NOAA MARINE MICROBES WORKSHOP REPORT

Hollings Marine Laboratory,
Charleston, SC,
November 29-30, 2011

Report produced by the Office of Oceanic and Atmospheric Research,
Office of Ocean Exploration and Research

Acknowledgments

NOAA thanks all the workshop participants as well as the NOAA employees and affiliates who contributed to the organization of the workshop and the production of this report. In particular, special thanks are due to Reginald Beach for shaping and leading the workshop, to Susan White for hosting the workshop at the Hollings Marine Laboratory, and to Nathalie Valette-Silver and Margot Bohan for co-leading the production of this report. A draft of this report was submitted twice for comment to all workshop participants, speakers and members of the NOAA Marine Microbes working group.

Table of Contents

Executive Summary

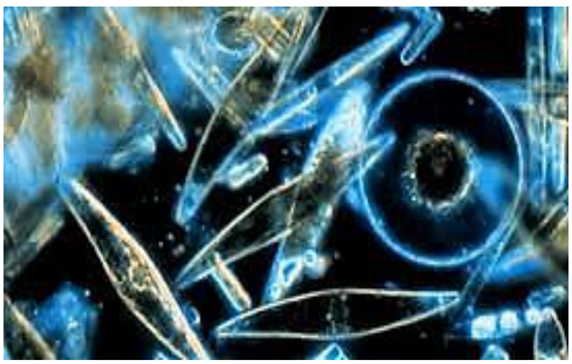
As we learn about the diversity of microorganisms and their associated biogeochemical processes, our view of the world's ocean ecosystems is being transformed and the relevance of microbes to the discussion of ocean resiliency and marine resource management is becoming unavoidable. Yet, contemplation of the sheer number of microorganisms, as well as their vast diversity and function in our ecosystems, has led to the realization that we only poorly understand how our planetary biogeochemical balance (or imbalance) is being realized, how emerging diseases are responding to global change (warming, acidification, coastal urbanization, pollution), and how microbial processes should be integrated into our biogeochemical and ecosystem health forecasts.

Given these relatively new insights into the breadth of the microbial realm, as well as the desire to know more about marine microbes, a community workshop was held in late November 2011. The aim of the workshop was to discuss ways to enhance NOAA's knowledge of the marine ecosystems' microbial components and to identify tools, insights and roles specific to microbial science that NOAA should consider embracing. Certainly, strengthening NOAA's holistic comprehension of the ocean's physical, biological, chemical and geologic components is critical to improving the agency's ability to conduct its stewardship role, foster ecosystems' resiliency and promote sustainable resource management.

Several programs within NOAA are actively supporting microbe projects, but the specifics of these activities and associated assets are not well known within individual line offices, and even less well known across line offices. This workshop was a 'kickoff,' in terms of providing a forum for cross-matrix NOAA discussions and for engagement with the wider microbial science community. The workshop established the important role of microbes in marine ecosystems and the strong role NOAA has to play in the marine microbial science arena, given NOAA's significant environmental sampling capabilities, its responsibilities for marine ecosystem health and ecological forecasting and its commitment to understanding biogeochemical cycles. The agency's dedication to these activities enables NOAA to better inform constituents on short- and long-term environmental status, trends and variability, as well as stewardship and management of our marine living resources. The workshop also illuminated the fact that, the microbial science community external to NOAA (other government agencies, industry and academia), has excess sequencing capabilities and capacity compared to ocean sample inventories. Community resources for analysis, sample storage (freezing) and cataloging genetic information appear to be well established and, generally, seem well supported; yet, due largely to cost and logistical difficulty of getting to sea, the community is ocean sample-limited. This workshop provided also an initial view into partnership possibilities. The time is ripe for engaging in what will undoubtedly be mutually beneficial partnerships

Introduction

Microbes are ubiquitous in the marine environment and play many varied roles in the ocean. As we learn more about the diversity of marine microorganisms (in the broadest sense – including microalgae, bacteria, protozoa and viruses) and their associated biological processes, our view of the functioning of world's ecosystems is being transformed. Equipped with new insights into the breadth of the microbial realm, we have the opportunity to enhance our understanding about how our planetary biogeochemical balance (or imbalance) manifests itself, how/why emerging diseases are responding to global change (warming, acidification, coastal urbanization and pollution), and how/why the study of microbial processes should be integrated into our biogeochemical and ecological health forecasts.



Microscopic plant-like organisms, called phytoplankton (pictured here), are the base of the marine food web. Diatoms are one of the most common forms of phytoplankton. Yet, certain species of phytoplankton can also be responsible for "red tides" or harmful algal blooms. Photo Credit: NOAA

Understanding Microbes & Their Communities

Most microbes live in highly organized and interactive communities that are versatile, complex, and difficult to analyze from many perspectives. Two of these challenges are outlined below.

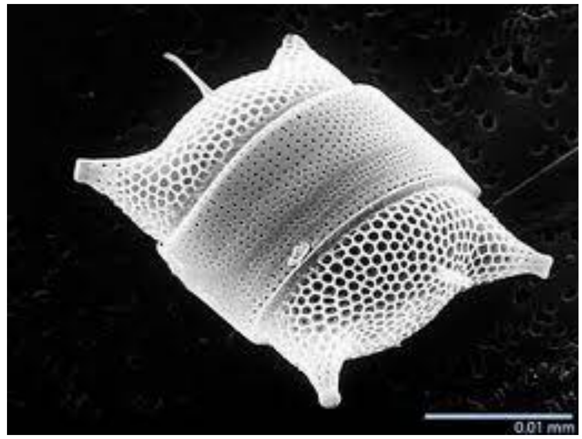
- *Microbes are exceedingly small—only 1/8000th the volume of a human cell and spanning about 1/100th the diameter of a human hair. Investigating processes within this size range is challenging.*
- *The microbial world encompasses millions of genes from thousands of species, with hundreds of thousands of proteins and multi-molecular machines operating in a web of hundreds of interacting processes in response to numerous physical and chemical environmental variables. Gene control is complex, with groups or "cassettes" of genes (operons) directing coordinated transcription and translation of genes into interacting proteins.*

NOAA is looking at ways to increase the agency's knowledge of the marine ecosystems' microbial components and to identify tools, insights and roles specific to microbial science that NOAA should embrace. NOAA has a strong role to play in the marine microbial science arena, given the agency's significant environmental exploration and sampling capabilities, its responsibilities with regard to marine ecosystem health, and its capacity in forecasting ecological/ biogeochemical cycles. This predictive capability is essential to better inform short- and long-term environmental status, trends and variability as well as stewardship and management of our marine living resources. Presently, NOAA is pursuing some aspects of microbial science. This workshop provided an opportunity to tie NOAA threads together, sharpen the focus of its activities and engage the broader microbial science community.

Background: Why Marine Microbes?

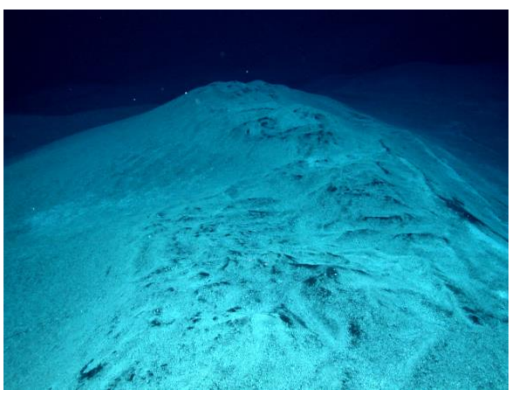
Scientific Motivation

Microbiologists know, by means of early studies using microscopic and other methods, microbes are everywhere -- in soil, water and air-- and comprise a significant portion of humans and all other organisms, both externally and internally. The world is quite literally bathing in microorganisms.



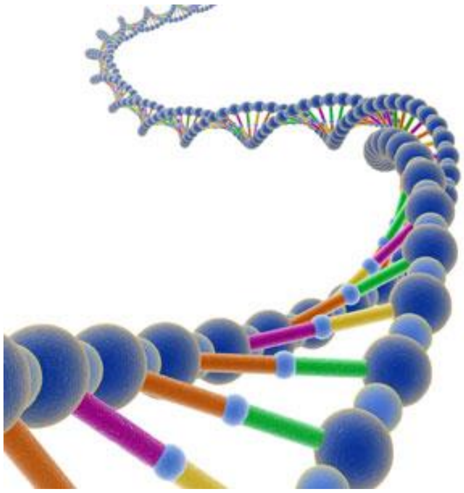
Diatoms are plant-like microbes that rely upon sunlight for energy. They are typically found in open water, although some live as surface films in the benthic marine environment or even under damp conditions. Photo Credit: NOAA

Relatively new genetic techniques, spurred by the Human Genome Project's goal of identifying the ~20,000-25,000 human genes, have drastically changed our viewpoint of Earth and Earth's composition and functioning. Results from applying the Project's genetic and subsequent sequencing approaches gave scientists insight into the identity of microbes and their possible metabolic functions.


Underwater microbial mats have been described as multi-layered sheets of micro-organisms (mainly bacteria & archaea) that live by exploiting and, to some extent, modifying local chemical gradients. Photo Credit: NOAA OER

Microbes and their communities underpin the function of the biosphere and are integral to all life on Earth. They are the earth's processing factory of biological, geological, and chemical (biogeochemical) interactions that make the earth habitable for humans.

These organisms are capable of existing in practically any environment and garnering energy from a variety of sources, from solar radiation to chemosynthesis (e.g., generated chemicals coming from the subsurface of the earth). They also play an essential role in marine ecosystems, driving and serving as indicators of change in the ocean. Dr. Rita Colwell suggested that microbes are "the canary in the coal mine" for the marine environment.



Prior to the technological advances of this millennium, such as DNA sequencing, the details of microbial function remained largely unknown. Photo Credit: Shutterstock/ Benjamin Albiach Gallan

Yet, the microbial world remains a largely unexplored frontier of truly confounding dimensions. For example, it is estimated that there are billions of times more individual bacteria on Earth than there are stars in the universe; a single drop of water contains millions of microbes. Given their adaptable nature and pervasiveness in the marine environment, microbes are organisms that can expand our comprehension of marine life processes at a whole-system level. Below are several discoveries [from projects recently funded by the National Science Foundation (NSF) and Department of Energy (DOE)] that underscore the ubiquity and indispensable value of some microbes in our ecosystems:

- Ocean phytoplankton, such as cyanobacteria *Prochlorococcus* and *Synechococcus*, account for about half the globe's photosynthesis, producing oxygen that sustains human and other life.
- Diatoms (ancient, intricately-shape microbes) represent one of the largest groups of organisms on Earth. They amass carbon in quantities comparable to that in all the earth's rainforests combined, and very likely, in geological time, influenced the earth's climate.
- Phytoplankton and other microbes are the primary producers in the ocean and form the base of all ocean food webs, leading ultimately to fish and marine mammals.
- In the Sargasso Sea—a body of water thought to be devoid of life—more than one million genes were discovered during DNA sequencing of microbe fragments recently collected there.

Microbial mats were the earliest form of life on Earth for which there is good fossil evidence. These microbes can use all types of metabolism and feeding strategies that have evolved on Earth. Chemotroph microbiol mats(pictured here) are abundant in deep and undersea volcanic environments. Photo Credit: NOAA OER

- Fungi also play an important role in the ocean processes and are a potentially important component in marine microbial food web.

This great diversity and sheer number of organisms has led to a "genomic revolution," a collective scientific realization that, while we thought we knew how the planet worked, there are still many realms to be studied and understood such as microbial processes, microbial community compositions and elemental pathways. Scientists are only beginning to explore this microbial world. Thanks to the latest technological development, the world's microbes and their unique biochemistries have begun to offer an essentially limitless store of benefits that can be applied to human needs, including energy and environmental missions. Understanding microbes in the context of whole living systems and harnessing their unparalleled capabilities will offer NOAA and the Nation new solutions to longstanding global stewardship challenges.

Programmatic Motivation

As a nation, we increasingly perceive the critical role of ocean processes in the functioning of basic Earth systems, yet our knowledge about these processes--many of which are mediated by microbial communities--remains limited. As we continue to experience extraordinary changes on our planet--changes that impact our lives and livelihoods-- the American public, coastal/ocean managers and public health officials are increasing their demands for predictive capabilities and early warnings of environmental changes and related impacts. These forecasts will help save lives, reduce human health risks, sustain healthy ocean and coastal ecosystems and preserve the economic benefits of these systems.

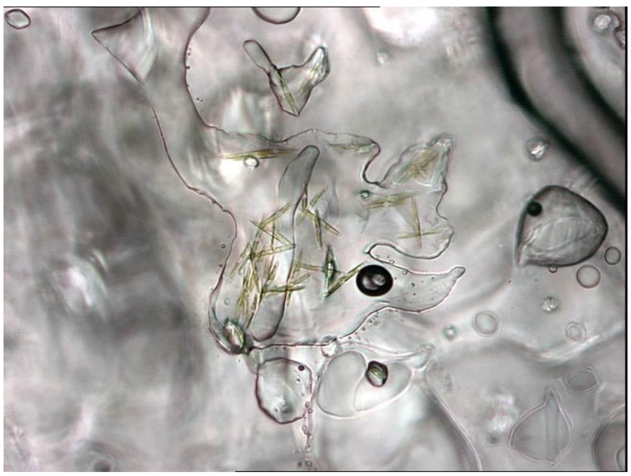


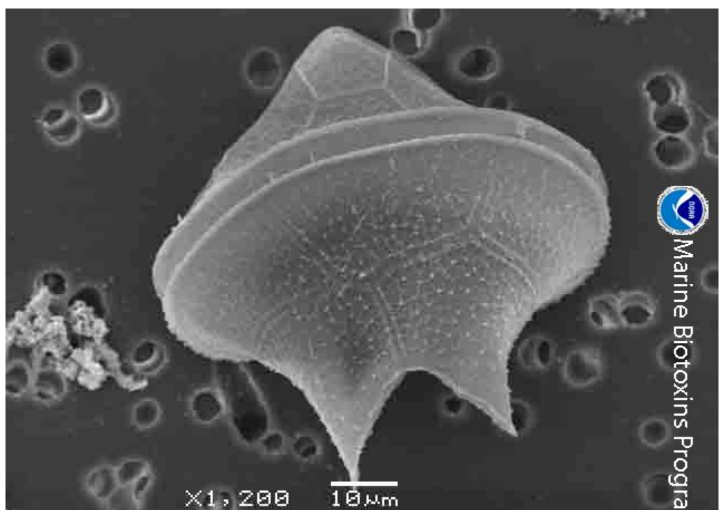
Image of Arctic phytoplankton. A recent study documented a massive Arctic phytoplankton bloom beneath the Arctic pack ice. Until recently, phytoplankton was thought to be restricted to waters free of sea ice. Photo Credit: NOAA

NOAA's vision of the future is one where societies and ecosystems are healthy and resilient in the face of sudden and/or prolonged change. Central to NOAA's mission is addressing many of the global environmental challenges we face, including the changing climate, the increasing number of natural and human-induced disasters, the threatened and degraded state of our ocean and coasts, and declining marine biodiversity. One of NOAA's primary mandates is to understand and use science to protect lives, property and resources and to address the dynamics of our ever-changing planet. This mandate includes characterizing ocean threats to human and ecosystem health, determining coastal water quality and stewarding living marine resources.

Harmful algal bloom in the Gulf of Mexico. Photo Credit: NOAA

Many believe that marine microbial science can provide the foundation for marine ecosystem forecasting, prediction and remediation. The agency's Healthy Ocean goal in NOAA's Next Generation Strategic Plan (NGSP), specifies the need for better comprehension of the microbial component of marine ecosystems within the context of the broader NOAA vision. Inherent in the NGSP Healthy Ocean Goal and NOAA's science mandate (referenced above) is the need to identify clear priorities for marine microbial science. This is particularly crucial, in light of the capacity of microbes to function as indicators (and drivers) of change in the ocean. Developing an ecosystem-level capability in NOAA to focus on microbial science will help us standardize, broaden and focus our investments.

NOAA is well-positioned, particularly given recent advancements in microbial science-specific tools and technology, to develop a robust strategy for incorporating microbial science in the agency's holistic approach to understanding the earth system. In the big picture, a better understanding of the role of microorganisms in ocean ecosystems will allow for improved monitoring of the overall health of the ocean and a more nuanced grasp of the ocean's role in regulating and responding to changes in global climate and other critical ecosystem processes.



Harmful algal blooms produce toxins that impact most marine organisms. The toxins, in turn, affect humans when they eat the contaminated sea food. Photo Credit: NOAA

Scope of Workshop

The goal of the workshop was to deliberate and discuss how we can strengthen NOAA's holistic understanding and management of ocean ecosystems by enhancing our knowledge of its microbial components and by identifying roles and opportunities best suited to NOAA. During his introductory remarks, OAR Acting Assistant Administrator, Craig McLean, said that a key to this gathering was to learn from the science community and to let their input guide NOAA's future engagement in microbial research. NOAA will continue to engage its partners from the wider research community and develop plans to integrate marine microbe research across its Line Offices, devising new approaches and incubating long-term-research in this important area. In advance of the workshop, the following challenges were imparted to all invited participants (see Appendices 1 and 2 for more details):

Microbes adapt rapidly in response to environmental change, an ability that underlies their survival for billions of years. For example, various species of "extremophile" microbes have adapted to great extremes of pressure, high surface-to-volume ratio enhances interactions and supports adaptation. Unlike animal cells, they have no protective nucleus for their DNA, which leaves it more vulnerable to alteration. Genes move easily among species. Moreover, microbial communities are awash in genetic material from viruses that confer additional genetic properties and expand their range of adaptability.

Workshop Challenge 1: Assuming NOAA would be inclined to improve its holistic understanding and management of ocean and coastal ecosystems by enhancing its knowledge of the microbial components of the marine ecosystem, *are there certain capacities or activities that will enable NOAA to meet this objective? Are there tools and insights that NOAA should have vis-à-vis microbes to better conduct its ocean stewardship activities?*

Workshop Challenge 2: Understanding that NOAA, the National Science Foundation, and the National Institute of Environmental Health Sciences currently conduct significant activities focused on Oceans and Human Health with some microbial emphasis, *what critical area(s) of study should NOAA specifically pursue to improve our understanding of marine microbes and their associated functions and services?*

Workshop Challenge 3: Acknowledging that NOAA and its partners conduct a significant portion of the total operational oceanic observations made daily, s*hould these observations include microbes? If so, what should the observational priorities be? For example, how important is it for NOAA to understand how microbes vary among habitats, photic zones, or water masses and whether/how microbes in habitats or water masses are linked? How important is it for NOAA to understand how the community* *composition and distribution of microbes respond to global changes (warming, chemistry, coastal urbanization and pollution)?*

These challenges served as foundational elements for consideration by discussants during the workshop.

The NOAA-hosted Marine Microbes Workshop was held at NOAA Hollings Marine Laboratory, Charleston, South Carolina. Photo Credit: NOAA

All told, 20 community participants (see Appendix 3) from academia and industry met at the NOAA Hollings Marine Laboratory in Charleston, South Carolina, with a comparable number of NOAA personnel, to share their expertise and perspectives on the current state of knowledge and technology regarding marine microbes and to help NOAA determine what its investment priorities should be in microbial research and technology. The workshop got underway with introductory remarks from Craig McLean (see Appendix 4) followed by microbial science and forecasting/modeling experts from NOAA Line Offices providing overviews about their programs'/offices' interests and emphases (see Appendix 5).

Following these overviews, subject matter experts from the external community gave presentations (see Appendix 6) focused on the following topics: 1) microbe observing tools and instruments, as well as biotechnology and natural products; 2) biogeochemical processes and cycling; 3) ecosystem health and emerging marine microbial diseases, organisms and human health; 4) forecasting microbial responses to global changes.

In addition, all workshop attendees participated in 3 topical breakout sessions (see Appendix 7 for more details). During each session, major challenges specific to the topic area, as well as tools/technologies, study methods, and opportunities were identified.

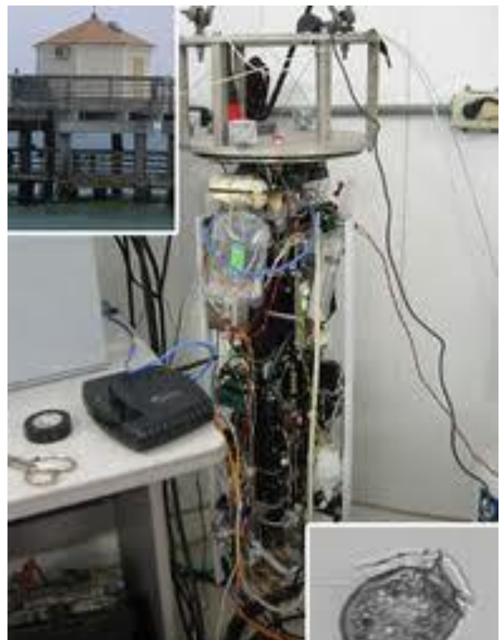
Finally, before the workshop wrapped up, a plenary of all the workshop participants was convened to formulate a list of major microbe-related science questions, as well as recommendations for near-term and longer-term activities/investments, for NOAA to consider (see Appendix 8). The workshop was followed the next day by a meeting of the NOAA workshop participants to discuss lessons learned and potential next steps (see Appendix 9).

Workshop Discussion Summaries

Following delivery of the introductory remarks and the charge to participants, the workshop began in earnest with short presentations by NOAA scientists.

Each presenter described ongoing activities related to marine microbe science in the National Marine Fisheries Service (NMFS), the Office of Oceanic and Atmospheric Research (OAR), the National Ocean Service (NOS) and the National Environmental Satellite Data and Information Service (NESDIS).

NMFS' presentation focused on projects supporting: the development of early warning systems for health hazards; the assessment of climate variation effects on ecosystem health; the sustainability of aquaculture and seafood safety; and the assessment of microbial impact on aquatic animal health.



Flow Cytobot, a tool used to optically detect marine microbes. Photo Credit: NOAA. Upper insert: Instrument deployment site in the Gulf of Mexico-Texas. Lower insert: Image of a toxic algae Dinophysis cf ovum. Photos: Lisa Campbell

OAR's presentation concentrated on ecosystem modeling. Earth System Models (ESMs), used in OAR, incorporate only rudimentary biodiversity information. OAR's fundamental message was that better constraints on microbial rates and biodiversity would help address many limitations of the present modeling framework.

NOS' presentation described activities aimed at the development of tools and methodologies to assess: microbial water quality; the use of molecular tools to identify the sources and distribution of pathogenic microbes in the marine environment; the relationship between nutrient and contaminant cycling and their impact on Harmful Algal Blooms (HABs); advancing HABs modeling tools; and human and ecosystem emerging diseases.

NESDIS' presentation addressed: the use of satellite products to indirectly assess, monitor, detect, and predict marine microbe presence and distribution; the development or modification of algorithms to improve the interpretation of satellite imagery in coastal waters; and NESDIS' capability to archive and distribute data and information collected in coastal and deep ocean areas.

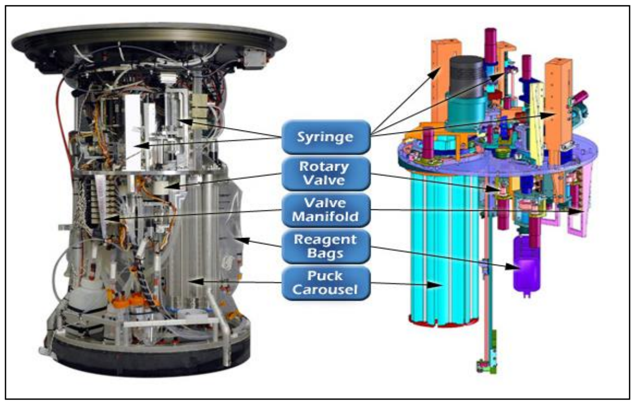
These presentations provided all workshop participants with a snapshot of NOAA's present investment in marine microbe-focused efforts.

EXPERT PRESENTATION - OBSERVING TOOLS, METHODOLOGIES, INSTRUMENTATIONS & APPROACHES

Dr. John Paul (Distinguished University Professor Biological Oceanography, University of South Florida) gave the first of four external community expert presentations, providing an overview of the suite of instruments and tools that are presently available to observe marine microbes.

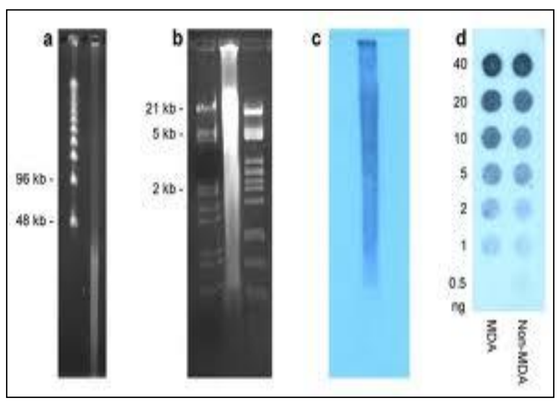
The following is a list of the tools and their various capabilities:

- Optical detection is made possible through the use of tools such as the *in vivo* pigment spectrometer or "Brevebuster" or the "Flow Cytobot," for *in-situ* flow cytometric analysis;
- The "Nucleic Acid Sequence Based Amplification" (NASBA) is used for genetic detection;
- The "Autonomous Microbial Genosensor" or the "Environmental Sample Processor" can be used to detect a wide range of organisms (e.g., microbes, harmful algae, and invertebrate larvae);



Environmental Sample Processor, Photo Credit: NOAA

- Single cell sequencing can be done using the Cytometric Sorting and Multiple Displacement Amplification (MDA) tool that is used for whole genome amplification;
- Meta-transcriptomics captures gene expression patterns in natural microbial communities and gives insights into the environmental biogeochemistry;
- Satellite imagery can be used to detect such things as the dinoflagellate *Karenia Brevis* in the Gulf of Mexico.



Multiple Displacement Amplification (MDA) conducts single cell genetic sequencing to enable identification of microbes. Photo Credit: Simková et al., 2008

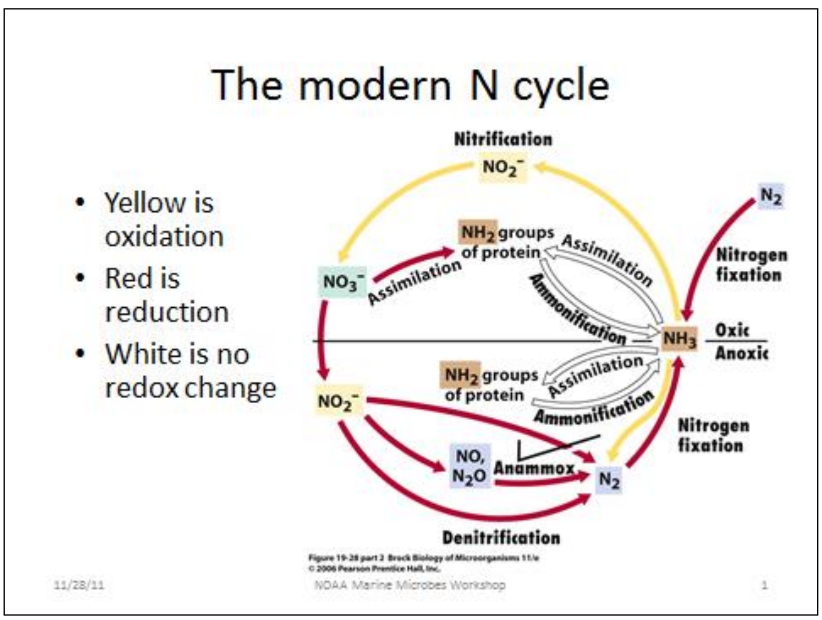
Dr. Paul concluded his presentation by suggesting the greatest potential for these tools and techniques would be to link them all together in the future. Autonomous genetic sensors show great promise but are costly. In fact, the "wish list" of marine biologists includes a "Microbial Detector Tricorder" that would provide scientists with insights into species identification, community composition, and activity for all microbes present in a sample. Something of this nature is not yet available, but it is something to strive for and likely would enable this field to make great leaps forward. (See Appendix 6-I for more detailed materials from Dr. Paul's presentation).

Dr. Paul's presentation was followed by a short discussion in plenary that highlighted: (a) the feasibility and need to develop a microchip to quickly identify marine microbes; (b) the important role of information technology and bio-informatics; (c) the need to train scientists and students in bio-informatics; and (d) the need to improve the nation's computational infrastructure. The topic of observing tools, methodologies, instrumentation and approaches was also integrated into the three workshop breakout session discussions that followed other expert presentations.

EXPERT PRESENTATION - BIOGEOCHEMICAL PROCESSES AND CYCLES

Dr. Margo Haygood (Distinguished Professor in Marine and Biomolecular Systems, Oregon Health and Science University) discussed the role of marine microbes in biogeochemical processes and cycles of key chemical elements. Biogeochemical processes control cycling of biologically important elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur as well as elements of lesser importance such as sodium, magnesium, potassium, calcium and chlorine.

Results from recent studies on the role of metals in biogeochemistry highlight the complexity of the nitrogen cycle. Nitrogen is the most common limiting factor in primary production. Microbes dominate processes and cycles of these key elements and are the foundation for primary production and for the formation of new organic matter. Microbe metabolisms are very diverse (e.g., heterotrophic, autotrophic, etc.) and



Schematic diagram extracted from Dr. M. Haygood's presentation microbes play a major role in the modulation of the biogeochemical processes that include all these important elements.

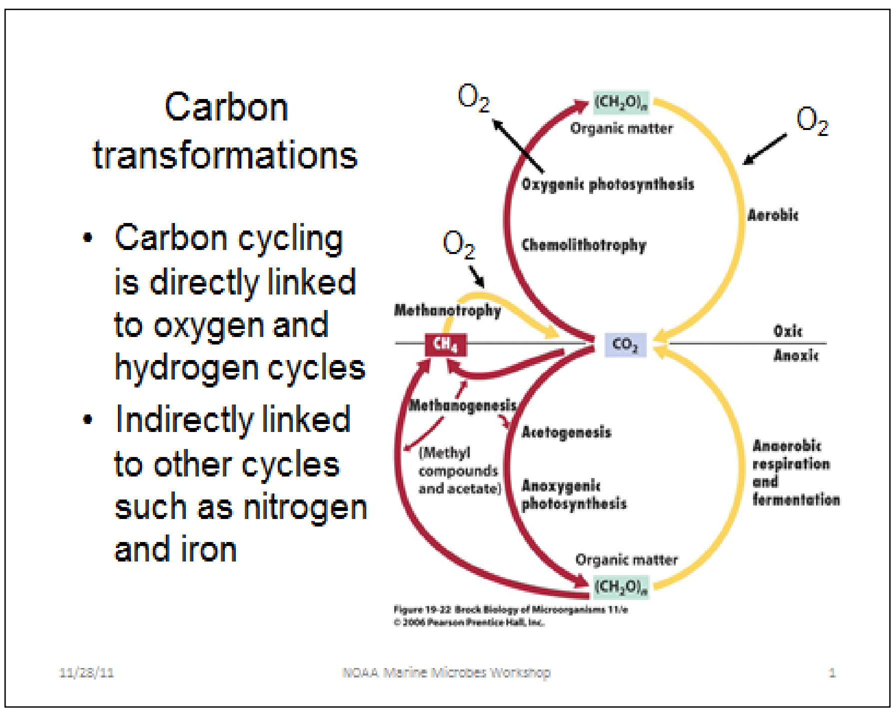
Factors of greater concern for ecosystem functioning are: warming of the atmosphere and of the ocean, increased seawater CO_2 concentration leading to acidification of the ocean and calcium carbonate minerals under-saturation that strongly influences calcification of marine organisms, and the expansion of low oxygen zones. The factors highlighted above reflect natural or anthropogenic events, will affect ocean processes and will have important effects on the health and functioning of marine ecosystems and the microbes existing within them (See Appendix 6-II for more detailed materials from Dr. Haygood's presentation).

BREAKOUT SESSION I: BIOGEOCHEMICAL PROCESSES AND CYCLES

The breakout session (see Appendix 7-I) that followed Dr. Haygood's presentation identified challenges and opportunities, needed tools and methodologies and the top science questions of interest to NOAA concerning biogeochemical processes/cycles and the role of marine microbes.

(a) Major Challenges and Opportunities

The natural or man-made changes observed in our environment, specifically in the ocean, represent both a challenge and an opportunity for the scientific community and for NOAA in particular. For example, the "rise of slime" or "microbialization" of the ocean will lead to habitat loss and/or changes and shifts of the elemental cycles without recovery to base. However, discovery of new habitats and new microbial functions will lead to the understanding of new processes/cycles.



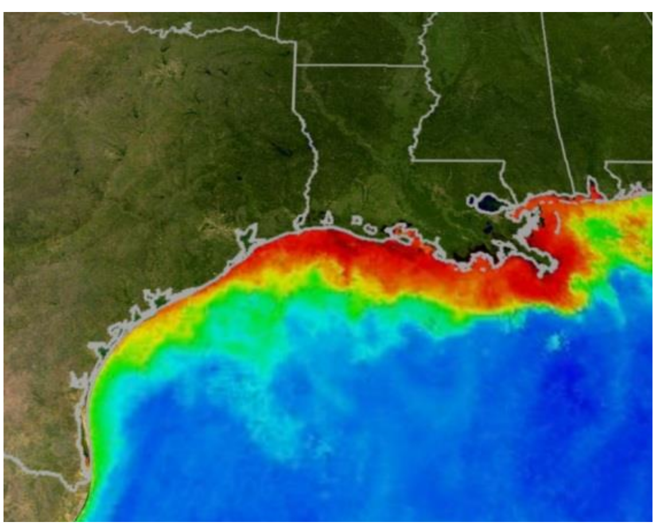
Schematic diagram extracted from Dr. M. Haygood's presentation

Increased urbanization of our coasts and associated nutrient loadings accelerate the pace of cycling. This change in nutrient input leads to changes in biological community structure and ecosystem function. Consequently, identification of "keystone species" is essential to understand the system's biological processes from the molecular to the ecosystem level. Improving our knowledge of the role of microbes in cycles and environmental resiliency is then indispensable. Workshop participants identified some noteworthy challenges concerning our comprehension of the marine microbe's role in biogeochemical processes and cycling including: (a) lack of funding to advance the needed research; (b) lack of communication and dialogue between the various groups working on that topic; (c) lack of general awareness of the importance of marine microbes in the oceans; and (d) the need to share and visualize the large amount of microbe-specific data that has been and will be gathered in the future.

Coral disease (an example is pictured here) occurs generally in response to biotic stresses, such as bacteria, fungi, and viruses and/or abiotic stresses, such as increased water temperatures and pollutants. Photo Credit: NOAA

Special attention must be paid to data set management and microbiologists must find new ways to visualize the large data sets available now and that will be acquired in the future. It is also essential to increase education and outreach to raise awareness of marine microbes to ocean.

(b) Tools, Methodologies, Instrumentation and Approaches



"Anoxic" or dead zone in the Gulf of Mexico off the Louisiana coast. Photo Credit: NOAA

The participants identified the need to focus on strategically selected regional studies. They recommended that NOAA provide the community broad access to its platforms for sampling, following appropriate sampling and preservation techniques. In addition, an effort should be made to develop new sensors to incorporate micro-biogeochemistry measurements into existing monitoring designs and to add instruments already deployed on observing stations.

According to workshop participants, identification of data archiving possibilities and samples repository capabilities are a must and NOAA needs to work in collaboration with other agencies and academic institutions nationally and internationally to improve archiving and access to both samples and data. Common access to new and archived data is essential to improving the community's modeling and forecasting capabilities.

(c) Top Science Questions and Opportunities for NOAA

- How are microbial communities structured, and what are their roles in the marine environment?
- How do nutrient loadings impact microbial community structure and what are the load tipping points?
- What are the sentinel species of microbes, including viruses, and their role in the various biogeochemical cycles?
- Which microbes are "indicators" of changes in biogeochemical cycles and ecosystem function? Which ones are the drivers of these cycles?
- What is the impact of a decreasing dissolved oxygen, ocean warming and acidification on these drivers?
- How can these "indicators" be used in mitigation efforts?

To respond to these questions in an organized way, attendees suggested the organization of a Gordon Conference on marine microbes to integrate across disciplines, regions and groups and to ease transition of basic marine microbe research results to applications.

Decline (and Fall) of
Pacific Island Shallow Coral Reefs

Dr. Rusty Brainard of NOAA's Pacific Islands Fisheries Science Center (PIFSC) and Dr. Forest Rowher of San Diego State University, have had a 5+ year partnership investigating the health of shallow coral reefs on remote Pacific islands. PIFSC has been providing berths on their island cruises to San Diego State University which has provided personnel and expertise to characterize microbial communities. This partnership has shown that the decline and disappearance of shark populations in proximity to increasing human populations and urbanization and increased nutrient levels, leads to a trophic imbalance and, ultimately, degraded reefs.

The trophic cascade goes something like this, reflecting multiple, cumulative effects: the coral grazer community increases due to fewer sharks, reduced algal communities (due to overgrazing) allow for an increased viral community. As the grazing community grows, so does the virus community, ultimately reaching a viral ecosystem health tipping point, such that viruses dominate and the reef ultimately dies. Without the microbial insight, especially viruses, this puzzle of dying coral reefs would not have been solved.

This government / academic partnership is a fine example of how NOAA can work with outside partners to benefit NOAA's stewardship mission, at little to no additional cost.

Expert Presentation - Emerging Diseases, Organisms and Ecosystem Health

Dr. Forest Rohwer (Professor of Biology, San Diego State University) discussed the impacts of ocean "microbialization" on coral reefs. (See Appendix 6-III for more details.) "Microbialization" means the increase in number and functions of microbes in the ocean. Most of the microbial diversity in the ocean is actually viral; ten million viruses exist per ml of seawater whereas the ratio for all the rest of the marine microbes is one million microbes per ml of seawater.

Interactions between corals and microbes are stress-specific and this association changes with the type of environment and habitat and with the type of stressors that can affect an area. Stressors can be local (e.g., overfishing or nutrient additions due to urbanization of the coasts) and/or global (e.g., ocean temperature and CO_2 increase with associated decrease in pH). Experimental work has been conducted to assess how stressors change the coral-virus communities. In one case, it was discovered that changes in temperature, pH and nutrients increase the relative proportion of pathogenic microbes (e.g., herpes) in the ocean environment.

During field studies to assess the health of Pacific Islands' coral reefs, it was observed that coral health depends on the number of inhabitants living on the island – specifically an increase in pathogens (viruses and microbes) is observed in overfished reefs.

Coral cover decreases and prevalence of diseases increases with the number of people living in the area. In healthy reefs (e.g., Kingman), the primary production supports fish and sharks, whereas, in degraded reefs (e.g., Xmas), the primary production supports microbes (e.g., "microbialization" of the reef). Viruses and microbes provide an amplified and early warning system for ecosystem shifts.

Black band disease in corals is characterized by a black concentric or crescent-shaped band that "consumes" live coral tissue on the colony surface, leaving behind a bare skeleton. Research suggests that the disease is primarily caused by cyanobacteria. Photo Credit: NOAA

Changes in stable states are maintained by changes in microbial communities. Although on coral reefs, microbial taxa analysis appears to be only partially correlated with alternative stable states, microbial growth rates and relative gene abundance are informative for determining ecosystem health.

Breakout Session II:
Emerging Diseases, Organisms and Ecosystem Health

The breakout session (see Appendix 7-II) that followed Dr. Rohwer's presentation identified challenges and opportunities, desirable tools and methodologies and the top science questions of interest to NOAA regarding emerging diseases and marine organism and ecosystem health.

(a) Major Challenges and Opportunities

One of the major challenges for the microbial science community is the fact that we do not understand clearly how virulence of pathogens is driven. There is an interaction between the virulence of the agent and the resilience of the host but we do not know what the tipping points are leading to diseases. We do not know how to slow down or mitigate the spread of pathogens and related diseases, and, in some cases, we even know nothing about specific pathogens. In particular, the detection and identification of small microbes is a problem and their habitats and roles in the environment are largely unknown.

Mesocosm Experiments. Photo Credit: NOAA

This lack of knowledge is a challenge, but it is also an opportunity to advance the science of microbial evolution with the tools we already have. We need to pursue the predictability challenge if NOAA wants to forecast the effects of population or ecosystem changes on the microbial community and disease transmission. Since we do not understand how ecosystems change and how climate variability affects pathogen distribution and virulence, or the host's susceptibility to the pathogens, there is a great opportunity for all scientists, including those in NOAA, to elucidate this problem.

(b) Tools, Methodologies, Instrumentation and Approaches

Prevention of diseases requires the development of new methods/techniques to forecast pathogens presence and their impacts on their host and on the environment. Existing models already used in forecasts should be adapted to marine microbes but new ones also need to be developed.

In addition, we need to improve the availability of assessment tools to understand the economic impact of diseases in the marine environment. New sensors need to be developed for the genetic detection of marine microbes including pathogens. For example, a gene-based "tool kit" with a microarray library for direct pathogen detection and gene virulence identification would be vital. Instruments such as the Environmental Sample Processor (ESP) would help in the *in situ* detection of marine microbes, harmful algae and associated biotoxins. Also, laboratory experiments using mesocosms could be very useful to address outstanding questions relating to the impact of stressors.

The Deepwater Horizon disaster motivated the concept of organizing a rapid, multidisciplinary science deployment team or "SWAT" team to respond to emergencies. Photo Credit: ABC News

One very important issue is the detection of introduced species. NOAA and its partners should initiate the organization of a rapid multidisciplinary science deployment team or "SWAT" team that would follow a series of prescribed protocols. This team would be used for rapid response to emergencies but also for capitalizing on new science opportunities.

The attendees emphasized the importance of reviewing current procedures used in aquaculture systems to ensure environmental, fish/natural resources and human health and safety. Finally, the role of education and outreach was also discussed, especially the potential for a "citizen science" component in sampling and observations.

(c) Top Science Questions and Opportunities for NOAA

- Should NOAA consider focusing on bioremediation and restoration approaches? In that case, NOAA would have to understand cause-effect relationships between host and pathogen and would need to be aware of the status of disease in NOAA's response effort to disasters (e.g., natural and man-made disasters, public health exposure).
- What is NOAA's role in epizootic events (or animal diseases epidemic events)?
- What is happening in deepwater systems? For example, what is the role of the "mobil-ome" (e.g., elements that can move around within the genome) in fish and other living resources' diseases (e.g., tuna diseases)?
- What are the reservoirs of diseases? (e.g., biofilms, sediments, organs, etc.)

- What is NOAA's role in forecasting/predicting ocean diseases? (e.g., detection, role of mechanistic drivers of diseases such as organism and environmental conditions, model development and specificity to NOAA's mission).
- Do we need to consider mechanistic modeling of emerging diseases (e.g., abiotic and biotic influence on disease, cause and effect)? How should transport models and atmospheric impacts be factored in? (e.g., role of vectors such as fungi, bacterial spores, aerosols, sea-spray).
- What are the socioeconomic impacts of diseases in term of costs and seafood safety? (For example: case of the human-focused cholera study in Calcutta, India).
- What is the role of disease in ecosystem functioning and are diseases changing in specific habitats (e.g., coral reefs, eelgrass, other plants and animals)? In that case, should microbiologists focus their monitoring on these special habitats?
- How do pathogens and microbes in general, adapt to changes in the environment? What makes the difference between presence of pathogens and presence/absence of disease?
- Do we need to evaluate the flow of pathogenic traits through microbial pathways, changes in host susceptibility, assessment of disease impacts across host life history and synergy of environmental factors?

EXPERT PRESENTATION - BIOTECHNOLOGY AND NATURAL PRODUCTS

Dr. Rita Colwell (Professor Emerita and Distinguished University Professor, University of Maryland) discussed advances in biotechnology and natural products extracted from the sea (see Appendix 6-IV for more details). She offered that the rate of progress is different between medical and environmental biotechnology, and marine biotechnology is faced with great resistance from the public (e.g., engineered salmon). Nonetheless, the value of natural products extracted from marine organisms, especially from marine microbes, is understood and appreciated. For example, a substance associated with the harmful algal blooms (HABs) of *Karenia brevis* could potentially be used to vaccinate animals in the wild against the toxin produced by these HABs. Unfortunately, these extractions are often difficult and costly to pursue.

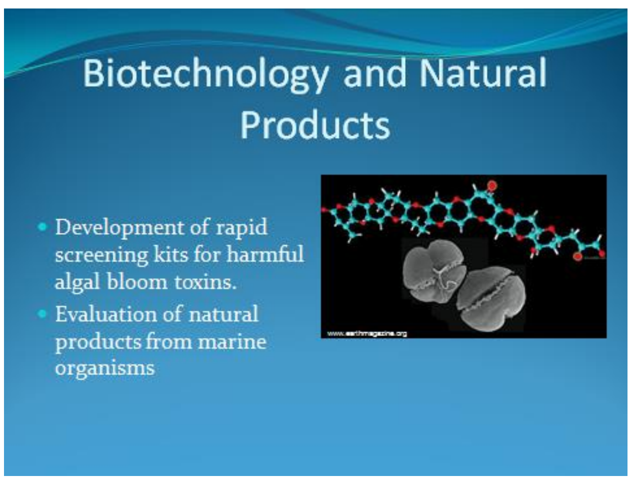


Illustration extracted from NOAA NOS' presentation

Dr Colwell highlighted the importance of research in natural products and in three other areas:

a) Study of the impact of environmental changes on vibrios: Pathogenicity of vibrios is impacted by the ecological function and changes in environmental parameters. For example, *Vibrio cholera* is dormant at temperatures below 15^0C. As the temperature of the ocean increases and water temperatures increase in more northern latitudes, it is expected that virulent forms of the bacterium could be detected in areas where they were previously unknown (e.g., vibrios in Chesapeake Bay are carried by copepods and their occurrence could migrate northward).

b) Research on antibiotic-resistant pathogens (resulting from human use of antibiotics): Research indicates the microorganisms in this category have been increasing in number deeper in the ocean water column. Unfortunately, as of now, we do not know why. It is obvious that more work needs to be done on ocean and human health.

c) Finally, some marine microbes can be very useful. For example, bacteria, able to degrade oil, were discovered in the Gulf of Mexico during the Deepwater Horizon catastrophe. We need to better understand the bacterial/microbial processes at play and how we can use these processes and microbe species in restoration activities.

Expert Presentation -Forecasting Microbial Responses to Global Changes

Dr. David Kirchman (Harrington Professor of Marine Biosciences University of Delaware) discussed the need to forecast microbial responses to global changes (see Appendix 6-V for more details). Global warming is not uniformly global. The poles, the Arctic and, to a lesser extent, the Antarctic have warmed more and faster than other latitudes, as expressed by the observed dramatic decrease of sea ice. Following the temperature increase, the composition of the phytoplankton in the Arctic appears also to be changing and the result is an increase in smaller phytoplankton cells. Even if the total biomass has not changed, this size change affects the food chain and ultimately the fish populations.

Warmer Arctic waters also accelerate microbial growth, but the observed rate changes cannot be explained by the temperature rise alone. Other factors, such as changes in the microbial physical environment, carbon fluxes and food webs are also at play and complicate the picture.

Microbial communities can extend in size from cubic millimeters (or smaller) to cubic kilometers. Even relatively simple communities can have millions of genes, giving them a genetic diversity substantially greater than that of higher life forms, even humans. Recent investigations have focused on collecting DNA fragments from environmental samples in the sea and other natural ecosystems. These "metagenomics" studies have given us a glimpse into the intricacies of these natural ecosystems and their diverse functions.

Dr. Kirchman emphasized three issues:
a) Ocean acidification: Higher concentrations of CO_2 in the atmosphere and in the ocean not only cause ocean warming but also ocean acidification. Ocean acidification directly impacts the nitrogen cycle. It is believed that pH affects ammonia oxidation, the first step in the transformation of ammonium to nitrates (nitrification), and a drop in 0.1 pH units that may decrease ammonia oxidation by as much as 40%.

b) Hypoxia: Anthropogenic sources of nitrogen, due to excess fertilizer use on land, result in increased frequency of harmful algal blooms (HABs) and associated coastal eutrophication and hypoxia. The rapid growth and multiplication of the microscopic algae implicated in the blooms often leads to the fast consumption of oxygen, causing hypoxia.

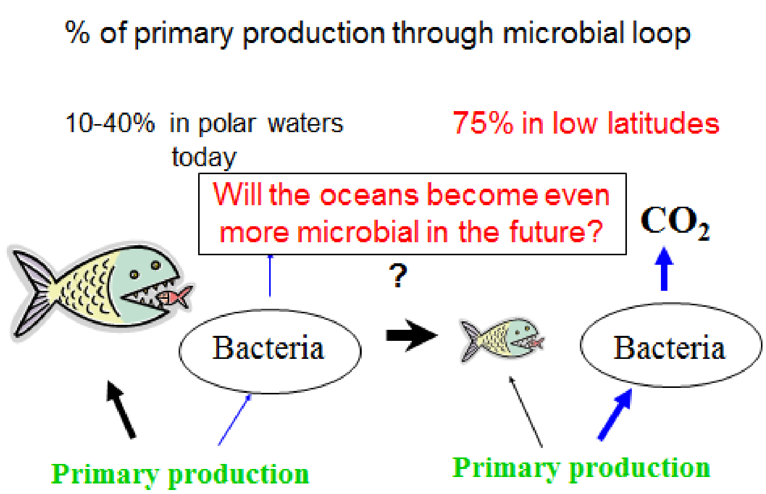


Diagram extracted from Dr. Kirchman's presentation.

c) Overfishing: Harvesting fish (e.g., overfishing of Atlantic cod) can be construed as a top-down controlled global human experiment on the food chain. The removal of top predators is believed to have a cascading effect on lower trophic levels. For example, declining stocks of cod in the Northeast United States caused the increase in forage fish, a decrease in large zooplankton (their prey) and an increase in phytoplankton concentrations. The impact of these trophic changes at the bottom of the food chain is not yet clear and more information is needed on the role of microbes in the functioning of ecosystems.

Breakout Session III: Forecasting Microbial Responses to Global Changes

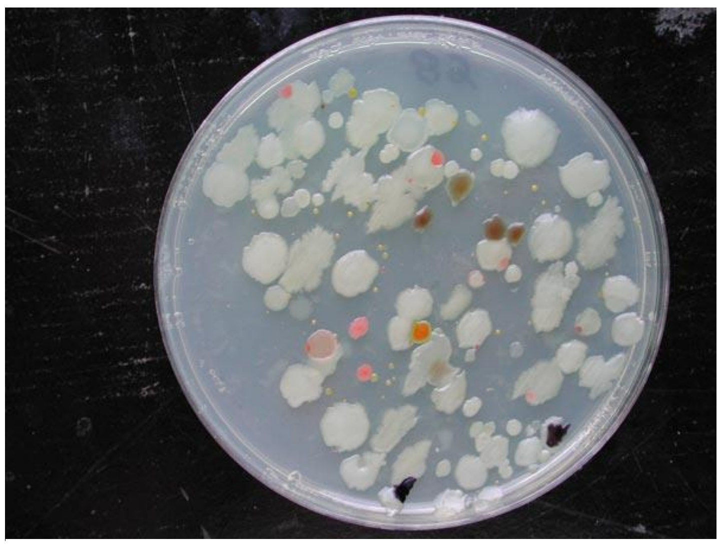
The breakout session (see Appendix 7-III), that followed Dr. Kirchman's presentation, identified challenges and opportunities, needed tools and approaches and the top science questions of interest to NOAA concerning the importance of forecasting microbial responses to global change.

a) Major Challenges and Opportunities

Among the major challenges discussed during the workshop were (a) the lack of baseline data against which to assess changes and (b) the lack of understanding about the most important biotic and abiotic drivers affecting microbial communities. Another related issue is the scale factor in both space (from millimeter to global) and time (from days to centuries).

This breakout session ultimately helped shape what appears to be a top research priority for the community -- the identification of various marine microbe linkages in biogeochemical processes and identification of the linkages most likely to change in response to global changes.

NOAA specifically has an opportunity to identify the microbial processes most relevant for fishery issues, especially the pathogen and virulence relationship in order to better understand macro-organismal health in living marine resources.

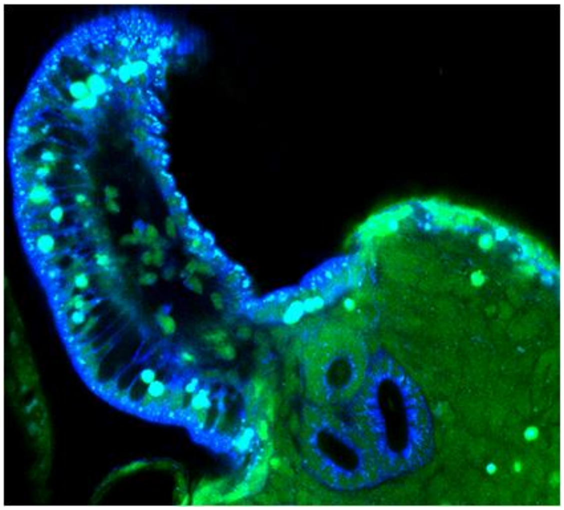


A short supply of marine microbe cultures, such as this one, may severely limit development of bio-discovery research. Photo Credit: NOAA

Another significant opportunity for NOAA would be to initiate regional pilot studies, possibly along the west coast and in the polar regions where climate changes may have the most severe consequences. In addition, NOAA could also address the oceanic increase in CO_2 through a better understanding of microbial processes. Finally, workshop participants highlighted once more, how NOAA could use its platforms (including drones) to increase the number of microbial samples collected and made available to the microbiological science community.

b) Tools, Methodologies, Instrumentation and Approaches

The development and use of new technologies that remove the need for the physical presence of personnel on board ships would considerably facilitate sampling efforts. New tools are needed to measure the dissolved organic carbon (DOC) including labile, semi-labile and refractory fractions. These data would be used to model the various DOC fractions and their role in the carbon cycle.



The symbiotic association between the squid, Euprymna scolopes, and the luminous bacterium, Vibrio fisheri, is one example of a beneficial animal-bacteria interaction. Photo Credit: Margaret McFall-Ngai

Another step in acquiring needed data would be to equip Argo floats and other mooring and buoys with O_2 and other biogeochemical sensors.

The inventory of existing data and samples, especially museum collections, will provide the community with the information needed to perform time-series analysis. It would be beneficial to have database and sample repositories and to develop new software to analyze sequence data more efficiently.

Finally, to understand the consequences of iron fertilization, the community needs new technologies for source identification and mesocosms experimentation.

c) Top Science Questions and Opportunities for NOAA

- What is the variability of microbial metabolism over time and space?
- What is the sensitivity of microbes to global changes and what could be the impact of these potential changes on living marine resources and human health? What influences this sensitivity?
- At what scale do we need to study microbes to be relevant to global changes and what are the effects of local forcings on the role of microbes in the ecosystem?
- What are the species and what is the role of microbes in biogeochemical cycles and how do both vary over time and space? Especially, what is the role of microbes in the carbon cycle?
- What are the roles and influence of microbial processes on eutrophication and hypoxia?
- How can microbes modulate climate through the release and interaction of aerosols with volatile organic carbon (VOC) and dimethyl sulfides (DMS)?
- Will global changes force genetic rearrangement within microbes responsible for diseases and cycles modulation?
- Could NOAA use the NSF Long-Term Ecological Research model to follow ecosystem changes?

Investment Priorities

At the end of the workshop, participants provided NOAA with general recommendations and suggestions for near- and long-term investment priorities (see Appendix 8 for more details).

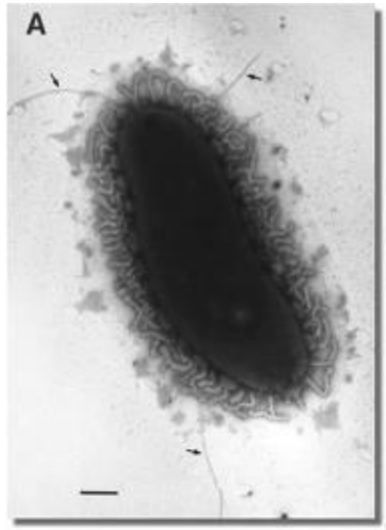


Image of a marine vibrio. Vibrio is a *genus of marine bacteria found in clams, shellfish, corals, and syngnathidae (e.g., seahorses).Vibrio can also infect humans (e.g. vibrio Cholera).Photo Credit: NOAA*

General Recommendations

NOAA needs to enhance its capabilities in genomics and other –"omics." To achieve this aim, in light of the earth rapidly changing environment, the general recommendations highlighted the need for NOAA to share, with its constituents and budgeters, the sense of urgency in understanding the role of microbes in disease and health. Marine microbiologists need to emphasize the fact that "microbes rule" most of the processes in the ocean and on land and that, because of rapid global changes, microbialization (e.g., the increase in marine microbes) of the ocean is increasing rapidly. It is essential for microbiologists to improve their marketing approach. Scientists need to explain why it is important, who should care and what would be the results of a more intense focus on marine microbes. More work on education and awareness of the role of microbes is needed and could be accomplished by developing a one-pager that could be titled: "Marine microbes: Did you know?"

Near-term Activities

What NOAA could do immediately to move forward and assist the scientific community would be related to sampling. One suggestion was to incorporate microbial sampling on all NOAA platforms. In particular, it would be important to add geochemical measurements (e.g., DOC, DNA, nitrates and other nutrients) to already existing parameters and perform direct count of microorganisms. These data would be used in health assessments.

NOAA needs to inventory its capabilities, identify external partners' capabilities and interests, link with existing databases (e.g., DOE) and create a data access portal that could be used in a regional pilot study (for example: enzyme discovery in the Gulf of Mexico). An effort should be made to identify and select core data-fields necessary to standardize existing and future data and to work on the visualization of existing data (for example using Google Earth). With these objectives in mind, NOAA also needs to prepare and validate QA/QC sampling and preservation protocols.

NOAA should initiate pilot studies in well-studied geographic areas to provide routine vibrio forecasts by region (e.g., Chesapeake Bay, Gulf of Mexico).

A NOAA Marine Microbes and Ecosystem Health Working Group should be created to establish connections of microbiologists across NOAA and with already existing programs and working groups such as the Ocean and Human Health Initiative, the NOAA One Health and the Ecological Forecasting working groups.

Long-term Activities

In the next 5 to 10 years, NOAA could either support or direct the efforts leading to the creation of a virtual manual for marine microbes study.

Marine Vibrios – Vibrio vulnificus causes seafood-related mortality (shown here). Vibrio cholerae infects humans, Vibrio coralliilyticus infects certain stony corals, and Vibrio spp. infects seahorses and other Syngnathids. There are also benign species, such as Vibrio fischeri Photo Credit: NOAA.

There is a great need to develop new technologies and tools to study marine microbes. In particular, it is essential to work towards a new technology, such as a microchip, that would allow for the *in-situ* sequencing of microbe genomes, similar to the coral reef microarray.

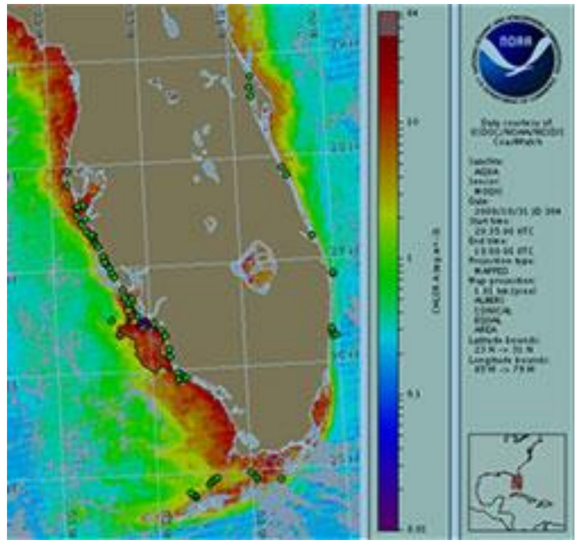
It is indispensable to develop and maintain a well-established and robust observing system for marine microbes. In particular, NOAA should use the National Estuarine Research Reserve System, Integrated Ocean Observing System and other regularly sampled stations (e.g., National Status and Trends, etc.) to add biogeochemical and microbes observations as well as microbial sampling to activities already in progress. The data obtained would directly be used for the development of a regional Earth model with location perspective.

Great advances could be achieved in NOAA by creating a Marine Microbe Program and a core facility for natural products derived from marine microbes,. This last item could be fee-based and would provide a standardized set of tests especially centered on enzymes of interest to NOAA.

Finally, in collaboration with DOE, NOAA should investigate the potential role of the National Oceanographic Data Center (NODC) in storing data and metadata regarding microbes and sequences for viruses and prokaryotes.

Partners

For all these proposed activities, NOAA should work collaboratively with academic institutions, especially cooperative institutes, and with other agencies (such as NSF, National Institute of Science & Technology (NIST), Department of Energy (DOE), Department of Interior (DOI), Department of Defense (DOD) and the Smithsonian Institution) to create a National Marine Microbe Program.



In the Gulf of Mexico off Florida's coasts, increased coastal urbanization and changes in nutrient loads trigger harmful algal blooms or "red tides" (in red) that consume the oxygen of the water and can create hypoxic to anoxic zones. Photo Credit: NOAA

In NOAA, partners include scientists and managers from OAR, NOS, NMFS and NESDIS. NWS is not yet involved but in light of their recent association with the Ecological Forecasting Working Group, an effort should be made to bring them into the fold of the NOAA Marine Microbes Working Group. Private sectors partners should be sought after to enlarge the circle of interested parties, especially entities dealing with biotechnology and pharmaceuticals.

Proposed Next Steps from NOAA Participants' Meeting

On December 1, 2011, NOAA participants met after the conclusion of the community workshop to reflect upon the workshop outcomes, discuss ways to move forward, and prioritize potential next steps that NOAA should consider. The following are the main discussion points (see Appendix 9 for more details):

- A workshop report should be prepared and shared with and reviewed by all workshop participants;
- A NOAA Marine Microbes Working Group should be established and should meet regularly to discuss what could be the primary focus of NOAA future activities, in particular revisiting potential investment areas recommended in the workshop, and to advise NOAA Leadership on needed investments. Priorities for the working group could be to decide how and where to collect and store samples and to develop a strategic plan for large scale sampling, preparation and archiving.
- NOAA programs that would benefit from knowledge of the role and function of marine microbes should be identified (e.g., stock assessment and marine mammal survey programs, NCCOS projects, NOAA Coral Program, Coral Diseases and Health Program, NOS National Status and Trends Program, etc.)
- An exit poll should be prepared for the attendees, asking them (a) to identify three priority activities for NOAA to pursue and (b) to submit a list of the work they are presently doing and the assets they are using. Within this context, it was also suggested that attendees be asked their opinions about preferred sampling protocols, as well as post-sampling storage protocols.

Metatranscriptomics sequences. Metatranscriptomics sequencing has the potential to give insights into environmental factors that regulate microbial activities. Photo Credit: NOAA

- Workshop attendees, particularly the external community experts, should be queried about what sort of scale-up of sampling they would recommend. To achieve this, NOAA attendees could compile a list of viable NOAA survey/sampling studies; the full suite of workshop attendees could then be engaged to identify where and how to enhance these NOAA studies with microbial components.
- Upon re-engaging the workshop participants, NOAA should also consider posing 4-5 relatively specific questions related to NOAA's mandates and ask how microbial input would be value-added.
- Request-for-proposal (RFP) mechanisms could be used to engage the extramural community for the longer term.

- Regional pilot studies and ecosystem assessments, including a microbial component, could be initiated to understand the microbial contribution to ecosystem function,
- Using NOAA Small Business Innovation Research (SBIR) or other financial vehicles, NOAA should invest in new sensor technology, looking into possibilities, such as developing a microarray or microchip to easily identify marine microbes, and invest in new sensor technology.

Conclusion

This workshop, organized specifically to engage experts of the microbial community, highlighted important gaps in the science community's knowledge of the marine world. It stimulated healthy debate about how marine microbial science should be exploited to meet NOAA's mandates and about how building on existing capabilities and taking on this new focus would enhance, and, in some cases, revolutionize our understanding of ocean ecosystems. It demonstrated that comprehension and better incorporation of microbial science into the NOAA research portfolio will enable the agency to find efficiencies and give insights into the marine environment that other ecosystem sampling/assessments have not revealed. The workshop provided NOAA with important direction for its future work on the topic.

NOAA should ensure its ecosystem priorities are aligned with the marine microbial science priorities delineated during the workshop and should consider enhanced investment in research leading to the discovery of marine natural products. In addition, efforts should be directed to understanding the role of microbes in biogeochemical processes and ultimately in ocean health. Marine microbes should be inserted in the program changes of the NOAA SEE process to increase support for the topic. This insertion could also highlight the linkage of the proposed work with the creation of jobs by maintaining ecosystem services (e.g., role of marine microbes in oil degradation, beach closures and seafood safety) and the potential role of marine microbes, especially bacteria and algae, in renewable energy.

Of course, NOAA, or any other single organization, cannot do everything on its own. The research and observation undertaken at NOAA, in partnership with academia, other federal agencies, not-for-profit organizations and the private sector, continuously improves our understanding of the earth as an interdependent system of ocean, air, land and living world. So, it is important to attain additional marine microbe-relevant capabilities and secure funds to support not only NOAA's intramural research but also that of the external community and to align NOAA's marine microbe science investment priorities with the Office of Science and Technology Policy (OSTP) goals related to ocean and human health.

Opportunities abound. One pervasive viewpoint, expressed many times in many ways during the workshop, was that NOAA needs to be involved in the genomic revolution to accomplish its mission or it will be left behind. Ultimately, NOAA needs to ensure that the momentum and concepts generated by this workshop will lead to fruitful synergies that benefit the marine microbial science community, the marine science field and the marine environment over which NOAA has stewardship.

APPENDICES

APPENDIX 1 – Workshop Agenda

Marine Microbes and NOAA
Scoping Science, Application and Observing Needs and Opportunities
Agenda

Hollings Marine Laboratory, 331 Fort Johnson Rd, Charleston, SC, Nov 29 – Dec 1, 2011

Workshop Challenge: Will NOAA improve its holistic understanding and management of ocean ecosystems, by enhancing knowledge of the microbial components of the marine ecosystem? If so, are there tools and insights that NOAA should have to better conduct its stewardship activities? Are there certain roles that are best suited to NOAA?

Tuesday, 29 November 2011

7:45–8:15 **ASSEMBLE/COFFEE**
8:15–8:35 **OPENING REMARKS –WELCOME** (Craig McLean, Rita Colwell, Susan White)

I. INTRODUCTION

8:35–8:50 Setting the Stage: One of NOAA's long-term goals is to achieve sustainable *marine fisheries, habitats, and biodiversity within healthy and productive ecosystems.** A grand science challenge is: *to assess and understand the roles of ecosystem processes and biodiversity in sustaining ecosystem services*** (*Craig McLean)*

II. NOAA LINE OFFICE ACTIVITIES WHICH CONSIDER MARINE MICROBES

8:50-9:05 **NATIONAL MARINE FISHERIES SERVICE** (NMFS, Rohinee Paranjpye)
9:05-9:20 **OCEANIC AND ATMOSPHERIC RESEARCH** (OAR, John Dunne)
9:20-9:35 **NATIONAL OCEAN SERVICE** (NOS, Fulton)
9:35-9:50 **NATIONAL SATELLITE AND INFORMATION SERVICE** (NESDIS, Chris Brown)
9:50-10:10 **Q&A**
10:10-10:30 **BREAK**

III. TOOLS, METHODOLOGIES, INSTRUMENATION AND NEW APPROACHES

10:30–11:00 **OVERVIEW OF EXISTING AND ANTICIPATED MARINE MICROBIAL OBSERVING TOOLS, METHODOLOGIES, INSTRUMENTATION AND APPROACHES** (John Paul)
11:00-11:30 **Q&A AND PLENARY DISCUSSION (30 MINUTES)** NOAA Marine Microbes Workshop
===

Tuesday, 29 November 2011(cont.)

IV. COMMUNITY PERSPECTIVES ON MICROBIAL OCEANOGRAPHY

11:30 – 12:00 **BIOGEOCHEMICAL PROCESSES AND CYCLING** (Margo Haygood)
12:00-12:15 **Q&A**

12:15–12:45 **LUNCH**

12:45-2:00 **BIOGEOCHEMICAL PROCESSES AND CYCLING: BREAKOUT GROUPS (2)**
(Facilitated by Rick DeVoe & Susan White*)*
Cross-Cuts:
1. Major Challenges & Opportunities by Habitat (shallow, deep, pelagic, benthic,+)
2. Tools, Methodologies, Instrumentation and Approaches
3. Top Science Questions and Roles for NOAA

2:05–2:30 **BREAKOUT GROUP SUMMARIES** (10 minutes per group, *Group Leads)*

2:30-3:00 **EMERGING DISEASES, ORGANISM AND ECOSYSTEM HEALTH** (Forest Rowher)
3:00-3:15 **Q&A**

3:15-3:30 **BREAK**

3:30-4:45 **EMERGING DISEASES, ORGANISM AND ECOSYSTEM HEALTH: BREAKOUT GROUPS (2)** (Facilitated by Rick DeVoe & Susan White)
Cross-Cuts (capture all, highlight those for NOAA):
1. Major Challenges and Opportunities by Habitat (shallow, deep, pelagic, benthic,+)
2. Tools, Methodologies, Instrumentation and Approaches
3. Top 5 Science Questions

4:55–5:20 **BREAKOUT GROUP SUMMARIES** (10 minutes per group, *Group Leads)*

5:20–5:30 Day 1, **WRAP–UP DISCUSSION / SUMMARY** (R. Colwell)

5:30-7:00 **RECEPTION SC DEPT NATURAL RESOURCES OUTDOOR CLASSROOM (ON HMLCAMPUS)**

Wednesday, 30 November 2011

7:45-8:15 **ASSEMBLE/COFFEE**
8:15-8:30 **OPENING REMARKS, DAY 1 REVIEW AND DAY 2 PREVIEW** (Rita Colwell, Susan White)

IV: COMMUNITY PERSPECTIVES ON MICROBIAL OCEANOGRAPHY (cont.)

8:30-9:00 **BIOTECHNOLOGY AND NATURAL PRODUCTS** (Chuck Merryman)
9:00-9:30 **Q&A AND PLENARY DISCUSSION (30 MINUTES)**

9:30-10:00 **FORECASTING MICROBIAL RESPONSES TO GLOBAL CHANGES**
(Warming, chemistry, pollution--Dave Kirchman)
10:00-10:15 **Q&A**

10:15–10:30 **BREAK**

10:30-11:45 **FORECASTING MICROBIAL RESPONSES TO GLOBAL CHANGES: BREAKOUT GROUPS (2)** (Facilitated by Rick DeVoe & Susan White)
Cross-Cuts (capture all, highlight those for NOAA):
1. Major Challenges and Opportunities by Habitat (shallow, deep, pelagic, benthic,+)
2. Tools, Methodologies, Instrumentation and Approaches
3. Top 5 Science Questions

11:50–12:20 **BREAKOUT GROUP SUMMARIES** (10 minutes per group, *Group Leads)*
12:25–1:00 **LUNCH**
==

V. ASSEMBLING A MICROBIAL OCEANOGRAPHY MATRIX -

1:00–2:00
PLENARY TO ASSEMBLE AND PRIORITIZE ACTIVITIES AND INVESTMENTS (Rick DeVoe)

	MAJOR CHALLENGES AND OPPORTUNITIES	TOOLS, METHODS, INSTRUMENTATION	TOP SCIENCE QUESTIONS
BIOGEOCHEMICAL PROCESSES AND CYCLING			
EMERGING DISEASES, ORGANISM AND ECOSYSTEM HEALTH			
BIOTECHNOLOGY AND NATURAL PRODUCTS			
FORECASTING MICROBIAL RESPONSES TO GLOBAL CHANGES			

2:00–2:15 **BREAK**

VI. ESSENTIAL NOAA PARTNERSHIPS

2:20–3:20 **PLENARY TO IDENTIFY NOAA INDUSTRY, ACADEMIA AND GOVERNMENT PARTNERSHIPS TO BEST ADDRESS NOAA ROLES IN MICROBIAL MATRIX** (Susan White)

3:20–3:40 **WRAP–UP DISCUSSION / REVIEW**

3:45 **THANK YOU FROM RITA AND SUSAN; ADJOURN**

* NOAA's Next Generation Strategic Plan http://www.ppi.noaa.gov/ngsp/
** Strengthening Science: Findings from the NOAA Science Workshop
http://www.nrc.noaa.gov/plans_docs/2010/Science_Workshop_WP_FINAL.pdf

==

Thursday, 1 December 2011
NOAA CONTINGENT ONLY

8:00–8:30 **ASSEMBLE/COFFEE**

I. 8:30-11:30 NOAA SYNTHESIS AND FUTURE ACTIVITIES

Look at results from the prior two days and prioritize activities and future steps.

APPENDIX 2 – Workshop Scope

Marine Microbes and NOAA: Scoping Science,
Application and Observing Needs and Opportunities
November 29-December 1, 2011,
Hollings Marine Lab, Charleston, SC

~~WORKSHOP SCOPE~~

NOAA research, undertaken in partnership with the wider national and international research community, provides the scientific knowledge base, sensing systems, products and services through which we understand and address the dynamics of our ever-changing planet.

In the years to come, we will continue to experience extraordinary changes in our world's ocean and atmosphere, with consequences that may dramatically change the way we live our lives. Strengthening our understanding of the ocean's physical, biological, chemical and geologic components is key to fostering system resiliency and promoting resource sustainability.

Our view of the world's ocean ecosystems is being transformed as we learn more about the diversity of microorganisms (in the broadest sense – including microalgae, bacteria, protozoa and viruses) and their associated biological processes; the relevance of microbes to the discussion of resiliency and resource sustainability is becoming more and more apparent.

The purpose of this workshop is to discuss how we can improve NOAA's holistic understanding and management of ocean ecosystems by enhancing our knowledge of the microbial components of these ecosystems. *What new technologies, research integration across disciplines, and collaboration with partners (in academia, the private sector, and across federal government) will be fruitful avenues for NOAA to pursue?*

NOAA, NSF, NIEHS currently conduct significant activities focused on Oceans and Human Health, which has a strong microbial component. Prior workshops and recent events, such as the Deepwater Horizon oil spill, highlight additional microbial roles to consider, including biogeochemical cycling, emerging diseases, organism and ecosystem health, biotechnology and natural products, to name several. *Aside from human health* *interactions, which of these or additional areas of study should NOAA pursue to improve its understanding of marine microbes and their associated functions and services?*

NOAA and its partners conduct a significant portion of the total operational oceanic observations made daily. *Should these include microbes? Are there tools and insights which NOAA should have vis-a-vis microbes to better conduct its ocean stewardship activities? Are there certain roles that are best suited for NOAA? How important is it for NOAA to understand how microbes vary among habitats, photic zones, or water masses and whether / how microbes in habitats or water masses are linked? Is it important for NOAA to understand how the composition and distribution of microbes respond to global changes (warming, chemistry, pollution)?*

NOAA's vision of the future is one where societies and their ecosystems are healthy and resilient in the face of sudden and prolonged change. We hope that you will participate in this microbial focused workshop to strengthen NOAA's understanding in this regard and ensure that NOAA's activities generate opportunities to foster resiliency, promote sustainable resource management and enhance ecosystem and human health.

APPENDIX 3 -- Workshop Participants List & Affiliations

Marine Microbes and NOAA:
Scoping Science, Applications, and Observing Needs and Opportunities
Charleston, SC – November 29 – 30, 2011

Participant List

NOAA Workshop Leadership
Rita Colwell, University of Maryland/Johns Hopkins
Craig McLean, OAR HQ
Susan White, NOS/NCCOS

External Community Experts
Doug Bartlett, Scripps Institution of Oceanography
Carl Cerniglia, National Center for Toxicological Research/USFDA
Dan Distel, Ocean Genome Legacy Foundation
Jack DiTullio, College of Charleston
Chris Dupont, Venter Institute
Jay Grimes, Gulf Coast Research Lab, University of Southern Mississippi
Margo Haygood, Oregon Health & Science University, Department of Environmental & Biomolecular Systems
Terry Hazen, Lawrence Berkeley National Lab/Department of Energy
John Heidelberg, University of Southern California
David Kirchman, University of Delaware
Maille Lyons, Old Dominion University
Rex Malmstrom, Department of Energy, Joint Genome Institute
Karen Nelson, Venter Institute (invited)
John Paul, University of South Florida
Carla Pruzzo, University of Genoa

External Community Experts (cont'd)
Forest Rowher, San Diego State University
Alyson Santoro, U of MD, Center for Environmental Science
Hal May, Medical University of South Carolina
Chuck Merryman, Venter Institute

NOAA Participants (at the Table)
Chris Brown, NESDIS/STAR
Rohinee Paranjpye, NMFS/NWFSC
Linda Rhodes, NMFS/NWFSC
Mike Fulton, NOS/NCCOS
Lisa May, NOS/NCCOS
James Morris, NOS/NCCOS
Cheryl Woodley, NOS/NCCOS
John Dunne, OAR/GFDL
Nathalie Valette-Silver, OAR/OER
Rik Wanninkhof, OAR/AOML
Michelle Wood, OAR/AOML

Observers/Facilitators
Rick DeVoe, NOS/SC Sea Grant
Janet Moore, NOS/NCCOS
Geoff Scott, NOS/NCCOS
Laura Webster, NOS/NCCOS
Reggie Beach, OAR/OER
Margot Bohan, OAR/OER
Paula Keener-Chavis, OAR/OER
Megan Mueller, OAR HQ
Michael Feldman, Ocean Leadership

APPENDIX 4 – Craig McLean's Opening Remarks

CRAIG MCLEAN
INTRODUCTORY REMARKS 8:50 — 9:05 AM (15 minutes)

Marine Microbes and NOAA:
Scoping Science, Applications, and Observing Needs and Opportunities
Charleston, South Carolina
Tuesday, November 29, 2011

Setting the Stage

- I am truly excited to hear what you all have to say and I don't want to spend a lot of time talking at you, but we thought it might be useful to provide some context for our discussions over the next two days by sharing some information on where NOAA is headed.

- We are and will continue to experience extraordinary changes on our planet that impact our lives and livelihoods. NOAA's mission is central to many of the challenges we are facing today, and will face in the future, from a changing climate and threatened or degraded oceans and coasts to declining biodiversity and an increasing number of natural and human-induced disasters.

- The research and observation undertaken at NOAA in partnership with academia, other federal agencies, not-for-profit organizations and the private sector continuously improves our understanding of the earth as an interdependent system of ocean, air, land and living world.

- Over the next two days we will focus on one small piece of the living world, but in the context of the broader ecosystem. Our challenge these next two days is:
    - Will NOAA improve its holistic understanding and management of ocean ecosystem by enhancing knowledge of the microbial components of the marine ecosystem?
    - And, if so, are there tools and insights which NOAA should have to better conduct its stewardship activities?
    - Finally, are there certain roles that are best suited to NOAA?

- These questions grew out of my conversations with Rita following Deepwater Horizon but they also resonate within the context of the efforts NOAA has undertaken recently to look at how we do business in a changing world.

NOAA Context

- Now I'd like to talk a little about three of those efforts – NOAA's Next Generation Strategic Plan, NOAA's Science Challenge Workshop and OAR's Strategic Plan.

- Some of you may have been involved in NOAA's efforts to develop a **Next Generation Strategic Plan - NGSP**, which outlined our vision for the future, the issues we must address, and the outcomes we want to help society realize.
    - NOAA's Healthy Oceans goal, identified in the NGSP, helps us understand some of the context in which we are examining NOAA's need to better understand the microbial component of marine ecosystems.
    - The Health Oceans goal calls for "*Marine fisheries, habitats and biodiversity sustained within healthy and productive ecosystems*."
    - Clearly, understanding microbes is a critical part of healthy and productive marine ecosystems.

- Also, part of the process of developing the NGSP included holding **NOAA's Science Challenge Workshop**. We brought together the leading scientists in NOAA and asked them to look at the grand science challenges facing NOAA and opportunities for NOAA to improve how we conduct our science.
    - The group identified an overarching, **grand science challenge** for NOAA to "*develop and apply holistic, integrated Earth system approaches to understand the processes that connect changes in the atmosphere, ocean, space, land surface, and cryosphere with ecosystems, organisms and humans over different scales*. "
    - Holistic understanding of the earth system has become an important recurring theme for NOAA.
    - The workshop group further identified several topic-specific science challenges for NOAA. Chief among them for our purposes here, "***assess and understand the roles of ecosystem processes and biodiversity in sustaining ecosystem services*** *and the connections*

among ecosystem condition, resilience and the health of marine organisms, humans and communities."

- Several science focal areas were identified for this challenge, including:
  - *developing system models to elucidate the cumulative consequences of changes in multiple ecosystem components on continued provision of ecosystem services;*
  - *the consequences of changes in biodiversity and habitats for the stability and magnitude of critical ecosystem services; and*
  - *understanding the market and non-market valuation of ecosystem benefits*
- In fact, the work we do here this week will contribute to a broader NOAA effort, which arose from the Science Challenge workshop, to develop our Ecosystem Research Agenda. An effort being undertaken in part by another group gathered in DC this week.
- Once again, I can't imagine how we are going to address this challenge without incorporating an understanding of marine microbes.

- NOAA's Next Generation Strategic Plan and the outcomes of the Science Challenge Workshop are both informing our efforts within OAR to chart our path forward.

- We've developed a new **OAR Strategic Plan**, aligned with the NOAA NGSP, which states that OAR's mission is to Innovate, Incubate and Integrate.
  - What do we mean by that?
    - We will apply innovative research and technology towards Earth-system discovery, understanding, and prediction.
    - We will incubate long-term research and extend knowledge that supports NOAA services and societal needs.
    - And, we will integrate research across NOAA, and with our external partners, to maximize NOAA's value to society.
  - Key to this gathering is the role of integrating marine microbe research across NOAA and with our partners in the wider research community. I hope we can do this in a way that allows us to innovate, find new approaches and incubate long-term research in this important area.

- This will help us achieve the OAR science goal for a "*holistic understanding and useful predictions of future states of the earth-system.*"
- The integration of the resultant scientific findings into NOAA science and services will ultimately enhance our stewardship of marine resources.

Charge to the group

- Marine Microbiology is a rich, diverse field of study and I hope the information I've shared on where NOAA is headed provides a context to help you frame your thoughts on the challenges before us at this workshop.

- NOAA's focus on a holistic approach to understanding the world around us is guiding our way ahead, and we cannot get there without your help.

- In addition to my role as the head of NOAA Research, I have the privilege of sitting on the **Interagency Working Group on Ocean Partnerships** and can assure you that the sharing of the results of this workshop will not be limited to NOAA.
  - For example, the ad hoc biodiversity group report on how the nation might attain an operational biodiversity network – BON - was well received. As a result, we are working to develop a National Oceanographic Partnership Program Broad Agency Announcement to implement certain BON case studies to monitor biodiversity status and trends across taxa - microbes to whales, and microbes in whales - and across geographic regions covering the ocean basins.

- This brings me back to **the questions we hope to be able to address** through this workshop:
  - Will NOAA improve its holistic understanding and management of ocean ecosystems, by enhancing knowledge of the microbial components of the marine ecosystem?
  - If so, are there tools and insights which NOAA should have to better conduct its stewardship activities?
  - Are there certain roles that are best suited to NOAA?

- Again, I want to thank everyone who had a hand in helping to put together this workshop – and all of you for joining us.

I look forward to hearing your perspective on the state of the science, major microbial challenges and opportunities, innovative approaches to studying microbes, the top science questions NOAA needs to address and who and how we should be collaborating with others to answer those questions.

APPENDIX 5 -- NOAA Line Office Presentation Highlights

APPENDIX 5-I

Current research related to marine microbes conducted in NMFS:

Northwest Fisheries Research Center (NWFSC), Alaska Fisheries Science Center (AFSC), Northeast Fisheries Science Center (NEFSC)

Rohinee Paranjpye

NOAA drivers: One Ocean, One Health report, Healthy Oceans goal, NOAA OHHI and National Ocean Policy: Water Quality Strategic Plan.

- Develop surveillance systems or predictive tools and early warning systems to forecast emerging threats to human health due to pathogens and toxins
- Ocean Health effects related to climate variation
- Ensure safe and sustainable seafood

Objective: Development of predictive tools for health early warning systems.
Specific projects at the NWFSC:

1) Investigation of the diversity of pathogenic *Vibrios* from the Pacific Northwest and comparison with distinct geographic locations (Mississippi, Louisiana, Maryland) and examination of the relationship to ecological factors.
2) Genetic and genomic differentiation (including whole genome sequencing) of several environmental and clinical *Vibrio parahaemolyticus* strains to identify virulence genes.
3) Developing and enhancing forecasting capabilities for harmful algal blooms, or HABs, (e.g. *Alexandrium cantenella*) and pathogens (*V. parahaemolyticus*) in Puget Sound and evaluating the potential impacts of climate change
4) Deployment of an advanced and autonomous biosensor to provide early warning of harmful algal blooms and pathogens that threaten shellfish and fish aquaculture
5) Collaborative monitoring program for HABs in the Pacific Northwest, Alaska and developing nations
6) Develop and refine methods for detection of emerging biotoxins (e.g. DSP)
7) Collect biotoxin exposure data for marine mammals from the US west coast

Objective: Assess effects on ecosystem health related to climate variation.
Specific projects at the NWFSC:

1) Multi-trophic assessment of pelagic food web as a measure of ecosystem health, including surveys of microbial communities, zooplankton biomass, fish abundance, & water quality
2) Modeling plankton & nutrients for trophic dynamics of Pacific salmon survival
3) Evaluation of structure & function of bacterial communities associated with chronic low dissolved oxygen

Specific projects at the AFSC

1) Monitor distribution and prevalence of *Hematodinium* in North Pacific crabs and *Ichthyophonus* in walleye Pollock that may be influenced by environmental conditions among other factors.

Objective: Ensure sustainable aquaculture and safe seafood.

Specific projects at the NEFSC:

1) Use of probiotic bacteria for use in molluscan shellfish hatcheries
2) Phylogenetic analysis of *Vibrio vulnificus* strains for biogeographic structure

Specific projects at the NWFSC:

1) Evaluation of the gut microbiome as an aspect of fish nutrition & sustainable feeds research.
2) Characterization of exposure risk and effects of chronic exposure to algal derived seafood toxins on health. Development of a biomarker for chronic algal toxin exposure in human blood.

Specific projects at the AFSC:

1) Effect of *Hematodinium* on the abundance and distribution of North Pacific crabs.
2) Effect of the parasite *Ichthyophonus* on prevalence and quality of walleye Pollock.

Objective: Assess the impact of microbes on aquatic animal health

Specific projects at the AFSC:

1) Disease ecology of parasitic dinoflagellates (*Hemotodinium* spp.) for population modeling of snow and tanner crab stocks

Specific projects at the NWFSC:

1) Research on genetic, immunologic, epidemiologic, & ecological aspects of an endemic bacterial pathogen of salmon (genome sequencing, genetic modifications, vaccine & drug testing, disease ecology).
2) Research on the effects of algal toxin exposure on marine mammals and development of biomarkers of exposure.

APPENDIX 5-II

OAR Activities in Marine Microbes

John Dunne

Extended Abstract:

Attempts to simulate global ocean biogeochemical and ecological cycles make broad use of the first part of Lourens Baas Becking's (1934) hypothesis that 'Everything is everywhere' in their general assumption that the proximate controls on microbially-mediated processes are resource- and biomass-limited chemical favorability rather than local microbial biodiversity. These efforts have far more trouble representing the complexity inferred from the second part of this hypothesis, that 'the environment selects' such that niche exclusion occurs to key microbes under many conditions, among other factors. A detailed understanding of controls on marine microbial response to environmental conditions and variability is critical to our ability to simulate ocean ecological and biogeochemical cycling. Better constraints on microbial rates and biodiversity would help address many limitations in the present modeling framework and inform efforts to project the future of ocean ecosystems.

As Earth System Models (ESMs) are the extensions of climate models, they are based on mechanistic geophysical understanding with geographically explicit atmospheric and oceanic circulation models, and land and sea ice dynamics. To these physical climate processes, an ESM adds interactive carbon dynamics and associated chemistry and ecology to represent interactions on timescales from minutes to millennia and explore Earth System behavior at both equilibrium and in transient. ESMs resolve coupled climate-carbon responses to diverse anthropogenic perturbations such as fossil fuel emissions, agriculture and forestry, and aerosol chemistry within a single, self-consistent system to allow investigation of ecological and biogeochemical feedbacks. Geophysical Fluid Dynamics Laboratory (GFDL)'s current ESMs represent only rudimentary functional biodiversity. In these current 'state of the art' marine ecological/biogeochemical models, plankton dynamics are represented through a suite of 'functional groups' each with a particular niche, including: small (prokaryotic and small eukaryotic picoplankton and nanoplankton) that maintain high growth rates under low nutrient conditions but are caught in a tight microbial loop with protists; larger eukaryotic microplankton that have high growth rates under nutrient replete conditions and more efficiently send material both up the food web and to depth in sinking material, plankton conducting opal, aragonite, and calcite formation, and diazotrophic phytoplankton. These models tend to represent a suite of elements to represent co-limitation by light, major nutrients like nitrogen and phosphate and micronutrients such as iron as well as bacteria and dissolved organic carbon dynamics.

The assumption that 'Everything is everywhere,' implies that biodiversity is great enough that chemically favorable transformations proceed if resources exist. One example of this is the idea that remineralization occurs via the most favorable electron receptor ($O_2 \rightarrow NO_3 \rightarrow Fe \rightarrow SO_4 \rightarrow CO_2$). 'But the environment selects' implies that biodiversity not being infinite may exclude many niches and exert critical controls on many processes. Examples of these controls include: light inhibition of nitrification allowing phytoplankton to compete for NH_4 under high light conditions; size, mineral ballast, aggregation and grazing ecology on sinking particle re-mineralization leading to deep penetration of sinking organic material into the ocean interior; the role of microbial ligands in modulating iron scavenging leading to relief of extreme iron limitation in an oxic ocean; the combined iron, light, temperature and ecological constraints on nitrogen fixation leading to maintenance of phosphorus surplus in broad areas of the tropical oceans; the necessity of growing in a population of methano-trophs after the 2010 Gulf Oil Spill in order to degrade the accumulating CH_4 in the deep ocean and its subsequent long lifetime before a rapid removal. In these cases as well as many others, the biogeochemical impact depends critically on the functional biodiversity of microbial dynamics.

Present limitations in efforts to project the future of ocean ecosystems include representation of: controls on the creation and cycling of refractory organic carbon; maintenance of hypoxia and denitrification; the response to climate warming and reorganization of circulation, and the response to acidification, both in CO_2 fertilization and $CaCO_3$ cycling. Key to the development of the next generation of global ecological/biogeochemical models will be conceptual description to address the biodiversity challenge of developing rules that guide how 'the environment selects' both when 'rare' events such as bloom succession, redox change, and toxin release happen, as well as when 'common' events such as nutrient utilization, local re-mineralization, and N_2 fixation don't happen, leaving the ecosystem with unutilized potential energy.

The microbial sensitivity to climate and CO_2 change is a major unknown. For example, in projecting geochemical implications of ocean acidification, the current GFDL ESM ignores ocean CO_2 fertilization of phytoplankton growth which has been suggested to potentially drive community shifts. The ESM does, however, include both calcite (forams, coccoliths) and aragonite (pteropods, corals) cycling, with aragonite being more soluble than calcite, and both production and dissolution assumed to be a function of saturation state. Our ESMs project a dramatic, two-thirds reduction in surface $CaCO_3$ production with both ocean interior and sediment feedbacks beginning to appreciably augment ocean CO_2 uptake over this century. Our ESMs also project significant changes to light, T, major and micro-nutrients, and oxygen in the marine environment under climate change and acidification. On the global scale, little change is projected in total primary production as decreases in nutrient supply are largely compensated by increases in temperature for small phytoplankton, while large phytoplankton and their less-efficiently-recycling food web that drives living marine resources are expected to decrease on the order of 5%. On the regional scale, however, we expect a diverse suite of intense changes as climate-induced enhancement of stratification, pole ward migration of the mid-latitude winds and other changes induce modulation of biomes.

APPENDIX 5-III

NOAA Work in Marine Microbes

National Ocean Service (NOS) - Summary

Michael Fulton & John Jacobs

The mission of the NOS is "to provide science-based solutions through collaborative partnerships to address evolving economic, environmental, and social pressures on our oceans and coasts". Microbiological research in NOS is broadly focused to address this mission.

One research focus in NOS is the development of tools and methodologies to assess microbial water quality. This includes the use of Earth Observing Systems to assess water quality, plankton, and harmful algal blooms (HABs). Another focus is the development and use of molecular tools to identify the sources and distribution of pathogenic microbes in the marine environment. NOS researchers are also studying the relationship between nutrient and contaminant cycling and how these relationships may affect HAB formation and persistence or impact marine organism health.

The issues of emerging diseases that may affect human and ecosystem health are also under investigation by NOS scientists. This includes work under the Oceans and Human Health Initiative (OHHI) that is examining the relationship between the health of the oceans and human health. Other research is focused on fish and coral disease, and the development of rapid water quality screening tests.

A final area of research is the use of advanced modeling tools to forecast human pathogen abundance and HABs, and how they may be affected by global climate change.

APPENDIX 5-IV

National Environmental Satellite, Data and Information Service (NESDIS) Work in Marine Microbes

Summary of NESDIS Activities and Interests Related to Marine Microbes

Presented by

Christopher Brown (NOAA/NESDIS/STAR)

At the workshop on Marine Microbes and NOAA: Scoping Science, Application and Observing Needs and Opportunities, Hollings Marine Laboratory, Charleston, SC, Nov 29 – Dec 1, 2011

The mission of the National Environmental Satellite, Data, and Information Service (NESDIS) is to provide timely access to global environmental data from satellites and other sources. In order to attain this goal, NESDIS acquires and manages the Nation's operational environmental satellites, operates the NOAA National Data Centers, supplies data and information services, performs official assessments of the environment, and conducts related research. Due to time restrictions, the talk only touched upon the satellite products generated by NESDIS, the research conducted by the Center for Satellite Applications and Research (STAR), and the functions served by the National Data Centers, specifically NODC, of relevance to marine microbes.

NESDIS generates and disseminates several satellite products that can be used directly or indirectly to assess, monitor, detect, and predict marine microbes, particularly marine phytoplankton. Though several atmospheric and land surface products are useful in these activities, e.g., precipitation and vegetative indices, oceanic products are most applicable, e.g., sea-surface temperature, sea-surface salinity, and ocean color radiometry (OCR). OCR products, in particular, are commonly used to understand and monitor marine microbes. Several OCR products are listed below, with asterisks indicating those operational and experimental products that are currently generated and distributed by NESDIS for US waters:

- Chlorophyll-a Concentration*
- Phytoplankton Carbon
- Primary Productivity*
- Turbidity / Suspended Sediment*
- Absorption by Colored Dissolved Material (CDM)
- Particle Size
- Presence of Coccolithophore Blooms*
- Calcite Concentration*
- Estuarine Sea-surface Salinity*

Scientists within STAR, the applied research Office within NESDIS, are developing and enhancing both satellite techniques, such as improving atmospheric correction for OCR imagery in coastal waters, and applications, such as detecting and predicting harmful algal blooms and water-borne pathogens, documenting phytoplankton phenology, tracking runoff plumes, and monitoring inland and coastal water quality.

The NESDIS Environmental Data Centers, consisting of NODC, the National Climatic Data Center (NCDC), and the National Geophysical Data Center (NGDC), archive and distribute data upon request. NODC archives and distributes oceanographic data and information, including physical, biological, and chemical measurements from *in situ* oceanographic observations, satellite remote sensing, and industrial oceanographic activities in coastal and deep ocean areas.

APPENDIX 6 -- External Community Experts' Presentations Highlights

APPENDIX 6-I

EXISTING AND ANTICIPATED MARINE MICROBIAL OBSERVING TOOLS, METHODOLOGIES, INSTRUMENTATION AND APPROACHES

John Paul, University of South Florida, College of Marine Science

Existing instruments available to observe marine microbes include:

- Optical Detection Gizmos
- Genetic Detection Widgets
- Single Cell Sequencing Juju
- Meta-Transcriptomics
- Satellite Wizardry

What we need is a "Microbial Detector Tricorder" that will give microbe identification, concentration, and activity for all microbes present in a sample

Optical Detection Gizmos:

These include:

1) In vivo pigment spectrometry such as the optical phytoplankton discriminator or "Brevebuster" developed by Gary Kirkpatrick at the Mote Marine Laboratory (FL).This instrument is mounted on a glider and used to study red tides. An example of the results obtained by the Brevebuster is given for the October 2011 red tide bloom. The instrument measured the pigments of *Karenia brevis*, the algae that produces the red tide.

2) Flow Cytobot was developed by Heidi Sosik and Rob Olson from Woods Hole Oceanographic Institution. This instrument is using the principles from conventional flow cytometry but is automated and submersible and is optimized for small cells (1-15um). Derived from the Flow Cytobot, this design has been improved by adding video and has also been optimized for detection of large cells (10 to 300um). Automated features that allow for standard analysis, bio-fouling control, real-time humidity sensing and intake valve control have been added to ensure up to 6 months extended routine unattended deployment. The instrument observational capabilities include enumeration, identification and cell sizing of thousands of individual plankton (nano/microplankton). The instrument was used at the Martha's Vineyard Coastal Observatory (MCVO) to assess community dynamics during seasonal to inter-annual events from 2006 through 2010. The instrument recorded over 400

million images of pico to microplankton. An automated image analysis and classification resulted in the identification of 27 diatoms taxa at MVCO. A similar study on the Texas Coast (Port Aransas) was used for specific-early warning and bloom dynamics.

Genetic Detection Widgets:

- One of the examples provided is the Nucleic Acid Sequence Based Amplification (NASBA) that uses the notion of molecular beacons: if a base is unbound to a target there will be no fluorescence but when the base binds to the target there is fluorescence. For example the Bioplex SE 300 is based on this principle that allows for the detection of assay output to be displayed on a laptop computer. A SE 1200 prototype was used on *Karenia brevis* RNA extract. The extract is NASBA amplified, transferred to a conjugate release pad, hybridized to capture probe, and detected with a detection probe linked to a dyed sphere.

- Autonomous Molecular biological platforms have also been developed.

  - The Autonomous Microbial Genosensor (AMG) was developed by the University of South Florida, Ecosystem Technology Group was tested on 9/6/11 on *Karenia brevis* and provided good results rapidly.

  - The Environmental Sample Processor (ESP) was developed by the Monterey Bay Aquarium Institute. This instrument includes: a Core ESP which is a sample processing and archival, and real-time probe array analyses (such as SHA or ELISA); External Sampling Modules for meeting special sampling requirements; Analytical Modules which are custom analytical devices fitted to the core ESP and require upstream sample collection and processing. The Core ESP provides housing, power, fluids (e.g., pre-processed sensors, reagents), and data/control communications and processing,

    The current functions of the ESP are: Real-time application of DNA and protein arrays (collect the sample, homogenize it, filter the lysate, then develop the array and image with CCD camera); Real time application of qPCR (collect the sample, homogenize the lysate, SPE for DNA and run series of qPCR reactions); sample archival (whole cell microscopy to fish, nucleic acids (DNA, RNA) and Phycotoxins). The ESP can be used to detect a wide range of organisms and target molecules such as marine microbes, harmful algae and invertebrate larvae. It also allows for combining rRNA probe arrays and real-time PCR. The instrument can be deployed on various platforms such as mooring, ROV, elevator/RCO, drifter or pier.

- Other ecogenomic sensors' trajectory are now under development to be housed in long- range AUVs and profiling floats.

Single Cell Sequencing Juju

- Single cell genomic sequencing Juju includes: isolate single cell by flow cytometric sorting, multiple displacement amplification (MDA) and assembly and informatics.

- MDA is a non-PCR based DNA amplification technique. This method can rapidly amplify minute amounts of DNA samples to a reasonable quantity for genomic analysis. The reaction starts by annealing random hexamers (primers) to the template: DNA synthesis is carried out by a high fidelity enzyme, preferentially Phi 29 DNA Polymerase, at a constant temperature. Compared to conventional PCR amplification techniques, MDA generates larger sized products with a lower error frequency. This method is currently used in whole genome amplification (WGA) and is a promising method for application to single cell genome sequencing and sequencing-based genetic studies. This technique has been used for single cell bacterium genomic sequencing and for single viral particle genomics. Examples include:

    - *Poribacteria*-uncultured bacteria from sponges capable of autotrophic and heterotrophic metabolism

    - *Hwanghaeicola aestuarii* gen. nov., sp nov., a moderately halophilic bacterium isolated from a tidal flat of the Yellow Sea

    - *Vibrio atypicus* sp. nov., isolated from the digestive tract of the Chinese prawn (*Penaeus chinensis O'sbeck*)

    - *Marinobacterium lutimaris* sp nov., isolated from a tidal flat

    - Viral suspensions are sorted via flow cytometry onto PTFE slides with 24 distinct wells containing agarose beads. Viral particles are then embedded within the agarose bead by overlaying with an additional layer of agarose. Lastly, MDA is performed *in situ.*

Meta-Transcriptomics

- In the River-Ocean Continuum of the Amazon (ROCA) example, the goal of the study is to relate the community biogeochemistry to gene expression. The USF part of the project is to analyze duplicate samples for eukaryotic metatranscriptome at 4 stations of the Amazon River and 8 stations in the plume (e.g., obtain 24 metatranscriptomes and over 250 million sequences)

- MG-RAST (the Metagenomics **R**apid **A**nnotation using **S**ubsystem **T**echnology) server is an automated analysis platform for metagenomes providing quantitative insights into microbial populations based on sequence data. The server provides web-based upload, quality control, automated annotation and analysis for samples up to 10GBp. Comparison between large numbers of samples is enabled via pre-computed abundance profiles. Presently the server includes: 47,956 metagenomes; 13.31 Tbp basepairs; 122.5 billion sequences and 10,056public metagenomes. The cloud version of the 3.1.2 MG-RAST is available. The MG-RAST provides a suite of tools for analysis and visualization of metagenomic data. The system is an adaptation of the RAST server system which was originally implemented to allow for high-quality annotation of complete microbial genomes using SEED data. The microbial SEED data are still used in the MG-RAST analysis pipeline; however, numerous other resources have been added to the system in order to enhance microbial sequence taxonomic and functional classification. More recently the greengenes, RDP-II and European ribosomal RNA Databases have been added to enable 16s rRNA classification of metagenomic data sets. The MG-RAST server is made available using technology established at Argonne National Laboratory and the University of Chicago. Registration with the site is required. User submission and analysis remain confidential, however it is possible to make your data ‘public’ and compare it with other public data sets. At its core, the system annotates individual sequence fragments, providing taxonomic and functional classification within a single metagenome and in comparison between multiple metagenomes. These data are presented using various visualization methods and are adjustable on the fly. Currently the server handles direct upload of files in fasta, fastq and sff format. Files larger than 50Mb can be uploaded in zip or gzip format. Both fasta and fastq need to be submitted in plain text ASCII format. Quality information can be supplied for fasta files by submitting it as a file with the same prefix followed by .qual. Multiplexed sequence data files can be parsed by submitting a descriptive multiplex identifier (MID) file in plain text ASCII format.

A high-throughput pipeline has been constructed to provide high-performance computing to all researchers interested in using metagenomics. The pipeline produces automated functional assignments of sequences in the metagenome by comparing both protein and nucleotide databases. Phylogenetic and functional summaries of the metagenomes are generated, and tools for comparative metagenomics are incorporated into the standard views. User access is controlled to ensure data privacy, but the collaborative environment underpinning the service provides a framework for sharing datasets between multiple users. In the metagenomics RAST, all users retain full control of their data, and everything is available for download in a variety of formats.

Satellite Wizardry

- USF satellite detection of *Karenia brevis* off Tampa Bay or Marco Island in Florida: Near real-time, updated every day, overlaid with Florida Fish and Wildlife Conservation Commission (FWC) cell counts, GE compatible using SeaWIFS

- Satellite detection of *Trichodesmium* blooms off Charlotte Harbor in Florida in optically complex waters example: Near real-time, updated every day, overlaid with Florida Fish and Wildlife Conservation Commission (FWC) cell counts, GE compatible using MODIS.

Summary

- *In situ* optical pigment analyses (Brevebuster) are powerful yet lack precise ID of genetic methods

- Seeing is believing-Imaging Flow Cytobot is very powerful

- Handheld genetic sensors useful for inshore spot testing by environmental managers

- Autonomous genetic sensors show great promise but are costly

- We are just learning how to relate gene expression to biogeochemical function

- Satellite monitoring gives largest spatial analysis

- Greatest potential is to link technologies together

APPENDIX 6-II

Biochemical Processes and Cycling

Margo Haygood
Oregon Health and Science University
Summary of the presentation to the NOAA Marine Microbes Workshop 2011

- The oceanographic processes of current concern for ecosystem function include: Warming of the atmosphere and oceans, increased seawater CO_2 concentration leading to acidification and associated calcium carbonate mineral undersaturation, and expansion of low oxygen zones. It is important to assess what is the interaction of normal biogeochemical processes with these recent phenomena.

- An integrative view of the role of microbes in marine ecosystems shows that microorganisms have important and varied roles in biogeochemical processes and cycling.

- Biologically important chemical elements include:
  1) Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus and Sulfur
  2) Sodium, Magnesium, Potassium, Calcium, Chlorine
  3) Manganese, Iron, Cobalt, Copper, Zinc
  4) Boron, Fluorine, Aluminum, Silica, Vanadium, Chrome, Nickel, Gallium, Arsenic, Selenium, Molybdenum, Tin, and Iodine.

- Microbes participate in many types of biogeochemical cycles. They can catalyze changes in elements (gaseous, dissolved, or solid/particulate, oxidation state or molecular form). For many of these elements, these microbial activities influence the distribution and reactivity of the elements. The availability of key elements governs biological activities and includes primary productivity that corresponds to the formation of new organic matter.

- The global influence of microbes is evident when looking at various planetary atmospheres. For example CO_2 is very high on Mars (95%) and Venus (95.6%) but very low on Earth (0.037%), whereas O_2 is high on Earth (21%) but inexistent on these other 2 planets. Nitrogen reaches 78% on Earth and ranges between 2.7% and 3.2% on the other planets. These differences reflect the fact that there is also widespread microbial activity on Earth.

- Microbes can exploit almost any thermodynamically favorable reaction, resulting in a myriad of metabolisms. Two types are specifically important for this discussion:

- Heterotrophs: In this case organic Carbon is the electron donor; the electron acceptor can be O_2 or any other oxidized species (e.g., NO_3^-, SO_4^{--}, MnO_2, etc.) and CO_2 is released at the end of the reaction.
- Autotrophs: Here, inorganic compounds are the electron donors such as water in the case of phytoplankton. Chemolitoautotrophs may use H_2S, NH_4^+, CH_4, reduced metals, etc. There are various electron acceptors and CO_2 is converted to organic carbon.

- Among all the elements of the periodic table C, H, O, N, P, S are the most important especially C, N and P.

- On Earth, the major carbon reservoir is composed of the rocks and sediments. They represent more than 99.5% of the total carbon on Earth. Besides this main reservoir, the oceans represent about 0.05% of total carbon on Earth (mostly inorganic C), methane hydrates about 0.014%, fossil fuels 0.006% terrestrial biosphere 0.003% and aquatic biosphere 0.000002%.

- The Carbon Cycle connects the atmospheric CO_2 to land plants and aquatic dissolved CO_2. On land, plants produce humus that leads to soil, rock and fossil fuels formation and connects to animals and microorganisms. All these activities release CO_2 to the atmosphere through human activities or natural processes. In the aquatic environment, the dissolved CO_2 is connected to the aquatic plants and algae that are used by aquatic animals. After death, plants and animals organic matter is mineralized in sediments and CO_2 is released to the environment.

- During the carbon cycle, carbon undergoes transformations. Carbon cycling is directly linked to oxygen and hydrogen cycles and indirectly linked to other cycles such as nitrogen and iron. Under oxic or anoxic conditions, CO_2 is generated from organic matter $(CH_2\ O)n$ by aerobic or anaerobic respiration and fermentation processes.

- The limitation of primary productivity in marine environments varies with the location, season and oceanographic conditions (e.g., advection upwelling). Several factors such as the amount of nitrogen, iron or light limit primary productivity in various locations. N is the most commonly limiting factor and nitrate NO_3^- is the most common form used by the phytoplankton. Carbon rarely limits primary production.

- Since among the biologically important elements nitrogen is the most commonly limiting factor in primary productivity, let us review what the major N reservoirs on Earth are.
  - Biologically available forms of fixed nitrogen are NH_4^+, NO_2^- and NO_3^-, but this pool is small (0.006%) compared with atmospheric reservoir, and reservoir comprising dead organic matter (~25%).

  - Pool sizes are inversely related to biological importance. Assimilation, mineralization and nitrification are quantitatively the most important processes linking inorganic reservoir with small and actively cycled reservoirs of living and dead organic nitrogen.

- Redox cycling between nitrogen compounds forms the basis for numerous microbial metabolisms.
- The modern N cycle depict several ways in which N_2 gas is fixed. There can be oxidation, reduction or no redox change.
    - Under oxic conditions, the fixation of N_2 produces NH_3. NH_3 can be assimilated directly to produce proteins or the nitrification of NH_3 can produce NO_2^- and NO_3. NO_3^- is then assimilated to form NH_2 groups of proteins. These proteins can in turn be transformed in NH_3 through ammonification.
    - If the reactions occur under anoxic conditions, NH_3 can be assimilated directly to produce NH_2 groups of proteins that ammonification can return to $NH_{3.}$ NO_3^- can be denitrified and reduced to N_2 by anammox that can be converted again into NH_3.

- The global ocean balance between N_2 fixation and the loss of fixed N through anammox and denitrification depends on the N:P ratio. If N:P >16, we will be in an oligotrophic environment with low primary productivity. If N:P is < 16 (for example in upwelling regions) there will be a high primary production and the environment becomes eutrophic. When N:P is <<16 there will be a very a high primary production that will produce high C flux. This will create low O_2 conditions that are favorable for denitrification and anammox.

- This explains why the very new N cycle proposed by Klotz in 2010 is getting ever more complex.

- This complexity increases when considering the role of metals. It is important to note that metals are important in every segment of the N cycle.

    - For example, in oxic conditions, Fe, Mo and V play a role in nitrogen fixation; Heme Fe, Fe and Cu are important in the nitrification process that converts NH_3 into NO_2^-, and Mo, Fe-S and Heme Fe regulate the conversion of NO_2^- into NO_3^-.
    - These same metals also play a role in the denitrification and nitrogen fixation processes occurring in anoxic conditions.

- Nitrogen inputs in marine systems drive the status of the environment.
    - In upwelling areas, such as the Eastern Boundary Upwelling Systems, the upwelled waters bring nutrients to the surface waters (e.g., California Current System, US West Coast).
    - Eutrophication is prevalent in areas where the runoff brings anthropogenically fixed N to coastal waters (e.g. Gulf of Mexico, East Coast estuarine systems)
    - Biological N_2 fixation is observed in oligotrophic offshore regions.

- Oceanographic processes of current concern for ecosystem function include:
    - Warming of the atmosphere and the oceans
    - Increased seawater CO_2 concentration leading to acidification and calcium carbonate mineral under-saturation
    - Expansion of low oxygen zones

- So at this point it is important to assess what is the interaction of normal biogeochemical processes with these recent phenomena.

- Possible effects of warming include:
    - A reduced solubility of O_2. This will lead to:
      – Expansion of low oxygen zones
      – Increased denitrification and reduced supply of nitrogen
    - Increased stratification
      – Inhibition of upwelling and mixing, reduced supply of nutrients
    - More extreme weather events
      – Increased upwelling and mixing, stronger blooms
      – Stronger phytoplankton blooms are associated with higher respiration, O_2 consumption at depth, and expansion of low oxygen zones.

- Possible effects of increased in CO_2 concentration will lead to:
    - Reduction in pH (increased [H+]): thermodynamic effects on reactions that include protons
    - Carbonate mineral saturation effects on organisms
    - Increased CO_2 concentration availability for primary production

- Possible effects of expanding low oxygen zones
    - Habitat loss for animals and effects on fisheries
    - Enhanced denitrification leading to a loss of N and a decreased primary productivity
    - Enhanced sulfate reduction associated with sulfide toxicity
    - Enhanced methanogenesis producing a stronger greenhouse effect

- Summary
    - Biogeochemical processes and cycles control the elements that are the foundation for marine primary productivity
    - Microbes dominate these processes and cycles
    - We need to understand how predicted global and local physical and anthropogenic changes will affect these processes and what alterations in ecosystems will ensue.

APPENDIX 6-III

Microbialization of Coral Reefs

Forest Rohwer
San Diego State University
Summary of presentation to the NOAA Marine Microbes Workshop

- Coral reefs are the location where the oceanic viruses and microbes meet the tropical shores. Most of the diversity in the ocean and the world is viral. There are about 10 million viruses (phages) and 1 million microbes per milliliter of seawater.

- Coral reef organisms include: corals, sponges, holothurians, microalga, and other species. They all directly interact with marine microbes that are the base of the food web..

- The interaction between corals and the microbial community are specific: the same bacterial community appears on the same coral species even when they are separated by 1000s of km. Different bacterial communities exist on different coral species. There are 100s of unique bacterial species per coral species and there are >500,000 species of bacteria on a 50 meter transect of coral reef**.**

- Coral holobiont include: protist, zooxanthellae, virus, bacteria, and archaea. It is predicted that coral-microbe-viral associations will change with environment and stress.

- The stressors to coral reefs can be:
  - Local: related to human habitation and resulting in coral diseases (overfishing, organic matter and nutrients additions)
  - Global: due to the increase of CO_2, temperature change and decreasing pH.

- Corals are extremely ancient animals with the first immune and neural systems. Mesocosm experiments using coral in aquaria give some information on how stressors change coral-associated viral communities. The impact of an increase in nutrient, temperature, organic matter, or a decrease in pH on the viral communities is assessed by determining changes in viromes. This is done by using DNA sequencing of isolate from viral communities. The results of these experiments demonstrate that stressors change the relationships between members of the holobiont. In particular, temperature, pH, and nutrients increase coral-associated *Herpes* viruses (e.g., induction of temperate *Herpes* viruses).

- Similar experiments conducted for the microbial communities show that all stressors increase the relative proportion of pathogenic microbes and the number of virulence factors.

- Each stressor changes the relative proportion of different virulence factors and specific pathogenic groups. In fact, you can "ask" the viruses and microbes what stressor is present.

- In a healthy reef, we observe: Large amount of coral and coralline crustose algae, and very small amount of turfs and macro-algae as well as the holobiont microbes and DOC. Grazing occurs by sea urchins and fish and predation by shark and shunt. All the processes are in quasi-equilibrium.

- When a reef is fished, the corals become diseased because the microbe population becomes more pathogen. There is an increase of turfs and macroalgae and a decrease in coralline crustose algae. This is due to a positive feedback between DOC, disease, algae and microbes that increase the space for algae. In those systems, the reefs are dominated by turfs and the grazing and predation decrease or disappear.

- Various coral health statuses are observable in the Pacific Islands where we can observe a change in reef health as a function of human presence. There is a strong gradient from pristine reefs such as in Kingman (zero inhabitants), then average health reefs like in Palmyra (about 15 inhabitants) and Fanning/ Tabuaeran (about 1000 inhabitants) and finally to a degraded reef like in Xmas/ Kiritimati (about 5,000 inhabitants).

- From this study it is obvious that when there are more people there are:
  - more viruses and microbes,
  - more super-heterotrophs and pathogens (such as *Enterobacteria, Staphy-Strepto-coccu, E. Coli,* Vibrio, etc),
  - coral cover decreases and
  - prevalence of coral disease increases

- In healthy reefs like Kingman, the primary production supports fish and sharks whereas in degraded reefs like Xmas, the primary production supports microbes. There is a “microbialization” of the reef.

- How generalizable is the microbialization model? In essence, the Metabolic Theory of Ecology (MTE) is an equation which suggests that most of the variation in the metabolic rate of an individual organism can be explained by the joint effects of body mass and temperature.

$$I = i_0 M^{\alpha} e^{-E/kT}$$

where:

- i of $_0$ = mass independent normalization constant that varies depending on the organismal group
- M = individual wet weight in grams
- Alpha = a scaling exponent that has also been shown to depend on the organismal group and physiological state
- E = activation energy (for this study I had assumed one value for respiration and photosynthesis) - (molecules must collide before they can react, and collisions must be sufficiently energetic to bring about bond disruption)
- K= Boltmann's constant
- T = temperature in degrees K (in this case the water temperature at the site at time of data collection)

In this talk metabolic rate energetic cost to the individual per unit time is measured in Joules per second or Watts. Boltzmann (or velocity distribution) distribution of / when the fraction of molecules with sufficient kinetic energy exceeds the activation energy (as the temperature is raised) the rate of reaction speeds up – exponentially. With MTE, much of the arguing in the last 10 years has been over the true value of the scaling exponent.

- Most recent efforts have been geared toward ground-truthing this theory for the organisms at the very small end of the body size spectrum, namely microbes. This equation was empirical and estimates are conservative.

- So, is more power drawn from a reef system by fish or by water-column associated microbes? Does this relationship change as a result of human impact?

- In the first slide I emphasized the bioenergetics standpoint that I am taking. By investigating these questions this study predicts how much energy is fluxing through (or consumed by) two different groups of organisms within Pacific coral reef systems.

- By comparison, this study encompasses 29 islands in 4 oceanographic regions across the Pacific:
  - Mariana
  - MHI
  - PRIAS – widest geographic region
  - American Samoa

- One of the questions casting doubt on the findings of these previous inter-island studies is whether the observed differences in microbial communities reflect human impacts associated with local land use and fishing or variation in oceanographic conditions.

- This map shows variation in Net Primary Production (NPP) (derived from satellite data) throughout the Pacific Basin.

- This yellow-green streak (which includes many of the Pacific remote islands and atolls) indicates higher NPP as a result of upwellings of the Equatorial undercurrent. In contrast, you can see that some of the lowest levels of NPP in equatorial Pacific occur in the Mariana region and one might expect NPP to be a significant factor driving variation in metabolic rates.

- The 29 islands and 99 sites included in this study were surveyed as part of NOAA's Coral Reef Ecosystem Division (CRED) and Pacific Reef Assessment and Monitoring Program (Pacific RAMP).

- The data I am presenting are at the island level. But, in general, the larger the island, the more sites were surveyed there.

- For the fish, the study includes fish data from all surveys (belt transect) performed at reef sites during the years 2001–2009. The fish data were provided at the island level for each fish family as mean biomass (g m^{-2}) and mean abundance (# individuals m^{-2}), from which the mean mass per individual (g) was calculated. Microbes were measured at about 1 m above the reef benthos at each site.

- Since surveys were carried out at an average water depth of 10 m, the mean abundances (individuals per m^2) represented the total number present in a 10 m^3 water column.

- Mass values for individual microbes were measured using a 0.2 micron anodisc stained with DAPI. That technique allows for measurement of:
  - Cell dimensions (l, w) were collected (ImagePro Software)
  - Cell volume (um^3) were calculated - all cells considered cylinders with hemispherical caps (Bjornsen, 1986)
  - Individual cell volumes converted to mass in wet weight (g) using previously established size-dependent relationships for marine microbial communities (Simon and Azam, 1989).

- Metabolic rate predictions for all individuals contained in a $10m^3$ volume of water were summed to obtain community–level energy flux (Watts). The total predicted metabolic rate (W $10m^3$) represents the Watts required by microbes plus the Watts required by the fish. The microbialization score is the ratio between the watts required by the microbes (W $10m^3$) over the total predicted metabolic rate (W $10m^3$).

- What is important to understand is that productivity does not predict "microbialization."

- There are two parameters which I want explain:
  - Microbialization score (%) is the microbial share of the "total" predicted metabolic rate. In this figure I have plotted the microbial share of the "total" predicted metabolic rate against the total energy use for each island. First I want to draw your attention to the x-axis – to point out that TPMR (fish + microbes) varied by ~ 1 order of magnitude throughout the Pacific (Rota in Mariana vs Oahu in MHI) – to put this amount of energy use in more familiar terms – I think about light bulbs – a 100W light bulb draws 1000 times more energy per second than the fish and microbes in a 10m3 column of reef water.

  Now, remember the first question? Is more power drawn from the reef system by fish or by water column-associated microbes?
  - The answer to this question is shown on the y-axis, as the microbialization score - which also varied widely – with the microbial share of the "total" predicted metabolic rate being lowest at Wake and highest at Oahu.

- Relative to other reef systems in the Pacific, the MHI are considered to be highly degraded - an interesting pattern that emerges from this figure is that, the more degraded islands within each region tend to fall out among the MHI region (blue dots). However, in order to relate microbialization to human impact (and answer the second question) we need a way to quantify human impact.

- The level of human impact was assessed using the cumulative global human impact map generated by the National Center for Ecological Analysis and Synthesis (NCEAS) which incorporates the following data: Fishing, inorganic and organic pollution, nutrient inputs, invasive species, ocean acidification, benthic structures, population pressure, commercial activity, sea surface temperature, ultraviolet insolation.

- Microbialization score is strongly correlated with the NCEAS Human impact score.

- The land mass of each island was converted into polygon format and the immediate 10 km of sea surface around the border of each polygon/island was used to calculate a mean impact score.
- When the microbialization score was plotted against this NCEAS Score we found a strong relationship between the microbial share of the "total" predicted metabolic rate and human impact with MHI among the highest and PRIAS among the lowest. So the answer to the second question is a resounding yes - the way energy is being allocated among these two organismal components is strongly correlated with degree of human impact.

- Mention JOH –nuclear testing, coral dredging to make runways, chemical weapons depot until 2000.

- Fished reef
  - favors super-heterotrophs /pathogens
  - increased coral-algae interaction zones
  - turfs are the bad guys

- Nutrient additions also impact the health of the reef and can lead to eutrophication. In that case iron concentrations play a role.

- The role of iron enrichment is being studied in the Line Islands eutrophication: it was observed that the top 1% of pristine coral reefs had very low concentrations of iron. For example in 6 of 12 Line Islands have black reefs (e.g., Kingman, Tabuaeran, Millennium). We can see an association between the presence of shipwrecks and black reefs where more than 1 km of reef was killed.

- Black Reef microbial communities are super-heterotrophs with increased virulence factors
  - isolate microbial communities pyro-sequencing BLASTx against SEED
  - relative abundance of subsystems
  - A little bit of iron goes a long way
  - ampicillin protects

Right nutrient in the wrong place...

- Local stressors - Human habitation results in coral diseases due to overfishing (organic matter) and nutrient additions.

- Global stressors - Increasing in CO_2 associated with temperature change and decreasing pH.

- The conjunction of both types of stressors leads to the microbialization of the reefs

Discussion points

1) Microbialization means less fish and more microbes (~500 g of fish = 1 g of microbes). This is probably happening in many ecosystems.
2) Viruses and microbes provide an amplified and early warning system for ecosystem shifts.
3) Alternate stable states are initiated by positive feedbacks mediated by viruses and microbes (e.g., DOC-Diseases-Algae-Microbes balance, bleaching, Coral Crustose Algaes (COA) and coral recruitment, etc.).
4) Alternative stable states are maintained by changes in microbial communities. Reversal (e.g., restoration) is still untested on coral reefs.
5) Microbial taxa analysis (e.g., *Vibrio*) is only partially correlated with alternative stable states on coral reefs. Growth rates and relative gene abundances are more informative for determining ecosystem health.

APPENDIX 6-IV

Biotechnology and Natural Products Summary

Rita Colwell
University of Maryland - College Park

- Medical biotechnology is increasing at a fast rate. Environmental biotechnology is increasing at a slower rate, and marine biotechnology is faced with road blocks. This is mostly due to the public opposition to genetic engineering products and food biotechnology. For example engineered salmons are not accepted by the public.
- Natural products from the sea are difficult to extract and slow to come on-line, but they are very useful. These are more acceptable, and this topic is a strategic path to take because the set of goals are more understandable and acceptable.
- Recently, scientists have observed more die-offs in the ocean and uncovered the source of many diseases. V_p and V_v are marine pathogens. The pathogenicity has an ecological function.
- All *Vibrios* degrade chitin. However, *Vibrio cholerae* (V_c) do not grow at temperatures under 15^{o}C; they have a dormant stage in sediments.
- It is now possible to predict, via satellite, the time of year and which estuaries in the Chesapeake Bay will have an increase in $V_{v.}$ Copepods carry 10,000 V_c million/ml, and cholera is a vector-borne disease. Mekalanos' research on V_c indicates that toxR confers *Vibrio* tolerance and creates pathogen adaptations. What is the role of the tetra toxins produced by *Vibrios*?
- Can we vaccinate animals in the wild against HAB toxins? It seems that this is becoming feasible.
- Research on human pathogens and antibiotic resistance has shown an increase as we go deeper in the water column and we do not know why.
- There are microbes that degrade oil, as seen in the Deepwater Horizon catastrophe. The marine microbiology community needs to understand the processes at play and the resulting natural attenuation, i.e., limitation of the action.
- NOAA should consider using NOAA's SBIR to support these kinds of project.
- NOAA should consider beefing up the NOAA Ocean and Human Health Initiative

APPENDIX 6-V

Forecasting Microbial Responses to Global Changes

Synopsis of a presentation at the NOAA workshop on microbes and climate change, November 29-30, 2011

David L. Kirchman
School of Marine Science and Policy
University of Delaware
Lewes, DE 19958

Global warming viewed from the poles:

The average global temperature has already increased about 1 ^{o}C, but this warming is not spread equally around the earth. Global warming is not necessarily global. In particular, the Arctic and the Antarctic to a lesser extent have warmed more so than lower latitude ecosystems. The effect of a warmer Arctic is most dramatically seen in the decrease in sea ice and in changes on land. However, the phytoplankton in the Arctic Ocean also appear to be changing. The average size of cells at the base of Arctic Ocean food webs is getting small, and one type of large phytoplankton class, the diatoms, seems to be decreasing (Li et al. 2009). Even if total phytoplankton biomass has not changed, changes in phytoplankton cell size have many ramifications for the rest of the food chain. Smaller phytoplankton means smaller herbivores and a longer food chain leading to fish. Furthermore, the number of bacteria has increased over the same time that phytoplankton cell size has decreased. These microbes, already abundant and important in the carbon cycle, may be even more so as the Arctic continues to warm.

Another consequence of warmer waters in the Arctic and perhaps elsewhere is faster microbial growth. Currently, rates of bacterial biomass production are lowest in the perennially cold waters of the Arctic Ocean and Antarctic seas (Kirchman et al. 2009). However, while bacterial growth and biomass production appears to be higher in warmer waters, the change in these rates is greater than can be explained by direct temperature effects alone. Other factors covarying with temperature are likely to have a much larger effect on microbial activity than the temperature increase predicted for the next century. These other factors include changes in the microbial physical environment (e.g., depth of the mixed layer in the surface ocean), carbon fluxes and food webs.

Work from the Arctic provides some clues to how the oceans in general may respond to warming and other changes in our climate. A key to understanding these changes is time series studies in which biogeochemical properties are examined regularly (at least monthly) over a long time at one location. However, equally important is a mechanistic understanding of the forces behind those changes.

The other CO_2 problem and the N problem. Global warming is caused by higher CO_2 concentrations in the atmosphere. The "other" CO_2 problem is ocean acidification. According to work at the Hawaiian Ocean Time Series (HOT) station, pH of the oceans which has been about 8.12, decreased to about 8.08 in 2009 due to higher atmospheric CO_2 and thus higher CO_2 partial pressure in the oceans (Doney et al. 2009). Both the National Science Foundation (NSF) and NOAA already have programs examining ocean acidification, but more work is probably needed. Ocean acidification may have more subtle, potentially more widespread effects than currently appreciated.

One example is the effect of pH on ammonia oxidation. This chemolithotrophic process is the first step in nitrification, which is the transformation of ammonium to nitrate. Nitrification, a crucial part of the nitrogen cycle, ultimately controls biological production in many ocean regions. Beman and colleagues argued that ammonia oxidation may decrease by 10 to 40% with a drop of 0.1 pH units (Beman et al. 2011). A lower pH affects ammonia oxidation rates by lowering concentrations of ammonia (NH_3), the actual substrate of ammonia oxidization. Ammonium (NH_4^+) is not oxidized, although it is a crucial N source for other members of plankton.

Another, probably larger impact on the N cycle is nitrogen from anthropogenic sources. Anthropogenic production of N in the form of fertilizer is now approaching natural rates of N_2 fixation. Although nitrogen-rich fertilizers are essential in modern agriculture in supporting today's growing population, too much of the nitrogen ends up in coastal oceans. In part due to higher N inputs, coastal waters are now being threatened by increasing coastal eutrophication, hypoxia, and harmful algal blooms (HABs). It is worth noting that these problems, which are already being studied by NOAA, are intrinsically microbial problems. The algae of HABs are microbes, and much of the oxygen consumption causing hypoxia problems is due to microbes.

Still, it could be argued that measuring oxygen concentrations is a problem in geochemistry, not microbial ecology or microbial oceanography. Yet, given the huge role of microbes in both producing and consuming oxygen, it seems obvious that a better understanding of hypoxia depends on a better understanding of microbes involved in oxygen reactions.

The other global experiment. Climate change is usually thought to affect "bottom up" factors, such as temperature, pH and other properties regulating growth of the marine biota. The release of CO_2 into the atmosphere has been called a global experiment; more accurately, it is a bottom-up global experiment. While this and other bottom-up experiments have been going on over the last hundred years, society has also been conducting a less well publicized experiment involving top-down controls, that is, grazing and viral lysis of marine organisms.

The experiment is the harvesting of fish in oceanic waters. The stocks of Atlantic cod, for example, and many others have decreased greatly over the last forty years.

While the problem of overfishing is well known, less well-understood is the trickle-down effect of removing a top predator from an ecosystem. The study of Frank and colleagues is an exception (Frank et al. 2011). They documented the trophic cascade caused by declines in Atlantic cod in coastal waters of the North Atlantic Ocean. As cod stocks declined, Frank and colleagues found that the next trophic level down, the forage fish, increased. This increase in turn caused a decline in their prey, large zooplankton, which in turn caused a rise in phytoplankton biomass. The measures of these changes in zooplankton and phytoplankton biomass were crude, leaving much room for more work. In particular, it is not clear if other biogeochemical processes and ecosystem services are also affected by overfishing. These trophic interactions raise the question of whether the recovery of Atlantic cod and of other fish depends on microbes and trophic interactions at the bottom of food chains.

An even more general message is that top-down factors cannot be ignored in thinking about the effect of global change on oceanic organisms.

What do we need to know? Marine microbes are at the heart of many climate change problems facing the oceans. In general we need to know more about these microbes in order to understand climate change and to predict the response of the oceans and other ecosystems to climate change. The challenge is to identify how much more we need to know.

One way to answer this is to consider the various levels at which microbes and the processes they mediate can be studied. At one extreme, we can examine biogeochemical processes, such as primary production or respiration, without any information about the microbes, in order to understand, for example, the spread of hypoxic areas in coastal waters. Examining total abundance and growth of microbes would provide more details about potential oxygen-producing and consuming microbes, but these measures are of the entire microbial assemblage; the assemblage is treated as a black box. That box can be opened up and the organisms can be identified with approaches built on 16S rRNA and other taxonomic marker genes. Finally, the metabolic potential of microbes can be deduced with data on the metagenomic and other ‘omic approaches. These approaches have already unleashed a dramatic flood of sequence data about bacteria, archaea, and to a lesser extent, protists thought to be important in global biochemical cycles. While these data have revealed much about marine microbes, microbial ecologists are still wrestling with extracting more information from the flood of sequence data. The flood will only grow as sequencing and other molecular technologies become cheaper and easier to use.

In short, many approaches can be taken to provide more information about microbes and their response to climate change. The appropriate level of detail will vary with the question: Which biogeochemical process and global change issue do we want to address? These processes and issues are likely best studied with some combination of approaches taken at various levels, ranging from rates of biogeochemical processes to metagenomic data. Using a combination of all approaches is now possible and is likely to be the most powerful in understanding and ultimately predicting the response of microbes to global change.

References

Beman, J. M., C.-E. Chow, A. L. King, Y. Feng, J. A. Fuhrman, A. Andersson, N. R. Bates, B. N. Popp, and D. A. Hutchins. 2011. Global declines in oceanic nitrification rates as a consequence of ocean acidification. Proceedings of the National Academy of Sciences **108**:208-213.

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean acidification: The other CO_2 problem. Annual Review of Marine Science **1**:169-192.

Frank, K. T., B. Petrie, J. A. D. Fisher, and W. C. Leggett. 2011. Transient dynamics of an altered large marine ecosystem. Nature **477**:86-89.

Kirchman, D. L., X. A. G. Moran, and H. Ducklow. 2009. Microbial growth in the polar oceans- role of temperature and potential impact of climate change. Nature Reviews Microbiology **7**:451-459.

Li, W. K. W., F. A. McLaughlin, C. Lovejoy, and E. C. Carmack. 2009. Smallest Algae Thrive As the Arctic Ocean Freshens. Science **326**:539.

APPENDIX 7 – Breakout Session Summaries

APPENDIX 7-I

Breakout Session I
Biogeochemical processes and cycles

Major Challenges and Opportunities by Habitats (shallow, deep, pelagic, benthic)

During the breakout session the participants identified several major challenges that included: the lack of funding to advance the needed research, the lack of communication and dialogue between the various groups to know who is doing what, the necessity to raise the awareness of the importance of marine microbes in the oceans, and the need to visualize the large amount of data that has been and will be gathered in the future.

The changes that are being observed in the ocean represent a challenge but also opportunities to better understand the role of micro-organisms in the ocean. For example, global warming and the associated change in the depth of the mixed layer will change the nutrient supply and its related effects. It is possible that the oxygen minimum zones will be expanding (at depth) and that the hypoxia problem will increase in shallow coastal waters. The increase in temperature and in CO_2 and related ocean acidification of the ocean will impact corals reef and lead to changes in pathogen distribution, virulence and biodiversity.

The "Rise of Slime" or microbialization is one of the major challenges facing the ocean today. This will lead to: habitat change and/or loss, shifting of the elemental cycles without recovery to base. Discovery of new habitats and new microbial functions will lead to the discovery of new cycles. These changes may or may not be expressed in permanent changes in microbial function or resiliency. Finally some of the major issues are related to time and space scales.

An increase in urbanization results in an increase in nutrient loadings that accelerate the pace of cycling. Excess nutrient in multiple habitats results in community structure and ecosystem function changes.

Identification of keystone species in multiple habitats is essential to understand the system’s biology going from the ecosystem to the molecular level. We need to understand the role of microbes in cycles with and without specific impacts on environmental resiliency.

As the knowledge on ocean microbes grows, it is important to think about data set management and applications. We need to determine the necessary "knowledge base" because of the large variety of data (i.e., acoustic) and find ways to visualize and integrate all these data. Designing a data portal for easy access will allow for the correlation of various microbial compositions, hindcast impacts of microbial changes, and relate microbial biogeochemistry to gene function and metabolism. It is essential to connect to and affiliate with other existing and future microbial datasets.

Increasing outreach and education is essential to raise awareness of the importance of marine microbes in ocean health. We must open the dialogue between the various groups to know who is doing what in order to share data and increase partnership and avoid duplication of efforts. In particular, connection between NOAA and NSF is desirable.

The opportunities reside in the improvement of the understanding of the relation existing between microbial biogeochemical processing and gene function (e.g., metabolism, virulence) and develop the proteomics tools necessary to fill this gap in understanding.

Tools, Methodologies, Instrumentation, and Approaches

NOAA must include extramural partners in a big way in the discussion of what tools and approaches are needed to study microbes and what are the priorities. We should focus our efforts on wise and strategic choice of regional studies.

The community is sample-poor so it is important to provide access to platforms for sample collection. This needs to be integrated with existing NOAA's efforts (e.g., SEAMAP/MARMAP) and engage external researchers into sampling activities. This aspect is important because it is indispensable to adhere to the appropriate sampling processing and preservation techniques in order to obtain a valuable sample. That effort will result in positive experiences but requires pre-planning and coordination. The research community needs to be able to take advantage of ships of opportunity and NOAA needs to be forthcoming on what NOAA resources (platforms) are available. One good way would be to expand sampling on all NOAA cruises to include biogeochemical sampling in addition to physical and chemical sampling.

At monitoring stations, NOAA should incorporate micro-biogeochemistry measurements and add "ARMS", settlement plates, and "biotraps" in observation areas such as coral reefs and on moorings. In particular, it would be good to use IOOS/OOI/3000 Argo floats. This will require an effort in sensor and technology development, in particular to measure biogeochemical rates and relations to molecular measurements.

The community needs to improve its molecular tools and increase its sequencing capabilities, data acquisition and integration. Scientists need to share existing sensors and technologies and develop new ones.

Platforms for data archiving and sample repository are essential. Appropriate sample processing and preservation protocols must be identified. There must also be consideration of the need for standardization and validation of sample and data processing. The only way this is likely to happen is to work across agencies and institutions, nationally and internationally. This should not be a NOAA-exclusive role, but the agency could play a coordinating role and should consider this need and foster the development of these tools, technologies and processes.

It is necessary also to offer training for graduate students and provide modeling opportunities that could lead to predictions and forecasts. Acquisition of good data contributes to the development of good models. These in turn assist in the identification of gaps in data needed to improve the models (e.g., HABs). In particular, there is a need to better study biofilms.

Top Science Questions & Opportunities for NOAA

The top science questions and opportunities for NOAA that have been identified by the workshop participants include:

- Determine what an “indicator” is or “sentinel” species is within the biogeochemical cycle and its roles in the processes.
- Identify microbes that can be seen as "indicators" and use them to understand changes in the biogeochemical cycle and ecosystem function. This can be done by identifying "keystone" microbe species, e.g., what are the microbes associated with organisms and land-based runoff.
- Discern the role of viruses in biogeochemical cycling.
- Identify those microbes that "drive" biogeochemical cycling and the effect of Dissolved Oxygen (DO) decrease, warming and calcification/ocean acidification upon these drivers.
- When microbes have been identified as indicators and drivers, determine how to leverage the microbe functions to assist in mitigation efforts. Identify which biogeochemical processes are most important to focus on for impact and resiliency.
- Collect baseline biogeochemical data by habitat (with focus on microbial diversity and function)
- Determine how to drive the dialogue about microbial science among and across the scientific community. In particular, the attendees highlighted the need to organize a GORDON conference on the topic. That will help in establishing a way to integrate

across disciplines, regions, agencies and institutions, and will ease transition of basic research on microbe research to applications.

- Figure out if microbial community structure matters in the marine environment and how microbial communities are structured.
- Resolve how nutrient loading impacts community structure and identify load "tipping points" in cycles, e.g., transition to hypoxia, going from NH_4 to NO_3^- and find "biomarker" targets that highlight changes in system.

Breakout Session II
Emerging Diseases, Organisms and Ecosystem Health

Major Challenges and Opportunities by Habitats (shallow, deep, pelagic, benthic)

There are specific pathogens we cannot do anything about (which ones? And are they the same across geographic regions, species, etc?). Should microbiologists do nothing about them and/or should they perhaps decide how to proceed once they know/understand the tipping points?

To understand diseases, it is imperative to identify how the susceptibility to pathogens is driven by virulence of the agent and/or resilience of "host." Doing so will advance our understanding of the science of microbial evolution. This goal can be achieved, through the study of pathogens, by using tools that already exist. Several lines of research must be followed to make progress:

- Determine how to slow down and mitigate the spread of pathogens and disease;
- Pursue a predictability challenge as to the effects of population and ecosystem changes on the microbial community and disease transmission;
- Discern how, when, and where the effects of pathogen and disease depend on the nature of the "system" under examination;
- Ascertain the maximum amount of 'X' one can take out or add to a system without seeing negative effects.

Because of the increase in human use of antibiotics and buildup of antibiotics in the ocean, we need to determine the effects across the environment of low levels of antibiotics and other xenobiotics on the development of antibiotic resistance, (e.g., farm animal production, medical application). How should bioavailability/cross-resistance be a part of this discussion?

In particular, one of the basic questions is related to the purity of cultures: Is there such a thing as a "pure" culture?

Another challenge, that could also be considered an opportunity, is gaining understanding of how ecosystem changes and climate variables affect pathogen distribution and virulence, as well as the host's susceptibility to pathogens. Understanding how adaptations of pathogens to the environment may establish disease is essential (e.g., type of habitat, identification of reservoirs for pathogens and of host susceptibility). In particular, we do not have information on the role of biofilms in the environment. We need to understand how virulence flows through microbe pathways and what the virulence factors are, so that we can create better markers.

In parallel, we need to understand what the drivers of environmental changes are that affect host susceptibility (e.g., temperature, immune comprise oxidation stress, salinity, etc.) and the role of novel hosts (via introductions, etc.) their distribution and abundance. What are the drivers of emerging disease? (e.g., feedback processes) What are the impacts of changes in temperature and other abiotic factors, such as the effect of ocean acidification on animal diseases?

It is important to establish the cause of disease and to assess presence of pathogens versus presence of disease. In particular, the role of microbes versus abiotic influence must be teased out.

The detection and identification of small microbes is a problem. Scientists do not know how to identify their habitat and role and their impact upon marine living resources. New microscopic tools need to be developed to achieve these objectives.

The detection and identification of fish disease is fairly well known in shallow and coastal areas, but it is completely unknown in deep ocean ecosystems. Genomics studies will provide better understanding of fish disease and disease impacts across life history stages.

Eukaryote microbial pathogens are less studied, (e.g., fungi), and the scientific community needs to improve its understanding of the physical transport and vectors. Consequently, incorporation of microbial ecological behavior within models would be very helpful.

Finally, one outstanding question is to determine what the role of disease is in ecosystem function.

Tools, Methodologies, Instrumentation and Approaches

To improve disease prevention, it is critical to develop methodologies and techniques to allow for the forecasting of pathogens presence and impacts in the environment (e.g., probiotics). To improve forecasts, model development should include driver identification (SST, salinity, etc.). Existing models should be adapted to the microbes and diseases or new models should be developed.

New sensors should also be developed for pathogen detection and for improving the limits of disease detection.

Adding field environmental sample processors (ESP) to the sensors would aid in the remote detection of marine microbes, especially harmful algae and biotoxins.

In addition, laboratory experiments and the use of mesocosms to test outstanding questions would be of great interest.

All approaches must take into account the degree and nature of the sensitivity of the studied system (e.g., natural systems, aquaculture systems, pristine systems) to be able to detect the introduction of new species.

A rapid, multidisciplinary science deployment team, e.g., a SWAT team, should be initiated and organized for rapid response to emergencies and for taking advantage of "science of opportunity". The SWAT team should establish and follow a series of prescribed protocols.

NOAA should strive to develop a reliable aquaculture system that will protect fish, and human and environmental health.

The development of a gene-based "tool kit" to detect the presence of microbes should be initiated as soon as possible; this could be a microarray library-specific and direct pathogen detection versus indicators. Determining ways to detect gene virulence identification is important.

Employing an epidemiological approach (e.g., beachgoers' survey & MSRA), as well as economic impact/assessment tool(s), would be very useful.

Finally, the role of education and outreach, as well as the role of "Citizen Science," should be investigated with the goal of increasing sampling capabilities (3/person).

Top Science Questions & Opportunities for NOAA

- Should NOAA consider focusing on bioremediation/restoration approaches? These approaches require understanding of cause and effect relationships between pathogens and diseases.
- Bluewater and deepwater systems: What is going on there? For example, in fish diseases (e.g., tuna) what is the importance of the mobilome?
- How do we improve understanding of microorganisms as "communities" in the ocean (see Rita Colwell's example of human cholera study in Calcutta)?
- What are the reservoirs of disease? (e.g., role of biofilms, sediments, organs)

- What is NOAA's role in forecasting/predicting disease? Consider the agency’s role regarding detection, identification of mechanistic drivers of disease (organism state and life stages, environment conditions), and model development that are specific to NOAA's mission.
- Consider mechanistic modeling of emerging disease, as a function of abiotic and biotic parameters that will detect cause and effect.
- Are diseases changing in particular habitats (e.g., coral, eelgrass, plants, animals)? To follow these trends, robust monitoring activities are indispensable.
- What is the role of disease in ecosystem function?
- What are the socioeconomic impacts of disease (quantified in $), e.g., seafood safety?
- What awareness does NOAA need to have, with regard to disease and public health exposure, in their response efforts to natural/industrial disasters?
- What is NOAA's role in response to epizootic events?
- How do transport models and atmospheric impacts need to be considered and what are the vectors of transmission? (e.g., fungii, bacteria spores, aerosols, sea spray).
- What are the adaptations of pathogens to specific environments?
- How does virulence flow through microbial pathways and what are the drivers of environmental changes that impact host susceptibility?
- What are the impacts of disease across life history stages?
- When does the presence of pathogens lead to disease?

APPENDIX 7-III

Breakout Session III
Forecasting Microbial Responses to Global Changes

Major Challenges and Opportunities by Habitats (shallow, deep, pelagic, benthic)

One of the major forecasting challenges is the issue of scale for both time (day-to-century) and space (millimeter-to-global). In addition to being able to follow changes associated with global changes, it is crucial to have good baseline data. Presently, we do not have access to the needed baseline data, but it would be feasible to obtain access since the samples are available. In addition, we do not have an inventory of the availability of platforms (all types, including drones) that could be used for sensing all parts of the spectrum.

One of the greatest opportunities for the science community would be to figure out a way to address the increase in CO_2 through microbial processes.
In this context, it is important to identify where the larger gaps are in our knowledge that would be suitable for NOAA investment. Particularly, what are the microbial processes that are most relevant over time to issues related to the role of micro-organisms in fisheries, living marine resources and human health?

Consequently, we need to identify the linkages that exist within and between the various processes and those that are most likely to change as our environment changes. For example, we need to understand what the most important biotic or abiotic drivers are that have an effect on microbial communities. Presently, we do not have a good understanding of what parameters lead to re-mineralization of Dissolved Organic Carbon (DOC) to CO_2 versus the re-mineralization of nutrients to Particulate Organic Carbon (POC).

Finally, NOAA should initiate regional pilots, especially on the West Coast and in the polar regions and include microbiologists on its ships. In this case, it is essential to be clear about the role and function of on-board microbiologists and how they could collect important samples without impacting the work originally planned.

Tools, Methodologies, Instrumentation, and Approaches

The following would be helpful in advancing marine microbial science and research:

- Time series information: source identification of microbes, database repository and comparison among habitats
- Inventory of existing (NOAA) data and samples and better ways to use the museum collections
- Time series analysis of existing data

- Data base repository and sample repository
- Mesocosm experimentation with iron fertilization, perturbation, etc.
- Mesocosm and molecular approaches to look at horizontal gene transfer and other ecosystem functions and to understand how to extrapolate from mesocosm to real world, to study adaptive capacity under changing environmental condition, and to conduct evolutionary process studies
- Development and use of new technology to sample on ships without the need for personnel physically present (e.g., role of graduate students)
- Development of software to analyze sequence data more efficiently
- Comparison of diverse habitats to assess microbial differences and similarities
- Development of new tools to measure the DOC pool and get the right sample to measure DOC. This will also require modeling of the various fractions (e.g., DOC labile, semi-labile and refractory)
- Augmentation of the ARGO floats and other buoys with appropriate sensors especially O_2 sensors.
- Observing Capabilities
    - sensor development for measuring and sampling
    - remote sensing products
- Community assembly models as a tool/approach (Get a group of experts together to) develop a prioritized list of observing/observational needs
- Bioinformatics tools
    - computational tools for data reduction, data mining, visualization
    - correlate function with phylotype; determine mechanisms of lateral gene movement

Top Science Questions & Opportunities for NOAA

- How can NOAA assist in improving understanding of the variability of microbial metabolism over time and space?
- How can the sensitivity of microbes (affiliated with humans and living marine resources) to global climate change be determined and can the factors that influence those sensitivities be identified? What is the degree of sensitivity to global climate change for microbes important to living marine resources (LMRs) and humans? And what influences those sensitivities?
- What is the role of microbes in the Carbon cycle? How has global change impacted that role?
- At what scale do scientists need to do microbiology studies to be relevant to studies relating to global changes? Do they need to assess the role of local forcing on microbial activities?

- What do microbiologists need to do to understand the modulation of climate through the release and interactions of VOC /DMS with aerosols? What is the role of marine microbes in this process?
- What are the type of microbes and their role in the biogeochemical cycles and how do those vary overtime?
- Through time-series data analysis, can we identify the conditions under which diseases emerge?
- Will global [climate] changes force genetic rearrangement within microbes responsible for diseases and biogeochemical cycles (time series)?
- What are the roles and influences of microbial processes on coastal eutrophication and hypoxia zones and the effects of global climate change over time?
- How should/can the NSF Long-Term Ecological Research (LTER) model be used in NOAA?
- How can microbiology improve the mechanistic robustness of ecological forecasts in the global climate change context?
- Do we have sufficient historical information available to enable the science community to make forecasts? (Without archived samples, scientists can't make forecasts.) (DOE had 10K isolates maintained for 20 years and sold it to a pharmaceutical company.)
    - need to estimate archive value to forecasts
    - long-term storage is critical
    - new tools for analysis in future will make these even more valuable
    - need to develop appropriate partnerships
- How can NOAA and its partners grow the understanding of gene transfer mechanisms that enable environments to adapt through change?
    - increase comprehension of horizontal gene transfer
    - enhance adaptive capabilities
- How does global change impact microbial/vector/symbiosis-host interactions? [This question is not just directed at human health, but also marine species health, e.g., corals, bacteria, symbiodynium, etc.]
- Do we have enough data to conduct needed forecasts? Do we need more information about physiological parameters? Are the biological parameters in global biogeochemical models understood? What is the role of biological variability?
    - biocrypt needs additional information; expertise in the field
    - need end-to-end impact decisions
    - need to understand intra-cellular biogeochemical process, as well
- How sensitive are microbial populations to environment change? Undoubtedly, microbes are extremely sensitive to change. Environmental microbe populations' sensitivity needs to be quantified--how/with what tools?

- How well can regional scale questions/models/forecasts be scaled up and down?
- How best can microbial components be incorporated into ecosystem assessments; prioritize measures needed.

APPENDIX 8 -- Investment Priorities Discussion Notes

PLENARY SESSION
Investment Priorities Discussion Notes

During the final plenary session of the workshop, the attendees discussed the investment priorities that would benefit the scientific community as a whole as well as the need for partnership and collaboration. They provided NOAA with general recommendations as well as near- and long-term specific activities that would allow for important progress in the field of marine microbiology.

General recommendations

- The community needs baseline information and data, including routine quantitative analysis of the type and distribution of microbes and microbial community composition (Pyro-sequencing of composition). NOAA should use NERRS, IOOS and other observing stations to add microbes to the observations and sampling suites already in action. In addition, NOAA should pursue additional sampling in different geographic areas, following patterns at different space and time scales. It would be advantageous if, at some point, scientists could map the microbiology of the ocean.
- To be able to discern trends, the community needs time series information and data. To accomplish this task, an inventory is needed of all the available data and locations for which there is information (e.g., inventory available data, data basis, and archives). For example, DOE has information and metadata about microbes that are available on line.
- It is essential to use platforms of opportunity, in addition to existing platforms (e.g., NOAA and UNOL vessels), to increase sampling capabilities and develop new sensors to identify microbial communities and their roles in the ocean.
- NOAA needs to enhance its capabilities in genomics and other "-omics", develop and use forecasting and modeling as a mechanism to answer questions and link the studies to ecosystem functioning and diseases of marine living resources and human.
- Marine microbiologists need to improve their marketing approaches and explain clearly: Why marine microbe studies are important? Who cares? So what? It is essential that all of us emphasize the fact that our world is under the "microbes rule" and that microbialization of the ocean is well underway. Scientists must share the sense of urgency in understanding the role of microbes in disease and health as our environment is changing at a rapid rate.
- To complement this outreach, researchers need to work on education and awareness of the role of microbes. Developing a one-pager, such as "Did you Know?," would be a great way to better publicize the importance of this topic.
- NOAA could pick up/resurrect/and build upon a formal program that was initiated at NSF on "Microbial interaction and associated processes."

Recommendations for Near Term Activities: What can NOAA can do immediately?

- Use NOAA platforms for sampling: add geochemistry measurements (DOC, DNA, Nutrients such as NO_3--) and direct count of organisms to be used in health assessments;
- Inventory NOAA's existing capabilities with an eye toward adding marine microbe science components;
- Identify external partners capabilities and interests with regard to marine microbiology;
- Link to other data bases (e.g. DOE) and create a data access portal to be used in a regional pilot study (for example: enzyme discovery in the Gulf of Mexico);
- Work on the visualization of existing data (for example using Google Earth);
- Prepare a QA/QC for sampling and archiving protocols;
- Use remote sensing data/color data to assess microbes distribution;
- Prepare an RFP to identify core field data necessary needs for marine microbial studies (minimum data standards);
- Provide routine forecasts of vibrio species by region (e.g., Gulf of Mexico, Chesapeake Bay);
- Initiate pilot demonstrations based on users inputs;
- Establish connection with the Ocean and Human Health Initiative and the NOAA One Health Working Group.

Recommendations for Long-Term Activities: What could NOAA do or support in the next 5 to 10 years?

- Create a Marine Microbe Program in NOAA;
- Create a digital, virtual manual for the study of marine microbes;
- Work toward developing a technology that allows for the *in-situ* sequencing, such as a microchip similar to the coral reef microarray;
- Develop and maintain a well-established robust observing system for marine microbes;
- Develop a regional Earth model with location perspective model;
- Create a core facility for natural products derived from marine microbes that could be fee-based and would provide standardized set of tests. NOAA would need to identify what enzymes are of interest to the agency;
- Investigate the potential role of NODC in storing data on microbes and sequences for viruses and prokaryotes.

Partners

- NOAA should work collaboratively with academic institutions and with other agencies to develop a National Marine Microbe Program;
- In NOAA, partners include OAR, NOS, NMFS, and NESDIS, and we should investigate NWS' potential role;
- NOAA must rely consistently on academic institutions and Cooperative Institutes to complement and leverage capabilities;
- Collaboration with other agencies also interested in the topic is essential. These agencies include but are not limited to: NSF, NIST, DOE, DOI, DOD, and the Smithsonian;
- Private sector partners should be sought after in order to make faster progress. In particular biotechnology and pharmaceutical entities could be highly valuable partners.

APPENDIX 9 -- Post-Workshop NOAA Participants' Discussion Notes

Summary of Participants' Discussion: How to Move Ahead?
December 1, 2011

After the community workshop, NOAA participants met on December 1, 2012 for a half-day to capitalize on the ideas suggested by the workshop participants and to identify next steps for the NOAA group.

General Actions

- Prepare a report to summarize workshop results (10-to-15 pages, plus appendices);
- Share the report with the workshop participants and publicize it in NOAA and in the interagency arena;
- Establish a NOAA Marine Microbe [and Ecosystem Health] Working Group that would meet every two months to exchange information on what is being done within NOAA and influence future directions;
- NOAA needs to be involved in the genomic revolution but how? It is important to define how. How can NOAA exploit this revolution to accomplish its mission?;
- Identify programs that rely on knowledge of marine microbes results (e.g. stock assessment, marine mammal survey, Jeff Hyland's program, coral program, Coral Disease and Health Program (CDHP), NOS National Status and Trends Program, etc.);
- Revisit NOAA's role in biogeochemistry and elemental cycles and make sure that ecosystem priorities are in sync with microbe priorities;
- Connect with DOE to learn about their samples and samples archive;
- Consult OSTP documents on bio-economics describing where to invest in biosciences. Specifically, assess intersections between OSTP recommendations and NOAA's interests in marine microbes, their role(s) in the biogeochemical cycle and processes and their linkages with natural products;
- NOAA cannot do everything, and collaboration is essential. However, funds are needed to support both NOAA internal research and the external community;
- NOAA should initiate regional pilots and ecosystem assessments that examine the role of marine microbes in ecosystem function and incorporate the study of microbe communities' status and trends. Using existing examples where marine microbes have been found to have a role in oil degradation, beach closures and seafood safety, NOAA can demonstrate the benefits to the Nation's economy and employment rate associated with this type of research. NOAA should endeavor to continue, where possible, to highlight the linkage with jobs: we need to maintain ecosystem health to retain and create jobs;

- NOAA should use the Magnuson-Stevenson Act to tie fishing effort data and ocean health data. This linkage will facilitate environmental change predictions and forecasts. NOAA needs to include microbial data to develop these forecast and prediction models. The models need to be validated by *in-situ* measurements. Microbe science can not only help us with remediation and predicting/forecasting changes in the ecosystem at multiple scales, but it can also help with magnitude-of-impact diagnoses, in the case of both natural and man-made disasters;
- NOAA should emphasize the value of natural products extracted from marine microbes and the potential for bacteria and algae to be used to produce energy;
- Letters to each Line Office AA and the direct supervisor of each NOAA workshop participant should be crafted for Craig McLean's signature, thanking them for committing their very able staff to the workshop;
- Finally, a thank you letter should be sent to the external attendees in appreciation for their input and participation.

Specific Areas of Potential Investment for NOAA:

Sampling

- Workshop participants want NOAA to collect samples and archive them. NOAA needs to develop a strategic plan for large scale sampling preparation and collection. However, many NOAA ships cannot take water samples, at present. NOAA should establish a pilot project to start and then, if the pilot proves successful, endeavor to put a CTD on every ship;
- Identify and target on-going programs to append a microbial component(s), e.g., stock assessment field surveys. Marine mammal surveys, Jeff Hyland's program, coral survey programs and the Nation Status &Trend program are conducted every year, and it would be good to piggy back on those. Ecosystem priorities should be in sync with microbe priorities. We could take a fish of each species or coral mucus samples and freeze them for subsequent analysis;
- NOAA should organize and maintain a microbe sampling program;
- All NOAA groups (and others too?) collecting sediments could do water samples and analyze for microbes, in addition to contaminant concentrations;
- Identify ongoing sampling plans and operations within NOS, NMFS and OAR; inventory who does what in each NOAA LO/Labs;
- NOAA needs to augment on-going sampling programs with a microbial component. NOAA should produce a list of the possible programs to approach, e.g., those who have an existing program and could benefit from adding a microbial component;
- National Marine Fisheries Service should determine what topics are national in scope and go across all fisheries centers;
- There is a need to develop standard operating procedures (SOPs) or identify good existing SOPs, e.g., Environmental Protection Agency methods, for collecting and

storing samples, particularly viruses;

- Revisit the potential investment areas suggested by the workshop participants. In particular, focus on how to best handle the community sampling needs:
  - Samples: How to collect and store them? Where?
  - Develop a strategic plan for large-scale sampling, preparation and collection.

Biogeochemistry and cycles

- The microbial science community does not understand the details of biogeochemical processes and cycles, yet new transformation processes and cycles are being discovered quite often in the course of other studies. How should NOAA sample to capture these biogeochemical changes and to answer the right questions?
- The Interagency Coastal Condition Report needs to include a microbial component;
- NOAA needs to focus on how the microbial community is changing with changing environmental conditions. Scientists need to get ecosystem baselines and conduct monitoring to enable them to respond to specific questions. Microbiologists need to be able to get time-series data that will come from long-term monitoring. This will allow them to extract information on status and trends in the microbe realm. This long-term monitoring is the backbone of an assessment tool;
- The increase of O_2 depleted zones (O_2 minimum zones) is stressing the marine biota that end up with more diseases and habitat contraction, as can be seen in the Chesapeake Bay. More consideration should be given to understanding the mechanism(s) leading to hypoxic/anoxic zones.

Linkage to job creation

- OSTP has interest in bio-economy and where to invest in biosciences; thus, they are following with great interest progress and discovery in the bio-medical arena and natural products. It would be advantageous for NOAA to connect with DOE regarding potential new energy sources derived from or linked to bacteria and algae;
- Knowing more about marine microbes will help in the creation of new jobs in such fields as ecosystem health, energy production, environmental resource management, the economy. NOAA and the science community at-large need to know how to assign a value to healthy ecosystems, oil spills, beach closures and seafood safety, etc., and comprehend the influence of microbes in that regard.

Technology development

- The microbial science community needs to move forward with the development of a microarray and identify what genes need to be included. The Beaufort Lab has been developing this technology for a single toxin (semi quantitative analysis). The development of a microchip is essential to automated analysis in the marine environment. One of the issues, besides the price (about $100K), is the number of parameters to include in it;

- NOAA needs to send out a request-for-proposals/announcement of funding opportunity to get answers to specific technology questions;
- NOAA should support better external partnerships in the genomics arena and develop microarrays to solve specific questions and apply newer technologies to solve other questions;
- Establish a sequence center in NOAA (at SWFSC?). Presently some NOAA sequences are sent to Japan.

Budget possibilities

- In these difficult budgetary times, one of NOAA's great challenges is finding the funds necessary to perform the science required to advance our knowledge of marine microbes. The NOAA Marine Microbes Working Group could strengthen the NOAA SEE "program change" document by adding microbes to the biodiversity piece and the topic could be raised at the Ecosystem Challenge workshop;
- In the NOAA SEE process, each AA has an annual opportunity to ask for money for 5 additional topics. For FY14, Craig McLean or Bob Detrick could add microbes or biodiversity as one of OAR's topics. NOAA could focus its efforts on the most relevant scientific questions that were discussed at the end to the community marine microbe workshop. Using the predictive mission of NOAA, we could develop a request/program change summary for budget activities that will include creation of jobs and ecosystem services, public health and environmental health, development of models to help get a mechanistic understanding of biodiversity. Input from all the NOAA Line Offices is important for the SEE Process;
- In the National Marine Fisheries Service, there are at least 2 programs currently conducting microbial research on regional scale, and money is available within this NOAA Line Office. For OAR, the source of funding is different, and this Line Office needs to seek additional funding;
- NOAA cannot do everything that needs to be done on its own, so the agency needs to find and fund support from the external community.

Next steps

- Develop an exit poll for attendees that could include some/all of the following questions:
  - Can you identify 4-5 ways microbial input would be value-added to ecosystem research & management?
  - What 3 near-term and 3 long-term priority activities would you like to see NOAA to pursue?
  - If you had your wish, what samples and/or existing data sets would you find most useful?
  - What kind of sample, that you could process, do you wish for?
  - What else can NOAA offer to its external partners, besides samples? Data sets?
  - What sort of scale-up of sampling across the agency would you recommend?
  - What are you currently doing in the field and what assets are you using?
  - Are there samples, data sets you would be willing to contribute to long-term monitoring studies?
  - Within this context, please share your opinions about preferred sampling protocols, as well as storage protocols post-sampling.
  - What did you, as a workshop participant, come away with at the workshop's completion?
- Conduct an inventory of marine microbial science capacities/capabilities available external to NOAA, as well as inside NOAA;
- Given OSTP's focus on bio-economies, looking at where to invest in biological science funding, we should be diligent to make the economic arguments for why microbial science needs support, e.g., healthy food/dinner plate/commercial fisheries argument, beach/healthy coast/tourism argument, etc.;
- Engage the extramural community for the longer term via request-for-proposals and request-for-information mechanisms;
- Identify potential intersections with other Federal and State agencies as well as academia and private sector programs.