

Woods Hole Sea Grant: 1998-2000 Projects

Molecular Biomarkers of Chemical Sensitivity

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A group of chemical contaminants known as PHAHs, or planar halogenated aromatic hydrocarbons, are persistent in the marine environment. Some of these contaminants accumulate in the blubber and other tissues of marine mammals. As a result, certain cetaceans and other marine mammals contain some of the highest levels of PHAHs reported in any wildlife groups. These contaminants may contribute to marine mammal mortality and morbidity. However, the magnitude of the risk that PHAHs pose to the health of marine mammals is controversial, in part because there is little direct information on the sensitivity of these animals to PHAHs. Because legal and ethical concerns preclude addressing these questions through direct testing of toxic chemicals on protected animals, alternate research approaches are required. In a previous Sea Grant study, these researchers studied PHAH sensitivity of beluga by cloning the gene for the beluga aryl hydrocarbon receptor (AhR) protein, which plays an important role in the mechanism of PHAH toxicity. This project will extend that work by examining the function of the AhR in intact cells, thus determining whether the results obtained with the associated AhR are reflected in real cellular events, including changes associated with toxicity. This project will lead to a better understanding of the risk to marine mammals exposed to PHAHs and may serve as a model for a new approach for assessing the risk of environmental contaminants to protected species. (R/B-151)

Biochemical Toxicology in Cetaceans

John J. Stegeman and Michael J. Moore, Woods Hole Oceanographic Institution

This project, related to the project described above, will attempt to address recent scientific evidence that establishes links between chemicals released into the marine environment and damage to fish, birds, and mammals, including humans, by disrupting hormone action and interfering with reproductive and developmental processes -- so-called "endocrine disruptors." These researchers have, over the years, conducted Sea Grant studies of biochemical toxicology in marine mammals using archived cetacean tissue samples from stranded animals and tissue biopsies. This project seeks to expand these efforts and to lay the foundation for a multi-institutional program focusing on molecular, histopathological, and gross-morphological features that may be linked to chemical effects in cetaceans. Such a concerted effort is essential for evaluating the susceptibility of cetaceans to different types of chemicals. Specifically, this project will obtain and archive cetacean tissue samples for analysis of enzyme and receptor systems relevant in chemical effects; establish a multi-investigator program to examine the properties of molecular, biochemical, and cellular systems that may participate in toxic mechanisms in cetaceans; describe histological features that may be linked to or that could corroborate biochemical results indicating chemical effects in these animals; and determine the concentrations of environmental chemical residues, including PAH and PHAH that may occur in liver and other organs of cetaceans. (R/B-152)

Identifying Wastewater-Derived Nitrogen in Aquatic Ecosystems: Tests of a Stable Isotope Tracer Approach

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Increasing nitrogen loading from watersheds is leading to eutrophication of coastal waters worldwide and is considered one of the most pervasive human-induced alteration of coastal ecosystems. To effectively manage and monitor these ecosystems, techniques for assessing nutrient-driven changes in coastal waterbodies must be developed. In previous Sea Grant studies, these investigators developed a stable isotopic approach for identifying wastewater-derived nitrogen in aquatic systems -- an approach that has proven useful for tracking septic tank-derived nitrogen from the Waquoit Bay watershed of Cape Cod, Massachusetts, into its surrounding estuaries. In this project, investigators will put their approach to the test. They will determine if the approach can be applied to a broader range of estuaries in the Cape Cod region, in estuaries where nitrogen loads enter from sources other than septic tanks, and in freshwater and brackish water systems. This information -- and this innovative approach -- will be useful to environmental managers as it will provide them with a sensitive, economic tool for identifying and monitoring the impact of anthropogenic nitrogen inputs on aquatic food webs. (R/M-40)

Detection and Quantification of Live Acanthamoebae in Natural Marine Ecosystems Using Molecular Genetic Methods

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This project, set to begin in 1999, will look at the free-living amoebae *Acanthamoeba*. *Acanthamoebae* can opportunistically infect humans. They do not generally pose a risk to healthy individuals, other than a small number of annual cases of *Acanthamoeba* keratitis, a painful corneal infection typically caused by improper care of soft contact lenses. *Acanthamoebae* can be isolated from soil, salt and freshwater (including tap water), and some marine sediments. In the marine environment, most *Acanthamoebae* have been obtained near sewage and waste dumps. Their presence at sewage dumps suggests that they may be useful as indicators of sewage contamination. However, analyzing the natural distribution and abundance of amoebae in general has been problematic. This project will attempt to implement detection and quantification methods that will provide valuable information on the presence and distribution of *Acanthamoebae* and provide a new set of tools for analysis of natural species. The development of such methods will enable ecologists to examine the mechanisms that affect the growth and aging of ecologically important organisms. (R/B-147)

The following four projects are part of a 7-project, multi-institutional initiative supported by the National Sea Grant College Program titled, "Chemical/Biological Interactions: Receptor Mediated Effects on Reproduction and Development in Aquatic Species."

Fish Cytochrome P450 Genes Involved in Chemical Effects

John J. Stegeman, Woods Hole Oceanographic Institution

In this study, researchers will establish a molecular basis for explaining and monitoring effects of anthropogenic or natural chemicals on

endocrine and developmental processes in fish. Through studying the salt marsh minnow *Fundulus heteroclitus*, investigators will clone and sequence genes for cytochrome P450 that may metabolize and activate or inactivate chemical compounds. These studies will provide a molecular foundation for understanding the mechanisms and monitoring the effects of diverse chemical pollutants. The results will have implications for and applications in ecology, toxicology and pharmacology of fish, and could provide new approaches for screening effluents and new chemicals for biological reactivity. (R/P-60)

Molecular Biological Approaches for Non-Destructive Assessment of Chemical Effects on Marine Mammals

John J. Stegeman and Michael J. Moore, Woods Hole Oceanographic Institution

Pollutants in the marine environment, such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, and furans, are believed to have adverse effects on marine populations, including marine mammals. Exposure to such pollutants may affect the physiology of marine mammals, including reproduction, immune defense, endocrine system functions, and, possibly, neural systems that control social and migratory behavior. Using molecular biological techniques, researchers in this study will focus on the pilot whale, studying biopsy samples of free-ranging populations as well as archived samples taken from stranded whales to evaluate the exposure of marine mammals to harmful chemical compounds. By quantifying expression of the cytochrome CYP1A gene, a sensitive biomarker of hydrocarbon effects, in the livers and other organs of the pilot whale, investigators will be able to characterize metabolic activity. The methods established in this study will provide a non-destructive means to study compounds that may pose a threat to fragile marine mammal populations. (R/P-61)

Impact of Environmental Contaminants on an Aquatic Bird Population

Mark E. Hahn, Michael J. Moore, and Constance A. Hart, Woods Hole Oceanographic Institution, and Ian Nisbet, I.C.T. Nisbet and Co.

Many questions surround the extent to which dioxin-like compounds and chlorinated pesticides disrupt endocrine functions -- such as reproductive and developmental processes -- in humans and aquatic animals, including birds. This project seeks to examine abnormalities, including gonadal feminization, in Common Tern embryos from two coastal Massachusetts sites with different levels of environmental contamination. Using chemical, biochemical, and immunohistochemical methods as well as cell-culture bioassays, researchers will measure exposure of the birds to a variety of environmental pollutants. Through a combination of field and laboratory-based studies, investigators hope to assess possible relationships between health effects and specific contaminants, including dioxin-like compounds and environmental estrogens. (R/P-58)

Identification of Bioactive Marine Natural Products Using a Fish Culture Bioassay

Mark E. Hahn, Woods Hole Oceanographic Institution

Marine organisms produce a variety of organic compounds (marine natural products), some of which display useful biological activities. Many of these products are structurally related to dioxins, PCBs, and other anthropogenic compounds that act through specific receptor mediated mechanisms. Investigators in this study will use a newly-devised cell culture bioassay system to determine marine natural products that stimulate or inhibit the same biochemical systems that respond to dioxins. Exploring the ability of marine natural products to mimic the effects of dioxins and PCBs will be important to understanding the biochemical and physiological changes that have been observed in animals living in contaminated environments. In addition, some of these natural compounds may be useful as future research tools or as pharmaceutical agents. (R/B-124)

The following project is part of a National Strategic Initiatives (NSI) competition in Marine Biotechnology, made possible by a special National Sea Grant College Program award.

Detection of Harmful Algal Species Using Molecular Probes: Field Trials

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Over the past two decades the economic and public health impacts from harmful algal blooms (HABs) have increased dramatically throughout the world. One result of this expansion is that regulatory officials and the fishing industry are faced with a broad array of affected species, spanning all levels of the food chain, many of which can be contaminated by several different toxins. These changes have forced a major reevaluation of strategies to monitor seafood products for marine biotoxins. Present techniques to identify phytoplankton species and to count toxin rely on manual microscope techniques that are time consuming and require trained specialists. This project represents the last phase of ongoing efforts to develop molecular probes and assay systems for several key HAB species. By refining and field testing nucleic acid-based probe assays that can be used in the laboratory and the field by personnel with limited technical expertise, this project will contribute an accurate, fast, and reliable tool for private, state, and federal monitoring programs for HAB species. (R/B-146)

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