Abstract or Summary of Proposed Research

RI DEM Ventless Trap Survey and Monitoring

The Rhode Island lobster fishery is one of the state's most valuable commercial fishery resources. Ex-vessel value of the commercial landings peaked in 1999 at $30 million. A recent decline in lobster abundance and landings reduced the value to $17 million by 2003. The decline in abundance was associated with heavy fishing pressure and an outbreak of shell disease. A large oil spill in 1996 caused extensive mortality of juvenile lobster and lead to reduced adult catches in later years. In order to maximize the chances of rebuilding this valuable fishery, management decisions must be based on sound data and analyses. The Department has initiated at-sea sampling in 1990 to monitor lobster abundance, growth, maturity, and mortality. Sampling areas consist of inshore waters of Rhode Island and offshore areas (mid-shelf and Canyons). Commercial lobster traps are size-selective due to the escape vent built in the traps, thus the potential bias in the commercial CPUE as a measure of abundance. An alternative sampling method for removing the bias is sampling with small mesh traps without escape vents. These traps known as "Vent-less" traps will be placed on commercial lobster vessels under contract for the project in areas consistent with the DEM at-sea sampling program. In addition, a port-sampling program will be conducted parallel to the sea sampling program to monitor the landings at-size by areas. The data from both sampling programs will aid in the assessment and management of lobster resources in Rhode Island.

Sampling will consist of a minimum of four (4) inshore fishery (Area 2) sea sampling trips every eight (8) weeks and one (1) offshore fishery (Area 3) sea sampling trip every 3 months (once per quarter). Data regarding size of lobsters, sex, shell condition, presence or absence of eggs, cull status, other shell/body damage, V-notch status, mortality, incidence and severity of shell disease, marketable catch in pounds and number, gear type, total traps hauled for trip, catch per trap haul, soak time, surface water temperature, and trap locations (LORAN). Also, calculations of length frequencies catch per unit of effort, fishing mortality, and size at sexual maturity will be made. Also, data will be presented on reproductive condition of females.

The Department of Environmental Management will be responsible for collection of data, computer data entry and analysis, and dissemination of information. In addition, the Department staff will participate in stock assessment workshops and plan development teams as necessary.

Maine Ventless Trap Monitoring Project

The Maine lobster fishery is the state's most valuable commercial fishery resource. Landings in 2005 were 67 million pounds with a peak ex-vessel value of $313 million. Reported landings have declined slightly (5%) from the peak of 70 million pounds in

2004 (the first year of mandatory landings reporting). However; estimates suggest landings may have declined as much as 25% since 2002 (Wilson et al 2004). With the dramatic decline in the lobster resource south of Cape Cod and subsequent impacts on the fishery, it is imperative to effectively monitor the relative distribution and abundance of the lobster fishery in near shore waters. The project provides the Maine Department of Marine Resources with the ability to effectively participate in the management of the lobster fishery in accordance with the Atlantic Coastal Fisheries Cooperative Management Act and enables the state to implement the lobster Fishery Management Plans enacted by the Atlantic State Marine Fisheries Commission. To address the need to develop a robust time series of relative abundance a cooperative random stratified Ventless Trap Survey has been designed to generate accurate estimates of lobster relative abundance and recruitment while attempting to eliminate the biases identified in conventional surveys. A fishery-independent survey, wherein scientists and contracted fishermen cooperatively collect the data, will provide greater control over the sampling design and data quality and quantity necessary to maintain a stratified sampling approach. Objectives of the study are: Develop a pilot project for a coast wide fishery independent monitoring program for American lobster; Accurately characterize relative abundance and size-distribution of American lobster from three statistical areas along the coast of Maine; Document the relative importance of depth and location as it pertains to American lobster abundance and distribution: Foster an improved relationship between the commercial lobster industry and fisheries scientists and managers in the interest of strengthening communication between science and industry; Foster industry "buy-in" to the lobster stock assessment process.

Microbiology of shell disease: environmental sources and diversity

Dr. Andrei Chistoserdov
Dr. Michael Tlusty
Dr. Roxanna Smolowitz
Dr. Darryl Felder

Elucidating the relationships between environmental sources and various forms of shell disease in lobster and various species of marine arthropods is the primary goal of the project. The following experimental objectives will comprehensively address this goal: (1) to elucidate a "natural reservoir" of bacteria associated with epizootic disease lesions in the Buzzards Bay, (2) to compare the composition of prokaryotic and eukaryotic communities found in lesions and on healthy carapaces of lobsters with epizootic, impoundment and cigarette burn diseases, (3) to compare the composition of prokaryotic and eukaryotic communities in lesions of shell disease in various decapods. We will use a combination of culture-dependent and culture-independent methods to characterize microbial communities in lesions of lobsters with various forms of shell disease, healthy carapace surfaces of lobsters and shell lesions of other arthropods. Histopathological evaluations will be carried out on all arthropods exhibiting shell disease. A distribution of potential pathogens in the environment will be investigated by PCR and FISH. This

project will answer specific questions regarding mechanisms of shell disease transfer among lobsters and the persistence of disease in the environment.

Characterization of the exoskeletal microbial communities and host immune responses associated with epizootic shell disease in lobsters

Dr. Alistair Dove
Dr. Bassem Allam
Dr. Anne McElroy
Dr. Gordon Taylor

We will use the disease triad of host–pathogen–environment as the basis for investigations of Epizootic Shell Disease (ESD) in lobsters, with the goal of evaluating the relative roles of each in the development of the disease. Our approach will focus on characterizing the composition of the exoskeletal microbial community of healthy and ESD lobsters using molecular tools from microbial ecology while at the same time assessing the status and response of the lobster's associated immune system. We will characterize ESD in lobsters collected from three locations, Eastern Long Island Sound (ELIS) where prevalence is high, Western LIS (WLIS) where prevalence is unexpectedly low, and at a remote reference site (Maine) were prevalence is also low. We will also characterize the temporal progression of ESD development in lobsters collected from ELIS. Laboratory experiments will be conducted wherein wild healthy and ESD lobsters will be subjected to temperature stress to measure the role of this dominant environmental factor in modulating the host-pathogen interaction. Through this approach we expect to gain significant insights into the etiology and environmental modulation of ESD, as well as optimizing tools for the study of crustacean diseases.

Etiological and mortality studies into epizootic shell disease of the American lobster

Dr. Jeffrey Shields
Dr. Patrick Gillevet

Characterization of the microbial community on lobsters with epizootic shell disease has relied on culture-dependent techniques. We propose to examine the entire bacterial community using a culture-independent technique. Objective 1 is to determine if different microbial communities are associated with healthy versus diseased lobsters and whether specific microbial communities are correlated with the epizootic disease syndrome (i.e., a polybacterial etiology). We will compare communities on lobsters with epizootic shell disease, lobsters with classical shell disease and lobsters without shell disease. Our second objective is to analyze carbonic anhydrase and phenoloxidase activity in the hemolymph and tissues of diseased and healthy lobsters as possible phenotypic markers of disease stress. Our third objective is to investigate the morbidity and mortality of lobsters with and without epizootic shell disease in a controlled laboratory experiment. In mortality studies, we will also monitor the progression of lesion formation using the

molecular methods in objective 1 as well as the enzyme markers. We ask whether the associated microbial community is indeed responsible for lesion formation, or whether it is a secondary effect. We will attempt to link the lesions to the presence of chitinolytic bacteria (vibrios) or to the lypolytic/proteolytic activity of alternate bacteria such as the Flavobacteriaceae.

The spread of lobster shell disease: genetic and social barriers

Dr. Jelle Atema
Dr. Roxanna Smolowitz
Dr. Steven Roberts
Dr. Gabriele Gerlach

Shell disease has crippled the lobster fisheries of Southern New England, but also in Nova Scotia, lobsters show far greater incidence of shell disease than elsewhere in the North. This could be due to regional environmental differences, such as temperature and pollution, and/or to regional stock differences in susceptibility, including resistance, tolerance, and behavioral avoidance. Given the difficulty of treating diseases of wild populations it is important to understand mechanisms involved in spreading disease. Social behavior, especially mate choice and stock preference, can create important barriers for gene flow and the spread of disease. Our preliminary data show that there are genetic and morphological differences between lobster stocks at a much smaller geographic scale than previously assumed. Additionally, stocks may show different disease susceptibility as a result of local adaptation such as temperature tolerance. We propose to investigate genetic and behavioral mechanisms that can limit or accelerate the spread of disease in lobsters. We will integrate our studies with the ongoing results from disease and stock monitoring programs, environmental studies identifying correlation with and perhaps causation of shell disease, and physiological studies of temperature adaptation in different lobster stocks.

A molecular approach to understanding lobster shell disease

Dr. Tim Verslycke
Dr. John Stegeman
Dr. Judith McDowell

Mechanistic understanding of animal physiology and its potential disruption by environmental stress (chemical or other) are critical research questions in environmental toxicology. The mechanisms behind stress-induced physiological disruption in crustaceans are largely unknown, hindering their prediction, prevention and remediation. We hypothesize that external stress on New England lobsters over the past years has increased and has resulted in physiological stress. Physiological disruption can lead to irreversible effects at the individual and population level, and may play an important role in lobster shell disease and lobster population declines. We further hypothesize that this

physiological disruption will be reflected by changes in gene expression. We propose to use global and targeted molecular approaches to identify genes that are differentially regulated in healthy versus shell-diseased lobster. We will use suppressive subtractive hybridization to identify candidate genes involved in shell disease, and quantitative real time polymerase chain reaction to quantify the expression of specific genes. Our proposed approach will determine the role (if any) of the hormone and immune systems, molting, energy and xenobiotic metabolism, and shell development in lobster shell disease. To the best of our knowledge, this will be the first study to use measures of gene expression to investigate lobster shell disease.

A possible role of alkylphenols in lobster epizootic shell disease

Dr. Hans Laufer

The main hypothesis to be examined is that alkylphenol concentrations found in lobsters may interfere in shell hardening (tanning, sclerotization) during molting, weakening cuticular structure, making it susceptible to microbial invasion. The main objective is the analysis of lobster shell disease (SD) and its relationship with anthropogenic environmental contaminant alkylphenols. They may produce defects in the shell by interfering with cuticle structure during tanning and sclerotization (shell hardening). Alkylphenols being antioxidants appear to interact with phenoloxidases and other proteins that are needed for shell hardening, by interfering with protein structure, crosslinking, calcium deposition and crystallization and chitin deposition. It is important to examine shell structure, mechanical and physical hardness, and measure the half-life of alkylphenols, their incorporation, metabolism and elimination, and their function in shell formation. We intend to determine how SD can be experimentally induced and how alkylphenols interact with epidermis and cuticle constituents during molting both in adult lobsters and in early larval and juvenile stages which may be most sensitive to chemical disruption.

Analysis of environmental contaminants in shell diseased versus non-shell diseased lobsters

Dr. Lawrence LeBlanc

Identifying causative agents is essential to designing strategies to prevent the spread of epizootic shell disease in the American lobster. We will examine whether contaminants may contribute to lobster shell disease by determining whether specific organic and trace metal contaminants co-occur with shell-diseased lobsters in a consistent fashion. Target analytes include bioaccumulative toxins and compounds known to have endocrine-disrupting properties such as polychlorinated biphenyls, polybrominated diphenyl ethers, organochlorine pesticides, alkyl phenols, naturally-occurring and synthetic estrogens and selected trace metals. These contaminants are widely distributed in aquatic environments, and up until now have not been systematically looked for in shell-diseased lobsters.

Lobster hemolymph and hepatopancreas tissues will be analyzed using a multi-residue method that will be adapted from existing protocols. The hepatopancreas tissue is of interest because it has been found to be the site of greatest concentration of metal and organic pollutants. Analysis of lobster hemolymph for contaminants has been minimal, but may prove useful in a rapid screening context because of potentially far less effort in sample extraction and cleanup prior to analysis. Expected products include the development of a rapid screening technique for organic pollutants and a multi-residue method for a wide variety of organic contaminants.

Environmental stress and susceptibility to shell disease – An assessment of structural deficiencies in shell formation at increased temperatures

Dr. Michael Tlusty
Dr. Roxanna Smolowitz
Dr. Andrei Chistoserdov

Observations of natural processes indicate that there is a positive correlation between the summer water temperature and the prevalence of shell disease in the following year. This research proposes to experimentally elucidate the temperature – shell disease relationship. Lobsters will be reared in the laboratory at one of three temperatures, and then will be assessed for cuticle formation, growth rate of cuticle, and susceptibility to shell disease. Cuticle formation will be investigated through histological and macro- and micro structural methods, while growth will be assessed through the rate of pigment deposition in the cuticle. The significance of this work is that it will address two of the priorities listed in the RFP, specifically how shell disease relates to the (2) the nature of the tissue within the context of (4) the role of environmental stress, where the stressor is increased temperatures. The results here will be useful for modelers and managers to predict future impacts to lobsters through increasing ocean temperatures. This work is of the utmost importance because, as global warming continues, lobster health will be significantly affected.

Mineralization of the lobster exoskeleton as a target of shell disease

Dr. Joseph Kunkel

We propose that shell disease is based on a temperature-enhanced microbial biofilm affecting the chemistry of cuticle chitin-protein-mineral composites. Shell disease involves the eventual demineralization of the cuticle; however it is not known if and where demineralization is involved in early phases of shell disease development. Mineralization and demineralization of crustacean cuticle is poorly understood due mainly to past difficulty in studying cuticle without first de-mineralizing. We will study normal and diseased cuticle minerals by maintaining the original composite form and content using plunge freezing during sampling and then treating it as a geological specimen. X-ray backscatter analysis will be used to analyze the mineral content and

micro-X-ray crystallography will be use to identify the crystalline nature of the mineral cuticle structure. A micro-coring tool will be used to obtain core transects of mineral content from the cephalo-thorax including the area of intense shell-disease prevalence in the dorsal triangle of cephalo-thoracic cuticle. We will map local cuticle surface proton and carbonate flux associated with cuticle demineralization during the etiology of epizootic shell disease. Methods will be developed to allow the early stages of epizootic shell disease to be mapped and related to the demineralization process.