*Connecting the Gulf of Mexico and Human Health*

A Workshop Sponsored by the University of Southern Mississippi and the Mississippi-Alabama Sea Grant Consortium

Long Beach, Mississippi

May 4-6, 2005

PARTICIPANTS

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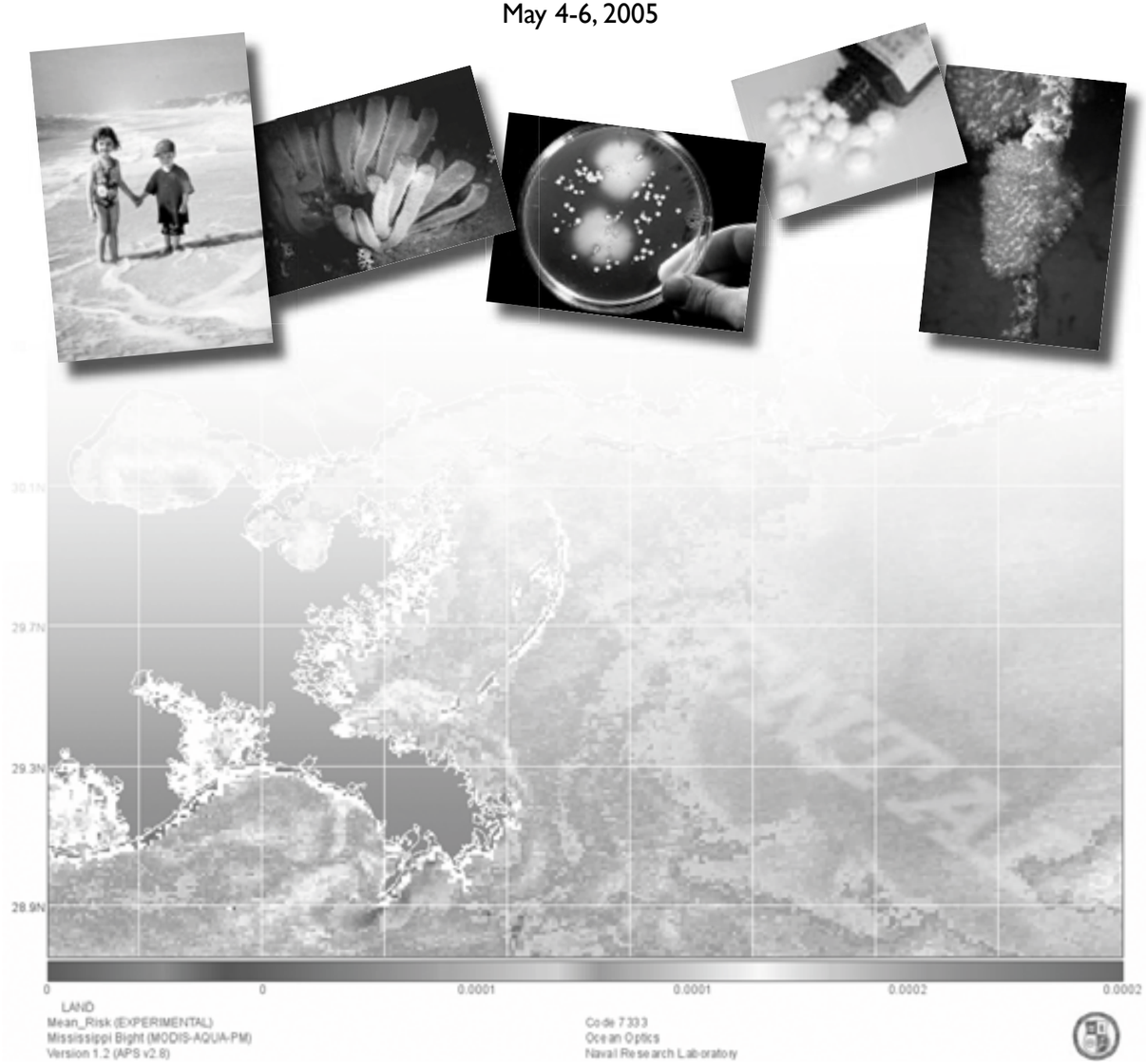

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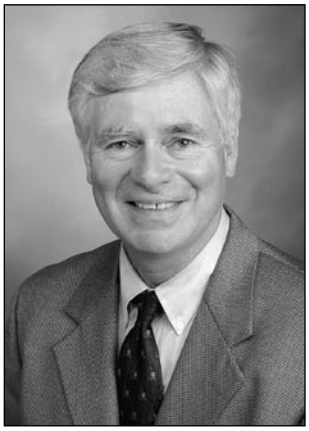
### D. Jay Grimes, Ph.D.
***The University of Southern Mississippi***



Dr. D. Jay Grimes is provost and vice president for academic affairs, professor of coastal sciences, and director of the Gulf Coast Research Laboratory at the University of Southern Mississippi. Grimes has served on the faculties of the University of Wisconsin-La Crosse (1971 to 1980), University of Maryland (1980 to 1987), and University of New Hampshire (1987 to 1990); he was also director of the New Hampshire Sea Grant College Program. In 1990, Grimes was selected for federal service as a microbiologist and program manager at the U.S. Department of Energy. In 1997, Grimes was appointed director of Southern Miss' Institute of Marine Sciences and of the Gulf Coast Research Laboratory; in 2001 he was instrumental in creating the College of Marine Sciences and became its first dean. Grimes is a fellow in the American Academy of Microbiology and in the American Association for the Advancement of Science, and he currently chairs the American Society for Microbiology's Communications Committee. He is past-president of the U.S. Federation of Culture Collections, served as vice chair of the Consortium for Oceanographic Research and Education, chair of the NASULGC Board on Oceans and Atmosphere, and served on the Science Advisory Panel to the U.S. Commission on Ocean Policy. Much of his research has focused on the ecology of waterborne human diseases, and he has published 46 papers, 27 book chapters, and three books. Most recently, Grimes has been investigating the applicability of remotely sensed data to predict human health risks from waterborne pathogens, especially *Vibrio* species. Grimes received his B.A. and M.A. in Biology from Drake University (1966 and 1968) and his Ph.D. in Microbiology from Colorado State University (1971).

### Amy E. Wright, Ph.D.
***Harbor Branch Oceanographic Institution***

Dr. Amy E. Wright received her Ph.D. in organic chemistry from the University of California at Riverside. She is currently director of the Division of Biomedical Marine Research at Harbor Branch Oceanographic Institution and the head of the Natural Products Chemistry Group. She is a member of the graduate faculty at the Marine Biomedicines and Environmental Sciences Center of the Medical University of South Carolina and the Department of Chemistry and Biochemistry at Florida Atlantic University. Her current research focuses on the discovery and biological investigation of novel marine derived compounds which may have utility in the treatment of cancer, infectious and neurodegenerative diseases from marine algae, invertebrates and microbes. A primary emphasis of the program is the discovery of compounds which act via disease-specific mechanisms. The majority of the organisms studied in the program come from deep-water habitats which the group collects using Harbor Branch's Johnson-Sea-Link human-occupied submersibles. Dr. Wright has more than 55 publications in the scientific literature and is an inventor on 21 U.S. patents.

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# *Connecting the Gulf of Mexico and Human Health*

## A Workshop Sponsored by the University of Southern Mississippi and the Mississippi-Alabama Sea Grant Consortium

### Long Beach, Mississippi

### May 4-6, 2005

## EXECUTIVE SUMMARY

The University of Southern Mississippi and the Mississippi-Alabama Sea Grant Consortium hosted a workshop entitled Connecting the Gulf of Mexico and Human Health at the university's Gulf Park campus in Long Beach, Miss., on May 4, 5 and 6, 2005. The purpose of the workshop was to follow up on human health related findings and recommendations of the U.S. Commission on Ocean Policy (USCOP). The meeting began with an overview by USCOP Commissioner Frank Muller-Karger. Dr. Muller-Karger discussed the findings of the Watkins Commission, with an emphasis on Chapter 23 – Connecting the Oceans and Human Health. Dr. Juli Trtanj next presented an overview of NOAA's new Oceans and Human Health Initiative (OHHI), including the OHHI Centers of Excellence, the individual investigator awards, and the Distinguished Scholars Program. The remainder of the meeting focused on the two main aspects of oceans and human health – natural products and diseases. Ten brief topical talks were presented on these two aspects, followed by detailed discussions in two breakout sessions. Dr. Amy Wright, Harbor Branch Oceanographic Institution, chaired the presentations and discussions on natural products, and Dr. Jay Grimes, the University of Southern Mississippi, chaired the presentations and discussions on diseases.

*The natural products breakout group discussed four topics:*

- unique opportunities for bioproduct discovery in the Gulf of Mexico (GOM);
- collaborations with Mexican scientists;
- creation of a GOM communications network; and
- a regional response to the President's Ocean Action Plan and the Congressional OHH Act.

Recommendations made by this group focused on these four topics.

*The diseases breakout group discussed three main areas:*

- educational needs and opportunities;
- better methods for the detection of pathogens and integration of such monitoring with ocean observing systems; and
- the concept of a GOM ecosystem in which indigenous pathogens play an integral role.

Eight recommendations were made by this group, all focusing on these three topics. Both breakout groups concluded that both scientific meetings and public information forums need to occur on a regular basis in order to better inform all stakeholders about issues relating to the GOM and human health.

## FRAMEWORK FOR THE WORKSHOP

The University of Southern Mississippi and the Mississippi-Alabama Sea Grant Consortium hosted a workshop entitled Connecting the Gulf of Mexico and Human Health at the university's Gulf Park campus in Long Beach, Miss., on May 4, 5 and 6, 2005. There were 52 registered participants at the conference (see list at the end of this report), as well as some "drop ins" who did not register. The purpose of the workshop was to follow up on human health related findings and recommendations of the U.S. Commission on Ocean Policy (USCOP).

The USCOP report, An Ocean Blueprint for the 21st Century, addressed the oceans and human health in Chapter 23 (see www.oceancommission.gov). Recommendations made in this chapter were focused on:

1. expansion of research and development efforts

related to basic marine biology and marine bioproducts;

2. federal support for microbiology and virology research;
3. federal support for toxin and pathogen monitoring and detection methods development;
4. establishment of a multi-agency oceans and human health initiative; and
5. federal and state agency action to better protect human health from contaminated seafood and coastal waters.

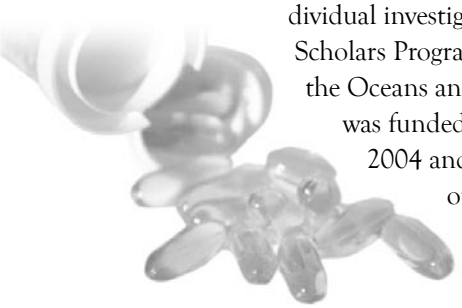
The chapter emphasized the potential of the oceans and its biodiversity to provide a vast array of chemical compounds that can be developed into useful biomedical and industrial products. It also emphasized the need to better understand oceanic sources of human illness, so that seafood contamination, harmful algal blooms and infectious diseases can be minimized.

The Connecting the Gulf of Mexico and Human Health Workshop was a forum to address ways in which scientists and managers from Alabama, Florida, Louisiana, Mississippi and Texas can be responsive to these Chapter 23 recommendations as they relate to the Gulf of Mexico. **Proceedings of this workshop, as well as most of the individual presentations (see below) can be found on the Mississippi-Alabama Sea Grant Consortium home page (www.masgc.org).**

## INTRODUCTION

The meeting began with an overview by Commissioner Frank Muller-Karger. Dr. Muller-Karger discussed the findings of the Watkins Commission, with an emphasis on Chapter 23 – Connecting the Oceans and Human Health. He also discussed the President's U.S. Ocean Action Plan (see http://ocean.ceq.gov) and concluded with a brief overview of the Gulf of Mexico Alliance.

Dr. Juli Trtanj next presented an overview of NOAA's new Oceans and Human Health Initiative, including the OHHI Centers of Excellence, the individual investigator awards, and the Distinguished Scholars Program. The OHHI was authorized by the Oceans and Human Health Act of 2005 and it was funded by Congressional direction in FYs 2004 and 2005. The OHHI is still not part of the president's budget and it was funded in the FY 2006 NOAA budget at $5 million (a $13 million reduction from FY 2005).



The remainder of the meeting focused on the two main aspects of oceans and human health – natural products and diseases. Brief topical talks were presented on these two aspects, followed by detailed discussions in two breakout sessions. Dr. Amy Wright, Harbor Branch Oceanographic Institution, chaired the presentations and discussions on natural products, and Dr. Jay Grimes, the University of Southern Mississippi, chaired the presentations and discussions on diseases. The topical talks and breakout session results are discussed in the following paragraphs.

## NATURAL PRODUCTS PAPERS

**Dr. Amy Wright**, Harbor Branch Oceanographic Institution, presented an overview entitled "Opportunities for Optimizing Natural Products Drug Discovery from the U.S. Gulf of Mexico." The talk began by briefly reviewing the recommendations of the USCOP report which outlined a need for expanding the research and development efforts related to basic marine biology and bioproducts. A brief discussion of what natural products are and why they represent a resource for the discovery of new medicines and bioprobes was presented. Successes to date, including the anti-inflammatory agents Prialt™ and the pseudopterosins, were discussed along with examples of compounds which are currently in clinical evaluation for the treatment of cancer. The talk then presented an overview of the process of marine drug discovery including: collection of materials, biological testing of materials, identification of the active chemical agents, pharmacology on the compounds, development of sustainable production methods for the compounds and the need for adequate patent protection to ensure development by the pharmaceutical or biotechnology industry. The talk proceeded to indicate that there is a strong correlation between habitat diversity, biological diversity and natural products diversity. The habitat and biological diversity of deep-water sites in the Gulf of Mexico were illustrated by detailing an expedition funded by the NOAA Ocean Exploration program to the southwestern and northeastern Gulf of Mexico. The expedition was conducted in September of 2003 aboard the R/V Ronald H. Brown using the Sonsub Innovator remotely operated vehicle for collections and documentation. Detailed topographic maps created using high resolution multibeam sea floor mapping technology were shown that illustrate the diversity of habitats within this area of the GOM. Photographs documenting

the diversity of benthic organisms at the sites were also shown. Final conclusions suggested that the GOM has a wealth of unique habitats, a wealth of unique organisms and, as exploration continues, a wealth of novel natural products with therapeutic potential that will be discovered.

**Dr. Carl Luer**, Mote Marine Laboratory, gave a short paper entitled "Identifying Potential Bioactive Agents from Elsamobranch Fishes." Marine fish represent a virtually untapped resource for drug discovery. This paper described ongoing research using elasmobranch fishes (sharks, skates and rays) as sources of compounds with therapeutic potential against human infectious diseases and cancer. One source of bioactive material is the culture media conditioned by short-term culture of shark immune cells. The resulting "conditioned medium" has been demonstrated to possess potent anti-proliferative activity against a variety of human tumor cell lines. Flow cytometric data indicate that inhibition of tumor cell growth occurs by inhibiting DNA synthesis and inducing apoptosis (programmed cell death). Induction of apoptosis appears to utilize a mitochondrial/caspase dependent pathway and may involve upregulation of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) receptors. Another source of bioactive material is stingray epidermal mucus and its associated microflora. Preliminary culture of mucus-associated bacteria resulted in at least 150 isolates, from which 16 potentially unique bacterial symbionts were subcultured. When exposed to a set of tester strain bacteria, these symbionts demonstrated detectable growth inhibition against methicillin-sensitive *Staphylococcus aureus* and *Enterococcus faecalis*. Studies are in progress to identify potential antibiotic producers as well as unique compounds from the mucus itself.

**Dr. Peter McCarthy**, Harbor Branch Oceanographic Institution, gave a short paper entitled "Marine Microorganisms in Drug Discovery." Marine invertebrates such as sponges and soft corals are a well-established resource for the discovery of new pharmaceuticals. In contrast, marine microorganisms, such as bacteria and fungi, are an underexplored resource. Within the marine environment there are many unique niches for microbial populations. One such niche is found within the tissues of marine invertebrates where we are only now beginning to understand the microbial community. Marine sponges harbor a large and diverse microbial population which may account for as much as 50% of the biomass. The majority of this population cannot be grown under laboratory conditions; however, the culturable population is highly diverse and has been shown to produce many bioactive natural products. The HBOI culture collection contains 17,000 diverse heterotrophic eubacteria and fungi isolated from many geographic locations. Approximately 76% of the collection has been derived from the tissues of marine invertebrates. Recent funding through the NSF Biotic Surveys and Inventories program has allowed a survey of 15% of the collection using molecular genetics methods: This has shown that our isolation techniques have resulted in the establishment of a truly diverse collection which contains both unique and previously uncultured microbes. Portions of these data are now available on a public Internet database, called the HBMMD - http://www.hboi.edu/dbmr/dbmr_hbmmd.html.

Small-scale fermentation of microbes is used to produce material that can then be tested for biological activity. Media are based on both "traditional" components such as starch, yeast extract and NZ amine as well as "marine" components such as dried kelp, fish extract and chitosan. Important questions remain: Can we determine which microbes are present in the unculturable sponge community? Can we develop methods to increase culturability? Can we discover pharmaceutically relevant natural products from these microbes? The answer to the first two questions is yes; molecular techniques have revealed details of the sponge-associated microbial community, and these techniques are now allowing us to explore variability between sponge genera. We can use this information in predicting ways in which some of these microbes may be cultured. We know that sponge-associated microbes produce novel compounds with significant biological activity. As such they are of interest for commercial development; further research is needed before any of these become marketable agents.

**Dr. Paul Sammarco**, Louisiana Universities Marine Consortium, gave a short paper entitled "The Search for, and Potential Mariculture of, Pharmaceutically Valuable Organisms on Offshore Platforms: Where Industry Meets Nature." Here, examples of chemical interactions (allelopathy) between marine species on the Great Barrier Reef were discussed. These included observations of abnormal skeletal and branching development in a scleractinian coral, *Pavona cactus*, or suppressed growth, which were found to occur in the vicinity of an octocoral – an alcyonacean soft coral – *Sinularia*

*flexibilis*. This led to the discovery of flexibilide – a highly toxic allelopathic agent with powerful cytotoxic capabilities. This compound was demonstrated to be effective at disabling or destroying the coral's zooxanthellae, nematocysts, success of fertilization, and embryonic development of larvae. An example was also given for the northern Gulf of Mexico, where the Flower Garden Banks are the only set of true coral reefs. It was shown how, since the 1940s, industry had introduced thousands of oil and gas platforms, providing the only new hard substratum in shallow water for coral community development since the late Pleistocene. It was shown that these platforms, particularly those at the edge of the continental shelf, are now allowing substantial reef communities to develop – communities which house certain invasive species and other species associated with pioneer communities that exhibit extraordinary competitive abilities, most likely chemically based. Preliminary laboratory tests on these organisms have revealed them to contain compounds with substantial antibiotic activity. These offshore platforms could serve as sites for scanning for natural products in new marine organisms. They can also be used to readily culture marine organisms known to produce valuable novel compounds which may be too difficult or expensive to synthesize in the laboratory.

**Dr. Mark Hamann**, the University of Mississippi, gave a short paper entitled "The Rational Design of Pharmaceutical Products from Marine Invertebrates and their Associated Bacteria: The Alkaloids as a Model Marine Drug Lead." In this presentation it was shown that important invertebrate-derived drug leads could be successfully produced from associated bacteria. This is highly important in regard to the commercialization and practical application of marine natural products to human health since the pharmaceutical and biotechnology industries are not equipped to develop and commercialize products directly from an invertebrate source. A microbial expression system allows for development and scale-up using existing infrastructure available to the pharmaceutical industry. Furthermore, the development of products from microbial expression systems allows for significant yield enhancements and, as a result, highly cost-effective production. This is of particular significance in the case of the manzamine alkaloids where the potential therapeutic applications fall in the area of malaria which affects millions living in the poorest regions of the world. The successful development of microbial expression systems from invertebrate hosts is a direct product of the application of hyphenated techniques involving molecular and microbiology with chemistry and spectroscopy. These techniques will play a critical role in the development of natural products from the Gulf of Mexico, which harbors a particularly high diversity of microbial communities living in associations with methane vents, methane hydrates, hypersaline and brackish environments.

## NATURAL PRODUCTS FOCUS SESSION

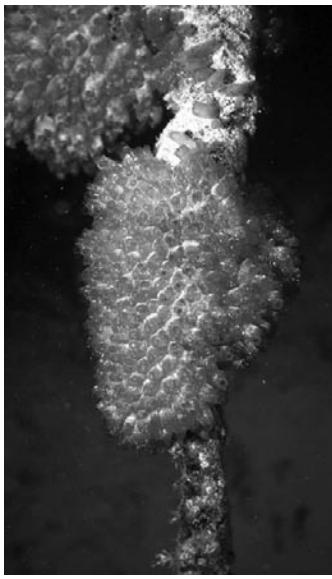
During the natural products breakout discussions, a number of topics were discussed including unique opportunities for marine natural products drug discovery in the GOM, the need for effective communication networks for scientists working in this field and the interest in a Center for Oceans and Human Health focusing on Gulf Coast opportunities and needs.

The discussants were challenged to define whether the Gulf of Mexico area has habitats that provide unique opportunities for bioproduct discovery. Further to this, the discussion focused on whether these resources would justify the creation of a Center for Oceans and Human Health focused on the Gulf Coast states. The discussants rapidly identified a number of opportunities for marine bioproduct discovery which are unique to the Gulf of Mexico area. The first opportunity identified was the diversity of habitats and organisms within the GOM. The GOM was identified as a highly productive area as witnessed by the second highest number of fish landings in the United States coastal fisheries. The GOM also has the widest continental shelf in the U.S. EEZ. The diversity of habitats include habitats such as the submerged surfaces of oil platforms, vent/seep communities; methane hydrates, deep-water pinnacle sites, deep-water hard bottom communities and deep water coral reefs throughout the GOM area, the Flower Garden Banks, unique deep-water fish that harbor unusual bacteria, the "Dead Zone", the Tortugas and Florida Keys, limestone outcrops off Sarasota, the zone where the Mississippi enters the GOM, and tropical habitats in Mexico. A second opportunity is the demographics of the Gulf Coast states which include a population which is relocating in high numbers to the coastal area as well as an increase in the average age of the population. Development of new products with efficacy against diseases associated with aging could be beneficial to states with these demographics.

The discussion next focused on whether closer collaborations with Mexican scientists would be beneficial as part of a program to discover new bioproducts. The consensus was that such relationships could be beneficial to both the United States and Mexico. A number of participants expressed interest in such collaborations, but have not proceeded to establish collaborations due to intellectual property issues. Unfortunately, the greatest impediment to such collaborations is the perception that it may be extremely time consuming and difficult for individual scientific groups to negotiate appropriate collaborative agreements for equitable sharing of proceeds from "bioprospecting." Collaborations between U.S. and Mexican scientists could be facilitated by the negotiation of a universal intellectual property (IP) sharing agreement between the Gulf Coast states and the Mexican government. Negotiation of such an agreement could be led by a U.S. agency such as NOAA or the NIH. The NIH has taken a leadership role in similar negotiations with Australia. The EPA has the Gulf of Mexico Accord, a program to coordinate work with Mexico, and perhaps this could be used as a model for a bilateral agreement between the U.S. GOM states and Mexico.

A third topic of discussion regarded the creation of a GOM communications network. The consensus was that such a network could be useful. It was suggested that there are a number of agencies and existing networks such as the Harte Institute (Gulfbase), COSEE, Sea Grant, NOAA NIEHS/NSF Centers, GCOOS, SECOORA, and NOAA OE which could, if coordinated, provide a unified platform for communication on GOM issues. Other suggestions included creating a network of researchers participating in a "GOM Bioproducts Roundtable" teleconference, similar to the "Mercury Roundtable" which brings together scientists interested in mercury pollution for an open discussion by teleconference on a monthly basis. Such a monthly conference call would require a coordinating party to organize it, and it was suggested that one of the organizations listed above would be best suited to organizing the calls.

A fourth topic of discussion regarded whether there should be a regional response to the President's OAP and the Congressional OHH Act. The consensus was that there should be a response. The response should include plans for the creation of a GOM regional OHH Center. There is a critical mass of scientists working on the discovery and development of commercially useful bioproducts in the Gulf Coast states. In addition to scientists working on the discovery of marine-described medicines, the discussants identified a number of researchers working in ancillary fields who could streamline the development of the materials. One example is a group which routinely conducts fish models of toxicology which could reduce the need for expensive mammalian animal models. The creation of a GOM OHH Center could bring these groups together, at least on a virtual basis, and provide regional core facilities for natural products drug discovery (possible cores: super critical fluid extraction facility, biological assay screening facility, a regional-use research vessel with submersible support for deep-water access, a metabionomics facility, a toxicology facility). The center could also encompass the discovery of other uses for marine-derived compounds such as the control of marine parasites and infectious diseases in marine fish and marine mammals.



*A series of six action items which would facilitate marine byproduct discovery and commercialization in the Gulf Coast states is summarized below:*

- Develop a strategic plan for creation of an Oceans and Human Health Center focused on the Gulf of Mexico region. This strategic plan would provide input to the National Science and Technology Council (NSTC) Interagency Oceans and Human Health Program Planning Process. Such a plan would assist the NOAA OHH and OE programs to coordinate their goals with respect to bioproducts discovery from the GOM region.
- Send a message to the governors of the Gulf Coast states, or possibly members of the Gulf of Mexico Alliance, prior to "The State of the Gulf of Mexico Summit" scheduled for March 28-30, 2006, indicating that marine natural products derived from Gulf of Mexico organisms represent a significant economic value to these states. The recommendation should be made to add the discovery of marine-derived bioproducts as a priority area for research and that these entities should support and nurture cooperation and collaboration between the various groups working in the field as well as assist in building the research and outreach capabilities in this field.
- Create a distributed network for coordination of information exchange and access to resources for marine-derived drug discovery

research efforts. (Note two organizations, the Gulf Coast Alliance in Florida and the Harte Institute in Texas, both support Internet-based information exchange networks). Other groups which could participate are the IOOS, CO-SEE, Sea Grant, NOAA, NIEHS-NSF OHH Centers; GCOOS, SECOORA, NOOA OE).

- Support retaining established oil platforms in the GOM as a resource for collection and aquaculture of biomedically important species.
- Support the creation of shared infrastructure for marine drug discovery in the GOM area. This could include creation of a Gulf Coast Regional Laboratory Core Facility for chemistry and biological testing of marine bioproducts as well as providing access to research vessels and submersibles for collection of organisms. Such a core facility could be part of a GOM Center for Oceans and Human Health.
- Support organizing a Summit for Oceans and Human Health in the Gulf of Mexico to be held for legislators and agency representatives of the Gulf Coast states.

## DISEASES PAPERS

**Dr. Jay Grimes**, the University of Southern Mississippi, presented an overview entitled "The Gulf of Mexico and Diseases." The five recommendations made in Chapter 23 of the Watkins Commission report were briefly discussed, in the context of waterborne and seafood-borne infectious diseases of concern from the Gulf of Mexico. Some disease agents, such as the enteric viruses, are introduced into the Gulf, whereas other agents of disease, such as the *Vibrio* species and toxic algae, are indigenous. An ongoing Southern Miss study, funded by the NOAA OHHI and basis for a Master of Science thesis project by Andrea Phillips, was discussed in detail. During Phase 1, archived data were used to compare observed *Vibrio parahaemolyticus* densities in oysters collected from Dauphin Island Bay and Cedar Point, Ala., with spatial and temporal environmental data, i.e., SST, ocean color (chlorophyll a), and turbidity, collected via remote sensing. Phase 2 involved water and oyster meat analyses for total and pathogenic *V. parahaemolyticus* enumeration from two stations, Dauphin Island, Ala., and Ocean Springs, Miss., using the colony lift technique and gene probe analysis. Real-time polymerase chain reaction (qPCR) analyses from enriched samples for presence/absence were also conducted in an MPN format for quantification. Water temperature, salinity, turbidity and chlorophyll concentration were also measured in situ. These data were compared with real-time remotely sensed ocean parameter data to determine the reliability of remotely sensed data. Correlations between *V. parahaemolyticus* density and environmental factors were also examined to determine if their incorporation would strengthen the U.S. Food and Drug Administration *V. parahaemolyticus* model for risk assessment. In Phase 1, it was determined that total *V. parahaemolyticus* was associated with remotely sensed temperature and salinity ($P<0.05$). In Phase 2, it was determined that both total and pathogenic *V. parahaemolyticus* residuals showed a highly significant association with turbidity ($P<0.01$) and that pathogenic *V. parahaemolyticus* residuals significantly associated with both turbidity and chlorophyll ($P<0.05$) at the Mississippi but not the Alabama stations.

**Dr. R.D. Ellender**, the University of Southern Mississippi, gave a short paper entitled "Can Fecal Pollution of the Coastal Zone be Prevented: Possible Remedies and Things We Cannot Change." The use of traditional bacterial source tracking methods has demonstrated the value of determining the source of aquatic fecal contamination. However, methods that have been developed during the last decade are time intensive and expensive. As a result we have evaluated the process of source tracking and developed a six-point plan, which introduces a new, qualitative method for determining the animal source of pollution. Together with other elements of the plan, the method attempts to delineate and detail the source and the level of pollution and expose modifications to remedy the problem. Furthermore, the plan recognizes problems that cannot be remedied and which must be accepted as fact. In addition, we have focused on rapid pathogen detection as part of the overall toolbox of methods. This research has direct application to the above-mentioned plan and is currently in development.

**Dr. Robin Overstreet**, the University of Southern Mississippi, gave a short paper entitled "Parasites Connecting the Gulf of Mexico with Human Health." Dr. Overstreet indicated that marine parasites from the Gulf of Mexico have historically been considered as incapable of infecting people. Recent investigations, however, demonstrate that

numerous species of marine digeneans (flukes), nematodes (roundworms), cestodes (tapeworms), coccidians, and other metazoan and protozoan parasites do or probably could utilize humans as final or intermediate hosts. More people in the Gulf of Mexico are getting infected, sometimes with previously unrecognized marine pathogens, because of 1) global spread and increased popularity of cuisines that include raw or undercooked seafood products, 2) an increase and translocation of people, 3) emphasis on different fishing grounds and different edible species, 4) regulations protecting specific marine animals, 5) climatic dynamics, and 6) reduced resistance in some people. In addition to acquiring parasites from eating infected products, people get infected with or affected by some agents from touching or occurring in the water with them. Depending on the parasitic species in question, each of the above circumstances generates a different risk. Reasons for this apparent increase in human infections also involve better diagnostic tests, more accurate identifications, more critical examinations, and improved media coverage.

**Dr. Erik Carlson**, the University of Southern Mississippi, gave a short paper entitled "Minnows as Models for Estuarine Health." The utility of small fish species as environmental sentinels, screening tools, and mechanistic models for xenobiotic, marine biotoxin, and pathogen exposure was discussed. The talk highlighted ongoing work at the University of Southern Mississippi Gulf Coast Research Laboratory (GCRL) on three model fish species which hold promise in both toxicological and biomedical research: Japanese medaka (*Oryzias latipes*), sheepshead minnows (*Cyprinodon variegatus*), and zebra fish (*Danio rerio*). One current goal at GCRL is the development of an extensive toxicological database for multiple endpoints at all levels of biological organization (i.e., population, individual, molecular). The establishment of the sheepshead minnow model, employing whole animal, cellular and molecular biomarkers of reproductive and immune status, was discussed in detail. These biomarkers could prove useful in assessing potential risks to both aquatic ecosystems and human health. Since sheepshead minnows are found in estuaries from Massachusetts to Central America, this species appears poised to serve as an excellent environmental sentinel.

**Dr. Asim Bej**, the University of Alabama at Birmingham, gave a short paper entitled "Detection of Vibrios in Shellfish Using Real-Time PCR and Beyond." A successful comprehensive ocean ecosystem management requires monitoring of ocean health as well as pathogens that affect human health. Our objective has been to develop molecular-based methodologies for the detection and monitoring of human pathogens, primarily *Vibrio* spp., indigenous to the Gulf of Mexico water and shellfish. To achieve this goal, we used a multiplexed real-time PCR targeting total and pathogenic strains of *V. vulnificus* and *V. parahaemolyticus* using SYBR Green I or dual-labeled Taqman probes. We have established a rapid and reliable detection of total and pathogenic *V. vulnificus* by selecting oligonucleotide primers and probes targeting vvhA (species-specific), and viuB (pathogenic-strain-specific) genes. For the detection of *V. parahaemolyticus*, we have developed a 4-plex Taqman probes-based real-time PCR targeting tlh (species-specific), tdh and trh (hemolysin producing pathogenic strains) and ORF8 (pathogenic pandemic strains O3:K6 serotype) genes. Both SYBR Green I and Taqman probe-based PCR exhibited high specificity to the targeted pathogens with the necessary sensitivity of an initial inoculum of 1 CFU in 5-h enriched 1 g of natural or seeded oyster tissue homogenate or 10 ml of Gulf of Mexico water. The standard curves from the real-time PCR cycle threshold values exhibited a strong correlation of the CFU in unenriched and enriched samples. The assay including the sample processing, enrichment and real-time PCR detection can be completed in 8 h making it a rapid single-day assay. In order to achieve detection of *V. vulnificus*, *V. parahaemolyticus* and *V. cholerae* in shellfish targeting species-specific and pathogenic-strain-specific genes, we have developed a single-color diagnostic oligonucleotide probe-based DNA microarray. Multiplexed PCR targeting the aforementioned genes for total and pathogenic strains of *V. vulnificus* and *V. parahaemolyticus*; and ompU, toxR, tcpI, and hlyA for *V. cholerae* were hybridized with the oligonucleotide probes, which were immobilized on an epoxysilane-derivatized, 12-well, Teflon-masked slides by using a MicroGrid II arrayer. Detection of the hybridized DNA on the microarray slides was achieved by using tyramide signal amplification with Alexa Fluor 546 fluorescent dye and imaged by using an arrayWoRx scanner. The detection specificity was 100% and the sensitivity on post-enriched seeded or unseeded natural oyster tissue homogenate was 1 CFU/g. Currently, we are developing a phage-displayed random peptide ligand-based detection of an array of *Vibrio* spp.

that inhabit the warm coastal waters of the continental United States and have been documented to cause human diseases. The selected peptide ligands that are specific for the pathogenic *Vibrio* spp. can potentially be used for "intact cell"-based detection avoiding purification followed by purification of cellular macromolecules such as DNA or proteins. The detection of pathogenic vibrios can be conducted in the field and completed in minutes. Rapid detection and monitoring of indigenous microbial pathogens, particularly vibrios, which are part of the ocean ecosystem, is necessary to protect human health, seafood industry, tourism and a prosperous economy for the U.S. coastal states.

## DISEASES FOCUS SESSION

Much of the diseases focus session was spent discussing educational needs and opportunities. Ideas discussed included using the GOM National Estuarine Research Reserves (NERRs) to inform the public about human health related topics, engaging the National Marine Educators Association, using the well-established networks of the four GOM Sea Grant programs, interacting with the ISSC, and promoting Sea Grant's John Knauss Fellowship Program. Other ideas discussed included establishing traveling museums/aquaria to better inform K-6 students about issues, better informing the GOM delegations about human health problems associated with the Gulf, introducing children in the heartland of the U.S. to coastal ocean problems and how rivers and drainage areas impact the coast, and how to target nonprofits for the funding of ocean-related education in schools and colleges.

Discussions next focused on methods for pathogen monitoring. Topics discussed included antiquated shellfish monitoring procedures, prediction of harmful algal blooms, the use of enterococci and *Escherichia coli* instead of fecal coliforms, use of polymerase chain reaction and microarrays for rapid analyses, and source tracking. It was agreed that not all tests can be applied to all sites – there are regional differences and there are different stakeholders (e.g., turbid waters in the Mississippi Sound versus clear waters along the Florida panhandle and molluscan shellfish bed versus recreational beach concerns). All agreed that we need to determine the contributions of marine mammals, migratory waterfowl, and ballast water to "pollution" for the GOM. It was generally agreed that these needs can be at least partially addressed through an integrated ocean observing system.

The concept that the GOM ecosystem is unique and that potential human pathogens are an integral part of this ecosystem was discussed in detail. These microorganisms play an important role in human health and the coastal economy, by virtue of causing a variety of waterborne and seafood-borne diseases and a variety of toxins associated with algae blooms. It is assumed, but not known, that these indigenous microbes also play an important role in the coastal ocean ecosystem, for example in decomposition and nutrient cycling. Therefore, a project that will allow a comprehensive study of occurrence and distribution of microorganisms in real time along the GOM, especially pathogens and toxic algae, will help predict possible disease incidence and algal blooms ahead of time. Also, such a project will help study microbial distribution in the GOM and microbial association with other micro- and macroflora and fauna.

*In summary of the focus session discussions, eight recommendations were developed:*

- Develop better wastewater treatment and disposal methods for coastal areas
- Establish no-discharge zones in and around molluscan shellfish growing areas
- Determine sources, fate and risk associated with methyl mercury in Gulf of Mexico fishes
- Increase baseline data on bacteria, viruses, protists and helminths that have a potential public health risk
- Support at every level education that is relevant to the Gulf of Mexico and the oceans
  - *Support smart growth for promotion of economic development and quality of life*
  - *Develop focused materials for educational targets, e.g., curricula for K-12 students*
  - *Hold regular symposia to exchange ideas and new information*
  - *Develop a marine education network focused on the* GOM
  - *Inform boards, legislatures and delegations about issue*
- Establish links between the developing Gulf of Mexico Coastal Ocean Observing System (GCOOS) and human health needs and opportunities
- Establish a Gulf of Mexico great waters-ecosystem approach to issues and opportunities with targeted objectives, research directions and services
- Develop better water quality monitoring technologies and applications (this could be part of GCOOS)

## POSTERS PRESENTED

*The National Institute for Undersea Science and Technology (NIUST)*
**Dr. Raymond Highsmith**
The University of Mississippi

*Plant/microbial filter septic system for fresh and salt water*
**Dr. Robert Woolsey**
Mississippi Mineral Resources Institute

*Investigating Vibrio parahaemolyticus in Gulf Coast oysters (Crassostrea virginica) and overlying waters*
**Andrea M.B. Phillips**
The University of Southern Mississippi

*A novel quantitative internal control system that is universally adaptable to any standard or real-time PCR assay*
**Dr. Angelo DePaola**
FDA, Gulf Coast Seafood Laboratory

*A real-time PCR method to detect toxigenic Vibrio cholerae*
**George Blackstone**
FDA, Gulf Coast Seafood Laboratory

*Fluorogenic PCR detection of viruses in waters*
**Dr. Y.C. Shieh**
FDA, Gulf Coast Seafood Laboratory

*Development of a quantitative multiplex RT-PCR assay for the detection of noro- and enteroviruses*
**Dr. William Burkhardt**
FDA, Gulf Coast Seafood Laboratory

*Comparison of 5 viral extraction protocols by qRT-PCR for enumeration of calicivirus in shellfish*
**Dr. William Burkhardt**
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*Mitigation of HAV in shucked oysters using high hydrostatic pressure treatment*
**Kevin Calci**
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## ABBREVIATIONS AND ACRONYMS

**CFU –** colony forming unit

**COSEE –** Center for Ocean Sciences Education Excellence

**DNA –** deoxyribonucleic acid

**EEZ –** exclusive economic zone

**EPA –** Environmental Protection Agency

**FDA –** Food and Drug Administration

**GCOOS –** Gulf of Mexico Coastal Ocean Observing System

**GCRL –** Gulf Coast Research Laboratory

**GOM –** Gulf of Mexico

**HBMMD –** Harbor Branch Marine Microbe Database

**HBOI –** Harbor Branch Oceanographic Institution

**IP –** intellectual property

**MPN –** most or mean probable number

**NERR –** National Estuarine Research Reserve

**NIEHS –** National Institute of Environmental Health Sciences

**NIH –** National Institutes of Health

**NIUST –** National Institute for Undersea Science and Technology

**NOAA –** National Oceanic and Atmospheric Administration

**NSF –** National Science Foundation

**NSTC –** National Science and Technology Council

**OAP –** Ocean Action Plan

**OE –** Ocean Exploration

**OHH –** Oceans and Human Health

**OHHI –** Oceans and Human Health Initiative

**ORF –** open reading frame

**PCR –** polymerase chain reaction

**qPCR –** quantitative polymerase chain reaction

**qRT-PCR –** quantitative reverse transcriptase-polymerase chain reaction

**R/V –** Research Vessel

**SECOORA –** Southeast Coastal Ocean Observations Regional Association

**SST –** sea surface temperature

**SYBR Green –** a DNA stain

**tdh –** thermostable direct hemolysin

**tlh –** thermolabile hemolysin

**TRAIL –** tumor necrosis factor-related apoptosis-inducing ligand

**trh –** thermostable related hemolysin

**USCOP –** U.S. Commission on Ocean Policy