OCEAN LIFE INSTITUTE Report Spring 2005

Message from OLI Director Larry Madin

Earth is defined by its ocean, where life on our planet emerged and still sustains its

greatest diversity and abundance. Understanding how life functions in the ocean is crucial to the health of modern human society and to the stability of global ecosystems. While the ocean once seemed an inexhaustible resource, we now realize that its capacity for lifesupport is finite. Now our task is to better comprehend its complexities in an effort to help maintain it.

The Ocean Life Institute (OLI) seeks to foster novel and interdisciplinary research in ocean biology and promote its application to solving pressing biological and ecological problems. Working under three broad themes – *Discovering Life*, *Sustaining Ecosystems*, and *Developing Tools* – OLI uses privatesector funds to support research projects and initiatives; fellows, postdoctoral scholars and graduate students; workshops, symposia and conferences.

Research Projects

OLI research covers a wide range of topics, from deciphering microbial genomes to listening to the heartbeat of whales. Three stories of exciting new research by OLI-funded scientists and students are highlighted in this report.

Graduate student Sheri Simmons and her advisor Katrina Edwards are investigating tiny living magnets – bacteria from Woods Hole waters that move through their muddy habitat following internal compasses. This research seeks to explain how this unusual ability relates to the physiological adaptation of the bacteria, and perhaps to shed light on how other animals might navigate by Earth's magnetic field.

Tiny photosynthetic cells in the water are the target of work by Rob Olson, Heidi Sosik and Scott Gallager, who describe new underwater devices to count and identify phytoplankton cells automatically, using both optical properties and mugshots. These detailed observations help us understand food production in the coastal ocean and detect the onset of red tides.

Man-made organic chemicals like pesticides can be dangerous pollutants in the ocean, but Mark Hahn and Chris Reddy have found that similar compounds also occur naturally. Distinguishing among them with sophisticated chemical analyses is essential to understanding how animals have evolved to respond to toxins and why some chemicals are more toxic than others.

Other OLI Activities

Broader in scope than individual projects, OLI *Research Initiatives* focus effort on well-defined and urgent problems. The North

Atlantic Right Whale Research and Conservation Initiative is a program of timely and practical research to help save this highly endangered species. In addition, the Coral Reef Connectivity and Conservation Initiative will join innovative WHOI science with international efforts to restore reef fish populations in the Caribbean.

Scientists supported by OLI have also begun research this year at the Liquid Jungle Laboratory, a tropical field station in Panama, where WHOI is a partner.

Important components of our outreach efforts include the discussion, communication and application of OLI research. In 2004, we sponsored a symposium on ecological impacts and future regulation of deep-sea fisheries, a workshop on the demographics of endangered albatross species, and a workshop on the evolution of deep-sea bacteria (co-sponsored with the Deep Ocean Exploration Institute). continued . . .

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In 2005, OLI will expand its work in the areas of tropical biology and conservation science, as well as continue its commitment to basic exploration and technology development. Although basic research is incremental, small discoveries can lead to great progress, and one of the goals of OLI is to provide a strong scientific basis for better conservation and management of the ocean.

Financial Information

Fundraising Campaign

Four Ocean Institutes were established by WHOI in 2000 to encourage early-stage, highpotential research—the kind of risk-taking work unlikely to receive government funding. This challenge called for a new funding paradigm, a form of venture philanthropy that could consistently leverage subsequent federal funding.

Woods Hole Oceanographic Institution

The goal of our comprehensive fundraising campaign is to raise enough funds to fully endow each of the four Ocean Institutes and our Access to the Sea program, so that high-promise science can be routinely supported at WHOI. Our total campaign target is \$200 million. As of December 31, 2004, we raised \$115,011,235.

Leveraging

OLI operates primarily on funds from private sources raised during the campaign in the form of endowment income and cur-

> rent-year gifts. This private support allows scientists the freedom and flexibility to try new ideas and take chances on research that could eventually, with proofof-concept, be funded by the federal government.

> Institute support has already led to large federally-funded programs at substantially higher levels than our original investment. For example, OLI support enabled Fellows Darlene Ketten, Heidi Sosik, Simon Thorrold, and Ken Buesseler to develop programs that were later funded for over \$3.5 million in total, a nearly ten-fold return

on the OLI investment in their science.

Even small sums can contribute to important results. A

This research into the fundamental characteristics and mechanisms of ocean life is essential if we are to understand and sustain our life-giving ocean in the future.

few thousand OLI dollars allowed Scott Gallager to test instruments that became the centerpiece of a \$1.2 million grant for deployment of an undersea observatory in the Antarctic.

Allocation of OLI Funds

OLI's Institute Advisory Committee determines which projects and personnel are funded each year through an internal peer-review process.

Between 2001 and 2004, OLI supported 7 fellows, 3 postdocs, 2 graduate students, 18 large research projects, 9 workshops, seminars or talks, and gave out 26 small discretionary grants for use in travel, equipment needs, etc.

This investment in the work of OLI totaled over \$2.5 million in the four-year period. None of this would have been possible without the vital support of our many donors.

Science Highlights

The Magnetotactic Bacteria of Woods Hole

Sheri Simmons

MIT/WHOI Joint Program in Biological Oceanography and **Katrina Edwards** Marine Chemistry and Geochemistry Department, WHOI

The microbes of Woods Hole, Massachusetts, are among the best studied in the world, and some of the strangest are undoubtedly those known as the magnetotactic bacteria. In a WHOI lab in the early 1970's, Richard Blakemore first watched tiny spherical bacteria (cocci) from Eel Pond mud swim persistently to one side of a drop of water and stay there. When he brought a bar magnet near the drop, to his great surprise, the cocci all swam toward the magnet's south pole (equivalent to Earth's north pole). When he turned the magnet around, they all swam in the opposite direction.

Since then, others have observed magnetotactic bacteria of all shapes and sizes around the globe, always in environments where oxygen is low or nonexistent. We now know that these bacteria contain chains of precisely shaped magnetic iron minerals and are, in effect, swimming compass needles. But why does this behavior exist?

Until very recently, it was widely thought that magnetotactic bacteria in the Northern Hemisphere always swam northwards when exposed to high oxygen (and vice versa in the Southern Hemisphere). Because of the way Earth's geomagnetic field is oriented, these responses would lead them deeper into the sediments and their preferred anoxic (or oxygen-free) habitat.

Our recent studies of magnetotactic bacteria dramatically contradict this explanation. We have found abundant bacteria in Salt Pond (in Falmouth) that swim towards geomagnetic south when exposed to high oxygen levels. This unexpected behavior will require a complete rethinking of why the bacteria orient to magnetic fields, so we are actively studying these "south-seekers."

We have also made significant advances in studying the ecology of the Salt Pond magnetotactic bacteria. In the summertime, Salt Pond is like a marine layer cake, with oxygenated water overlying a deep layer high in hydrogen sulfide. Interestingly, magnetotactic bacteria are most abundant at the interface between these layers.

We have been using a new and highly sensitive hybridization technique (called CARD-FISH) to determine and confirm the

identity of several unique types of magnetotactic bacteria in Salt Pond. These include a highly unusual chain-forming bacterium, which also happens to be a "south-seeker," and a large rodshaped cell, which is the first magnetotactic bacterium identified in the gamma subdivision of the Proteobacteria, one of the largest bacterial groups.

The size and proximity of Salt Pond make it an ideal environment for us to work out exactly how water chemistry determines the abundance and distribution of magnetotactic bacteria. This knowledge will help scientists use magnetotactic bacteria as "recorders" of the early Earth environment and perhaps even indicators of life on other planets.

Magnetic compass senses appear to be important to many other organisms, including fish and birds that migrate long distances. These bacteria may help scientists understand how this magnetic orientation operates at the most fundamental level. So the magnetotactic bacteria of Woods Hole are, once again, pointing the way to future discovery.

> Light microscope images of magnetotactic bacteria from Salt Pond in Falmouth, Mass. Left, "many-celled magnetotactic prokaryote" (MMP); and right, the "barbell," a unique chainforming bacterium we discovered in Salt Pond. The rightmost image shows the bacteria collecting on the magnetic south side, and the left image shows bacteria collected on the magnetic north side of a drop of water.

Submersible "Imagingin-Flow" Instruments to Monitor Phytoplankton in the Ocean

Robert Olson, **Heidi Sosik**, and **Scott Gallager** Biology Department

Ecosystems in the sea are just as complex and productive as those on land, but much more difficult to study. Reaching most ocean environments is expensive and time-consuming. Despite the visibility of large animals like whales, we know that the vast majority of the biomass in the oceans is composed of extremely small organisms that cannot be easily observed. These include thousands of species of small or weakly swimming organisms (the plankton) that drift with the currents. The smallest planktonic cells are less than a millionth of a meter in diameter but can occur at concentrations of up to a billion cells per liter.

Within this community, photosynthetic cells (the phytoplankton) produce nearly half of the Earth's oxygen and nearly all of the food for planktonic animals (zooplankton), which, in turn, feed higher trophic levels such as fish. The species composition of the plankton community affects higher trophic levels (including the fish available for human consumption), and phenomena such as harmful algal blooms are caused by particular species of plankton, so it is vital to be able to identify individual species in the marine environment and understand how their populations are regulated.

Although we are beginning to appreciate the potential effects of climate change and human activities (such as increasing temperature and nutrient supply) on marine community structure, a fundamental understanding of the complex interaction between physical and biological factors rests on detailed and sustained observations.

Only now is it becoming possible to acquire these critical observations. With funding from OLI and other internal sources, our research group at WHOI developed and deployed specialized instruments that can continuously monitor individual organisms in the plankton's native environment.

We can now measure and count the smallest phytoplankton cells using a submersible flow cytometer (FlowCytobot), in which optical properties of individual suspended cells are recorded as they pass through a focused laser beam. However, FlowCytobot cannot sample much larger cells, and these often dominate the phytoplankton in coastal waters.

These larger cells (below) often have recognizable shapes, so

we have developed a second submersible flow cytometer that incorporates an imaging system (Imaging FlowCytobot) that actually takes pictures of the cells. In the future we will use advanced image-processing techniques to make cell identifications based on shape and other cell features.

Both instruments are designed to operate unattended for months at a time. They are connected to the shore laboratory by power and data cables, so we can monitor results and modify sampling procedures from our lab.

FlowCytobot has been deployed at the Martha's Vineyard Coastal Observatory for the past two years, sampling continuously and sending data back to shore. Imaging FlowCytobot is undergoing field tests now in Woods Hole Harbor, and we plan to deploy it alongside FlowCytobot in 2006. With these combined approaches, we will be able to observe changes in phytoplankton community structure over a wide range of cell sizes and types, and elucidate the processes and interactions that control the life cycles of individual species.

Selected images of phytoplankton cells analyzed by Imaging FlowCytobot.

Sources and Biological Activity of Natural and Industrial Organohalogen Compounds in the **Oceans**

Mark Hahn Biology Department and **Chris Reddy** Marine Chemistry and Geochemistry Department

Halogenated organic hydrocarbons such as polychlorinated biphenyls (PCBs) and the pesticide DDT are well-known contaminants of marine environments. These compounds were synthesized during the past century and were originally considered wonder chemicals because of their chemical stability (PCBs) and pesticide activity (DDTs). Unfortunately, both groups of compounds displayed undesirable biological activity (toxicity) and this, in combination with their persistence, global distribution, and bioaccumulation into organisms, led to successful efforts to phase out their use in most countries.

Despite the fact that PCBs and DDT are no longer produced, and even as the environmental concentrations of these compounds decline, the concentrations of new classes of halogenated pollutants are increasing. These so-called "emerging contaminants" include the brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), which are currently in production and use around the world. Like PCBs and DDT, PBDEs are lipophilic (fat loving)

and metabolically resistant, making them long-lived and bioaccumulative. Their content in human milk has increased dramatically during the past 20 years, and they may be the "new PCB problem."

6-MeO-BDE-47

This compound is produced naturally by one sponge in the Indian Ocean but found in much higher latitudes in a variety of animal tissues.

Confounding this increase in the PBDEs in human and animal tissues is another class of compounds that are called methoxylated PBDEs (MeO-PBDEs - see structures above). It is possible that these compounds could be derived from the flame retardant PBDEs. Alternatively, some of these compounds can be synthesized naturally, mainly by marine sponges. Oddly enough, the known natural sources of these compounds are generally limited to equatorial or tropical regions of the oceans, but we see MeO-PBDEs in much higher latitudes and even in freshwater systems such as the Detroit River.

Compounds like 6-MeO-BDE-47 that are made by sponges are not necessarily rare. There are many known halogenated organic compounds that are produced by plants and animals. Current estimates are that more than 3,000 such "natural organohalogens" exist. Many are made by marine species that use

these compounds for chemical defense.

Chemists interested in finding new drugs from the sea have been responsible for finding many of these compounds. Most have been found by prospecting

A flame retardant that is the major one found in animal tissues. It is possible that it could be transformed into 6-MeO-BDE-47.

animals that do not have a hard shell or move fast and, therefore, rely on chemicals to defend themselves. However, very little is known about how long these naturally-produced compounds last in the environment and how they behave. Environmental chemists have become interested in them, because they have structures and properties similar to those of industrial compounds (see structures above).

Currently, we are trying to determine the sources of MeO-PBDEs by measuring the radiocarbon content of these compounds. Radiocarbon is a radioactive isotope of carbon that has a half life of 5,730 years. It is incorporated into all living organisms, at a level reflecting the environment in which that organism formed. Upon death, no more radiocarbon is incorporated, and the amount decreases over time.

Most industrial halogenated

Science Highlights (continued)

organic compounds are synthesized from petrochemicals, which contain no radiocarbon. Hence, the flame retardant PBDEs are what we call "radiocarbon dead" or "radiocarbon free." In contrast, naturally-produced compounds should have modern or contemporary levels of radiocarbon. Using accelerator mass spectrometry (AMS), it is very easy to determine if a compound is radiocarbon free or modern. (The hard part is getting the material for the measurement. In our case, it took 6 months to isolate enough material at high purity for a recent AMS measurement). In the laboratory, we call this approach the "Dead or Alive Theory." Industrial chemicals made from petrochemicals are "radiocarbon dead" and natural products are "radiocarbon alive."

We recently determined that several MeO-PBDEs in a True's beaked whale were actually produced naturally. This was an incredibly laborious task that employed classical chemistry procedures and advanced instrumentation. We started with 10 kg \sim 22 lb) of blubber and isolated about 1 mg each of these compounds. In many respects, this was like finding a needle in a haystack since we removed 10,000,000 mg of other whale material, mainly fats, to get our compounds of interest in very high purity $(99+%).$

We are now trying to continue this work on other animals and also trying to determine how these compounds move from sponges to whales. This work will provide invaluable insight on how both natural and industrial

compounds cycle in the environment.

Despite the recent progress in understanding the source and environmental behavior of natural organohalogens, we know very little about their biological activities. The similarities between the chemical structures of synthetic and natural organohalogens suggest that their biological effects may also be similar. Thus, it is possible to ask specific questions about whether natural products mimic the effects of industrially-produced chemicals such as PCBs, and to apply existing bioassays to address these questions. It is also necessary to develop novel bioassays that may provide additional information about the effects of natural compounds.

PCBs, chlorinated dioxins and other synthetic organohalogens cause toxicity through multiple mechanisms. Some of these involve interactions with specific receptor proteins that exist in most cells of vertebrate animals (fish, birds, mammals). One of these receptors, the aryl hydrocarbon receptor (AHR), is well known as the protein through which the highly toxic 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD, sometimes called "dioxin") causes its effects. For TCDD and other synthetic organohalogens, such as certain PCBs, the toxic potency of each chemical compound is determined in part by the strength with which it interacts with the AHR protein. Similarly, the ability of a natural organohalogen to interact with the AHR protein can be used as a measure of its potential toxicity. Thus, the

natural organohalogens isolated from whale blubber and other sources are being examined for their ability to bind to the AHR. These assays are being performed with human and whale AHR proteins that are synthesized in a test tube, using AHR genes that have been isolated and characterized.

Another measure of potential toxicity of an organohalogen is its ability to stimulate gene expression. The activity of genes (i.e., the use of a gene to synthesize the protein it encodes) is normally tightly regulated within a cell. PCBs and dioxins can "turn on" gene expression through their interaction with the AHR, which acts as a molecular switch. Using mouse cells that have been engineered to synthesize a green fluorescent protein (GFP) when exposed to chemicals that can interact with the AHR protein, we are examining the ability of natural organohalogens to stimulate gene expression. This sensitive bioassay provides additional information about the biological activity of these compounds.

In addition to the established assays mentioned above, we hope to develop a novel cell culture bioassay that will combine the use of species-specific AHR proteins with a GFP-based measure of gene expression to produce a sensitive and species-specific assessment of potential toxicity.

Our study employs unique tools to understand the health of marine ecosystems by comparing natural and anthropogenic chemicals in the same animals, using novel bioassays and new recombinant cell lines to assess effects on marine mammals.