OCEANS AND HUMAN HEALTH

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ROUNDTABLE REPORT

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December 17-18, 2001
National Institute of Environmental Health Sciences
And
National Science Foundation

Research Triangle Park, North Carolina

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EXECUTIVE SUMMARY

On December 17-18, 2001, the National Institute of Environmental Health Sciences (NIEHS) and the National Science Foundation (NSF) co-hosted a scientific roundtable on oceans and human health (OHH). The two agencies invited experts in the fields of epidemiology, pharmacology, biological oceanography, geochemistry, and biochemistry to provide guidance to the agencies as they confirmed shared research interests. Based upon existing literature and past meetings, the roundtable was organized around three central research fields: Harmful Algal Blooms (HABs), Marine Pharmaceuticals, and Water- and Vector-borne Diseases. A session leader with expertise in the respective field provided a brief overview of the state of the science before leading a discussion that included the identification of research gaps. This document summarizes each of the three themes and the recommendations offered.

Harmful Algal Blooms (HABs)
Session Leader: Daniel Baden, University of North Carolina, Wilmington
HABs represent the most notorious marine hazard to humans and animals alike. It is estimated that over 60,000 individual cases and clusters of human intoxication occur annually in the US alone. Health effects in humans range from acute neurotoxic disorders to chronic and persistent diseases. Areas requiring further research include:

- Biosynthesis and function
  A complete matrix description of the families and classes of organisms that produce HABs is required. A description of the biochemical pathway(s) by which distinct organisms produce toxins is necessary.
- Extrapolated materials
  In order to precisely develop analytical detection methods and for all types of toxicological and transport studies, specifically labeled toxin derivatives are necessary.
- Exposure and effect assessment
  Toxins produce their effects at concentrations that approach limits of detection in many cases. Biomarkers of exposure are crucial for diagnosis, treatment, prognosis, and epidemiological evaluation of these toxins and their possible human health effects.
- Prevention and Control
  Cleanup and remediation require intervention in our foods, water, and air.

Water- and Vector-borne Diseases
Session Leader: Jed Fuhrman, University of Southern California
It has been estimated that human pathogens in the marine environment lead to significant health problems and annual losses of billions of dollars of income worldwide. Types of pathogens include bacteria, viruses, and protists. Survival and persistence of various pathogens is strongly influenced by environmental conditions. Within this field, additional studies may address:

- Detection and quantification
  New tests are needed to detect waterborne pathogens more quickly and efficiently.
  Interdisciplinary research is needed to examine the potential for global climate change to affect the spread of human diseases.

- Pathogenicity
  Use of genomics and proteomics to better understand pathogenicity. Characterization of factors controlling pathogen survival to enhance our understanding of the fates of pathogens released into the sea.
- Prevention
  Epidemiological studies are needed to evaluate risks of various pathogens. Rapid, accurate, and affordable tests are needed. Nearshore physical and geological oceanography can be applied to study the transport of pathogens.

Marine Pharmaceuticals
Session Leader: William Fenical, Scripps Institute of Oceanography
Multidisciplinary research is required to investigate marine species that potentially are a source for new drugs and to identify the marine environment that will support cultivation of these organisms. Areas requiring further investigation are:

- Marine biodiversity and organisms
  Multidisciplinary research and training are needed to increase our knowledge of marine organisms and their value for new therapies.
- Molecular mechanisms of natural marine toxins
  By understanding molecular mechanisms of toxins, researchers will be better equipped to develop drugs that can block the biological activity of a toxin. New methods for detecting toxins in seafood can also be developed.
- Techniques for culturing marine organisms
  New techniques for culturing such organisms are needed to fully understand their therapeutic potential.

In addition to providing summaries of these discussions, this meeting report contains a list of reading materials suggested by meeting participants. For more information on this meeting, please contact:

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CHAPTER 1

HARMFUL ALGAL BLOOMS

Session Leader:
Daniel Baden

Worldwide, harmful algal blooms cause a variety of acute, sub-acute, and chronic diseases in humans, as well as in other mammals, fish, and birds. Based on public health, commercial fishery, recreation and tourism, and monitoring and management costs, researchers estimate that the 15-year capitalized impact from HABs in the US to exceed $400,000,000. Health effects in humans range from acute neurotoxic disorders (such as saxitoxin, brevetoxin, and ciguatera toxin poisonings like paralytic shellfish poisoning, neurotoxic shellfish poisoning, and ciguatera finfish poisoning) to chronic and persistent diseases (such as amnesic shellfish poisoning and chronic liver disease caused by the cyanobacterial toxins, the microcystins). Disease caused by exposure to the environmental chemicals produced by harmful algal bloom organisms initiates with consumption of contaminated seafood or the inhalation of the toxins entrapped in sea spray. These are the two currently recognized mechanisms for environmental toxin exposure. The oral route of intoxication is by far the better understood and more commonly recognized, and coastal states all have public health surveillance and monitoring systems in place to prevent human intoxications. However, exposure to aerosolized particles in Florida red tide (and putative Pfiesteria outbreaks) is not uncommon and is an intoxication route which is much more difficult to quantify or control. It is conjectured, but unproven, that the mechanism of intoxication and death is the same for man and animal.

While readily recognized that HABs produce toxins that affect living systems, and for several prominent types of HAB poisonings the major toxins have been characterized, there are major gaps in our knowledge.

<u>Biosynthesis</u>
Although species that produce toxins responsible for amnesic shellfish poisoning, ciguatera fish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, and paralytic shellfish poisoning are known, it cannot be certain that these are the only species producing these toxins. Nor is it certain that these are the only toxins that cause ocean-related maladies. In fact, brevetoxin discovered in a previously undescribed species of *Chattonella* expands our brevetoxin-producing species into the *Raphidophytes*. So presently, toxic species are taxonomically classified into diatoms, dinoflagellates, blue-green algae (cyanobacteria), and raphidophytes. New toxin types such as the azaspirulides and cylindrospermopsin expand the number of distinct chemical entities in at least two areas. Additional toxins are known that are produced within marine vectors like shellfish, including the pectenotoxins and yessotoxin.

- A complete (and evolving) matrix description of the families and classes of organisms that produce HABs is required. What is the broad distribution of toxin-producing capability?
- Are there species/species interactions?
- A description of the biochemical pathway(s) by which distinct organisms produce toxins is necessary. Are pathways convergent, identical, unialgal, or more complex in derivation?

- Are there specific conditions, nutrient profiles, or physical parameters that support maximal toxin synthesis? Is toxin production constitutive or inducible?
- What is the nature of the ecology, genetics and enzymology that confer toxigenicity?

Extrapolated Materials or New Agents

Toxins as produced by HAB organisms are needed in high purity, spectroscopically characterized, in adequate supply, and of reproducible potency as standards. New toxic organisms in new areas represent new threats. In order to precisely develop analytical detection methods and for all types of toxicological and transport studies, specifically labeled toxin derivatives are necessary. Designer toxins, developed through organic manipulation, genetic manipulation of HAB genome, and metabolic manipulation through the use of designer substrates, hold potential for producing many new tools for research and diagnostic uses. In this regard, HAB research is in synergy with marine natural products work, and controlled synthesis through genetics or metabolism is promising.

- What types of derivatives might be useful for metabolism studies? For transvector characterization? For specific test development? For therapeutics?
- What types of tests are relevant? At what sensitivity? At what specificity? For what forms of the toxins?
- How can standards, derivatives, and tests be made readily available to investigators?
- Are there test batteries in academia or industry that can be employed to gain knowledge of the full range of effects those toxins, their metabolites, and derivatives possess?
- As new epizootics or environmental hazards become evident, what are the toxins that contribute to the events?

Exposure & Effect Assessment

Toxins produce their effects at concentrations that approach limits of detection in many cases. Principal in exposure assessment studies are developing a concept of what it is that should be measured. Biomarkers of exposure are crucial for diagnosis, treatment, prognosis, and epidemiological evaluation of these toxins and their possible human health effects. In general, the epidemiology of the harmful algal blooms has been poorly described, often dependent on case reports and case series associated with specific outbreaks. The marine toxin diseases in human and other populations are highly under reported (per CDC only 2 to 10% are actually reported). Only acute rather than chronic health effects have documented in most of these diseases. There has been almost no evaluation of chronic health effects in persons affected acutely with high doses of toxin, nor of persons exposed to chronic low levels of toxins. Health effects in sensitive subpopulations (such as children or persons with underlying neurological or immune disease) have not been assessed. Finally, once exposure has been assessed and perhaps quantified, therapeutic steps must be implemented to alleviate toxicity

- What types of samples are required to assess exposure? Amounts? Preparation?
- Can traditional clinical-type testing formats suffice for detection? Or, can association of ligand/receptor complexes or their result be developed into quantitative or qualitative biomarkers?
- Since exposure precedes disease, what types of agents detection is necessary in field situations to preclude exposure, and prevent disease? Can mass mortality events, physiological changes, or molecular lesions be used for monitoring?

- Can these exposure levels be correlated with sub-clinical health effects?
- Are there susceptible/resistant populations of man or animal?
- Are there specific molecular targets that can be exploited for assessment? Are all subtypes of receptors equally affected? What are the structure/activity relationships that govern interaction with molecular targets for each toxin type?
- Do toxins work in synergy or antagonism?
- At what level of toxin do chronic or acute effects become important, and under what suite of environmental conditions? Can acute poisonings result in chronic health effects
- Can health effects be mitigated and/or prevented? Are there antidotes or can they be developed? Can molecular therapeutics be addressed?

Biochemical Function and Potential Role in the Biosynthetic Species

The question of why aquatic microalgae produce such potent exocrines has provided for much discussion. Hypotheses ranging from the anthropomorphic "produced to kill" to just "pure serendipity" have been offered. The former hypothesis begs an explanation of justification and represents only one use, i.e., as "poisons"; the latter hypothesis is derived from a probability based on the number of unique essential receptors that exist in living systems and number of unique ligands that could potentially bind to those receptors---a certain number of pairing would be complementary just based on chance, and some of those binding events would be deleterious.

An additional explanation is a regulatory function in the progenitor that has a homologous regulatory potential in the exposed species. An example of this might be an enzymatic function in a dinoflagellate that regulates through an association/dissociation mechanism (the well recognized regulatory and catalytic subunits concept). The regulator in the system affects the same enzyme in higher organisms that have evolved a permanently condensed allosteric regulatory mechanism instead. Both mechanisms are highly efficient, and achieve the same result through different mechanisms. Placing an association/dissociation regulator (toxin) in the regulatory site of an allosteric enzyme is certain to result in improper modulation of the enzyme, and vice versa.

- Do toxins play a role in intermediary metabolism in progenitors? As regulators? Can these roles be used to extrapolate molecular mechanism of toxicity to intoxicated species?
- Are toxins sequestered in HAB organisms or packaged?
- Are toxins endocrines or exocrines?
- Can synthesis be regulated, stimulated, or halted?

Sentinel Organisms

In animal populations, a number of major epizootics have been documented in the last two decades, involving whales, manatees, bottlenose dolphins, sea turtles, sea lions, sea otters, finfish (e.g., tuna, menhaden, flounder), shellfish (e.g., softshell clams, blue crabs, bay scallops), and pelicans and other seabirds. HABs represent the most notorious marine hazard to humans and animals alike, and it is estimated that over 60,000 individual cases and clusters of human intoxication occur annually in the U.S alone. Sea creature deaths can encompass large percentages of local populations: in some cases the species affected is an endangered or protected species. These episodes, too, are apparently on the rise.

- Are there critical species for study (both accumulators and symptomatic species) as sentinels?

- Is the mechanism of human illness and animal malady the same or different?
- Can key therapeutic intervention be developed to broadly treat animals and humans afflicted with marine toxin illnesses?

Cleanup/Remediation/Control

Cleanup and remediation require intervention in foods, water and air. Presently, cleanup and remediation pertain to disposal of animal carcasses that exist after an epizootic HAB event, or foods that must be decontaminated by natural depuration (breakdown of toxins), or closure of areas that pose a danger to humans from toxins that remain for short periods in the water and overlying air until monitoring indicates that the affected areas are safe for human contact. Improved remediation/control techniques are needed for most harmful algal species.

- What types of cleanup are necessary? Do existing technologies suffice to address cleanup issues?
- What is the natural progression of HAB events? Do they exhibit toxic potential at all stages?
- Are there environmental parameters that can be altered to modulate HAB initiation/maintenance/dissolution?
- Are these HAB events predictable and preventable?
- Can oceanographic and biomedical technologies be coupled to produce smart sensors that detect developing blooms, together with relevant ecological and physical data?

The entire field of harmful algal bloom research has expanded in the past quarter century from a small group of investigators who met at the First International Conference in November of 1974. The Tenth International Conference will convene in October of 2002 (St Petersburg, FL). The number of active attendees is expected to exceed the attendance of the First Conference by over six-fold. Areas of expertise represent taxonomy, ecology, physiology, chemistry and biochemistry, oceanography, toxicology and pharmacology. Remarkable progress has been made in the general field over the past 25 years, and the first generation of students trained in "Harmful Algal Bloom" interdisciplinary research are assuming lead research roles in the field. The oceanographic and biomedical sciences have both been represented since HAB research inception. There is a general recognition that the field would not have progressed as rapidly as it had without the constant dialog between biomedical and marine scientists.

The questions the field will ask in the future will be many. They will be based in molecular sciences for both toxicological mechanism and therapeutic work. Genomics of harmful algal species should be developed for many applications, from improved systematics and molecular detection techniques to determination of the genes controlling toxin production, toward developing methods to suppress toxicity. Improved biomarkers for toxin exposure are critically needed for human epidemiology studies which – although fundamental in understanding chronic/sublethal and acute impacts of harmful algae – remains a pioneer area in this field. New technologies will be required to enhance detection in progenitors, exposed individuals, and food and air. Existing technologies will need to be refined to target detection of toxic strains and to quantify toxins in environmental samples, which should be developed for combined use with remote sampling techniques such as automated platforms, drifters, and fixed moorings.

CHAPTER 2

VECTOR - & WATER-BORNE DISEASES

Session Leader:
Jed Fuhrman

It has been estimated that human pathogens in the marine environment lead to significant health problems and annual losses of billions of dollars of income worldwide. Although it has been an area of research for decades and progress in several areas, there is still much we do not know about this topic. This outline covers basic information on the subject, with a focus on areas ripe for future research. The different aspects are (1) human exposure to vector and water-borne diseases from the ocean, (2) the types and sources of pathogens, (3) detection and quantification, (4) microbiological standard development, and (5) prevention.

The two main routes of human exposure to marine water-borne disease are (1) eating fish, especially shellfish, and (2) recreational contact, such as swimming or surfing. There is little question that both routes are the cause of illness in the United States and globally. Shellfish are of particular concern because they are filter-feeders, and efficiently remove extremely small particles from seawater. Some even feed directly on bacteria, but most target slightly larger algae and suspended detritus that may contain numerous attached bacteria and viruses. Also, many shellfish are eaten whole, including gills and stomach, and sometimes they are eaten raw. These both increase the chance of disease transmission. In recreational contact, illnesses can come from skin or wound infections, ear, sinus, and respiratory infections, or GI and systemic infections from swallowing seawater.

Types of pathogens include bacteria, viruses, and protists. Bacteria include native marine organisms (e.g. *Vibrio vulnificus, V. parahemolyticus*), but mostly those originating from humans and terrestrial animals. Because viruses tend to have a limited host range, human pathogenic viruses in seawater most probably come from human sources (primarily via fecal transmission, but possibly blood-borne via blood in the wastewater stream). Protists can include native forms, such as toxin-producing dinoflagellates (discussed under the topic of harmful algal blooms) and possibly also others like *Cryptosporidium* and *Giardia*.

Sources and Fates. The human- and terrestrial animal-derived pathogens in seawater primarily enter the sea from sewage and runoff, including agricultural runoff, although it is also possible to have transmission between swimmers (particularly at very crowded beaches). Sewage (usually treated in developed countries) enters the ocean via submerged outfalls and sometimes rivers, while runoff is rarely treated and enters via rivers or directly onto the shore. Raw sewage can enter the sea from runoff via leaking and aging sewers, improperly functioning septic systems, or locations where sewage treatment is lacking or inadequate. When pathogens are introduced into seawater, most are inactivated and broken down by a variety of natural processes, presumably of the same type that lead to the turnover of native marine bacteria, viruses, and protists. Native marine bacteria and viruses are extremely abundant, typically $>10^9$ bacteria and $>10^{10}$ viruses per liter, and they have natural turnover times of approximately a day. These native microbes are inactivated or removed by protists, sunlight, enzymes, adsorption to particles, and other processes, although all the details are not fully worked out and there is undoubtedly a great deal of variation in space and time. We know

relatively little about the fates of most pathogens in marine environments. A better understanding of the processes involving the native microbes will help to understand the general fates of pathogens released into the sea. A more focused understanding of the fates of particular pathogens would call for specific studies of target pathogens, or surrogates, in selected environments of interest. One relevant question is how pathogens may become attached to particles and sink to the sea floor, possibly to be resuspended later. Sediments may protect the pathogens and serve as a long-term reservoir. The various aspects of particle-pathogen interactions are also particularly relevant to under-standing how the pathogens may be taken up by shellfish or swimmers, and how they are transported.

Survival and persistence of various pathogens is strongly influenced by environmental conditions, and global change has the potential to alter significantly the existing patterns. For example, pathogens now largely limited to tropical areas are likely to move poleward under a general warming scenario. Therefore, the current pathogen types found at coastal U.S. cities may change in the near future, and organisms like pathogenic *Vibrio cholerae* may become a problem. It is also important to realize that microorganisms undergo gene transfer, including genes coding for virulence factors and antibiotic resistance. Therefore, relatively benign forms may become pathogenic and/or resistant to antibiotics, and pathogenic forms may become more prevalent. As we experience increased human population pressure in coastal areas and changes in environmental conditions, this may lead to an increased importance of marine-borne pathogens.

Detection of these pathogens can be done a few ways - cultivation, DNA or RNA detection, and immunological detection. None are fully satisfactory. This is an area where biotechnological advances have much impact. Cultivation is the most well-established method, but for pathogens like viruses it is too slow by itself to provide results meaningful for saying if shellfish are safe for an oyster bar or if a beach is safe for swimming (results can take >1 week). Even for bacteria, cultivation takes a day or more, during which time the contamination situation may change. Many pathogens are not readily culturable, or may enter a viable but not cultural state. Some methods are a hybrid, such as brief growth of viruses in tissue culture followed by rapid genetic detection. It is generally thought that the extremely low level of pathogens that may cause a health problem, compared to levels of native harmless organisms, calls for methods that involve amplification, through growth or PCR. PCR-based methods are being developed for some pathogens in shellfish and water samples, but these generally need further refinements and standardization before they are suitable for routine use. It may be possible to use direct detection if systems have suitable sensitivity and discrimination - perhaps something like atomic force microscopy coupled with immunological probes. No such systems are close to development at this time.

An important issue in this area is the use of “indicator”organisms, as opposed to direct detection of known pathogens. Monitoring currently is based on indicators. For example, fecal coliforms are used as an indicator of fecal contamination that may include bacterial or viral pathogens; coliphages are sometimes used as an indicator of fecal-derived viruses. Bacterial indicators that are most commonly used are total coliforms, fecal coliforms, and enterococci; others are sometimes used as well. Recent results suggest that that bacterial indicators are not necessarily suitable for viruses, and vice versa, because the biological and physical properties of viruses and bacteria are disparate enough to cause them to “go different ways” in the environment. Indicators permit more sensitive detection for the very reason that they may include harmless organisms from problematic sources.

When genetic methods are used to detect pathogens, such as enteroviruses or hepatitis A, the viral RNA may be considered as an indicator of viable viruses, because one does not know if the source virus was infective at the time of collection. Some indicators are much better than others. For example, "total coliforms" includes many naturally-occurring marine bacteria.

A topic of much interest is source identification. Can one tell if an indicator comes from a human source or an animal one? It is particularly important when considering that some pathogens have a wide host range (some bacteria) while others have a narrow one (e.g. most viruses). When setting standards, one would expect to have a greater tolerance for, say, fecal contamination from birds than from humans. However, the most commonly used current indicators are bacteria that can come from any warm-blooded animal, and there is a widespread concern (particularly among people losing money from having their fishing area or beaches closed) that some high bacterial readings are due to "natural" animal-based contamination from birds rather than from sewage contamination. Some recent approaches, typically called "bacterial fingerprinting," are being used experimentally in research studies in an attempt to characterize sources. The idea is to identify strains or species of bacteria characteristic of certain sources. Some of these approaches require culturing the bacteria and testing individual clones (e.g. antibiotic sensitivity patterns, pulsed field gel electrophoresis), while others examine mixed natural samples directly (e.g. terminal restriction fragment length polymorphism). Further development and verification of such approaches is called for. Related to this question, the finding of human pathogenic viruses, like hepatitis A, is direct evidence of a human source of contamination. Several kinds of viral pathogens are now detectable by genetic methods (enteroviruses, hepatitis A, rotaviruses, adenoviruses, astroviruses, etc.), and some effort is needed to assess whether the detection systems are seeing only human versions of these viruses, because there may be closely related viruses that infect animals yet cross-react in the assays.

Related to this source identification is the question of pinpointing locations of contamination. When a beach or fishing area is contaminated, can one use biotechnological techniques to track the contamination back to its source? Often this may be a matter of extensive sampling and good identification of the indicators.

A problem in this field is the large volumes of seawater required for detection of pathogenic viruses by currently used techniques. Typically, tens or hundreds of liters are needed. This makes many kinds of studies impractical, and also makes routine monitoring of beaches a difficult proposition. It would be a big advantage if methods required a few liters or less.

Quantification. Practical application of these measurements requires that they are quantitative, but this is very difficult. Even for something that seems straightforward like counting indicator bacteria, it turns out that recovery of viable cells is highly dependent on the condition of the bacteria at the time of collection. Sunlight damage to these cells (which may be repaired by the bacteria), or co-occurring pollutants, can cause highly variable recovery. Genetic methods, such as PCR tests, involve collection and extraction steps that can have highly variable recovery of viruses or bacteria, and "messy" natural samples usually include substances that can inhibit genetic amplification. Some of these variables can be corrected for, such as with internal standards in quantitative PCR. However, variable collection and extraction efficiency is harder to deal with, and more work is needed to improve these steps.

There are definite trade-offs in detection and quantification. The easiest and least expensive methods, like cultivation of indicator bacteria, are not the fastest nor most sensitive, but their low cost permits covering many locations and frequent sampling. Methods that identify indicators precisely may be too slow to assist management of recreational waters. However, it seems possible to develop relatively inexpensive biotechnological methods that are both fast and practical. The ideal system would be portable for field use and yield results in a matter of minutes or at most an hour. One could imagine the use of automated moored versions of such systems to monitor sites and radio the results to users, avoiding the need for manual sampling and analysis.

Along these lines, some of the systems being developed (commercially and by the military) to detect bioterrorism may turn out to be suitable, or modified without a great deal of effort, for marine applications. With a large market, such as shellfish testing labs and water quality agencies, prices of such systems may become reasonable.

Setting Standards. Modern techniques permit exquisitely sensitive detection of pathogens and indicators, meaning that even relatively harmless levels of contamination can be detected. What is an acceptable level of hepatitis A or rotaviruses in shellfish or at a beach? As new methods come on line to detect possible hazards, standards must be set to permit their use by managers of the resource. This is one of the more difficult issues in this area. Epidemiology is one obvious way to develop standards. Good quantitative methods are needed first.

Prevention of problems. How can water borne marine diseases be prevented? There are a few obvious ways. Education is important, such as letting the public know where and when not to fish or swim, or explaining of the risks of raw shellfish. Testing of shellfish and recreational beaches needs to be comprehensive, with good standards and enforcement. Along the lines of developing infrastructure, improved treatment of wastewater, diversion of storm waters to a treatment facility, and proper placement of outflows may prevent contaminating locations with high human exposure. This is mostly an engineering problem, but its solution requires coordination with environmental and health scientists as well as physical oceanographers. There is also the possibility of disinfection of shellfish or runoff, e.g. with radiation. It is important to coordinate any changes in treatment with the relevant monitoring and detection methods. Testing procedures may need to be modified to accommodate any such treatment; e.g. radiation may inactivate viruses or bacteria but not destroy their RNA, DNA or surface antigens, so genetic or immunological tests may become unsuitable.

Specific goals for research under this initiative may include:

- Learning what are the marine waterborne human pathogens, including emerging and unculturable ones. What are the illnesses? Are many unknown? However, without needing to identify them all, a priority list of currently-known pathogens can be selected for immediate research.

- Use of genomics and proteomics in order to better understand pathogenicity and survival of marine-borne pathogens and to target genetic and immunological detection approaches (for this initiative, focus new efforts on important pathogens unique or special to marine habitats, as most marine-borne pathogens are transmitted more often by non-marine mechanisms and fall under other research programs)

- Development of rapid, accurate, affordable tests, requiring relatively small sample volumes, to detect and quantify the various pathogens in water and shellfish

- Characterization of factors controlling pathogen survival in seawater, and spatial/temporal patterns of dispersal, transport, and partitioning into suspended particles and sediments.

- Assessment of indicators vs. direct pathogen detection, bacterial and viral

- Survey animal viruses for relatives of human marine-borne pathogens, to avoid false positives in genetic or immunological tests.

- Epidemiological studies to evaluate risks of the various pathogens (and/or connection to indicators)

- Evaluation of alternative wastewater or runoff treatment or disinfection methods under realistic conditions

- Application of near shore physical and geological oceanography to understand transport of pathogens where human exposure occurs, for evaluating problems with existing systems, and to assist in siting and design of new or replacement sewage and runoff outfalls.

- Evaluation environmental conditions regulating native marine organisms that are related to human disease.

- -Evaluation/prediction of effects of global change on the distribution of various pathogens.

CHAPTER 3

MARINE PHARMACEUTICALS

Session Leader:
William Fenical

Marine Drug Discovery: Examining a Major Biomedical Resource

Nature has traditionally provided the chemical diversity required for the discovery of new drugs. Early civilizations soon learned that their botanically-rich environments could be used as a source of natural medicines. This successful activity, still in use today in much of the developing world, provided the foundation for the modern pharmaceutical industry. Early products such as morphine, aspirin, and quinine, underscored the fantastic array of natural medicines found in the diversity of life where we live. Historically, the proximity to plant diversity facilitated the discovery of a complex "materia medica," a treasure chest of natural drug formulas that continue to be used today. Terrestrial plant sources continue to be a significant resource for the discovery of new drugs, in particular anticancer drugs, Taxol® being a prime example. Although the diversity of land-based plants and animals is high, it is interesting to note that the oceans are, by far, our most biodiverse resource. On land, 17 of the approximately 34 Phyla (the fundamental division of life) exist, while the oceans contain 32 of the 34 Phyla. Marine biodiversity is immense, however because the oceans are more difficult to explore, this biomedical resource was not examined until the 1970's. The development of safe underwater breathing devices, and "in the ocean" sampling technologies, allowed the scientific community to examine the enormous biodiversity of coral reefs throughout the world's oceans. In the beginning, the goal of these early explorations was to answer a simple question: Do the plants and animals in the sea produce diverse chemical compounds that can be used to develop drugs? Within ten years of study, the answer was clearly, yes. Marine organisms evolved in a completely separate environment, an aqueous milieu in which competition for resources was intense. Chemists uncovered a fantastic array of novel chemical compounds, presumably used as defensive agents, which had not been observed before (Faulkner, 2001). These compounds were not only chemically unique, but they were produced by biosynthetic processes that had not been previously observed. Some of the simple elements of seawater, the halogens bromine chlorine and iodine, for example, were incorporated into elaborate natural compounds. It became clear that marine life was genetically distinct from life on land, and that these genetic differences were also expressed by the unique chemical compounds they produce. Indeed, the vast biomedical potential of the marine environment began to be understood, and small academic programs to discover drugs began to be established in the mid 1980's.

Efforts to discover new marine drugs began slowly. The National Cancer Institute, part of the US National Institutes of Health, in many ways led the exploration of the oceans in their quest to find new drugs to treat cancer. The US Department of Commerce's Sea Grant Program, although small in magnitude, funded some of the most basic of research, which was particularly important in setting the stage for success today. Industry, however, was slow to follow as they viewed this source as outside of their capabilities. Thus, academic researchers began the difficult job of searching the world's oceans to find new medicines. Progress has understandably been slow, since the challenges of developing drugs for the 21st century are far more demanding than those realized in these programs during the last 60 years. Nonetheless, major advances are now being made in illustrating

that the marine environment is a new frontier in drug discovery. At this time, there are at least six new marine-derived anticancer agents being developed with the assistance of the National Cancer Institute. One such example is the unique molecule Bryostatin-1, a potent anticancer agent discovered in chemical studies of the bryozoan (moss animal) *Bugula neritina. B. neritina* is a common marine invertebrate found most frequently fouling ship bottoms and pier pilings. Bryostatin-1 is a potent inhibitor of cancer cell growth, but this compound is also an effective activator of the immune system. Bryostatin-1, which is currently in clinical trials for various types of cancer, may find considerable utility in treating this difficult disease. Another recent example is Ecteinascidin-743, a potent anticancer agent extracted from the Caribbean ascidian (sea squirt) *Ecteinascidia turbinata.* In clinical trials, Ecteinascidin-743 is showing excellent results in the treatment of some of the most difficult cancers. Other examples of compounds in development include the anticancer agents dolastatin-10, spongistatin, discodermolide, and many others.

In other cases, sustainable products have already been developed from coral reef organisms. One example is a new anti-inflammatory product from the soft-coral *Pseudopterogorgia elisabethae,* an animal, which occupies selected areas of the coral reefs in the Bahamas Islands. This animal produces the Pseudopterosins, a class of anti-inflammatory agents that have found commercial application in skin creams. The yearly requirement for this animal is in excess of 2,000 Kg, an amount, which if simply harvested would quickly decimate the populations of this animal. Through a careful study of the reproduction and regrowth of *P. elisabethae*, researchers discovered that natural populations of the soft-coral, when carefully "pruned," would fully regrow in less than 18 months. With the goal of creating a sustainable resource, a program was created in the Bahamas Islands to manage the coral reefs in proximity of Grand Bahama Island. This successful program, now in its tenth year, has convincingly demonstrated that coral reef organisms represent a significant biomedical resource when harvesting is carefully scientifically managed and controlled. This program can only be successful, however, if the quality of the coastal ocean in the Bahamas is maintained. Without the natural balance of these regions intact, it is clear that biodiversity will diminish and our access to a diversity of natural resources will no longer be available.

Have the effects of a diminishing biodiversity in the oceans been observed? Without question, the answer is yes. During an exploration of the marine invertebrates in the Central Philippines, a unique ascidian, identified as a *Diazona* sp., was collected and examined as part of an NCI project. This animal was shown to contain an exciting new class of anticancer agents called the Diazonamides. These compounds are potent inhibitors of cancer cell growth and they appear to act on cancer cells by an entirely new mechanism of action. This exciting preliminary discovery was quickly followed up, but the animal was nowhere to be found for recollection. Either this was a very rare species or there were human influences disturbing the diversity of Philippine coral reefs. Three expeditions to recollect this animal were unsuccessful despite returning to the exact location of the initial study. This disturbing result virtually destroyed the development of the Diazonamides as anticancer drugs. Although small collections were found seven years later, there remained insufficient amounts of the Diazonamides for development. Synthetic chemists, world wide, have been attempting to synthesize these compounds, but to date none have been successful. Specific organisms, once collected, may no longer exist even within short periods of time. Regional oceanic areas, especially coastal regions and coral reefs, can experience rapid changes due to the activities of humankind.

The success in discovering these new potential drugs was based upon the high diversity of life, including many rare species, in the world's oceans. Programs to discover treatments for cancer and other diseases encompass literally thousands of species that are first collected in small amounts, tested, and then re-collected in sufficient amounts for development. This process obviously requires access to regions of high biodiversity and an unchanging marine environment, conditions less likely to exist as the activities of humankind create negative effects on the world's oceans. It is now frequent that biodiversity is being observed to diminish.

Despite these difficulties, many of the resources in the world's oceans continue to be robust and diverse, and to support the discovery of new pharmaceuticals. Very often marine derived compounds possess unique biological properties that render these compounds of use in basic biology. These compounds, often called molecular probes, are may even be more important than new drugs. Discovery of the potent marine toxin Tetrodotoxin led to a much more refined understanding of the receptors for human pain. Similarly, the Red Tide toxin Okaidaic Acid and the sponge metabolite Illimaquinone are now in use to probe basic cellular processes. Since the 1970's, studies have focused on macroscopic plants and animals, and in particular the high diversity of life found on coral reefs. This continues today with productive studies demonstrating the value of these marine resources. However, we are now realizing that the oceans are also a fantastic source for microorganisms, relatives of the terrestrial fungi and bacteria that since the 1940's led to the development of over 120 drugs for the treatment of infectious diseases and cancer. Ordinary seawater contains up to one million microorganisms per drop! These microbes are so unusual, so biologically diverse, that we are currently struggling to isolate them, understand their roles in the ecology of the ocean, and place them in culture. Seawater is one source, but the surfaces and internal tissues of plants and animals provide specific homes for an incredible diversity of microscopic life in the process called symbiosis (living together). The roles of symbiotic microorganisms in protecting their hosts and in the production of a wide array of bioactive compounds is just now being investigated. Similarly, the bottom of the world's oceans, like the soil on land, provide a sink in which organic matter must ultimately settle. Because of this, marine sediments are home to a diversity of microbial life. The identities of these microorganisms and their roles in the ocean remain to be fully understood. In the 1950's, microbiologists believed that marine microorganisms were essentially unculturable. This view, based upon the fact that classical Pasteur-style methods failed, cannot be accepted today. What is clear is that old methods, essentially fashioned after the chemical composition of the human body, do not apply to a saline, aqueous environment in which microorganisms evolved in a separate and specific process. Today, as a result of studies analyzing DNA directly extracted from seawater, we know that a fantastic diversity of life exists there. Yet, we still lack the insight and understanding to effectively culture the majority of these unique microbes. Likewise, the microbes associated with marine plants and animals are poorly known and appear to possess nutritional requirements, which have made culture difficult. These microorganisms are largely oligotrophic (adapted to very low and possibly unknown nutrients) and clearly live in an environment difficult to reproduce under laboratory conditions. Given the size of the world's oceans, their geographic and climatic variations, and the fact that they are three dimensional, leads to the conclusion that marine microbial diversity must be immense.

How do we begin to access the microbial diversity of the sea? This question will increase in importance as the potential of marine microbial drug discovery becomes more fully realized. Certainly, the answer lies in an orchestrated analysis of the nutritional requirements of marine

bacteria and fungi. We need to use every method available, including molecular probes and direct DNA extraction to catalog the genetic diversity present. But, just having knowledge of diversity does not lead to the use of these microbes for the production of new drugs. Culture studies must be undertaken which involve creative use of marine nutrients and novel isolation methods. Access to even the most remote of ocean environments must be made feasible, and most importantly, the value of this undeveloped resource must be appreciated and conserved.

Over the past five years, the field of marine microbial drug discovery has been established as one of the most exciting frontiers in natural drug discovery. Over 100 papers each year demonstrate the discovery of new chemical compounds with impressive biological activities. The actinomycetes are a class of filamentous bacteria, common in the soil, that are recognized as arguably the most prolific source of new drugs every discovered. While originally found in the soil in the early 1900's, recent findings show that entirely new groups of actinomycetes are found in marine sediments. One group, the *Salinospora*, which was discovered in 2001, is now recognized to produce a large diversity of bioactive compounds likely to yield new antibiotics and anticancer drugs. One such example is a *Salinospora* strain CNB-384, which was found to produce Salinosporamide A, a potent and highly selective inhibitor of cancer cell growth.

Clearly, in the overall sense, the world's oceans are a storehouse of undiscovered new drugs and new products of many types including enzymes, agrochemicals, and gene products. It is our last major natural resource; we need to realize its importance and plan for the future.

<u>Research Needs & Future Needs in Marine Pharmaceuticals Discovery</u>
Studies in marine pharmaceuticals research are most effective when they involve collaborative programs that integrate marine natural products chemistry with broad, therapeutically justified pharmacological screening activities. Effective programs should be responsive to new frontiers in marine pharmaceutical discovery that may include, but not be limited to, the following areas of (unprioritized) need:

- Studies of plants and animals in less favorable environments (polar oceans, deep ocean environments, etc.).

- Studies of uncollectible, but culturable plants and animals from diverse, and underinvestigated taxonomic groups.

- Phylogenetic analyses to confidently define invertebrate taxa, at the species level, thus facilitating studies of rare and poorly described species.

- Applications of new, innovative bioassays to even well studied plants and animals.

- The development and utilization of new approaches in marine cell culture, aquaculture and biochemical methods for the production of marine pharmaceuticals.

- The innovative application of novel sampling methods to access marine organisms for biomedical investigation.

- The applications of molecular genetics to isolate and express biosynthetic genes and to express these pathways in expression hosts for the production of marine pharmaceuticals.

- The broad-scale development of marine microorganisms as a novel resource for the discovery of new pharmaceuticals by investment in understanding the basic microbiology of the oceans.

- The development of new technologies to facilitate the saline cultivation and large-scale fermentation of marine microorganisms.

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ROUNDTABLE AGENDA

December 17-18, 2001 National Institute of Environmental Health Sciences

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Monday, December 17, 2001		**Conference Room 101-A**
8:30 a.m.	Welcome & Introductions	Dr. Kenneth Olden, NIEHS Dr. Anne Sassaman, NIEHS
9:00 a.m.	NSF Introduction	Dr. Don Rice, NSF
9:05 a.m.	Charge to participants NSF/NIEHS vision	Dr. Don Rice, NSF Dr. Allen Dearry, NIEHS
9:15 a.m.	Vector- & Water-Borne Diseases A. Introduction (15 minutes) B. Discussion (2 hours) C. Summary (15 minutes)	Moderator: Dr. Jed Fuhrman, Univ. Southern California
12:00 p.m.	Lunch	Cafeteria
1:00 p.m.	Harmful Algal Blooms A. Introduction (15 minutes) B. Discussion (2 hours) C. Summary (15 minutes)	Moderator: Dr. Dan Baden, UNC-Wilmington
3:45 p.m.	Marine Pharmaceuticals A. Introduction (15 minutes) B. Discussion (1 hour)	Moderator: Dr. William Fenical, Scripps Inst. of Oceanography
5:00 p.m.	Adjourn	
6:30 p.m.	Dinner	DoubleTree

Tuesday, December 18, 2001 **Conference Room 101-B**

8:30 a.m.	Marine Pharmaceuticals	Moderator: Dr. William Fenical, Scripps Inst. of Oceanography
	A. Discussion (1 hour) B. Summary (15 minutes)	
9:30 a.m.	Other Issues	Open Discussion
	There are many other scientific areas of importance related to oceans and human health beyond the three fields identified for the purposes of this meeting. The goal of this hour is to highlight and discuss briefly these other key topics relevant to research priorities of NSF and NIEHS.	
10:30 a.m.	Break	
10:45 a.m.	Session Summaries	Session Leaders
	A. Vector/Water-borne Diseases B. HABs C. Pharmaceuticals	(15 minutes) (15 minutes) (15 minutes)
11:45 a.m.	Wrap Up	Dr. Allen Dearry, NIEHS
12:00 p.m.	Adjourn	
12:00 p.m.	**Session leader discussion with agency staff regarding report development**	
2:00 p.m.	Adjourn	

SUGGESTED READING LIST

GENERAL:

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