Annual Report for Period: 05/2004 - 05/2005
Submitted on: 02/25/2005
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.

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.

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

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

Activities and Findings

Research and Education Activities:
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

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

Other Specific Products

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.

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.

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.

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.

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.

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.

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.

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 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.

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
Any Product

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 has overseen the inauguration of the Center, and facilitated the successful initiation of the Research Projects and the Pilot Project program. Inter-center communication has been established and put on a regular solid footing. The Center has leveraged additional private funds. The Administrative Core has served as the focal point for all Center activities.

1. The Administrative Core objectives are in general to ensure 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. To these ends, we established the Center Office and oversaw the implementation of each of the components, worked toward developing a cohesive structure that would have visibility in and impact 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. After funding was received, the budgets for each component were adjusted in two steps, one to reflect the decrease funding from the NIEHS and one to reflect the requirement by the NSF that ship costs be incorporated into the science portion of the budget from NSF. Revised budgets were received and subcontract awards made and project accounts established. 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 were initiated at the time accounts were established and funding began. All principle and associated investigators in the projects and cores were involved in these meetings. Meetings were held bi-weekly for the first three months, and monthly thereafter. The meetings address all aspects of the Center interactions and communications, internally as well as externally.
4. We have established an External Advisory Committee, which will review the Center programs and advise the Director and Deputy Director. Members who have been engaged are Dr. Michael Gallo, University of Medicine and Dentistry of New Jersey, and Dr. Gerald Plumley, of the Bermuda Biological Station for Research. Additional members have been invited.
5. The Administrative Core oversaw the operation of the successful Pilot Project program. To achieve the objectives outlined above, we established a Pilot Project Program and issued a call for pilot project proposals in June of 2004.

Anderson – Project 1

Research and Education Activities:

Project 1 has the following specific aims:

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;
3. Characterize the relationships between toxicity, physiological variability and genotype in *Alexandrium* spp. from the Gulf of Maine;
4. Track changes in the relative abundance of *Alexandrium* genotypes in Bay of Fundy source population through time;
5. Track changes in the genotypic diversity of *Alexandrium* populations through time throughout the Gulf of Maine;
6. Investigate the relationship between *Alexandrium* population structure and the quantity and composition of toxins in the plankton.

Findings:

Studies to date have focused on Aim 1, identifying the genetic markers that we will use in subsequent Aims. As stated in the proposal, we initially targeted the internal transcribed spacer (ITS) region of the ribosomal RNA operon as a region that might reveal the presence of distinct genotypes within the *Alexandrium fundyense* and *Alexandrium ostenfeldii* species in the Gulf of Maine. Sequencing of the ITS region, as well as the D1-D3 and D8-D10 regions of the large subunit (LSU) ribosomal gene, has been completed for nine strains of *A. fundyense* and 13 strains of *A. ostenfeldii*. For both species, the sequences of all three of the regions proved to be less than 0.5% different over ~2700 bp. Thus, no genotypic differentiation is apparent at the level of ribosomal genes.

Fortuitously, a group of Japanese researchers recently published a paper describing the development of microsatellite markers for *Alexandrium tamarense*, a closely related species.

Microsatellite markers are gene regions containing strings of tandemly repeated nucleotides, for instance CACACACA (dinucleotide repeat) or GGCGGCGGCGGC (trinucleotide repeat). The identification of such markers is laborious and time-consuming, so we were encouraged by the report of existing markers for a congeneric species. We have since tested all of the published microsatellite primers, and nearly all of them work well with *A. fundyense* and show variation between strains. In addition, some of the microsatellite primers also amplify *A. ostenfeldii* DNA. At present, we are completing microsatellite analysis of a group of 68 recent *A. fundyense* isolates from the Gulf of Maine to ascertain the extent of genotypic variability within this region. In parallel, we are refining the procedures for microsatellite amplification and analysis from *A. ostenfeldii*.

In the Fall of 2004, a graduate student joined the project. His experimental work has focused on characterizing the microsatellite loci in *Alexandrium*, in particular the variation amongst individual cells in a clonal culture and the mutation rate of the loci in a population. An understanding of both of these rates has implications for using these microsatellite loci to determine intra-population genetic variation. He is also involved in the development and testing of a quantitative PCR method for enumeration of *Alexandrium ostenfeldii*, which is a crucial component of the field work scheduled for May of 2005, as described below.

Planned work in the upcoming project year will focus on Aims 1-3 of the proposal. We will continue to characterize the microsatellite primers that amplify *A. ostenfeldii*. The next step is to investigate sampling methods that would allow us to perform microsatellite analysis on a large number of samples. Traditionally, microsatellites are used on individuals, which in our case means a single cell. Single cell PCR is not uncommon with dinoflagellates, so we should be able to circumvent the need to establish hundreds of cultures to obtain sufficient DNA. In the next months we plan to investigate the feasibility of using flow cytometry to sort individual cells for microsatellite PCR, or for establishing clonal cultures for microsatellite analysis. Likewise, we will evaluate the possibility of sorting cysts by flow cytometry, again for single-cell PCR.

Our first field sampling is planned for May 9-18 2005. This cruise will include a large number of stations covering the extent of the Gulf of Maine. During this cruise, we will collect samples for microsatellite analysis, to determine the extent of genetic variability present in the Gulf of Maine (Aim 2). We will also collect cells and cysts to establish new cultures for genotyping. When we finish our current analysis of our present cultured Gulf of Maine strains, we will begin the physiological characterization (Aim 3). The physiological experiments will be expanded to include new genotypes that may be discovered from the cruise cultures. The physiological data will also be used by Dr. McGillicuddy's group (Project 3) to begin adapting their model.

McGillicuddy – Project 2

Research and Education Activities:

Thus far our year one effort has been devoted to testing the hydrodynamic nowcast-forecast-hindcast system to be used in acquisition and interpretation of the WH-COHH *Alexandrium fundyense* surveys. We are very fortunate to have a recent data set (R/V *Oceanus* Voyage 391, May-June 2003) suitable for this purpose. Underway current measurements from the shipboard ADCP reveal both tidal and sub-tidal variability, with magnitudes as large as 1m s^{-1} (Figure 1). Currents from three fixed moorings (B, E and I) of the Gulf of Maine Ocean Observing System (GoMOOS) were also collected and depth-averaged. Buoy B is located in the western Maine shelf to the northwest of the Wilkinson Basin, whereas buoys E and I are located offshore of Casco Bay and Penobscot Bay, respectively. Both sources of velocity data were assimilated into the hydrodynamic model.

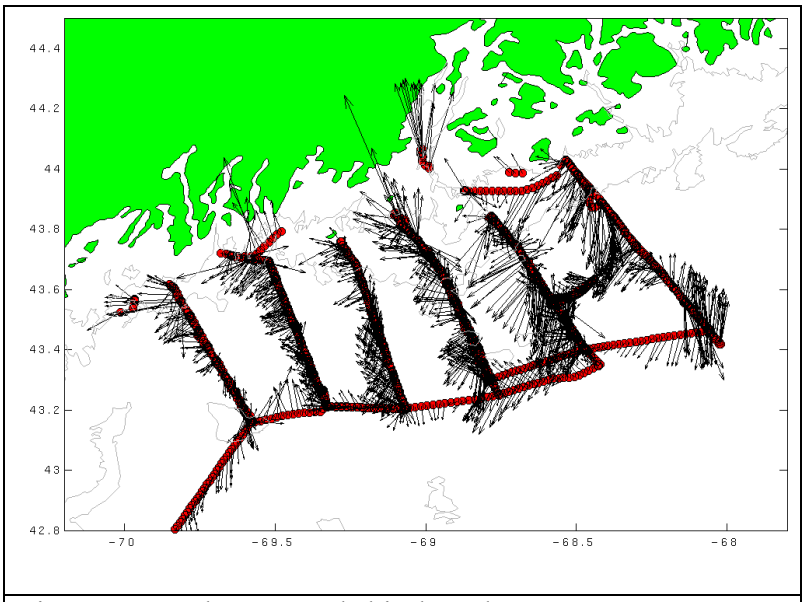

Figure 1. Depth-averaged ship-board ADCP current vectors measured in the survey.

To hindcast the coastal circulation, the forward model Quoddy is initialized with the objective analyzed temperature and salinity fields. At the model surface, 6-hourly optimally interpolated wind fields are applied. The model is cold-started on May 22nd, spun up for 2 days to allow the tidal dynamics to develop and stabilize, and then integrated forward in time for another 14 days before stopping on June 7th. Model solutions of state variables are saved and compared with in-situ current and coastal sea level observations collected between May 28th and June 7th at each individual observation location; misfits between the model and data drive the inverse models to produce sea level adjustment along the model seaward open boundary (OB), which subsequently drive another forward model run.

The adjoint inverse models treat tidal and sub-tidal sea level inversions separately. This is done by two adjoint inverse sub-models: Truxton (Lynch et al., 1998) and Casco (Lynch et al., 2001), respectively. Truxton is a frequency domain inverse model that uses observations to improve the accuracy of tidal amplitude and phase specifications along the OB. Casco is a time domain inverse model that makes use of interior observations to adjust the time-dependent sub-tidal boundary elevations. At the end of each iteration of the inverse procedure, both tidal (from Truxton) and sub-tidal (from Casco) elevation adjustment are added to the prior boundary elevations to form more accurate elevation specifications along the seaward OB.

Findings:
Sub-tidal sea level adjustment is important as it determines the sub-tidal circulation that is often more relevant to the material property transport in the coastal water. Casco provides time-dependent sub-tidal open boundary sea level adjustments at the specified interval. For example, a snapshot of the 6-hourly time series of Casco-derived surface elevation at the seaward OB, along with domain wide model solutions of sub-tidal surface elevation and surface currents is shown (Figure 2).

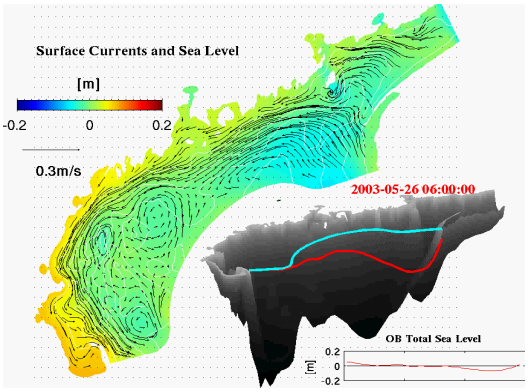

Figure 2. A snapshot of the sub-tidal sea level (inverted by Casco, denoted by red curve) at the model seaward open boundary, along with the modeled surface elevation and surface current at this particular time in the upper left panel. Note that the blue curve overlaid on the 3-d bathymetry indicates the location of model seaward open boundary, and the open boundary sea level (red curve) on the same plot is exaggerated to show its spatial pattern. The real value of OB sea level is shown in the lower right panel, where the left (right) end of the x-axis is Cape Code (Cape Sable).

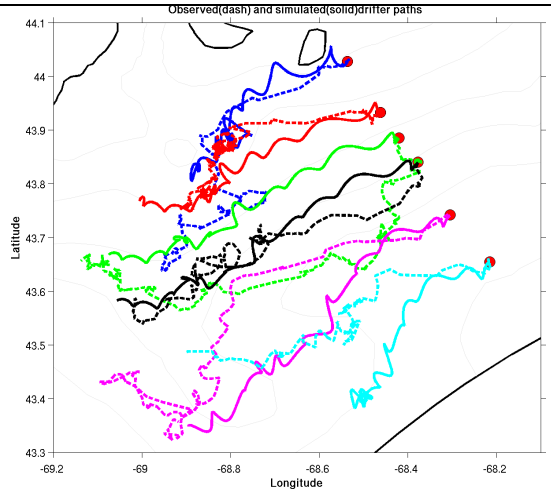

Figure 3. Comparisons between the modeled (solid lines) and observed (dash lines) drifter trajectories. Release at most onshore location was a surface drifter, and the remaining 5 were drogued at 15 m.

At this particular time, sea level at OB subsides between the Jordan Basin and the Cape Sable with amplitude of about 6 cm. The resultant pressure gradient thus drives cyclonic circulation that is observable in the surface current map (Figure 2, left panel). The sub-tidal sea level variation along the OB is a manifestation of the coastal ocean response to remote forcing, including the basin-scale wind field, coastal trapped waves, and offshore momentum and buoyancy forcing of upstream Scotian shelf and deep Atlantic Ocean.

To evaluate the data-assimilative hindcast model performance, we quantify the accuracy with which the model can predict the trajectories of 6 drifters that were released off Penobscot bay along the easternmost transect of the ship survey. Among them, the most onshore release was a surface drifter drogued at 1 m. The other 5 were drogued at 15 m. All drifters were equipped with the Global Position System (GPS) and were tracked by the satellites in the real time. Collectively, these drifter trajectories provided useful observations as to where the material property may have been transported by the coastal circulation. Since the drifter trajectory information has not been assimilated, they act as an independent dataset to evaluate the skill of the data assimilative model hindcast. To do that, numerical particles are released at the same time, location and water depth of each drifter deployment. Trajectories of these numerical particles are then tracked with the Fourth-order Runge-Kutta scheme using the modeled velocity fields (Blanton, 1993). The numerical particle tracking starts from May 31st (Year day 151) when the drifters were released to June 7th (Year day 158) when the field survey completed. During this 7-day tracking period, the modeled and observed drifter trajectories agree nicely (Figure 3), both showing significant displacements as the result of the MCC transport. To quantitatively assess the model tracking skill, the time series of modeled and observed drifter divergence is produced and the mean drifter divergence rate is calculated by averaging divergence rates of all 6 drifters. The mean divergence rate is found to be 1.78 km/day, equivalent to the rms current error of about 0.02m s^{-1}. In previous Georges Bank data assimilative model forecast experiment, where only ship-board ADCP current were assimilated, the mean divergence rate was found to be 3.4 km day^{-1} (Lynch et al., 2000). Thus our present results are very encouraging.

Gast – Project 3

Research and Education Activities:
This proposal examines the diversity, distribution and persistence of human pathogens in the coastal marine environment of Mt. Hope Bay, Massachusetts. This estuarine environment has been heavily impacted by human activity, including significant thermal pollution, several sewage outfalls and the effects of past industrial activities. Monitoring for bacterial contamination occurs in the shellfish areas, but outside of these regions, very little is known about the occurrence of either bacterial pathogens or protistan parasites that are likely introduced into the Bay through sewage or runoff. The potential impact of the thermal output of the Brayton Point Power Plant on these microbial populations has not been examined at all. We hypothesize that both bacterial and protistan human pathogens are present in Mt. Hope Bay and nearby rivers, and that their distribution and persistence are affected by physico-chemical condition in the Bay. To examine this hypothesis we propose to accomplish the following specific aims:
Aim 1: Determine the distribution and persistence of human protistan pathogens in Mt. Hope Bay.

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.

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.

Findings:
Establishment of fieldwork. The summer was spent doing preliminary assessments of the field sites. In July samples of water and sediment were collected using boats supplied by Roger Williams University at two sites in Mt Hope Bay to optimize extraction methods and to establish a summer baseline. One site was at the underwater sewage outfall near the Braga Bridge and the other site was within the discharge plume from the Brayton Point Power Plant. Sites further up the Taunton River were explored to determine optimal locations to sample for warm and cool freshwater samples. In November 2004 a second sampling trip was undertaken to sample at all four of our chosen sites and to begin documenting water column parameters using the YSI datasonde.

In addition to establishing sampling grids, connections were made with researchers at Roger Williams University. Future sampling will be conducted using RWU small boats, and preliminary processing of samples will be accomplished at laboratory facilities there as well. Furthermore, there are several opportunities to collaborate with scientists and undergraduate students who are conducting chemical and physical studies of the bay.

Our seasonal sampling will continue in February (if possible), March, April, May, July and September.

Results. Two different protocols were used to extract nucleic acids from water samples. Both yielded amplifiable DNA. As we are interested in obtaining bacterial nucleic acids as well, we will follow the extraction protocol that utilizes protease digestion in future water extraction. Sediment sample nucleic acids were extracted using the commercial soil extraction kit from MoBio. The sediments also yielded amplifiable material. DGGE analyses were run and determined that the extent of variation between samples from the same site was small in November. Differences between sites were large. Bacterial, archaeal and eukaryotic ribosomal clone libraries were created and sequenced from one of the Brayton Point sediment samples. Most of the clones Blast as unknown environmental clones in the database. Amoeba cultures have been established from all four sites, and screening for endosymbiotic bacteria will begin.

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 chosen three sampling sites in our model estuary to carry out our initial survey of Vibrio diversity. These sites represent the salinity regime under which different Vibrio populations/species have been reported in nature. We are using culture-dependent and independent approaches to determine the types of vibrios that occur. We have constructed several clone libraries with Vibrio-specific 16S rRNA gene targeted primers and sampled these to establish the co-existing diversity of Vibrio populations. This has shown that both our target

species are components of the microbial community in the water column and provides us with the necessary sequence information to establish effective quantitative PCR tools for our planned quantitative monitoring of the different Vibrio populations. We have further begun to assemble a strain collection at the high salinity end of the estuary (representative of the coastal ocean). Since the spring of 2004, we have sampled monthly, which has provided us with a collection of different Vibrio isolates including a large number of one of our target species, V. parahaemolyticus. We have characterized the diversity of these strains by sequencing of several genes (16S rRNA, Hsp60, SodA, ChiA). This provides an important foundation for Aim 3 where we seek to define the bounds of gene flow in co-existing pathogenic and non-pathogenic populations by multii locus sequence typing (MLST). In October 2004, we begun our first sampling of the lower salinity sites in the estuary with the goal of also building up a V. vulnificus strain collection. We have isolated ~5,000 strains from the water column and from potential animal hosts (including oysters) and will characterize their diversity during the winter months.

Findings:

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

To differentiate different Vibrio variants that are free-living or attached to particles (or other environmental compartments), we have begun adaptation and development of a polony amplification technique, which will allow us to carry out in situ amplification and differentiation of different Vibrio strains. This will provide a foundation to correlate abundance and distribution of different Vibrio populations/species with changes in physio-chemical conditions on a variable spatial and temporal scale.

Physical/biological field studies will begin in March, 2005. We are preparing instrumentation for small boat surveys of the estuary in the spring and summer of 2005, to map out the salinity, temperature, chlorophyll and nutrient fields in the estuary, in coordination with the Vibrio sampling, as conditions change seasonally from early spring to late fall. We are planning to coordinate our sampling with the field efforts of Plum Island Estuary LTER researchers.

In addition, we have begun to set up a three-dimensional hydrodynamic primitive equation numerical model (finite volume coastal ocean model; FVCOM) for the Plum Island Estuary domain to study the circulation within the estuary, determine the residence time for tracers, and determine the rate of water mass exchange between the estuary and the coastal ocean. These simulations will allow us to understand how the estuary responds to changes in forcing (e.g., changes in river flow, tidal amplitude, and coastal ocean conditions) and help us interpret our field measurements within the broader context of the physics controlling the Plum Island Estuary circulation and the spatial and temporal structure of salinity and temperature.

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.

As mentioned above, we have obtained additional funding from the Moore foundation, which

will provide us with two complete genome sequences of our isolates. We anticipate that these genomes will greatly enhance the aims of this study and they come at no cost to the project. For example, we will determine the presence and diversity of potentially mobile elements in the genomes and will then carry out screens in our strain collections. One of our target will be integron and phage-related genes, which have high potential to be transferred between strains, but others will include specific pathogenicity gene s (e.g., type III secretion systems, haemolysin genes), which we will map onto the strain collection to determine their prevalence and potential combinations of occurrence.

Sogin – Genomics Facility Core

Research and Education Activities:
The genomics core provides DNA sequencing services and computational support to investigators in the Woods Hole Center for Oceans and Human Health (WHCOHH). The level of DNA sequencing and data analysis will ramp up during years two thru five. Many of the Center's projects will take advantage of our strengths in molecular microbial ecology and the bioinformatics required for molecular phylogenetics. To accommodate anticipated demands of the WHCOHH research projects, we have expanded the capacity of our core laboratory for high-throughput DNA sequencing and phylogenomics within the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution (JBPC) at the Marine Biological Laboratory (MBL). We have purchased additional robotics for high-throughput liquid handling and a second ABI 3730Xl DNA sequencing platform (using a combination of institutional resources and funding from the NIH NCRR Shared Instrumentation Program - NIH Grant 1 S10 RR021045). These instruments will double our DNA sequencing capacity. For data storage, we have added 3.5 terabytes of disk space (as outlined in our original proposal) and a data archiving tape library system that will protect the integrity of data generated by the WHCOHH.

Center investigators are currently able to submit a request for sequencing using a web-based form that provides the core facility staff with information on the type of sample, primers desired, and post-processing steps requested (http://jbpc.mbl.edu/pubforms/WHCOHHSvc.html). When samples are received, they are sequenced on one of two available Applied Biosystems 3730XL sequencers within 1-2 days. After the automated pipeline is run on a project dataset, the investigator may view graphs depicting data quality, e.g. range of high quality bases or number of singlets vs. assembled contigs, and statistics for each project are presented in a table derived from the underlying database. With the recent recruitment of a dedicated bioinformatics specialist (Susan Huse), we will be able to expand this pipeline to provide automated data analysis functions that can be requested via the web-based pipeline. Staff of the Bay Paul Center for Comparative Molecular Biology and Evolution has considerable expertise in pure statistics, genome assembly, astrophysical modeling, gene expression, phylogenetics, information management, data acquisition pipelines, and databasing. The combination of this expertise with our advanced computational resources will meet anticipated needs for each of the projects supported by The Woods Hole Center for Oceans and Human Health. Dr. Sogin has provided advice to PIs of individual projects on the use of appropriate computer algorithms including alignment algorithms CLUSTALX and where appropriate phylogenetic analysis.

Findings:
To date, we have used a small fraction of our DNA sequencing budget. Due to the staggered arrival of the awards from NIH and NSF, the subcontract to the parent NSF WHCOHH award did not arrive at the MBL until May. Therefore, this report summarizes only nine months of activity (late May through January). For similar reasons, the sample collection plans for the primary WHCOHH research projects rescheduled their sample collection activities. To date, the Genomics core facility has provided services to the Anderson laboratory, which is exploring population structures for toxic dinoflagellates, and the Polz laboratory at MIT, which is exploring vibrio species. A total of 4032 sequencing reads have been provided to those two projects over the last nine months or about 25% of the anticipated volume on an annualized basis. Because of the initially low sequencing volumes, we deferred the hiring of the bioinformatics programmer and the dedicated technician to January, 2005. The primary WHCOHH projects have now scaled up their activities and several pilot projects with sequencing requirements are underway. The unexpended funds for sequencing reagents will allow us to support much higher levels of activity during the second year of the project. By shifting the bioinformatics hiring to January, we will be in a better position to meet the data processing demands associated with increased levels of data generation over the next 18 months.

Hajduk - Pilot Project Program

Research and Education Activities:
The specific objectives of the Pilot Project Program are:

- 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.
- To support the collection of preliminary data that can be used to generate full proposals to NSF, NIH, or other agencies or organizations.
- 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.
- To stimulate collaborative and interdisciplinary research within the center.
- To foster the application of new technologies and experimental approaches to questions concerning the impact of oceanic processes on public health.
- To ensure the ability of the center to respond rapidly to new scientific information and emerging challenges in this field.
- 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:
To achieve the objectives outlined above, we established a Pilot Project Program and issued a call for pilot project proposals in June of 2004.
The request for proposals was distributed by email to all faculty and research staff at WHOI, MBL, and MIT. Shortly after the call for pilot project proposals was issued, we held informational meetings at MIT (for MIT faculty) and in Woods Hole (for WHOI and MBL

faculty). At these meetings, the structure and objectives of the overall Oceans and Human Health program were described, and the objectives of the Woods Hole/MIT Center were presented. The Pilot Project Program, including application and review processes, was described and potential applicants were given the opportunity to ask questions.

Thirteen one-page pre-proposals were received by the deadline of July 15, 2004. These pre-proposals requested a total of $938,557 ($615,230 direct costs). Five were from WHOI, 4 were from MBL, 3 were from MIT, and one was cross-institutional. The applicants came from multiple departments at the three institutions. The pre-proposals were reviewed and scored by the Pilot Project Program Director, Pilot Project Program Deputy Director, Center Director, Center co-Director, and Genomics Core leader. All 13 applicants were given feedback from the review.

Six of those submitting pre-proposals were invited to submit full proposals. The following six full proposals, requesting a total of $414,352 ($263,924 direct), were received by August 15, 2004:

- Characterization of a cyanobacterial anti-algal compound
- Cnidarian toxins against voltage-gated Ca2+ channels
- Marine phage as vectors of gene transfer between marine bacteria and bacterial pathogens
- Development and Use of Gene Transfer to Understand the Molecular Basis of Toxin Production in the pennate diatom Pseudo-nitzschia multiseries
- Assessing and Monitoring Microbial Communities Associated with Blue Mussels Growing at an Offshore Site
- Development of a PCR-based method for quantifying grazing rates on harmful Algae.

The six full proposals were reviewed and scored by members of the Internal Advisory Committee, according to the following criteria: Scientific merit, Relevance and Potential Impact, Innovativeness, Investigators, Facilities, and Broader impacts. Three proposals were selected for funding:

- 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
  (Jon King, MIT)

These pilot projects were supported using the funds provided by NIH and NSF as part of the COHH Center grant ($126,174), supplemented by additional institutional commitment provided by WHOI ($31,447), for a total of $157,621.
The funded pilot projects address important questions regarding the interactions between the oceans and human health. Each of the projects represents a new direction for the Center, expanding the scope of our research efforts. Significantly, two of the projects address questions concerning marine natural products, one of the three focal areas of the COHH program and the only one not represented in our original proposal. The projects also serve to

add four new scientists as participating members of the center, expanding our representation at all three member institutions. All three of the pilot projects involve postdoctoral researchers or graduate students, and will thus contribute to the training of the next generation of researchers in this area.

Training and development:

There will be a variety of training opportunities through an REU program, as well as in graduate education. This will be an important part of the activities of the Center.

It is our goal to establish interactions with the Miami COHH center regarding the possibility of conducting a small survey of undiagnosed pneumonias and gastroenteritis cases in the Mt Hope Bay region from hospital records and to compare those with records from a similar region without the stresses found in Mt Hope Bay (eg. Wellfleet).

Outreach: The Center's activities have substantial importance for local, State and Regional public health concerns. As well, the studies will be of interest to local teachers and students.

WHOI publishes **Oceanus**, a semi-annual report on current WHOI research projects, written by WHOI scientists and edited for a lay audience. Each issue provides several articles on a single theme, such as ocean circulation, biodiversity, and climate. Three articles concerning Harmful Algal Blooms have been published in the latest issue of Oceanus.

Invited speaker for MBL Science Writers workshop, June 8, 2004. Title: "Microbial Life in Extreme Environments" – included a presentation on Mt. Hope Bay and Thermal Pollution.

MBL LabNotes article (Volume 14, Number 2) entitled "Fighting the Tide; New Center for Oceans and Human Health tackles small but powerful human health threats" - featuring

Radio Station Interview with Bridgett Ennis from Finger Lakes Productions International: Nov 22, 2004 - 90-second radio feature titled Our Ocean World – hasn't aired yet.

NE-COSEE participation with New Bedford Global Learning Charter School. Presentation to the 8th grad class regarding the project in Mt Hope Bay, September 2004.

The COHH genome core has built a website that will be used by its investigators for processing molecular data and it will be linked to a new initiative funded by the Sloan Foundation, "The International Census of Marine Microbes" (ICoMM) (http://icomm.mbl.edu). Investigators from the COHH project and the University of Washington are members of the Scientific Advisory Group for ICoMM, which will have a significant component relating to identification and distribution of human pathogens in marine environments.