Annual Report for Period: 05/2006 - 04/2007
Submitted on: 01/30/2007
Principal Investigator: Stegeman, John J.
Award ID: 0430724
Organization: Woods Hole Ocean Inst
Title:
The Woods Hole Center for Oceans and Human Health

Project Participants

Senior Personnel

Name: Stegeman, John
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Stegeman is responsible for the oversight of all of the activities of the Center, and for facilitating communication within and among the Centers.

Name: McGillicuddy, Dennis
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. McGillicuddy's responsibilities include overall project oversight, design of population dynamics model for the various A. fundyense genotypes, execution of the physical oceanographic component of large-scale survey operations during years 2 through 4, design of the coupled physical-biological simulations and interpretation and publication of the results.

Name: Hahn, Mark
Worked for more than 160 Hours: No
Contribution to Project:
Senior Scientist and Deputy Director of the Pilot Project Program

Name: Anderson, Donald
Worked for more than 160 Hours: Yes
Contribution to Project:
Senior Scientist and PI, is responsible for project oversight, and management.

Name: Erdner, Deana
Worked for more than 160 Hours: Yes
Contribution to Project:
Research Associate and Co-PI, is responsible for technical oversight of experimental work and sampling efforts, project management, and
data review and interpretation.

Name: Gast, Rebecca
Worked for more than 160 Hours: Yes
Contribution to Project:
CO-PI, supervised the pathogen detection,cultured amebas from sediment samples.

Name: Amaral Zettler, Linda
Worked for more than 160 Hours: Yes
Contribution to Project:
Co-PI, supervised the microbial community surveys, participated in sample collection and processing.

Name: Polz, Martin
Worked for more than 160 Hours: No
Contribution to Project:
PI coordinates the project and participates in data analysis as well as dissemination of results.

Name: Lerczak, James
Worked for more than 160 Hours: No

Contribution to Project:
Co-PI is overseeing the collection and analysis of the physical measurements within Plum Island Estuary and is responsible for providing the physical estuarine context for the interpretation of Vibrio population dynamics.

Name: Sogin, Mitchell
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Sogin directs activities in the Molecular Core including phylogenetic and associated bioinformatics activities.

Name: Morrison, Hilary
Worked for more than 160 Hours: Yes
Contribution to Project:
Co-director of the Core Facility, manages the day to day operation of the sequencing lab and data flow for the COHH projects. This is a highly automated process but it requires almost daily maintenance.

Post-doc

Name: Kirkup, Benjamin
Worked for more than 160 Hours: No
Contribution to Project:
Dr. Kirkup has recently joined the project in the Polz lab. He holds a Ph.D. from Yale in ecology and evolution of enteric bacteria. His background in mathematical modeling and genetic analysis is an ideal fit for the current project since he is able to interface with both the biological and physical modeling.

Name: Hackett, Jeremiah
Worked for more than 160 Hours: No
Contribution to Project:
Worked on Alexandrium genomics, with a focus on the identification of genes involved in saxitoxin biosynthesis. Hackett was supported by a WHOI Postdoctoral Scholar award, but supplies and other support were provided by the COHH grant.

Name: Ralston, David
Worked for more than 160 Hours: No
Contribution to Project:
Responsible for processing and analysis of the various data sets, seagoing operations, running the coupled models, and visualizing the results

Name: Carr, Jennifer
Worked for more than 160 Hours: No
Contribution to Project:
Responsible for sampling and development of genetic system for model organisms.

Name: Wildschutte, Hans
Worked for more than 160 Hours: No
Contribution to Project:
Responsible for genomics and O-antigen characterization of the vibrios.

Graduate Student

Name: Brosnahan, Michael
Worked for more than 160 Hours: No
Contribution to Project:
Is responsible for characterization and application of microsatellite markers used for population studies.

Name: Koid, Amy

Worked for more than 160 Hours: No

Contribution to Project:
REU student

Name: Osborn, Deborah

Worked for more than 160 Hours: No

Contribution to Project:
Hollings Fellow

Name: Willard, Eric

Worked for more than 160 Hours: No

Contribution to Project:
Northeastern University co-op student

Name: Blossom, Hannah

Worked for more than 160 Hours: No

Contribution to Project:
Northeastern University co-op student

Name: Tully, Benjamin

Worked for more than 160 Hours: No

Contribution to Project:
REU student, summer 2006.

Name: Hunt, Dana

Worked for more than 160 Hours: No

Contribution to Project:
Researching the environmental distribution of vibrios in the water column.

Name: Preheim, Sarah

Worked for more than 160 Hours: Yes

Contribution to Project:
Researching the environmental distribution of vibrios among animal hosts.

Name: Xue, Xong

Worked for more than 160 Hours: No

Contribution to Project:
Identification of isolates, growth dynamics of vibrios over tidal cycles.

Name: Hasegawa, Yuko

Worked for more than 160 Hours: No

Contribution to Project:
Working on the development of PCR primers for the V-tag sequencing studies.

Undergraduate Student

Name: Bobb-Semple, Aisha

Worked for more than 160 Hours: No

Contribution to Project:
Sophomore, biology. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Han, Jing

Worked for more than 160 Hours: No

Contribution to Project:
Sophomore, EECS. Participated under the auspices of the MIT UROP program; involved with strain characterization,

environmental sampling and sequence analysis.

Name: Buchwald, Carolyn

Worked for more than 160 Hours: No

Contribution to Project:

Senior, EAPS. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Ngo, Lynn Ly

Worked for more than 160 Hours: No

Contribution to Project:

Graduated 2006, CE. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Chang, Sarah

Worked for more than 160 Hours: No

Contribution to Project:

Wellesley junior, history. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Proehl, Sarah

Worked for more than 160 Hours: No

Contribution to Project:

Sophomore, chemistry. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Smith, Sarah

Worked for more than 160 Hours: No

Contribution to Project:

Sophomore, chemistry. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Name: Schieffer, Stella

Worked for more than 160 Hours: No

Contribution to Project:

Sophormore. Participated under the auspices of the MIT UROP program; involved with strain characterization, environmental sampling and sequence analysis.

Technician, Programmer

Name: Keafer, Bruce

Worked for more than 160 Hours: No

Contribution to Project:

Research Associate, is responsible for field work planning and execution, as well as with sampling and sample analysis.

Name: McCauley, Linda

Worked for more than 160 Hours: Yes

Contribution to Project:

Research Assistant, is responsible for testing and application of molecular methods for the population genetic studies,

phytoplankton culture and characterization, and data review and analysis.

Name: Kosnyrev, Valery

Worked for more than 160 Hours: Yes

Contribution to Project:

Research Associate and is assisting Dr. McGillicuddy in processing and analysis of the various data sets, seagoing operations, running the coupled models, and visualizing the results.

Name: Anderson, Laurence

Worked for more than 160 Hours: No

Contribution to Project:

Research Associate and has significant seagoing experience, and will participate in the large-scale survey cruises and will support post-cruise processing of the data.

Name: Dennett, Mark

Worked for more than 160 Hours: Yes

Contribution to Project:

Assisted with sample collection and processing.

Name: Moran, Dawn

Worked for more than 160 Hours: Yes

Contribution to Project:

Processed samples and ameba cultures, pathogen detection, cloning, and sequencing.

Name: Rocca, Jennifer

Worked for more than 160 Hours: Yes

Contribution to Project:

Works in the core facility preparing DNA templates and sequencing reactions for members of the COHH team.

Name: Huse, Susan

Worked for more than 160 Hours: Yes

Contribution to Project:

Susan has been promoted to a Research Associate and she has been developing methods to eveluate error rates associated with Pyrosequencing technology.

Name: Libera, Katherine

Worked for more than 160 Hours: No

Contribution to Project:

Responsible for testing and application of molecular methods for the population genetic studies, phytoplankton culture and characterization, and data review and analysis.

Name: del Castillo, Erika

Worked for more than 160 Hours: No

Contribution to Project:

Responsible for microbial community survey library construction and analysis.

Other Participant

Research Experience for Undergraduates

Organizational Partners

Massachusetts Institute of Technology

Marine Biological Laboratory

Other Collaborators or Contacts

University of Washington

Pacific Research Center for Marine Biomedicine at the University of Hawaii

University of Miami

Harvard Medical School

Fisheries Research Agency of Japan, National Research Institute of Fisheries and Environment of Inland Sea

Woods Hole United States Geological Survey

Drs. Uwe John and Allan Cembella, Alfred Wegener Institute, Bremerhaven Germany

Dr. Satoshi Nagai, National Research Institute of Fisheries and Environment of the Inland Sea, Japan

Plum Island Sound Estuary Long Term Ecological Research (PIE-LTER) program in collaboration with Charles Hopkinson and Hap Garritt (MBL)

Dr. Wayne Wurtsbaugh, Utah State University

Dr. Philip Roberts, Georgia Tech

Dr. Sandra McLellan at the Great Lakes WATER Institute in Milwaukee,WI

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)
See attached file.

Findings: (See PDF version submitted by PI at the end of the report)
See attached file.

Training and Development:
See attached file.

Outreach Activities:
See attached file.

Journal Publications

Erdner, D.L. and D.M. Anderson, "Global transcriptional profiling of the toxic dinoflagellate Alexandrium fundyense using Massively Parallel Signature Sequencing.", BMC Genomics, p. 1, vol. 7:88, (2006). Published

Nagai, S., L. McCauley, N. Yasuda, D.L. Erdner, D.M. Kulis, Y. Matsuyama, S. Itakura and D.M. Anderson., "Development of microsatellite markers in the toxic dinoflagellate Alexandrium minutum (Dinophyceae)", Molecular Ecology Notes, p. 756, vol. 6, (2006). Published

Anderson, D.M., Keafer, B.A., McGillicuddy, D.J., Mickelson, M.J., Keay, K.E., Libby, P.S., Manning, J.P., Mayo, C.A., Whittaker, D.K., Hickey, J.M., He, R., Lynch, D.R., Smith, K.W., "Initial observations of the 2005 Alexandrium fundyense bloom in southern New England: General patterns and mechanisms", Deep Sea Research II, p. 2856, vol. 52, (2005). Published

Martins, C.A., "Functional genomics of a non-toxic culture of Alexandrium lusitanicum.", Ph.D. Thesis, MIT/WHOI Joint Program in Oceanography, p. , vol. , (2006). Ph.D. Thesis

Ralston D.K., McGillicuddy, D.J. and D.W. Townsend, "Asynchronous vertical migration and bimodal distribution of motile phytoplankton", Journal of Plankton Research

, p. , vol. , ( ). Submitted

Acinas, S.G, Sarma-Rupavtarm, R., Klepac-Ceraj, V., Polz, M.F., "PCR induced sequence artifacts and bias: insights from two 16S rRNA clone libraries constructed from the same sample. Appl. Environ. Microbiol.", Appl. Environ. Microbiol., p. 8966, vol. 71(12), (2005). Published

Klepac-Ceraj, V., Ceraj, I., Polz, M.F., "CLUSTERER: extendable java application for sequence grouping and cluster analyses", Online J. Bioinf., p. 15, vol. 7(1, (2006). Published

Hunt, D.E., Klepac-Ceraj, V., Acinas, S.G., Gauthier, C., Bertilsson, S., Polz, M.F., "Evaluation of 23S rRNA PCR Primers for use in Phylogenetic Studies of Bacterial Diversity.", Appl. Environ. Microbiol., p. 2221, vol. 72(3), (2006). Published

Marcelino, L., Backman, V., Donaldson, A., Steadman, C., Thompson, J.R., Paccocha-Preheim, S., Lien, C., Lim, E., Veneziano, D., Polz, M.F., "Accurate identification of low abundant targets amidst similar sequences by revealing hidden correlations in oligonucleotide microarray data.", Proc. Natl. Acad. Sci. USA

, p. 13629, vol. 103(37), (2006). Published

Polz, M.F., Hunt, D.E., Preheim, S.P., Weinreich, D.M., "Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. Phil. Trans. R. Soc. Lond. B.", Phil. Trans. R. Soc. Lond. B., p. 2009, vol. 361, (2006). Published

Veneziano, D., Klepac-Ceraj, V., Polz, M.F., "Likelihood estimation of richness and species abundance distribution in microbial communities.", Journal of Theoretical Biology, p. , vol. , ( ). Submitted

Sogin, M.L., H.G. Morrison, J.A. Huber, D.Mark Welch, S.M. Huse, P.R. Neal, J.M. Arrieta, and G.J. Herndl., "Microbial diversity in the deep sea and the under-explored "rare biosphere.", Proc. Natl. Acad. Sci. USA, p. 12115, vol. 103(32), (2006). Published

Messerli, S.M. and Greenberg, R.M., "Cnidarian toxins acting on voltage-gated ion channels.
", Marine Drugs, p. 70, vol. 4, (2006). Published

Ehrenreich, I.M., J.B. Waterbury, and E.A. Webb, "The Distribution and Diversity of Natural Product Genes in Marine and Freshwater Cyanobacterial Cultures and Genomes.", Appl. Environ. Microbiol., p. 7401, vol. 71, (2005). Published

Books or Other One-time Publications

Thompson, J.T., Marcelino, L., Polz, M.F., "Diversity and sources of human bacterial pathogens and overview of methods of their detection and quantification.", (2006). Book, Published
Editor(s): Shimshon Belkin and Rita Colwell
Bibliography: Ocean and Health: Pathogens in the Marine Environment. Springer. p. 464.

Martins, C.A., "Functional genomics of a non-toxic culture of Alexandrium lusitanicum.", (2006). Thesis, Published
Collection: MIT/WHOI Joint Program in Oceanography
Bibliography: N/A

Web/Internet Site

URL(s):

http://www.whoi.edu/science/cohh/whcohh/index.htm

Description:

Additional Web Sites:
http://www.whoi.edu/sbl/liteSite.do?litesiteid=3230&articleId=13371
http://science.whoi.edu/users/ruoying/Redtide_05/movie.html
http://science.whoi.edu/users/ruoying/Redtide_06/
http://science.whoi.edu/users/mcgillic/cohh/oc412/data/
www.whoi.edu/people/rgast

Future web site:
Genomics Facility Core: We are working on a web site principally with funding from the Sloan Foundation to make available the 454 sequence tags including taxonomic breakdown. This web site (MICROBIS) will be released sometime during the first or second quarter of 2007.

Other Specific Products

Product Type:

Poster presentation

Product Description:

04/06: Initial Observations of the 2005 Alexandrium fundyense Bloom in southern New England: General Patterns and Mechanisms. Symposium on Boston Harbor and adjacent coastal waters. New England Estuarine Research Society. Nantasket, MA.

11/06: D.M. Anderson, B.A. Keafer, D.J. McGillicuddy, and R. He. The 2005 and 2006 Alexandrium red tides: A tale of two blooms. Wellfleet Harbor Conference.

Sharing Information:

Presented at meetings.

Product Type:

Software (or netware)

Product Description:

We are currently collaborating with colleagues in Austria and Germany to develop widely applicable microarray software based on our paper Marcelino et al. (2006).

Sharing Information:

Will be available to interested researchers.

Product Type:

Software (or netware)

Product Description:

Env454 database schema; in-house scripts to assemble reads generated from the same PCR clone with different sequencing primers (?clonebyclone?) and to estimate the taxonomic distribution of clones amplified from a particular sample (?clone2taxbreak?).

Sharing Information:

Service using database will be available to interested researchers.

Contributions

Contributions within Discipline:

Project 1:
Our genetic analysis of the 2006 Alexandrium bloom gives an unprecedented dataset with which we can evaluate hypotheses about how toxic dinoflagellate blooms form and change. Population genetic analysis of phytoplankton is becoming more common, and there are genetic descriptions of both toxic and nontoxic blooms in the literature. However, the availability of 2 successive years of data, both covering major

regional blooms, will allow us to assess whether the genetic relationships and changes that we observe are generalizable û i.e. do they describe the way that blooms always progress, or does the pattern change year-to-year. The occurrence of a huge bloom in 2005, following a decade with no major blooms or toxicity in southern New England waters, and the subsequent bloom in 2006 argue strongly for a recurrence in 2007. We have an extensive field season planned through the COHH in 2007, and hope to add another year of data to this dataset that will increase our analytical power. This information will greatly contribute to our understanding how blooms form and how they persist. Incorporation of this knowledge into the existing physical-biological models of Dr. McGillicuddy is a primary objective of this work, with the intent of enhancing our predictive capabilities for harmful bloom events.
With regard to human resource development, we have trained numerous undergraduate, graduate, and post-graduate students during this project. In the latter category, two of these individuals have moved on to faculty positions: J. Hackett to the Ecology and Evolutionary Biology Department at the University of Arizona, and D. Erdner to the Marine Science Institute of the University of Texas at Austin.
D. Anderson served as discussion leader for the National Council for Science and the Environment (NCSE) 7th International Conference on Science, Policy and the Environment: Integrating Environment and Human Health breakout session on Oceans and Human Health.

Project 3:
We have been able to provide standardized methods for the collection and rapid processing of numerous environmental water samples. These methods were used by multiple SGER projects collaboratively examining the effects of Hurricane Katrina on the microbes of Lake Pontchartrain.

Project 4:
We have obtained funding from the Moore Foundation to sequence the genome of several of the strains isolated in this project. This is a significant step in providing marine microbiology with the necessary core dataset for future research. Annotation and correlation with data from the other ongoing sequencing efforts will provide additional value to the users of these genomes.

Genomics Facility Core:
The tag sequencing technology has the potential to impact all of the Centers of Oceans and Human Health. It provides a new tool for detailed monitoring of microbial populations in marine environments. This will allow detection of a wide range of human pathogens even before they become significant fractions of the analyzed populations. We are preparing proposals for submission to the NSF/NIH Ecology of Infectious Disease Program that will characterize entire microbial populations in an investigation that will identify new indicator organisms and differential persistence of microorganisms associated with sewage pollution as a function of environmental conditions including physical forcings.

Pilot Project Program:
The funded pilot projects address important questions regarding the interactions between the oceans and human health. Many of the projects represent new directions for the Center, expanding the scope of our research efforts into, for example, marine natural products and the economics of pathogens and toxins. The projects also serve to add new scientists as participating members of the center, expanding our representation at all three member institutions. Many of the funded or proposed pilot projects involve postdoctoral researchers or graduate students, and will thus contribute to the training of the next generation of researchers in this area.

Contributions to Other Disciplines:

Administrative Core:
To other disciplines of science or engineering: To improve public health through an enhanced understanding of how oceanic processes affect the distribution and persistence of human pathogens and toxin producing organisms.

Genomics Facility Core:
The tag sequencing technology can be applied to any microbial population structure study including marine, terrestrial and even the human biome.

Contributions to Human Resource Development:

Project 1:
We have trained numerous undergraduate, graduate, and post-graduate students during this project. In the latter category, two of these individuals have moved on to faculty positions: J. Hackett to the Ecology and Evolutionary Biology Department at the University of Arizona, and D. Erdner to the Marine Science Institute of the University of Texas at Austin.

D. Anderson served as discussion leader for the National Council for Science and the Environment (NCSE) 7th International Conference on Science, Policy and the Environment: Integrating Environment and Human Health breakout session n Oceans and Human Health.

Project 3:
A talk on 'Oceans and Human Health' was given at an MBL Writer's workshop June 2006. A presentation entitled 'Research on Human Pathogens in Coastal Marine Environments at the Woods Hole Center for Oceans and Human Health' was presented at a Knight Fellows (science writer's) lecture Oct 2006.

Project 4:
Our laboratory is already providing trained undergraduates with developed talents, a firm grounding in laboratory protocols and scientific ethics to other laboratories in the field. Their training and enthusiasm makes them desirable laboratory members and has earned them invitations to these institutions. They will be able to contribute critical expertise to newly founded laboratories at other universities by participating in short-term research programs.

Genomics Facility Core:
Sue Huse and Phil Neal are database specialists and as part of the development of bioinformatics of tag sequencing they have received a 'crash' course in molecular evolution. Yuko Hasegawa, a graduate student at Brown University will join the genomic core and develop a thesis project relevant to COHH that takes advantage of the tag sequencing strategies.

Contributions to Resources for Research and Education:

Project 3:
L.Amaral-Zettler has been very active in teacher workshops and classroom interactions over the history of this project. Recently she participated as a science advisor in a webquest exercise. A webpage of the results from that workshop is available at http://serc.carleton.edu/microbelife/topics/pontchartrain/index.html

Genomics Facility Core:
We are developing high capacity molecular sequence analysis software and curated sequence tag databases that will be valuable to the entire microbiology community.

Contributions Beyond Science and Engineering:

Project 2:
Observations and models point toward a cyst deposition event as the causative factor leading to the historic New England red tide of 2005. These results suggest that cyst maps provide a basis for predictions of interannual variations in bloom severity, which could prove to be an extremely valuable tool for resource managers and agencies charged with protection of public health. Indeed, cyst observations in the fall of 2005 indicate persistent high abundance in the western Gulf of Maine cyst bed. This suggests the region could be susceptible to similarly severe blooms in the coming years. Observations from 2006 collected via a companion study funded by NOAA are consistent with that hypothesis.

Project 3:
This work is generating a baseline of microbial diversity for Mt Hope Bay that includes sequences of potential human pathogens that has not been described before. This information may be valuable to current discussions regarding potential dredging of a channel at the mouth of the Taunton River (nearby our sewage outfall and the power plant sites) and pending rulings against the power plant and its thermal discharge.

Project 4:
The creation and intense characterization of this strain collection will permit it to be used in testing technologies meant to identify and enumerate pathogens from among environmental isolates, allowing the precise cause of false positives and negatives to be discerned by associating them with well known environmental strains.

Genomics Facility Core:
The tag sequencing strategy has the potential to idenfity new indicator organisms and develop targets for DNA microarrays that will allow for more comprehensive tracking of microbes introduced into the environment through anthropogenic activity.

Special Requirements

Special reporting requirements: None
Change in Objectives or Scope: None
Unobligated funds: less than 20 percent of current funds
Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Activities and findings

Stegeman – Administrative Core

Research and Education Activities:

1. Foster the communication, planning, integration and interaction among Center members, thereby assuring that the goals developed in the Center vision are achieved.
2. Provide and maintain an administrative structure to oversee and monitor the financial aspects of the Center, including grants management.
3. Establish a structure that will support effective communication and planning with contribution from all Center Investigators.
4. Establish an External Advisory committee, which will review the Center programs and advise the Director and Deputy Director.
5. Oversee the operation of and work toward a successful Pilot Project small grant program.
6. Establish and maintain public and private web pages for the dissemination of information and for data transfer and communication.
7. Pursue interactions between the COHH and other Centers within the three Institutions, as well as with other COHH and EHS Centers, in order to expand the reach and impact of the center, and to expand the research base on issues relevant to the Center mission and themes.
8. Encourage the involvement of center investigators in community outreach and education efforts.

Findings:

The Administrative Core continues to oversee the Center, and facilitate the success of the Research Projects and the Pilot Project program. Inter-center communication continues on a regular basis. The Center has leveraged additional federal and private funds for this activity. The Administrative Core has served as the focal point for all Center activities.

1. The Administrative Core continues to foster the research and other activities of the Woods Hole Center for Oceans and Human health, to integrate the various components of the Center and to foster the success of the component projects and cores in the Center. The Center Office oversees each of the components, achieving a cohesive structure with visibility through all three of the component institutions, the Woods Hole Oceanographic Institution (WHOI), the Marine Biological Laboratory (MBL) and the Massachusetts Institute of Technology (MIT).
2. The Center Director and Biology Department staff (administrative professionals), and the respective Grants Management offices at the Woods Hole Oceanographic Institution and other institutions are monitoring the financial accounts of the Center grants.
3. Center Investigator meetings have been held on a regular monthly basis. The meetings take place on the first Friday of each month. All principle investigators in the projects and cores are involved in these meetings. Likewise, pilot project investigators also participate in the meetings. At these meetings we address all aspects of the Center interactions and communications, internally as well as externally.

4. The External Advisory Committee includes Dr. Michael Gallo, University of Medicine and Dentistry of New Jersey, and Dr. Gerald Plumley, of the Bermuda Biological Station for Research, and Dr. Margaret (Peg) Riley, University of Massachusetts, Amherst. The External Advisory Committee reviewed the Center programs in detail at a full day meeting held in Woods Hole on 13 October, 2006. The review included all aspects of the Center. The committee also met jointly with the Director of the MBL and the Associate Director of Research at WHOI.
5. The Administrative Core continues to oversee the Pilot Project program. In 2006, two projects were selected for full funding, and a third received partial funding, with the remainder of that request being leveraged from private internal sources at WHOI. These pilot projects involve investigators at WHOI and MBL. This brings to nine the number of pilot projects funded. Notably, one of the projects that we funded this year is a collaborative project between the Woods Hole and the Miami Centers for OHH.
6. The combined Centers Web site established at WHOI continues to serve as a site for all four centers (http://www.whoi.edu/science/cohh/index.htm).
7. The interactions with the other COHH have been highly productive. A) All the Woods Hole Investigators and the Director of the Core Facility attended the second joint Center Directors' meeting, in Seattle (April, 2006), with travel supported by the Admin Core. B) Since June 2006, we have been the organizers of the monthly conference calls among the leadership of the four COHH, to discuss points in collaboration in all aspects of the Centers' activities. C) Interactions between the WH-COHH and other NIEHS Centers: Members of the WH-COHH presented posters at the MIT CEHS Annual Meeting, held in Cambridge. We are continuing to explore opportunities for jointly sponsored enrichment activities.
8. **Outreach and impact:** Two major activities took place this year. A) WH-COHH investigators Gast, Polz and Amaral-Zettler, and Pilot Project co-recipient Chris Reddy continued studies of the microbial populations and hydrocarbon contaminants in Lake Ponchartrain, in the wake of Hurricane Katrina. Dr. Gast of the Woods Hole Center applied for and won internal WHOI funding to host a colloquium to bring all the COHH investigators together to assess findings. This colloquium was held in November 2006. B) A proposal for a new Gordon Research Conference on Oceans and Human Health was prepared, with Dr. Stegeman of the Woods Hole Center taking the lead. This proposal received enthusiastic support from the community and has been approved by the GRC board. The new GRC on OHH is scheduled for summer of 2008.

Training and development:

The Center has supported two undergraduates in 2006, through the NSF REU grant. The two supported in 2005 were Amy Koid, Franklin and Marshall College, who worked in the laboratory of Don Anderson on a project entitled "Differential gene expression in *Alexandrium fundyense*, a saxitoxin-producing dinoflagellate", and Benjamin Tully (Rutgers) who worked with Becky Gast and Linda Amaral-Zettler on a project entitled "Monitoring current and emerging pathogens in Mt. Hope Bay". COHH support also was used for a graduate student, Claudia Martins. Other students worked on COHH projects in the labs of Mitch Sogin and Martin Polz.

Anderson- Project 1

Research and Education Activities:
Work in Year 3 has focused on Specific Aim 5 of the project: *track changes in the genotypic diversity of Alexandrium populations through time throughout the Gulf of Maine.*

Findings:

In 2005, we used microsatellite markers for *Alexandrium tamarense* to monitor the genetic composition of an unprecedented bloom of *Alexandrium fundyense* in the northeastern U.S. Results of that study indicated that the bloom populations show high genetic diversity, and that the composition of the populations changes significantly on the time scale of about one month. We were able to conduct a similar microsatellite analysis in 2006 by leveraging event response ship time provided by NOAA for *Alexandrium* monitoring in the Gulf of Maine (GOM). We collected and analyzed a time course of samples collected at one geographic location (near Cape Ann, MA) as well as samples collected from the wider GOM during a single, large-scale research cruise. The latter samples included an offshore sample from near George's Bank, which provides a valuable snapshot of offshore populations of *Alexandrium*. The microsatellite dataset is complete, and we are currently processing and analyzing the data. Comparison of the 2005 and 2006 data sets will allow us to examine the interannual variability in bloom populations, to determine if the observed population dynamics are a consistent phenomenon or whether they change year-to-year. Also in 2006, we collected a time course of samples from an *Alexandrium* bloom in a Cape Cod salt pond. Because this bloom is self-contained, analysis of these samples will provide a picture of bloom development in the absence of external sources of cells.

During this project year we also developed a novel method for generating individual DNA samples for microsatellite analysis. In 2005, we isolated single cells to provide isolates for genotyping, and subsequently waited several months for the single cells to grow up to full-sized cultures. In 2006, we developed a "mini-culture" technique, where we grew the single cells in small volumes of medium to generate just enough cells for DNA extraction. The use of this method greatly increased our throughput and decreased our overall analysis time. As a result, in 2006 we were able to genotype more than 600 individuals from our combined analyses.

In our continued collaboration with Dr. D. McGillicuddy of the Woods Hole COHH, we have provided our field data (cell counts, cyst maps, hydrographic information) for use in numerical model runs that have focused on the 2005 and 2006 bloom seasons. The results of these "hindcast" analyses are of great importance (see progress report from McGillicuddy), leading us to the conclusion that we have entered a "new regime" that will have more frequent and more intense outbreaks of paralytic shellfish poisoning (PSP) in southern New England for the next decade or more. We are now working on a manuscript that will be submitted to Nature on these findings.

Our microsatellite efforts have led to two international collaborations. The first is with Dr. Satoshi Nagai, of the National Research Institute of Fisheries and Environment of the Inland Sea, Japan. Dr. Nagai developed microsatellite markers for *Alexandrium* species in Japan and has worked with us to apply these markers to GOM populations. The second collaboration is with Drs. Uwe John and Allan Cembella at the Alfred Wegener Institute in Bremerhaven, Germany. One of the populations from the 2005 bloom has been used in a comparison of global

Alexandrium population relationships, and this data was presented at the International Harmful Algal Bloom meeting in Copenhagen in September 2006.

During the course of our work, we have involved a number of graduate and undergraduate students, as listed above. These students have worked on several aspects of this project, including the cruise sample collection and isolation of the *Alexandrium* cultures for microsatellite analysis.

Outreach:

Presentations by D.M. Anderson:

04/06 Overview of the 2005 *Alexandrium* bloom: A regional perspective. MIT Sea Grant, Cambridge, MA.

05/06 The 2005 New England red tide: mechanisms and implications for the future. New England Shellfish Sanitation Association annual meeting, Port Jefferson, NY.

11/06 Invited seminar, "The 2005 and 2006 red tides", Atmospheric, Marine and Coastal Environmental Science, Hong Kong University of Technology, Hong Kong.

Presentations by B.A. Keafer

09/06 Recent *Alexandrium fundyense* "red tide" blooms in the Gulf of Maine: Mechanisms and future implications. National Marine Life Center, Buzzards Bay, MA.

Posters:

04/06 Initial Observations of the 2005 *Alexandrium fundyense* Bloom in southern New England: General Patterns and Mechanisms. Symposium on Boston Harbor and adjacent coastal waters. New England Estuarine Research Society. Nantasket, MA.

11/06 D.M. Anderson, B.A. Keafer, D.J. McGillicuddy, and R. He. The 2005 and 2006 *Alexandrium* red tides: A tale of two blooms. Wellfleet Harbor Conference.

McGuillicuddy- Project 2

Research and Education Activities:
Our originally proposed specific aims are to:

1) Formulate a suite of population dynamics models for the various genotypes of *A. fundyense*. Model formulation will be guided by existing observations, as well as laboratory experiments to be conducted in Project 1 (Anderson).

2) Incorporate the ensemble of population models into existing models of Gulf of Maine coastal hydrodynamics.

3) Use the coupled physical-biological models to construct hindcast simulations of *A. fundyense* survey observations to be collected jointly with Project 1 (Anderson).

4) Diagnose the simulations to determine the processes regulating the space/time expression of the different genotypes in terms of *A. fundyense* abundance.

5) Utilize toxigenicity data for the various genotypes (provided by Project 1 (Anderson)) together with the coupled physical-biological models (Aim 3) to make predictions of shellfish toxicity along the coast. Toxicity predictions will be tested with observations from ongoing shellfish monitoring programs.

Findings:
Our effort this year was focused on hindcasting the historic harmful algal bloom that occurred in New England in 2005 (Anderson et al. 2005). Three potential contributing factors have been identified: (1) increased cyst abundance off mid-coast Maine, (2) favorable transport conditions that arose from two unusually strong storms with NE winds, and (3) favorable growth conditions caused by delivery of nutrients via anomalously high river discharge. We have run several numerical experiments to quantify these factors. Our results suggest that:

(A) The high abundance of cysts in the western GOM was the main cause of the 2005 bloom. The massive bloom condition would have occurred regardless of the anomalous wind conditions and riverine discharge of 2005.

(B) Wind forcing was an important regulator, in the form of both episodic bursts of northeast winds and the downwelling-favorable mean condition, causing onshore advection of offshore populations.

(C) The anomalously high river runoff enhanced alongshore transport near the coast, but had limited impact on the gulf-wide bloom distribution.

We are in the process of composing a manuscript describing these results in detail.

We have also begun an investigation of the influence of vertical migration on the vertical distribution of motile phytoplankton. Our results suggest that asynchronous migration can

potentially explain the bimodal vertical distribution of *A. fundyense* that is sometimes observed in the Gulf of Maine (Ralston et al., submitted).

Outreach:
09/06 Lecture: *Red tides in the Gulf of Maine*. Ocean Science Journalism Fellowship Program, Woods Hole Oceanographic Institution, Woods Hole, MA.

10/06 Lecture: *Toxic algae in the Gulf of Maine: observations and models*. WHOI Topics in Oceanography workshop for middle-school and high-school science teachers, WHOI Exhibit Center.

11/20/06 Together with Don Anderson, I participated via digital videoconference: Symposium on red tides, Alexandria University, Alexandria, Egypt.

1/12/07 Interview with Wanda Curtis, Working Waterfront magazine re: red tides.

Gast-Project 3

Research and Education Activities:

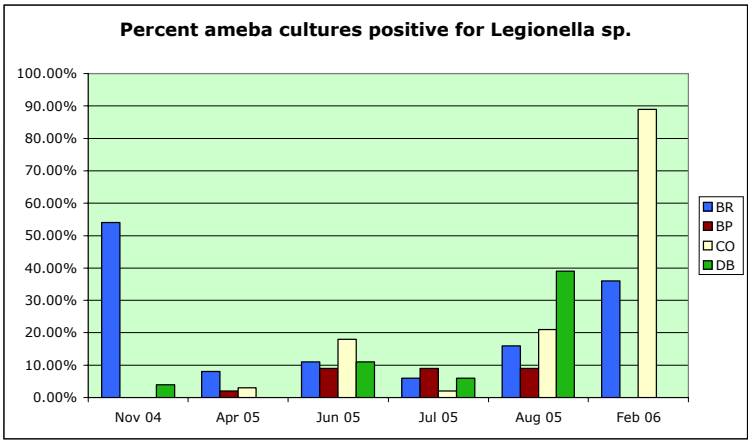
This proposal examines the diversity, distribution and persistence of human pathogens in the coastal marine environment of Mt. Hope Bay, Massachusetts. This estuarine environment has been heavily impacted by human activity, including significant thermal pollution, several sewage outfalls and the effects of past industrial activities. Monitoring for bacterial contamination occurs in the shellfish areas, but outside of these regions, very little is known about the occurrence of either bacterial pathogens or protistan parasites that are likely introduced into the Bay through sewage or runoff. The potential impact of the thermal output of the Brayton Point Power Plant on these microbial populations has not been examined at all. We hypothesize that both bacterial and protistan human pathogens are present in Mt. Hope Bay and nearby rivers, and that their distribution and persistence are affected by physico-chemical condition in the Bay. To examine this hypothesis we propose to accomplish the following specific aims:
Aim 1: Determine the distribution and persistence of human protistan pathogens in Mt. Hope Bay. We will conduct a molecular survey of human pathogens within the Mt. Hope Bay estuarine system, including sewage outfalls near and removed from thermal point sources, contaminated shellfish beds and shorebird nesting grounds, to better characterize the types of protistan pathogens present in this coastal environment. Our project will specifically target Giardia, Cryptosporidium, Naegleria, and Acanthamoeba, but will also perform general microbial eukaryotic and prokaryotic diversity assessments to determine whether there are novel sequence types present that are related to known pathogens (might represent unidentified human pathogens).
Aim 2: Determine whether naturally occurring marine amoebae can serve as reservoirs for pathogenic bacteria, and look for evidence of the natural occurrence of these associations in Mt. Hope Bay. We will test whether locally collected marine amoebae are capable of harboring the pathogenic bacteria Vibrio parahaemolyticus, Vibrio vulnificus, and Legionella pneumophila. The research will test for the induced presence and replication of bacteria in actively growing and dormant phases of the amoebae, and will utilize both PCR-based and in situ hybridization

based methods to detect naturally occurring associations.
Aim 3: Establish physico-chemical parameters associated with pathogen presence in Mt. Hope Bay and test predictive capability of water quality models regarding these correlations.
We will correlate measurements of water and sediment physico-chemical parameters with microbial community structure, including the presence of human pathogens. These measurements and our biological data will be used to assist in establishing and validating a water quality model for Mount Hope Bay that may also be used to understand and predict the potential presence of pathogens.

Findings:

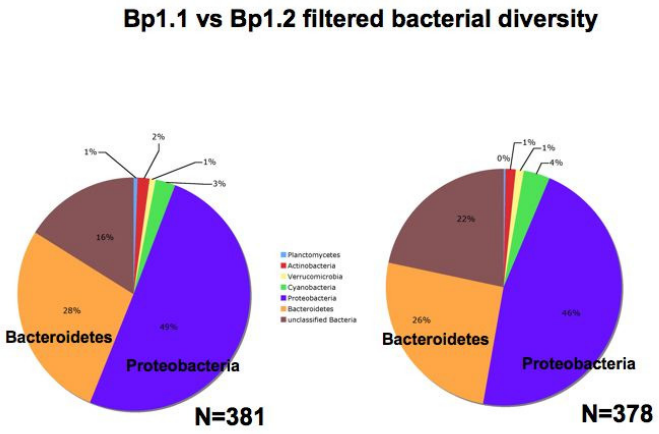
Four sites in Mt. Hope Bay were selected for our survey of human parasites and the effect of physico-chemical parameters on their distribution and persistence. Our first is located within the thermal plume of the Brayton Point power plant outlet. The size and temperature of this plume varies depending upon plant operations. The second site (Braga Bridge) is located at an underwater sewage outfall that releases secondarily treated sewage into the Taunton River just a bit north of the Bay proper. A third site is farther up the Taunton River (Dighton Bridge) at a brackish water/marsh site, and the fourth site (Common Fence) is near the bottom of the Bay at an area not directly impacted by the thermal plume or the sewage outfall. Sediment and water samples were collected in triplicate at all four sites. Our seasonal sampling officially began in November 2004, and continued through April, June, July, August, November 2005 and February 2006. An additional sampling was carried out in June 2006 in response to large amounts of precipitation and increased runoff. In addition to our four sites we also conducted two transect sampling trips in August of 2005 and February of 2006. Each time our transect began at our Brayton Point site and included 7 stations along a direct line to our control site Common Fence. Transect sampling included water samples (collected at the surface and at depth just above the sediment) and sediment samples. Sediment and water samples were again also obtained and processed from five sites in the Great Salt Lake, Utah.

Results. Ameba cultures. Amebas were enriched from sediment samples collected at each of the four sites during the year, and from the Great Salt Lake sites. These were accomplished using agar plates made of 5 different media types; non-nutrient seawater, non-nutrient freshwater, minimal media seawater, minimal media freshwater and minimal media brackish water. To date, over 150 amoeba cultures have been recovered and analyzed for the presence of legionellae. There appears to be an increase in the percent of ameba cultures positive for Legionella species during the warmer months, with all of the stations yielding positive amebas, whereas during the winter, only two sites yield positive ameba cultures (see graph below).

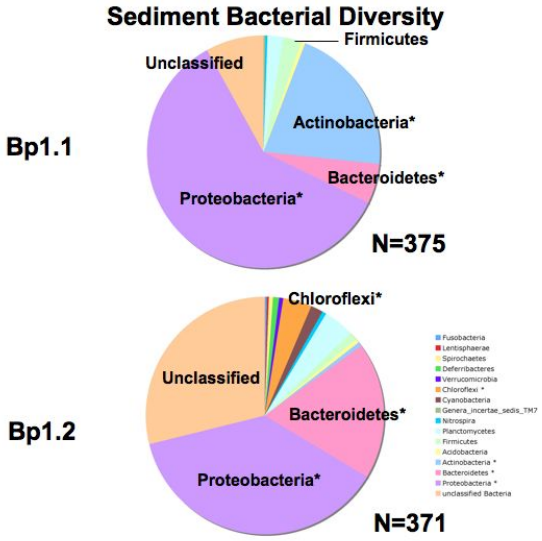


Nine of the recovered ameba cultures were positive for the presence of L. pnuemophila by amplification with a nested mip (macrophage infectivity potentiator) gene method. These results indicate that marine amebas do harbor the human pathogen, and although the number was less than 10% of the recovered amebas, they all occurred in samples collected during the summer. It was also of interest to note that none of the freshwater ameba cultures were found to carry L. pneumophila. Our future work with these cultures will be to determine whether particular ameba species or genera harbor legionellae, and to determine whether the Legionella sequence types present in amebas are also prevalent in the environmental samples. We are also pursuing methods of pathogenicity associated gene primers to determine whether any of the novel legionellae carried by amebas are potentially pathogenic.

Clone library analyses. We have compiled the first comprehensive (eukaryal, bacterial, archaeal) data from small-subunit ribosomal RNA gene clone libraries for samples collected near the thermal plume and underlying sediments of the Brayton Point Power Plant. We have partial sequences of nearly 4,000 clones from 2 different sites and have further sequenced 1,000 unique clones from these to full-length. Not surprising, our findings reveal a highly diverse consortium of the three domains including relatives of sludge bacteria, polyaromatic hydrocarbondegrading bacteria, and representatives related to the genera Staphylococcus, Streptococcus, and Clostridium. Comparisons between overall diversity in water samples for two replicate samples collected at the Brayton Point Power Plant site yielded very similar phylum-distributions shown in the pie chart below.



Our phylum-level comparisons between sediment samples, however, revealed significant differences in the microbial assemblages recovered (charts below, indicated by asterisks).

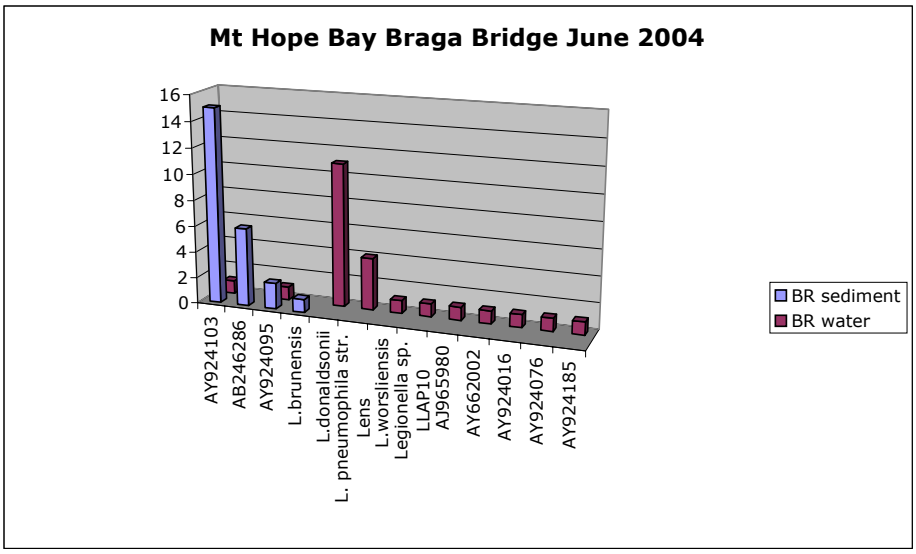


Preliminary phylogenetic placement of these environmental sequences revealed that the largest number of potential human pathogens were detected in sediment samples and not in the watercolumn samples. SARST analysis of sediment samples. Our clone library constructs provide us with a reference library of full-length sequences for our Brayton Point Power Plant site. This serves as a baseline dataset with which to compare sequences obtained from higher-throughput methods that target short variable regions of small-subunit rRNA genes. Given that our initial phylogenetic analyses identified the largest number of potential human pathogens in sediment samples, we sought to further explore the microbial diversity in these samples using methods that would allow us to sample more deeply. One such method that we have begun applying to our sediment samples is called SARST (Serial Analysis of Ribosomal Sequence Tags). This high-throughput method allows us to increase our data recovery by up to 10-fold by

sequencing a series of concatenated tags for each clone sequenced. We currently have a total of 2,365 tags from the V6 region of SSU rRNA from one of our Brayton Point sediment samples. 513 of these possessed 100% matches to known organisms in GenBank. Of these we further identified potential pathogens belonging to the following bacterial genera that possess pathogenic representatives: Shigella, Shewanella, Pseudomonas and Francisella. The last of these has especially caught our attention because there have been recent outbreaks of Francisella tularensis on the nearby island of Martha's Vineyard. Furthermore, Francisella tularensis has been shown to associate with free-living amoebae such as Acanthamoeba. The occurrence of Francisella in estuarine environments is not well-documented. As a result of this finding, we will begin to screen our samples for this pathogen in both environmental samples and ameba cultures.

Detection of specific pathogens. We are currently in the process of setting up amplification arrays with our extracted samples for use with organism specific primers. These include primers for Giardia, Cryptosporidium, Acanthamoeba, Naegleria and Legionella. The Legionella primers were used in nested amplifications of our seawater, sediment and ameba culture samples. Sediment and ameba cultures from Mt. Hope Bay showed positive amplification results, and there appears to be a seasonal pattern to the prevalence of Legionella-like species in amebas. All of the water and sediment samples from the Great Salt Lake were positive using the Legionella amplification method. Both MHB and GSL samples are not often positive for L. pneumophila, but it has been detected. Giardia and Cryptosporidium amplifications of MHB samples were carried out as a multiplex, but the results were difficult to interpret. We also found that our detection using the direct, multiplex amplification was relatively low (we needed 20- 80 cells per liter of water). We have revised our amplification strategy to include nested methods for both Giardia and Cryptosporidium, and will retest our water and sediment samples.

Legionella sequences. Samples that were positive for the presence of legionellae were amplified, cloned and sequenced. Initial sequencing of PCR products directly indicated that a diverse collection of species were present, similar to what is seen in freshwater environments, but had not been previously reported for marine environments. We pursued cloning prior to sequencing our environmental PCR products because it was likely that a mixture of species were present. From this approach we still observe a diverse collection of sequence types, representing strains (99% similarity to known sequences), species (>97% similarity to known sequences) and potentially novel genera (<95% similarity) that are sister groups to the legionellae. At a site, the sequence types present, and prevalent, change over time. The abundant sequence types in sediment are not necessarily the same as the overlying water, although they can be on occasion. In this work we have now newly described both the significant diversity of legionellae in the marine environment and have documented the seasonal pattern of their abundance. The following graph is an example of the diversity of Legionella-like sequences recovered, and the differences between sample types at the same site. Note that L. pneumophila was detected in the water at this site – the sewage outfall.



Significance:

We have completed most of our seasonal sampling within the Mt. Hope Bay system and have been processing these samples for pathogen presence and microbial community structure. Although the detection of legionellae in marine environments has been documented, the extensive presence of Legionella-like sequences in Mt Hope Bay was unexpected. This is also true for the Great Salt Lake samples. These organisms appear to be fairly prevalent in amebas recovered from the sediments of these environments, and the number of sites that they were recovered from increased as the year progressed. This suggests that legionellae not only persist there, but they can also spread. Through our combined clone library and SARST approaches, we have directly detected and documented the presence of potential human pathogens present in Mount Hope Bay. This approach has the advantage of identifying both known and emerging pathogens. We have also started SSU rRNA V6 tag-sequencing of samples from our summer and winter transects off the thermal plume using a newly acquired pyrosequencing (454) technology available at the MBL through the MBL Keck genomic facility that serves the Wood Hole COHH. Preliminary results from the first run have yielded thousands of tags for each of the eight samples run thus far. The tags are currently being processed though the V6 database pipeline at the COHH genomics core facility that will aid in identifying the closest relatives of the tags to known taxa in GenBank.

Outreach:

Mentoring of undergraduate students through REU programs, informal epidemiology training and participation in a California beach study.

The investigators have participated in several interviews with local newpapers, and were interviewed by the local NPR radio station (December 18, 2006, The Point, hosted by Mindy Todd). We have also begun informal interactions with physicians who have contacted us regarding monitoring for pathogens in coastal environments (Dr. John Harries, Sanibel Island, FL).

Polz – Project 4

Research and Education Activities:

AIM 1. To characterize and model dynamics and reservoirs of *V. vulnificus* and *V. parahaemolyticus* populations over seasonal cycles

We now have samples along the salinity gradient from the entrance of Plum Island Sound to the Parker River dam from the fall of 2004 to the spring of 2006. A total of eight surveys were completed over this period with similar tidal conditions (high tide). Vertical profiles of temperature and salinity were measured at each sampling station. Surface water samples will now be analyzed to determine major inorganic nutrients, strain diversity (by isolation) and culture independent quantification (by QPCR-CDCE). Three of the surveys were conducted in coordination with water chemistry sampling conducted by the Plum Island Estuary Long Term Ecological Research Site (PIE-LTER) PIs in which a suite of water chemistry, nutrient, chlorophyll, suspended sediment and light penetration measurements were made (see http://ecosystems.mbl.edu/pie/). In addition, we are using USGS river gauge data to document temporal variations in river discharge and PIE-LTER long-term time series of salinity, temperature, dissolved oxygen, fluorescence and turbidity at three locations within the estuary to document variability in estuarine physics and water chemistry on various time scales (tidal, diurnal, event, seasonal, and interannual).

Large seasonal and storm-driven variations in salinity and temperature were observed along the estuary. In addition the residence time – the time water-borne material is retained in a region of the estuary before being flushed into the open ocean – varies seasonally and with location within the estuary (Vallino and Hopkinson, 1998). For example, residence time in the upper Parker River ranges from a few days during periods of high river discharge typically occurring in the spring and early summer to more than a month during low discharge conditions typically observed in late summer and early fall. In contrast, residence time within Plum Island sound is a day or less for a wide range of river discharge.

AIM 2. To test the link between estuarine physics, nutrient and particle abundance and growth patterns of Vibrio species over tidal cycles.

Samples were collected roughly once per hour over a 24-hour period from July 26-27, 2006 in order to document short timescale variations in estuarine physics, water chemistry and corresponding *Vibrio* abundance and strain diversity. Samples were collected from a small boat while moving up and down the main estuary channel with the tidal currents tracking a water mass with a constant surface salinity. Physical measurements were made over the entire water column, while water samples were collected near the surface and near the bottom. In addition, PIE-LTER measurements at three locations in the estuary, spanning our sampling locations, will be used to document variations in dissolved oxygen, fluorescence, and light attenuation during our sampling period. The samples are now in the process of being analyzed for flow cytometric cell counts (bacteria, eukarya, viruses), community production rates, diversity and abundance of vibrios (by QPCR-CDCE). Moreover, we have for the first time applied a new in situ fitness assay, which we have recently developed to measure the growth rate of different vibrios under different environmental conditions (Yu and Polz, unpublished). This assay employs spiking of BrdU into natural water samples where the BrdU is taken up by active cells and incorporated into DNA when they grow. The labeled DNA can then be captured by magnetic beads and thus purified away from DNA of cells that did not respond by growth under the given conditions. The captured DNA can serve as a template for PCR based assays, such as cloning of specific genes or QPCR-CDCE, and thus allows finely tuned differentiation of how strongly different groups of

organisms grew under specific conditions (by comparison or relative abundances at the beginning and end of incubation).

AIMS 3 and 4. To determine the 'rules' of recombination, gene transfer and clonality in co-occurring V. vulnificus and V. parahaemolyticus and related vibrios AND To assess the diversity, mobility and evolutionary history of genes implicated in pathogenicity.

We have established a core set of strains, which we have characterized by multilocus sequence analysis (MLSA). This has revealed the presence of clusters of strains at different phylogenetic hierarchies. The MLSA dataset is now serving as input for a new technique, which we are currently designing, to estimate the homologous recombination rates among strains with different sequence divergence. This will allow us to estimate to what extent clusters are genetically isolated (i.e., have low enough recombination rates with other clusters) so that they are evolutionarily stable populations, which are free to diverge phenotypically. We regard our new method as a significant advance since MLSA data are rapidly building up for many organisms but estimation of recombination rates are still difficult to achieve with current methods.

Findings:

Several important findings were made during 2006. We have characterized the **homologous recombination rate** among environmental vibrios providing estimates of gene flow among potential pathogens and non-pathogens. This required the development of a new method, which will be widely applicable to multilocus sequence data. Further, we have discovered that **microdiverse vibrios are differentially distributed in the water column**. Different genotypic clusters within named species are either preferentially attached to particles and zooplankton or are found free-living. This shows that microdiverse taxa represent ecologically differentiated populations despite potential for high migration between the different compartments. Finally, we have developed a technique, which allows **estimation of in situ fitness differences among genotypes**. The method is based on BrdU spiking into environmental samples. The label is incorporated into bacterial DNA proportional to growth. The DNA can subsequently be captured on magnetic beads and the frequency of different genotypes be determined by QPCR or clone libraries. We have applied he technique for the first time to field samples during our 24-hour tidal cycle sampling.

Finally, we are continuing the analysis of our strain collection, which has already been mined for several important insights, particularly that the genomes contained in it are largely unique, even within clusters of bacteria that share common 16S rRNA sequences. This is particularly important when the evolution and maintenance of pathogenicity determinant genes is considered. We have now shown that these genes are much more widespread among related strains than anticipated; further, we have identified clear cases of gene transfer and losses indicating that pathogenicity genes may be part of a mobile gene pool, which is frequently exchanged among related strains.

Training:

This project has been undertaken in a manner that provides intense and varied training opportunities. Each of the postdoctoral researchers and graduate students listed has been

involved in developing new methods and adopting computational, microbiological, or molecular methods new to them.

The undergraduate training in conjunction with this project is a continuing effort to integrate individual initiative with broad education in a working research atmosphere. Each of the undergraduates receives many hours of personal tutelage in methods, experimental design, scientific ethics and data analysis from a senior graduate student or postdoctoral associate. They learn basic microbiology, basic molecular biology, and the statistics and modeling necessary. Several students have learned basic Matlab programming to assist them with their data processing and analysis. All their skills are taught in a general fashion, so as to be portable to other research environments. In addition, each undergraduate has ownership over an intellectually coherent portion of the research program, and are engaged in experimental design as well as experimental conduct. This is made more significant because many of the undergraduates involved in the research have been underclassmen for whom this is their first research experience.

Outreach:

Outreach is being conducted jointly with the Museum of Science to develop a program of education regarding environmental microbiology. The population in Boston is primarily aware of marine bacteria as causing beach closings, having a negative impact on shellfishing, and in relation to the large bio-reactors that process sewage in the harbor. This awareness is positive because the public, particularly boaters and fishermen, are eager to know about the microbiology of their coastal waters. However, it also brings with it a sense of negativity about bacteria that is ill-founded and can be remedied through education.

A new outreach component, which focuses on vibrio-phage interactions is the planned establishment of a phage hunting club at MIT. We are actively participating in this endeavor.

Sogin- Genomics Facility Core

Research and Education Activities:

a. Facility operations and administration:

The genomics core provides DNA sequencing services and computational support to investigators in the Woods Hole Center for Oceans and Human Health (WH-COHH). Many of the Center's projects take advantage of our strengths in molecular microbial ecology and the bioinformatics. Over the past calendar year, the genomics core facility has provided WH-COHH projects with just over 18,000 reads corresponding to ~12.5 million base pairs. The total usage over the first three years of the center's operation is 44,000 reads generating a total of 30 million basepairs. **Figure 1** Shows the usage pattern for the genomics core and **Figure 2** describes the use of the Genome Core Facility by different WH-COHH projects.

Figure 1. WH-COHH sequencing usage pattern.

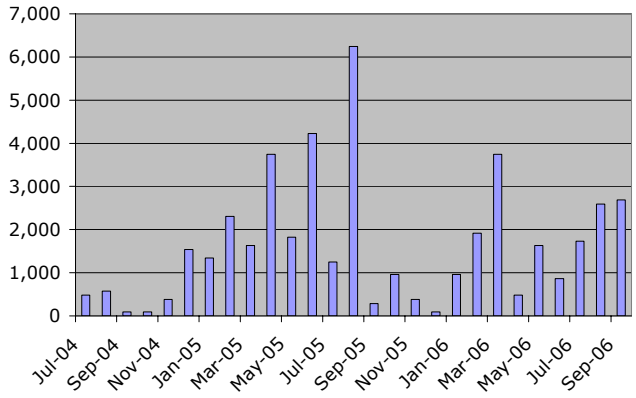
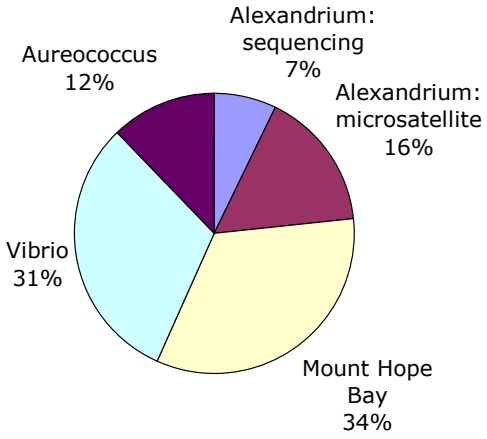


Figure 2. WH-COHH project sequencing activity.



Sequencing costs have been ~20% higher than originally projected because of increased costs of reagents for operating the ABI 3730 XL and failure of the RevPrep system for automatically producing very lost cost sequencing templates. Instead of templates costing 10 cents, we estimate each template now costs 30 cents using a protocol run on a new Biomek platform. This is still a relatively inexpensive template production cost. Other equipment additions included 20 new high performance nodes to our cluster computing environment (using institutional funds) and a Roche Genome Systems20 (GS20) pyrosequencing system funded by a new award titled ***Microbial population structure of the world's oceans*** from the W.M. Keck Foundation to the Marine Biological Laboratory at Woods Hole. We anticipate that this system will become a key element of future COHH investigations. A project under consideration is to use the GS20 to generate draft genome sequences for 20 *Vibrio* strains from Martin Polz's laboratory. Funding for that project has been provided to Martin Poltz of MIT by the Gordon and Betty Moore Foundation.

b. Scientific and technology developments in the genome core.

Using the GS20 sequencer the core facility developed a new, massively parallel DNA sequencing approach to characterize microbial populations with unprecedented level of sensitivity for detecting low abundance organisms. The International Census of Marine Microbes (ICOMM) provided the test samples and funds for reagents and the WH-COHH provided computational support for analysis of the data. Using samples from a TRANSAT cruise that followed the deep water circulation of the North Atlantic and samples from Axial, a seamount off the coast of Oregon, we developed a tag sequencing strategy for exhaustively monitoring microbial populations in the marine samples. We exploited the massively parallel, pyro-sequencing capability of the GS20 system which employs 454 Life Sciences technology, to sample hypervariable regions (rapidly evolving sequences that can record differences between both divergent and closely related organisms) from 200,000 rRNAs in a

single sequencing run. Using primers that flank the V6 hypervariable region in bacterial rRNAs, we generated PCR amplicons from environmental DNA preparations. With the GS20 system we were able to sequence many thousands of PCR amplicons from each environmental library without requiring the construction of recombinant clones or sequencing templates. Individual amplicons bound to beads in a PicoTiterPlate™ directed the pyro-sequencing reactions.

Each sequence tag served as a proxy for a specific rRNA phylotype. Matches to hypervariable regions from known phylotypes (compiled from molecular sequence databases with nearly 200,000 entries) provided information about taxonomic identity and microbial diversity. Enumerating the frequency of individual tags also provided a first-order description of the relative occurrence of specific microbes in a population. Rarefaction and non-parametric estimators such as Chao1 and the abundance-based coverage estimator ACE provided estimates of species richness.

As part of this project we have also determined that the error rate of 454 Life Science Sequencing can be as low as 0.25% when we remove reads that contain even a single undetermined base and reads that are either too short or too long. On average, this process leads to the elimination of ~20% of the reads but the remaining sequences are significantly more accurate than traditional capillary sequencing. A manuscript describing this result is in progress.

Findings:

The results of these pilot studies are impressive. Rather than estimating only a few hundred OTUs (Operational Taxonomic Units) as do most studies of marine microbial diversity, we were able to detect the presence of minor members of microbial populations and obtain more accurate estimates of total microbial diversity. The samples 53R, 55R, 112R, 115R, 137 and 138 in the table below are water column samples from the North Atlantic Flow. The FS samples come from diffuse flows of Axial Seamount. Tag sequences were collected and the program DOTUR was used to estimate rarefaction and numbers of species (both ACE and Chao1) estimators. Based upon comparisions of unique sequences (distance of 0) we estimate nearly 60,000 OTUs in the diffusive flows and tens of thousands of OTUs in the water column samples. This provides a remarkably comprehensive picture of Bacterial populations

Table 1. Diversity estimates of V6 Tags from marine samples.

Dist		0			0.03		
ID	Reads	OTU	**ACE**	Chao1	OUT	**ACE**	Chao1
53R	4,995	2,595	**16,488**	14,565	1,954	**6,826**	6,271
55R	13,906	7,006	**46,134**	40,638	5,480	**21,051**	19,252
112R	9,270	5,459	**36,213**	31,948	4,238	**15,620**	13,223
115R	10,987	5,595	**37,944**	32,428	4,209	**14,276**	12,499
137	13,898	6,594	**41,117**	36,007	4,782	**15,707**	13,782
138	14,375	7,055	**51,102**	43,875	5,666	**25,627**	23,342
FS312	4,849	2,661	**15,020**	12,905	1,968	**5,910**	5,676
FS396	17,731	8,376	**59,231**	49,964	6,568	**25,908**	23,081

and its application to projects in the WH-COHH have enormous potential. Rather than focusing upon detection of a single kind of organism or being constrained by budgets which allow the sampling of only a few hundred sequences, this application of 454 technology will allow us to develop detailed descriptions of microbial populations never before possible. The tag sequencing technology has the potential to impact all of the Centers of Oceans and Human Health. It provides a new tool for detailed monitoring of microbial populations in marine environments. This will allow detection of a wide range of human pathogens even before they become significant fractions of the analyzed populations. More recently we developed methods that allow the multiplexing of this very high throughput tag sequencing strategy so that it can be applied to multiple samples from near-shore sites that are of interest to the WH-COHH program. Our initial characterizations relevant to COHH focused on comparisons of the Mount Hope Bay environment with Eel Pond in Woods Hole. We see very significant differences in the population structures of these two systems. We have also have developed improved bacterial primers and new multiplex primers for Archaea. Protists primers will be developed when our system is upgraded from a GS20 to a GSFLX capable of longer reads during the first quarter of 2007. Over the next few months we will apply the tag sequence strategies to many samples from the Mount Hope Bay site as well as a comprehensive community analysis of microbes so that we can track point sources and transport of microbial communities associated with human sewage.

Training:

Through the genome core activities we have assisted Kevin Lin, a local high school student, in the development of his science fair project. We will also be training a graduate student, Yuko Hasegawa in the use of advanced genomic techniques in the analysis of microbial communities associated with anthropogenic activities.

Hahn- Pilot Program Project

Research and Education Activities:

a. Specific Aims

The specific objectives of the Pilot Project Program are:

A. To assess the feasibility of new areas of study, especially those that are not currently represented in our Research Project base but would contribute to the overall goals of the center.

B. To support the collection of preliminary data that can be used to generate full proposals to NSF, NIH, or other agencies or organizations.

C. To recruit scientists not currently involved in research on oceans and human health, but who may have expertise in one of these areas, to become participating members of the center and interact with other center investigators.

D. To stimulate collaborative and interdisciplinary research within the center.

E. To foster the application of new technologies and experimental approaches to questions concerning the impact of oceanic processes on public health.

F. To ensure the ability of the center to respond rapidly to new scientific information and emerging challenges in this field.

G. To contribute to the training of future researchers in the field of Oceans and Human Health through enhancement of graduate and postdoctoral training opportunities.

b. Studies and Findings:

Completed pilot projects (see list of publications in publication section)

• *Characterization of a cyanobacterial anti-algal compound* (Eric Webb and Chris Reddy, WHOI).

• *Cnidarian toxins against voltage-gated Ca2+ channels* (Robert Greenberg, MBL).

• *Marine phage as vectors of gene transfer between marine bacteria and bacterial pathogens* (Peter Weigele and Jonathan King, MIT).

Progress on pilot projects funded in year 2:

• *Transcriptome profiling in the harmful alga Aureococcus anophagefferens.* (Sonya Dyhrman, WHOI). *Aureococcus anophagefferens* is a widespread HAB species that has had severe and negative impacts. *A. anophagefferens* is allelopathic and is thought to produce a suite of natural products including a water soluble neuroactive metabolite, or toxin, that has been implicated in dose-dependent mortality and health decline in model shellfish. The goal of this COHH pilot project is to sequence three Long- SAGE (serial analysis of gene expression) libraries for this organism to examine the *A. anophagefferens* transcriptome and how it changes with external

stressors. To date, over 110,00 Long-SAGE tags have been sequenced. The ongoing analysis of these data suggests that the harmful alga *Aureococcus anophagefferens* has a robust transcriptional response to both phosphorus and nitrogen starvation. Tag annotation is ongoing. This is the first study to examine global transcriptional patterns in this organism.

• *Beach Pathogens* (Steve Elgar, Britt Raubenheimer, & Rebecca Gast, WHOI). Pathogens in coastal sediments pose a serious health risk to users of America's beaches, but the effects of waves, currents, and changes in beach sediment on pathogen distribution are not understood. The researchers hypothesize that sediments contaminated by pathogens (eg, from sewage) can be exposed when wind, waves, and currents cause changes in the beach configuration (eg, erosion or accretion), potentially creating additional human health hazards, via both direct contact with contaminated sand and exposure to pathogens carried by spray from breaking waves in the surf. Enteroccocci, a proxy for fecal contamination, were surveyed in ocean beach sands before and after large waves from a hurricane, and after a rain storm that occurred as hurricane waves were diminishing. Beach core samples were collected at 3 locations along a cross-shore transect between the high- and mid-tide lines near Kitty Hawk, NC on the Outer Banks of North Carolina in September 2006. The redistribution of enterococci was observed when beaches erode or accrete. Additional samples from beaches near outfalls will also be surveyed to determine whether they have the potential to contaminate recreational areas.

• *Names-based cyberinformatics tools for rapid response communications and outreach during event management – a pilot based on harmful algal blooms in NE US coastal waters* (David J. Patterson, MBL, and Don Anderson, WHOI). Algal blooms are increasing in frequency, extent and significance. The objective of this proposal is to promote human health by applying new informatics technologies for biology to improve communication among the public and stakeholders in response to a bloom event. The primary deliverable will be a pilot template for a web site that can rapidly call upon expert sources of information, inherit previously known but relevant information, can add local content and will combine the information dynamically in a very flexible environment. Current activity has involved the *Encyclopedia of Life* project, which will complement the web site to be developed in this project.

Third round of pilot projects

The third call for pilot project proposals was issued in September of 2006 by email to all faculty and research staff at WHOI, MBL, and MIT. Proposals were due November 1, 2006. Thirteen proposals were received and subsequently were reviewed and scored by members of the Internal Advisory Committee. The proposals requested approximately $430,000 in total costs (~$275,000 direct costs). Ten were from WHOI (multiple departments), two were from MBL, and one was from MIT. All applicants were provided with written reviews. Four projects were selected for full or partial funding:

• *Anthropogenic impacts and profiling fecal microbial populations at a salt marsh* (Mitch Sogin, MBL)
Fecal coliforms are indicator organisms that warn of possible fecal contamination and its potential impact on human health. Coliform surveillance activities generally rely upon

cultivation assays or molecular Microbial Source Tracking (MST) technology to assay for the presence of a particular indicator organism in environmental samples. This project will employ a massively parallel DNA tag sequencing strategy to profile entire microbial communities in the Little Sippewissett Salt Marsh and the barrier Woodneck Beach. This study site is surrounded by ~40 homes, most of which are occupied on a seasonal basis and all are serviced by septic systems. The marsh communicates with Buzzards Bay through a tidal inlet. Multiple times each summer high coliform counts indicate dangerous conditions for recreational use. Possible coliform sources include human waste from failed septic systems, bird populations or other animals. The objective is to use the tag sequence data to locate specific sources of fecal contamination and identify suites of genes that could serve as multi-species indicators of human pollution. The experimental strategy takes advantage of rapidly evolving hypervariable regions in ribosomal RNAs and our ability to generate many thousands of short DNA tag sequences using 454 Life Science's pyrosequencing sequencing technology on a Roche Genome Sequencer 20 System. Samples will be collected throughout the summer of 2007 and tag sequence will be determined form those microbial populations that were harvested on days corresponding to high coliform count measurements provided by the Barnstable Health Department. Because the technique returns quantitative information about most if not all members of a microbial community, these researchers will be able to track the location of contamination and map its distribution through the marsh.

• *Transcriptional Markers of Life Cycle Transitions in Harmful Algal Blooms* (Don Anderson, WHOI)
Bloom dynamics of the red tide dinoflagellate, *Alexandrium fundyense* are driven in large part by transitions in its life cycle. While these stages are well documented, the biological and oceanographic forces that trigger transitions between the stages are not. A major obstacle to determining the conditions that trigger these transitions is our inability to rapidly identify sexual stage cells. Here, a novel transcriptome experiment will be used to discover molecules that are uniquely expressed by conjugating gamete cells and by germinating cysts. This experiment will utilize sequencing-by-synthesis technology that is newly available through the Bay Paul Center. Data from the experiment will be analyzed in a fashion that is directly analogous to SAGE. Results will be used to leverage a larger, multi-year proposal to verify and validate transcriptional markers discovered through the proposed work.

• *The Economic Effects of Harmful Algal Blooms: A pilot project to estimate the costs of human respiratory ailments associated with aerosolized brevetoxins* (Porter Hoagland and Di Jin, WHOI; Lora Fleming, Miami)
This study will estimate the costs-of-illness associated with human respiratory ailments that arise as the consequence of the aerosolization and coastal to inland transport of brevetoxins from blooms of the marine dinoflagellate, *Karenia brevis*, in the Gulf of Mexico. The research will develop models to link the occurrence of HAB events in the coastal-ocean with exposures to aerosolized brevetoxins. The researchers will compile datasets and develop models of illness rates that would permit historical estimates of these kinds of impacts and the simulation of future potential impacts. This is a proof-of-concept Pilot Project designed to develop an analytical framework that can be used on a larger scale, using more extensive datasets in the future. It is critical that we understand the costs of natural hazards such as HAB events for at least two reasons. First, the nature of the costs (their effect) and their incidence (who is affected and at

what rate) will enable the characterization of feasible actions to mitigate the costs. Second, the scale of the costs will help resource managers, scientists, and the general public to gauge the levels of and need for potential mitigation.

• *The Economics of Human Health Risks from Pathogens and Toxins in the Marine Environment* (Hauke Kite-Powell and Porter Hoagland, WHOI)
These researchers will produce an "order of magnitude" estimate of the annual human health cost imposed on residents of the United States by exposure to pathogens and toxins from the marine environment. The estimate will be derived from a review and synthesis of information in the existing literature on (1) the spatial and temporal prevalence of marine pathogens, (2) the pathways by which they affect humans and the potentially exposed populations, (3) the human health effects of exposure, and (4) the economic cost of resulting medical conditions. The estimate will inform future research on pathogens in the marine environment, allowing scholars and public officials to target pathogens and settings where improved scientific understanding is most likely to produce significant economic benefits, and setting the stage for focused economic analyses. (co-funded with the WHOI Marine Policy Center)

The funded pilot projects address important questions regarding the interactions between the oceans and human health. Many of the projects represent new directions for the Center, expanding the scope of our research efforts into, for example, marine natural products and the economics of pathogens and toxins. The projects also serve to add new scientists as participating members of the center, expanding our representation at all three member institutions. Many of the funded or proposed pilot projects involve postdoctoral researchers or graduate students, and will thus contribute to the training of the next generation of researchers in this area.

During the next project period, we will continue the Pilot Project Program, issuing a new call for proposals and funding 2-3 new projects. We also will follow-up the projects funded this year and last year, to gauge their progress and success in achieving research goals and obtaining externally funded grants using preliminary data generated in the pilot projects.

Activities and findings

Stegeman – Administrative Core

Research and Education Activities:

1. Foster the communication, planning, integration and interaction among Center members, thereby assuring that the goals developed in the Center vision are achieved.
2. Provide and maintain an administrative structure to oversee and monitor the financial aspects of the Center, including grants management.
3. Establish a structure that will support effective communication and planning with contribution from all Center Investigators.
4. Establish an External Advisory committee, which will review the Center programs and advise the Director and Deputy Director.
5. Oversee the operation of and work toward a successful Pilot Project small grant program.
6. Establish and maintain public and private web pages for the dissemination of information and for data transfer and communication.
7. Pursue interactions between the COHH and other Centers within the three Institutions, as well as with other COHH and EHS Centers, in order to expand the reach and impact of the center, and to expand the research base on issues relevant to the Center mission and themes.
8. Encourage the involvement of center investigators in community outreach and education efforts.

Findings:

The Administrative Core continues to oversee the Center, and facilitate the success of the Research Projects and the Pilot Project program. Inter-center communication continues on a regular basis. The Center has leveraged additional federal and private funds for this activity. The Administrative Core has served as the focal point for all Center activities.

1. The Administrative Core continues to foster the research and other activities of the Woods Hole Center for Oceans and Human health, to integrate the various components of the Center and to foster the success of the component projects and cores in the Center. The Center Office oversees each of the components, achieving a cohesive structure with visibility through all three of the component institutions, the Woods Hole Oceanographic Institution (WHOI), the Marine Biological Laboratory (MBL) and the Massachusetts Institute of Technology (MIT).
2. The Center Director and Biology Department staff (administrative professionals), and the respective Grants Management offices at the Woods Hole Oceanographic Institution and other institutions are monitoring the financial accounts of the Center grants.
3. Center Investigator meetings have been held on a regular monthly basis. The meetings take place on the first Friday of each month. All principle investigators in the projects and cores are involved in these meetings. Likewise, pilot project investigators also participate in the meetings. At these meetings we address all aspects of the Center interactions and communications, internally as well as externally.

4. The External Advisory Committee includes Dr. Michael Gallo, University of Medicine and Dentistry of New Jersey, and Dr. Gerald Plumley, of the Bermuda Biological Station for Research, and Dr. Margaret (Peg) Riley, University of Massachusetts, Amherst. The External Advisory Committee reviewed the Center programs in detail at a full day meeting held in Woods Hole on 13 October, 2006. The review included all aspects of the Center. The committee also met jointly with the Director of the MBL and the Associate Director of Research at WHOI.
5. The Administrative Core continues to oversee the Pilot Project program. In 2006, two projects were selected for full funding, and a third received partial funding, with the remainder of that request being leveraged from private internal sources at WHOI. These pilot projects involve investigators at WHOI and MBL. This brings to nine the number of pilot projects funded. Notably, one of the projects that we funded this year is a collaborative project between the Woods Hole and the Miami Centers for OHH.
6. The combined Centers Web site established at WHOI continues to serve as a site for all four centers (http://www.whoi.edu/science/cohh/index.htm).
7. The interactions with the other COHH have been highly productive. A) All the Woods Hole Investigators and the Director of the Core Facility attended the second joint Center Directors' meeting, in Seattle (April, 2006), with travel supported by the Admin Core. B) Since June 2006, we have been the organizers of the monthly conference calls among the leadership of the four COHH, to discuss points in collaboration in all aspects of the Centers' activities. C) Interactions between the WH-COHH and other NIEHS Centers: Members of the WH-COHH presented posters at the MIT CEHS Annual Meeting, held in Cambridge. We are continuing to explore opportunities for jointly sponsored enrichment activities.
8. **Outreach and impact:** Two major activities took place this year. A) WH-COHH investigators Gast, Polz and Amaral-Zettler, and Pilot Project co-recipient Chris Reddy continued studies of the microbial populations and hydrocarbon contaminants in Lake Ponchartrain, in the wake of Hurricane Katrina. Dr. Gast of the Woods Hole Center applied for and won internal WHOI funding to host a colloquium to bring all the COHH investigators together to assess findings. This colloquium was held in November 2006. B) A proposal for a new Gordon Research Conference on Oceans and Human Health was prepared, with Dr. Stegeman of the Woods Hole Center taking the lead. This proposal received enthusiastic support from the community and has been approved by the GRC board. The new GRC on OHH is scheduled for summer of 2008.

Training and development:

The Center has supported two undergraduates in 2006, through the NSF REU grant. The two supported in 2005 were Amy Koid, Franklin and Marshall College, who worked in the laboratory of Don Anderson on a project entitled "Differential gene expression in *Alexandrium fundyense*, a saxitoxin-producing dinoflagellate", and Benjamin Tully (Rutgers) who worked with Becky Gast and Linda Amaral-Zettler on a project entitled "Monitoring current and emerging pathogens in Mt. Hope Bay". COHH support also was used for a graduate student, Claudia Martins. Other students worked on COHH projects in the labs of Mitch Sogin and Martin Polz.

Anderson- Project 1

Research and Education Activities:
Work in Year 3 has focused on Specific Aim 5 of the project: *track changes in the genotypic diversity of Alexandrium populations through time throughout the Gulf of Maine.*

Findings:

In 2005, we used microsatellite markers for *Alexandrium tamarense* to monitor the genetic composition of an unprecedented bloom of *Alexandrium fundyense* in the northeastern U.S. Results of that study indicated that the bloom populations show high genetic diversity, and that the composition of the populations changes significantly on the time scale of about one month. We were able to conduct a similar microsatellite analysis in 2006 by leveraging event response ship time provided by NOAA for *Alexandrium* monitoring in the Gulf of Maine (GOM). We collected and analyzed a time course of samples collected at one geographic location (near Cape Ann, MA) as well as samples collected from the wider GOM during a single, large-scale research cruise. The latter samples included an offshore sample from near George's Bank, which provides a valuable snapshot of offshore populations of *Alexandrium*. The microsatellite dataset is complete, and we are currently processing and analyzing the data. Comparison of the 2005 and 2006 data sets will allow us to examine the interannual variability in bloom populations, to determine if the observed population dynamics are a consistent phenomenon or whether they change year-to-year. Also in 2006, we collected a time course of samples from an *Alexandrium* bloom in a Cape Cod salt pond. Because this bloom is self-contained, analysis of these samples will provide a picture of bloom development in the absence of external sources of cells.

During this project year we also developed a novel method for generating individual DNA samples for microsatellite analysis. In 2005, we isolated single cells to provide isolates for genotyping, and subsequently waited several months for the single cells to grow up to full-sized cultures. In 2006, we developed a "mini-culture" technique, where we grew the single cells in small volumes of medium to generate just enough cells for DNA extraction. The use of this method greatly increased our throughput and decreased our overall analysis time. As a result, in 2006 we were able to genotype more than 600 individuals from our combined analyses.

In our continued collaboration with Dr. D. McGillicuddy of the Woods Hole COHH, we have provided our field data (cell counts, cyst maps, hydrographic information) for use in numerical model runs that have focused on the 2005 and 2006 bloom seasons. The results of these "hindcast" analyses are of great importance (see progress report from McGillicuddy), leading us to the conclusion that we have entered a "new regime" that will have more frequent and more intense outbreaks of paralytic shellfish poisoning (PSP) in southern New England for the next decade or more. We are now working on a manuscript that will be submitted to Nature on these findings.

Our microsatellite efforts have led to two international collaborations. The first is with Dr. Satoshi Nagai, of the National Research Institute of Fisheries and Environment of the Inland Sea, Japan. Dr. Nagai developed microsatellite markers for *Alexandrium* species in Japan and has worked with us to apply these markers to GOM populations. The second collaboration is with Drs. Uwe John and Allan Cembella at the Alfred Wegener Institute in Bremerhaven, Germany. One of the populations from the 2005 bloom has been used in a comparison of global

Alexandrium population relationships, and this data was presented at the International Harmful Algal Bloom meeting in Copenhagen in September 2006.

During the course of our work, we have involved a number of graduate and undergraduate students, as listed above. These students have worked on several aspects of this project, including the cruise sample collection and isolation of the *Alexandrium* cultures for microsatellite analysis.

Outreach:

Presentations by D.M. Anderson:

04/06 Overview of the 2005 *Alexandrium* bloom: A regional perspective. MIT Sea Grant, Cambridge, MA.

05/06 The 2005 New England red tide: mechanisms and implications for the future. New England Shellfish Sanitation Association annual meeting, Port Jefferson, NY.

11/06 Invited seminar, "The 2005 and 2006 red tides", Atmospheric, Marine and Coastal Environmental Science, Hong Kong University of Technology, Hong Kong.

Presentations by B.A. Keafer

09/06 Recent *Alexandrium fundyense* "red tide" blooms in the Gulf of Maine: Mechanisms and future implications. National Marine Life Center, Buzzards Bay, MA.

Posters:

04/06 Initial Observations of the 2005 *Alexandrium fundyense* Bloom in southern New England: General Patterns and Mechanisms. Symposium on Boston Harbor and adjacent coastal waters. New England Estuarine Research Society. Nantasket, MA.

11/06 D.M. Anderson, B.A. Keafer, D.J. McGillicuddy, and R. He. The 2005 and 2006 *Alexandrium* red tides: A tale of two blooms. Wellfleet Harbor Conference.

McGuillicuddy- Project 2

Research and Education Activities:
Our originally proposed specific aims are to:

1) Formulate a suite of population dynamics models for the various genotypes of *A. fundyense*. Model formulation will be guided by existing observations, as well as laboratory experiments to be conducted in Project 1 (Anderson).

2) Incorporate the ensemble of population models into existing models of Gulf of Maine coastal hydrodynamics.

3) Use the coupled physical-biological models to construct hindcast simulations of *A. fundyense* survey observations to be collected jointly with Project 1 (Anderson).

4) Diagnose the simulations to determine the processes regulating the space/time expression of the different genotypes in terms of *A. fundyense* abundance.

5) Utilize toxigenicity data for the various genotypes (provided by Project 1 (Anderson)) together with the coupled physical-biological models (Aim 3) to make predictions of shellfish toxicity along the coast. Toxicity predictions will be tested with observations from ongoing shellfish monitoring programs.

Findings:
Our effort this year was focused on hindcasting the historic harmful algal bloom that occurred in New England in 2005 (Anderson et al. 2005). Three potential contributing factors have been identified: (1) increased cyst abundance off mid-coast Maine, (2) favorable transport conditions that arose from two unusually strong storms with NE winds, and (3) favorable growth conditions caused by delivery of nutrients via anomalously high river discharge. We have run several numerical experiments to quantify these factors. Our results suggest that:

(A) The high abundance of cysts in the western GOM was the main cause of the 2005 bloom. The massive bloom condition would have occurred regardless of the anomalous wind conditions and riverine discharge of 2005.

(B) Wind forcing was an important regulator, in the form of both episodic bursts of northeast winds and the downwelling-favorable mean condition, causing onshore advection of offshore populations.

(C) The anomalously high river runoff enhanced alongshore transport near the coast, but had limited impact on the gulf-wide bloom distribution.

We are in the process of composing a manuscript describing these results in detail.

We have also begun an investigation of the influence of vertical migration on the vertical distribution of motile phytoplankton. Our results suggest that asynchronous migration can

potentially explain the bimodal vertical distribution of *A. fundyense* that is sometimes observed in the Gulf of Maine (Ralston et al., submitted).

Outreach:
09/06 Lecture: *Red tides in the Gulf of Maine*. Ocean Science Journalism Fellowship Program, Woods Hole Oceanographic Institution, Woods Hole, MA.

10/06 Lecture: *Toxic algae in the Gulf of Maine: observations and models*. WHOI Topics in Oceanography workshop for middle-school and high-school science teachers, WHOI Exhibit Center.

11/20/06 Together with Don Anderson, I participated via digital videoconference: Symposium on red tides, Alexandria University, Alexandria, Egypt.

1/12/07 Interview with Wanda Curtis, Working Waterfront magazine re: red tides.

Gast-Project 3

Research and Education Activities:

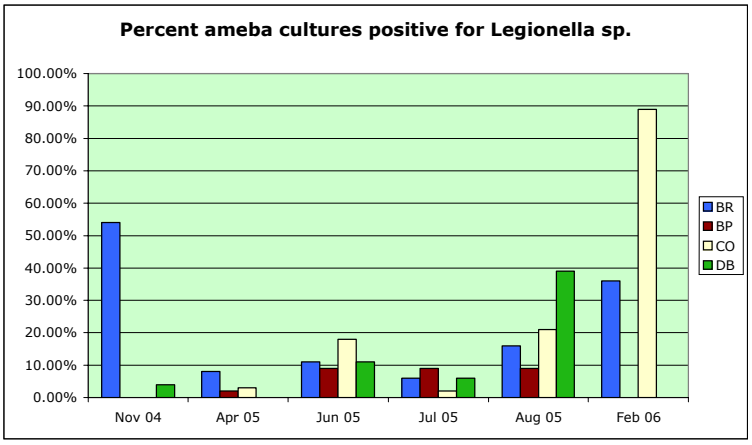
This proposal examines the diversity, distribution and persistence of human pathogens in the coastal marine environment of Mt. Hope Bay, Massachusetts. This estuarine environment has been heavily impacted by human activity, including significant thermal pollution, several sewage outfalls and the effects of past industrial activities. Monitoring for bacterial contamination occurs in the shellfish areas, but outside of these regions, very little is known about the occurrence of either bacterial pathogens or protistan parasites that are likely introduced into the Bay through sewage or runoff. The potential impact of the thermal output of the Brayton Point Power Plant on these microbial populations has not been examined at all. We hypothesize that both bacterial and protistan human pathogens are present in Mt. Hope Bay and nearby rivers, and that their distribution and persistence are affected by physico-chemical condition in the Bay. To examine this hypothesis we propose to accomplish the following specific aims:
Aim 1: Determine the distribution and persistence of human protistan pathogens in Mt. Hope Bay. We will conduct a molecular survey of human pathogens within the Mt. Hope Bay estuarine system, including sewage outfalls near and removed from thermal point sources, contaminated shellfish beds and shorebird nesting grounds, to better characterize the types of protistan pathogens present in this coastal environment. Our project will specifically target Giardia, Cryptosporidium, Naegleria, and Acanthamoeba, but will also perform general microbial eukaryotic and prokaryotic diversity assessments to determine whether there are novel sequence types present that are related to known pathogens (might represent unidentified human pathogens).
Aim 2: Determine whether naturally occurring marine amoebae can serve as reservoirs for pathogenic bacteria, and look for evidence of the natural occurrence of these associations in Mt. Hope Bay. We will test whether locally collected marine amoebae are capable of harboring the pathogenic bacteria Vibrio parahaemolyticus, Vibrio vulnificus, and Legionella pneumophila. The research will test for the induced presence and replication of bacteria in actively growing and dormant phases of the amoebae, and will utilize both PCR-based and in situ hybridization

based methods to detect naturally occurring associations.
Aim 3: Establish physico-chemical parameters associated with pathogen presence in Mt. Hope Bay and test predictive capability of water quality models regarding these correlations.
We will correlate measurements of water and sediment physico-chemical parameters with microbial community structure, including the presence of human pathogens. These measurements and our biological data will be used to assist in establishing and validating a water quality model for Mount Hope Bay that may also be used to understand and predict the potential presence of pathogens.

Findings:

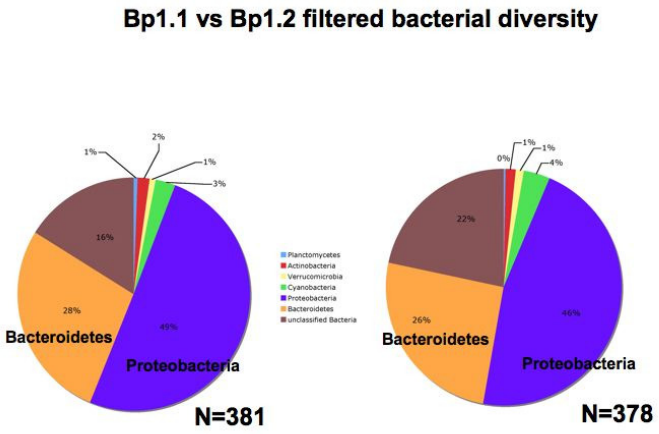
Four sites in Mt. Hope Bay were selected for our survey of human parasites and the effect of physico-chemical parameters on their distribution and persistence. Our first is located within the thermal plume of the Brayton Point power plant outlet. The size and temperature of this plume varies depending upon plant operations. The second site (Braga Bridge) is located at an underwater sewage outfall that releases secondarily treated sewage into the Taunton River just a bit north of the Bay proper. A third site is farther up the Taunton River (Dighton Bridge) at a brackish water/marsh site, and the fourth site (Common Fence) is near the bottom of the Bay at an area not directly impacted by the thermal plume or the sewage outfall. Sediment and water samples were collected in triplicate at all four sites. Our seasonal sampling officially began in November 2004, and continued through April, June, July, August, November 2005 and February 2006. An additional sampling was carried out in June 2006 in response to large amounts of precipitation and increased runoff. In addition to our four sites we also conducted two transect sampling trips in August of 2005 and February of 2006. Each time our transect began at our Brayton Point site and included 7 stations along a direct line to our control site Common Fence. Transect sampling included water samples (collected at the surface and at depth just above the sediment) and sediment samples. Sediment and water samples were again also obtained and processed from five sites in the Great Salt Lake, Utah.

Results. Ameba cultures. Amebas were enriched from sediment samples collected at each of the four sites during the year, and from the Great Salt Lake sites. These were accomplished using agar plates made of 5 different media types; non-nutrient seawater, non-nutrient freshwater, minimal media seawater, minimal media freshwater and minimal media brackish water. To date, over 150 amoeba cultures have been recovered and analyzed for the presence of legionellae. There appears to be an increase in the percent of ameba cultures positive for Legionella species during the warmer months, with all of the stations yielding positive amebas, whereas during the winter, only two sites yield positive ameba cultures (see graph below).

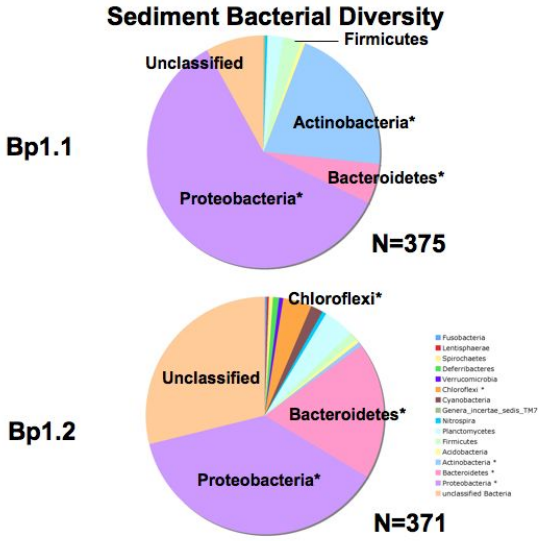


Nine of the recovered ameba cultures were positive for the presence of L. pnuemophila by amplification with a nested mip (macrophage infectivity potentiator) gene method. These results indicate that marine amebas do harbor the human pathogen, and although the number was less than 10% of the recovered amebas, they all occurred in samples collected during the summer. It was also of interest to note that none of the freshwater ameba cultures were found to carry L. pneumophila. Our future work with these cultures will be to determine whether particular ameba species or genera harbor legionellae, and to determine whether the Legionella sequence types present in amebas are also prevalent in the environmental samples. We are also pursuing methods of pathogenicity associated gene primers to determine whether any of the novel legionellae carried by amebas are potentially pathogenic.

Clone library analyses. We have compiled the first comprehensive (eukaryal, bacterial, archaeal) data from small-subunit ribosomal RNA gene clone libraries for samples collected near the thermal plume and underlying sediments of the Brayton Point Power Plant. We have partial sequences of nearly 4,000 clones from 2 different sites and have further sequenced 1,000 unique clones from these to full-length. Not surprising, our findings reveal a highly diverse consortium of the three domains including relatives of sludge bacteria, polyaromatic hydrocarbondegrading bacteria, and representatives related to the genera Staphylococcus, Streptococcus, and Clostridium. Comparisons between overall diversity in water samples for two replicate samples collected at the Brayton Point Power Plant site yielded very similar phylum-distributions shown in the pie chart below.



Our phylum-level comparisons between sediment samples, however, revealed significant differences in the microbial assemblages recovered (charts below, indicated by asterisks).

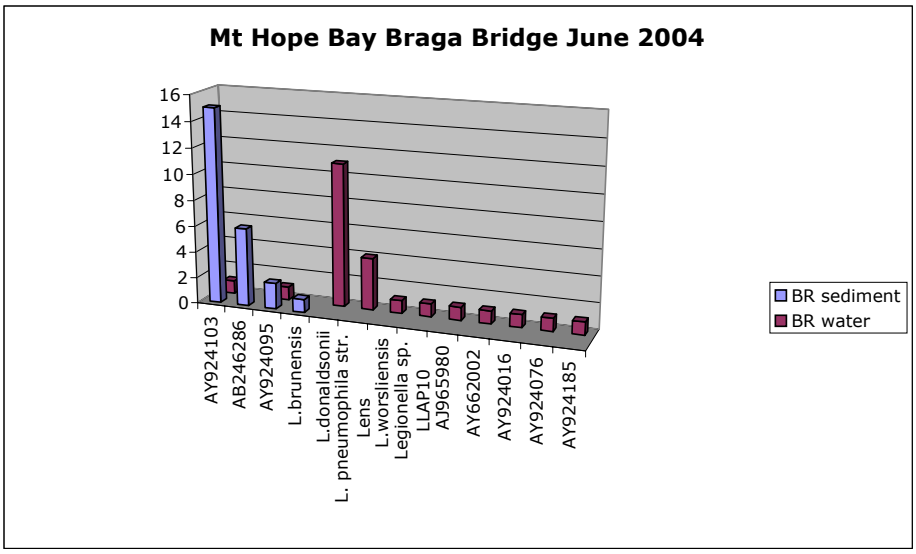


Preliminary phylogenetic placement of these environmental sequences revealed that the largest number of potential human pathogens were detected in sediment samples and not in the watercolumn samples. SARST analysis of sediment samples. Our clone library constructs provide us with a reference library of full-length sequences for our Brayton Point Power Plant site. This serves as a baseline dataset with which to compare sequences obtained from higher-throughput methods that target short variable regions of small-subunit rRNA genes. Given that our initial phylogenetic analyses identified the largest number of potential human pathogens in sediment samples, we sought to further explore the microbial diversity in these samples using methods that would allow us to sample more deeply. One such method that we have begun applying to our sediment samples is called SARST (Serial Analysis of Ribosomal Sequence Tags). This high-throughput method allows us to increase our data recovery by up to 10-fold by

sequencing a series of concatenated tags for each clone sequenced. We currently have a total of 2,365 tags from the V6 region of SSU rRNA from one of our Brayton Point sediment samples. 513 of these possessed 100% matches to known organisms in GenBank. Of these we further identified potential pathogens belonging to the following bacterial genera that possess pathogenic representatives: Shigella, Shewanella, Pseudomonas and Francisella. The last of these has especially caught our attention because there have been recent outbreaks of Francisella tularensis on the nearby island of Martha's Vineyard. Furthermore, Francisella tularensis has been shown to associate with free-living amoebae such as Acanthamoeba. The occurrence of Francisella in estuarine environments is not well-documented. As a result of this finding, we will begin to screen our samples for this pathogen in both environmental samples and ameba cultures.

Detection of specific pathogens. We are currently in the process of setting up amplification arrays with our extracted samples for use with organism specific primers. These include primers for Giardia, Cryptosporidium, Acanthamoeba, Naegleria and Legionella. The Legionella primers were used in nested amplifications of our seawater, sediment and ameba culture samples. Sediment and ameba cultures from Mt. Hope Bay showed positive amplification results, and there appears to be a seasonal pattern to the prevalence of Legionella-like species in amebas. All of the water and sediment samples from the Great Salt Lake were positive using the Legionella amplification method. Both MHB and GSL samples are not often positive for L. pneumophila, but it has been detected. Giardia and Cryptosporidium amplifications of MHB samples were carried out as a multiplex, but the results were difficult to interpret. We also found that our detection using the direct, multiplex amplification was relatively low (we needed 20- 80 cells per liter of water). We have revised our amplification strategy to include nested methods for both Giardia and Cryptosporidium, and will retest our water and sediment samples.

Legionella sequences. Samples that were positive for the presence of legionellae were amplified, cloned and sequenced. Initial sequencing of PCR products directly indicated that a diverse collection of species were present, similar to what is seen in freshwater environments, but had not been previously reported for marine environments. We pursued cloning prior to sequencing our environmental PCR products because it was likely that a mixture of species were present. From this approach we still observe a diverse collection of sequence types, representing strains (99% similarity to known sequences), species (>97% similarity to known sequences) and potentially novel genera (<95% similarity) that are sister groups to the legionellae. At a site, the sequence types present, and prevalent, change over time. The abundant sequence types in sediment are not necessarily the same as the overlying water, although they can be on occasion. In this work we have now newly described both the significant diversity of legionellae in the marine environment and have documented the seasonal pattern of their abundance. The following graph is an example of the diversity of Legionella-like sequences recovered, and the differences between sample types at the same site. Note that L. pneumophila was detected in the water at this site – the sewage outfall.



Significance:

We have completed most of our seasonal sampling within the Mt. Hope Bay system and have been processing these samples for pathogen presence and microbial community structure. Although the detection of legionellae in marine environments has been documented, the extensive presence of Legionella-like sequences in Mt Hope Bay was unexpected. This is also true for the Great Salt Lake samples. These organisms appear to be fairly prevalent in amebas recovered from the sediments of these environments, and the number of sites that they were recovered from increased as the year progressed. This suggests that legionellae not only persist there, but they can also spread. Through our combined clone library and SARST approaches, we have directly detected and documented the presence of potential human pathogens present in Mount Hope Bay. This approach has the advantage of identifying both known and emerging pathogens. We have also started SSU rRNA V6 tag-sequencing of samples from our summer and winter transects off the thermal plume using a newly acquired pyrosequencing (454) technology available at the MBL through the MBL Keck genomic facility that serves the Wood Hole COHH. Preliminary results from the first run have yielded thousands of tags for each of the eight samples run thus far. The tags are currently being processed though the V6 database pipeline at the COHH genomics core facility that will aid in identifying the closest relatives of the tags to known taxa in GenBank.

Outreach:

Mentoring of undergraduate students through REU programs, informal epidemiology training and participation in a California beach study.

The investigators have participated in several interviews with local newpapers, and were interviewed by the local NPR radio station (December 18, 2006, The Point, hosted by Mindy Todd). We have also begun informal interactions with physicians who have contacted us regarding monitoring for pathogens in coastal environments (Dr. John Harries, Sanibel Island, FL).

Polz – Project 4

Research and Education Activities:

AIM 1. To characterize and model dynamics and reservoirs of *V. vulnificus* and *V. parahaemolyticus* populations over seasonal cycles

We now have samples along the salinity gradient from the entrance of Plum Island Sound to the Parker River dam from the fall of 2004 to the spring of 2006. A total of eight surveys were completed over this period with similar tidal conditions (high tide). Vertical profiles of temperature and salinity were measured at each sampling station. Surface water samples will now be analyzed to determine major inorganic nutrients, strain diversity (by isolation) and culture independent quantification (by QPCR-CDCE). Three of the surveys were conducted in coordination with water chemistry sampling conducted by the Plum Island Estuary Long Term Ecological Research Site (PIE-LTER) PIs in which a suite of water chemistry, nutrient, chlorophyll, suspended sediment and light penetration measurements were made (see http://ecosystems.mbl.edu/pie/). In addition, we are using USGS river gauge data to document temporal variations in river discharge and PIE-LTER long-term time series of salinity, temperature, dissolved oxygen, fluorescence and turbidity at three locations within the estuary to document variability in estuarine physics and water chemistry on various time scales (tidal, diurnal, event, seasonal, and interannual).

Large seasonal and storm-driven variations in salinity and temperature were observed along the estuary. In addition the residence time – the time water-borne material is retained in a region of the estuary before being flushed into the open ocean – varies seasonally and with location within the estuary (Vallino and Hopkinson, 1998). For example, residence time in the upper Parker River ranges from a few days during periods of high river discharge typically occurring in the spring and early summer to more than a month during low discharge conditions typically observed in late summer and early fall. In contrast, residence time within Plum Island sound is a day or less for a wide range of river discharge.

AIM 2. To test the link between estuarine physics, nutrient and particle abundance and growth patterns of Vibrio species over tidal cycles.

Samples were collected roughly once per hour over a 24-hour period from July 26-27, 2006 in order to document short timescale variations in estuarine physics, water chemistry and corresponding *Vibrio* abundance and strain diversity. Samples were collected from a small boat while moving up and down the main estuary channel with the tidal currents tracking a water mass with a constant surface salinity. Physical measurements were made over the entire water column, while water samples were collected near the surface and near the bottom. In addition, PIE-LTER measurements at three locations in the estuary, spanning our sampling locations, will be used to document variations in dissolved oxygen, fluorescence, and light attenuation during our sampling period. The samples are now in the process of being analyzed for flow cytometric cell counts (bacteria, eukarya, viruses), community production rates, diversity and abundance of vibrios (by QPCR-CDCE). Moreover, we have for the first time applied a new in situ fitness assay, which we have recently developed to measure the growth rate of different vibrios under different environmental conditions (Yu and Polz, unpublished). This assay employs spiking of BrdU into natural water samples where the BrdU is taken up by active cells and incorporated into DNA when they grow. The labeled DNA can then be captured by magnetic beads and thus purified away from DNA of cells that did not respond by growth under the given conditions. The captured DNA can serve as a template for PCR based assays, such as cloning of specific genes or QPCR-CDCE, and thus allows finely tuned differentiation of how strongly different groups of

organisms grew under specific conditions (by comparison or relative abundances at the beginning and end of incubation).

AIMS 3 and 4. To determine the 'rules' of recombination, gene transfer and clonality in co-occurring V. vulnificus and V. parahaemolyticus and related vibrios AND To assess the diversity, mobility and evolutionary history of genes implicated in pathogenicity.

We have established a core set of strains, which we have characterized by multilocus sequence analysis (MLSA). This has revealed the presence of clusters of strains at different phylogenetic hierarchies. The MLSA dataset is now serving as input for a new technique, which we are currently designing, to estimate the homologous recombination rates among strains with different sequence divergence. This will allow us to estimate to what extent clusters are genetically isolated (i.e., have low enough recombination rates with other clusters) so that they are evolutionarily stable populations, which are free to diverge phenotypically. We regard our new method as a significant advance since MLSA data are rapidly building up for many organisms but estimation of recombination rates are still difficult to achieve with current methods.

Findings:

Several important findings were made during 2006. We have characterized the **homologous recombination rate** among environmental vibrios providing estimates of gene flow among potential pathogens and non-pathogens. This required the development of a new method, which will be widely applicable to multilocus sequence data. Further, we have discovered that **microdiverse vibrios are differentially distributed in the water column**. Different genotypic clusters within named species are either preferentially attached to particles and zooplankton or are found free-living. This shows that microdiverse taxa represent ecologically differentiated populations despite potential for high migration between the different compartments. Finally, we have developed a technique, which allows **estimation of in situ fitness differences among genotypes**. The method is based on BrdU spiking into environmental samples. The label is incorporated into bacterial DNA proportional to growth. The DNA can subsequently be captured on magnetic beads and the frequency of different genotypes be determined by QPCR or clone libraries. We have applied he technique for the first time to field samples during our 24-hour tidal cycle sampling.

Finally, we are continuing the analysis of our strain collection, which has already been mined for several important insights, particularly that the genomes contained in it are largely unique, even within clusters of bacteria that share common 16S rRNA sequences. This is particularly important when the evolution and maintenance of pathogenicity determinant genes is considered. We have now shown that these genes are much more widespread among related strains than anticipated; further, we have identified clear cases of gene transfer and losses indicating that pathogenicity genes may be part of a mobile gene pool, which is frequently exchanged among related strains.

Training:

This project has been undertaken in a manner that provides intense and varied training opportunities. Each of the postdoctoral researchers and graduate students listed has been

involved in developing new methods and adopting computational, microbiological, or molecular methods new to them.

The undergraduate training in conjunction with this project is a continuing effort to integrate individual initiative with broad education in a working research atmosphere. Each of the undergraduates receives many hours of personal tutelage in methods, experimental design, scientific ethics and data analysis from a senior graduate student or postdoctoral associate. They learn basic microbiology, basic molecular biology, and the statistics and modeling necessary. Several students have learned basic Matlab programming to assist them with their data processing and analysis. All their skills are taught in a general fashion, so as to be portable to other research environments. In addition, each undergraduate has ownership over an intellectually coherent portion of the research program, and are engaged in experimental design as well as experimental conduct. This is made more significant because many of the undergraduates involved in the research have been underclassmen for whom this is their first research experience.

Outreach:

Outreach is being conducted jointly with the Museum of Science to develop a program of education regarding environmental microbiology. The population in Boston is primarily aware of marine bacteria as causing beach closings, having a negative impact on shellfishing, and in relation to the large bio-reactors that process sewage in the harbor. This awareness is positive because the public, particularly boaters and fishermen, are eager to know about the microbiology of their coastal waters. However, it also brings with it a sense of negativity about bacteria that is ill-founded and can be remedied through education.

A new outreach component, which focuses on vibrio-phage interactions is the planned establishment of a phage hunting club at MIT. We are actively participating in this endeavor.

Sogin- Genomics Facility Core

Research and Education Activities:

a. Facility operations and administration:

The genomics core provides DNA sequencing services and computational support to investigators in the Woods Hole Center for Oceans and Human Health (WH-COHH). Many of the Center's projects take advantage of our strengths in molecular microbial ecology and the bioinformatics. Over the past calendar year, the genomics core facility has provided WH-COHH projects with just over 18,000 reads corresponding to ~12.5 million base pairs. The total usage over the first three years of the center's operation is 44,000 reads generating a total of 30 million basepairs. **Figure 1** Shows the usage pattern for the genomics core and **Figure 2** describes the use of the Genome Core Facility by different WH-COHH projects.

Figure 1. WH-COHH sequencing usage pattern.

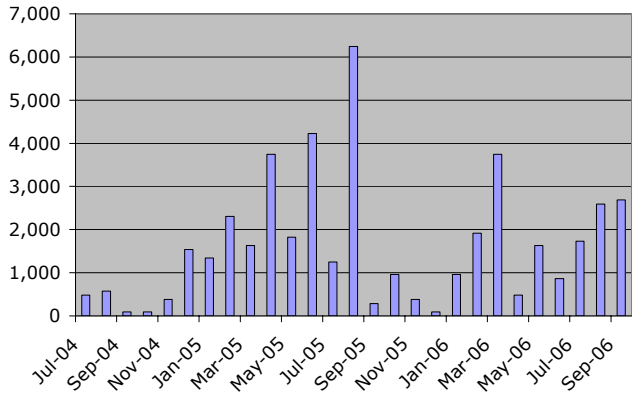
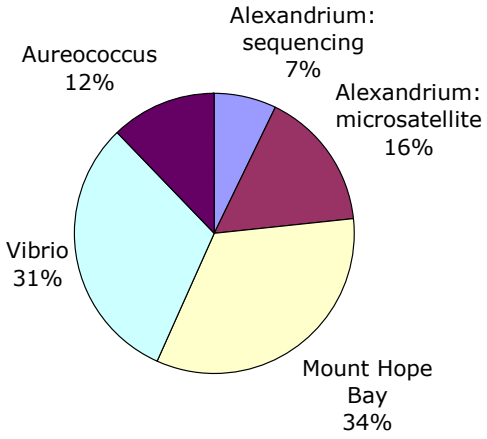


Figure 2. WH-COHH project sequencing activity.



Sequencing costs have been ~20% higher than originally projected because of increased costs of reagents for operating the ABI 3730 XL and failure of the RevPrep system for automatically producing very lost cost sequencing templates. Instead of templates costing 10 cents, we estimate each template now costs 30 cents using a protocol run on a new Biomek platform. This is still a relatively inexpensive template production cost. Other equipment additions included 20 new high performance nodes to our cluster computing environment (using institutional funds) and a Roche Genome Systems20 (GS20) pyrosequencing system funded by a new award titled ***Microbial population structure of the world's oceans*** from the W.M. Keck Foundation to the Marine Biological Laboratory at Woods Hole. We anticipate that this system will become a key element of future COHH investigations. A project under consideration is to use the GS20 to generate draft genome sequences for 20 *Vibrio* strains from Martin Polz's laboratory. Funding for that project has been provided to Martin Poltz of MIT by the Gordon and Betty Moore Foundation.

b. Scientific and technology developments in the genome core.

Using the GS20 sequencer the core facility developed a new, massively parallel DNA sequencing approach to characterize microbial populations with unprecedented level of sensitivity for detecting low abundance organisms. The International Census of Marine Microbes (ICOMM) provided the test samples and funds for reagents and the WH-COHH provided computational support for analysis of the data. Using samples from a TRANSAT cruise that followed the deep water circulation of the North Atlantic and samples from Axial, a seamount off the coast of Oregon, we developed a tag sequencing strategy for exhaustively monitoring microbial populations in the marine samples. We exploited the massively parallel, pyro-sequencing capability of the GS20 system which employs 454 Life Sciences technology, to sample hypervariable regions (rapidly evolving sequences that can record differences between both divergent and closely related organisms) from 200,000 rRNAs in a

single sequencing run. Using primers that flank the V6 hypervariable region in bacterial rRNAs, we generated PCR amplicons from environmental DNA preparations. With the GS20 system we were able to sequence many thousands of PCR amplicons from each environmental library without requiring the construction of recombinant clones or sequencing templates. Individual amplicons bound to beads in a PicoTiterPlate™ directed the pyro-sequencing reactions.

Each sequence tag served as a proxy for a specific rRNA phylotype. Matches to hypervariable regions from known phylotypes (compiled from molecular sequence databases with nearly 200,000 entries) provided information about taxonomic identity and microbial diversity. Enumerating the frequency of individual tags also provided a first-order description of the relative occurrence of specific microbes in a population. Rarefaction and non-parametric estimators such as Chao1 and the abundance-based coverage estimator ACE provided estimates of species richness.

As part of this project we have also determined that the error rate of 454 Life Science Sequencing can be as low as 0.25% when we remove reads that contain even a single undetermined base and reads that are either too short or too long. On average, this process leads to the elimination of ~20% of the reads but the remaining sequences are significantly more accurate than traditional capillary sequencing. A manuscript describing this result is in progress.

Findings:

The results of these pilot studies are impressive. Rather than estimating only a few hundred OTUs (Operational Taxonomic Units) as do most studies of marine microbial diversity, we were able to detect the presence of minor members of microbial populations and obtain more accurate estimates of total microbial diversity. The samples 53R, 55R, 112R, 115R, 137 and 138 in the table below are water column samples from the North Atlantic Flow. The FS samples come from diffuse flows of Axial Seamount. Tag sequences were collected and the program DOTUR was used to estimate rarefaction and numbers of species (both ACE and Chao1) estimators. Based upon comparisions of unique sequences (distance of 0) we estimate nearly 60,000 OTUs in the diffusive flows and tens of thousands of OTUs in the water column samples. This provides a remarkably comprehensive picture of Bacterial populations

Table 1. Diversity estimates of V6 Tags from marine samples.

Dist		0			0.03		
ID	Reads	OTU	**ACE**	Chao1	OUT	**ACE**	Chao1
53R	4,995	2,595	**16,488**	14,565	1,954	**6,826**	6,271
55R	13,906	7,006	**46,134**	40,638	5,480	**21,051**	19,252
112R	9,270	5,459	**36,213**	31,948	4,238	**15,620**	13,223
115R	10,987	5,595	**37,944**	32,428	4,209	**14,276**	12,499
137	13,898	6,594	**41,117**	36,007	4,782	**15,707**	13,782
138	14,375	7,055	**51,102**	43,875	5,666	**25,627**	23,342
FS312	4,849	2,661	**15,020**	12,905	1,968	**5,910**	5,676
FS396	17,731	8,376	**59,231**	49,964	6,568	**25,908**	23,081

and its application to projects in the WH-COHH have enormous potential. Rather than focusing upon detection of a single kind of organism or being constrained by budgets which allow the sampling of only a few hundred sequences, this application of 454 technology will allow us to develop detailed descriptions of microbial populations never before possible. The tag sequencing technology has the potential to impact all of the Centers of Oceans and Human Health. It provides a new tool for detailed monitoring of microbial populations in marine environments. This will allow detection of a wide range of human pathogens even before they become significant fractions of the analyzed populations. More recently we developed methods that allow the multiplexing of this very high throughput tag sequencing strategy so that it can be applied to multiple samples from near-shore sites that are of interest to the WH-COHH program. Our initial characterizations relevant to COHH focused on comparisons of the Mount Hope Bay environment with Eel Pond in Woods Hole. We see very significant differences in the population structures of these two systems. We have also have developed improved bacterial primers and new multiplex primers for Archaea. Protists primers will be developed when our system is upgraded from a GS20 to a GSFLX capable of longer reads during the first quarter of 2007. Over the next few months we will apply the tag sequence strategies to many samples from the Mount Hope Bay site as well as a comprehensive community analysis of microbes so that we can track point sources and transport of microbial communities associated with human sewage.

Training:

Through the genome core activities we have assisted Kevin Lin, a local high school student, in the development of his science fair project. We will also be training a graduate student, Yuko Hasegawa in the use of advanced genomic techniques in the analysis of microbial communities associated with anthropogenic activities.

Hahn- Pilot Program Project

Research and Education Activities:

a. Specific Aims

The specific objectives of the Pilot Project Program are:

A. To assess the feasibility of new areas of study, especially those that are not currently represented in our Research Project base but would contribute to the overall goals of the center.

B. To support the collection of preliminary data that can be used to generate full proposals to NSF, NIH, or other agencies or organizations.

C. To recruit scientists not currently involved in research on oceans and human health, but who may have expertise in one of these areas, to become participating members of the center and interact with other center investigators.

D. To stimulate collaborative and interdisciplinary research within the center.

E. To foster the application of new technologies and experimental approaches to questions concerning the impact of oceanic processes on public health.

F. To ensure the ability of the center to respond rapidly to new scientific information and emerging challenges in this field.

G. To contribute to the training of future researchers in the field of Oceans and Human Health through enhancement of graduate and postdoctoral training opportunities.

b. Studies and Findings:

Completed pilot projects (see list of publications in publication section)

• *Characterization of a cyanobacterial anti-algal compound* (Eric Webb and Chris Reddy, WHOI).

• *Cnidarian toxins against voltage-gated Ca2+ channels* (Robert Greenberg, MBL).

• *Marine phage as vectors of gene transfer between marine bacteria and bacterial pathogens* (Peter Weigele and Jonathan King, MIT).

Progress on pilot projects funded in year 2:

• *Transcriptome profiling in the harmful alga Aureococcus anophagefferens.* (Sonya Dyhrman, WHOI). *Aureococcus anophagefferens* is a widespread HAB species that has had severe and negative impacts. *A. anophagefferens* is allelopathic and is thought to produce a suite of natural products including a water soluble neuroactive metabolite, or toxin, that has been implicated in dose-dependent mortality and health decline in model shellfish. The goal of this COHH pilot project is to sequence three Long- SAGE (serial analysis of gene expression) libraries for this organism to examine the *A. anophagefferens* transcriptome and how it changes with external

stressors. To date, over 110,00 Long-SAGE tags have been sequenced. The ongoing analysis of these data suggests that the harmful alga *Aureococcus anophagefferens* has a robust transcriptional response to both phosphorus and nitrogen starvation. Tag annotation is ongoing. This is the first study to examine global transcriptional patterns in this organism.

• *Beach Pathogens* (Steve Elgar, Britt Raubenheimer, & Rebecca Gast, WHOI). Pathogens in coastal sediments pose a serious health risk to users of America's beaches, but the effects of waves, currents, and changes in beach sediment on pathogen distribution are not understood. The researchers hypothesize that sediments contaminated by pathogens (eg, from sewage) can be exposed when wind, waves, and currents cause changes in the beach configuration (eg, erosion or accretion), potentially creating additional human health hazards, via both direct contact with contaminated sand and exposure to pathogens carried by spray from breaking waves in the surf. Enteroccocci, a proxy for fecal contamination, were surveyed in ocean beach sands before and after large waves from a hurricane, and after a rain storm that occurred as hurricane waves were diminishing. Beach core samples were collected at 3 locations along a cross-shore transect between the high- and mid-tide lines near Kitty Hawk, NC on the Outer Banks of North Carolina in September 2006. The redistribution of enterococci was observed when beaches erode or accrete. Additional samples from beaches near outfalls will also be surveyed to determine whether they have the potential to contaminate recreational areas.

• *Names-based cyberinformatics tools for rapid response communications and outreach during event management – a pilot based on harmful algal blooms in NE US coastal waters* (David J. Patterson, MBL, and Don Anderson, WHOI). Algal blooms are increasing in frequency, extent and significance. The objective of this proposal is to promote human health by applying new informatics technologies for biology to improve communication among the public and stakeholders in response to a bloom event. The primary deliverable will be a pilot template for a web site that can rapidly call upon expert sources of information, inherit previously known but relevant information, can add local content and will combine the information dynamically in a very flexible environment. Current activity has involved the *Encyclopedia of Life* project, which will complement the web site to be developed in this project.

Third round of pilot projects

The third call for pilot project proposals was issued in September of 2006 by email to all faculty and research staff at WHOI, MBL, and MIT. Proposals were due November 1, 2006. Thirteen proposals were received and subsequently were reviewed and scored by members of the Internal Advisory Committee. The proposals requested approximately $430,000 in total costs (~$275,000 direct costs). Ten were from WHOI (multiple departments), two were from MBL, and one was from MIT. All applicants were provided with written reviews. Four projects were selected for full or partial funding:

• *Anthropogenic impacts and profiling fecal microbial populations at a salt marsh* (Mitch Sogin, MBL)
Fecal coliforms are indicator organisms that warn of possible fecal contamination and its potential impact on human health. Coliform surveillance activities generally rely upon

cultivation assays or molecular Microbial Source Tracking (MST) technology to assay for the presence of a particular indicator organism in environmental samples. This project will employ a massively parallel DNA tag sequencing strategy to profile entire microbial communities in the Little Sippewissett Salt Marsh and the barrier Woodneck Beach. This study site is surrounded by ~40 homes, most of which are occupied on a seasonal basis and all are serviced by septic systems. The marsh communicates with Buzzards Bay through a tidal inlet. Multiple times each summer high coliform counts indicate dangerous conditions for recreational use. Possible coliform sources include human waste from failed septic systems, bird populations or other animals. The objective is to use the tag sequence data to locate specific sources of fecal contamination and identify suites of genes that could serve as multi-species indicators of human pollution. The experimental strategy takes advantage of rapidly evolving hypervariable regions in ribosomal RNAs and our ability to generate many thousands of short DNA tag sequences using 454 Life Science's pyrosequencing sequencing technology on a Roche Genome Sequencer 20 System. Samples will be collected throughout the summer of 2007 and tag sequence will be determined form those microbial populations that were harvested on days corresponding to high coliform count measurements provided by the Barnstable Health Department. Because the technique returns quantitative information about most if not all members of a microbial community, these researchers will be able to track the location of contamination and map its distribution through the marsh.

• *Transcriptional Markers of Life Cycle Transitions in Harmful Algal Blooms* (Don Anderson, WHOI)
Bloom dynamics of the red tide dinoflagellate, *Alexandrium fundyense* are driven in large part by transitions in its life cycle. While these stages are well documented, the biological and oceanographic forces that trigger transitions between the stages are not. A major obstacle to determining the conditions that trigger these transitions is our inability to rapidly identify sexual stage cells. Here, a novel transcriptome experiment will be used to discover molecules that are uniquely expressed by conjugating gamete cells and by germinating cysts. This experiment will utilize sequencing-by-synthesis technology that is newly available through the Bay Paul Center. Data from the experiment will be analyzed in a fashion that is directly analogous to SAGE. Results will be used to leverage a larger, multi-year proposal to verify and validate transcriptional markers discovered through the proposed work.

• *The Economic Effects of Harmful Algal Blooms: A pilot project to estimate the costs of human respiratory ailments associated with aerosolized brevetoxins* (Porter Hoagland and Di Jin, WHOI; Lora Fleming, Miami)
This study will estimate the costs-of-illness associated with human respiratory ailments that arise as the consequence of the aerosolization and coastal to inland transport of brevetoxins from blooms of the marine dinoflagellate, *Karenia brevis*, in the Gulf of Mexico. The research will develop models to link the occurrence of HAB events in the coastal-ocean with exposures to aerosolized brevetoxins. The researchers will compile datasets and develop models of illness rates that would permit historical estimates of these kinds of impacts and the simulation of future potential impacts. This is a proof-of-concept Pilot Project designed to develop an analytical framework that can be used on a larger scale, using more extensive datasets in the future. It is critical that we understand the costs of natural hazards such as HAB events for at least two reasons. First, the nature of the costs (their effect) and their incidence (who is affected and at

what rate) will enable the characterization of feasible actions to mitigate the costs. Second, the scale of the costs will help resource managers, scientists, and the general public to gauge the levels of and need for potential mitigation.

• *The Economics of Human Health Risks from Pathogens and Toxins in the Marine Environment* (Hauke Kite-Powell and Porter Hoagland, WHOI)
These researchers will produce an "order of magnitude" estimate of the annual human health cost imposed on residents of the United States by exposure to pathogens and toxins from the marine environment. The estimate will be derived from a review and synthesis of information in the existing literature on (1) the spatial and temporal prevalence of marine pathogens, (2) the pathways by which they affect humans and the potentially exposed populations, (3) the human health effects of exposure, and (4) the economic cost of resulting medical conditions. The estimate will inform future research on pathogens in the marine environment, allowing scholars and public officials to target pathogens and settings where improved scientific understanding is most likely to produce significant economic benefits, and setting the stage for focused economic analyses. (co-funded with the WHOI Marine Policy Center)

The funded pilot projects address important questions regarding the interactions between the oceans and human health. Many of the projects represent new directions for the Center, expanding the scope of our research efforts into, for example, marine natural products and the economics of pathogens and toxins. The projects also serve to add new scientists as participating members of the center, expanding our representation at all three member institutions. Many of the funded or proposed pilot projects involve postdoctoral researchers or graduate students, and will thus contribute to the training of the next generation of researchers in this area.

During the next project period, we will continue the Pilot Project Program, issuing a new call for proposals and funding 2-3 new projects. We also will follow-up the projects funded this year and last year, to gauge their progress and success in achieving research goals and obtaining externally funded grants using preliminary data generated in the pilot projects.