Florida Marine Biotechnology: Research, Development and Training Capabilities to Advance Science and Commerce

Sea Grant
Florida

March 2001

TP-110

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Florida Marine Biotechnology: Research, Development and Training Capabilities to Advance Science and Commerce

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Sea Grant Technical Paper Number 110

March 2001

$3.00 for Printed Version, or
Free at website www.flseagrant.org

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Preface

This document represents the first census of Florida's strong and productive network of collegiate faculty involved in research and education in Marine Biotechnology. It was compiled in autumn 2000, thanks to the efforts of an ad hoc committee and the campus coordinators for Sea Grant. Those individuals include P. Anderson, UF*; N. Blake, USF; J. Cato, UF; R. Dodge, NSU; N. Ehrhardt, UM; K. Haddad, FMRI; J. Fourqurean, FIU; W. Jeffrey, UWF; D. Hanisak, HBOI; R. Kerr, FAU; J. Lin, FIT; K. Leber, MML; B. Mashburn, NSU; N. Marcus, FSU; C. Rafalski, UWF; J. Paul, USF; L. Robinson, FAMU; S. Pomponi, HBOI; A. Rossi, UNF; W. Seaman, UF; P. Walsh, UM; G. Tolley, FGCU; L. Walters, UCF.

This initial listing identifies 78 faculty. For each, areas of scientific expertise and research and teaching focus are described. Further, potential applications and benefits to Florida are indicated. Some submissions were edited to conform to the one-page maximum limit. The form given on page 87 was used for gathering information. We encourage faculty not yet listed to register for the update.

The first publicity of this material was at the Florida Marine Biotechnology Summit II, held October 16-17, 2000. From that meeting came the concept of establishing an electronic network to link faculty, students, laboratories and classrooms across Florida. This document is starting point for such a network. For an electronic version, go the website www.flseagrant.org.

Although Florida Sea Grant organized this document and the effort to assemble it, clearly the efforts of many individuals are absolutely essential if it is to have a positive impact on developing Florida's potential in marine biotechnology. We thank the colleagues involved to date, and invite others to join the work.

* Abbreviations of institutions are: FAMU, Florida A&M University; FAU, Florida Atlantic University; FGCU, Florida Gulf Coast University; FIT, Florida Institute of Technology; FIU, Florida International University; FMRI, Florida Marine Research Institute; FSU, Florida State University; HBOI, Harbor Branch Oceanographic Institution; MML, Mote Marine Laboratory; NSU, Nova Southeastern University; UCF, University of Central Florida; UF, University of Florida; UM, University of Miami; UNF, University of North Florida; USF, University of South Florida; UWF, University of West Florida.

Contents

Purpose

The level of activity and interest in "marine biotechnology" among Florida university faculty and allied laboratory scientists is reported in this document. The information will be used to (1) promote networking and collaboration in research and education, (2) inform industry of possible academic partners, (3) identify contacts interested in potential new sources of funding, and (4) assist development of funding for a statewide marine biotechnology research, training and development program.

This document is the first of its kind. Institutions of higher learning were given the opportunity to contribute both an overview of campus capabilities and individual faculty Expressions of Scientific Interest. They are listed in the table of contents.

Disciplines

While cellular and molecular sciences will prevail among the disciplines addressing these subjects, interests in engineering, policy, law and economics (which might identify and address opportunities and constraints in education, science and commercialization) also are pertinent.

Subjects

For purposes of the survey, biotechnology topics of interest include: (a) **marine bioproducts**, focused on the discovery of drugs, bioadhesives, biominerals, and other economically important products, the development of new models and screens for product discovery, the identification of new drug targets and mechanisms-of-action of marine-derived drugs, the development of sustainable production methods for marine bioproducts and the development of novel methods for marine by-products utilization; (b) **marine animal health**, focused on marine pathogens, diagnostics, treatments, drug delivery systems, and immunology, physiology, and pharmacology of both wild and cultured marine animals; (c) **aquaculture**, focused on cell and molecular techniques to improve size, growth rate, disease resistance, survivability, and reproductive yields of aquacultured organisms; (d) **coastal human health risks**, focused on the development of new diagnostic tools to assess seafood pathogens and water-borne pathogens and pollutants; (e) **coastal habitat restoration**, focused on remediation, and molecular and cellular approaches to strain improvement, hybrid development, and production technology for submerged and coastal aquatic vegetation; (f) **forensics and monitoring**, focused on bioforensics for identification of threatened and endangered species, seafood identification, evaluation of health risks, and regulatory issues as related to economic fraud, and the development of new biosensors.

Potential

Marine biotechnology has been defined as the development of goods and services derived from marine organisms and processes. Examples include:

- Pharmaceuticals for the treatment of cancers and diseases such as arthritis, and many others
- Technologies that ensure the quality of the seafood we eat
- Safe and effective chemicals for use in agriculture
- Technologies for a marine veterinary industry for aquaculture and oceanaria
- New varieties of plants for coastal restoration
- Technologies for detection of toxins in the environment

A growing network of academic-industry-governmental partners sees a bright future for marine biotechnology in Florida. The faculty and organizations described in this report have a vital role to play in serving and nurturing this sector.

The long-term goal of promoting scientific advancement, commercial development, and job growth through a "Florida Marine Biotechnology Research, Training and Development Program" builds on the following rationale:

Why Florida?

- Florida has the longest coastline of any marine state in the "lower 48"
- It has an enormous variety of marine habitats including deep sea, coral reefs, estuaries and mangroves, each with its own array of organisms that could yield useful products
- Florida has an established statewide marine research program representing all ten public universities, three private universities, and two not-for-profit research laboratories
- Despite the state's recent entry into this field, accomplishments made by Florida scientists include discovery, licensing and development of a potent anti-cancer compound; development and licensing of a process to manufacture anti-flammatory agents from local corals; and genetic selection of sea oats that will allow commercial procedures to prevent erosion of coastal dunes

Why Now?

- Marine biotechnology is a multi-billion dollar industry with markets projected to grow 15-20% annually over five years
- Florida lags far behind other states and countries in marine biotechnology
- This effort is important to the whole state of Florida; it is akin to the business and academic research synergy that has so successfully propelled the dynamic economic development of Silicon Valley and North Carolina's Research Triangle

What Benefits Are Projected to Florida?

- Create and attract new, clean, high technology industries in Florida
- Provide high paying jobs
- Create opportunities that keep Florida-trained students in the state
- Sustain and restore aquatic habitats

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Seabird Immunology and Physiology: current focus is to understand immunological processes of long-lived seabirds as a function of age; changes in reproductive effort as a function of age; indentify critical mechanisms that protect seabirds from infectious agents. Previous work conducted with Leach's Storm Petrel in New Brunswick and Common Terns in Massachusetts. On-going projects involve Masked Boobies and Waved Albatrosses in the Galapagos Islands. Potential future work will involve Sooty Terns in the Dry Tortugas.

Immunological reagents for identifying marine biotoxins: develop polyclonal and monoclonal antibodies that are specific for toxins produced by marine dinoflagellates in collaboration with Kathleen Rein, FIU Chemistry.

(10) Potential Applications and Benefits:

Seabird Immunology: current research aids our understanding of the demography of seabirds, which is critical for their conservation as well as fisheries management.

Immunological reagents against biotoxins: assist public health specialists and wildlife biologists in screening algal blooms for potentially lethal toxins.

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for a
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We are interested in the discovery of new bioactive metabolites from marine organisms. Currently our new compounds are examined for activity as anti-cancer, anti-inflammatory and anti-tuberculosis agents. Also, we have interests in the microbial transformation of bioactive marine natural products. These experiments are designed to identify the probable metabolic fate of these pharmaceuticals in animals and to produce analogues of these drugs that may have improved properties.

(10) Potential Applications and Benefits:

The applications of this research clearly lie in the pharmaceutical/biotechnology industry.

EXPRESSION OF SCIENTIFIC INTEREST
Florida Marine Biotechnology Research, Development and Training Program

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Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

This Summer Semester (2000) Florida Gulf Coast University sent forward to the Florida Board of Regents (BOR) a proposal for a new biotechnology program to be housed in our College of Arts and Sciences. BOR approval to develop a new program is a key step in the process of program development in the State University System (SUS) because many requests are turned down as a result of budgetary constraints or because they duplicate existing programs within the SUS. We were fortunate to obtain BOR approval to develop such a program, and have immediately begun the process of hiring a molecular biologist to design and submit the program for final approval, hopefully by next Fall 2001. Our plan is to have our new Biotechnology program work closely with our new Marine Science program and our existing College of Health professions. Given the interest which Florida Sea Grant has in seeing further application of biotechnology to marine science, we feel that there are a number of activities that we are actively engaged in which could result in future proposals from us to Florida Sea Grant.

We are acutely aware that undergraduate research experience greatly enhances the quality of graduates from undergraduate science programs. As a science faculty, we have endeavored to provide these experiences within our undergraduate science curriculum. We have found that the resources available from within the SUS limit our ability to provide faculty development time and equipment to engage in this crucial activity. Below are some specific undergraduate research activities that would benefit greatly from external funding:

(1) FGCU faculty are developing a project to test the toxicity and biodegradability of biodiesel as part of the Biochemistry, Organic II and Environmental Toxicology classes. Biodiesel is synthesized by the students from various plant lipids, including marine algae, and the fuel is subsequently tested on marine organisms.

(2) FGCU faculty are interested in identification of biopharmaceuticals from the marine environment, specifically the bacteriostatic properties of naturally occurring marine peptides. Faculty and students will screen peptides for antimicrobial activity on marine pathogens.

(3) Our environmental chemistry class places heavy emphasis on the analytical chemistry of locally important pollutants and toxins. This class is linked with the Toxicology course mentioned above, so that students analyze, then investigate, the toxicity of pollutants of interest.

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for a
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education

My research focuses on human use and management of tropical plant resources, including mangroves and sea grasses. Working with Dr. Martin Quirke, an analytical organic chemist, we use electrospray mass spectrometry to identify secondary plant constituents and, in collaboration with Dr. Kelsey Downum, a phytochemist, we use a series of bioassays to identify active Secondary constituents.

(10) Potential Applications and Benefits:
Our research identifies and characterizes secondary compounds that may have application in medicine and helps preserve and validate traditional knowledge regarding plant resources. In addition, we employ matrix models to determine the effects of harvesting plant resources on future populations.

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Research and Education

Our research group has been studying immunity in selected marine animals including, currently: (1) marine mammals, dolphins and manatees (with Dr. G. Bossart, Harbor Branch/U.M.); (2) several sponge species and (3) two species of gorgonian coral (with Dr. C. Shaffer & Dr. Sylvia Smith). Building on our earlier cellular work with dolphins and manatees, we have studies underway examining bottlenose dolphin peripheral lymphocyte cytokine gene sequence and expression in response to various stimuli. Because tissue interactions in sessile marine animals, such as sponges and corals, happen as normal occurrences, not just surgical artifacts, these intraspecific recognitions have importance in understanding health and disease, ecology and the development of management strategies as well as elucidating basic principals that might be applicable across a broad phylogenetic spectrum.

Over the last three decades we have demonstrated the existence of a highly discriminating adaptive alloimmunity in the gorgonian coral, *Swiftia exserta.* Histocompatibility and polymorphism have been major topics of long standing in immunology and varying degrees of *H* gene (histocompatibility) diversity have been proposed for marine invertebrates, ranging from limited to very polymorphic. This has been controversial in recent years and has been difficult to resolve with the techniques available in the past. Because the animals in question can reproduce asexually as well as sexually, the relationship of collected animals is not easy to resolve based on morphological bases. We have studies underway to correlate molecular fingerprinting with the allografting and wound healing data in the coral and hope in the future to extend that study in a similar fashion to our local sponge model. Our recent, on-going examinations of gorgonian genes have discovered at least one member of an important immune gene family.

Because these animal also routinely receive tissue trauma (bites, cuts, abrasion, etc.) wound healing is important for their health. We have determined the normal processes of wound healing in two species of gorgonian coral (one with zooxanthellae and one without). That work has now been extended into examinations of the effects of “non-lethal” environmental perturbations, e.g. salinity and temperature, on wound healing and tissue interactions.

Our studies of cell death, killing, and apoptosis have focused on sponges and corals, utilizing cytology, flow cytometry and limited molecular techniques. This includes studies of normal processes, experimental situations and mass mortalities.

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for a
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My primary research interest is the conservation genetics and phylogeography of marine organisms. These interests encompass the following:

1. Population structure of marine animals. In the past this has included studies of sea turtles and manatees, but current interests are more tightly focused on fish. One major initiative involves range-wide genetic inventories for selected Atlantic reef fishes.
2. Identification of population-specific and species-specific genetic markers to trace animals through migratory circuits and marketplaces.
3. Assessment of genetic diversity and cryptic evolutionary partitions in aquatic organisms.
4. Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).
   Corresponding to the research interest above:
5. Populations are the fundamental units of species management, and their resolution is a prerequisite for informed management of commercially-exploited species. In addition, we are examining the role of mid-Atlantic ridge islands as stepping stones for trans-Atlantic colonization. If these islands are essential for colonization from East to West Atlantic, then they have an enormous influence on the biodiversity of coastal ecosystems.
6. Population specific markers have allowed us to identify which sea turtle nesting populations are impacted by oceanic fisheries. In the East Atlantic, for example, about 90% of turtles killed in driftnets are derived from nesting colonies in the southeast United States. In another recent paper, we demonstrated that about 25% of the turtle meat sold in New Orleans is actually alligator meat.
7. Our surveys often uncover evidence of cryptic species. In the Caribbean bonefish (believed to be one species; *Albula vulpes*), genetic assays demonstrate the presence of two species. Globally, we have uncovered evidence for five additional species of bonefishes. The presence of cryptic species has clear implications for realignment of management practices for bonefishes, which comprise one of the most popular recreational fisheries in the world.

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Fish endocrinology, hormones and receptors in fish, fish larval development and embryology. Confocal microscopy. I had a sabbatical in Sweden attempting to become a molecular biologist and to do gene expression work, but I am still inclined more to work with students and collaborators in this area than to claim any kind of expertise myself.

(10) Potential Applications and Benefits:

Numerous applications of endocrine & genetic manipulations in aquaculture and fisheries, training opportunities for overseas students in technical and applied areas.

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

My laboratory has the world's largest repository of marine natural products that bind to and manipulate the actin cytoskeleton. We have characterized the cell biological and biochemical properties of hundreds of marine natural products and their chemical derivatives as they relate to effects on actin polymerization and cytoskeletal modulation. We have devised high-throughput methods for screening additional marine natural products for similar activities. We study the effects of these marine natural products on properties of the cytoskeleton that are directly relevant to both tumor cell survival and immunosuppression.

(10) Potential Applications and Benefits: Marine natural products that we have characterized are now in various stages of development as therapies for cancer, cystic fibrosis, and autoimmune disease.

EXPRESSION OF SCIENTIFIC INTEREST
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

I am a wetland ecosystem ecologist. As such, I have the ability to work on wetland restoration efforts, including specific habitat restoration questions. I have been involved in salt marsh restoration efforts in the past, largely through the development and use of ecological models. My involvement in Everglades research has considerable focus on the restoration of this landscape.

(10) Potential Applications and Benefits:

All benefits and applications of my research center on the restoration of wetland habitats and ecosystems, particularly regarding the functioning of restored systems in the context of their adjoining landscapes.

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education

My primary research interests are in understanding patterns of genetic variation within and among populations of marine species, and determining relationships among species. This includes understanding the factors that affect genetic diversity, the geographic distribution of genetic diversity, and the derivation of significant population parameters from molecular data. The ultimate goal of this research is to understand the evolutionary mechanisms that control the maintenance or decline of genetic diversity, and ultimately, biodiversity. My laboratory is equipped for carrying out state-of-the-art genetic and molecular analyses. My doctoral research, postdoctoral training, and current research are all focused on using molecular techniques to address evolutionary and population genetic questions.

(10) Potential Applications and Benefits:

Population genetic studies of marine organisms are useful in the development of models for management and recovery of economically important or endangered marine species. Genetic marker data, for example, may be used for identification and tracking of pelagic larvae. This information can be used to identify source populations, the optimal size and spacing of conservation areas based on dispersal, populations that are particularly low in diversity and in danger of local extirpation, or high in genetic diversity, and therefore especially high priority for conservation. These genetic data can be used for stock identification, determination of genotype-specific survivorship, fecundity, or resistance to disease, and forensic identification of threatened or endangered species at risk for poaching. Determination of relationships among populations or species can be critical for development of strain improvement or hybrid development. Molecular data can also be useful for identification of introduced species and pathogens. The identification of source populations of introduced species and pathogens may often prove critical for control, management and prevention of further introductions.

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
(Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

Coastal human health risks: we are looking for bacteriophage that will help to identify oysters infected with highly virulent strains of Vibrio vulnificus and could possibly be used in their purification. We also have shown that bacteriophage can be used therapeutically in a mouse model of V. vulnificus infection and hope to develop this for human therapy in cases of antibiotic resistant V. vulnificus or develop its use as a preventive measure for the treatment of wounds acquired in a marine environment.

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(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Marine bioproducts: Fungi are, economically and medically, among our most important micro-organisms. Their role in food fermentation is legendary, they produce a variety of industrial and medically important compounds; and they are becoming increasingly significant in diseases of man and other animals due to their opportunistic character and their resistance to anti-bacterial drugs. Their role in the natural environment is equally important: they function in the food web as organic recyclers and as a food source for many animals. Interest in fungi is accelerating as a result of searches for new organic compounds for industrial and pharmaceutical uses. Although there have been approximately 50.000 species of fungi described, this number probably represents less than 1% less of the species that occur in nature. This raises a critical issue: many of the environments are rapidly degrading and as the organic constituents of these habitats change so do the fungal populations, consequently many fungal species are being lost. One of the frontiers for exploration for new and unique commercially exploitable fungi is the marine environment. RSMAS has one of the world's leading laboratories in marine mycology that provides the capabilities of isolating and characterizing these fungi by traditional and molecular methods as an initial step in determining their commercial value.

Human and animal health; forensics and monitoring: Our laboratory has developed a large molecular database for the identification of fungal species including human and animal pathogens. An important aspect of this research has been the development of a molecular array system for the rapid and accurate identification of fungal species. The method employs a hybridization probe technique that was designed for use in the average laboratory, where expensive equipment is not available. The method is quick, reliable and inexpensive.

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(9) Area of Scientific Interest and Expertise in Marine Biotechnology Research and Education

(a) Collagenolytic behavior of crab collagenase versus matrix metalloproteinases. The metastatic process involves tumor cell adhesion to other cells and protein ligands, and eventual invasion through basement membranes. In many cases, invasion requires the dissolution of basement membrane (type IV) collagen by proteases. The matrix metalloproteinase (MMP) family of proteases has been implicated in the invasion and metastatic processes. One possible approach for inhibiting the metastatic process is to develop therapeutics targeted for MMPs. While the MMP family is most often associated with collagen catabolism, a number of other unrelated proteases are capable of cleaving collagen triple-helices. A variety of serine proteases possess collagenolytic activity, such as *Uca pugilator* fiddler crab collagenase 1 and trypsin. The domain structures for some of these proteases differ greatly from MMPs; for example, fiddler crab collagenase 1 appears to have a collagen binding “groove” not obvious in MMPs. We are presently examining MMP and serine protease hydrolysis of triple-helical peptides (THPs) to further define the mechanisms by which proteases catabolize extracellular matrix components. This, in turn, could lead to novel developments in protease inhibitor design.

(b) Identification of novel conopeptides. Members of the genus Conus have been studied worldwide. Areas of geographical analysis include Australia, Hawaii, the Red Sea (C. textile), the coast of California and the Indo-Pacific. The venom of one cone snail from Atlantic has been analyzed to date (C. ermineus). Three peptides (EI, EIVA and EIVB) have been isolated and characterized from C. ermineus. There are no extensive biochemical studies of Atlantic cone snails to date. In collaboration with the laboratory of Dr. Frank Marí, we are engaged in the first exhaustive investigation of the properties and distribution of potential pharmaceutical agents from Atlantic cone snails.

(10) Potential Applications and Benefits:

The development of MMP inhibitors offers a promising strategy to modulate metastasis and arthritis. Conopeptides have already found utility as therapeutics for chronic pain.

EXPRESSION OF SCIENTIFIC INTEREST
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology
Research and Education:

> I am a marine botanist and biogeochemist with a special interest in the factors regulating the persistence and productivity of seagrass beds. Among other interests, I study the regulation of planktonic and benthic biomass by nutrient supply rates, the factors that control species composition and growth rate of seagrasses, and the factors that control the uptake and storage of elements (nutrients and metals) in marine plants.

(10) Potential Applications and Benefits:

I have been developjng the knowledge required to use seagrasses as biosensors of environmental conditions, especially the availability of light, nutrients and metals. I have also been instrumental in the design and implementation of benthic monitoring programs designed to provide ecological information necessary to answer questions concerning management and regulation of the coastal environment in south Florida.

EXPRESSION OF SCIENTIFIC INTEREST
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My current research interest is in isolation and cultivation of microalgae from various environments. Microalgae are known as potential source of many commercially valuable products such as: extracellular polysaccharides, lipids, pigments, bioflocculants, vitamins, and bioactive compounds. Bioactive compounds include substances that show antibacterial, antifungal, antiviral and antitumor activities. Microalage are also the source of specialty chemicals (e.g. straight-chain polyunsaturated fatty acids associated with lessened incidence of heart diseases), "health foods", and aquaculture feeds. I am particularly interested to study the extracellular polysaccharide production as well as to screen the algal strains for their antimicrobial activity.

We are in process of setting up an algal culture collection at FIU that will consist of algal strains isolated from the various environments in South Florida. The culture collection will be made available to everyone interested in basic research of algae, or their commercial application.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Anaerobic Microbiology
Isolation and characterization of new anaerobic bacterial species
Biodegradative interactions in microbial communities.
Anaerobic microbial biodegradation of xenobiotics
Anaerobic bacteria as possible diagnostic tool for monitoring human pollution of aquatic environment

Anaerobic bacterial physiology
Investigations into catabolic pathways in anaerobic bacteria

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

a) Marine animal health and diagnostics: Ultrastructural and cytochemical applications of histology and electron microscopy are applied to diagnosis and description of marine pathogens in corals and related organisms (e.g., Richardson, Goldberg and 7 others. Florida's mystery coral killer identified. Nature 392: 557-558. 1998).

b) Coastal habitat restoration: coral reefs. Reef repair and cleanup techniques have been applied to damaged reefs in the Caribbean (e.g., 1999. Goldberg, Walter M and A, Caballero. Reef damage by large vessel impact and its mitigation by site cleanup: methods and results after one year. Intl. Conf. on Sci. Aspects of Coral Reef Assessment, Monitoring and Restoration (Abstract). Manuscript in preparation.

(10) Potential Applications and Benefits:

The PI will work with resource management and conservation agencies to repair and mitigate damage to coral reefs, and identify pathogens and their effects on their coral hosts.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

(1) Name: **Kelly Goodwin**
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Pollutant Bioremediation:

I am interested in applying marine biotechnology research to the field of environmental microbiology, particularly in the area of pollutant remediation. Coastal waters and sediments are burdened with a variety of contaminants. Many of these compounds are produced naturally or are analogs to compounds produced naturally in marine systems (*e.g.*, halocarbons, petroleum products). Therefore, marine systems are a rich source for pollutant-degrading bacteria. For example, we recently isolated a marine bacterium that degrades the halocarbon fumigant, methyl bromide (MeBr). Isolation of the bacterium was carried out using traditional microbiological methods, and the bacterium was identified using molecular biological methods (PCR amplification of 16S rRNA genes). I am presently working on isolating the gene(s) responsible for carrying out the pollutant degradation. This gene system is promising because the organism has a tremendous substrate range. Purification of key enzymes in the methyl bromide degradation pathway will allow us to study pollutant degradation in detail and to better assess the commercial applications for these organisms and/or its genes. We have similar work in progress for a marine isolate that degrades carcinogenic polycyclic aromatic hydrocarbons (PAHs).

Utilizing Marine Biotechnology in Human Pathogen Assays and *in situ* Sensors:
A number of coastal problems can impact human health, such as sewage pollution and contamination of shellfish with *Vibrio* bacteria. I hope to use marine biotechnology to overcome the drawbacks of traditional microbiological assays. I am interested in designing a human pathogen assay system that would give rapid, species-specific identification of both *Vibrio* bacteria and human fecal contamination. The assay would utilize immobilized, flurophore-labeled, species-specific probes.
(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Protecting coastal systems from pollution, sewage contamination, and *Vibrio* infection is important for protecting human and ecosystem health, which in turn is critical for fishing and tourism industries. The study of marine pollutant-degrading bacteria can lead to biotechnology that protects and remediates polluted environments. Development of biotechnology that can be used shipboard or deployed on moorings would allow for accurate, near real-time monitoring of problem organisms. Such technology would be highly valuable to agencies

responsible for water quality monitoring and could find wide use in the field of microbial ecology.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Malacobranchiology: A Functional Analysis of the Clam Gill

The basic function of the gill in bivalved molluscs (i.e., clams, mussels, oysters, and scallops) is to produce – through ciliary activity – a water current that delivers oxygen to the animal. But such gills are very complex organs, so in addition to respiration, they participate in filter feeding and waste management, osmotic and ionic regulation, and reproduction. In all of these activities, the flow of water past the gill filaments and the movement of filtered particles on the gill surface are controlled by several distinct ciliated tracks, by muscles of various types, and by mucus secreting cells. Moreover, these structures and their interactions with sensory structures and the central ganglia are controlled by a network of neurons that communicate via a rich blend of neurotransmitters and modulating agents.

The objectives of this and collaborating laboratories areas are: to identify physiological signal molecules by chemical purification of extracts; to analyze the effects of these molecules in pharmacological studies; to localize the molecules within the clam gill by immunohistochemical and the methods of molecular genetics; and thereby to determine how the component actions are integrated to produce the various organized behaviors shown by the whole gill in the clam. Experiments are being carried out on the commercially valuable hard clam *Mercenaria mercenaria*.

(10) **Potential Applications and Benefits of Malacobranchiology**

The clam gill – like the mammalian airway – is an interface between the animal's internal milieu and its external environment; that is the external respiratory (and feeding) current passes through the gill in channels that are intimate with the branchial blood vessels that perfuse the gills. Therefore the gill is highly sensitive to, and responds to critical chemical cues in the external environment; but it is also the first line of defense against dangerous chemicals and pathogenic organisms.

(a) Because the gill is central to the physiology of bivalve molluscs, a thorough knowledge of the function of this organ would benefit the **aquaculture** of clams and oysters in Florida, augmenting both **productivity** and the **health** of these animals.

(b) Because the muscles, various cilia, and secretory cells of the gill respond differently to chemicals in the water, the gill can serve as a novel bioassay with which to screen various **marine bioproducts** and to analyze their mechanisms of action. The gill could also be used to **monitor coastal waters** for dangerous chemicals and pathogens.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

Rational drug design against critical components of parasite neuromuscular systems holds the promise of providing safe and effective agents to combat a variety of fish parasitic diseases. My laboratory's interest is in defining the structural and functional properties of such potential target molecules in a variety of parasitic flatworms. We plan to extend this strategy to gill flukes, flatworms that are major pathogens of marine animals in the wild and in culture. Our major focus is on voltage-gated calcium channels, which are critical components of excitable cells. Calcium channels open in response to a change in membrane potential, allowing calcium to flow down the electrochemical gradient and into the cell. In so doing, they serve as regulators of intracellular calcium, an important second messenger in cells. We are cloning calcium channel cDNAs from a variety of flatworms, and we are determining the physiological and pharmacological properties of these flatworm channels using heterologous expression. We hope to define specific structures within these channels that may serve as potential target sites for new, rationally designed, antiparasitic agents. We are also beginning a new set of experiments to examine the properties and roles of nitric oxide synthases (NOS) in parasitic flatworms. NOSs synthesize the gaseous intercellular messenger, nitric oxide, which is likely to play a crucial role in the realization of flatworm life cycles.

The methods we use include DNA cloning, PCR, DNA sequencing, heterologous expression of cRNAs, electrophysiology, capillary electrophoresis, HPLC, and various biochemical and pharmacological protocols.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

These projects have the potential to enhance the productivity of the aquaculture industry in Florida and throughout the world. The use of rational drug design against critical components of parasite neuromuscular systems should result in safe and effective agents to combat a variety of fish parasitic diseases. These efforts may also have positive impact on the tropical fish industry in Florida.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
Our long-term goal is to discover microbial secondary metabolites with biomedical applications, by screening natural populations and by genetically manipulating biochemical pathways to create laboratory strains of microbes that make compounds not found in the natural environment. Current projects develop tools needed to fulfill that goal. Specific objectives of our current work are to: 1) test the hypothesis that microbes associated with invertebrates that make bioactive products are enriched with bacterial species which produce antimicrobial metabolites; 2) identify, isolate, and determine the structure of bacterial metabolites that have potential as lead compounds for the design of new antibiotics; to identify genes encoding enzymes in the metabolite's biosynthetic pathways; 4) to develop cloning vehicles capable of replicating in both *E. coli* and in common marine bacteria .

Bacteria are isolated from marine invertebrates and the water column off the coast of Florida and other locations representing polar, temperate, and tropical environments. Bacteria are cultured in commercial media. Identification of bacteria is done by analysis of 16S rRNA gene sequences. Chemical extraction of cultures using solvents of differing polarities provides crude extracts for activity assays done by industrial collaborators. Purification of metabolites is done by chromatography, and structure elucidation is done using NMR, IR, UV and mass spectroscopy. Genes in metabolic pathways are identified using insertional mutagenesis. Construction of cloning vehicles uses standard recombinant DNA techniques.
(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).
Use of antibiotics to treat infectious diseases has increased since mid-century. Concurrently, pathogens have become more resistant to therapy, such that currently over 10% of clinical isolates of pathogenic bacteria are resistant to most available antibiotics. One solution to drug resistance is to identify novel antimicrobial compounds, which can serve as leads in drug discovery programs. Marine microbes are an exciting potential source of lead compounds. Our projects for the near future are focused on developing three products (microbes, vectors and bioactive natural products) to aid in antibiotic discovery and production. Benefits of this research will impact: 1) the pharmaceutical industry, who will evaluate our products as lead compounds for drug discovery; 2) public health, since the objectives of the project are the development of new drugs; and 3) the Florida economy since new industrial concerns and/or new industrial processes will translate into employment opportunities. Tools generated by current projects will lead to development of genetically-engineered laboratory strains of marine

microbes. These will ultimately help conserve Florida Coastal resources, by providing the opportunity to create new compounds without excessive "bioprospecting".

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Current Research: My research activities are oriented towards microbial physiology and microbial ecology. Specifically they can be grouped into two areas, (1) basic physiology of nitrifying bacteria and (2) the structure and function of natural planktonic bacteria with emphasis on the factors that regulate microorganism community structure. I am currently involved in a DOE-funded project that uses *in situ* detection techniques for the visualization of genes and/or their expression in marine bacteria. Specifically, I have used chemoautotrophic bacteria to simultaneously look at the gene and gene activity for carbon fixation and ammonia oxidation. I am also collaborating with a group interested in marine algal toxins. My work with this group is aimed at clarifying the role of epiphytic bacteria on the growth of toxic dinoflagellates.
Methods: In situ methodologies that involve the use of fluorescent dyes incorporated on intact cells thru FISH (fluorescent *in situ* hybridization), IS-PCR (*in situ* polymerase chain reaction), ISRT (*in situ* reverse transcription) and RT-PCR (reverse transcription PCR) reactions. Final analyses after the *in situ* procedures is carried out by epifluorescence microscopy coupled to image analysis and is now being combined with flow cytometry. Similar techniques along with metabolic fluorescent probes will be used in microcosm experiments to study the dinoflagellate-bacteria association in toxic algae.

(10) Potential Applications and Benefits

Traditional ways to measure bacterial abundance (i.e. microscopy) or the whole community process approach to measure activity do not lend themselves to any type of large-scale or monitoring studies. That is why microbial ecologists like myself are moving toward methods that reduce the time of analysis (i.e. flow cytometry), increase the level of resolution (i.e. fluorescent or radioactive probes) and that in addition provide new insights into the structure and function of microbial communities (i.e. in situ methodologies on whole cells). These new generation of methods will not only facilitate

the study of bacteria belonging to a given taxa or phylogenetic group without the need for cultivation but also has enormous practical advantages for monitoring pathogenic, indicator or bioengineered species in clinical and environmental studies

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Discovery of antitumor and anti-inflammatory compounds from marine organisms and structure activity relationship studies to develop these compounds into potential pharmaceutical products.

(10) Potential Applications and Benefits:

The marine derived biologically active natural products generally have complicated molecular structures and usually occur in very low yields. Re-supplying material for complete biological activity studies has been the main hurdle to develop these compounds into useful pharmaceutical products. There are several ways to overcome the supply issue. Total synthesis has been one of the successful ways to overcome the supply issues for structurally simpler compounds. However, the synthesis of complex molecules is complicated and not economical. An alternative and simplest method is to study the structure activity relationships, which determine the functional groups and the molecular fragments responsible for their biological activities. These data could be combined to provide a structurally simplified molecule for total synthetic studies. The discovery of a simpler analog via structure activity studies conveniently exclude any future dependence for the marine organisms which would likely to get eradicated due to over collection.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Basic and applied research on marine plants (seaweeds and seagrasses). HBOI is an international leader in the experimental cultivation of seaweeds with 25 years of experience. A more recent focus has been seagrass cultivation.

(10) Potential Applications and Benefits:

Seaweed Aquaculture
Applications include agar production, bioconversion, food and aquacultural feeds, biomedical compounds, and wastewater treatment. Much of this research has focused on the red seaweed *Gracilaria*, which is a major source of the commercially important compound agar. A mutant strain of *Gracilaria* was isolated at HBOI and patented for its high-quality agar properties; this patent was the first awarded for a seaweed strain anywhere. An important future direction is integration of seaweed cultivation in marine polyculture systems.

Seagrass Cultivation
The impacts of coastal development have resulted in a loss of seagrass in many areas. A high priority for coastal managers is restoration of seagrass beds. Traditional restoration efforts of transplanting seagrass from an established bed to other locations damage the donor bed and contradict the growing management practice of no loss of seagrass habitat. The development of a seagrass nursery requires practical application of the ecology and physiology of seagrasses. An innovative nursery approach to seagrass restoration and creation would play a significant role in the re-establishment of seagrass habitat in Florida's coastal waters.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Immunology, development of immunoassays. Bioremediation of waterways using marine microorganisms.

(10) Potential Applications and Benefits:

The development of rapid, accurate, immunoassay field identification kits will allow fisheries officers to ascertain whether a butchered fish is a federally-protected billfish or not; it will also allow fisheries biologists to obtain more accurate billfish catch statistics. Prototype kits have been used in the apprehension and litigation of individuals violating laws protecting billfish.

Studies on the use of living marine organisms to remove man-made pollutants in the Lake Worth estuary and thus improve water clarity and quality.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Organic Biogeochemistry of estuarine and coastal environments. Analytical methods development for the characterization of natural and anthropogenic organic matter in water, suspended solids and sediments/soils.

(10) Potential Applications and Benefits

Areas of Scientific Interest and expertise in Marine Biotechnology Research and Education: Environmental assessment; coastal processes; oil spill fingerprinting and environmental fate.

developed from catfish purified GST-ð and the other antibodies are commercially available. If positive results are found for GST-µ or GST- we will develop fish antibodies to these proteins.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

If we can demonstrate that expression of one or more forms of GST is highly responsive to exposure to a chemical pollutant, this could be further developed as a diagnostic tool for monitoring exposure in the field. This approach has been successfully used with CYP1A to monitor for polycyclic aromatic compounds, and for vitellogenin for exposure to estrogenic chemicals.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Current efforts are centered around detecting changes in microbial community structure and function in response to environmental stress. Microbial community structure is monitored using PCR amplification of 16S-rRNA genes and phylogeny determined using Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphism (TRFLP). Further identification of important species is provided by DNA sequencing and reference to genetic databases. These techniques may also be used to monitor changes in the presence and expression of important genes involved in biogeochemcal cycles. Changes in community structure may lead to changes in ecological function of the microbial ecosystem.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology
Research and Education:

I am a microbial ecologist who specializes in nutrient cycling and water quality research. Much of my work involves studying the mechanisms by which excess nutrients and pollutants affect microbial processes in wetlands and coastal environments.

Currently I am conducting research on phosphorus cycling and the mobilization and accumulation of mercury in the Florida Everglades, Florida Keys and Florida Bay. Methods used include a wide variety of analytical chemistry techniques and microbial enzymatic and functional assays.

(10) Potential Applications and Benefits:

The microbes which are the focus of my research are the one-celled bacteria that have the capability of cycling and/or breaking down nutrients such as nitrogen, phosphorus and ammonium. This process, called recycling, makes nutrients available for plants and other organisms in the ecosystem to use, and contributes to the life cycle of the system. However, in our modern world, humans produce and dispose of tons of nutrients each day. The microbes can only work so fast to deal with the overload. My research looks at what happens to these bacteria and their ability to break down the nutrients in the environment, along with the effects on the plants and animals. The data collected will be one of the keys to understanding long-term effects of agricultural and/or urban runoff on wetlands and the long-term effectiveness of constructing water-cleansing facilities called stormwater treatment areas and protective buffer zones.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Use of genetic markers to characterize genetic diversity within population structure and between marine coastal dune plants used for dune restoration/stabilization. *Uniola paniculata* (sea oats) is currently used as a model. We have conducted a molecular study using Random Amplified Polymorphic DNA (RAPD) for Florida sea oats populations in the years of 1996 and 1997 with the specific objectives of studying the spatial patterns of overall genetic variation both within and between sea oats populations. Two populations each from the Gulf of Mexico (Egmont Key and St. George) and the Atlantic coast (Sebastian Inlet and Anastasia State Park) in Florida were used. Our study has revealed a high genetic variability both within and between populations as well as between the coasts. Populations that were in the same coast generated a significantly lower genetic differentiation among populations compared to the ones obtained from the two coasts. Especially, a very low genetic differentiation was found between populations sampled from the Atlantic coast. Seven sea-oats genotypes from each site were clonally propagated using micropropagation technology. Common garden and reciprocal transplant studies using these genotypes are being completed to determine relationships between the adaptive significance the observed genetic diversity.

(10) Potential Applications and Benefits:

Ecologically, it is important to determine the relationship between geographic source, genotype and growth performance of plants used for dune stabilization. These results will provide a system to select both elite and commercially valuable dune plant genotypes for specialized or enhanced function, especially dune restoration/stabilization and creation for wildlife habitat. Micropropagation technology will be used to alleviate current limitations associated with commercial production of sea oats using traditional propagation methods.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Our interest lies in the area of marine natural products chemistry. Specifically we focus on biosynthetic, enzymology and molecular biology studies of biologically active natural products. In addition, we have a focused program directed at the identification of novel active drugs / drug leads from marine sources.

(10) Potential Applications and Benefits:

The overall goals of the projects underway in the Kerr lab are to use the biosynthetic, enzymology and genetic information to develop biotechnological production methods. Marine organisms have proven to be a prolific source of novel chemicals many with exciting biomedical applications. However, the development of many of these new pharmaceuticals has often been hampered by lack of available compound. Currently the only source of such marine natural products is from the collection of the source organism. Our efforts are aimed at the development of production methods that will be applicable for "scale-up" to meet projected market demands. Current projects involve potent anti-cancer and anti-inflammatory agents.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Marine *Vibrio* and chitin degradation; bacterial pathogenesis; toxin production and modes of action; carbohydrate metabolism; signal transduction and applications for marine and natural products.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

The focus of the research in my lab is on the horseshoe crab, *Limulus polyphemus*. The research projects in my lab focus on two areas. The goals of the first area are: 1) To develop a synthetic medium that can be used to culture horseshoe crab cells *in vitro.* 2) To isolate stem cells that can be grown in culture and induced to differentiate into mature blood cells. The second focus in the lab is on the embryonic development of the horseshoe crab. Our goals in this area are: 1) To fill in the gaps in our knowledge of the embryonic development of this animal. 2) To determine why *Limulus* needs four sets of the HOX genes (genes that regulate early development), when all other arthropods that have been studied have only a single set of these genes. Methods: Physiological analysis techniques, cell culture, standard histological and microscopy techniques (including light, fluorescence and EM), and molecular techniques especially those used in analysis of gene expression.

This is a new line of research for me. My background is in Genetics, Cell, Molecular and Developmental Biology, with most of my research experience in *Drosophila* Development and Genetics.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Limulus Amebocyte Lysate (LAL) is produced from the blood cells of adult horseshoe crabs. Thousands of adult crabs are collected, and up to 200 ml of blood is taken from each animal. The animals are returned to the ocean after bleeding, but about 10% do not survive the procedure. This is contributing to the decline in *Limulus* populations along the U.S. east-coast. The establishment of horseshoe crabs cell lines that can be induced to differentiate into mature blood cells *in vitro* will provide a renewable source of blood cells for the production of LAL and will relieve one of the pressures that is causing the population declines.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Marine Animal Health: "Etiology of Marine Turtle Fibropapillomatosis". In the last decade, a serious new threat to sea turtles has emerged in the form of an epizootic disease, fibropapillomatosis (FP). FP is characterized by the development of multiple tumors on the skin and occasionally internal organs, most frequently the lungs and kidneys. These tumors interfere with swimming, eating, breathing, seeing, and reproduction; turtles with heavy tumor burdens become severely debilitated and die. FP has seriously impacted green turtles, *Chelonia mydas*, in Florida and Hawaii, and is now emerging as a significant threat to the loggerhead, *Caretta caretta*, in Florida. The prevalence of FP in the Indian River Lagoon of Florida is >70%. Through long-term transmission studies in captivity, we have clearly demonstrated that FP is caused by an infectious subcellular agent, most likely an enveloped virus. We have demonstrated a unique chelonian herpesvirus in more than 97% of experimentally induced and spontaneous fibropapillomas using electron- microscopic, molecular, and serological techniques. This virus is now a leading candidate for the etiology of the disease. We have also demonstrated a strong association between the antibody response to this herpesvirus in green turtles and the development of clinical fibropapillmatosis, and are developing a diagnostic test for this disease. The potential role of environmental co-factors is also being explored. **Methods:** Immunoassay development, PCR, histopathology, virus/pathogen isolation and cell culture.
Forensics and Monitoring: We are interested in and capable of developing molecular and serological diagnostics for infectious diseases of marine animals in the wild, in captivity, or in aquaculture.
(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).
The host range of the virus(es)/pathogens we study is unknown. It is unclear whether such viruses can affect fish populations or even extend to marine mammals. In this era of emerging infectious diseases, it is important to be able to study and monitor the occurrence and routes of spread of infectious diseases in marine animal populations in order to protect our marine resources. Vaccine development methodologies need to be explored for application in this area.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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9.1. Osmosensory signal transduction in cells of euryhaline animals:

The objectives are to 1) identify the mechanisms used by cells of euryhaline animals routinely exposed to salinity stress in their natural habitat to sense the setpoint and changes in environmental osmolality and 2) get insight into the molecular contingencies that enable cells to carry information about osmotic changes in their environment from molecular sensors to osmoprotective effectors. We are using biochemical and molecular genetic methods to investigate these processes in gill cells of euryhaline fishes, including Fundulus heteroclitus.

9.2. Cellular protection against environmental change/ stress:

The objective is to investigate the biochemical basis for the ability of cells from euryhaline animals routinely exposed to salinity stress in their natural habitat to adapt to and resist such stress. We are using a biochemical and genetic methods repertoire to identify how adverse effects of changes in the micromolecular milieu on macromolecular structure are repaired and counteracted under such conditions.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Aquaculture management; Assessment of impact of environmental change on marine organisms; Biomonitoring; Information processing technology.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Our laboratory is interested in how nutrition and environmental factors influence gene expression in marine organisms. Diet and the nutrients in that diet are a major environmental influence on any organism. In the area of marine animal health we are interested in how the scope of this influence is affected by the availability of those nutrients. Using a marine hepatocyte model, our goal is to characterize the pathways linking nutrient availability to gene regulation and illustrate how cells respond to their environment through metabolite control of gene expression.

Many aquatic organisms are resistant to environmental pollutants and we are studying their natural mechanism of clearance that may be due to the function of inherent multi-drug resistant protein extrusion pumps. This mechanism of multixenobiotic resistance is similar to the mechanism of multidrug resistance (MDR) exhibited in chemotherapy resistant human tumor cells in which overexpression of a membrane protein pump facilitates drug clearance. We are evaluating the expression of marine MDR extrusion pumps in terms of marine animal health and as ecosystem "biomarkers" to assess environmental quality and ultimately human health. We expect to clone protein extrusion pumps from liver tissue of marine organisms and to characterize their function by comparing results from *in vivo* and *in vitro* experiments.

(10) Potential Applications and Benefits:

Presently, it is now recognized that nutrients are modifiers of gene expression in all animals. The liver is a major organ for substrate metabolism, protein synthesis and detoxification. Therefore, in terms of marine animal health, hepatic function is dependent on substrate availability and understanding how cells respond to their environment through metabolite control of gene expression is critically important.

We want to demonstrate that the expression of the marine extrusion pumps established from toxin treated cultured primary hepatocytes experiments will complement *in vivo* assays using toxin treated fish and ultimately, complement assays using animals from the wild. The intention is not only to establish a criteria of predicting early-on environmental quality but also to develop an analysis assay that can be used in the field. In the context of ecosystem indicators, the possibility of establishing a diagnostic "biochemical marker" designed to assess early-on, long-term environmental quality would be potentially significant to the study of pollution effects, environmental monitoring of organisms, and risk assessment needed in regulatory policy. This also would allow evaluation of biologically relevant exposure, as well as pathobiological effects of such exposure. These studies not only impact coastal human health evaluation but also show potential in forensic monitoring of health risks.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods). Aquaculture – evaluation of the production and economic feasibility of alternative species (freshwater and marine). Current projects include sturgeon, redfish, bull minnow (Fundulus grandis) evaluations.
(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes). Program efforts target existing industry and potential producers and provide specific production guidelines, economic analysis and marketing strategies.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).
Microbially produced biosurfactants allow microbes to interact more intimately with hydrophobic microenvironments and enhance the bioavailability of hydrophobic substances both to the microbe that produces the surfactant and to much of the marine biota as well. Increased bioavailability may enhance biodegradation rates of compounds whose biodegradation rate is limited principally by high hydrophobicity. A downside of biosurfactant effects is that they may increase the apparent dissolved concentrations the aqueous phase of toxic materials that would otherwise be relatively inert in that they would be sequestered into hydrophobic milieu. Biosynthesis of biosurfactants is often triggered by exposure of the microbe to hydrophobic environments, particularly if the hydrophobic materials can be utilized as a carbon substrate for growth. However, there are numerous other environmental cues that stimulate biosurfactant production and seem unrelated to either hydrophobicity or to carbon nutrition, and these mechanisms are not well understood. Marine microbes that produce biosurfactants will affect their ability to colonize hydrophobic surfaces and may improve competition for hydrophobic niches. Biologically produced surfactants exhibit a tremendous variety of chemical structures and are equivalent or better to synthetic surfactants in their ability to emulsify oils and to lower surface- and interfacial tension. Biosurfactants as a class are less toxic than their synthetic counterparts. Marine biosurfactants are poorly characterized. We have characterized the physiology and regulation of a number of marine bacteria that produce biosurfactants.
(10) Potential Applications and Benefits:
Understanding the regulation of biosurfactant production will allow fundamental understanding of their role in microbial ecology and physiology of marine environments. Industrial and other uses of biosurfactants are the same as those of their synthetic counterparts. Surfactants are widely used in industry generally where multi-phase systems are in use. Applications involve emulsification, detergency, flocculating, foaming, wetting, phase dispersion, de-emulsification, or solublization There is concern, however, that the use of synthetic surfactants may have deleterious effects on the environment as synthetic surfactants in high concentrations can inhibit microbial growth when added to the environment. Biosurfactants have specific application in cosmetic and pharmaceutical formulations, food processing, promoters of bioremedial processes for crude oils, polycyclic aromatic hydrocarbons. Recently, the capacity

of biosurfactants to facilitate mobilization of heavy metals from soils that are high in humic acid content and contaminated with a range of hydrophobic pollutants has been exploited.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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A major area of research in our laboratory is the use of fiber optic biosensors for rapid detection of microbial pathogens in marine waters. Evanescent wave, fiber optic biosensors are an innovative, cutting edge technology capable of real time/near real time detection of microbial pathogens. We have used these biosensors to detect and identify pathogens such as *E. coli* O157:H7, *Salmonella, Vibrio cholerae,* and *Cryptosporidium* from various sources including sewage, seawater, apple cider/apple juice, and ground beef. Current biosensor assays are based on antibody-antigen interactions, although we are in the process of developing nucleic acid probe-based biosensor assays. The biosensor assays are sensitive, specific, and rapid. Furthermore, the biosensor unit is portable and can be used in the field by minimally-trained personnel. This biosensor research currently is supported by $1,000,000 in annual funding from the Department of Defense and the Water Environmental Research Foundation.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Fiber optic biosensors have many potential applications in marine biotechnology. Unlike slower conventional microbial monitoring techniques, which are based on indicator microorganisms and do not provide information on the presence or absence of specific pathogens, biosensors can be used for real time/near real time detection of specific microbial pathogens. Such rapid information can be useful in determining the immediate health of marine waters and environments as well as for rapidly screening marine life and seafood for microbial pathogens and determining point sources of contamination. Biosensors, which also are capable of detecting toxins, can potentially be used to detect red tide and other harmful marine conditions.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Marine Bioproducts: Specifically, the discovery of new anti-cancer compounds from marine natural products through the screening of crude extracts, their chemical separation and the identification of their mode(s) of action. Current research involves 1) the search for analogs and derivatives and mechanism of action of the marine natural product, discodermolide, a compound which was discovered at HBOI as an anticancer compound and is currently licensed by a pharmaceutical company for development as an anticancer compound 2) mechanism of action studies on a marine derived compound, dictyostatin, which possesses anticancer activity and 3) development of new screening assays for the rapid discovery of new anticancer agents from marine extracts.

(10) Potential Applications and Benefits

The commercial benefit of our drug discovery work lies in the discovery and eventual commercialization of a particular marine derived compound as an anticancer agent to treat human cancer; a direct benefit to mankind.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Molecular evolutionary study of secondary metabolite (polyketide -PK) biosynthetic pathways in marine invertebrates and/or associated microbial isolates. Some of the objectives in this research include:

- Comparing sequences and predicted enzyme structures of "paralogous" modules (within a single PK pathway) and correlating these with corresponding PK metabolite or PK intermediates and infer possibility of gene duplications
- Comparing orthologous (between different bacteria and different pathways) PKS enzyme module sequences and structures to obtain correlations with PK metabolite end products and also to investigate the possibility and frequency of horizontal gene transfer

Applying molecular tools - e.g. DNA sequencing, RFLP analyses - for the clarification of taxonomic and systematics identities of marine invertebrates and microorganisms.
Applying molecular biology tools to optimize in vitro invertebrate cell culture.

Education - Molecular Studies of Marine biodiversity- college level training in molecular techniques applied to assessing and characterizing biological diversity in natural populations of marine organisms. Recently endorsed as an International Biodiversity Observation Year (IBOY) project by Diversitas.

(10) Potential Applications and Benefits:

a. With respect to marine bioproducts, our current projects will contribute to biotechnological solutions (gene cloning, enhanced cell culture) to the meet the supply of promising bioactive natural products and reagents. With genetically engineered solutions, pressure on threatened and stressed coastal habitats and their organisms may concomitantly be alleviated.
b. Several of the molecular methods (DNA fingerprinting and sequencing) in our laboratory can be applied in a forensics context, to identify samples at from the individual to the species or higher taxonomic levels.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Training
and Development Program,
Fiscal Year 2001 and Beyond

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

a) Cellular Immune Function in Sharks, Skates, and Rays - Research objectives include studies to identify the immune cells produced by sharks and their relatives, characterize the sites of immune cell production, define functions for the various immune cells, and understand how these functions are regulated. Methods include hematological and histological techniques, *in vitro* cell culture, RNA *in situ* hybridization, cell proliferation assays, tumor cell growth inhibition assays, chromatography, isoelectric focusing, and polyacrylamide gel electrophoresis.

b) Angiogenesis Inhibitors from Shark Tissues and Cells - Research objectives include studies to identify biomolecules that inhibit neovascularization. Sources of material include extracts from cartilage and conditioned media from *in vitro* cell cultures. Methods include ion exchange and size exclusion chromatography, polyacrylamide gel electrophoresis, isoelectric focusing, collagenase/gelatinase inhibition assays, and capillary endothelial cell assays.

c) Elasmobranch Models for Endocrine Disruption - Research objectives include studies of environmental effects on endocrine disruption of reproduction and embryogenesis in elasmobranch species representing placental viviparity, aplacental viviparity, and oviparity. Methods include histology, immunocytochemistry, immunoblotting, immunogold, and ELISA.

(10) Potential Applications and Benefit: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

a) Cellular Immune Function in Sharks, Skates, and Rays - Goals include the isolation and characterization of novel cell regulatory factors that possess anti-tumor or anti-bacterial activity.

b) Angiogenesis Inhibitors from Shark Tissues and Cells - Goals include purification of active inhibitors with potential for synthetic or genetically engineered versions to be developed into viable therapies benefitting from inhibition of angiogenesis. Applications include solid tumors, diabetic retinopathy, rheumatoid arthritis, and psoriasis.

c) Elasmobranch Models for Endocrine Disruption - Goals and applications include development of representative elasmobranchs as sentinel species for assessment of environmental contaminants on reproductive capacities in vertebrate animals.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Research is directed towards the isolation, fermentation and taxonomy of marine-derived microorganisms. Extracts from fermentation broths are provided to both the in-house screening program and external collaborators for drug discovery programs. We are especially interested in the microorganisms that are associated with deep-water macroorganisms such as sponges. However, it is estimated that over 95% of marine microorganisms cannot be cultivated in the laboratory using conventional techniques. Specific projects include the development of new isolation techniques for marine microorganisms, the development of new fermentation methods and the study of the taxonomy and inter-relationships of the microorganisms in our collection of 13,000 strains.

Other research interests include the discovery of new antifungal agents and the study of the mechanism of action of antifungal agents, with emphasis on compounds affecting the fungal cell wall.

(10) Potential Applications and Benefits:

The Harbor Branch microbial collection represents a unique resource for the discovery of novel natural products and enzymes of industrial importance. Methods being developed for the isolation of unusual microbes may have significance in other areas of microbiology.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Red Tide- Harmful algal blooms have significant environmental and economic impacts on the State of Florida. Mitigation strategies for red tide blooms can best be developed and implemented when the origin of a bloom can be determined and the organism's growth and dispersal can be monitored. A major focus of our work is to develop specific probes to identify, quantify, and examine the relatedness of *Gymnodinium breve* isolates found at traditional bloom sites and, with this information, to locate the origins and to monitor the dynamics of blooms.

We are using two approaches to develop these biomarkers:

I- oligonucleotide primers appropriate for real time PCR analyses are being developed which will allow the identification and quantification of *G. breve in situ.*

II-Secondly, antibodies have been developed to cell surface markers of *Gymnodinium* which will not only be useful in detecting low levels of organisms but will also be invaluable in examining unusual life cycle stages which are problematic in the environment. Together, these complementary reagents will be used in the identification and comparison of geographic isolates.

Manatee serology/disease - Both monoclonal and polyclonal antibody reagents have been developed to manatee IgG. These manatee-specific antibody reagents will be used not only to evaluate sera for antibodies to pathogenic agents/toxins, including red tide organisms, but will also allow fundamental questions concerning manatee immunology to be addressed.

Genetics-Experiments to resolve the population genetic structure of the West Indian manatee using mitochondrial DNA control region sequences have been completed for eight locations across the western Atlantic region. These genealogies have been used to illuminate aspects of evolution and biogeography that are pertinent to sirenian systematics, ecology, and wildlife management programs. Current work focuses on the development of microsatellite markers for resolution of populations at the level of pedigree analyses.

Potential Applications and Benefits: The environmental relevance of this work is self-evident. The significant public health, economic, and ecosystem impacts of harmful algal blooms outbreaks are severe. Blooms in some coastal areas have caused the virtual collapse of ecosystems with accompanying serious economic impacts. For example, economic losses in the U. S. from harmful algal blooms over several decades likely exceed one billion dollars due to the need for toxin monitoring programs, closures of shellfish beds, fish and shellfish mortality, disruption of tourism, threats to public and coastal resource health, publication of watershed, health, and seafood advisories, and medical treatment. The cost of the environmental impact of threats to the endangered Florida manatee is incalculable. Our work has applications which benefit the economy and endangered species of the State of Florida.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Bioluminescence and chemiluminescent reactions, molecular detection of luciferase control genes and luciferase kinetics of marine luminous bacteria, and mass spectrometry of homoserine-lactones (major regulaltor of quorum sensing genes).

(10) Potential Applications and Benefits:

Bioluminescence/chemiluminescence are amongst the most sensitive methods known, often surpassing radioactive methods. Thus, there could commercial relevance in the development of diagnostic kits. The environmental relevance is understanding how lumious marine bacteria function in fish guts, light organ symbioses and in seawater.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Training
and Development Program,
Fiscal Year 2001 and Beyond

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

Diagnosis and treatment of disease of dolphins, whales, and sea turtles - Research objectives of this ongoing project include monitoring disease processes found in live stranded animals and the determination of appropriate treatments for each disease seen. Methods include appropriate diagnostics such as serological testing, radiology, ultrasonography, endoscopy, MRI, CT scans, cytology, microbiological culture, viral isolation, as well as other standard medical tests. Treatments are tested as felt appropriate to control the disease process.

(10) Potential Applications and Benefit: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

The potential benefit and application of this study relates to marine animal health as well as coastal human health risks. Many of the species that we work with may be considered sentinels for aquatic health as well as human health. The more that is learned about the diseases that affect these animals that share the coastal marine habitat with humans, the better we can treat the diseases and eventually prevent them before they occur. By monitoring these diseases, we may be able to prevent any effects on the human inhabitants of the coastal areas. One additional benefit is in monitoring the impact of human activity on these species.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Isolation and characterization of marine-derived peptides. NMR is the primary tools for determining the 3-dimensional structure of peptides.

(10) Potential Applications and Benefits:

The project involves a search for novel conopeptides from cone snails from the Caribbean region. The conopeptides will be characterized at FAU using modern NMR methods and Cognetix will perform bioassays related to the relief of chronic pain.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education

I am a microbial molecular biologist. My project involves understanding the role of Pseudomonas aeruginosa infection among patients with cystic fibrosis. This will be addressed at a molecular level by looking at (i) the mechanisms that promote spontaneous conversion of nonmucoid Pseudomonas aeruginosa to a fatal mucoid or alginate-producing form, (ii) the mechanisms involved in spontaneous reversion back to nonmucoid form observed under laboratory conditions, (iii) the role of alginate in biofilm formation and (iv) the mechanisms responsible for overexpression of b-lactamase genes that contributes to antibiotic resistance. (v) therapeutic use of ginseng as an alternative medicine against bacterial infection

My recent interest include coral diseases caused by bacterial pathogens and looking for anti-fouling agents produced by marine organisms.

(10) Potential Applications and Benefits:

The anti-fouling agents produced by marine organism have therapeutical value against bacterial infection, cancer, etc.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
My area of research is in open ocean aquaculture and the development of new and automated aquaculture systems.

(10) Potential Applications and Benefits:
The research advances in open ocean aquaculture will lead to a significant increase in the existing fishery supplies. Successful open ocean aquaculture practices will generate widespread realization of environmentally friendly commercial aquaculture.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Marine microbiology/ microbial ecology

Current interests involve trying to increase recoverability of marine microorganisms associated with deep-water sponges and determining the nature and evolutionary implications of their interaction. Sponge-microbial associations have long been documented but relatively little is known about the nature of the interactions. The amount of bacteria residing in sponge species varies dramatically but can constitute up to 60% of the biomass, suggesting that they may play a crucial role in determining the nutrition, health, and chemical defenses of the host sponge. Because water column microorganisms provide a food source for the sponge, differentiating between truly associated species and food species is difficult. We have performed numerous cultivation studies using various media compositions and media additions in an attempt to better characterize the entire microbial community, as well as the specifically associated microorganisms. Early results indicate that we can increase the number of colony forming units recovered by employing media additions that have been shown to increase aerotolerance. Low nutrient media appears to be more effective for increasing microbial recovery than higher nutrient counterparts. Additional studies remain to be done to determine the nature of the invertebrate-microbial interaction. Preliminary results suggest that the host sponge is not providing some required growth factor for its associated microbes, indicating that the interaction is not based on nutritional requirements.

Microbial fermentation processes

Microbes have been found to produce secondary metabolites of interest but their expression is thought to vary dramatically with environmental conditions. Studies have been undertaken to determine what nutritional additions and growth conditions will optimize production of secondary metabolites under laboratory conditions.

(10) Potential Applications and Benefits:

HBOI has access to deep-water marine invertebrate samples that are not readily available to other researchers. This provides an advantage in terms of novel microbial isolations and characterizations. Additionally, in-house capabilities to perform marine based fermentations

from the recovered microorganisms provides information on secondary metabolite production and potential pharmaceutical applications.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
The design and efficacy of marine reserves; Sustainable fisheries; Coral reef restoration.
Overfishing and habitat degradation threaten sensitive coastal marine habitats and are of vital importance to fisheries management as laid out, for example, in The Sustainable Fisheries Act of 1996 and the amended Magnuson-Stevens Fishery Conservation and Management Act. Effective management requires integration and application of innovative technological advances in the biological sciences. These include not only molecular and biochemical tools, but also statistical and mathematical tools. Our long-term research program aims to apply these tools to the restoration of coral reefs and the development of sustainable fisheries. Our program will develop and use: mathematical models (spatially structured individual-based models, ordinary differential equations, dynamic optimization models); statistical approaches (e.g., non-linear estimation, BACIPS assessment models); field techniques (micro-tagging, habitat enhancement via deployment of artificial reefs, chemical induction of settlement of coral larvae, foodweb manipulations); and lab techniques (imagine analysis, laser-ablation ICPMS otolith analysis, DNA sequencing). We apply these tools using the Florida Keys ecosystem as our experimental system. We currently have funding from National Sea Grant to design an innovative study of habitat enhancement as a technique to augment fisheries production. The study develops mathematical models, statistical designs, and fieldwork to facilitate the management of marine ornamentals (fishes and invertebrates harvested in the aquarium trade).

(10) Potential Applications and Benefits:
Our research aims to enhance the management of sustainable marine resources through better understanding of the processes that affect dynamics, quantification of the effects of human activities on coral reef ecosystems, and application of this knowledge to the restoration of these systems. The effective management of marine ornamentals and coral reef systems is critical because marine ornamentals represent a significant industry in Florida and because the Florida Keys is a site of tremendous state and national environmental importance. We have already established ties with industry (marine collectors and suppliers), extension (e.g., the Tropical Aquaculture Laboratory, University of Florida), and state and federal agencies (e.g., National Marine Fisheries Service), which will enhance the impact of this work on marine environmental policy and the marine ornamental industry.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

1. Development of a Marine Prophage Induction Assay. Because of the escalating pollution in our nearshore environments, it is necessary to have a simple method for the detection of potential mutagenic activity for marine samples. Additionally, such an assay could be used to monitor products for human use for potential mutagenic or teratogenic activity. Current methods use animals assays which are time consuming, expensive, and of concern because of animal rights issues. We are developing a mutagenesis assay based upon prophage induction using a marine lysogenic bacterium. Lysogens are bacteria that contain a silent viral infection. The virus genome survives in the host and is silently replicated along with the bacterial chromosome. Upon encountering a DNA damaging agent, the viral DNA becomes activated and viral production and cell lysis occurs. The assay takes advantage of this fact and uses viral production as an indication of mutagenic activity. Our objectives are to screen marine bacteria as potential candidates for use in the prophage induction assay, to cross calibrate the assay with the -Microscreen assay, and to test environments suspected to contain carcinogens with our method. So far two bacteria have been used with success in the Marine Prophage Induction Assay (MPIA). Our research is environmentally relevant because it has provided basic information on the process of lysogeny in the marine environment. It has become apparent that many bacteria in the ocean are lysogenic, and it may be that prophage induction is a major mechanism for viral production in these environments (particularly bays and estuaries which receive pollutants regularly). The application of this assay is the production of a commercially viable assay for mutagen detection, in environmental samples and products for human use.

2. Molecular Methods for the Detection and Identification of Human Pathogenic Viruses in Florida's Coastal Waters. Because of the development in the coastal zone, there is an increasing amount of wastewater impacting our beaches and shorelines. Wastewater contains over 100 pathogenic viruses. We have previously found a high incidence of enterovirus in Florida's coastal waters, and to gather more information on this phenomenon, we have developed methods for the rapid quantitation and identity of these viruses. This research has provided information on the types of enteroviruses present in Florida's coastal waters, and will be the basis for a testing business. Enteroviruses are being evaluated as a wastewater indicator that might replace or supplement the current indicator, fecal coliform bacteria.

EXPRESSION OF SCIENTIFIC INTEREST
for a
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
Marine Bioproducts

- Drug discovery, particularly for the treatment of cancer, inflammation, and infectious diseases. Our multi-disciplinary research program includes the collection of unique deep water samples using our fleet of research vessels and manned submersibles; the development and implementation of new models to screen extracts of these organisms; the discovery and licensing of novel marine-derived compounds, and the development of methods (fermentation, cell culture, aquaculture) for sustainable use of these marine resources.
- Development of a marine invertebrate cell culture model to study the role of marine natural products in nature, their application to understanding and treating human diseases, and in vitro production of bioactive compounds. We are using current methods in gene array technology to probe the genome of a model sponge species, identify genes involved in the production of bioactive compounds by the sponge, and determine the role of the bioactive compound in nature. This research is being conducted with funding from the Florida Sea Grant College Program, with matching support provided by our industrial collaborator, Research Genetics, Inc. (Huntsville, AL).
- The training of students (undergraduates, graduates, and post-doctoral fellows) in each of these research areas.

(10) Potential Applications and Benefits:

- Harbor Branch licensed discodermolide, a novel antitumor compound isolated from the deep water sponge *Discodermia*, to Novartis Pharmaceutical Corporation in April 1998. The drug is in advanced pre-clinical trials and is expected to advance to clinical (human) trials by mid-2001.
- Other compounds in preclinical trials include the topsentins, a series of bisindole alkaloids with potent antiinflammatory activity, derived from the deep water sponges *Spongosorites* spp., and the lasonolides, antitumor macrolides derived from the deep water sponge *Forcepia* sp. Research on both of these series of compounds continues both in our laboratories as well as in the laboratories of our industrial collaborators.
- Marine organisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Marine bioproducts- Discovery of novel compounds from marine invertebrates, algae and microorganisms for the potential development of new therapeutic agents or biomedical research tools: Senior Research Specialist for the Collections/Taxonomy Program within the Division of Biomedical Marine Research at HBOI. Chief Scientist in charge of organizing and supervising worldwide expeditions for the collection of marine organisms to develop pharmaceutically active compounds. Responsible for curation of 25,000 specimens of marine organisms in taxonomic museum collection. Diving Safety Officer for all diving activities from Harbor Branch vessels and by a staff of 60 research divers. Mr. Reed's research on the deep-water Oculina coral banks off Florida since 1976 has resulted in the establishment of a 300 sq.mi. sanctuary for these reefs

(10) Potential Applications and Benefits:

Potential development of new therapeutic agents or biomedical research tools.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology
Research and Education:

My laboratory is investigating the biosynthesis of polyketides by dinoflagellates. Polyketides are an important class of secondary metabolites represented by numerous commercially important compounds such as erythromycin, tetracyclin and lovestatin (a cholesterol-lowering drug). Recent advances in our understanding of the biosynthetic pathways for these secondary metabolites have led to strategies for manipulating these pathways to generate new polyketides. Thus chemical diversity can be achieved via the new strategy of "combinatorial biosynthesis". Biosynthetic pathways have not been identified for dinoflagellate (marine protists) derived polyketides and this is the goal of our work. We are studying both the biosynthesis of known polyketides and using polymerase chain reaction (PCR) to screen for the expression of biosynthetic genes in order to identify new polyketides. My work is currently supported by two grants; NIH Molecular Genetics of Dinoflagellate Polyketide Biosynthesis

The major goal of this project is to develop methodology that may be used to identify and clone the biosynthetic gene cluster for the synthesis of okadaic acid from the dinoflagellate *Prorocentrum lima*. This includes the development of a transformation system for *P. lima*.

NIH SBIR (with CalBio Marine) New Drugs from Symbiotic Marine Dinoflagellates
The major goal of this project is to identify new drugs from cultured symbiotic dinoflagellates. Traditional bioassay guided fractionations will be combined with molecular techniques to identify new compounds as well as biosynthetic potential

(10) Potential Applications and Benefits:

Okadaic acid (a dinoflagellate derived polyketide) is an inhibitor of protein phosphatase and has been used extensively to identify cellular processes that are regulated by phosphorylation. A MEDLINE search of okadaic acid returned over 3500 entries. It is isolated in minute quantities from dinoflagellate cultures and sells for $118 for 50µg. Heterologous expression of the biosynthetic pathway could provide significant quantities of okadaic acid for research. Polyketides include numerous commercially important antibiotic, anti-fungal, immunosupressant, anti-tumor and anti-cholesterol drugs. Dinoflagellates may be a new source of commercially important drugs. Furthermore, the polyketides derived from dinoflagellates are truly unequalled in terms of their size and structural complexity. Harnessing this biosynthetic capability will add unprecedented tools to increase the repertoire of reactions available for combinatorial biosynthesis.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

A) Isolation, identification, and characterization of pathogenic microorganisms and pathogenic microbial consortia on coral reefs
B) Hyperspectral remote sensing of coastal marine aquatic ecosytems (phytoplankton assemblages, seagrass, coral community, etc.)

(10) Potential Applications and Benefits:

A) Pathogens: coastal management
B) Remote sensing

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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1. *Diagnosis*
   Standardize reverse-transcriptase polymerase chain reaction (RT-PCR) assays for the rapid diagnosis of morbillivirus infection in tissues of marine mammals.

2. *Immunology*
   Cloning of immune cytokines of marine mammals.

3. *Molecular Virology*
   Isolation and molecular characterization of viruses from marine mammals.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(1) Name: **John Scarpa**
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Marine Bioproducts: Aquaculture methods for organisms with pharmaceutical and nutriceutical compounds. Previous research in culturing the tunicate, *Ecteinascidia turbinata*, in land-based systems.

Marine Animal Health: Examining the environmental (especially ionic) and nutritional requirements off Pacific white shrimp, *Penaeus vannamei*, in fresh water. Examining the shrimp pathogen IHHNV for virulence and vaccine development for shrimp culture.

Aquaculture: Delineating culture methods and increasing disease resistance through breeding and genome manipulation (e.g., polyploidy, gynogenesis) of aquatic organisms, primarily oysters, clams, marine shrimp, fish and lower invertebrates. Current research is examining the resistance of the Caribbean oyster, *Crassostrea rhizophorae*, to the oyster disease Dermo (*Perkinsus marinus*), as a genetic resource for American oyster, *C. virginica*, breeding programs.

(10) Potential Applications and Benefits:

Marine Bioproducts: Decrease dependence on “wild” stock. Manipulate environment for increased synthesis of target compound.

Marine Animal Health: Once environmental ionic requirements for growing marine shrimp in fresh water are elucidated, potential exists in Florida and other inland areas for the culture of shrimp. Vaccine development for viral pathogens would increase shrimp survival and profitability of enterprises. If an avirulent form of the virus can be found, genetic analysis would allow for understanding on how to disable the mechanism of attack.

Aquaculture: Decrease dependence on “wild” stock, increase growth rates, decrease poor quality of oysters in summer, and increase overall survival.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

(1) Name: **Michael C. Schmale, AND Patrick D.L. Gibbs**
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

We have two principle areas of interest in the field of marine biotechnology: Diseases of marine organisms and construction of optimized vectors for production of transgenic fish.

a) Diseases of marine organisms – We are currently investigating an unusual virus-like agent which appears to be the cause of naturally occurring tumors in one species of fish on coral reefs in South Florida (Damselfish Neurofibromatosis). We believe this agent may a unique type of oncogenic virus that has unusual abilities to transform neuroectodermal cell types. This agent should be a valuable tool for understanding carcinogenesis in these cell types and as such has human health as well as marine animal health implications. We have developed probes for DNA and RNA associated with this agent are using molecular and virological techniques to investigate the mechanisms of infection, replication and cell transformation by this agent.

b) Development of vectors and transgenic fish for use as model systems for understanding function of vertebrate gene promoters and as biosensors for environmental monitoring. We have developed vectors which produce high level, stable, multi-generational expression of a green fluorescent protein (GFP) reporter throughout the life cycle of the zebrafish. While numerous labs have achieved transient or low-level expression (detectable only in eggs and larvae) these constructs allow easy scoring of gene expression in adult fish. We are currently expanding this research into several new areas. We are testing the ability of certain regions of DNA to act as boundary element sequences (enhancing expression of the constructs in a position independent manner). We are developing constructs containing inducible promoters to facilitate experiments to determine how vertebrate promoters function and to develop transgenic lines of fish which can be used as biosensors of toxicants in the environment. Finally, we will be attempting to transfer these constructs and transgenic technology from the test species, the zebrafish, to other species, such as Fundulus.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Analyses of the virus-like agent involved in damselfish neurofibromatosis should yield important information relevant to human health and as such could be considered a marine bioproduct.

Development of the transgenic fish model system will yield fish and DNA constructs which can be used in environmental, aquaculture and human health related research. Patents

have been applied for to cover several of these constructs which may yield marketable products in the form of both transgenic fish and DNA vectors.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Research interests include determination of the biogenetic origin of bioactive metabolites from tropical deep water sponges and the use of invertebrate cell culture and microbial fermentation as renewable sources of marine natural products with pharmaceutical potential. Other research interests include the role of bioactive metabolites in the marine environment (chemical ecology) and the potential use of natural products in sponge systematics.

Techniques employed include dissociation, differential centrifugation, density gradient fractionation, flow cytometry, confocal and electron microscopy and immunohistochemistry. Also used are chemistry techniques such as hplc with diode array detection.

(10) Potential Applications and Benefits

Potential development of alternative, renewable sources for marine natural products with pharmaceutical or cosmetic potential; sustainable use of marine resources.

EXPRESSION OF SCIENTIFIC INTEREST
For Florida Marine Biotechnology Research, Training and Development,
Fiscal Year 2001 and beyond

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(1) Name: **Steven Serfling, Ken Leber**
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(9 & 10) Areas of Scientific Interest & Expertise In Marine Biotech Related Research & Education, and Potential Applications & Benefits:
A. AQUACULTURE:
1. Non-surgical, biotechnology methods (e.g. blood, slime chemistry changes) for identification of sex, stage of maturation of ovary/testes, and optimal timing for egg removal and fertilization, in marine fish and sturgeon.
2. Methods/products for controlling and accelerating maturation and spawning of captive broodstock (time release hormones, special diet supplements/lipids, water chemistry, etc.), and for improving quality and quantity of eggs.
3. Genetic identification of desired traits (growth rate, size, disease resistance, etc.) in different strains, species, offspring of certain marine fish and sturgeon, to accelerate selective breeding programs.
4. Improved methods for preserving, storing, transporting and shipping live sperm, eggs, and larvae, for collecting and preserving unique or endangered genetic stocks, creating hybrids, and to have viable sperm and eggs available at the same time for year-round spawning.
B. MARINE ANIMAL HEALTH:
1. Develop simple methods to measure state of health/stress in marine fish and sturgeon in response to different culture conditions, water quality, diets, etc. (e.g. blood chemistry changes).
2. Probiotic methods and species/strains for natural disease prevention and control.
3. Dietary supplements (non-injection methods) for increasing immune system and ability of cultured fish to recover from handling, shipping and stress.
4. Develop and/or evaluate "natural", non-toxic, non-stressful products/chemicals fordisease prevention and control, i.e. products that do not weaken the immune system or health of the species being treated, to eliminate use of antibiotics and toxic/stressful chemicals drugs, and meet FDA approval for food production.
C. FORENSICS & MONITORING:
1. Develop DNA and/or biochemical markers (ratio or presence of certain lipids, minerals, nutritional supplements, etc.) for distinguishing wild versus cultured fish products, and fish raised for stock enhancement. Markers must be natural and acceptable to consumers and FDA.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

In a one-year pilot project supported by the Florida Department of Health, we are monitoring fecal bacterial contamination in the Pensacola area recreational waters. We began exploring the use of PCR based technologies to identify the profiles and sources of bacterial contamination. Based on promising preliminary results we are proposing an in depth investigation where we will test two strategies for simultaneous detection and identification of fecal bacteria. We intend to investigate an anaerobic-bacterial 16S-TRFLP based species ratio and an Enterococcus repetitive element sequence-based (rep) PCR fingerprinting approach as an indication of human or animal fecal source contamination of water and sediments. We will validate these PCR based monitoring strategies through comparison to traditional water quality monitoring methods.

As part of this process we will utilize a novel ion exchange membrane for the concentration and purification of environmental nucleic acids. Our first strategy focuses on unique sequence PCR targets of dominant fecal bacteria. Since anaerobes are by far the most numerous single class represented here they are a logical target indicator group, which is rarely utilized because they are either dead or difficult to culture. Five fecal bacteria will serve as targets for their respective dominance in either human or animal feces. Four anaerobes: Bacteroides thetaiotaomicron, B. vulgatus, B. distasonis, and Clostridium clostridiiforme, and E. coli were chosen. Detection and quantification of these sources will be through competitive PCR terminal-restriction-fragment-length polymorphic (TRFLP) (16S-rDNA or unique sequence regions) analysis. The Enterococcus population approach is intended to focus efforts on a more diverse source of information than typing single indicators like E. coli. Our aim instead, is to characterize a more complex survivor assemblage based on 17 species of Enterococcus in water and sediments and compare to the same assemblages from likely source organisms (humans, cattle, waterfowl, and marine animals). Survivor fingerprints will be evaluated by both standard electrophoresis and TRFLP methods. We will determine the availability and turnover of cell-free nucleic acids in bayou, beach and bay waters. To do this we must concentrate DNA and RNA from large volumes of water. To evaluate the DNA from filterable cells, as well as extracellular nucleic acids we will employ a novel prototype membrane filter (provided by PALL Corporation) capable of binding and selectively releasing high quality DNA and RNA for PCR applications. Because this membrane is capable of concentrating DNA and RNA from water quickly, we intend to examine its potential for detecting pathogens (enteric

viruses (Hepatitis A, Norwalk and Polio) and a protozoan pathogen (Cryptosporidium parvum)) known to be present in coastal waters.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

1. Molecular approaches to forensic and diagnostic applications
2. Conservation Biology
3. Biotechnological approaches to fisheries management
4. Molecular ecology and evolution

Current research activities are focused in the following areas:

a) Development of genetic markers for use in forensic, fisheries management, conservation, seafood diagnostics, and ecological contexts.
b) Population genetics and stock identification of fishes (emphasis on elasmobranches).
c) Molecular approaches to the study of mating systems in fishes.
d) Basic research in molecular biology and evolution (emphasis on elasmobranches).

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

Our research in forensics and diagnostics has direct applications in regulatory issues pertaining to monitoring of seafood trade, detection of fraud in seafood commerce, and fisheries management.

Our research in stock identification and basic research in ecology and evolution has direct application to issues of marine conservation, biodiversity assessment, and fisheries management.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and expertise in Marine Biotechnology Research and Education:

My current research involves the use of molecular approaches to investigate microbial populations of ecological (carbon and nitrogen cycling) and public health interest (harmful algal bloom species). My work involves the development of geneprobe assays, gene amplification assays, genetic fingerprinting, and *in situ* genetic labeling of whole cells combined with epifluorescent microscopy and cell-sorting flow cytometry

(10) Potential applications and benefits:

The development of molecular techniques for the examination of microbial processes such as carbon fixation, nitrification, and of toxin production (e.g. ciguatera fish poisoning, paralytic shellfish poisoning, neurotoxic shellfish poisoning) provide additional tools for the sensitive and rapid monitoring of microorganisms involved in these activities. The integration of these techniques (genetic fingerprinting, geneprobes, in situ PCR and in situ reverse transcription, microscopy, and cell-sorting flow cytometry) with traditional biogeochemical research may help to better understand how environmental and anthropogenic factors affect microbial nutrient cycling and toxin-producing phytoplankton. Because all these molecular techniques depend on whether enough sequence data is available, it is expected that the abovementioned methods will apply to a wide variety of genes of ecological, biomedical or industrial interest.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and expertise in Marine Biotechnology Research and Education:
The use of natural products in alternative medicine. Evolution of innate immunity with emphasis on complement and antibacterial peptides.
Experimental animal: Nurse shark (*Ginglymostoma cirratum)*. Ten adult animals have been maintained at the Keys Marine Laboratory (KML) since 1980.
Education Activities: Every Summer since 1994 the PI has team taught, with Dr Charles Bigger, a Marine Comparative Immunology Workshop at the KML. The 3-day laboratory-based workshop introduces students to a comparative approach to the study of various aspects of the innate and adaptive immune system in a diverse group of marine organisms, including invertebrates and vertebrates.
Research Activities:
I Isolation and characterization of antibacterial proteins/peptides from shark leukocytes. Two distinct types of antibacterial activity have been shown to be present in shark leukocyte lysates. In addition to lysozyme-like activity, bactericidal activity was demonstrated against a marine organism, *Planococcus citreus* . Current studies focus on (a) the purification of the bactericidal peptide to obtain amino acid composition and sequence data; and (b) the assessment of antibacterial activity against clinically significant bacterial strains, such as *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, and Escherichia coli.* The potential medical application of antibacterial peptides is their use against drug-resistant bacteria/organisms (i.e., “super bugs”).
II Purification and characterization of shark complement proteins, and cloning of corresponding genes. Elucidation of complement activation pathways and study of the bioactivity of complement-derived anaphylatoxins, complement control proteins, and complement receptors. Project involves molecular biological techniques, protein purification and analysis (e.g., SDS-PAGE) methods, and development of functional assays specific for the shark system.
III Study of the *in vitro* effect of shark cartilage on normal immune function of human cells (i.e., leukocytes). This pre-clinical study examines the bioactivity of an acid-extract of shark cartilage by assessing its ability to stimulate specific cellular responses such as cytokine production, up-regulation of antibody synthesis and release, induction of apoptosis, oxidative burst, expression of surface markers, and cell proliferation. Study data could be used to predict the *in vivo* effects of long-term prophylactic use of cartilage on normal individuals who take it as a protective measure against cancer, chronic degenerative disease, AIDS, etc. The study will attempt to identify the active component(s) and determine whether (a) it is contraindicated for certain individuals, such as those suffering from hypersensitivity or

autoimmunity, and (b) it can be obtained from alternative sources (cartilage from the beef and poultry industry), thereby preserving and protecting the shark species that are currently being indiscriminately slaughtered.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
The design and efficacy of marine reserves; Sustainable fisheries; Coral reef restoration.
Overfishing and habitat degradation threaten sensitive coastal marine habitats and are of vital importance to fisheries management as laid out, for example, in The Sustainable Fisheries Act of 1996 and the amended Magnuson-Stevens Fishery Conservation and Management Act. Effective management requires integration and application of innovative technological advances in the biological sciences. These include not only molecular and biochemical tools, but also statistical and mathematical tools. Our long-term research program aims to apply these tools to the restoration of coral reefs and the development of sustainable fisheries. Our program will develop and use: mathematical models (spatially structured individual-based models, ordinary differential equations, dynamic optimization models); statistical approaches (e.g., non-linear estimation, BACIPS assessment models); field techniques (micro-tagging, habitat enhancement via deployment of artificial reefs, chemical induction of settlement of coral larvae, foodweb manipulations); and lab techniques (imagine analysis, laser-ablation ICPMS otolith analysis, DNA sequencing). We apply these tools using the Florida Keys ecosystem as our experimental system. We currently have funding from National Sea Grant to design an innovative study of habitat enhancement as a technique to augment fisheries production. The study develops mathematical models, statistical designs, and fieldwork to facilitate the management of marine ornamentals (fishes and invertebrates harvested in the aquarium trade).
(10) Potential Applications and Benefits:
Our research aims to enhance the management of sustainable marine resources through better understanding of the processes that affect dynamics, quantification of the effects of human activities on coral reef ecosystems, and application of this knowledge to the restoration of these systems. The effective management of marine ornamentals and coral reef systems is critical because marine ornamentals represent a significant industry in Florida and because the Florida Keys is a site of tremendous state and national environmental importance. We have already established ties with industry (marine collectors and suppliers), extension (e.g., the Tropical Aquaculture Laboratory, University of Florida), and state and federal agencies (e.g.,

National Marine Fisheries Service), which will enhance the impact of this work on marine environmental policy and the marine ornamental industry.

EXPRESSION OF SCIENTIFIC INTEREST
Florida Marine Biotechnology Research, Development and Training Program

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(1) Name: **Ian R Tebbett**
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Areas of expertise

The Analytical Toxicology Core Laboratory within the Center for Environmental and Human Toxicology provides state of the art analytical support to researchers throughout the U.S. Our key areas of expertise lie in the analysis of drugs, and environmental toxicants in biological specimens. We provide analytical support to UF's Superfund program project (Health effects of Chlorinated Hydrocarbons), as well as to many other groups of researchers studying environmental contaminants in wildlife including marine species. Among our studies in this area, we have developed assays for pesticide residues in shark livers and muscle, fish fillets, crabs, alligator tissue and eggs and tissues from sea birds. In addition to Superfund researchers, current clients include the St Johns River Authority, DEP and Mote Marine research station.

Benefits

We are one of a very few laboratories in the country that has the capabilities to develop, validate and perform analyses for toxicants in marine biological specimens.

EXPRESSION OF SCIENTIFIC INTEREST
Florida Marine Biotechnology Research, Development and Training Program

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Name: **Aswani Volety**
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Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:
Marine bioproducts: My interests lie in understanding host-parasite interactions and focus on how the hosts defend themselves against pathogenic organisms. Of particular interest to me are the lysozyme(s) in bivalves including other anti-microbial peptides. Lysozymes are very effective against bacterial pathogens. I was successful in partial purification of lysozyme from plasma of oysters. Preliminary investigation of molecular characterization of lysozyme indicates that it may be a dimmer of 19kd. Lysozyme activity in oysters is negatively correlated with prevalence of *Perkinsus marinus* in oysters. We are currently in the process of investigating the role of lysozyme in the defense of oysters against various bacterial (vibrios) and protozoan parasites (*Perkinsus marinus*).

Marine animal health: Mechanisms of how parasites evade host defense and how hosts defend against pathogenic parasites are of particular interest to me. I have used the interactions of *Perkinsus marinus* and oysters as a model. Results indicate that *P. marinus* suppresses/
inhibits production of reactive oxygen species (ROS) by oyster hemocytes, thereby escaping ROS mediated killing of hemocytes. I would like to investigate the role of pathogenicity and environmental factors in modulating immunological and physiological responses of aquatic organisms.

Coastal human health risks: Coliform bacteria have been traditionally used to investigate the contamination of coastal and estuarine waters. However, using Enterococcus as an indicator organism in assessing risk to humans has been suggested. In collaboration with the Environmental Protection Agency, we have developed a delayed incubation medium for Enterococcus sps. In collaboration with Mr. David Fries, Center for Ocean Technology at the University of South Florida, we would like to develop molecular tools for rapid and sensitive detection of Enterococcus sps.in coastal waters.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(1) Name: **Patrick J. Walsh**
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(8) Website: www.rsmas.miami.edu
(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).

Aquaculture. Much of our biotechnology oriented research falls under the general area of aquaculture. We have been studying the impact of stress on the nitrogen metabolism and excretion patterns of bony fish for the past two decades. Stress in a laboratory or commercial aquaculture setting can be induced in fish by relatively slight disturbances, including air-exposure during netting, overcrowding, and other stressors. It is accompanied by a classical stress response in which the stress hormone, cortisol, is released, and a variety of physiological changes are induced. Many of these changes are problematic to hatchery operations because they adversely affect the health and growth of animals. We propose to use biotechnological approaches to both better understand stress, and to prescribe protective and ameliorative actions that can be taken in the aquaculture industry. Specifically, we would adapt DNA microarray technology to fish species of interest in Florida aquaculture (e.g., redfish, snook, etc.) to better understand the gene level changes that occur during stress. These findings would ultimately be used in the development of genetic engineering methods to produce strains of fish that are resistant to stress.

(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).

The research has ultimate and direct relevance to improving the commercial applicability of aquaculture.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(1) Name: **Amy Wright**
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Division of Biomedical Marine Research
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

My current research program focuses on the discovery of novel marine natural products with potential use as human therapeutic agents or as lead structures for rational drug design. Although compounds are evaluated in a broad spectrum of disease models, our primary emphasis is on the discovery of cancer therapeutics which act via non-traditional mechanisms. Compounds are obtained from marine plants, invertebrates and microorganisms associated with marine invertebrates using bioassay guided purification. The structures of new compounds are determined through spectroscopic means with an emphasis on the use of nuclear magnetic resonance spectroscopy. A new collaborative research project in my group focuses on the production of “un-natural” natural products through the recombination of genes responsible for secondary metabolite biosynthesis. Ancillary interests include evaluation of the ecological role of marine natural products; applications of natural products chemistry to systematics of the Porifera and deep water Gorgonacea; study of the role of microbial associates in the production of therapeutically interesting natural products; and development of micro-analytical methods for monitoring the production of therapeutically important marine natural products in aquacultured and cell cultured organisms.

(10) Potential Applications and Benefits:

The organisms that live in deep-sea habitats have not been fully investigated for their biosynthetic potential and represent a unique source of compounds to assist in treatment of diseases such as cancer, arthritis and neurodegenerative disease. By coupling HBOI’s unique deep-sea collection capabilities with the high-throughpout screening capabilities found both at HBOI as well as with partners in academia, biotechnology and large Pharma, and our ability to purify and define the structures of active compounds, we are discovering novel marketable agents with utility in treating the diseases that continue to plague our aging population.

EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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(1) Name: **Anita C. Wright**
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(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education:

Coastal human health: Research interests focus on the genetics of virulence factors for foodborne pathogens, particularly on *Vibrio* species found in oysters and clams. Our laboratory is developing several molecular probes for detection of pathogens in the environment and food products, including a *Vibrio vulnificus* probe that has been adopted by the F.D.A. for evaluation of product safety. Currently, we are also investigating the genetics and regulation of the capsular polysaccharide expression of *V. vulnificus* in order to better understand its pathogenesis and microbial ecology.

Marine animal health: We are looking at the prevalence and distribution of *Perkinsus marinus* in clams and oysters from the Gulf of Mexico. We are using PCR for detection of this protozoan parasite that has devastated oyster beds in the Chesapeake Bay and elsewhere. We will be monitoring the potential impact of this parasite on the growing clam industry in the State of Florida.

(10) Potential Applications and Benefits: These studies should provide tools for more rapid and quantitative detection of pathogens that are relevant to both human and marine animal health. Molecular probes developed from this research can be used to evaluate environmental conditions that promote growth or expression of virulence factors of these species. These data also may be used to develop products that promote attenuation or elimination of pathogenic variants. Commercial applications of our research include the potential development of molecular probes for diagnostic analysis of pathogens associated with seafood consumption. We are optimizing protocols for detection and enumeration of *Salmonella* spp., *V. cholerae, V. parahaemolyticus, V. vulnificus, Escherichia coli* O157, *Listeria moncytogenes* and others. These probes have the potential benefit of producing a safer product for the consumer.

STATEMENTS OF ORGANIZATIONAL CAPABILITIES IN MARINE BIOTECHNOLOGY

- Florida Atlantic University
- Florida Gulf Coast University
- Florida International University
- Florida Sea Grant College Program
- Florida State University
- Harbor Branch Oceanographic Institution
- Mote Marine Laboratory
- Nova Southeastern University
- University of Florida
- University of Miami
- University of West Florida

Note: Future edition of this document may include other organizations, where marine biotechnology activity either exists but was not reported or is just emerging. For this version, each contributor used their own particular format to report information. Future versions may include more details, and in a more standardized format. (In a few cases, the compilers edited memos to condense information.)

Marine Biotechnology at Florida Atlantic University
Charles E. Schmidt College of Science

Marine biotechnology activities at FAU cover a broad spectrum of disciplines. Research activities range from the biotechnological development of new anticancer drugs from sponges to the development of field identification kits for billfish. In addition to the vigorous research programs involving undergraduate and graduate students as well as postdoctoral fellows, a wide selection of courses are offered to students at all levels. Student enrollment at both the undergraduate and graduate levels in marine science continues to expand, reflecting the enormous growth the Charles E. Schmidt College of Science currently enjoys.

The Department of Chemistry and Biochemistry's new Ph.D. program involves faculty from a number of different departments (Biological Sciences and Biomedical Sciences as well as Chemistry and Biochemistry) and includes scientists at Harbor Branch Oceanographic Institute's Division of Biomedical Marine Research. A focus of this multidisciplinary graduate program is marine biotechnology. The combined resources of these groups of scientists will provide for an unparalleled training opportunity in a variety of disciplines in the marine biotechnology field at the undergraduate, graduate and post doctoral levels.

Gumbo Limbo Marine Science Center: FAU's Gumbo Limbo Marine Science Center is located only minutes from the Boca Raton campus on the Intracoastal Water Way in the heart of Boca Raton. This facility provides scientists with indoor and outdoor tanks containing running seawater directly adjacent to modern laboratory areas providing an ideal environment for students and faculty to conduct experiments with live marine organisms in a controlled manner. FAU's facility at Gumbo Limbo is shared with the City of Boca Raton which provides an educational resource to the public. The placement of FAU research projects amongst City exhibit areas has exposed the public to ongoing research programs at FAU; this has proved to be a very positive experience for all concerned.

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Florida Gulf Coast University
Statement of Marine Biotechnology Capability

Florida Gulf Coast University (FGCU) would like to express its interest in the establishment of a Florida Marine Biotechnology Research, Development and Training Program. FGCU is currently developing an undergraduate degree program in Biotechnology and has several faculty members that are interested in research questions related specifically to marine biotechnology. Science education at FGCU is founded upon interdisciplinary approaches to learning and the incorporation of undergraduate research as part of the educational process. The new degree program in biotechnology supports both of these ideals and could serve to link academic programs in the Colleges of Arts and Sciences with those in the College of Health Professions.

Faculty Expertise

- José Barreto, Associate Professor (Biochemistry) - membrane physiology, medical biochemistry
- David Brown, Assistant Professor (Environmental Health, Molecular and Clinical Sciences) - molecular biology, biochemistry
- Nora Demers, Assistant Professor (Biology) - innate immunity, molecular biology
- Joseph Kakareka, Associate Professor (Chemistry) - analytical chemistry, environmental chemistry
- Clifford Renk, Professor (Environmental Health, Molecular and Clinical Sciences) - immunology, microbiology
- Mike Savarese, Associate Professor (Marine Science) - physiological ecology, geobiology
- Greg Tolley, Associate Professor (Marine Science) - marine ecology, fish biology, physiological ecology
- Aswani Volety, Assistant Professor (Marine Science) - immunotoxicology, pathophysiology, shellfish diseases

Facilities

- Whitaker Center for Science, Mathematics, and Technology Education (Spring 2001)
  - Research Laboratories:
    - Ecology
    - Environmental, Molecular and Clinical Science
    - Marine Science
    - Organic Chemistry
    - Physiology
  - Teaching Laboratories:
    - Analytical Chemistry
    - Ecology
    - Environmental, Molecular and Clinical Science
    - Marine Science
  - Support:
    - Acid Lab

Animal Housing
Aquarium Room/Web Lab
Walk-in Freezer/Cooler

Equipment

Atomic-absorption:	Perkin-Elmer Graphite Furnace-Atomic Absorption Spectrometer-Analyst 100
	MDA-80 Mercury analyzer
Autoanalyzer:	Skalar Aqua Water Analyzer
Autoclave:	Sterilmatic sterilizer
Centrifuges:	Micro MB, 14,000 rpm
	Clinical, 7,100 rpm
Chambers:	Lab-Line, Biotronette Mark III
	Lab-Line CO_2 incubator
Clean Room:	Mini-, with UV irradiation
Fluorometers:	Turner Designs 10-AU
	Fluorometer, filter, digital, lab-based
Gas Chromatograph:	Hewlett-Packard Model 5890 with autosampler, electron-capture, flame ionization detector, computer-assisted (shared with UF-IFAS)
	GowMac Series 350, thermal conductivity detector
	Hewlett-Packard Model 5890 Series II with autosampler
Mass Spectrometer:	Mass spectrometer detector, computer-assisted (shared with UF-IFAS)
HPLC:	Hewlett-Packard Model 1010 HPLC with autosampler, post-column reaction system, diode array detector, fluorescence detector, computer-assisted (shared with UF-IFAS)
PCR:	Perkin-Elmer with 2400 thermal cycler
Ovens:	VWR Furnace, muffle
	Precision drying oven
Refrigerators:	Freezer, ultra-low temperature
	Refrigerator, general purpose, lab
	Refrigerator/freezer, explosion-proof
Spectrophotometers:	Spectronic 21, Model D
	Mini-Spectronic 21
Water bath:	Precision, Shaking

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Capabilities of Florida International University
for Scientific Research and Education in Biotechnology

Florida International University (FIU) is a Ph.D. granting, Research I university, with an active research program in a broad range of marine science and the life sciences. Research and teaching activities in the area of marine Biotechnology are concentrated in 3 units of the university. These units are:

1. Department of Biological Sciences
   Department Chair: Dr. John Makemson
   Phone: (305) 348-2201
   http://www.fiu.edu/~biology

35 faculty with research and teaching interests that include marine ecology, coastal habitat assessment, marine animal health and diagnostics, microbiology, marine immunology, molecular genetics, molecular physiology, aquaculture, and drug discovery.

2. Department of Chemistry
   Department Chair: Dr. Ken Furton
   Phone: (305) 348-2292
   http://www.fiu.edu/orgs/chemistry

21 faculty with research and teaching interests that include chemical forensics, analytical instrumentation development, organic geochemistry, environmental fate of toxic substances, oil spill fingerprinting, biosynthesis of biotoxins, and drug discovery.

2. Southeast Environmental Research Center
   Director: Dr. Ron Jones
   Phone: (305) 348-3095
   http://serc.fiu.edu

18 faculty with research interests that include coastal monitoring and assessment, marine ecology, marine biogeochemistry, molecular genetics, stable isotope biogeochemistry and environmental toxicology.

18 Faculty responded to the Sea Grant survey on expertise in Biotechnology.

Florida Sea Grant College Program
Marine Biotechnology Research, Training and Development

As the only statewide, university-based coastal research, extension and education effort in Florida, Sea Grant sponsors faculty research and training of graduate students. One of the largest research theme areas of Florida Sea Grant is marine biotechnology. The goal is to use marine biotechnology to create and enhance products and processes from Florida's coastal resources. Efforts relate to developing and sustaining sources of supply for marine bioproducts, improving health and production of marine organisms, promoting human health and environmental quality, and facilitating informed consumer, business and technical decisions.

In addition to being the only coherent source of marine research funding for faculty in Florida, Florida Sea Grant works with industry through the BIOFlorida trade association. It also educates the public about biotechnology issues, and facilitates technical exchange via statewide "summits" and national meetings. This document was organized by Sea Grant.

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Marine Biotechnology at Florida State University

The request you sent for “Expressions of Scientific Interest” identified six areas of interests. This summary is based on those responses and my own grasp of research being conducted at FSU. Presently there is little activity at Florida State University that can be grouped under the rubric of marine biotechnology. We have one faculty member, Dr. Lita Proctor, Department of Oceanography who is actively engaged in research that relates to coastal habitat restoration. With funding from the Department of Energy and in collaboration with researchers at FAMU, Dr. Proctor has been studying the *role of microorganisms in the degradation of hydrocarbons*. My own research, funded by Florida Sea Grant focuses on *expanding the variety of fish and invertebrates, specifically marine ornamentals that can be cultured by the aquaculture industry*. It targets the dietary needs of the early feeding stages of fish larvae. However, the work at present does not rely on the use of cell and molecular technologies.

The University has the potential to contribute significantly in the area of marine bioproducts research. To the best of my knowledge no faculty are actively engaged in research on marine bioproducts however, the important drug taxol was discovered and synthesized by Dr. Robert Holten a member of our faculty. Thus we have the capability to make important headway in a drug identification and synthesis once bioactive compounds are identified. In addition we have faculty and well equipped laboratories that could readily address important problems in bioforensics relating to seafood identification and the development of new biosensors.

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Biotechnology at HARBOR BRANCH Oceanographic Institution

Biotechnology at HBOI is primarily concentrated in two research divisions, Aquaculture and Biomedical Marine Research. Our **Aquaculture Division** has established itself as a leader in the development of culture technologies for new aquatic species. Research and education programs address the culture of molluscs, crustaceans, marine ornamentals, food fish, seaweed, and biomedical species. Aquaculture development and service programs support industry expansion in Florida and around the world. In collaboration with academic, government and private research institutions, the Aquaculture Division has designed and implemented culture systems for a variety of marine animals and plants. Facilities, equipped with salt and freshwater systems, include laboratories, hatcheries, nurseries, covered raceways, a variety of greenhouses and biosecure buildings, and teaching laboratories and classrooms. The 60-acre Aquaculture Development Park is an incubator for new businesses that are testing concepts and developing pilot-scale facilities. The Park provides opportunities to develop new aquaculture technologies, collaborate with a broad variety of private sector commercial partners, and receive training in a wide range of aquaculture disciplines.

The mission of the **Division of Biomedical Marine Research (DBMR)** is to address critical human health problems through chemical and biological research on marine organisms and their chemical constituents. Implementation of the mission is achieved through a multidisciplinary team approach consisting of sample acquisition, cell culture, and molecular biology of marine organisms, and chemical and biological studies of chemical components derived from these organisms that show potential as new therapeutic agents or biomedical research tools. Using Harbor Branch's JOHNSON-SEA-LINK submersibles, DBMR has collected unique samples of marine organisms from depths of 150-3000 feet. The Division has assembled an inventory of marine invertebrates (primarily sponges, gorgonians, and tunicates), algae and microorganisms which comprise a broad spectrum of phylogenetic, geographic, temporal and chemical diversity. Key components of DBMR include:

Screening Laboratory and Flow Cytometry/Confocal Microscopy Facility - Research focuses on mechanism of action studies of marine-derived compounds in the areas of immunology and tumor biology and design and development of novel screening assays for the high throughput laboratory.

Fermentation Laboratory - The goals of the Fermentation Laboratory are to isolate microorganisms such as bacteria and fungi from the marine environment and to ferment these microorganisms to provide extracts for drug discovery programs.

Natural Products Chemistry Laboratory - The goal of this laboratory is to isolate and determine the structures of marine-derived natural products with pharmaceutical potential.

Invertebrate Cell Culture Laboratory - The Invertebrate Cell Culture Group is one of the few groups worldwide addressing the potential of in vitro production of marine invertebrate-derived natural products with pharmaceutical potential.

Molecular Genetics Laboratory - Current projects include a) application of genetic fingerprinting and DNA sequence analysis for identification of marine invertebrate cell types grown in vitro; b) cloning of species-specific cell growth factors; c) molecular evolutionary analyses of secondary metabolite biosynthetic pathways; d) sponge-microbe symbioses; and e) marine invertebrate molecular systematics and population genetics.

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Mote Marine Laboratory
Statement of Organization's Capability in Marine Biotechnology

Mote Marine Laboratory (MML) is an independent, nonprofit, international center for marine and estuarine research, scientific collaboration, and education founded in 1955. MML capabilities in the area of Marine Biotechnology include innovative and multi-disciplinary research in the following areas:

A. Expertise: Doctoral scientists trained in Protein Biochemistry, Immunology, and Fish Physiology, growth and reproduction, disease resistance, cytology.

B. Capabilities: Protein isolation and purification, cell culture, tissue processing and histology, RNA *in situ* hybridization, immunochemical techniques, fish and elasmobranch husbandry, serological testing, radiology, ultrasonography, endoscopy, MRI, CT scans, cytology, microbiological culture, viral isolation.

C. Facilities: Protein biochemistry laboratory, histology laboratory, tissue and cell culture laboratory, animal holding facilities, controlled environment experimental aquarium laboratories, more than 80 tanks and biofilter systems.

D. Collaborators:

1. Gary Litman, Ph.D., Department of Pediatrics and Institute for Biomolecular Sciences, University of South Florida College of Medicine, Children's Research Institute, St. Petersburg, FL.
2. Robert Langer, Ph.D., Department of Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA.
3. Yoni Zohar, Ph.D., Center for Marine Biotechnology, University of Maryland, Baltimore, MD (molecular and endocrine mechanisms regulating reproduction, development and growth).
4. John Trant, Ph.D., Center for Marine Biotechnology, University of Maryland, Baltimore, MD (PCR-RT, immunoblotting).

Contact: Kumar Mahadevan
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Nova Southeastern University Oceanographic Center's
Capability in Marine Biotechnology

The Nova Southeastern University Oceanographic Center has several faculty with interest and active research programs in various areas of marine biotechnology. Current research activities and capabilities include:

1) Cutting-edge molecular genetic approaches to questions and problems in marine forensics, fisheries management, seafood diagnostics, conservation biology and biodiversity assessment (Dr. Mahmood Shivji).

2) Extensive microbiology and chemistry research capabilities for culture and assessment of marine microbial biodiversity and microbial natural products (Drs. Andrew Rogerson and Veljko Dargojlovic).

3) Research in development and use of biofilms for enhancing settlement and recruitment on artificial reefs for reef restoration (Drs. Andrew Rogerson, Richard Spieler, and James Thomas).

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Summary of Marine Biotechnology Capability for University of Florida Faculty

A total of 18 *Expressions of Interest* were received from University of Florida faculty, covering all areas identified in the announcement (a break down is provided below). An examination of the specific areas identified by the respondents reveals that the single most popular category is Marine Animal Health, in part reflecting the presence of the School of Veterinary Medicine, closely followed by Aquaculture. A few *Expressions* crossed disciplinary boundaries.

The number of replies likely represents a small percentage of the total number who would respond to a request for proposals in the field. Replies were received from many different areas of the university, representing six different colleges and one non-affiliated unit, the Whitney Laboratory. The home departments of the respondents are listed below.

Affiliation of Respondents

Coll. Vet. Medicine (2) - Molec. Virology (Romero); Analytical Toxicology Core Lab. (Tebbett)

College of Medicine (3) - Medicine (Bubb); Pathology (Klein); Biochemistry (McGuire)

Pharmacy (1) - Medicinal Chemistry (James)

Liberal Arts and Sciences (2) - Zoology (Osenberg, St. Mary and Bolker 1 & 2)

Engineering (1) - Mechanical Engineering (Niezrecki)

IFAS (4) - Fish. & Aquatic Sci. (Bowen, Lazur); Microbiology & Cell Sci. (Keyhani); Environmental Hort. (Kane)

Whitney Laboratory (4) - R. Greenberg, M. Greenberg; Kultz; Laine

Areas of Interest

Marine Bioproducts (2) - Bubb; Keyhani

Marine Animal Health (6) - M. Greenberg*; R. Greenberg; Klein; McGuire; Romero; Tebbett

Aquaculture (4) - M. Greenberg*; Kultz; Lazur; Niezrecki

Coastal Human Health Risks (1) - McGuire

Coastal Habitat Restoration - Osenberg, St. Mary and Bolker 1 & 2; Kane

Forensics and Monitoring (3) - James; Laine; Bowen

*cross disciplinary

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Overview of the Marine Biotechnology Capability of the University of Miami's Rosenstiel School of Marine and Atmospheric Science

The Marine Biotechnology capability at UM/RSMAS is primarily focused on microbes (fungi and bacteria) and bony fish.

Marine Microbiology:

Marine Bioproducts, Forensics and Monitoring, Coastal Human Health Risks

In the marine microbial area, investigators are examining fungi for their use in the generation of novel marine bioproducts for pharmaceutical and other uses. Furthermore, since fungi are typically so difficult to rapidly identify from the taxonomic standpoint, investigators are developing rapid molecular methods. These are especially important related to human pathogenic fungi, that are carried by food or other pathways. In a third area of interest, investigators are determining the mechanisms of pollutant degradation by microbes, and ways to potentially utilize these organisms in bioremediation activities.

Faculty/Staff: Fell, Goodwin

RSMAS is currently conducting a search to fill two marine microbiologist positions which could add to our capability in this area.

Marine Fish Pathology and Molecular Biology:

Aquaculture, Marine Animal Health, Coastal Human Health Risks

Primarily using Marine Bony Fish, investigators are examining the effects of stress on fish as it relates to aquaculture, including ways to mitigate the effects of stress. Marine animal health is being examined as it relates to the mechanisms of transmission of virally-transmitted diseases. This research could have direct benefit to Florida aquaculture operations. In a third area of research, the basic mechanisms and limits to the production of transgenic fish is being tested. While this research could potentially impact the number of strategic topics of interest for Florida, it is primarily applicable via the use of biosensors in the detection of marine toxicants.

Faculty/Staff: Walsh, Schmale, Gibbs

RSMAS is currently conducting a search to fill an aquatic toxicologist position which could add to our capability in this area.

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The University of West Florida (UWF) received legislative authorization and special funding to create the Center for Environmental Diagnostics and Bioremediation (CEDB) in 1990. The Wetlands Research Laboratory (WRL) was created by UWF in 1989 to address the analysis of pollution in regional waters. The CEDB-WRL is staffed by faculty with backgrounds in crustacean neurohormones, microbial genetics, microbial biochemistry and bioremediation, protozoology, marine biology, ecology and environmental chemistry. Faculty interests are reflected by major research programs in bioremediation, environmental health, DNA chemistry, and ecological surveys. These all reflect the overall mission of the CEDB-WRL to secure extramural research support, recruit uniquely talented environmental scientists, and establish a regional resource offering not only analytical services but scientific oversight and evaluation as well. CEDB-WRL also seeks to enrich the educational/training opportunities for students at UWF through practical job experience.

Areas of expertise include estuarine ecology, molecular diagnosis for environmental contamination, ultraviolet radiation effects on bacterial populations, bioremediation of hydrocarbon and halogenated hydrocarbon pollutants, diagnosis and management of problems associated with urban waters, environmental toxicology, analytical Chemistry and environmental bacteriological testing.

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EXPRESSION OF SCIENTIFIC INTEREST
for a
Florida Marine Biotechnology Research, Development and Training Program

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Instructions: (1) Use no more than one page, with a minimum 12-point type size, to provide information according to the 10 headings below. (The font used here is 12-point.) (2) Please return to Florida Sea Grant at electronic mail address: jhw@mail.ifas.ufl.edu.

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(1) Name:
(2) Position:
(3) Affiliation:
(4) Mailing Address:
(5) Telephone:
(6) Fax:
(7) Electronic Mail:
(8) Website:
(9) Areas of Scientific Interest and Expertise in Marine Biotechnology Research and Education: (Note: provide a brief paragraph for each area; e.g., synopsis of current research objectives, methods).
(10) Potential Applications and Benefits: (Note: identify commercial or environmental relevance of your work; e.g., products, processes).