophus californianus*) are a marine mammal species susceptible to a variety of stressors, including marine toxins, infectious diseases, malnutrition, and oil spills. These stressors can greatly affect the health of the animals. Traditional methods of diagnosis and health assessment involve veterinary examination and standard blood chemistry measurements. Approaches that can more accurately diagnose and monitor the condition of sea lion health in stranded and recuperating animals are needed. A canine microarray (Agilent Technologies) is being tested to determine if the array can be used to diagnose and monitor the health of the sea lion. The canine microarray was chosen because the dog is the closest relative to the sea lion for which a sequenced genome and a commercial microarray are available. We hypothesize that sea lion RNA will hybridize to the dog microarray with sufficient affinity to make the canine microarray a useful tool for distinguishing the transcriptomic profiles of various tissues and between exposures to various stressors. In order to test the canine microarray, total RNA was isolated from blood, bone marrow, brain, liver and kidney from sea lions exposed to domoic acid, a marine biotoxin, and from sea lions without documented domoic acid exposure. These are all potential target organs for the impact of known stressors in the sea lions' environment. RNA was labeled with Cy3 and Cy5 dyes and hybridized to a 4x44k canine oligonucleotide array. We observed that sea lion RNA from blood, bone marrow, brain, kidney, or liver hybridized with 30-40% efficiency to the canine microarray. In addition, we observed that the gene expression patterns of the various tissues could be distinguished using this array. The results are being analyzed to determine which dog genes are informative of gene expression in the sea lion. The canine microarray will be redesigned in light of these findings and used to test its effectiveness as a diagnostic and prognostic tool for sea lion health.

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ANALYSIS OF DIURNAL CHANGES IN PROTEIN ABUNDANCE IN THE DINOFLAGELLATE, *Karenia brevis*

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The red tide dinoflagellate, *Karenia brevis*, is a eukaryotic protist responsible for harmful algal blooms (HABs) that occur annually in the Gulf of Mexico. The mechanisms controlling gene expression in this dinoflagellate are therefore of interest. Our laboratory has recently demonstrated post-transcriptional regulation of gene expression in several key processes generally under transcriptional control in eukaryotes, including the cell cycle and acute stress responses. We have also identified in *K. brevis* an unusual RNA trans-splicing mechanism that suggests the presence of constitutive transcription of mRNA as found in trypanosomes. This suggests that gene expression may be regulated largely at the translational or post-translational level. In order to assess whether translation plays the major role in regulation of gene expression in *K. brevis*, we have employed proteomic analysis of global protein expression over the diurnal cycle, a primary regulator of physiology in photosynthetic organisms. In the current study, we collected cells from triplicate cultures at four points over the diurnal cycle, circadian times (CT) 2, 8, 14 and 22 (where CT0 is the time lights come on). Total protein was extracted using Trizol reagent, followed by two-dimensional gel electrophoresis. Samples (30 *u*g) were focused using 4-7 isoelectric focusing strips and analyzed by 2-dimensional electrophoresis on 8-16% gels, and stained using Sypro Ruby. PDQuest software was used for spot matching and intensity analysis. Approximately 17% of proteins examined in this study appear to be significantly changing (>2-fold) over the cycle. Several different patterns of expression were apparent, with the most frequent pattern being downregulation at night. Attempts to identify some of these changing proteins using mass spectrometry are underway. Once proteins are identified, protein abundances will be compared with their transcript levels previously determined by microarray analysis.

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Role of trace elements and nutrients in cardiomyopathy in pygmy sperm whales (*Kogia breviceps*)

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Pygmy sperm whales are the second most frequently stranded toothed whale along the U.S. Atlantic and Gulf coasts. More than half the cases documented exhibit signs of cardiomyopathy. Many factors may contribute to the development of idiopathic cardiomyopathy in pygmy sperm whales, including genetics, infectious agents, chemical toxins, contaminants, and nutritional abnormalities. This study will focus on how trace elements and nutrients relate to the disease state. Nutritional deficiencies of a key trace element (selenium) and/or antioxidant vitamins, in combination with a diet high in polyunsaturated fatty acids (PUFAs) and free radicals, have been shown to contribute to cardiomyopathy. We hypothesize that the pygmy sperm whale diet consisting mainly of squid imparts a high dose of PUFAs that require effective antioxidant biochemistry to regulate free radical formation. Vitamin E (alpha tocopherol), vitamin B_1 (thiamine), and selenium (Se) will be examined in liver, blood, and heart tissue due to the roles these analytes can play in antioxidant biochemistry and protein formation. The contaminant mercury (Hg) will also be studied to determine if the Se/Hg detoxification pathway inhibits the bioavailability of Se. Se status may be impacted by sequestration chemistry wherein Se binds Hg making the Se less bioavailable for various biochemical processes, including selenium/antioxidant chemistries and selenoprotein formation. The goal of this research is to assess trace elements, vitamins, selenoproteins, and metabolites in a comparative context between animals exhibiting or lacking idiopathic cardiomyopathy to gain insight about the pathways driving the disease.

Funding for this study is provided by the National Institute of Standards and Technology 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 pygmy sperm whales in partnership with NOAA's Marine Mammal Stranding and Life History Project and Harbor Branch Oceanographic Institute's (HBOI) Marine Mammal Research and Conservation Program.

Localization of Facilitated Urea Transporters to Tubular Segments in the Bundle and Sinus Zones of the Kidney of the Euryhaline Stingray, *Dasyatis sabina*.

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The principle component of the osmoregulatory strategy of marine elasmobranchs is the maintenance of high concentrations of urea in their body fluids. The reabsorption of filtered urea by the renal tubules is the primary mechanism underlying the retention of urea. Urea movement across the renal tubular epithelium occurs, at least in part, via specific phloretin-sensitive, facilitated transport proteins. We have identified two members of a urea transporter (UT) family from the kidneys of the Atlantic stingray, *Dasyatis sabina*. To clarify the role of these UTs, we utilized immunohistochemistry to identity the tubular sites at which they are expressed. Stingrays were maintained in harbor water (850 mOsmol/kg H_2O) and fed a diet of shrimp for at least 2 weeks prior to study. They were anesthetized with MS-222 in buffered harbor water and perfused with elasmobranch Ringer's followed by 4% paraformaldehyde. The kidneys were blocked in paraffin. Five-micron sections were incubated with an affinity-purified antiserum generated to a sequence common to the 2 UTs (strUT-1 and strUT-2). Localization of UT expression was visualized using DAB stain. The specificity of the signal was confirmed by incubation of adjacent sections with the antiserum preincubated with the immunizing peptide. We also examined the expression of 2 other membrane transporters. Tubular segments were identified from the criteria reported by Lacy and Reale (1985). Numerous positively stained tubular segments were observed in both bundle and sinus zones in the presence of strUT. In the sinus zone, strong immunoreactive signal was observed in the Proximal-III tubular segment and in the Intermediate VI tubular segment. Weak staining was found in the Intermediate I tubular segment. In the bundle zone, immunoreactive signal was observed in Neck I and II, Proximal I, and Distal-I segments. In contrast, Na^+-K^+-ATPase was localized to Neck I and II as well as Proximal I and II in the bundle zone. In the sinus zone, staining was found in both Intermediate I and VI. The Intermediate segments in the bundle zone still require further study to determine their immunoreactivity to both antibodies. Our findings indicate that the strUTs are expressed in tubular segments in both the bundle and sinus zones. The expression of UTs in the bundle zone supports a role for countercurrent exchange in urea reabsorption. The mechanism(s) by which urea is reabsorbed via UTs in segments in the sinus zone remain to be identified.

The impact of environmental stressors on the vitamin D3 pathway within the skin of the Atlantic bottlenose dolphin (*Tursiops truncatus*)

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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. We previously established cell strains and cell lines from dolphin skin as an *in vitro* tool for measuring the molecular-level impact of the environment on this marine mammal. These cell models are being used to investigate the role of the vitamin D3 pathway within dolphin skin. Vitamin D is of interest because of its acknowledged chemopreventative, antimicrobial, and immunomodulatory properties within terrestrial animals. Within the skin, UVB radiation stimulates the conversion of 7-dehydrocholesterol into the active, hormonal form of vitamin D3: 1,25-dihydroxyvitamin D3 (1,25D3). The primary route through which this hormone exerts a biological function is via interaction with the nuclear vitamin D receptor (VDR), a potent ligand-activated regulator of gene transcription widely expressed in many organs. Whether aquatic mammals also possess this pathway and gain the same immune benefits from vitamin D3 as terrestrial mammals is unknown. We have detected expression of VDR in dolphin skin cells and found the cells to be sensitive to exogenous 1,25D3 administration, similar to humans and other animals. We are interested in whether environmental stressors interfere with the vitamin D3 pathway in dolphin skin, proposing a possible mechanism for the detrimental impact of environmental fluctuations on marine mammal health. Very few studies have investigated the impacts of environmental factors on the vitamin D3 pathway within any animal model. We have found that certain stressors including hypoxia, increased ambient temperature, and fuel oil alter levels of VDR and the expression of various VDR gene targets in dolphin skin cells. Such findings may help elucidate the role of vitamin D on innate immunity in dolphin skin.

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METHOD VALIDATION FOR MEASURING PERFLUORINATED CONTAMINANTS

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Perfluorinated compounds are an emerging class of organic contaminants with worldwide distribution and cause adverse toxicological effects. Therefore, it is important to be able to measure these compounds with accuracy in order to determine the potential risks of these compounds in humans and wildlife species. To measure contaminant concentrations in a sample, the sample undergoes 1) extraction, 2) removal of proteins and biomolecules, and 3) measurements of compounds. We have optimized a method for measuring perfluorinated contaminants using an automated solid-phase extraction system with weak anion exchange cartridges. The extraction system also removes proteins and biomolecules from the effluent. Liquid chromatography tandem mass spectrometry is used to measure the concentration of perfluorinated compounds in samples. We have validated the use of a second analytical column in the liquid chromatograph, which separates contamination that can be introduced from the instrument during analysis, and compound-specific standards for concentration measurements. This approach increases extraction efficiency, decreases contamination, and increases the accuracy of measurements.

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A Novel Hollow Fiber Model System to study Gene and Protein Regulation during Stress in the Alveolar Epithelium of Marine Mammals

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Studies of the alveolar epithelium are extremely difficult due to the complex nature of lung architecture that prevents easy access. Marine mammals have a respiratory system that is physiologically similar to terrestrial mammals with the exception of an increased respiratory air exchange volume and an increased flow rate. While there are a number of obvious morphological differences, the alveoli of marine mammals are similar to those of terrestrial mammals in that they posses alveolar epithelial type I and type II cells, which function similarly in surfactant production.

One of the major differences with regards to marine mammalian lungs is the ability to completely collapse the lung under circumstances such as swallowing and diving. This results in the removal of all air from the alveoli and prevents the absorption of compressed gas by the blood and, subsequently, protects the animal from Caisson disease (the bends). During deep diving activities and increasing hyperbaric pressure, the lung alveoli of marine mammals collapse and experience low oxygen tension in blood and tissue. Pulmonary surfactant is crucial in allowing for proper reinflation and restoration of lung oxygen tension upon surfacing. Recent observations suggest that marine mammals have an incredibly high surfactant turnover production rate during these times of hypoxic stress. These observations in coordination with data from our laboratory confirm a link of hypoxia to surfactant production as well as the role of oxygen sensitive genes, including HIF-2α and hemoglobin, in such surfactant synthesis and/or secretion. Thus, studies of the marine mammal alveolar epithelium could prove highly useful in further elucidating the complex mechanisms that play a role in surfactant regulation, especially during times of hypoxia.

Current cell models using cultured alveolar cell systems do not accurately mimic the *in vivo* cellular microenvironment lacking air-liquid interface and dynamic stretching characteristics of native lung tissue, which are important for normal phenotypic gene expression and cellular function. Thus, there is a critical need for the development of new model systems, particularly for marine mammals. We have established a novel, selectively semi-permeable hollow fiber membrane-based model system that more accurately mimics the microenvironment of the mammalian (both marine and terrestrial) alveolar epithelium. 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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Assessment of the Prokaryotic Diversity Associated with Selected Caribbean Corals

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Coral reefs are among the most productive and speciose ecosystems on the planet, supporting one quarter of all marine species. Unfortunately, these systems are being degraded on a global scale due to infectious disease, anthropogenic disturbance, and climate change. Microorganisms account for most of the biodiversity in coral reefs, yet most remain unidentified. Corals naturally form associations with assemblages of microorganisms, and these assemblages are thought to play vital roles in coral ecology as symbionts or as pathogens; however, the biodiversity within these communities and the potential changes in microbe/coral interactions, particularly with respect to the global degradation of coral ecosystems, are poorly understood. Our current research is a survey of the diversity of microalgae, archaea, and bacteria associated with three species of Caribbean corals: *Montastrea faveolata*, *Acropora palmata*, and *Pseudopterogorgia americana*. This work incorporates culture-based isolation and culture-independent molecular techniques to evaluate and compare the diversity and composition of microbial assemblages associated with the surface mucopolysaccharide layer (SML) and tissues of these corals. Moreover, we are assessing the potential differences in microbial communities between healthy coral colonies and colonies that are compromised by disease or bleaching. The work presented here focuses on determining the community structure and biodiversity of the bacteria present in the SML and tissues of *M. faveolata* using denaturing gradient gel electrophoresis and cloning/sequencing of 16S rDNA genes. Additionally, we are beginning construction of metagenomic libraries that will enable us to simultaneously examine the metabolic capabilities and phylogenetics of coral-associated microbial assemblages. We are particularly interested in comparing the bacterial communities among healthy and diseased colonies of *M. faveolata* to elucidate potential changes in bacterial communities that are related to the declining health of the host organism.

This work is supported by the National Science Foundation Biotic Surveys and Inventories Program.

The *IGH* Eμ3' Enhancer of the Channel Catfish: Can We Extrapolate Knowledge of Structure/Function Relationships to Other Teleost Species?

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The core region of the Eμ3' transcriptional enhancer that drives the expression of the teleost *IGH* locus has been characterized functionally in two species, the catfish (*Ictalurus punctatus*) and the zebrafish (*Danio rerio*). These studies have suggested important differences: whereas the catfish enhancer acts through an E-box and two octamer motifs, the zebrafish enhancer exerts its major effects through two E-box motifs alone. In this study, the function of the catfish enhancer was re-examined in a broader comparative context within the teleosts. Electrophoretic mobility shift assays of motifs from catfish, zebrafish and *Fugu* were conducted to determine their ability to bind catfish E-protein and Oct transcription factors. Transient expression assays were conducted using a region of the catfish core enhancer that includes a newly-described hybrid octamer/E-box motif. Alignments of sequences (phylogenetic footprinting) homologous to the Eμ3' enhancer region from six teleosts were conducted to determine conserved regions. These studies allowed the following conclusions to be drawn: 1) the important 3'E-box motif described in the zebrafish corresponds, in the homologous region of the catfish enhancer, to an Oct motif with a newly-described negative regulatory function; 2) comparison of the Eμ3' enhancer sequences of six teleosts indicates that while a variety of octamer and E-box motifs are found in this region, strict evolutionary conservation of the important functional elements of the teleost Eμ3' enhancer has not occurred.

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Isolation, purification, structural determination, and monitoring of Goniodomin A produced by the red tide-associated dinoflagellate, *Alexandrium monilatum*

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Abstract.
The chain-forming dinoflagellate *Alexandrium monilatum,* formerly known as *Gonylaux*, was first observed in 1936 on the Texas coast in Offats Bayou where it caused annual formation of red colored water, as well as severe fish mortality. According to Connell and Cross, local residents of that area observed red water and dead fish virtually every year 15-20 years prior to their 1949 report. It has also been reported to be associated with widespread discolored water and increased fish mortality in the Mississippi Sound, off the eastern and western coasts of Florida, and in the Gulf of Nicoya, Costa Rica. Early studies have found that *A. monilatum* produced a harmful substance(s) that is predominantly contained in the cell mass and increases toxicity when the organism cytolyses. Our studies corroborate with other research findings demonstrating that the toxin has low water solubility, casting doubt on the presence of more typical water soluble saxitoxin-like toxins that are water soluble. Using sophisticated chemical, chromatographic, and analytical chemistry techniques, we have successfully purified and identified the molecular structure of the toxin produced by *A. monilatum*. To solve the molecular structure of the toxin, we utilized a 500MHz NMR equipped with the following experiments: 1H, 13C, COSY, HSQC, HMBC. In addition, mass analysis utilized ESI-MS, MALDI-TOF MS, and Q-TOF MS. The toxin represents a polyether macrolide with an empirical formula of C43H60O12. This toxic compound is identified as Goniodomin A, identical to the one produced by the rock pool-blooming dinoflagellate, *Alexandrium pseudogoniaulax*. This compound had not been previously isolated from *A. monilatum*. We have successfully solved the structure of a toxin that has caused fish mortalities in the Gulf of Mexico for over 60 years.
Current studies into environmental impacts and toxin detection/monitoring are underway. We have established mass spectrometric standard curve using our own internal standards for the toxin. This method will now be used to monitor toxin production over the growth cycle of the dinoflagellate. This will provide information regarding any variances in toxin production over the growth of the dinoflagellate.

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FUNCTIONAL GENOMICS OF THE NORTH ATLANTIC RIGHT WHALE: THE SKIN TRANSCRIPTOME AND ITS POTENTIAL USE IN THE STUDY OF HEALTH AND DISEASE.

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The North Atlantic right whale (*Eubalaena glacialis*) is a highly endangered marine mammal whose populations have failed to recover despite 70 years of protection from whaling. The inability of the species to rebound may be due to a combination of several factors including environmental changes, increased interaction with humans, and compromised health. Efforts have been initiated to develop the functional genomic tools needed to study right whale health at the molecular physiological level. An expressed sequence tag (EST) cDNA library has been constructed from a skin biopsy, and 2496 randomly selected clones have been sequenced. In addition, 96 genes identified as potentially responsive to stress and immune challenge have been cloned by targeted RT-PCR from skin cDNA. The analysis of the EST collection (archived at www.marinegenomics.org and GenBank) showed that the library was 31.85% redundant, yielding 1511 unigenes. A Gene Ontology analysis of the unigene collection indicated that the skin is a rich source of expressed genes with diverse function, suggesting an important role in diverse physiological processes including the inflammatory response. The unigenes derived from the analysis of the EST collection have been combined with the PCR-cloned sequences to design an oligonucleotide-based microarray for investigations of changes in the expression of genes that should be indicators of immunological health of *E. glacialis.* The role of the skin as an immune organ in marine mammals is not well understood, but knowledge derived from the skin transcriptome and its application in a microarray should lead to advances in this area.

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Rhodopsin palmitylation and removal by hydroxylamine

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Sensory proteins are very important to cellular systems from bacteria to humans. G-protein coupled receptors are a common family of sensory proteins used to detect environmental changes in all studied terrestrial and marine organisms. Rhodopsin, the visual pigment of rod photoreceptors, is the prototypical G-protein coupled receptor; it is the only protein in this family with its crystal structure resolved. In humans, rhodopsins and rhodopsin like proteins, are implicated in diseases such as diabetes, AIDS, cancer, age related macular degeneration as well as many others. Common structural features in these g-protein coupled receptors are its seven transmembrane spanning regions as well as post-translational modifications. Each structural feature of rhodopsin is thought to provide its own unique attribute to the protein. Post-translational modifications on rhodopsin and most other G coupled protein receptors are glycosidation, phosphorylation and palmitylation. Palmitylation is the focus of this study. Hydroxylamine (NH_2OH) removes palmitate modifications from rhodopsin in an unknown fashion, here native palmitylation and removal by hydroxylamine are analyzed. Molar ratios (palmitylation per rhodopsin) are used to compare rhodopsins in different retinal locations and conditions. Observations of bovine rhodopsin from outer and inner retinal segments, and treatments with hydroxylamine are analyzed.

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THE FUNCTIONAL POTENTIAL OF CORAL-ASSOCIATED MICROBIAL COMMUNITIES IN *PSEUDOPTEROGORGIA AMERICANA* FROM LA PAGUERA, PUERTO RICO

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Coral-associated microbial communities are increasingly recognized as important components of the coral holobiont that influence coral health and disease. It has been suggested that these communities contribute to the health of the coral ecosystem through energy and nutrient cycling; others suggest that microbial communities provide corals with protection against infectious disease. To date however, there are few studies that directly address the functional role of these communities. In the present study, we examined the functional potential of the coral-associated microbial community found in the surface mucopolysaccharide layer (SML) of a common Caribbean coral, *Pseudopterogorgia americana*. The SML samples were collected in March of 2006 off the coast of La Paguera, Puerto Rico. DNA was extracted and amplified for use in a functional gene array, the GeoChip, which contains 24,000 probes targeting 10,000 functional genes involved in approximately 150 functional groups associated with biogeochemical processes. We examined the presence of these genes in community DNA from the SML of healthy *P.americana* colonies. Our preliminary data indicate that this microbial community possesses 1189 genes known to play a role in numerous biogeochemical processes. These processes include carbon degradation/fixation (159 genes), dissimilatory sulfite reduction (94 genes), metal homeostasis (203 genes), methane generation and oxidation (50 genes), nitrogen fixation/reduction (145 genes), ammonification (94 genes), and organic chemical degradation (444 genes). Our data, although preliminary, suggests that the coral-associated microbial community found in the SML of *P. americana* may play an active role in maintaining the coral holobiont through cycling of key nutrients, metals and organic contaminants. In on-going studies, we are evaluating the functional potential of healthy as well as diseased *P. americana* samples in triplicate using both SML and tissue samples.

This research is supported by the College of Graduate Studies and a National Science Foundation Biodiversity Surveys and Inventories grant.

The mucous samples were collected in March of 2006 off the coast of La Paguera, Puerto Rico and stored in 5M GIT Buffer at -20C. The community DNA was extracted using a CTAB/Phenol/Chloroform protocol and amplified using the Amersham Biosciences TempilPhi amplification kit (25-6400-01).

Category	Gene Category	# Genes	% Total	Example Genes
CDEG	Carbon Degradation	126	10.60	cellulase, chitinase, laccase
CFIX	Carbon Fixation	33	2.78	cdhE, cooF, ACSAB
DSR	Dissimilatory Sulfite Reduction	94	7.91	dsrA, dsrB
MET	Metal Homeostasis	203	17.07	Ni, Cd, Co, Zn, Pb, Al, Hg
MGEN	Methane Generation	19	1.60	mcr, mcrA, mcrG
MOX	Methane Oxidation	31	2.61	mmo, mmoA, pmo, pmoA
NFIX	Nitrogen Fixation	48	4.04	nifD, nifH
NIT	Ammonification, Ammonia Assimilation	94	7.91	amoA, amoB, amoC, gdh
NRED	Nitrogen Nitrate/ Nitrite Reduction	97	8.16	nir, nirK, nirS, norB, narB
ORG	Organic Chemical Degradation	444	37.34	benzene, parathion
		1189	**100.00**	

CHARACTERIZATION OF THE VIRULENCE OF A *VIBRIO AESTUARIANUS* STRAIN PATHOGENIC TO THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

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In France, annual mass mortalities of *Crassostrea gigas* oysters have been reported during summer since the 1980's. Several studies on this subject have demonstrated that these mortality outbreaks resulted from complex interactions between the physiological and/or genetic status of the oysters, environmental factors and one or more infectious agents, among which are bacteria of the genus *Vibrio*. *Vibrio aestuarianus* was the most frequently encountered species isolated from the hemolymph of moribund and healthy oysters. Interestingly, these bacterial isolates exhibited variable virulence following experimental challenge of adult animals, this variation being apparently linked to the toxicity of bacterial extracellular products (also called ECPs).

As these data implicated some *V. aestuarianus* strains in mortality events, this work was aimed at investigating pathogenicity mechanisms of *V. aestuarianus* strain 01/32, which induced the highest oyster mortality after an experimental challenge. Studies of both *in vitro* and *in vivo* interactions between this strain and oyster immune cells established the central role of the ECPs in the pathogenesis. ECPs from *V. aestuarianus* 01/32 were indeed shown to be lethal when injected into oysters and to inhibit hemocyte adhesion and phagocytosis. Accordingly, biochemical and genetic approaches were further implemented to identify the major source of ECP toxicity. These two complementary approaches led to the characterization of a gene encoding a zinc-dependent metalloprotease and to the demonstration of its involvement in the lethal effect of ECPs. When expressed in a heterologous system, the metalloprotease conferred a toxic phenotype on the ECPs of the transconjugant and caused inhibition of hemocyte adhesive and phagocytic activities.

Taken together, these results demonstrate the critical role played by the metalloprotease in pathogenicity mechanisms of *V. aestuarianus* 01/32 during experimental infection of *C. gigas* oysters.

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GENE REGULATION IN *KARENIA BREVIS*: INSIGHTS FROM MICROARRAYS AND SEQUENCE ANALYSIS

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Karenia brevis is a dinoflagellate whose expressed genome is of significant interest because of its role in producing harmful algal blooms. The longevity of a *K. brevis* bloom is dependant on the cell's ability to adapt to the coastal environment. Transcription of general stress response genes is known to be a key regulatory step in the development of a broad stress resistance. To gain a better understanding of gene regulation in *K. brevis*, we developed a DNA microarray containing 10,265 unigenes. To identify if a general stress response program exists in *K. brevis*, the microarray was used to measure transcriptional changes in response to acute heat, peroxide, lead, paraquat, or sodium nitrite. Consistent with a general stress response that includes a transient shut-off of general mRNA transcription, genes involved in ATP driven processes were downregulated following each of the treatments. However, transcription of stereotypical heat shock proteins and other stress related genes, known to be induced at the protein level in *K. brevis*, were not seen, implicating post-transcriptional regulation of these mRNAs.

The lack of transcriptional regulation found following acute environmental stress along with the distinctive nuclear organization in dinoflagellates suggests that *K. brevis* may have evolved alternative regulatory mechanisms for regulating gene expression. There are several similarities between the gene organization of dinoflagellates and their sister group, the trypanosomes, including polycistronic transcription and the lack of identifiable transcriptional regulators suggesting that dinoflagellate RNA transcription may be similar to trypanosomes. In trypanosomes, long stretches of chromosome are constitutively transcribed into polycistronic units with concurrent splice leader *trans*-splicing and polyadenylation of the nascent chains. The presence of this regulatory mechanism in a closely related genus prompted us to investigate trypanosome-like *trans*-splicing in *K. brevis*. Following sequence analysis of our *K. brevis* unigene set, we identified several mRNA sequences that contain a consensus 22-bp leader sequence. Furthermore, we isolated a gene from genomic DNA encoding a potential SL RNA. This gene shares several key features to the previously characterized SL RNAs. This study provides evidence for the occurrence of SL *trans*-splicing in a dinoflagellate and lends tremendous insight into regulation of gene expression in these early branching eukaryotes.

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Temperature Effects on Sea-Ice Diatoms Intracellular DMSP Levels

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Dimethylsulfoniopropionate (DMSP) production by marine phytoplankton is a fundamental component in the global sulfur cycle and the earth's radiation budget. Furthermore, it is believed to have important intracellular roles as a cryoprotectant and antioxidant. Polar ice-diatoms have been implicated as major producers of DMSP, yet little work has looked at the environmental factors influencing DMSP production within these organisms. It was hypothesized that changes in growth temperature would affect DMSP production in polar diatoms. To test this hypothesis two dominant members of sea-ice algal communities, *Navicula glaciei* and *Fragilariopsis cylindrus*, were grown at 0° C and 4° C. Intracellular DMSP concentrations and photosynthetic efficiency (Fv/Fm) were compared between temperatures. While photosynthetic efficiency was found to decrease in both diatoms in response to increased temperature, DMSP to chloryphyll *a* ratios (DMSP:Chl *a*) decreased in *F. cylindrus*, but increased in *N. glaciei*. These findings were duplicated in a second, independent set of experiments. The results indicate that there are species-specific responses in intracellular DMSP levels to changing growth temperatures. Future work will utilize a proteomics approach to identify regulatory components of sea-ice diatom DMSP production.

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PATTERNS AND PREDICTIONS FROM DOLPHIN (*Tursiops truncatus*) DIFFERENTIAL GENE EXPRESSION.

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The use of cDNA microarrays in functional genomics approaches, especially in an organism like the dolphin where the knowledge base is small, can greatly accelerate both novel gene discovery and the study and understanding of molecular physiological responses, in both controlled experimental situations and in wild populations. In this study, the transcriptome analysis of 20 wild dolphin blood samples from different geographical locations in U.S. waters (Charleston, SC and Indian River Lagoon, FL) has been conducted using a species-specific peripheral blood leukocyte (PBL) cDNA microarray. Total RNA extracted from blood leukocytes of wild dolphins was analyzed to investigate such variables as sex, location, age, health status and environmental stress. Two different machine learning approaches were used: Artificial Neural Networks (ANN) and Support Vector Machines (SVM), and different sets of genes were used as classifiers. In such supervised learning methods, the algorithm uses transcriptional profiles from samples of known classification to predict the classification of new samples. Here we show the results from ANN for the prediction of the "location" (Charleston, SC and Indian River Lagoon, FL) confirmed by SVM and cluster analysis, a more traditional microarray analysis approach. The results show that such gene expression analysis can provide insight into the natural history and physiological status of wild dolphins.

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IMMUNOLOGICAL RESPONSE OF DISTAL LUNG CELL LINES TO BREVETOXINS

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Brevetoxins, produced by *Karenia brevis*, are marine algal toxins associated with Florida red tides. Brevetoxin exposure can occur through consumption of contaminated shellfish or toxin inhalation. Various ailments have been documented following brevetoxin inhalation, including lung irritation, cough, wheezing, and congestion. While little is known on how brevetoxins exert these effects in the lung, previous studies have suggested that brevetoxins may have an impact on the lung immune system. In this study, various immunological responses were examined in mouse alveolar epithelial or mouse alveolar macrophage lung cell lines following exposure to 0.5-2 μg/ml brevetoxin-2. Western blotting for surfactant protein-A, a protein involved in lung innate immunity, identified that brevetoxin-2 decreases the amount of secreted SP-A. Cytokine antibody arrays primarily identified a T_H1 response following brevetoxin-2 exposure. Microscopic imaging of macrophages incubated with fluorescently labeled particles indicated that macrophage phagocytosis increases after brevetoxin-2 exposure. These results suggest that brevetoxin-2 alters the immune response in the lung and enhances inflammation. Future work will aim to identify the pathways leading to these altered responses.

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Novel structure of polyketide synthase gene transcripts in the Florida red tide dinoflagellate, *Karenia brevis*

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Karenia brevis is the Florida red tide dinoflagellate responsible for detrimental human and environmental health effects through production of brevetoxins. Brevetoxins are polyketide compounds thought to be synthesized by a modified polyketide synthase (PKS) complex, but the gene cluster for this PKS has yet to be identified. Further, because axenic cultures are unavailable, the origin of PKS sequences obtained from dinoflagellates remains controversial. Here, eight PKS transcripts were identified in *K. brevis* by high throughput screening of two *K. brevis* cDNA libraries. Phylogenetic analysis of PKS transcripts encoding ketosynthase domains and subsequent analysis of their gene expression in bacteria-free cultures confirmed these sequences to be encoded by *K. brevis*. A spliced leader sequence identified at the 5′ end of the PKS transcripts indicates that the sequences described are full length transcripts, which was further confirmed by Northern blot analysis. Although most similar to type I modular PKS, sequence analysis determined that seven of the transcripts encode single catalytic domains, six KS domains and one KR domain, an unexpected organization for a type I modular PKS. Presence of the spliced leader on PKS transcripts also suggests post-transcriptional control of PKS genes in *K. brevis*. This is the first study to describe full-length PKS transcripts in a dinoflagellate and sheds light on the structure of these genes in a toxic dinoflagellate.

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Membrane Vesicles Play a Role in Metal-Microbe Interactions

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Membrane vesicles (MV) are extracellular formations derived from the outer-membrane of Gram-negative bacteria. Their biological roles include transport of virulence factors, protein and DNA exchange, cell-cell communication and biofilm formation. Although it is known that metals interact with the bacterial membrane, how MVs modulate this interaction is unknown. The Gram-negative bacterium *Burkholderia vietnamiensis* $PR1_{301}$ (PR1) has been used as a model microorganism to study metal-microbe interactions and is 20-fold more resistant to Zn at pH 5 versus pH 7. The mechanism of pH-dependent metal resistance in PR1 has not been identified. Recently, we have found that MVs may be playing a role in metal-microbe interactions. When PR1 was grown in the presence of 250 mg L^{-1} Zn at pH 6, Zn was found localized to MVs but not cells. For this reason, we are investigating the possible involvement of MVs in pH-dependent metal resistance in PR1. To accomplish this, first we developed a method using filtration and differential centrifugation to isolate and quantify MV production in PR1. At pH 6, it was found that a maximum production of MVs (3 μg protein mL^{-1} or 2 % of total culture protein) occurred at early stationary phase. This sample point will be used to sample for MV production at pH 5 and 7 (with and without sub-lethal Zn concentrations) to evaluate differential MV production and characterize their protein content and fatty acid composition. Additionally, field-flow fractionation (FFF) coupled to static light scattering was used to fractionate MVs and determine their geometric radius and absolute number. This was conducted at pH 6 with and without 75 mg L^{-1} Zn. Results demonstrate that relative to cells unamended with Zn, MVs produced when PR1 was exposed to Zn are 10 ± 2.8 % larger and 45 ± 21 % less abundant during mid-exponential phase growth, though at late stationary phase they are not statistically different. Further analysis of collected MV fractions will be done using inductively coupled plasma mass spectrometry (ICP-MS) to quantify changes in Zn content. The results of these studies will demonstrate whether MVs containing Zn are a novel mechanism of metal resistance or whether they harm the microorganism by allowing Zn to be more bioavailable to the microorganism. This will highlight the significance of a key overlooked aspect of metal-microbe interactions.

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Comparative analysis of microbiota associated with healthy and diseased acroporid corals

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Acropora spp. corals have suffered region-wide declines throughout the Caribbean during the past two decades, prompting their recent designation as 'threatened' under the U.S. Endangered Species Act. A diverse microbiota is associated with the surface mucus layer of these corals and is thought to participate in health and disease processes. Previous examinations of these microbial communities have yielded conflicting results. This study used multiple approaches to ascertain whether a predictable microbial assemblage is associated with healthy and/or diseased corals. Replicate samples were collected from healthy and diseased acroporid colonies at six sites in the Florida Keys and Dry Tortugas during a mortality event in 2003. Three different sample processing methods were used in parallel during sample collection. Individual 16S rRNA gene libraries were generated from each sample (~20,000 usable clone sequences from 69 libraries). In addition, over 600 bacterial isolates were identified from these samples and each sample was directly screened by PCR for known coral pathogens. Our results indicate that sampling methodology introduces significant variability into the microbial community composition detected, perhaps explaining the conflicting results in earlier studies. Analysis of healthy coral mucus revealed consistent microbial composition between samples, with only nominal differences introduced by geographic locale and species. Members of the families Pseudomonadaceae and Xanthomonadaceae were consistently present in healthy corals. Diseased samples from the northern Florida Keys had unique microbial profiles, including increased prevalence of Vibrionaceae and Flavobacteriaceae, when compared to healthy corals. Diseased corals from the southern sampling sites were not significantly different from reference corals. This concurs with the histopathology that indicated disease signs were distinct from those of corals at the northern sites, thus suggesting two different disease pathologies occurred during this outbreak event.

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Vibrio vulnificus and _V. parahaemolyticus_ Densities in Oyster Samples in the Charleston, SC Area

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Vibrio vulnificus and *Vibrio parahaemolyticus* are Gram-negative, motile, curved, rod-shaped bacteria. *Vibrio* infections have been associated with the consumption of raw or undercooked seafood (i.e. shellfish) and exposure of open wounds to contaminated waters. The objective of the present study was to determine the numbers of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oysters 28 South Carolina Estuarine and Coastal Assessment Program (SCECAP)/SC-DNR Long-term Disease Sites near Charleston, SC the during summer 2006 (Aug.-Oct.) and winter 2007 (Jan.-Feb.). Two non-radioactive alkaline phosphatase (AP) labeled probes were used to detect the presence of target genes (*vvh* for *V. vulnificus* and *tlh* for *V. parahaemolyticus*) in oyster samples. This hybridization technique is used to determine total numbers of *V. vulnificus* and *V. parahaemolyticus* in oyster samples. *Vibrio* bacteria were present in all samples. The concentrations of *V. vulnificus* and *V. parahaemolyticus* during the summer months ranged from 40 CFU/g to 690 CFU/g and 40 CFU/g to 2000 CFU/g respectively. In the winter months, the concentration of *V. vulnificus* ranged from the limit of detection ([LOD] 10 CFU/g) to 600 CFU/g. The concentration of *V. parahaemolyticus* ranged from the LOD to 105 CFU/g. Overall the data showed that *V. vulnificus* and *V. parahaemolyticus* densities were higher during the summer months than during the winter, as would be expected.

Novel chlorinated compounds isolated from the toxin producing cyanobacteria, *Trichodesmium thiebautii.*

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Trichodesmium is a toxin producing non-heterocystous cyanobacteria ubiquitous in tropical, subtropical, and temperate seas. *Trichodesmium* is known for its ability to fix nitrogen and for its massive blooms; as a result, it is considered the major component of oceanic primary production and global nitrogen cycling. The toxin(s) produced by this cyanophyte has been observed as a potential cause of death of fish, crabs and bivalves pan-globally. In addition, *Trichodesmium thiebautii* cells have demonstrated neurotoxic effects in laboratory studies, as well as caused respiratory distress and contact dermatitis of humans at collection sites. However, to date, a *T. thiebautii* toxin has not been isolated or structurally characterized. Here we report the extraction of a toxin(s) from *T. thiebautii* cell mass. We have established a purification method with several chromatographic techniques; demonstrated cytotoxic activity of purified *T. thiebautii* toxin using GH4C1 rat pituitary cells and N2A mouse neuroblastoma cells; and completed a chemical structure using nuclear magnetic resonance (NMR), mass spectroscopy (MS) and fourier transformed-infrared spectroscopy (FT-IR). The structure is a novel compound with a mass of 318 m/z and a molecular formula of $C_{20}H_{27}ClO$. We are currently working to elucidate the structure of a second chlorinated compound that has a mass of 341 m/z. These compounds are the first small molecule natural products isolated and characterized from *Trichodesmium.*

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Mortality and Infection Following *in vivo* dsRNA Knockdown of Crustins in the Pacific Whiteleg Shrimp, *Litopenaeus vannamei*

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As invertebrates, penaeid shrimp rely solely upon an innate, non-adaptive immune response to survive in marine environments under a variety of stresses, including bacterial, fungal, and viral infection. The invertebrate immune response includes both cellular responses, such as phagocytosis, and humoral responses, such as the secretion of antimicrobial peptides. The crustin family of putative antimicrobial peptides was identified via shrimp EST libraries. Sequence homology to an 11.5kD antimicrobial peptide from the shore crab, as well as the discovery of a putative serine protease inhibitor domain (whey acidic protein) made crustins excellent candidates for further analysis. In previous experiments these peptides have been shown to play a role in the shrimp immune response. In this study, the activity of crustins is being assessed following immune challenge with *Vibrio penaeicida* and *Fusarium oxysporum*, both are known shrimp pathogens. In order to focus on the function of crustins *in vivo*, the knockdown of crustin by injection of dsRNA was undertaken in our bioassay facilities. Successful knockdown was demonstrated (lasting approximately 7 days post injection). In further experiments, crustins were knocked down followed by subsequent challenge with either bacteria or fungi, and changes in mortality were determined. Other shrimp control genes (both immune and non-immune) have been knocked down, and do not cause any change in mortality compared to negative controls. This study shows a different response to infection by bacteria and fungi. It appears that crustins are providing some immune protection within the shrimp.

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ANTI-LIPOPOLYSACCHARIDE FACTOR (ALF): A BROAD SPECTRUM ANTIMICROBIAL PEPTIDE ESSENTIAL FOR SHRIMP IMMUNITY AGAINST BACTERIAL AND FUNGAL INFECTIONS

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Antimicrobial peptides are an essential component of the innate immune system of most organisms. Expressed sequence tag analysis from various shrimp (*Litopenaeus vannamei*) tissues revealed transcripts corresponding to two distinct sequences (ALFLv1 and ALFLv2) with strong sequence similarity to anti-lipopolysaccharide factor (ALF), an antimicrobial peptide originally isolated from the horse shoe crab *Limulus polyhemus*. Full-length cloning revealed a 528 bp transcript with a predicted open reading frame coding for 120 amino acids in ALFLv1, and a 623 bp transcript with a predicted open reading frame coding for 93 amino acids in ALFLv2. Transcript abundance in the various cDNA libraries, suggests that ALFLv1 is expressed at significantly higher levels than ALFLv2, thus ALFLv1 was selected for further analyses. A reverse genetic approach was implemented to study the *in vivo* role of ALFLv1 in protecting shrimp from bacterial, fungal and viral infections. Injection of double-stranded RNA (dsRNA) corresponding to ALFLv1 gene into the shrimp resulted in a significant reduction of ALFLv1 mRNA transcript abundance as determined by qRT-PCR. Following knockdown, shrimp were challenged with low pathogenic doses of *Vibrio penaeicida*, *Fusarium oxysporum* or white spot syndrome virus (WSSV) and the resulting mortality curves were compared with those obtained from injecting either saline, non-specific dsRNA (catfish gene) or a non-immune shrimp gene followed by experimental infection. A significant increase of mortality in the ALFLv1 knockdown shrimp was observed in the *V. penaeicida* and *F. oxysporum* infections when compared to controls, showing that this gene has a role in protecting shrimp from both bacterial and fungal infections. In contrast, ALFLv1 dsRNA activated the sequence-independent innate anti-viral immune response giving increased protection from WSSV infection. These results have demonstrated the usefulness of reverse genetics for the functional characterization of genes *in vivo*.

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Anti-microbial activity of *Pseudopterogorgia americana* associated microorganisms against the coral pathogen *Vibrio coralliilyticus* and closely related phylotypes

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Vibrio coralliilyticus (ATCC BAA-450) is a temperature-dependent coral pathogen originally isolated from a coral reef off the coast of Zanzibar in the Indian Ocean. This coral pathogen has been found to induce coral-bleaching in *Pocillopora damicornis* through lysing of the zooxanthellae and coral tissue at temperatures higher than 24.5°C. Currently, the genome of this coral pathogen is being sequenced and on-going studies are using proteomics to examine the influence of temperature on the virulence of this organism. In this study, we compare the ATCC strain of *V. coralliilyticus* with related strains isolated from the surface mucopolysaccharide layer (SML) of visually-diseased *Pseudopterogorgia americana* corals off the southern coast of Puerto Rico. First, using 16S rDNA sequence analysis from the samples analyzed thus far, we identified five bacterial strains from the diseased *P. americana* samples (PaD1) that phylogenetically clustered with *V. coralliilyticus*. The healthy samples (PaH1) did not have any bacterial isolates that clustered with this coral pathogen. Second, a genomic profiling technique, REP-PCR, revealed that one of the five strains was similar to ATCC BAA-450, while four of the strains were different. Third, we examined whether the bacteria isolates from both healthy and diseased *P. americana* are able to inhibit the growth of ATCC BAA-450 when grown at two different temperatures, one where *V. coralliilyticus* is not pathogenic (24°C) and one where pathogenicity has been observed (27°C). We used a modified agar-overlay anti-microbial assay to screen *P. americana* bacterial isolates against *V. coralliilyticus* for their ability to inhibit the coral pathogen. We will also examine whether the five PaD1 coral isolates that are homologous to *V. coralliilyticus* show similar results in the anti-microbial assay. This study, as well as future studies that will incorporate additional strains of *V. coralliilyticus* found in the Caribbean, will contribute to our understanding of this coral pathogen and its role in the coral disease process as seawater temperatures increase.

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Factors influencing exposure and availability of brominated flame retardants in free-ranging bottlenose dolphins (Tursiops truncatus)

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Brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), are chemical additives to consumer products which reduce their flammability and the risk of fire. Piscivorous marine mammals, including bottlenose dolphins, feed at top trophic levels within aquatic ecosystems and are therefore vulnerable to accumulating heavy burdens of many different organic contaminants, including PBDEs. Assessing the potential health effects of contaminant exposure in wild marine mammals has proven difficult, partly as a result of their chronic exposure to extremely complex contaminant mixtures. To assess the toxicity of these biologically relevant contaminant mixtures using lab based tests, a measure of exposure in wild marine mammal populations is needed and the variability of contaminant mixtures between individuals of a wild population must be understood to determine whether all individuals are exposed to comparable mixtures or whether subsets of the population are exposed differentially. To assess exposure to brominated flame retardant mixtures in a wild marine mammal population, 14 PBDE congeners were measured in 106 blubber biopsies, 40 plasma samples and 18 milk samples collected from free-ranging bottlenose dolphins during capture and release health assessments in Sarasota Bay, FL between 2000 and 2005. Total PBDE levels ranged from 20 to 1508 ng/g wet mass in blubber. Concentrations were detectable, but lower in plasma and milk. No relationship between age and total PBDE blubber concentrations was evident for male bottlenose dolphins. However, upon reaching sexual maturity at 10 years of age, PBDE levels in females appear to drop and remain low for life, suggesting substantial offloading of PBDEs during parturition and lactation. PBDE congener mixtures also appeared to vary with age and sex. Shifts in PBDE mixtures in male bottlenose dolphins may be a result of age related metabolism or ontogenetic changes in prey preference or location. In females, shifts in PBDE mixtures may result from a differential offloading of PBDE congeners during lactation as a comparison of PBDE profiles in milk and reproductive female blubber suggest that larger, higher brominated PBDEs are retained in blubber while smaller, less lipophillic PBDE congeners are offloaded through the milk.

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