**Annual Report for Period:** 05/2005 - 05/2006
**Submitted on:** 03/09/2006
**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:** No
**Contribution to Project:**
Senior Scientist and PI, is responsible for project oversight, data review and interpretation, and preparation of manuscripts.

**Name:** Erdner, Deana
**Worked for more than 160 Hours:** No
**Contribution to Project:**
Research Associate and Co-PI, is responsible for technical oversight of experimental work and sampling efforts, data review and interpretation, and preparation of manuscripts.

**Name:** Gast, Rebecca
**Worked for more than 160 Hours:** No
**Contribution to Project:**
Associate Scientist and Co-PI - responsible for project oversight.

**Name:** Amaral Zettler, Linda
**Worked for more than 160 Hours:** Yes
**Contribution to Project:**

**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.

**Name:** Hajduk, Steve

**Worked for more than 160 Hours:** No

**Contribution to Project:**

Director of the Pilot Project Program

**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:** Marcelino, Luisa

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Microarray technolgy development for monitoring of thousands of co-occurring bacterial species in environmental samples.

Support provided by NSF.

**Graduate Student**

**Name:** Brosnahan, Michael

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Graduate Student, is responsible for characterization of microsatellite markers used for population studies and the development and testing of quantitative PCR methods.

**Name:** Benoit, Jennifer

**Worked for more than 160 Hours:** No

**Contribution to Project:**

Ms. Benoit is a first year graduate student in the WHOI-MIT joint program and resides in the Polz lab. She has started to participate in characterization of strains and in determination of genetic diversity of the Vibrio isolates. Her interests lie in the ecology and evolution of pathogenic bacteria. Ms. Benoit can draw on ample experience collected during two years as a technician in a fish pathogenicity laboratory.

**Name:** Nardello, Ilaria

**Worked for more than 160 Hours:** No

**Contribution to Project:**

Guest student from the University of Viterbo, participated in the 2005 Alexandrium survey summer cruise in the Gulf of Maine.

**Undergraduate Student**

**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:** No

**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:**

**Name:** Moran, Dawn

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

**Name:** Laatsch, Abby

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

**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:**

A database specialist and scientific programmer, works under the direction of Dr. Sogin to build a web-based high throughput pipeline for processing DNA sequencing data. This pipeline will streamline the process of constructing phylogenetic trees from the molecular data sets generated by members of the COHH team.

**Other Participant**

**Research Experience for Undergraduates**

**Name:** Mahoney, Brenna

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Brenna Mahoney is a Cornell University student, participated in the project as a REU student in the summer of 2005. Her work focused on the establishment and analysis of clonal isolates from a 2005 bloom population of A. fundyense.

Support provided by NSF.

**Years of schooling completed:** Junior

**Home Institution:** Same as Research Site

**Home Institution if Other:** Cornell University

**Home Institution Highest Degree Granted(in fields supported by NSF):** Associate's Degree

**Fiscal year(s) REU Participant supported:**

**REU Funding:** REU supplement

**Name:** Halliday, Elizabeth

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

An REU student - worked in the summer of 2005 and participated in sampling and analysis of Legionella. Her project was selected as a poster for the 2005 Ocean Sciences meeting and as a result of her research experience Elizabeth has decided to pursue graduate studies in marine science.

Support provided by NSF.

**Years of schooling completed:** Sophomore

**Home Institution:** Same as Research Site

**Home Institution if Other:** University of Maryland

**Home Institution Highest Degree Granted(in fields supported by NSF):** Associate's Degree

**Fiscal year(s) REU Participant supported:**

**REU Funding:** REU supplement

## 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

Roger Williams University

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

Woods Hole United States Geological Survey

Naval Research Laboratory

United States Department of Agriculture

## Activities and Findings

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

**Findings:**
See attached file.

**Training and Development:**
See attached file.

**Outreach Activities:**
See attached file.

## Journal Publications

He, R., McGillicuddy, D.J., Lynch, D.R., Smith, K.W., Stock, C.A., and J.P. Manning., "Data Assimilative Hindcast of the Gulf of Maine Coastal Circulation.", Journal of Geophysical Research, p. , vol. , (  ). Submitted

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

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

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", PNAS, p. , vol. , (  ). Submitted

## Books or Other One-time Publications

## Web/Internet Site

**URL(s):**
http://www.whoi.edu/science/cohh/whcohh/index.htm
**Description:**
Woods Hole Center for Oceans and Human Health - web site

2 Additional Web Sites:

www.whoi.edu/people/rgast

http://www.whoi.edu/devel/lori/cohhNEW/whcohh/genomics/about.htm

Data from R/V Oceanus voyage 412 are available at:
http://science.whoi.edu/users/mcgillic/cohh/oc412/data/

## Other Specific Products

**Product Type:**
**Physical collection (samples, etc.)**
**Product Description:**
Strain collection - transferring to the Ocean Genomes Legacy Foundation (Polz Lab - MIT)
**Sharing Information:**
Will be available to interested researchers.

**Product Type:**
**Software (or netware)**
**Product Description:**
Microarray analysis software development (Marcelino, Polz Lab MIT)
**Sharing Information:**
When available will share with the ocean science community

**Product Type:**
**Software (or netware)**
**Product Description:**
Analysis of gene transfer events software (Friedman, Polz Lab - MIT)
**Sharing Information:**
Will share when complete

**Product Type:**
**Poster presentation**
**Product Description:**
3 Posters presented at the 2005 Ocean Sciences meeting (Gast, Amaral-Zettler, Halliday)
**Sharing Information:**
Was on display at above meeting.

## Contributions

**Contributions within Discipline:**
Contributions

To the development of the principal discipline of the project:

Project 1:
To the development of the principal discipline of the project: We have demonstrated through microsatellite analysis that a significant amount of intraspecific genetic diversity exists in both A. fundyense and A. ostenfeldii in the Gulf of Maine, despite their apparent homogeneity at the level of ribosomal DNA sequence. This is one of the few examples where intraspecific variability has been explicitly demonstrated in a marine phytoplankton species.

This genetic diversity exists in both 'background' level populations as well as bloom populations. Samples collected from the large bloom of

2005 allowed us to assess changes in the genetic composition of Alexandrium over the course of a bloom, demonstrating that the populations that make up the bloom change over time, all while maintaining a high genetic diversity among individual cells. These findings support our primary hypothesis that bloom populations are heterogeneous, and they provide the first in-depth analysis of the way that a harmful algal bloom evolves over time. This information brings us another step closer to understanding how blooms form and how they persist. Incorporation of this knowledge into the existing physical-biological models of Dr. Dennis McGillicuddy is a primary objective of this work, with the intent of enhancing our predictive capabilities for harmful bloom events.

Project 2:
To the development of the principal discipline of the project: We hypothesize that the aggregate distribution of A. fundyense is composed of a mosaic of genetically distinct subpopulations, each with different physiological and/or behavioral responses to environmental conditions. The goal of this project is to understand the hydrodynamic and biological controls on these populations, their toxin production, and how these factors ultimately determine fluctuations in shellfish toxicity.

Project 3:
To the development of the principal discipline of the project: We have extensive experience using molecular methods with various types of environmental samples, but it is important to establish that the methods being used work with the types of samples being collected. In particular, that the volume of water being collected is adequate for obtaining good representation of the organisms present in the environment, and that the DNA can be reliably amplified. Sediment samples and water samples that have large amounts of detritus have compounds (primarily humic acids) that co-purify with DNA and strongly inhibit amplification. Our results from the July samples indicate that we can reliably extract and amplify using our protocols and that we have collected appropriate amounts of material for analysis.

Project 4:
To the development of the principal discipline of the project: It is increasingly recognized that pathogens evolve from harmless variants via lateral gene transfer in the environment. Thus, our explicit goal of understanding what factors select for the emergence and persistence of pathogenic strains in the environment is relevant to a wide range of bacteria of medical importance. In this sense, the study takes an exemplary character.

The sequencing of environmental Vibrio genomes 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:
To the development of the principal discipline of the project: The project will provide an automated mechanism for processing raw sequencing data, annotating sequences and inferring phylogenetic trees that will then be cross-mapped to geographical distribution of pathogens and closely related non-pathogens.

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.

Pilot Program Core:
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.

**Contributions to Other Disciplines:**
Contributions

To other disciplines of science or engineering:

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.

Project 1:
To other disciplines of science or engineering: In addition, we are developing a method û quantitative PCR û for the identification and enumeration of Alexandrium ostenfeldii. Molecular methodologies for the quantification of harmful algal species are becoming increasingly important in public health monitoring programs and, as such, this methodology should be of direct use to scientists and coastal managers in many parts of the world.

Project 4:
To other disciplines of science or engineering: The project will also contribute significantly to the general fields of microbial ecology and evolution, environmental engineering and oceanography of naturally occurring pathogen populations. Specifically, we will provide some of the first comprehensive measures of rates and bounds of lateral gene transfer and population diversity of free-living bacteria. These have significant relevance for interpretation of microbial diversity in natural and engineered systems.

Genomics Facility Core:
To other disciplines of science or engineering: COHH results in the form of distribution and persistence of human pathogens in marine environments will be integrated into the ICoMM initiative.

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

Pilot Program Core:
The funded and proposed 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. The projects also serve to add four 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 Human Resource Development:**
Contributions

To the development of human resources:

Project 4:
To the development of human resources: Currently, the project is partially supporting the efforts of one postdoc and one graduate student. We are also committed to involvement of undergraduate researchers in the project and typically have two undergraduates in the laboratory.

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:
To the development of human resources: Potential opportunity for training undergraduate and graduate students in the new MBL/Brown University Joint Program.

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.

**Contributions to Resources for Research and Education:**
Contributions

To physical, institutional, and information resources that form the infrastructure for research and education:

Project 4:
To physical, institutional, and information resources that form the infrastructure for research and education: We are preparing a strain database, which will be publicly accessible. Our collection of strains from the site is currently unique in its scope and size. We are collaborating with the Ocean Genomes Legacy Foundation, which is considering housing the strains.

Genomics Facility Core:
To physical, institutional, and information resources that form the infrastructure for research and education: Databases of molecular sequences and phylogenetic inferences will be posted on publicly available websites.

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:**

Contributions

To the public welfare beyond science and engineering:

Project 2:
To the public welfare beyond science and engineering: The study of Harmful Algal Blooms (HAB) and pathogens is essential to determine their potential to impact human health. Blooms of the toxic dinoflagellate Alexandrium fundyense are annually recurrent phenomena in the Gulf of Maine during the spring and summer months. Toxins produced by A. fundyense accumulate in the tissues of filter-feeding shellfish such as mussels and clams. Human ingestion of these contaminated shellfish can lead to Paralytic Shellfish Poisoning (PSP), a potentially fatal illness. Understanding the factors that determine the distribution and abundance of A. fundyense is therefore of considerable economic and public health interest.

Project 3:
The widespread diversity, distribution and persistence of legionella-like species in the marine environment is unexpected, but provides support for the hypothesis that L. pneumophila may be present in these environments, especially in amoebae. The unexpected diversity of these organisms in the marine environment raises the question of whether they may be pathogens of other organisms as well as humans.

Project 4:
To the public welfare beyond science and engineering: The last few years have seen rising concern about the emergence of new variants of pathogens and spread of existing pathogens due to local or global environmental change. This has focused attention on the ecological context of pathogens in both the human body and the environment. Our project seeks to explore fundamental aspects of the emergence and persistence of pathogens in the environment and is thus of high relevance for monitoring, predicting and possibly preventing pathogen outbreaks and ensuring the safety of seafood and aquaculture.

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.

## 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:

Any Book

**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 continues to foster the development 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 and 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 these accounts.
3. Center Investigator meetings have been held on a regular basis, approximately monthly. All principle and associated investigators in the projects and cores are

involved in these meetings. The meetings 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 next meeting with the EAC to review the Center programs will occur in summer 2006.
5. The Administrative Core continues to oversee the Pilot Project program. Three projects were selected for funding in 2004, and three in 2005. The pilot projects involve investigators at all three institutions, WHOI, MBL and MIT. We coordinated our call for proposals with the MIT Center for Environmental Health Sciences.
   - *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).
   - *Transcriptome profiling in the harmful alga Aureococcus anophagefferens.* (Sonya Dyhrman, WHOI).
   - *Beach Pathogens* (Steve Elgar, Britt Raubenheimer, & Rebecca Gast, WHOI).
   - *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 (D.J. Patterson, MBL, and D. Anderson, WHOI).
6. The combined Centers Web site was established at WHOI and incorporates all four Centers (http://www.whoi.edu/science/cohh/index.htm).
7. The interactions with the other COHH have been highly productive. A successful Center Directors Meeting was held in Miami, attended by all members of all four centers. The Centers joined forces, obtaining SGER grants from NSF to address microbial populations and pathogen issues in Lake Pontchartrain after hurricane Katrina. The Woods Hole Center has hosted conference calls dealing with the sampling and analyses in the Katrina effort.
8. **Outreach and impact:** The Center's activities have had substantial importance for local, State and Regional public health concerns. Of particular importance, WH Center investigators identified the massive red tide in the Massachusetts Bay and the Gulf of Maine, in the spring of 2005. The discovery allowed a timely alert and monitoring and closure of shellfish beds.

   Outreach also involved the participation of the Director in the IOOS Public Health Workshop in St. Petersburg in January 2006.

**Training and development:**

The REU grant associated with the WH-COHH supported two students in the summer of 2005. Megan Jamison, Humboldt State University, did a study of the role of tides in exchanging water (and stuff in it) between estuaries and the coastal ocean. Brenna Mahoney, Cornell University, did a study of Intra-population genetic variation of *Alexandrium fundyense* in the Gulf of Maine using microsatellite DNA.

**Anderson – Project 1**

**Research and Education Activities:**

Work in Year 2 has focused on Specific Aims 1 and 2 of the project:

1. Identify a genetic marker capable of distinguishing different genotypes within *A. fundyense* and *A. ostenfeldii* populations;
2. Determine the extent of natural genetic diversity of *Alexandrium* spp. in the Gulf of Maine.

**Findings:**

For Aim 1, our initial work examined the ribosomal DNA genes, to determine sequences that would allow us to differentiate between *Alexandrium* genotypes in the Gulf of Maine (GOM). The ribosomal regions proved to be highly similar, so at the end of Year 1 we had begun to characterize a set of recently published microsatellite markers for *Alexandrium tamarense* (*A. tamarense* and *fundyense* belong to the same species complex). In Year 2 we demonstrated that these markers work very well in *A. fundyense*, and used them to characterize the genetic diversity of a set of 55 clonal A. *fundyense* strains that were isolated from the GOM in 2003 (Task 1.1). We have observed very high genetic diversity among these isolates (93% unique genotypes). We are at present analyzing the toxin composition of these isolates, which will complete the data we need for publication of this work.

Efforts to define a suitable genetic marker for *Alexandrium ostenfeldii* have proved more difficult. As in *A. fundyense*, the ribosomal DNA sequences of *A. ostenfeldii* strains from the GOM are highly similar. We also tested the *A. tamarense* microsatellite markers with *A. ostenfeldii*, but they proved unsuitable for this species. The microsatellite markers have proved to be a useful tool for *A. fundyense*, therefore we have initiated a collaboration with Dr. Satoshi Nagai of the National Research Institute of Fisheries and Environment of the Inland Sea in Hiroshima, Japan, to isolate microsatellite markers for *A. ostenfeldii*. Dr. Nagai's group published the *A. tamarense* microsatellite markers, and has identified microsatellite sequences in a number of other harmful algae. Dr. Nagai visited our lab for two weeks this October, and will return to Japan with the materials necessary for the microsatellite identification. We hope to have the markers available for testing before the end of 2006.

We have also made significant progress towards Aim 2: Determining the Natural Genetic Diversity of *Alexandrium* in the GOM. From May 9-18 2005, we had our first research cruise covering the geographic extent of the Gulf. During the cruise, we realized that we were sampling the beginning of an *Alexandrium* bloom, which turned out to be the largest bloom in the region in at least three decades. From plankton samples from the cruise and subsequent sampling, we isolated several hundred clonal *Alexandrium* cultures and genotyped them using the microsatellite markers described above. These isolates are derived from 6 distinct samples taken throughout the first 6 weeks of the bloom. Overall, these samples also show very high genetic diversity (>98%), like our 2001 isolates. Most notably, the microsatellite results indicate that the southernmost populations, collected late in the bloom, are genetically distinct from the earlier populations sampled during the research cruise.

Brenna Mahoney, a Cornell University student, participated in this work as an REU student through the Center. She participated in research cruises, and assisted in the isolation and genotyping of the cultures from the research cruise. Brenna's project was submitted to a competition among REU projects, and she was selected to present her work at the recent AGU/ASLO meeting in Honolulu, HI February 20-24, where it was very well received.

**McGillicuddy – 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 a large-scale oceanographic survey of *A. fundyense* in the Gulf of Maine, voyage 412 of R/V Oceanus (Aim 3). The station grid consisted of a set of transects spanning from Massachusetts Bay to the Bay of Fundy. Given the surprisingly high abundance of *A. fundyense* in Massachusetts Bay, that area was resurveyed at the end of the cruise. During the one-week time period in between Massachusetts Bay snapshots, the /A. fundyense/ population increased substantially. High-resolution profiles were taken at selected stations to examine genetic structuring of the population with depth. One profile in the Bay of Fundy, one near Casco Bay, and two in Massachusetts Bay were obtained. Sampling strata was 0-30m with 3m nominal resolution. Vertical interval chosen on the basis of cell profiles from Martin et al. (2005), Townsend et al. (2005) and Townsend et al. (2001) which show very few cells below 30m. Moreover, SHA profiles from the present cruise indicate comparable numbers at the surface and 10, with background levels at 20m.

Drifters were deployed along three transects: Cape Ann (3 surface drifters), Penobscot

Bay (6 surface drifters), and the Bay of Fundy (9 drogued drifters). The Cape Ann drifters transited southeast offshore of the back side of Cape Cod. The Penobscot Bay drifters went southwest in a generally along-coast direction. The line of drifters in the Bay of Fundy behaved quite differently, with the northwest part of the line moving to the southwest and the southeast part of the line moving to the northeast. This cyclonic shear is consistent with the hypothesized gyre at the mouth of the Bay, but the drifters have not been in the water long enough to close the loop.

Hydrography, shipboard ADCP velocity measurements, GoMOOS mooring observations, and coastal tide gauges were assimilated into a numerical model to provide the hydrodynamic context in which to interpret the biological observations. The fidelity of the circulation fields was quantified by evaluating the ability of the model to simulate the trajectories of drifters deployed during the survey. Comparison of the simulated and observed trajectories from the Cape Ann line indicates that the model drifters generally go in the right direction, but their speed is too large. In contrast, the simulated drifters in the Penobscot Bay line move slower than observed, and do not turn offshore as much as the real drifters. The westernmost drifters in the Bay of Fundy move to the southwest as the real drifters do, but the easternmost drifters compare poorly with observations: the real drifters do northeast, whereas the simulated drifters go west. The nature of these discrepancies will be investigated in a hindcasting study.

Our observations during voyage 412 of R/V Oceanus in May 2005 were the first measurements of what turned out to be an extraordinary bloom event, reported in detail by Anderson et al. (2005). The outbreak eventually closed shellfish beds from central Maine to Massachusetts, including Nantucket Island and portions of Martha's Vineyard, and resulted in the closure of 40,000 km2 of offshore federal waters as well. The coastal *A. fundyense* bloom was exceptional in several ways: high toxin levels were measured farther south than ever before in New England; levels of toxicity in many locations were higher than previously observed at those stations; for the first time toxicity at some locations was above quarantine levels; cell concentrations far exceeded those observed in the coastal waters of southern New England in the past; and for the first time, the governors of Maine and Massachusetts officially declared the red tide to be a disaster, clearing the way for federal assistance.

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**Gast – Project 3**

**Research and Education Activities:**

1. Describe major research and education activities of the project

**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 and 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:**

2. Describe major findings

   To date we have collected 8 sets of water and sediment samples throughout the different seasons from our 4 sites in Mt Hope Bay.

Aim 1: We have completed our first comprehensive (eukaryal, bacterial, archaeal) 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 of life including relatives of sludge bacteria (*Comamonas*, *Acinetobacter*, *Atopostipes*, and hits to uncultivated sludge bacteria), polyaromatic hydrocarbon-degrading bacteria, and representatives related to the genera *Staphylococcus*, *Streptococcus*, *Clostridium* and *Legionella*. We are also obtaining hits to harmful algal bloom species related to *Pseudo-nitzchia, Heterocapsa* and *Alexandrium*. Phylogenetic analyses will further unveil the relationships of many of these clones and determine whether they are related to known pathogens and may possibly represent undescribed taxa.

Aim 2: We have used a nested PCR approach to screen our Mt Hope Bay water and sediment samples, and amoeba cultures for the presence of *Legionella* species. The following two charts summarize the data. Briefly, water and sediment samples are positive for *Legionella* species at all times of the year (Figure 1). A portion of the amoeba cultures were also positive, with more of the recovered amoebae having *Legionella* species in the summer (Figure 2). None of the sequences from the PCR fragments recovered were identical to *L. pneumophila*, but only 34 of the sequences could be grouped as similar to each other. The other 24 sequences were different from each other and from other *Legionella* species in the database. This indicates a large diversity of *Legionella* species in the marine environment, either as free-living organisms or present within amoebae. The samples will also be screened with primers specific for *pneumophila*.

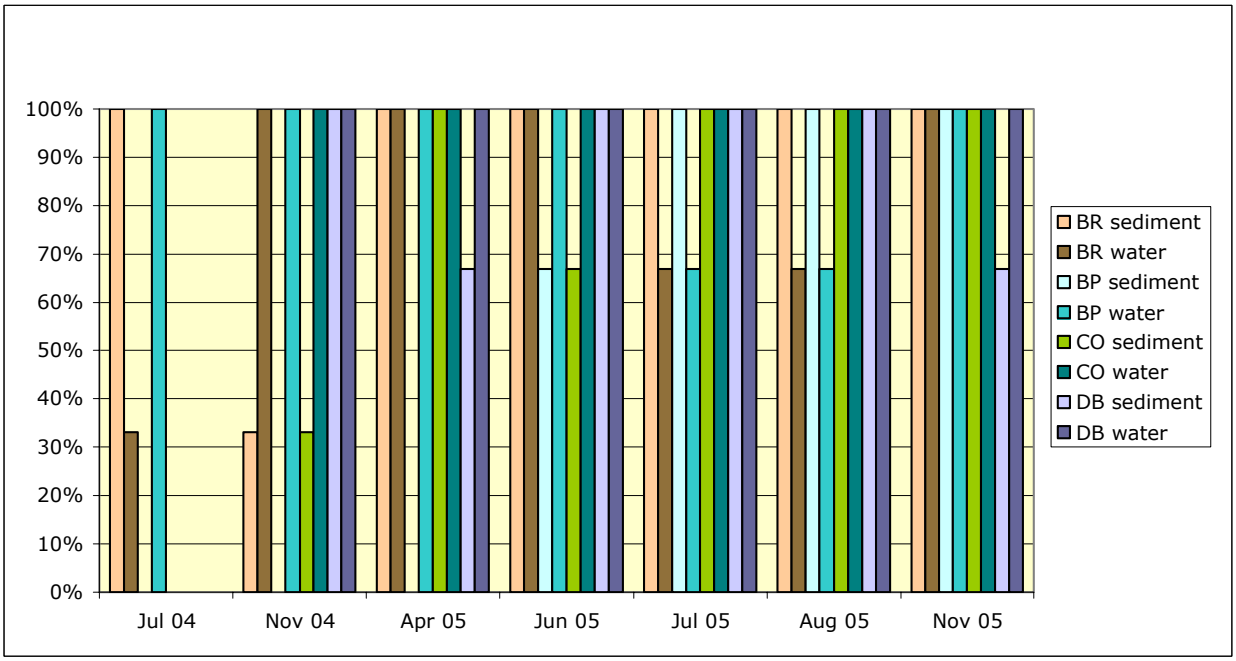


Figure 1. Percent water and sediment samples positive for *Legionella* species.

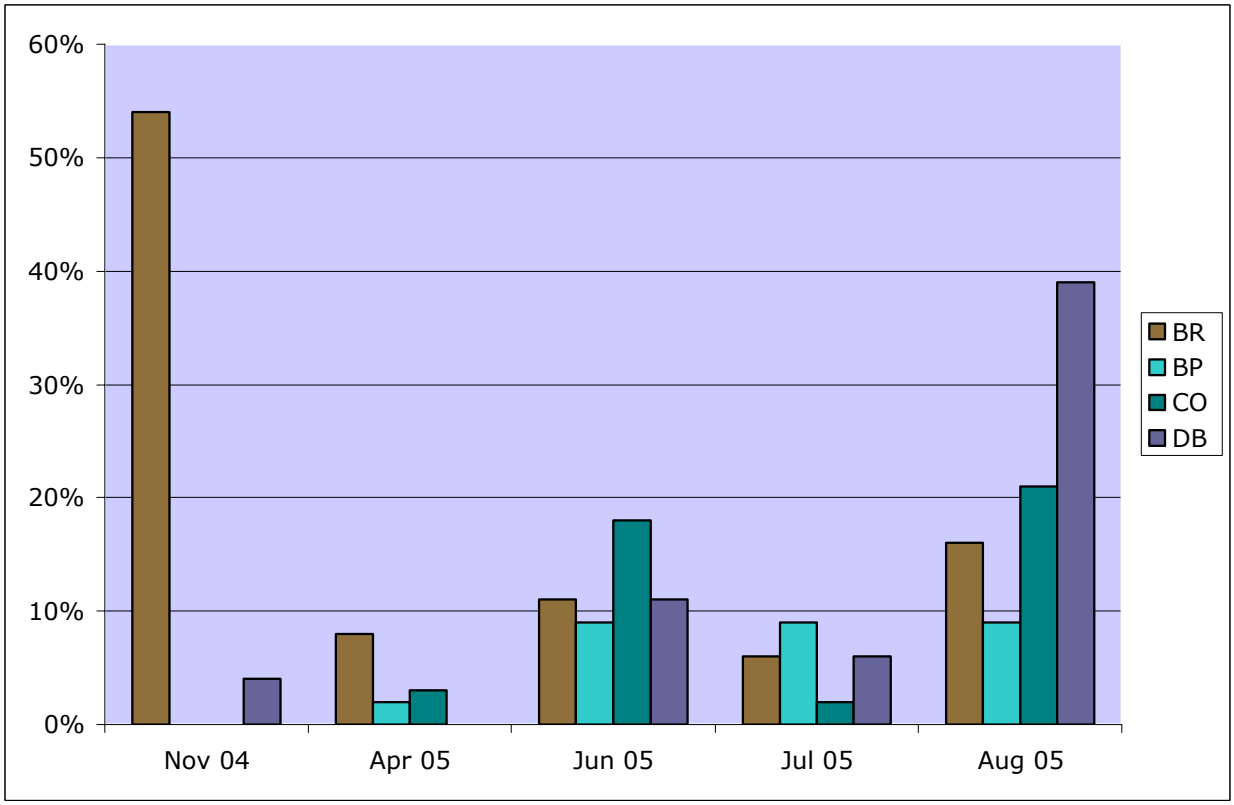


Figure 2. Percent amoeba cultures positive for *Legionella* species.

In addition to our Mt Hope Bay samples, we have examined a set of water and sediment samples from the Great Salt Lake in the lower portion of the lake where numerous sewage outfalls from the city are present. These samples were collected in August of 2005 by Dr. Wayne Wurtsbaugh and his undergraduate students. All of these samples were also positive for *Legionella* species, as were many of the amoeba cultures. None of these sequences were identical to *L. pneumophila*, but the samples have not been screened with pneumophila-specific primers. The sequences were distinct from the ones we recovered from Mt Hope Bay, and overall tended to cluster together in similarity analyses. This further confirms the unexpected diversity of *Legionella* species in saline environments that we have discovered.

3. Describe opportunities for training provided by your project
   An undergraduate REU student worked with us during the summer of 2005 and participated in sampling and analysis for *Legionella*. Her project was selected as one of the invited REU posters at the 2005 Ocean Sciences meeting. As a result of her research experience this summer, Elizabeth has decided to pursue graduate studies in marine science.
   We also mentored a teacher from Danbury Connecticut (Bob Weinheimer) that was participating in the ISIS (Southern Connecticut State University's Sixth Year in Education Certificate) Program. As part of this program he

participated in both field sampling and lab work centered around water and sediment sampling of Mount Hope Bay. We hope to maintain ties with this teacher and work towards developing curricula that will take advantage of the data collected during our field seasons.

4. Describe outreach activities
   Linda - iVisit

**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 have now completed the sampling of vibrioplankton over seasonal cycles in the PIE estuary, which we use as our model site. Data collection included temperature, salinity, nutrients, and cells for culturing and DNA extraction. DNA extracted from the environment will be analyzed by QPCR to obtain culture-independent measures of vibrio population dynamics. Moreover, several hundred *Vibrio* strains are being characterized in a variety of fashions to create a unique resource. Analyses include sequencing of 16S rRNA and six housekeeping genes to determine phylogenetic identity, presence/absence of pathogenicity determinant genes, and metabolic and physiological diversity. To provide a background for additional genomic information, the complete genomes of two isolates have been sequenced. Further, efforts have begun to provide the response of growth parameters to variation in salinity and temperature. Sequencing of potential regulation genes that control these responses is planned.

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

Preparations for AIM 2 have been completed and the actual sampling will be carried out during the 2006 season. We have developed and tested a new method for assaying in situ growth of specific bacterial populations (Yu, Thompson and Polz, in preparation); this combines BrdU labeling of actively growing bacteria and immunocapture of DNA with subsequent quantitative measurement of specific alleles by QPCR-CDCE, or by cloning and sequencing. Because genomic DNA is captured it is possible to assay growth responses and/or differences on varying scales of genetic differentiation (from rRNA alleles to specific functional genes).

**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 made considerable progress in both aims. First, sequencing of six protein coding genes for ~300 strains provides a picture of recombination and discontinuity in the phylogeny of coexisting strains. Software has been developed to assist in exploring this very complex data. So far, we were able to show that effective bounds of recombination, consistent with observed population structure exist.

A study of virulence factors (which may have ecological roles beyond causing vertebrate disease, and thus may represent an ecological reservoir of pathogen genes) is underway. These are studied both using classical phenotypic and molecular methods. Contacts that provide access to new microarrays developed for identifying *Vibrio* virulence and antibiotic resistance factors have also been formed. Finally, studies that integrate an understanding of these strains with their relationships to primary producers (photosynthetic bacteria and algae) are in the pilot stage. These studies may bring the metabolic, phylogenetic and competition/communication data together in a picture of ecological associations across a broad taxonomic range.

**Findings:**

2. Describe major findings

- **microarray development** (manuscript submitted): Pathogen monitoring and detection requires simultaneous analysis of thousands of co-existing sequence types in environmental samples. Yet such applications are hampered by theoretical and technological limitations because differentiation of similar sequences, which are at low abundance remains difficult. We develped a general method to accurately identify low abundant targets in systems containing complex mixtures of homologous targets. We combined a new analytical predictor of non-specific probe-target interactions (cross-hybridization) with a new optimization algorithm, which iteratively deconvolutes true probe-target signal from raw signal affected by spurious contributions (cross-hybridization, noise, background and unequal specific hybridization response). The method was capable of quantifying with unprecedented specificity and accuracy ribosomal RNA (rRNA) sequences in artificial and natural communities. Controlled experiments with spiked rRNA into artificial and natural communities demonstrated the accuracy of identification and quantitative behavior over different concentration ranges. Finally, we illustrated the power of this methodology for accurate detection of low-abundant targets in natural communities. We accurately identified *Vibrio* taxa in coastal marine samples at their natural concentrations (<0.05% of total bacteria) despite the high potential for cross-hybridization by hundreds of different co-existing rRNAs. This suggests that this methodology should be expandable to any system requiring accurate identification of low abundant targets amidst pools of similar sequences.

- ***Vibrio* strain analysis**: The strain collection has already been mined for a 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.

Studies of recombination rates also suggest that there should be some persistent pattern and segregation of diversity. Though this does not necessarily imply mechanistic causation, recombination rates drop as sequence identity declines. Perhaps the declining rates of successful homologous recombination reinforce a biological species, or perhaps this is the side-effect of other barriers to horizontal genetic exchange.

**3. Describe opportunities for training provided by your project**

This project has been undertaken in a manner that provides intense and varied training opportunities. Each of the postdoctoral researchers listed has been involved in developing new methods and adopting computational, microbiological, or molecular methods new to them. The graduate students have also been involved in significant training, particularly the rotation of first year graduate students.

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.

**4. Describe outreach activities**

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.

**Sogin – Genomics Facility Core**

**Research and Education Activities:**

1. **Describe major research and education activities of the project**

   The COHH Genome core at the MBL is responsible for service sequencing samples from members of the WH-COHH and the development of new technology that will influence research by the WH-COHH. 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 explored a tag sequencing strategy for exhaustively monitoring microbial populations in the marine samples. We exploited the massively parallel, pyro-sequencing capability of Roche Applied Science's Genome Sequencer 20 (GS20) system based upon 454 Life Sciences pyro-sequencing 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 hypervariable regions in 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 (using a molecular sequence database with as many as 200,000 entries that span a targeted hypervariable region) provided information about taxonomic identity and microbial diversity. Enumerating the frequency of individual tags also provides 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.

**Findings:**

**2. Describe major findings**

The results of these pilot studies are impressive. Rather than estimating only a few hundred OTUs (Organism 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 Cha1) 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. Clearly this provides a remarkably comprehensive picture of Bacterial populations and its application

| **Dist** | | **0** | | | **0.03** | | |
|---|---|---|---|---|---|---|---|
| **ID** | Reads | OTU | **ACE** | Chao1 | OTU | **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 |

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.

3. **Describe opportunities for training provided by your project**
   Through the genome core activities we have assisted Kevin Lin, a local high school student, in the development of his science fair project.

**Hajduk – 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.

**Findings:**
Progress of initial pilot projects

• *Characterization of a cyanobacterial anti-algal compound* (Eric Webb and Chris Reddy, WHOI).
*Microcoleus* PCC7420, a filamentous, non-diazotrophic cyanobacterium isolated from a Woods Hole salt marsh, produces compounds that inhibit the growth of other cyanobacteria. *Microcoleus* contains two gene families that have been implicated in natural product synthesis: non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS). The objective of this research is to purify and structurally characterize the growth-inhibiting compound and determine the environmental cues that regulate its expression. We have begun characterization of the compound produced by pure cultures of *Microcoloeus*. This has involved developing new culturing techniques aimed at optimizing the production of the compound.

• *Cnidarian toxins against voltage-gated Ca2+ channels* (Robert Greenberg, MBL).
Cnidarians such as jellyfish and sea anemones produce venoms that are comprised of a variety of toxins. Several of these toxins have been characterized and are targeted against specific receptors and ion channels in excitable cells. The goals of this project are to screen cnidarian venoms for effects on voltage-gated $Ca^{2+}$ channels, to isolate the specific

toxins that interact with voltage-gated $Ca^{2+}$ channels, and to obtain the amino acid sequence of these purified peptide toxins. To date, we have screened venoms from the anemone *Metridium sessile*, and the jellyfish, *Cyanea capillata* against the human $Ca_v2.3$ voltage-gated $Ca^{2+}$ channel expressed in *Xenopus* oocytes. The results with the *Metridium* venom were somewhat variable; in some experiments, we saw a 15-20% reduction in current, and a slowing of inactivation. However, there were problems with reproducibility. We are currently moving to a mammalian expression system for these studies, which may prove more reliable in part because of fewer confounding effects from endogenous channels.

• *Marine phage as vectors of gene transfer between marine bacteria and bacterial pathogens* (Peter Weigele and Jonathan King, MIT).
Bacterial infections continue to be a major source of disease and mortality worldwide. A diverse set of genes and gene clusters necessary for bacterial pathogenesis have been documented. Some of these virulence genes are found encoded within facultative pathogens by bacteriophages in the integrated, or lysogenic state (prophages). However, very little is known of the sources and environmental distribution of phage-encoded virulence genes; the ocean may be an important reservoir of these genes and may be an active site for their phage mediated exchange between bacterial populations. The overall goals of this project are to investigate whether phage-borne genes or gene clusters associated with virulence effects in bacterial pathogens of humans are also found among phages infecting marine photosynthetic bacteria. To screen for gene sequences of interest carried by phage in marine and estuary water samples, we developed a procedure to concentrate phage particles from water samples taken in the field. Using this procedure, we screened water samples were from the Parker River and the Charles River, the latter corresponding to times of sewage discharges. Phage particles were isolated on a cesium chloride density gradient and examined in the transmission electron microscope using negative stain, revealing plentiful and morphologically diverse phage particles. We are now ready to isolate DNA from these samples and probe them with sequences of interest.

<u>Second call for pilot project proposals.</u>
The second call was issued in September of 2005 by email to all faculty and research staff at WHOI, MBL, and MIT. Seven one-page pre-proposals were received by the deadline of October 1, 2005. These pre-proposals requested approximately $395,000 in total costs ($236,000 direct). Four were from WHOI, two were from MBL, and one was from MIT. The applicants came from multiple departments at the three institutions. The pre-proposals were reviewed by the Pilot Project Program Director, Pilot Project Program Deputy Director, Center Director, and Center co-Director. All 7 applicants were given feedback from the review.

Five of those submitting pre-proposals were invited to submit full proposals by Nov. 8, 2005. The titles are:

- Transcriptome profiling in the harmful alga *Aureococcus anophagefferens*
- Role of bacterial cell-cell signaling in coastal biofilms
- Beach Pathogens

• Vibriosis in zebrafish : Infection and Immune protection

• Rapid response information centers to assist in crisis management – pilot based on red tides

The five full proposals were reviewed and scored by members of the Internal Advisory Committee. In addition, we coordinated our call this year with that of the MIT Center for Environmental Health Sciences. (CEHS) We exchanged information about proposals received and we considered joint funding of projects that were relevant to both centers.

Three proposals were selected for full or partial funding:

• **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. The researchers will sequence roughly 30-40,000 tags per library to build a comprehensive view of the transcriptome.

• **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 authors 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. To examine how physical forces may impact sewage-associated microbes on beaches, they will investigate the effects of waves, currents, and changes in beach sediment on the quantity and distribution of *Legionella* spp. *Legionella* species will be surveyed using PCR amplification before and after an early-fallseason large-wave event (when significant sediment erosion is likely). *Legionella* in samples of sand from the beach and the surfzone will be correlated with wave conditions and changes in sediment levels (*eg*, erosion and accretion).

• **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** (formerly titled: Rapid response information centers to assist in crisis management – pilot based on red tides). **(D.J. Patterson, MBL, and D. 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. At the core of this project lie original internet services that use the names of organisms to discover and manage biological information. Taxonomic indexing is a biologically informed suite of services that uses taxonomic knowledge and awareness of nomenclatural conventions to bring together information that has been cataloged under different names. Around such services the authors are assembling modular software that allow them to combine distributed and local knowledge in flexible, interoperable, and scaleable web environments called STAR*sites. The pilot site will exploit the 2005 NE US *Alexandrium* bloom to demonstrate the feasibility of rapidly combining expert information from multiple sources with locally generated data.

**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 continues to foster the development 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 and 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 these accounts.
3. Center Investigator meetings have been held on a regular basis, approximately monthly. All principle and associated investigators in the projects and cores are

involved in these meetings. The meetings 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 next meeting with the EAC to review the Center programs will occur in summer 2006.
5. The Administrative Core continues to oversee the Pilot Project program. Three projects were selected for funding in 2004, and three in 2005. The pilot projects involve investigators at all three institutions, WHOI, MBL and MIT. We coordinated our call for proposals with the MIT Center for Environmental Health Sciences.
   - *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).
   - *Transcriptome profiling in the harmful alga Aureococcus anophagefferens.* (Sonya Dyhrman, WHOI).
   - *Beach Pathogens* (Steve Elgar, Britt Raubenheimer, & Rebecca Gast, WHOI).
   - *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 (D.J. Patterson, MBL, and D. Anderson, WHOI).
6. The combined Centers Web site was established at WHOI and incorporates all four Centers (http://www.whoi.edu/science/cohh/index.htm).
7. The interactions with the other COHH have been highly productive. A successful Center Directors Meeting was held in Miami, attended by all members of all four centers. The Centers joined forces, obtaining SGER grants from NSF to address microbial populations and pathogen issues in Lake Pontchartrain after hurricane Katrina. The Woods Hole Center has hosted conference calls dealing with the sampling and analyses in the Katrina effort.
8. **Outreach and impact:** The Center's activities have had substantial importance for local, State and Regional public health concerns. Of particular importance, WH Center investigators identified the massive red tide in the Massachusetts Bay and the Gulf of Maine, in the spring of 2005. The discovery allowed a timely alert and monitoring and closure of shellfish beds.

   Outreach also involved the participation of the Director in the IOOS Public Health Workshop in St. Petersburg in January 2006.

**Training and development:**

The REU grant associated with the WH-COHH supported two students in the summer of 2005. Megan Jamison, Humboldt State University, did a study of the role of tides in exchanging water (and stuff in it) between estuaries and the coastal ocean. Brenna Mahoney, Cornell University, did a study of Intra-population genetic variation of *Alexandrium fundyense* in the Gulf of Maine using microsatellite DNA.

**Anderson – Project 1**

**Research and Education Activities:**

Work in Year 2 has focused on Specific Aims 1 and 2 of the project:

1. Identify a genetic marker capable of distinguishing different genotypes within *A. fundyense* and *A. ostenfeldii* populations;
2. Determine the extent of natural genetic diversity of *Alexandrium* spp. in the Gulf of Maine.

**Findings:**

For Aim 1, our initial work examined the ribosomal DNA genes, to determine sequences that would allow us to differentiate between *Alexandrium* genotypes in the Gulf of Maine (GOM). The ribosomal regions proved to be highly similar, so at the end of Year 1 we had begun to characterize a set of recently published microsatellite markers for *Alexandrium tamarense* (*A. tamarense* and *fundyense* belong to the same species complex). In Year 2 we demonstrated that these markers work very well in *A. fundyense*, and used them to characterize the genetic diversity of a set of 55 clonal A. *fundyense* strains that were isolated from the GOM in 2003 (Task 1.1). We have observed very high genetic diversity among these isolates (93% unique genotypes). We are at present analyzing the toxin composition of these isolates, which will complete the data we need for publication of this work.

Efforts to define a suitable genetic marker for *Alexandrium ostenfeldii* have proved more difficult. As in *A. fundyense*, the ribosomal DNA sequences of *A. ostenfeldii* strains from the GOM are highly similar. We also tested the *A. tamarense* microsatellite markers with *A. ostenfeldii*, but they proved unsuitable for this species. The microsatellite markers have proved to be a useful tool for *A. fundyense*, therefore we have initiated a collaboration with Dr. Satoshi Nagai of the National Research Institute of Fisheries and Environment of the Inland Sea in Hiroshima, Japan, to isolate microsatellite markers for *A. ostenfeldii*. Dr. Nagai's group published the *A. tamarense* microsatellite markers, and has identified microsatellite sequences in a number of other harmful algae. Dr. Nagai visited our lab for two weeks this October, and will return to Japan with the materials necessary for the microsatellite identification. We hope to have the markers available for testing before the end of 2006.

We have also made significant progress towards Aim 2: Determining the Natural Genetic Diversity of *Alexandrium* in the GOM. From May 9-18 2005, we had our first research cruise covering the geographic extent of the Gulf. During the cruise, we realized that we were sampling the beginning of an *Alexandrium* bloom, which turned out to be the largest bloom in the region in at least three decades. From plankton samples from the cruise and subsequent sampling, we isolated several hundred clonal *Alexandrium* cultures and genotyped them using the microsatellite markers described above. These isolates are derived from 6 distinct samples taken throughout the first 6 weeks of the bloom. Overall, these samples also show very high genetic diversity (>98%), like our 2001 isolates. Most notably, the microsatellite results indicate that the southernmost populations, collected late in the bloom, are genetically distinct from the earlier populations sampled during the research cruise.

Brenna Mahoney, a Cornell University student, participated in this work as an REU student through the Center. She participated in research cruises, and assisted in the isolation and genotyping of the cultures from the research cruise. Brenna's project was submitted to a competition among REU projects, and she was selected to present her work at the recent AGU/ASLO meeting in Honolulu, HI February 20-24, where it was very well received.

**McGillicuddy – 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 a large-scale oceanographic survey of *A. fundyense* in the Gulf of Maine, voyage 412 of R/V Oceanus (Aim 3). The station grid consisted of a set of transects spanning from Massachusetts Bay to the Bay of Fundy. Given the surprisingly high abundance of *A. fundyense* in Massachusetts Bay, that area was resurveyed at the end of the cruise. During the one-week time period in between Massachusetts Bay snapshots, the /A. fundyense/ population increased substantially. High-resolution profiles were taken at selected stations to examine genetic structuring of the population with depth. One profile in the Bay of Fundy, one near Casco Bay, and two in Massachusetts Bay were obtained. Sampling strata was 0-30m with 3m nominal resolution. Vertical interval chosen on the basis of cell profiles from Martin et al. (2005), Townsend et al. (2005) and Townsend et al. (2001) which show very few cells below 30m. Moreover, SHA profiles from the present cruise indicate comparable numbers at the surface and 10, with background levels at 20m.

Drifters were deployed along three transects: Cape Ann (3 surface drifters), Penobscot

Bay (6 surface drifters), and the Bay of Fundy (9 drogued drifters). The Cape Ann drifters transited southeast offshore of the back side of Cape Cod. The Penobscot Bay drifters went southwest in a generally along-coast direction. The line of drifters in the Bay of Fundy behaved quite differently, with the northwest part of the line moving to the southwest and the southeast part of the line moving to the northeast. This cyclonic shear is consistent with the hypothesized gyre at the mouth of the Bay, but the drifters have not been in the water long enough to close the loop.

Hydrography, shipboard ADCP velocity measurements, GoMOOS mooring observations, and coastal tide gauges were assimilated into a numerical model to provide the hydrodynamic context in which to interpret the biological observations. The fidelity of the circulation fields was quantified by evaluating the ability of the model to simulate the trajectories of drifters deployed during the survey. Comparison of the simulated and observed trajectories from the Cape Ann line indicates that the model drifters generally go in the right direction, but their speed is too large. In contrast, the simulated drifters in the Penobscot Bay line move slower than observed, and do not turn offshore as much as the real drifters. The westernmost drifters in the Bay of Fundy move to the southwest as the real drifters do, but the easternmost drifters compare poorly with observations: the real drifters do northeast, whereas the simulated drifters go west. The nature of these discrepancies will be investigated in a hindcasting study.

Our observations during voyage 412 of R/V Oceanus in May 2005 were the first measurements of what turned out to be an extraordinary bloom event, reported in detail by Anderson et al. (2005). The outbreak eventually closed shellfish beds from central Maine to Massachusetts, including Nantucket Island and portions of Martha's Vineyard, and resulted in the closure of 40,000 km2 of offshore federal waters as well. The coastal *A. fundyense* bloom was exceptional in several ways: high toxin levels were measured farther south than ever before in New England; levels of toxicity in many locations were higher than previously observed at those stations; for the first time toxicity at some locations was above quarantine levels; cell concentrations far exceeded those observed in the coastal waters of southern New England in the past; and for the first time, the governors of Maine and Massachusetts officially declared the red tide to be a disaster, clearing the way for federal assistance.

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**Gast – Project 3**

**Research and Education Activities:**

1. Describe major research and education activities of the project

**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 and 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:**

2. Describe major findings

   To date we have collected 8 sets of water and sediment samples throughout the different seasons from our 4 sites in Mt Hope Bay.

Aim 1: We have completed our first comprehensive (eukaryal, bacterial, archaeal) 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 of life including relatives of sludge bacteria (*Comamonas*, *Acinetobacter*, *Atopostipes*, and hits to uncultivated sludge bacteria), polyaromatic hydrocarbon-degrading bacteria, and representatives related to the genera *Staphylococcus*, *Streptococcus*, *Clostridium* and *Legionella*. We are also obtaining hits to harmful algal bloom species related to *Pseudo-nitzchia, Heterocapsa* and *Alexandrium*. Phylogenetic analyses will further unveil the relationships of many of these clones and determine whether they are related to known pathogens and may possibly represent undescribed taxa.

Aim 2: We have used a nested PCR approach to screen our Mt Hope Bay water and sediment samples, and amoeba cultures for the presence of *Legionella* species. The following two charts summarize the data. Briefly, water and sediment samples are positive for *Legionella* species at all times of the year (Figure 1). A portion of the amoeba cultures were also positive, with more of the recovered amoebae having *Legionella* species in the summer (Figure 2). None of the sequences from the PCR fragments recovered were identical to *L. pneumophila*, but only 34 of the sequences could be grouped as similar to each other. The other 24 sequences were different from each other and from other *Legionella* species in the database. This indicates a large diversity of *Legionella* species in the marine environment, either as free-living organisms or present within amoebae. The samples will also be screened with primers specific for *pneumophila*.

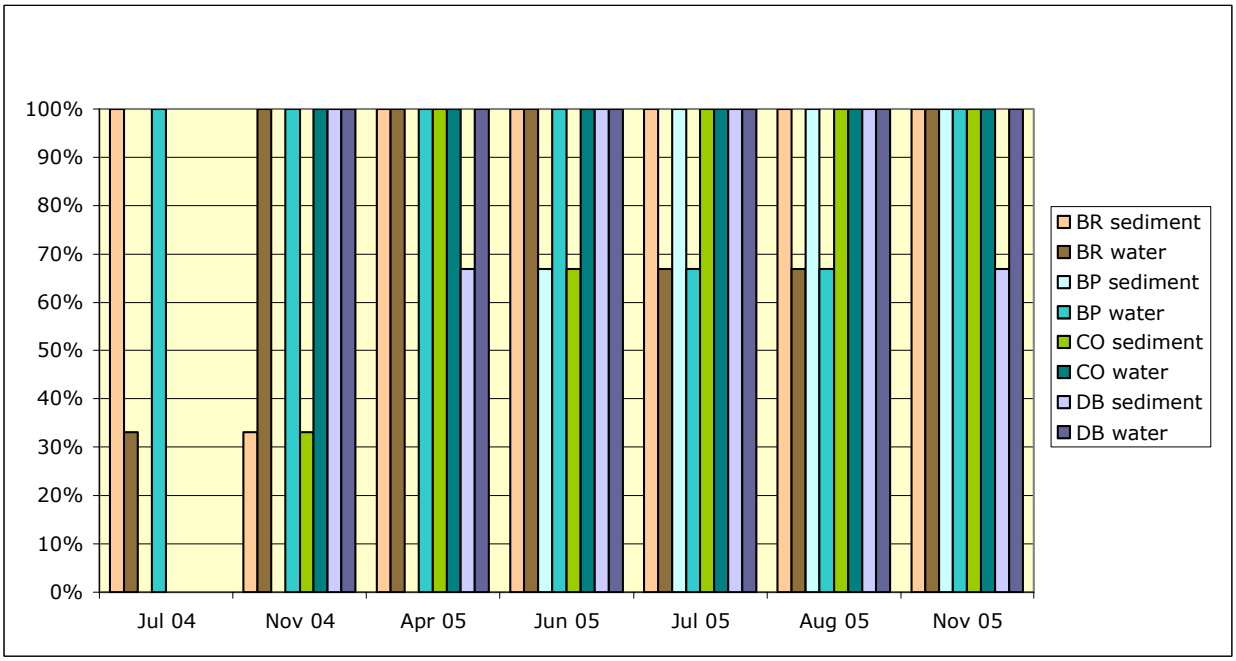


Figure 1. Percent water and sediment samples positive for *Legionella* species.

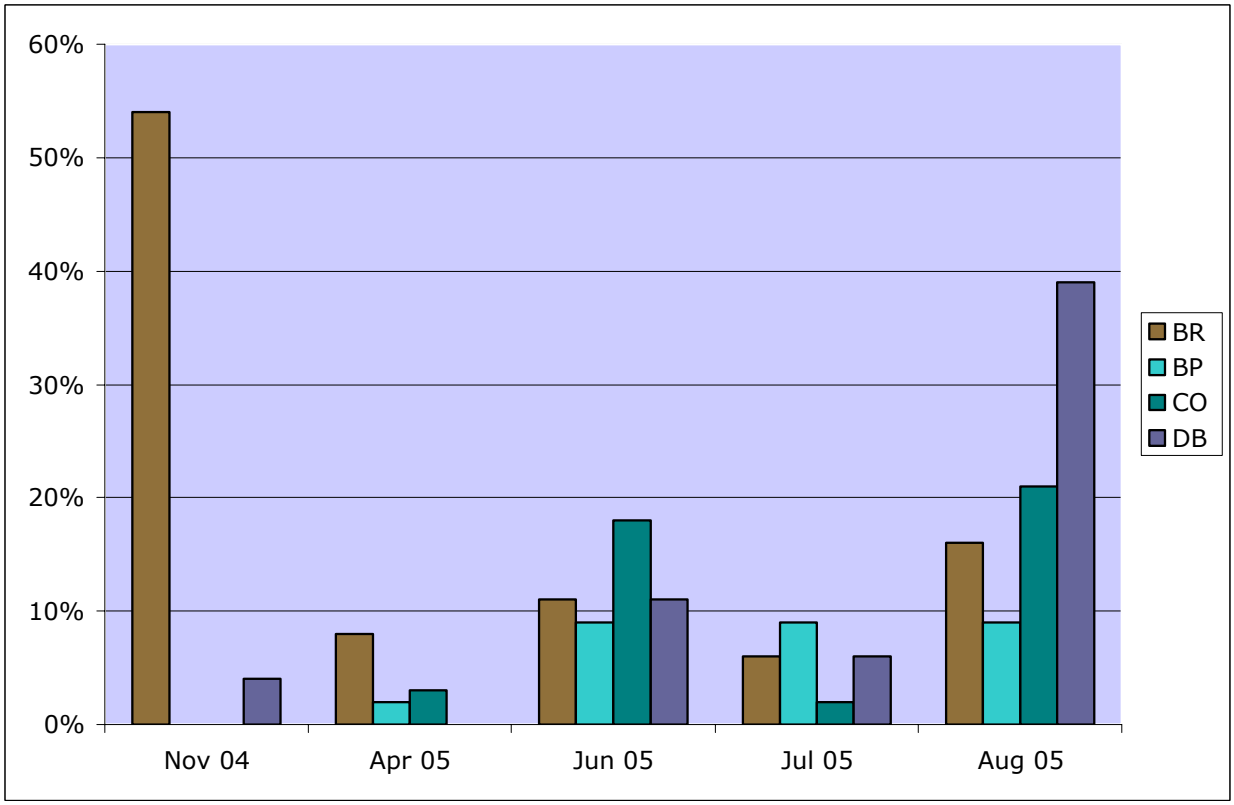


Figure 2. Percent amoeba cultures positive for *Legionella* species.

In addition to our Mt Hope Bay samples, we have examined a set of water and sediment samples from the Great Salt Lake in the lower portion of the lake where numerous sewage outfalls from the city are present. These samples were collected in August of 2005 by Dr. Wayne Wurtsbaugh and his undergraduate students. All of these samples were also positive for *Legionella* species, as were many of the amoeba cultures. None of these sequences were identical to *L. pneumophila*, but the samples have not been screened with pneumophila-specific primers. The sequences were distinct from the ones we recovered from Mt Hope Bay, and overall tended to cluster together in similarity analyses. This further confirms the unexpected diversity of *Legionella* species in saline environments that we have discovered.

3. Describe opportunities for training provided by your project
   An undergraduate REU student worked with us during the summer of 2005 and participated in sampling and analysis for *Legionella*. Her project was selected as one of the invited REU posters at the 2005 Ocean Sciences meeting. As a result of her research experience this summer, Elizabeth has decided to pursue graduate studies in marine science.
   We also mentored a teacher from Danbury Connecticut (Bob Weinheimer) that was participating in the ISIS (Southern Connecticut State University's Sixth Year in Education Certificate) Program. As part of this program he

participated in both field sampling and lab work centered around water and sediment sampling of Mount Hope Bay. We hope to maintain ties with this teacher and work towards developing curricula that will take advantage of the data collected during our field seasons.

4. Describe outreach activities
   Linda - iVisit

**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 have now completed the sampling of vibrioplankton over seasonal cycles in the PIE estuary, which we use as our model site. Data collection included temperature, salinity, nutrients, and cells for culturing and DNA extraction. DNA extracted from the environment will be analyzed by QPCR to obtain culture-independent measures of vibrio population dynamics. Moreover, several hundred *Vibrio* strains are being characterized in a variety of fashions to create a unique resource. Analyses include sequencing of 16S rRNA and six housekeeping genes to determine phylogenetic identity, presence/absence of pathogenicity determinant genes, and metabolic and physiological diversity. To provide a background for additional genomic information, the complete genomes of two isolates have been sequenced. Further, efforts have begun to provide the response of growth parameters to variation in salinity and temperature. Sequencing of potential regulation genes that control these responses is planned.

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

Preparations for AIM 2 have been completed and the actual sampling will be carried out during the 2006 season. We have developed and tested a new method for assaying in situ growth of specific bacterial populations (Yu, Thompson and Polz, in preparation); this combines BrdU labeling of actively growing bacteria and immunocapture of DNA with subsequent quantitative measurement of specific alleles by QPCR-CDCE, or by cloning and sequencing. Because genomic DNA is captured it is possible to assay growth responses and/or differences on varying scales of genetic differentiation (from rRNA alleles to specific functional genes).

**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 made considerable progress in both aims. First, sequencing of six protein coding genes for ~300 strains provides a picture of recombination and discontinuity in the phylogeny of coexisting strains. Software has been developed to assist in exploring this very complex data. So far, we were able to show that effective bounds of recombination, consistent with observed population structure exist.

A study of virulence factors (which may have ecological roles beyond causing vertebrate disease, and thus may represent an ecological reservoir of pathogen genes) is underway. These are studied both using classical phenotypic and molecular methods. Contacts that provide access to new microarrays developed for identifying *Vibrio* virulence and antibiotic resistance factors have also been formed. Finally, studies that integrate an understanding of these strains with their relationships to primary producers (photosynthetic bacteria and algae) are in the pilot stage. These studies may bring the metabolic, phylogenetic and competition/communication data together in a picture of ecological associations across a broad taxonomic range.

**Findings:**

2. Describe major findings

- **microarray development** (manuscript submitted): Pathogen monitoring and detection requires simultaneous analysis of thousands of co-existing sequence types in environmental samples. Yet such applications are hampered by theoretical and technological limitations because differentiation of similar sequences, which are at low abundance remains difficult. We develped a general method to accurately identify low abundant targets in systems containing complex mixtures of homologous targets. We combined a new analytical predictor of non-specific probe-target interactions (cross-hybridization) with a new optimization algorithm, which iteratively deconvolutes true probe-target signal from raw signal affected by spurious contributions (cross-hybridization, noise, background and unequal specific hybridization response). The method was capable of quantifying with unprecedented specificity and accuracy ribosomal RNA (rRNA) sequences in artificial and natural communities. Controlled experiments with spiked rRNA into artificial and natural communities demonstrated the accuracy of identification and quantitative behavior over different concentration ranges. Finally, we illustrated the power of this methodology for accurate detection of low-abundant targets in natural communities. We accurately identified *Vibrio* taxa in coastal marine samples at their natural concentrations (<0.05% of total bacteria) despite the high potential for cross-hybridization by hundreds of different co-existing rRNAs. This suggests that this methodology should be expandable to any system requiring accurate identification of low abundant targets amidst pools of similar sequences.

- ***Vibrio* strain analysis**: The strain collection has already been mined for a 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.

Studies of recombination rates also suggest that there should be some persistent pattern and segregation of diversity. Though this does not necessarily imply mechanistic causation, recombination rates drop as sequence identity declines. Perhaps the declining rates of successful homologous recombination reinforce a biological species, or perhaps this is the side-effect of other barriers to horizontal genetic exchange.

**3. Describe opportunities for training provided by your project**

This project has been undertaken in a manner that provides intense and varied training opportunities. Each of the postdoctoral researchers listed has been involved in developing new methods and adopting computational, microbiological, or molecular methods new to them. The graduate students have also been involved in significant training, particularly the rotation of first year graduate students.

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.

**4. Describe outreach activities**

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.

**Sogin – Genomics Facility Core**

**Research and Education Activities:**

1. **Describe major research and education activities of the project**

   The COHH Genome core at the MBL is responsible for service sequencing samples from members of the WH-COHH and the development of new technology that will influence research by the WH-COHH. 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 explored a tag sequencing strategy for exhaustively monitoring microbial populations in the marine samples. We exploited the massively parallel, pyro-sequencing capability of Roche Applied Science's Genome Sequencer 20 (GS20) system based upon 454 Life Sciences pyro-sequencing 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 hypervariable regions in 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 (using a molecular sequence database with as many as 200,000 entries that span a targeted hypervariable region) provided information about taxonomic identity and microbial diversity. Enumerating the frequency of individual tags also provides 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.

**Findings:**

**2. Describe major findings**

The results of these pilot studies are impressive. Rather than estimating only a few hundred OTUs (Organism 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 Cha1) 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. Clearly this provides a remarkably comprehensive picture of Bacterial populations and its application

| **Dist** | | **0** | | | **0.03** | | |
|---|---|---|---|---|---|---|---|
| **ID** | Reads | OTU | **ACE** | Chao1 | OTU | **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 |

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.

3. **Describe opportunities for training provided by your project**
   Through the genome core activities we have assisted Kevin Lin, a local high school student, in the development of his science fair project.

**Hajduk – 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.

**Findings:**
Progress of initial pilot projects

• *Characterization of a cyanobacterial anti-algal compound* (Eric Webb and Chris Reddy, WHOI).
*Microcoleus* PCC7420, a filamentous, non-diazotrophic cyanobacterium isolated from a Woods Hole salt marsh, produces compounds that inhibit the growth of other cyanobacteria. *Microcoleus* contains two gene families that have been implicated in natural product synthesis: non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS). The objective of this research is to purify and structurally characterize the growth-inhibiting compound and determine the environmental cues that regulate its expression. We have begun characterization of the compound produced by pure cultures of *Microcoloeus*. This has involved developing new culturing techniques aimed at optimizing the production of the compound.

• *Cnidarian toxins against voltage-gated Ca2+ channels* (Robert Greenberg, MBL).
Cnidarians such as jellyfish and sea anemones produce venoms that are comprised of a variety of toxins. Several of these toxins have been characterized and are targeted against specific receptors and ion channels in excitable cells. The goals of this project are to screen cnidarian venoms for effects on voltage-gated $Ca^{2+}$ channels, to isolate the specific

toxins that interact with voltage-gated $Ca^{2+}$ channels, and to obtain the amino acid sequence of these purified peptide toxins. To date, we have screened venoms from the anemone *Metridium sessile*, and the jellyfish, *Cyanea capillata* against the human $Ca_v2.3$ voltage-gated $Ca^{2+}$ channel expressed in *Xenopus* oocytes. The results with the *Metridium* venom were somewhat variable; in some experiments, we saw a 15-20% reduction in current, and a slowing of inactivation. However, there were problems with reproducibility. We are currently moving to a mammalian expression system for these studies, which may prove more reliable in part because of fewer confounding effects from endogenous channels.

• *Marine phage as vectors of gene transfer between marine bacteria and bacterial pathogens* (Peter Weigele and Jonathan King, MIT).
Bacterial infections continue to be a major source of disease and mortality worldwide. A diverse set of genes and gene clusters necessary for bacterial pathogenesis have been documented. Some of these virulence genes are found encoded within facultative pathogens by bacteriophages in the integrated, or lysogenic state (prophages). However, very little is known of the sources and environmental distribution of phage-encoded virulence genes; the ocean may be an important reservoir of these genes and may be an active site for their phage mediated exchange between bacterial populations. The overall goals of this project are to investigate whether phage-borne genes or gene clusters associated with virulence effects in bacterial pathogens of humans are also found among phages infecting marine photosynthetic bacteria. To screen for gene sequences of interest carried by phage in marine and estuary water samples, we developed a procedure to concentrate phage particles from water samples taken in the field. Using this procedure, we screened water samples were from the Parker River and the Charles River, the latter corresponding to times of sewage discharges. Phage particles were isolated on a cesium chloride density gradient and examined in the transmission electron microscope using negative stain, revealing plentiful and morphologically diverse phage particles. We are now ready to isolate DNA from these samples and probe them with sequences of interest.

<u>Second call for pilot project proposals.</u>
The second call was issued in September of 2005 by email to all faculty and research staff at WHOI, MBL, and MIT. Seven one-page pre-proposals were received by the deadline of October 1, 2005. These pre-proposals requested approximately $395,000 in total costs ($236,000 direct). Four were from WHOI, two were from MBL, and one was from MIT. The applicants came from multiple departments at the three institutions. The pre-proposals were reviewed by the Pilot Project Program Director, Pilot Project Program Deputy Director, Center Director, and Center co-Director. All 7 applicants were given feedback from the review.

Five of those submitting pre-proposals were invited to submit full proposals by Nov. 8, 2005. The titles are:

- Transcriptome profiling in the harmful alga *Aureococcus anophagefferens*
- Role of bacterial cell-cell signaling in coastal biofilms
- Beach Pathogens

• Vibriosis in zebrafish : Infection and Immune protection

• Rapid response information centers to assist in crisis management – pilot based on red tides

The five full proposals were reviewed and scored by members of the Internal Advisory Committee. In addition, we coordinated our call this year with that of the MIT Center for Environmental Health Sciences. (CEHS) We exchanged information about proposals received and we considered joint funding of projects that were relevant to both centers.

Three proposals were selected for full or partial funding:

• **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. The researchers will sequence roughly 30-40,000 tags per library to build a comprehensive view of the transcriptome.

• **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 authors 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. To examine how physical forces may impact sewage-associated microbes on beaches, they will investigate the effects of waves, currents, and changes in beach sediment on the quantity and distribution of *Legionella* spp. *Legionella* species will be surveyed using PCR amplification before and after an early-fallseason large-wave event (when significant sediment erosion is likely). *Legionella* in samples of sand from the beach and the surfzone will be correlated with wave conditions and changes in sediment levels (*eg*, erosion and accretion).

• **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** (formerly titled: Rapid response information centers to assist in crisis management – pilot based on red tides). **(D.J. Patterson, MBL, and D. 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. At the core of this project lie original internet services that use the names of organisms to discover and manage biological information. Taxonomic indexing is a biologically informed suite of services that uses taxonomic knowledge and awareness of nomenclatural conventions to bring together information that has been cataloged under different names. Around such services the authors are assembling modular software that allow them to combine distributed and local knowledge in flexible, interoperable, and scaleable web environments called STAR*sites. The pilot site will exploit the 2005 NE US *Alexandrium* bloom to demonstrate the feasibility of rapidly combining expert information from multiple sources with locally generated data.