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Woods Hole Sea Grant: 2000-2002 Projects

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

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Acanthamoeba is a genus of free-living amoebae present in soil, saltwater, and freshwater, including tap water. Although Acanthamoeba does not appear to be a human health threat in the ocean, very little is known about the distribution of the potential pathogens in the environment. Their presence at sewage dump sites suggests that acanthamoebae may be useful as indicators of sewage contamination because it appears that the presence of detectable numbers of Acanthamoeba in marine sediments is due to human activity, and because the presence of the amoebae potentially corresponds with that of coliform bacteria. Until now, the analysis of the natural distribution and abundance of amoebae in general has been problematic, due in part to their small size and an inability to reliably identify them in natural samples. This project has implemented novel molecular tools and techniques -- a reverse dot-blot method -- to better assess populations of organisms once thought to be 'ambiguous eukaryotic microorganisms,' due to a lack of knowledge about the species abundance and diversity. Having just completed the first year of a two-year project, the investigator has successfully used the dot-blot technique to detect and identify acanthamoebae in the marine environment. Efforts are now underway to characterize the natural distribution and abundance of Acanthamoeba ribotypes. The determination of what ribotypes are abundant in the environment and how they change over time, or due to human action, may provide information as to why Acanthamoebae infections are so rarely seen from marine environments as opposed to freshwater. Refinement of the ribotype work is now underway. (R/B-147)

Estradiol Dynamics: A Molecular Basis for Potential Endocrine Disruption in Marine Mammals

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Marine mammals are known to have large lipid stores and long lifespans. And, residing at the top of the marine food web, they may be the ultimate sink for persistent chemicals, whether those chemicals are released on land or at sea. Understanding the effects of chemicals on marine mammals can provide a critical point of reference for judging whether such effects are of concern on a global basis. This project seeks to establish a molecular foundation by which to evaluate whether exposure of marine mammals to so-called endocrine disruptors (such as polychlorinated biphenyls (PCBs) and the chlorinated dioxins) poses threats to the reproduction or development of these organisms. Among the key hormonal systems thought to be involved in chemical effects on reproduction is one that controls the levels and action of estradiol. Successful reproduction requires close control over this system in adults. Also, the effects of chemicals on estradiol control during development may play a role in reproductive dysfunction and/or disease later in life. Investigators will examine genes and gene products that control estradiol synthesis, action, and degradation, in a comprehensive approach that, heretofore, has not been accomplished in any marine species. (R/B-162)

Bivalve Dispersal as Indicated by Shell Trace Element Composition

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For decades, researchers have been searching for a marker that will help identify the source habitat of larvae settling into a benthic population. Bivalve larvae spend up to 60 days in the planktonic state. During that time, the larvae can be transported substantial distances in coastal currents, making it difficult to determine the geographic origin of the larvae. Ultimately, these dispersal processes have an important impact on the magnitude and variation of recruitment. Using Mya arenaria, the soft-shell clam, this project will seek to develop a marker for bivalves by focusing on trace element incorporation by the larval shell. Bivalve larvae incorporate distinctly elevated amounts of trace elements into their shells when they are spawned in water with elevated concentrations of those elements. Because the larval shell is retained as part of the juvenile shell, each bivalve carries a record of the trace element composition of its larval environment: a unique, location-specific fingerprint, of sorts. The trace element composition of water, and overlying sediments of coastal habitats, varies geographically along the coast. To determine whether this variation translates into a useful marker in the bivalve shell, investigators will compare field-collected individuals with those spawned in the laboratory under controlled trace element conditions. The resultant marker will give researchers a powerful and direct tool for tracking larval dispersal in coastal waters, and for understanding the interaction of hydrodynamics and behavior in the dispersal process. (R/O-32)

Impact of Environmental Contaminants on Aquatic Birds: The Molecular Basis of Differential Dioxin Sensitivity

Mark E. Hahn, Woods Hole Oceanographic Institution, and Sean Kennedy, Environment Canada, Canadian Wildlife Service

While environmental contaminants, such as dioxins and related planar halogenated aromatic hydrocarbons (PHAH) are known to be highly toxic to many vertebrate animals, there are dramatic differences in sensitivity, both within and among vertebrate classes. These differences are a major limitation in ecological risk assessment, which often requires extrapolation among species. This project seeks to assess the mechanistic basis for such differences among three species of birds, including common and roseate terns, two coastal Massachusetts species that are at risk from contaminants. Herring gulls will also be included in the study. The investigator will test the hypothesis that differential sensitivity to dioxin-like compounds in birds is due, at least in part, to differences in the characteristics or expression of the aryl hydrocarbon receptor (AhR), an intracellular protein that mediates most dioxin effects. This protein has been well studied in mammals, but has not been extensively characterized in non-mammalian vertebrates. The approaches to be used in this project -- cloning, in vitro expression, and analysis of protein function -- represent a new way to study the impact of environmental contaminants on protected species. (R/P-64)

Determining Reproductive Success of Commercially Valuable Squid in New England with DNA Fingerprinting

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Worth $30 million per year in the Northeast U.S., the short-fin squid, Loligo pealei, is now being heavily exploited by fishers who traditionally harvested groundfish. Squid live for only six to fifteen months and heavy fishing pressure on squids during their inshore

migration to spawn could have adverse affects on recruitment. This project seeks to provide solid biological data on reproductive success in Loligo pealei. These data would allow investigators to address the following question -- one that is vital to fisheries management: is there multiple paternity in many, or most, individual egg capsules laid by female Loligo pealei? Female squid lay egg capsules, each containing approximately 150 eggs. Behavioral evidence suggests that multiple paternity may be extensive, but genetic proof of paternity is required because mating success often does not equate to fertilization success due to sperm competition, which occurs in many species including squids. The investigator will apply DNA fingerprinting techniques to egg capsules to measure the degree of multiple paternity within individual egg capsules. These egg capsules are the result of complex mating trials conducted in the laboratory by the investigator. Additional laboratory trials will be conducted on sexual selection and mating to answer related questions on multiple paternity as the results from the DNA fingerprinting tests become available. Field samples of egg capsules will also be collected and analyzed and results will be verified and compared to laboratory findings. (R/B-163).

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Novel Biomarkers of Dioxin Effects

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Environmental contaminants, including planar halogenated aromatic hydrocarbons, or PHAH, are widely distributed in the world's oceans. The highest concentrations of these chemicals are often found in urban harbors and other coastal areas; however, PHAH have been documented in remote locations as well, including open ocean, polar regions, and in the deep sea. These chemicals pose a well-documented risk to marine organisms and ecosystems. In order to more accurately measure the impact of PHAH in marine environments, there is a need to identify responses that can serve as sensitive indicators, or biomarkers, of adverse effects in marine animals. Existing biomarkers, such as cytochrome P4501A (CYP1A) have not been closely linked to toxic endpoints, despite extensive research. Though useful as markers of exposure to contaminants, CYP1A may be less useful as an indicator of toxic effects. This project seeks to identify genes whose expression is induced or repressed by TCDD in killifish and that are directly related to changes in cell growth and proliferation -- the hallmarks of PHAH toxicity. In addition, the investigator will clone and sequence selected candidate genes potentially linked to toxicity, and test the value of these genes as biomarkers by measuring their expression in animals exposed to PHAH in the laboratory and in the marine environment. (R/P-64)

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