State of lobster science

Lobster shell disease workshop

assessing research priorities for understanding how lobster biology and health issues impact productivity

*University of Massachusetts, Boston,
March 12th and 13th, 2005*

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Proceedings of a workshop "State of Lobster Science: Lobster shell disease – assessing research priorities for understanding how lobster biology and health issues impact productivity" held March 12 - 13, 2005 at the University of Massachusetts Boston, USA.

This workshop was sponsored by the Policy Center for Marine Biosciences and Technology, University of Massachusetts Boston, MA, The New England Aquarium, Boston, MA, and The Marine Biological Laboratory, Woods Hole, MA .

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New England Aquarium

Aquatic Forum Series
Final Report 05-1

Citation for this report:

Electronic Copies Available at:
www.neaq.org/scilearn/research/landing.php?Link name=lobster

Printed in the United States of America

Front cover photos by: A Dove (top) and M Tlusty (bottom, a backlit section of a molt demonstrating how much shell can be removed by bacteria).

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PREFACE

The workshop on the "State of Lobster Science" was designed to bring scientist from the Northeast region together to:

1. Discuss the current state of research being conducted on lobster shell disease;
2. Set research priorities to understand the relationship between lobster shell disease and lobster biology; health issues, and the environment, and how this does and can affect fishery productivity;
3. Establish new collaborations to better understand and more effectively research shell disease; and
4. Develop a framework for a regional effort to support this plan of action.

The American lobster (*Homarus americanus*) is the most commercially important fishery in the northeast. This lobster is found on the east coast of North America from Newfoundland to North Carolina. In the Northeast it is one of the few fishery resources that are considered to be generally healthy. In 2003 landings the landings of lobster were valued at $285.6 million. In Maine alone, in 1999 the dockside value of the catch was a record $185 million, with an estimated impact on the state economy of $500 million. As reported in this workshop, it is generally accepted that the inshore fishery for American lobster is recruitment based and therefore vulnerable to disruptions in larval supply and survival of pre-recruit lobster.

However, disease and environmental problems are severely reducing population sizes in Long Island Sound and south of Cape Cod. In 1998, the Long Island Sound fishery caught over 8 million pounds of lobster with a dockside value of approximately $29 million. The population crash of 1999 eliminated fishing in many parts of the sound impacting 1300 fishermen, and causing losses in excess of $16 million. While a variety of factors are implicated in the Long Island Sound stock crash, disease issues are pervasive, and indicative that long-term health of the lobster stock may be compromised.

One of the more noticeable diseases affecting lobster in eastern long island sound was shell disease. Shell disease results from bacterial invasion of the carapace, but the reasons for this increase in the ability of bacteria to penetrate the carapace are unknown. This disease is reportedly increasing in prevalence into the more productive waters off northern Massachusetts, New Hampshire, and Maine (which accounted for 88% of lobster landings in 2003). Thus it is imperative to understand why shell disease has become epizootic and if it can be transferred up the coast thus affecting all populations of the American lobster.

While shell disease is not the only disease impacting lobster health, it is a key disease to understand. It appears the prevalence and severity of the disease can be influenced by the pathogen (type, density, pathogenicity), internal lobster factors (shell quality, nutritional status), and the environment (ocean temperatures, current patterns, microbial communities). Only by concomitantly assessing these three areas will researchers full understand how this and other diseases will affect lobster populations, and the management methods necessary to control the spread of lobster disease.

This workshop is intended to develop the cross collaborations necessary to advance research on lobster health. Roundtables have been structured to review:

1. Causes of Disease
2. Animal Responses
3. Population Responses
4. Management Implications.

Finally the final chapter and goal of this workshop is "Priority Setting", which is intended to provide to decision makers research priorities to assist this important fishery industry.

Harlyn Halvorson
Wood's Hole, MA

Chapter 1 Position Papers

1. **Epizootic shell disease in the American lobster, *Homarus americanus***
   Roxanna Smolowitz, Andrei Y. Chistoserdov, and Andrea Hsu

2. **A microbiological assessment of epizootic shell disease in the American lobster indicates its strictly dermal etiology**
   Andrei Y. Chistoserdov, Sai Laxmi Gubbala, Roxanna Smolowitz and Andrea Hsu

Epizootic shell disease in the American lobster, *Homarus americanus*

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Previously, three types of shell disease had been recognized. Impoundment shell disease occurs in American lobsters and was described by Smolowitz et al. (1992). Early lesions are bilaterally symmetrical and are centered around setal cores primarily on the dorsum of the animal carapace (Bullis, 1989). Lesions consist of round, blacken, focal erosions that overlap as the disease worsens thus increasing the affected surface area. Only, in its most severe form do the lesions cover the entire surface of the carapace (Smolowitz et al., 1992). A common histological characteristic of impoundment shell disease is the scooped out appearance of the eroded cuticle. The onset of the disease is related to overcrowding, poor water quality, and inadequate diets associated with winter impoundments (Fisher, 1976: Smolowitz et al., 1992; Prince et al., 1995). Bacteria were the most common organisms identified on the surface of the lesions (Smolowitz et al., 1992).

Burnt spot, or rust spot shell disease appears as individual, circular, blackened lesions at various locations on the body, and is attributed to invasions by several different fungi (Stewart, 1980; Burns et al., 1979) and/or bacteria (Rosen, 1970; Sindermann, 1979). Burnt spot has been reported in American lobsters in offshore canyons at prevalence's up to 8 % in some areas (Ziskowski et al. 1996) and may be similar to early forms of impoundment shell disease. It is thought that many of the lesions seen in this disease begin at the pores and pits in the carapace (Mallory, 1978). Researchers have suggested that burn spot shell disease could be used as an environmental health indicator as it is commonly associated with pollution (Ziskowski et al., 1996; Weis et al., 1987).

Shell disease of inshore, wild populations of lobsters has existed at low to rarely moderate levels in the *H. americanus* population for decades. Severe erosive epizootic shell disease affecting the carapace of *Homarus americanus* was first noted approximately 8 years ago. The range of this epizootic form of shell disease originally extended from eastern Long Island Sound to the near shore waters of southeastern Massachusetts. Ongoing surveys (Castro and Angell, 2000; CT Dept of Environmental Protection Bureau, 1999; Estrella 1991) found that the prevalence of shell disease in 1996 and 1997 was relatively low (0-5.6%) in southern New England. Yet by the year 2000, the percentage of diseased lobsters approached or exceeded 20% in near shore coastal areas from southern Massachusetts to eastern Long Island Sound. Percent of affected animals in Rhode Island rose to 42.9% by the year 2001. Areas with severely diseased populations were localized to Rhode Island and eastern Long Island Sound (22.7% in 2001), and Buzzards Bay (11.6% in 2001). Recently, still low, but possibly increased, numbers of animals effected by the disease have been seen in Cape Cod Bay and more northern waters along the coast of Massachusetts. Most recently, high levels of affected lobsters were noted in Kittery, Maine.

In order to better understand this disease, we evaluated the epizootic shell disease seen in lobster populations from along the coast of New England using gross and microscopic methods. Bacteria sampling was also conducted as part of this study and is presented in a separate paper.

Sampling:

American lobsters (*Homarus americanus*) with lesions representative of epizootic shell disease were collected for sampling from areas reported to contain high prevalence's of shell diseased lobsters. Twenty-five animals were collected from Eastern Long Island Sound, Buzzards Bay, MA, and Vineyard Sound, MA Lobsters were held in flowing seawater for one to two weeks before necropsy. Necropsies occurred on April 5, 2001 and June 29, 2001. As a part of A. Hsu's Masters thesis work, an additional 27 lobsters were sampled from various locations along the New England Coast line from May 2002 to August 2002. They were kept cool and were necropsied immediately upon arrival at the laboratory.

Necropsy:

At necropsy, each animal was evaluated for lesion occurrence and severity using the methods develops By Bruce Estrella, MA Dept. of Marine Fisheries (Estrella 1991; Estrella, Lobster Shell Disease Workshop, Millstone, CT, June 15, 2000). Digital photographs were acquired of the dorsal carapace of each lobster necropsied. One half of the carapace with underlying connective tissues was carefully removed and fixed in 10% formalin in seawater. Additionally, samples of lesions on other areas of the carapace such as the dorsal abdomen and rostrum were removed, with the attached underlying epithelium and connective tissues, and fixed immediately. Internal organs were examined grossly and any abnormalities were noted. Internal tissues selected for histological examination included any potential abnormalities in any organ and sections of gonad, hepatopancreas, kidney, neural cord, gill, pyloric stomach and abdominal muscle.

Processing Tissues:

After fixation of at least 1 week, the carapace samples were decalcified in a formic acid solution. All tissues were trimmed to an appropriate size for processing and were processed into paraffin blocks. Six µm sections were cut from each block and stained with hematoxylin and eosin stains (Bullis and McCafferty 1995). Sections of selected blocks were also stained with tissue Gram or Gomori Methalamine silver stain (Luna 1968).

Microscopic Evaluation:

Lesion appearance/progression was evaluated microscopically and divided into stages. Other tissues of the body were histologically evaluated for occurrence of any lesions or other abnormalities. Normal appearing portions of the hard carapace from the same lobsters with lesions and sections of hard carapace from unaffected lobsters were used as controls for this work. Normal carapace in C4/DO is composed of several layers (Aiken 1980) (Fig. 1). The outermost layer, the epicuticle, is divided into the cement layer (deposited post ecdysis by the tegmental glands of the subcutis) and a wax layer,

which is deposited just before ecdysis by secretions from the epidermal cells. Both an outer and an inner porous trilaminarate make up the internal portions of the epicuticle.

The wax secretions, thought to be produced by underlying epithelial cells, arrive at the surface by way of the wax canals. Wax canals are small tributaries that originate from larger pore canals. Pores canals transverse the inner layers of the cuticle. Both wax and pore canals are formed by the epidermal cells in a vertical fashion as the cuticle is produced.

The exocuticle is the only other layer produced before ecdysis (but calcified after ecdysis). The layers internal to the exocuticle consists of the calcified endocuticle, uncalcified endocuticle and finally the membranous layer. These are laid down in a sequential manner after ecdysis (Aiken 1980). The uncalcified endocuticle is laid down in C2/C3 and the membranous layer is secreted in stage C4. The membranous layer is absorbed before ecdysis allowing separation between the old carapace and the newest portions of the new carapace. All of these layers (except the epicuticle) can be simplistically described as being composed of a woven, spiraling lattice of chitin crystals within which a protein matrix is deposited. The configuration of the chitin crystals and the types of protein forming the matrix between the chitin crystalline lattice varies within the layers of the carapace. Importantly, the entire carapace is laid down between molts by the simple columnar epithelium that underlays the cuticle.

The inner epicuticle contains extracellular dihydrophenols, which were deposited as it was produced. Phenol oxidases (also deposited during formation in the epicuticle as an extracellular enzyme) can oxidize the phenols resulting in "tanning" of the cuticle (forming a melanized brown/black hardened cuticle) when it is scratched or abraded. Melanizing proteins are also present to varying extent in the exocuticle and calcified endocuticle of the carapace (Neville 1975). Melanization of inner layers of cuticle occur by deliverance of substrate and the phenol oxidase activator through the pore canals (Unestam and Ajaxon 1976).

Gross Morphological Findings:

Grossly, epizootic shell disease was characterized by irregular dorsal midline erosions into the carapace of the cephalothorax (Fig. 2). In severe cases, lesions extend laterally and irregularly from the midline to cover the opercula and/or extend along, and laterally from, the dorsal midline of the abdominal segments. Lesions affected the hard portions of the carapace and not the arthrodial membranous joints that separate them. Rarely, lesions were noted on the claws and on the ventral carapace. Erosions were characterized grossly by brown/tan/black, irregular, granular surfaces. Granular tissues at the base of the erosive lesions varied from firm/hard to rubbery to soft and thin in texture. Underlying internal soft connective tissues were not seen from the surface of the lesions in almost all cases.

Microscopic Findings:

Evaluation of lesions from all animals shows a progressive pattern of erosion formation from the surface into the deeper layers of the carapace. Most of the animals examined in this study were in C3 or C4/DO stages of the molt cycle.

Erosions were microscopically grouped into three general categories based on depth of the erosions. Associated characteristic inflammatory responses were determined

for each category. True ulceration of the carapace (total destruction of the carapace and its epithelium with exposure of the underlying connective tissues) was very rarely identified.

Microscopic Erosion Classification:

Category 1: Shallow epicuticular and exocuticular lesions.
　　　　Shallow erosions in the epicuticle and exocuticle showed either multifocal shallow to deep pitting erosions which extended from the surface into the underlying, normal appearing exocuticule.   Most commonly, the leading edge of the infection appeared to extend from the surface erosions into the exocuticle through the cuticular pores/wax canals rather than setal canals or tegmental gland ducts (Fig. 3).
　　　　The erosions edges were brown/gold in color, which indicated activation of the melanistic (phenolic) inflammatory response. Bacteria often appeared as colonies (mostly appearing as stacks of short rods) were commonly noted within the erosions of the crystalline chitin lattice and were at the leading edge of the lesions.

Category 2: Moderately deep erosions into the calcified endocuticle (Fig. 4)
　　　　Moderate lesions consisted of erosions into the calcified endocuticle. Bacteria, often present in large amounts, were the primarily organisms found at the leading edge of the lesions. The bacteria invaded the carapace by replacing the protein matrix between the lattice crystals forming "pillars" of remaining lattice that projecting from the floors (i.e. leading edge) of the lesions.
　　　　The underlying cuticular epithelium was hyperplastic and hypertrophic. Moderate numbers of inflammatory cells consisting of both granular and agranular hemocytes were present in the underlying connective tissues.   In animals in C4/DO, a membranous layer of varying thicknesses (up to 160 mm) composed of pale, eosinophilic lamellar tissue, similar to the uncalcified cuticle/membranous layer, was produced by the cuticular epithelium and was present between the epithelium and the older uncalcified endocuticle/membranous layer. This *de novo*, inflammatory cuticle was not present in tissue sections of adjacent normal carapace or in areas with mild carapace erosions.
　　　　The most commonly identified secondary organisms in the category two erosions were small protistans. When present, histologically, this organism appeared to invade after, or (in low numbers) with the bacteria. In rare cases and in some areas of erosion in individual animals, the organisms appeared to invade ahead of the bacteria once the bacteria had initially eroded the epicuticle and portions of the exocuticle. The protistians, and other secondary organisms, disrupted the eroded remaining crystalline lattice producing a Swiss cheese-like effect.

Category 3: Severe deep erosions into the uncalcified endocuticle/membranous layer (Fig. 4).
　　　　Erosions in this category were characterized by extension of the lesions into the deeper uncalcified layers of the endocuticle with loss of most of the overlying carapace. Crystalline lattice pillars, in general, were not present on these severely eroded and melanized surfaces. Inflammatory cuticle was often present in these deep lesions and, in some cases, was the only tissue remaining between the cuticular epithelium and the

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external environment. The cuticular epithelium was hyperplastic and hypertrophic and large numbers of both granulocytic and agranulocytic hemocytes were often present in the sub-epithelial connective tissues. No pathogens were identified in the inflamed connective tissues.

Ulceration:
Ulcerations of the carapace where characterized by total loss of all cuticular material and the cuticular epithelium, thus exposing the connective tissues of the body to the environment. In such areas hemocytic infiltration was extensive. Degranulated hemocytes at the surface of the wound formed a melanized pseudomembrane over the exposed connective.

Internal Lesions
In only 3 animals contained internal lesions. These lesions were noted in the connective tissues distant from the carapace erosions. No other pathogens were noted histologically in any lobster examined.

The Cause of Shell Disease:
The predominate organisms seen at the leading edge/interface between degraded carapace and underlying intact carapace were bacteria. This finding was most prominent in tissues from animals examined in the early spring. Abundant colonies of bacteria (mostly appearing as stacks of short rods) were noted on the surface, and within deep erosions into the crystalline lattice of the carapace. Tissue gram stains showed that the bacteria were gram negative.

Other organisms identified in the lesions including free-living nematodes, filamentous algae, large and small protozoa, and barnacles. The second most commonly identified secondary organisms were small protistans. These were associated with portions of the lesions in many of the animals and occurred in eroded carapaces of animals from all areas sampled (including Maine). They were commonly associated with animals collected in the later periods of the spring and in the summer. Unlike bacteria, which were found at all levels of erosion, secondary organisms were very rarely found in the lesions when the lesions extended into the uncalcified endocuticle or membranous layers. These secondary organisms (especially the small protistans), while not primarily responsible for the lesions, did contribute, sometimes markedly, to the ongoing degeneration of the epi- and exocuticles of the infected carapaces.

Molting of Eroded Carapaces:
Histological examination of one animal in molt showed that the animals could form a new epicuticle and exocuticle internal to the eroded carapace and the associated inflammatory cuticle when it occurred.   Histological sections showed a lytic space did form between the inflammatory cuticle and the new epicuticle thus allowing for molt to occur.

Conclusions:

Epizootic shell disease may represent an extreme presentation of enzootic shell disease. But, epizootic shell disease is characterized by severe irregular deep erosions that appear to begin at the dorsal midline of the cephalothorax, primarily affecting the cephalothorax, the abdominal segments and the rostrum. Other portions of the animals carapace were affected but to a lesser extent. Arthrodial membranes (joints) were rarely affected. Interestingly, at the histological level the carapaces appeared normal (lesions did not result from poorly formed carapace) and the disease resulted from invasion of the carapace from the outer surface (not from an internal disease or abnormality). True ulceration was rare.

Bacteria were identified as the primarily cause of the carapace erosions. The occurrence of pillars composed of chitin crystalline lattice formed by removal of the matrix proteins and lipids suggested that the invading bacteria were not primarily chitin feeders, but were attracted to the matrix proteins and lipids. Secondary organisms were noted with the lesions, but were either rare, or when commonly present, invaded primarily in areas of previously bacteria infection. However, and potentially importantly, secondary infections did contribute to the erosive event, at least in the epicuticular and exocuticular and calcified endocuticular layers.

The production of the inflammatory cuticle in combination with the stubble of the remaining eroded original cuticle resulted in the soft rubbery feel of the pitting erosions. The inflammatory, hemocytic response was abundant in erosions classified as categories 2 and 3, and demonstrated that the hemocytic response was functioning well in these animals. The lack of any abnormalities in other organs of the animals body indicate this disease may be due to infection of the carapace by bacteria that do not survive in soft tissues.

The reasons for the occurrence of epizootic shell disease are still not understood. Environmental factors may play a roll. Certainly bacteria have been identified as the cause of shell disease in previous work (Smolowitz et al. 1992; Fisher 1988; Getchell 1989; Malloy 1978). The potential for acceleration of the growth of bacteria on the shell possibly due to increased environmental temperature, vs. the ability of the lobster to remove such bacteria effectively, many are important. The primary locus of the initial lesions (dorsal cephalothorax) occurs in an area where the lobsters may be less able to effectively clean the carapace. Also, it is possible that increased temperatures may slow down the lobsters cleaning response (thermal stress).

The bacteria colonizing the surface of the carapace and causing the lesions may be more aggressive than the normal flora that resides on the surface. Such changes in pathogenicity might occur through plasmid or phage transfer between bacteria. Changes in environment might also produce increased invasions by secondary organisms resulting in increased degeneration of the superficial layers of the eroded carapace. While the carapace and its inflammatory response appear normal in the histological sections, it is possible that at the molecular level there may be an abnormality in formation of the cuticle (especially in the laying down of the protein/lipid matrix) or in melanizing responses). Any one or a combination of these possible causes could be at work in this disease. These possibilities warrant further study, not only to answer the important questions about shell disease in lobsters but to better understand how changes in the marine environment interact with an animal, such as a lobster, to cause disease.

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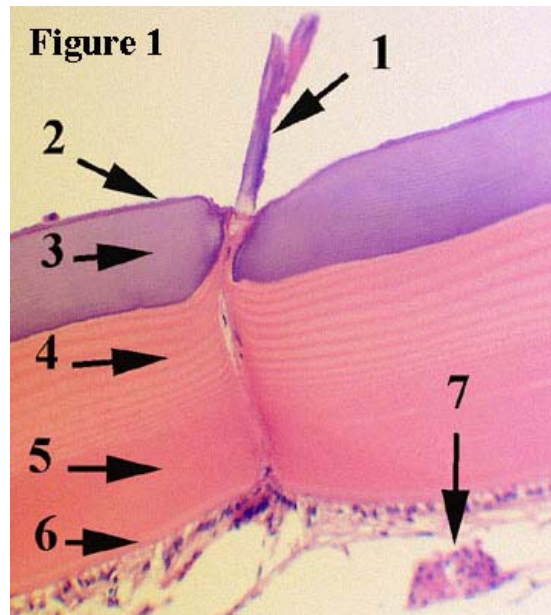
Figure 1: Photomicrograph of a histological section of normal lobster carapace in stage C4. (1, Seta; 2, epicuticle; 3,exocuticle; 4, calcified endocuticle; 5, uncalcified endocuticle; 6, membranous layer; 7, tegmental gland) (6 µm paraffin section, hematoxylin and eosin stain, 25x).
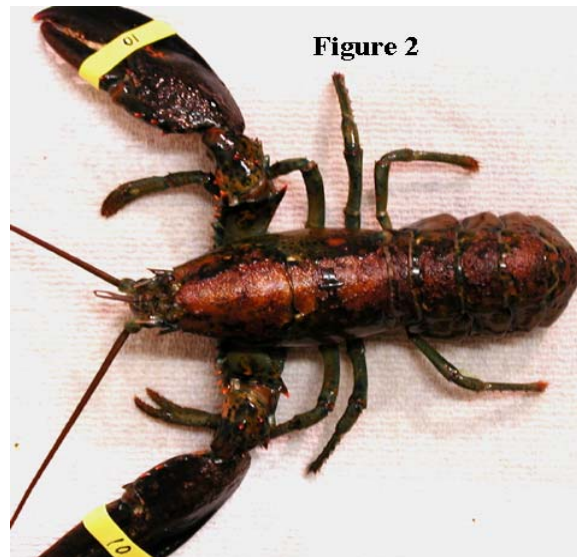
Figure 2: An American lobster with severe epizootic shell disease.
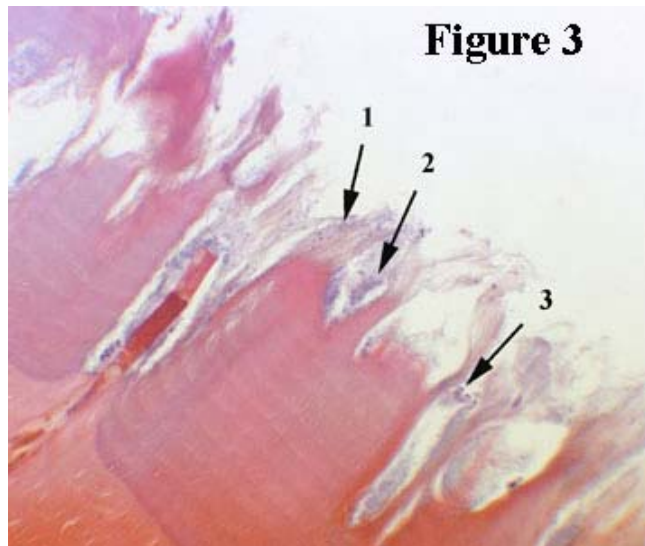
Figure 3: Epizootic Shell Disease, Category 1: Erosions into the epicuticle. Pillars of chitin latice are observed (1). Erosions occur through pores (2) and setal canals. Bacteria are present at the leading edge of the lesions (2). Rarely other organisms, such as protistians are also present in the lesions (3). (6 µm paraffin section, hematoxylin and eosin stain, 400x).
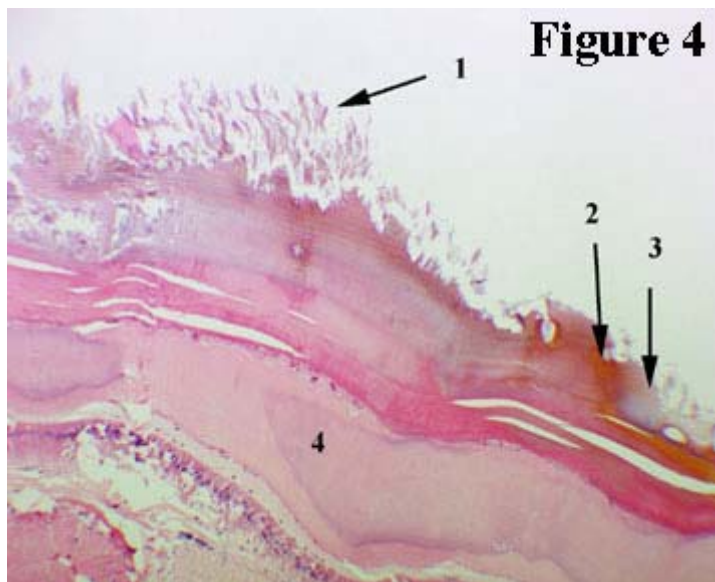
Figure 4: Epizootic Shell Disease, (A mixed category lesion) Category 2: Erosions extend into the calcified endocuticle forming "pillars" of chitin crystalline lattice (1) and invade down pores causing melanization (2). Sections of the lesion show loss of the calcified endocuticle and exposure of the melanized ucalcified cuticle (3). Necrotic debris and hemocytes are noted between the old uncalcified cuticle/membranous layer and the new inflammatory cuticle (4) (25x). (6 µm paraffin section, hematoxylin and eosin stain, 100x).

A microbiological assessment of epizootic shell disease in the American lobster indicates its strictly dermal etiology

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Introduction:

        Epizootic shell disease is a newly recognized disease of the America lobster, *Homarus americanus.* (Smolowitz et al., 2005a; Smolowitz et al., 2005b). It is distinctly different in its pathology, epidemiology and etiology from another shell disease of the American lobster, impoundment shell disease (Smolowitz et al., 1992). Shell diseases, which have been described for other species of *Crustacea*, appear to be more similar in pathology and etiology to impoundment rather than epizootic shell disease (Bullis et al., 1988; Noga et al., 1994; Goarant et al., 2000; Porter et al., 2001; Vogan at al., 2002). Our earlier investigations indicate that bacteria belonging to *Flavobacteriaceae* and perhaps unknown representatives of α-proteobacteria, rather than representatives of *Vibrio* spp., are likely culprits of epizootic shell disease (Chistoserdov et al., 2002; Chistoserdov et al., 2005). *Vibrio* spp. are isolated from epizootic shell disease lesions only occasionally, whereas members of this genus are routinely isolated from and suggested to be culprits of impoundment shell disease is the American lobster (Fisher, 1977; Malloy, 1978; Stewart, 1980 and Getchell, 1989) as well as shell diseases of several other species of *Crustacea* (Bullis et al., 1988; Noga et al., 1994; Goarant et al., 2000; Porter et al., 2001; Vogan at al., 2002).

        As with most other infections diseases, interplay between the environment, the host and the pathogen (Tlusty, this volume) likely plays a key role in the epizootic shell disease epidemics. The environmental reasons for its emergence or re-emergence are not clear, but speculations suggests that a general warming of seawater in Long Island Sound and adjacent embayments may put stress on the lobster population (Wilson et al., 2003). A thermal stress, in turn, may effect the ability of lobster immune system to withstand an infection. Thus, epizootic shell disease appears to be a newly emerging or re-emerging wildlife infectious disease connected with the global change.

        It is possible that in addition to bacteria, which directly attack shell of lobsters, additional internal pathogens may weaken the immune system of lobsters preventing effective defense against lobster epizootic shell disease. If such pathogens existed they would likely to cause a latent and lethal infection. Therefore, two groups of pathogens must potentially be considered: cuticular or dermal pathogens, which directly cause

lesion formation, and internal (most likely hemolymph) pathogens or parasites, which affect the general health of lobsters.

The goal of this paper is to review our present knowledge of the role, which microorganisms may play in the etiology and epidemiology of shell disease, and also to present the findings of our laboratories on characterization of microbial communities associated with diseased lobsters. So far, we have failed to detect any concomitant internal infection in lobsters with epizootic shell disease implying that the pathogenesis of the disease is strictly dermal.

METHODS
Sampling:

The data presented here are based on analysis of microbial communities in lesions and hemolymph of 14 lobsters collected from eastern Long Island Sound (ELIS), 5 lobsters collected from the coastal waters of central Long Island Sound (CLIS), 10 lobsters collected from Buzzards Bay (BB) and 10 lobsters collected at Kittery, Maine (KME). Additional lobsters used in this work were from independent batches and came from Eastern Long Island Sound (CT, n=4), Rhode Island (RI, n=9), Buzzards Bay (MA, n=3,) Cape Cod Bay (CCB, n=3), Maine (ME, n=4), and offshore waters of New Hampshire (NH, n=5) and were used only in some experiments. Seven additional lobsters from Maine, three from Kittery and four from Northern Maine and five lobsters from CLIS, which appeared "healthy", were used as "negative" controls. Microbiological material form lesions and healthy carapace surfaces was collected by scraping with a sterile razor blade and suspended in sterile seawater. Hemolymph was drawn directly from hearts of each lobster into sterile Vacutainer® tubes with anticoagulant and refrigerated or immediately plated on Petri dishes with appropriate media.

Abundances of bacteria were determined according to Taylor et al. (1986) using an Olympus BX51 epifluorescent microscope.

Culture-dependent microbiological analyses of microbial communities in shell lesion material:

Four media were tested and Difco™ Marine Agar 2216 (MA; Becton-Dickinson, MD) and Seawater Agar II (SAII; seawater with 1.7% of agar, 0.1% peptone, 0.01% Tween 80 and vitamin mix) were selected for the routine culturing. Serial dilutions of lesion material were prepared in sterile seawater and plated on Petri dishes with the two media in triplicates. Plates were incubated at room temperature (22°C) and growth observations were made every 24 hours. Individual colonies were picked with sterile toothpicks and re- streaked several times on new plates with appropriate media to isolate/verify that pure cultures were obtained.

Culture-dependent analysis of hemolymph samples:

Five µl and 100 µl of hemolymph were plated on two sets of Petri dishes containing Brain Heart Infusion Agar with 10% sheep blood or Rabbit Blood Agar (both from Hardy Diagnostics, CA) and incubated at two different temperatures, 22°C and 37°C. Growth observations were made every 24 hours.

16S rRNA analysis of bacterial isolates:

DNA from isolated bacteria and lesion were purified using the procedures by Marmur (1961) and Xu and Tabita (1996), respectively. The 16S rRNA gene from isolates was amplified using either the primers fD1 and rP3 with modifications (Weisburg et al., 1991) for RFLP analysis or the primers 503F and 1494R for sequencing as described by Borneman and colleagues (1996). On average a 700 bp portion of 16S rRNA gene was sequenced (approximately bases 600 through 1300, *E. coli* numbering). Searches for closest phylogenetic neighbors were carried out in the RDP and GenBank databases. The phylogenetic trees were constructed using the PHYLIP package, version 3.5c.

Denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the composition of microbial communities from lesions was carried out according to Muyzer et al. (1993).

Infection experiments were carried out at the Flax Pond Marine Laboratory. Groups of healthy lobsters (5 individual each) were exposed to various isolates for 24 hours at 14°C. The carapace of two out of the five lobsters in each experiment was mechanically breached. In a separate experiment, four healthy lobsters (epicuticle of two of them was mechanically damaged) have also been kept in the same tank with two lobsters with shell disease. Exposure experiments were run for close to six months.

Result and Discussion:
Healthy carapace surfaces have much fewer bacteria than lesion surfaces:

Microbiological material was collected from lesions and healthy surfaces of four lobsters from CLIS and numbers of bacteria in this material was compared using two methods. First, bacteria were enumerated by plating on MA plates. The number of colony forming units per 1 cm^2 of the lesion surface was 6×10^9, whereas the number of colony forming units per 1 cm^2 of the healthy carapace was only 4×10^5. Total DNA was also isolated from lesion and healthy carapace samples of four lobsters. Figure 1 shows agarose gel electrophoresis of this DNA. A photograph of the gel was scanned and quantities of DNA determined. No DNA can be isolated from healthy carapace surfaces of one of the lobsters (line 7 in Figure 1). DNA from healthy carapace in lines three, five and nine (Figure 1) was visible in the gel, but the quantity was two orders of magnitude lower than in respective lanes loaded with DNA isolated from lesions (i.e., lanes 2, 4 and 8). Thus, both methods indicate that the number of bacteria present on the healthy carapace is substantially lower than that of the lesions, although the difference varies from two to four orders of magnitude, depending on the method.

Similar experiments were carried out with lobsters collected during the 2003 outbreak of epizootic shell disease in Kittery, Maine. DNA was successfully isolated from all 13 lobsters (i.e., 10 diseased and 3 "healthy") and only for one "control" lobsters the DNA yield was noticeably lower. No DNA could be isolated from carapaces of three out of four "control" lobsters from a northern location in Maine. A likely interpretation of these data is that lobsters, which appear healthy but contain bacteria on the carapace in quantities comparable to those of lesions, are in initial stages of shell disease development.

Epizootic shell disease lesions contain a very simple community:

Two types of media, MA and SAII, were used to isolate bacteria from lesions of ELIS, CLIS and BB lobsters. Bacterial colonies were separated into groups based on colony morphology and counted. The numbers of colonies recovered on SAII and MA were equal and were approximately 2-20x10^9 cells per cm^2 for lesions and 2-30x10^5 per cm^2 for healthy carapace surface. Bacteria colonies from lesions fall into 5 to 9 morphological classes. At least two morphological classes, attributed to *Pseudoalteromonas* spp. and a bacterium belonging to *Flavobacteriaceae* were present in all animals from LIS and BB samples, however, only *Pseudoalteromonas* spp. were the dominant in the CLIS lobsters. The colonies of *Pseudoalteromonas* and the *Flavobacteriaceae* isolates from CLIS lobsters appeared identical to those recovered from BB and ELIS lobsters. RFLP and 16S rDNA-sequencing analyses confirmed that all *Pseudoalteromonas* isolates are identical and likely belong to strains of one species, *Pseudoalteromonas gracilis*. The *Flavobacteriaceae* isolates were related to several related genera (*Lacinutrix* sp., *Psychroserpens* sp., *Aquimarina* sp.) and, thus, we proposed to name them the "lobster *Flavobacteriaceae* species complex" (Chistoserdov et al., 2005).

We also attempted to cultured chitinolytic bacteria from the ELIS and BB lobsters using selective media containing crude chitin powder from crab shells. Petri dishes with SAII medium were overlaid with 5 ml of "soft" or "hard" chitin suspension. Generally, shell lesion material from most lobsters contained chitinolytic bacteria, albeit their counts were six orders of magnitude lower than the total viable count of bacteria. Isolation of pure cultures of chitinolytic bacteria was difficult, due to a gliding motility of non-chitinolytic bacteria and a long time response in the development of positive reactions (sometimes up to two weeks). However, we managed to isolated pure cultures of chitinolytic bacteria from five lobsters one of which was from ELIS and four from BB and one horseshoe crab. They also fall into two groups: the lobster *Flavobacteriaceae* species complex (*Aquimarina* sp., *Cellulophaga* sp.) and *P. gracilis*.

Diseased and healthy lobsters from Kittery, Maine, were analyzed in a similar fashion. Bacteria colonies from lesions fell into 3 to 7 morphological classes. DNA was isolated from 47 isolates, which represent all individual colony morphologies from all 13 lobsters. The isolates were grouped using RFLP analysis of 16S rDNA products with *Hae*III, *Hin*fI and *Hha*I. A portion of 16S rRNA gene was sequenced for a representative of each group. All other members of each group were assumed to have identical 16S rDNA sequences. Sequence information allowed us to identify the isolates. One morphological class of colonies with two slight variations was attributed to the lobster *Flavobacteriaceae* species complex and was isolated from each single diseased lobster and from one healthy lobster. Bacteria belonging to this complex were loosely related to each other and shared 16S rRNA similarities with *Aquimarina*, *Cytophaga*, *Glaciecola*, *Flexibacter*, and *Leucobacter* spp. as well as 16S rRNA sequences from uncultured members of *the Cytophaga-Flexibacter-Bacteroides* clade. Additional isolates were related to *Vibrio* spp. (three animals) and *Alteromonas marina* (one animal). Bacteria isolated from carapaces of one of the three healthy animals were related to an Antarctic bacterium R-9217 and the - to *Bacillus horikoshii*. Two "healthy" animals contained bacteria identical to those found in lesion of diseased lobsters, albeit in lower numbers.

Unlike lesion material from LIS lobsters, not a single *Pseudoalteromonas* spp. isolate was found in the Maine lobsters.

DGGE analysis of microbial communities in lesions generally supported our cultivation experiments. Figure 2 shows that the composition of microbial community is quite simple. Only two bands are present in lesions of all lobsters and are indicated in the figure with letters A and B. DNA in band A belongs to a member of the *Flavobacteriaceae* family, which is closely related to the *Flavobacteriaceae* chitinoclastic isolates. DNA in band B belongs to as yet uncultured α-proteobacterium.

Hemolymph of lobsters with shell disease may contain bacteria but there is no correlation between shell disease and bacterial hemolymph infection.

Cultivation of bacteria from lobster hemolymph was successful for nine diseased lobsters out of 51 lobsters tested (both diseased and "healthy"). We never detected the presence of bacteria in hemolymph of "healthy" lobsters by cultivation. Only one lobster was heavily infected, with bacterial counts exceeding 2×10^6 bacterial cell of *Brochothrix thermosphacta* per one milliliter of hemolymph. In all cases, each lobster harbored a bacterium of only one type. Same type bacteria were isolated only from two lobsters from the same batch. We detected bacteria belonging to *Corynebacterium jeikeium*, *C. fastidiosum*, *B. thermosphacta*, *Pseudomonas fragi*, *Pseudomonas* sp. and four as yet unidentified bacteria. It is interesting that bacteria encountered in relatively larger numbers in the hemolymph are common spoilage bacteria (*B. thermosphacta*, *Pseudomonas fragi*, *Pseudomonas* sp.), whereas rare isolates (*C. jeikeium*, and *C. fastidiosum*) are human communal/opportunistic pathogens.

Shell disease cannot be easily transmitted from one lobster to another lobster, additional environmental factors or stressors are required:

A series of infection experiments were conducted at the Flax Pond Marine Laboratory. Groups of healthy lobsters (5 individual each) were exposed to isolated *P. gracilis* and individual chitinolytic isolates strains (10^6 cells of each per liter of seawater) for 24 hours at 14°C. The carapace of two out of the five lobsters in each experiment was mechanically breached. To elucidate transmittance of shell disease, in a separate experiment, four healthy lobsters (epicuticle of two of them was mechanically damaged) have also been kept in the same tank with two lobsters with shell disease. Duplicate experiments were run with combinations of *Pseudoalteromonas gracilis* and strains belonging to the lobster *Flavobacteriaceae* species complex. In tanks 1 and 2, healthy lobsters from CLIS were exposed to *P. gracilis*, strain 19b1white and *Cellulophaga baltica* 11a2. In tanks 5 and 6, healthy lobsters from CLIS were exposed to the same *P. gracilis* isolate and *Aquimarina mulleri* 18a. In tank 8a separate experiment was run wherein healthy lobsters (both not damaged and with mechanically breached carapace) were housed with lobsters from CLIS exhibiting severe shell erosion in the carapace and tail. Tanks 3, 4, and 7 served as controls in which no pathogens were introduced to the system. There was no lesion development on any of the healthy lobsters during the 6 months of incubation. An additional infection experiment was carried out at UL Lafayette at room temperature (22°C). Unfortunately, lobsters died within few days without developing of infection.

Conclusions:

- Epizootic shell disease is different in its pathogenesis, etiology and epidemiology from other shell diseases in *Crustacea* including impoundment shell disease of the American lobster.
- Microbial communities found in lesions of lobsters from different locations appeared to be similar to each other. Several related bacteria belonging to the lobster *Flavobacteriaceae* species complex were isolated from lesions of every studied lobster. They can also be present on carapaces of some but not all "healthy" lobster (a prelude for infection?).
- No correlation has been found between the numbers and activity of chitinoclastic bacteria and the incidence of epizootic shell disease as well as between epizootic shell disease and hemolymph infection.
- PCR-DGGE analyses of the microbial communities from lobster lesions generally confirm our culture-dependent data. 16S rDNA sequences from the lobster *Flavobacteriaceae* species complex and an unknown α-proteobacterium were successfully amplified from all lesions.
- Groups of healthy lobsters were exposed to isolated *P. gracilis* and two species belonging to the lobster *Flavobacteriaceae* species complex. No transmission of the disease was detected suggesting that transmission of epizootic shell disease in lobster populations, in addition to the presence of the pathogen, depends on one or several as yet unidentified environmental factors

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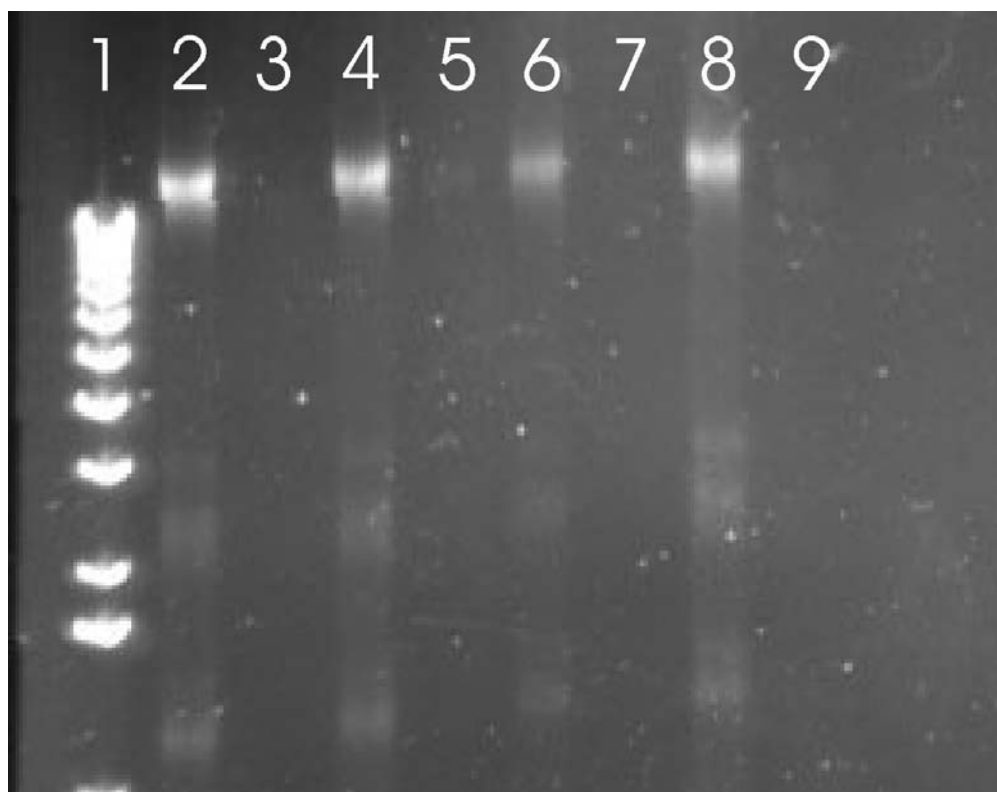
Figure 1: Agarose gel electrophoresis of DNA isolated from epizootic shell disease lesions (lanes 2, 4, 6 and 8) and healthy carapace surfaces (lanes 3, 5, 7, 9).
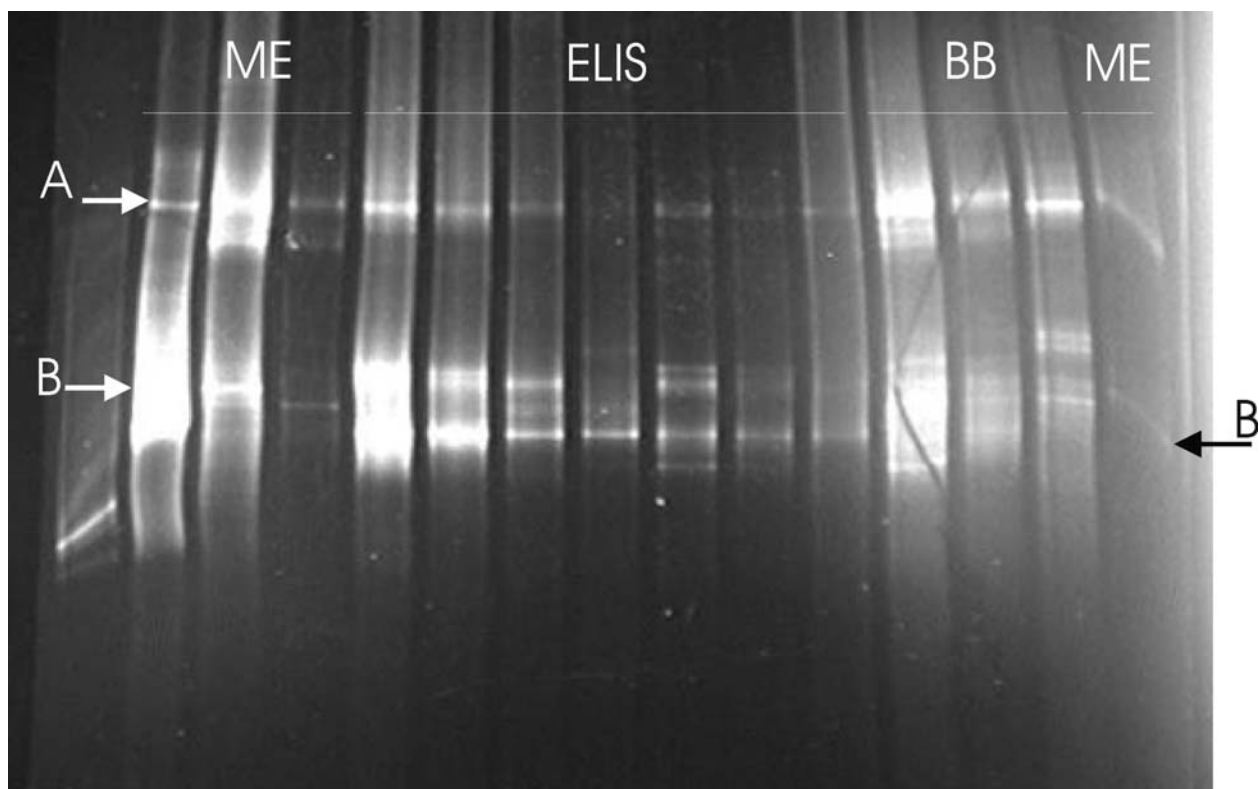
Figure 2: DGGE of 16S rDNA from microbial communities found in lesions of representative lobsters from various locations. ME - Kittery, Maine; ELIS - eastern Long Island Sound; BB- Buzzards Bay. For A, and B see explanation in text.

Chapter 2 Causes of Disease

During the discussion period, the panel discussed the following questions, and the discussion was transcribed and is presented following the submitted papers.

1. **Are the bacteria present at the leading edge of the lesions a specific species/strain or can similar species/strains cause the same lesions?**

2. **Have changes in the environment increased the occurrence of pathogenic bacteria in the water and/or on the lobster surface?**

3. **What is the role of viral phages or plasmids in increasing the pathogenicity of shell disease bacteria?**

4. **Are there other pressures (such as amoebic grazing, or inability of the weak/heat stressed lobsters to clean the dorsal carapace) that promote the growth of the bacteria?**

5. **How do these lesions compare with lesions in other animals caused by similar species/strains bacteria?**

The lobster back biofilm: possible role of the total microbial community in lobster shell disease

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The community of microbes (biofilm) on the lobster carapace is a complex one, and few of its components have been investigated in any detail. We undertook a preliminary investigation of these microbes on healthy and shell-diseased lobsters in Maine, funded by the Maine Department of Marine Resources.

In July and August of 2004, student intern Ms. Glorya Laughton (University of New England) traveled with DMR personnel, particularly Mr. Mark Gosselin, to lobster landing sites throughout Maine, and sampled haphazardly-selected animals for amoebae and other microbes in conjunction with DMR dock surveys. Sampled animals were scored for carapace character (soft vs. hard) and for the presence and state of shell disease. No animals with limp lobster syndrome were encountered. The carapaces were swabbed with sterile cotton, and the swabs were streaked onto agar plates and returned to the Bigelow Laboratory for cultivation of the associated microbes. Strain isolation and cultivation procedures were conducted primarily by technician Ms Wendy Bellows.

From these plates, some 70 strains of microbes have been obtained, of which at present 42 are being retained for further work. Most of these strains are amoebae with cells less than 10 micrometers in length/diameter (Fig. 1). From their growth characteristics in culture, we estimate that there are at least 15 species of amoebae represented in the cultures, most of which are new to science (Fig. 2).
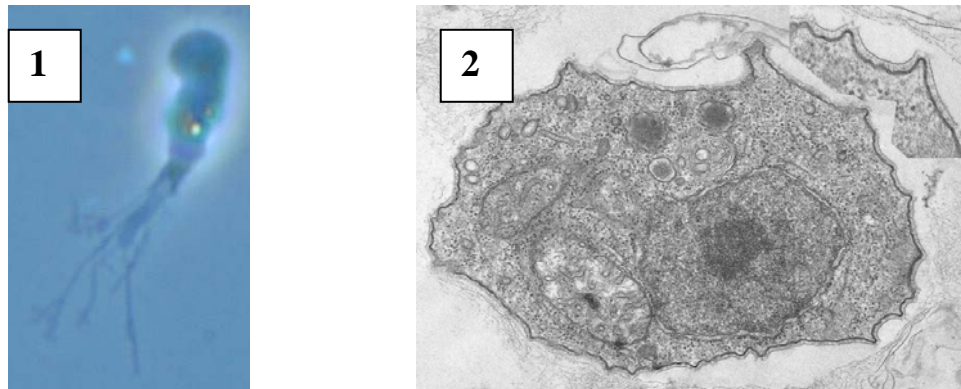
Figure 1: Unidentified amoeba, strain 5-5A1, from a stage-2 shell disease lobster. The cell is 10 micrometers long, excluding the fine threads ("uroidal filaments"), which are not recorded from known amoebal species.

Figure 2: Electron micrograph of this amoeba. The surface coat (inset) is not recorded from known amoebal species.
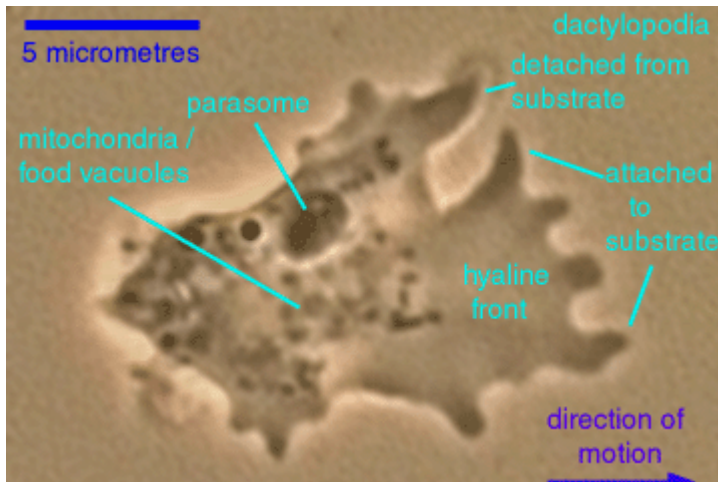
Figure 3: *Neoparamoeba pemaquidensis*: a single cultured cell as it appears under the microscope when attached to a surface and in motion.

The most commonly observed identifiable amoeba in the cultures was *Neoparamoeba pemaquidensis*. This species causes amoebic gill disease in Atlantic salmon and other marine finfish, especially in aquaculture settings, and a wasting disease of sea urchins, and there is strong evidence to suggest that it is the cause of the "limp lobster syndrome" resulting in the collapse of the lobster fishery in western Long Island Sound. Most strains of this species are harmless, eating bacteria not lobster. We know from the salmon and urchin disease systems that *N. pemaquidensis* can switch from being harmless to being pathogenic, but we do not know what causes this switch. We now know that *N. pemaquidensis* is common and intimately associated with lobster carapaces, at least during the summer months, in the Gulf of Maine.

Many of the amoeba strains we have isolated will burrow into agar, indicating that they can break down complex polysaccharides. We have also observed this behavior in other protists isolated from lobster carapaces, including net slime molds (*Labyrinthula* spp.) and colorless nonphotosynthetic diatoms. The diatoms can use the agar as their sole food (carbon) source. We cannot yet rule out the possibility that these microbes contribute to the initiation of shell disease lesions in some way, perhaps acting in synergy with the bacteria present.

We found:
1) Both the density and diversity of microbes, including bacteria as well as protozoa and algae, was greater on hard-shelled lobsters than on soft-shelled lobsters.

2) The same amoeba species were isolated from animals across the Gulf of Maine; no differences could be traced to geography.

From both healthy and shell-diseased lobsters, we isolated, in addition to the amoebae, several strains of bacteria that were capable of growth on agar to which no significant amounts of organic material (food; carbon) were added. These bacteria fed the amoebae that arose in the cultures. Included among these isolates were several strains that formed yellow colonies. These bacterial strains were inconspicuous on the original plates, being overgrown by other bacterial species. However, when we attempted to

render some of our amoeba strains axenic (no bacteria present), these yellow bacteria frequently survived, and amoebae would not grow in their presence. Most but not all of these yellow bacterial strains have come from animals with shell disease. These cultures appear to represent Flavobacteriaceae (e.g. *Cytophaga* spp.), similar to those isolated from shell disease lesions in southern New England. The molecular identity of some cultures has been investigated in the laboratories of Roxanna Smolowitz (Marine Biological Laboratory) and Andrei Chistoserdov (University of Louisiana, Lafayette). Resistance of *Cytophaga*-group bacteria to protistan grazing has been reported in marine planktonic ecocysystems (Beardsley C et al., *Applied and Environmental Microbiology* 69: 2624, 2003).

Summary:
1) Lobster carapaces support a diverse community of amoebae and other types of protozoa and algae. The density and diversity of this community appears to increase as a function of age of the carapace, but not as a function of location of the sample within Maine; these qualitative preliminary observations need to be quantified, preferably through the development of appropriate molecular-level sampling methods.

2) The amoeba *Neoparamoeba pemaquidensis* is a common constituent of the lobster carapace microbial community. The factors that induce pathogenesis in *N. pemaquidensis* are unknown, but should they be present, the reservoir of infection for amoebic limp lobster syndrome (paramoebiasis) is on the back of the lobster itself.

3) Some of the protozoa and algae on lobster carapaces, including *N. pemaquidensis*, are capable of breaking down complex polysaccharides (i.e., agar). This ability, if it extends to lobster carapace constituents, may play a role in the initiation and/or propagation of shell disease lesions.

4) Some isolated strains of yellow bacteria do not support amoebal growth. These strains may be similar to, or identical with, bacterial strains isolated from shell disease lesions in Long Island Sound and Massachusetts Bay. These observations suggest that there may be a link in nature between a bacterium's ability to escape from protistan grazing and its ability to initiate and propagate shell disease lesions.

Immune functions: importance in disease?

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A lobster (*Homarus americanus*) die-off significantly affected fisheries in the fall of 1999, especially in western Long Island Sound. While the exact cause(s) of the die off are still not precisely known, dead and dying lobsters in the initial phase of the die-off were diagnosed with infections with paramoeba, a newly recognized disease condition of lobsters (Mullen et al. 2004). As the immune system represents the first line of defense against disease-causing agents, and is one of the most sensitive systems to environmental stressors, we recently developed new assays to quantify the immune system of lobsters (De Guise et al. 2005a). Flow cytometry allowed the discrimination of different populations of hemocytes based on their relative size and complexity. Also using flow cytometry, natural killer (NK) cell-like activity and its stimulation by human recombinant interleukin 2 (IL-2) were described for the first time in lobsters, as was the expression of TLR2, a pattern recognition receptor, on granular hemocytes. Apoptosis was also measured for the first time in lobster hemocytes, in higher proportion in non-granular hemocytes than in granular hemocytes. It was also shown that circulating hemocytes failed to proliferate upon stimulation, suggesting that they are terminally differentiated and originate from a separate hematopoietic organ. Overall, several new assays were developed to allow the quantitative evaluation of disease-relevant immune functions. It was also recently shown that relatively low concentrations of diverse pesticides could affect immune functions in lobsters (De Guise et al. 2004, 2005b). Studies in our laboratory also demonstrated the sensitivity of oyster immune functions differences in water temperature and salinity (Goedken et al. 2005). While it is not known if cellular immune functions are involved in the resistance to shell disease, the new assays developed in our laboratory could be relevant for future use in health assessment and effects of environmental changes on health of the American lobster.

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Defining the Etiology of Epizootic Lobster Shell Disease: The Importance of Genetic Investigations of the Associated Bacterial and Viral Ecology

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Introduction:

When insight into management of any disease is sought, whether it be a disease of the American lobster (*Homarus americanus*) like epizootic shell disease or of human beings like AIDS, truly effective measures typically must derive from understanding of disease causation and the associated biology. Epidemiologic concepts of interactions of host, agent, and environment and postulates of causation including Koch's and others (17) must be considered and application of epidemiologic methods typically applied to human disease (28) may also be important. Koch's postulates have been considered previously (http://www.seagrant.uconn.edu/LHN2.PDF) in relation to lobster health issues including the epizootic shell disease of the American lobster that seriously compromises the integrity of the crustacean exoskeleton and has emerged to high prevalence in recent years in lobster populations of Southern New England (10, 48). The diverse speculation regarding shell disease etiology may, in fact, reflect a reality that disease causation is complex. Factors contributing to initiation of disease may not be as readily defined as those most evident at later stages of disease. In the case of AIDS, for example, opportunistic pathogens are almost always the proximal cause of morbidity and mortality, but there are few scientists who doubt that human immunodeficiency virus initiates disease and is the etiologic agent. In developing effective management it has been relevant to understand both infection with HIV and the associated secondary pathogens. Understanding environmental and societal conditions promoting disease transmission globally and that were critical to emergence of the new disease has also been important.

With respect to shell disease, the AIDS example is not used here to make any direct comparison, but rather to suggest that in investigation of any emerging disease there are likely to be many complexities to encounter in understanding the biological and environmental correlates of disease. While a number of important observations have been made, understanding of lobster shell disease is nascent, at best, but it is probable that relevant information for understanding the disease etiology will be derived from a variety of disciplines and perspectives. The viewpoint presented below emphasizes the need to better understand the genetics and interactions of shell disease lesion-associated microbes and their viruses, but is submitted with realization of the likely importance of environmental factors including the anthropogenic climate change that is now evident globally as data of warming penetrating to greater depths continues to increasingly support prominent models of the effects of greenhouse gases (1, and an update presented by T.P. Barnett at the February 2005 American Association for the Advancement of Science Annual Meeting, Washington, D.C.). Anthropogenic changes in climate, ocean salinity, and CO_2 uptake are considered to have many impacts on marine ecosystems including the emergence of diseases in marine environments (22).

The perspective presented below that investigation of microbial and viral ecology will be critical in understanding shell disease etiology assumes that microbial and viral populations and expression of their genomes will reflect the influences of anthropogenic environmental changes. Investigations of bacterial and viral interactions within the microbial assemblages of lobster shell disease lesions are an opportunity to study an emerging disease-associated microbial community and possibly to discern critical genetic and environmental factors in establishment of these consortia. Investigations of microbes grown in pure cultures have provided most of the fundamental knowledge base of microbiology, but pure cultures of organisms grown in suspension culture do not represent the natural environment and growth conditions of the vast majority of microorganisms which are typically found in surface adherent interactive mixed species assemblages and are quite different from their planktonic counterparts in their gene expression and vulnerability to environmental stresses such as antibiotics (5). While microbes comprise more than 60% of the Earth's biomass (with cellulose accounting for another 30%), it is estimated that more than 99% of microbes have not been cultured in the laboratory but are increasingly being studied using genomic technologies (13, 6, 42). Thus understanding microbes in their great diversity and interactions within natural environments together with their great impacts upon natural and human systems is increasingly recognized as a major frontier in the biological sciences (45). Viruses clearly are powerful forces in shaping host genetics and evolution and virology has a rich history of providing tools that illuminate the molecular functioning of cellular hosts (32). It is proposed here that virological studies will be critical to understanding the ecology of the polymicrobial assemblages inhabiting shell disease lesions and may also provide valuable tools for gaining insight into the etiology of the disease.

Background:

The emergent epizootic shell disease, which is histologically distinct from another serious shell disease syndrome frequently observed during winter impoundment of lobsters (47, 23), has remained uncommon in the more northern coastal lobster habitats of the United States and the Canadian maritime provinces but clearly is a potential threat. Initial scanning electron micrographic and histological observations of epizootic shell disease lesions on lobsters have shown exoskeletal erosion by a mixed community of microbes in which prokaryotes predominate (23, 49). Denaturing gradient gel electrophoresis (DGGE) data have shown significant consistency in the species composition of microbial populations in shell disease lesions (12) suggesting that the microbial communities present may be interactive and structured microbial consortia. The study included an effort to demonstrate disease transmission under defined laboratory conditions. Lack of transmission from diseased to unaffected lobsters in this initial study suggests that etiology of the disease may depend upon the interaction of environmental and genetic factors in a complex polymicrobial context.

Interactions of bacteria with bacterial viruses (bacteriophages) in the environment are important in nutrient cycling and in regulating population dynamics of microbial life (59). Epizootic lobster shell disease presents an opportunity to study bacterial and viral interactions within the context of interesting microbial consortia colonizing the exoskeleton of American lobsters. Viruses are the most common biological agents in marine environments (18) and bacteriophages are the most abundant and genetically

diverse biological entities on Earth with tailed phages alone estimated to number up to 10^{31} (4, 40, 44). Bacteriophages are potent agents of horizontal gene transfer and are a major driving force in bacterial evolution (36, 37, 29, 16, 38). The importance of gene transfer events such as acquisition of pathogenicity islands and lysogenic conversion by bacteriophages has been repeatedly demonstrated and bacteriophages are frequently associated with the virulence and toxigenicity of the bacterial pathogens they infect (11, 54, 2, 15, 26, 8, 3, 35).   The genetic differences between *Escherichia coli* K12 and O157:H7, for example, are accounted for by prophage DNA (39). Despite the enormous diversity and ecological importance of viruses in marine and other environments and the powerful influences of bacteriophages on bacterial evolution, investigation of the impacts of viral interactions within sessile microbial communities has not been extensive (55, 24, 25, 20). The many precedents for phage-mediated gene transfer associated with disease suggest that viral interactions with microbes in shell disease lesions warrant investigation. Furthermore, bacteriophages are increasingly being studied for a variety of biological control strategies targeting specific bacteria (46, 50, 33, 52, 31).

Bacteriophages clearly have major impact on the ecological balance of microbial life and in some cases upon bacterial virulence. The ability to facilitate interspecies transfer of bacterial genes by means of phage transduction within both sessile and planktonic prokaryotic populations may be responsible for significant contributions to the diversity and development of these communities (21). The precise roles of environmental conditions in the processes of lysogenic phage induction and genetic transduction and, thereby, in lateral gene transfer is unclear (56) but variations in nutrient concentration (43, 58, 53), season (58), and phage genotype (57) are likely to have an effect. The sequencing of bacterial genomes has revealed the presence of a number of intact phage genomes, phage genes and remnants of phage genomes among bacterial genes (9). Clearly the degree of homology detected between diverse genomes can most easily be explained by extensive lateral transfer of genes not only between phage and bacteria but also between the bacterial, archaeal, and eukaryotic domains of life as well (9, 27). It is understood that lateral gene transfer occurs in the natural world by means of transduction, transformation, and even conjugation and that sometimes-favorable traits are transferred such as UV resistance, resistance to antibiotics, and enhanced recombination (37).

Understanding microbial components of shell disease may involve concepts that are at the forefront of thought in microbiology. Increasingly it is recognized that bacteria behave much differently when assembled at surfaces in organized interactive communities or biofilms than when growing in pure culture (5, 19). Lobster shell diseases will only be well understood through learning much more about interactions within the microbial communities that inhabit the lobster exoskeleton. The roles of bacteriophages in microbial communities are thought to be very important but remain largely undefined although intriguing studies are present in the literature (14, 55). For several decades bacteriophages were largely regarded as important for their role in the origins and early understanding of molecular biology (7, 51) but now there is greatly revived interest in these most abundant of all biological entities due to increasing recognition of their enormous genomic diversity, their ecological importance, and their potential practical applications in medicine and other fields. The dichotomous lytic and lysogenic replication cycles of bacteriophages are now classic models of genetic regulation and virus replication control (41), but understanding of how these mechanisms

interact with bacterial physiology in diverse natural environments has only begun to be explored in studies of diverse organisms and under more ambiguous genetic programs such as pseudolysogeny (57, 43) and under environmental stresses that may even alter the intracellular persistence of lytic phages (34, 14). While intercellular communication, such as quorum sensing, among bacteria is being actively explored by many researchers with significant advances being frequent (e.g., 30), comparatively little is known about how the powerful selective and genome altering forces of bacteriophage infection at work in microbial populations may influence these communication networks. Epizootic lobster shell disease lesions provide a definable and relatively tractable microbial consortium that is progressively invasive on the lobster exoskeleton. Disease lesions provide a framework for exploring and beginning to define the natural history of phage interactions with a distinct marine microbial consortium along with the genetic responses to environmental variables that may influence bacterial and viral functioning within the community.

Research in Progress:
        Establishing the foundations for investigating microbial and viral interactions in shell disease lesions depends first upon identification and, as possible, isolation and cultivation of the microbes and viruses present. Detection and isolation of bacteriophages has begun primarily using bacterial hosts that have been kindly provided by Roxanna Smolowitz (Marine Biological Laboratory) and by Deanna Prince (Lobster Institute, University of Maine). It is expected that efforts to detect integrated temperate phages (or their remnants) together with exploration of bacterial interactions with lytic phages from marine environments will lead to new insights and tools for understanding structure and function of shell disease microbial consortia.
        In virological and genomic studies of environmental and genetic interactions of bacteriophages with microbial communities of epizootic lobster shell disease the central hypotheses are that: (1) Bacteriophages capable of infecting bacteria in shell disease lesions are present in marine environments and have critical interactions that influence structural and functional relationships within the microbial communities; and (2) virus-mediated gene transduction or other viral interactions with bacteria in these marine microbial consortia may contribute to emergence of increased bacterial invasiveness within epizootic shell disease lesions. Current research is focused on detection, isolation and characterization of bacteriophages that will infect bacteria that have been isolated from shell disease lesions. The bacteriophages being isolated and detected will then be used to selectively probe and possibly perturb the lesion-associated microbial community. At present several lytic phages have been isolated and morphological and genomic characterizations have begun.
         Enrichment, detection and isolation of lytic bacteriophages were achieved using the enrichment procedure illustrated below. Briefly bacterial isolates from shell disease lesions are cultivated to log-phase, water samples are incubated with the specific bacteria and the presence of phages is indicated by clearing in the bacterial cultures. Phage lysates are centrifuged and supernatants are tested for the presence of phages by serially diluting and plating onto a lawn of the specific bacteria isolated from lobster shell disease lesions as shown below. Individual plaques are then collected, diluted, and plated in at least three repetitions of the isolation procedure to obtain pure bacteriophage isolates.

Phage genomic libraries are being cloned, sequenced and phage isolates are being further analyzed by transmission electron microscopy.
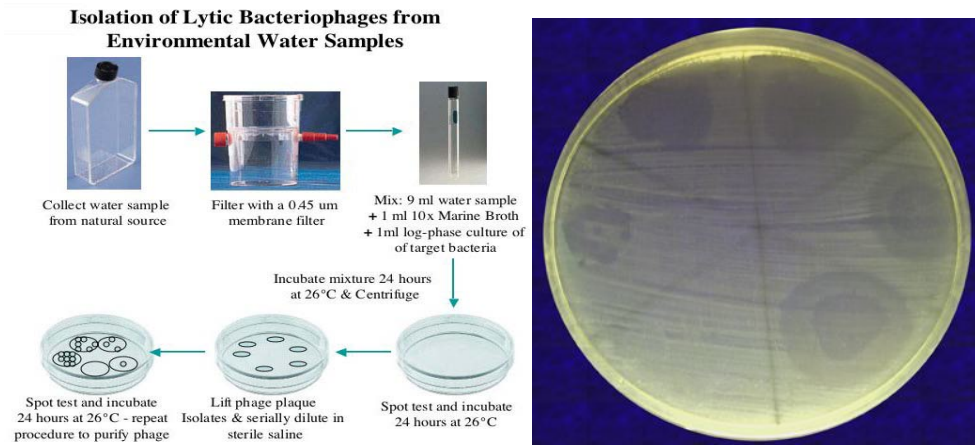
Detection of lysogenic phages using ultraviolet light or mitomycin C induction is also being pursued and application of molecular methods and electron microscopy is anticipated. Results presented here are preliminary data for bacteriophage isolates that lytically infect some of 13 bacterial isolates derived from epizootic shell disease lesions. Bacteria used include 3 *Vibrio* species and 10 strains that are currently being further characterized. Among the phages detected, four distinct phages have now been plaque-purified for further study. Dilution for isolation of a phage designated KLW that infects a bacterium identified as *Vibrio ED4* is shown above. EcoRI / HindIII digests of DNA purified from several independent phage isolates infecting *Vibrio ED4* appear to be identical as shown on the left below suggesting that perhaps this phage is abundant in various environments. As shown on the right below, distinct lytic phages have also been detected by enriching with *Vibrio ED4,* another strain identified as *Vibrio Sr3*, and the shell disease associated bacterium from the Smolowitz laboratory that has been designated through 16S rRNA gene analysis as *Brachybacterium arcticum.* Results of restriction digests of phage DNA extracted using Qiagen Lambda DNA purification kits are shown below. Clearly a phage infecting *Vibrio ED4* that is distinct from KLW has been isolated as well as a different phage that infects *Vibrio Sr3*. The lack of visible DNA from the *Brachybacterium arcticum* specific phage (labeled AY/BB below) may suggest a RNA genome or may reflect relatively low viral titers of this putative phage.

Initial DNA sequencing results for the phage KLW genome indicated that this phage has not previously been sequenced. Considering the great diversity of phages in marine environments, it is expected that many phage isolates will be found to be unique. While most genomic sequences of phage KLW show no significant matches in the databases, predicted open reading frames when translated frequently show similarity to proteins of phages of enteric bacteria that have been sequenced.   Isolation and characterization of bacteriophages infecting bacteria found in epizootic lobster shell disease lesions is continuing and will be greatly expanded as funding permits.
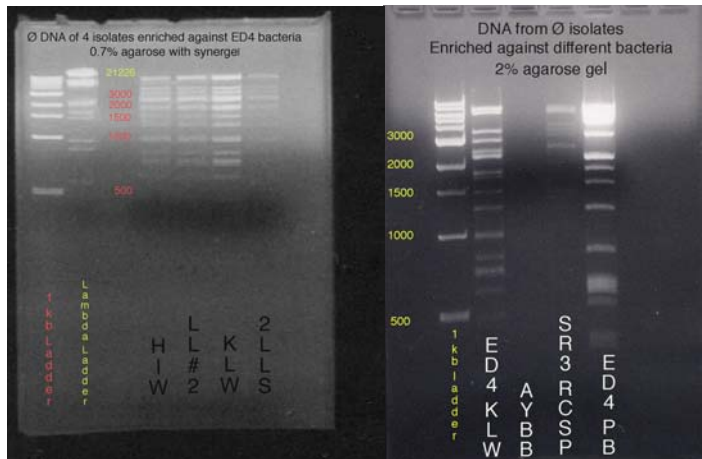
Morphological characterization of the isolated phages infecting *Vibrio ED4* (panels A and B) or *Vibrio Sr3* (panel C) reveal phages of three distinct morphotypes. Phage KLW (panel A) has appearance consistent with classification in the family *Podoviridae*, or short tailed T7-like phages. In contrast the phage PB (shown attached to *Vibrio ED4* in panel B) has morphology consistent with the family *Myoviridae*, T4-like tailed phages with contractile tail structures. The phage designated Phage RCSP (panel C) has no visible tail structure and thus will be assigned to a distinct viral family. TEM along with culture based methods will also be used in detecting and characterizing temperate phages harbored by shell disease lesion-associated bacteria. While a variety of methods may help in identifying which bacteria are associated with the leading edge of shell disease lesions, visualization of phage attachment via electron microscopy may contribute valuable evidence. If lytic phage infection occurs within the lesion context, it will be interesting to observe the effects upon lesion structure and progression.

A   B   C
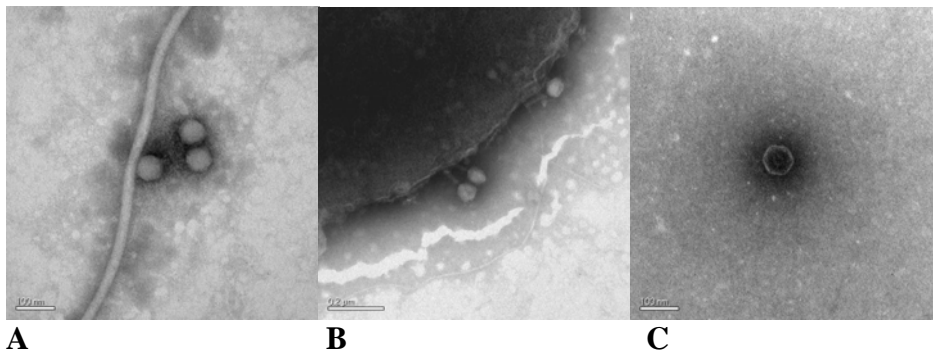
Discussion:

The best currently available evidence suggests that understanding the composition and interactions of microbes associated with epizootic shell disease lesions on lobster exoskeletons will be essential for defining the etiology of this newly emerged disease and for identifying factors that contribute to either spread of containment of the disease. While bacteriophages and other viruses exist at a nanoscale that can readily be ignored, the powerful interactions of bacteriophages in shaping the genetic characteristics and evolution of bacterial populations could prove to be critical in explaining the more

aggressive presentation of the epizootic form of lobster shell disease. Establishing the etiology of lobster shell disease appears to be a problem in biocomplexity both at the level of complex microbial associations and interactions and in relation to a complex and changing environment. While microbiological studies will be critically important, a variety of disciplines and approaches may make important contributions. Genomic and perhaps even metagenomic studies of the microbial communities associated with the disease lesions may be needed to understand the emergence and etiology of epizootic lobster shell disease. It is highly likely that viral sequences will be encountered and that bacteriophages will prove to be important to the ecology of the microbial consortia. It is also possible that new bacteriophages discovered and characterized will be valuable tools for research and may contribute to development of strategies for disease management.

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Molecular Approaches to Characterize Bacterial Communities and Populations Associated with Lobster Shell Disease

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The first presentation of the workshop by Dr. Andrei Chistoserdov focused on the amplification, separation, and sequencing of 16S rDNA in the bacteria for the identification of bacterial communities associated with lobster shell disease (LSD). Because less than one percent, at best, of the bacteria present in most environments cannot be cultivated, a major advantage of 16S rDNA-based approaches for characterizing bacterial communities is that they are culture-independent (Muyzer 1998). Here, additional genetic fingerprinting approaches are described that complement 16S rDNA-denaturing gradient gel electrophoresis (DGGE) and can provide a wealth of additional ecological and epidemiological information on the bacterial community dynamics and population structure of potential LSD pathogens infecting lobster shells and their distribution in the surrounding environment.

Methods to Profile Bacterial Communities:

Ideally, to observe dynamics of bacterial communities in the environment, methods should not be culture-dependent. In addition, they should be amenable to processing many samples as is necessary to accurately characterize community changes in both time and space. The 16S rDNA-DGGE approach, while yielding valuable phylogenetic information about the bacterial community members, is too labor-intensive for high-throughput needs. Therefore, several other DNA-based non-culture-dependent methods have been developed. Among the most widely used and field-tested are amplified ribosomal intergenic spacer analysis (ARISA) and terminal restriction fragment length polymorphism (T-RFLP) analysis. Both can be adapted to automated analysis, which provides both high-throughput and machine-based reproducibility.

ARISA is a highly reproducible technique that provides a ribosomal intergenic spacer (IGS)-based fingerprint of the bacterial community (Daffonchio et al. 1998). The transcribed IGS between the 16S- and 23S-rDNA genes typically encodes tRNAs and is useful for differentiating between bacterial species because of length heterogeneity. For the ARISA method, the IGS is amplified by PCR using a fluorescently-labeled forward DNA primer and it is automatically detected on a DNA analyzer (Fisher and Triplett 1999). The method has seen increasing use for the study of both aquatic and soil bacterial communities (Ranjard et al. 2003; Anderson and Cairney 2004; Hewson and Fuhrman 2004; Yannarell and Triplett 2004).

T-RFLP allows significantly increased throughput as compared to gel-based community profiling techniques (Marsh 1999). The IGS-PCR products are terminally labeled with a fluorescent dye during the amplification process. The PCR products are digested with a restriction enzyme and analyzed on an automated DNA sequencer. Advantages of the technique are the high resolution of DNA band separation, the potential to quantify bands, and the ability to use an internal fluorescent standard for improved sample-to-sample comparison. Thus the method has been used to examine community dynamics in environments as diverse as activated sludge and termite guts

(Liu et al. 1997). It has even been used to study marine Archaea in the flounder (van der Maarel et al. 1998).

Methods to Profile Bacterial Populations:

Once suspected pathogens and associated bacteria of LSD have been identified (see Chistoserdov et al.), characterizing their populaton structure and dynamics can provide significant insight into the nature of the disease. Methods for this purpose should be culture-independent, capable of fine resolution of strains at the subspecies level of phylogeny, and have the capacity to process many samples. As in the characterization of bacterial communities, 16S rDNA-DGGE is an excellent first approach, followed by one or more of the emerging population profiling methods describe in brief below. As an example of their utility, considerable effort using these methods is currently underway to distinguish among fecal contamination indicator bacteria in environmental waters at the strain level, and potentially, among human and non-human sources (Dombek et al. 2000; Carson et al. 2001).

The three most commonly used genetic based methods are pulsed field gel electrophoresis (PFGE), repetitive element PCR (rep-PCR), and ribotyping. All three methods are currently culture-dependent. Therefore, bacteria must first be grown and isolated. All three methods result in banding patterns on agarose gels. PFGE is based on whole chromosome extraction, followed by specific cleaving of the chromosome into smaller fragments with a restriction endonuclease enzyme. Ribotyping is similar, except that the endonucleases used provide more fragments per genome than in PFGE. The ensuing gel is specifically stained with a fluorescently tagged oligonucleotide to yield a DNA fingerprint. PFGE is considered the gold standard for use in epidemiological studies (Arbeit et al. 1990; Olive and Bean 1999), but both methods are highly reproducible, provide high resolution at the subspecies level, and can be automated (Fontana et al. 2003). The third method, rep-PCR, uses PCR primers to target highly repetitive DNA elements (repetitive extragenic palindromic DNA) in bacterial chromosomes. Subsequent gel electrophoresis of the PCR products yields banding patterns that allow discrimination of bacterial strains (McLellan et al. 2003). However, rep-PCR is much less labor-intensive than PFGE and ribotyping, but not as reproducible (Myoda et al. 2003).

Analysis of the relatively polymorphic 16S-23S rDNA intergenic spacer region has become a more common tool for bacterial identification. Due to the location of the IGS between conservative DNA regions, the length of 16S-23S rDNA intergenic spacer regions (IGS) are specific to bacterial species based on a genotypic species concept (Gürtler and Stanisich 1996; García-Martínez et al. 1999). Thus, polymorphic lengths of IGS can be used to identify bacterial species without intensive culture-dependent biochemical tests (Jensen et al. 1993; Scheinert et al. 1996; Perez et al. 1998; Bennasar et al. 2000).

Because most of the IGS region is non-coding, its sequence is also polymorphic within the same species and even among the multiple *rrn* operons contained on a single bacterial chromosome. For example, *Escherichia coli* has seven *rrn* operons which contain 16S-23S IGS regions that vary in size from 354- to 446 bp (García-Martínez et al. 1996). Restriction fragment length polymorphism (RFLP) analysis (Riffard et al. 1998; Liveris et al. 1999; Guasp et al. 2000; Barsotti et al. 2002; Kabadjova et al. 2002; Ranka

et al. 2004) and heteroduplex analysis of the IGS (Jensen and Hubner 1996; Daffonchio et al. 1999; Baudart et al. 2000) have been used to differentiate among closely related bacterial isolates, but these methods are typically not sensitive enough to detect just a few differences among base pairs, which is necessary for higher resolution at the strain-level (Hall 1994; Zavaleta et al. 1996). Thus, a major consideration for the development of a high-resolution analytic tool to assay strain-level diversity is the capacity to discriminate among small differences in DNA sequence. DNA fragments with sequence differences as small as one base pair (Myers et al. 1985), can be resolved by DGGE. Similar resolving power can be achieved on a non-gradient gel by applying a temporal temperature gradient during the course of the electrophoresis (i.e., thermal gradient gel electrophoresis or TGGE). Consequently, combining PCR of the IGS with gradient gel electrophoretic analysis has been well established as a useful tool to differentiate bacteria at the subspecies or strain levels (Buchan et al. 2001; Casamayor et al. 2002; Janse et al. 2003; Yasuda and Shiaris 2005).

IGS-DGGE approaches are typically culture-dependent because strains are visualized as individual banding patterns of the often complex multiple operon structure of bacterial species (Acinas et al. 2004). By developing species-specific, and even operon-specific primers, the species population structure can be observed (Yasuda and Shiaris, unpublished data). Thus, population-level DNA fingerprinting methods can be automated and provide detailed information on population distribution and dynamics.

Finally, more sophisticated but labor-intensive methods, such as fluorescence *in situ* hybridization (FISH), potentially allow the simultaneous visualization, identification, enumeration and localization of individual bacterial cells directly on lobster shells. FISH not only allows the detection of culturable microorganisms, but also of yet-to-be cultured bacteria, and can therefore help in understanding complex microbial communities (Moter and Gobel 2000). FISH detects nucleic acid sequences by a fluorescently labeled probe that hybridizes specifically to its complementary target sequence within the intact bacterial cell. For example, a lesion on a lobster shell would be fixed, pretreated, hybridized with the specific oligonucleotide probe, washed, and visualized by epifluorescence or confocal microscopy. FISH can also be combined with other methods like microautoradiography, microsensors, and immunolabeling to yield valuable information about the bacterium and its function in the environment.

LSD Research Questions:
In summary, there are a variety of powerful molecular-based approaches for studying bacterial community structure and bacterial population biology. They can be used to address important questions about the microbial ecology and epidemiology of potential pathogens involved in lobster shell disease. For example:

- Are there specific strains of the LSD-associated bacteria that preferentially colonize the lobster cuticle? Is yes, then what are the genetic traits that allow them to exploit this niche?
- Is there a relationship between LSD-associated bacteria and surrounding water, particles, and sediments? What environmental conditions promote their colonization and lesion-formation?

- What are the community dynamics of the LSD-associated bacteria? Does the community structure change with time and growth of the lesions?
- Are there season changes in the lesions and their LSD-associated bacteria?

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The American lobster EST sequencing project and implications for shell disease gene regulation studies

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In 1999 the Mount Desert Island Biological Laboratory (MDIBL) opened the Marine DNA Sequencing and Analysis facility. The work being conducted at MDIBL is not directly related to lobster shell disease; however, our lobster Expressed Sequence Tag (EST) project provides significant, publicly accessible genetic data that can be used for basic biological investigations of *Homarus americanus*. ESTs represent randomly selected cDNA clones that are sequenced in a single pass. This contribution will outline the lobster EST project and describe curation and bioinformatics efforts.

The MDIBL Marine DNA Sequencing and Analysis Center uses state of the art equipment to investigate biomedical and basic biological questions on marine species. EST projects are sequenced using two ABI 3100 capillary-electrophoresis sequencing platforms. High throughput capabilities are enabled through the use of an automated colony picker, a Beckman-Coulter liquid handling workstation and DNA microarray printing and scanning equipment. In addition the center utilizes real-time quantitative PCR analysis.

The *Homarus* EST project is an outgrowth of MDIBL's ongoing interests in marine physiology including, epithelial membrane biology, ion transport, gill function, and osmoregulation. A variety of crustacean projects are ongoing at MDIBL including ion regulation in crustacean gills (Lucu & Towle, 2003) neurogenesis in lobsters (Sullivan & Beltz, 2004), molt dynamics in blue crabs (Pierce, Butler & Roer, 2001), Melatonin and biological rhythms in intertidal crustaceans (Tilden et. al., 2003) and escape dynamics of Calanus copepods (Lenz, Hartline & Davis, 2000).

In 2003 a normalized cDNA library from mixed tissues of the lobster *Homarus americanus* was constructed. Mixed tissues included the gill, epipodite, branchiostegite, testis, heart, brain, antennal gland, hepatopancreas, and flexor muscle. cDNA library construction is a four-step process. After tissues are harvested mRNA is immediately extracted using standard chemical techniques. mRNA is then used as the nucleic acid template for generation of complementary DNA (cDNA) using reverse transcriptase. In order to generate a high quality library with low redundancy of clones, MDIBL used Invitrogen Inc. custom library services to normalize the cDNA library. Invitogen uses a proprietary subtraction hybridization technique to reduce abundant sequences up to one-hundred-fold without significantly altering the average cDNA insert size or abundance of rare sequences. Subtractive hybridization both increases the likelihood of discovery of novel sequences that represent genes expressed at low levels in the initial tissues and decreases the likelihood of redundantly sequencing common cDNAs. The normalized set of cDNAs, representing the genes being expressed in the initial tissues harvested, is returned within plamids transformed into a bacterial clone library.

Sequencing lobster ESTs from the cDNA library is a three-step process. After growth on standard bacterial media, individual colonies are picked, grown overnight and robotically prepped for PCR based DNA sequencing. All reactions are carried out in

standard 96 well plate formats. To date approximately 5600 clones from the lobster cDNA library have been sequenced.

Prior to submission to Genbank (NCBI), MDIBL staff curates DNA sequence data. Curation involves identification and deletion of plasmid vector sequence and complete deletion of low quality sequencing reactions. After these primary steps sequence data is translated six-fold into predicted amino acid sequences and compared to NCBI databases using BLASTx (Altschul, et. al. 1990). BLAST results are used to initially match the expressed sequence tags to known proteins. Such tentative, first-pass assignments to proteins are a coarse filter and are not homology assessments. After first-pass assignments are made each processed EST data file is submitted to GenBank (www.ncbi.nlm.nih.gov). In late 2004 MDIBL initiated automated first pass curation of sequence using trace2dbest software.

Currently the lobster EST dataset is being more carefully scrutinized. In this second phase of data curation EST BLAST searches are refined and adjusted. Three types of analyses are now being conducted on the dataset, refined BLASTp searching, nucleotide-to-nucleotide searching, and Pfam high throughput searches (Pfam is a Wellcome Trust/Sanger Institute Protein family/domain database). Nucleotide and protein search refinements include changing weight matrices thereby altering the stringency of searches. EST clones that exhibit > 50% similarity to a described metazoan gene are particularly investigated. All lobster ESTs have been searched and aligned to the protein domains within the Pfam database. The goal of the Pfam inquiry is to assess the relative numbers of common protein domains within the current set of lobster derived amino acid sequences.

The secondary data curation effort has uncovered several ESTs representing genes that may be involved in lobster innate immune responses (Table 1). Currently the complete EST dataset has been crosschecked against the Pfam data. The secondary curation of EST nucleic acid and amino acid sequences using BLAST is only ~10% complete.

Table 1: Lobster ESTs Potentially involved in Innate Immune Responses

Clone Accession #	Tentative Protein type	Identification Basis
CN852485	Prophenoloxidase activating factor 3	Sequence similarity
CN852780	Peroxinectin	Sequence similarity
CN854275	Animal haem peroxidase	Pfam domain similarity
CN949947	Natural resistance-associated macrophage protein	Pfam domain similarity

The ESTs in Table 1 may represent genes that would be differentially regulated upon pathogen challenge and uncovering such differential regulation would be relatively straightforward through the use of Q-PCR testing. Gene discovery using EST sequencing will not provide data necessary to alleviate an outbreak of epizootic shell disease; however, it will be possible to rapidly enhance our understanding of the lobster's natural response to pathogen challenge through the Q-PCR or microarray investigations. MDIBL is investigating the possibility of printing select EST sequences on a microarray chip that could become a screening platform for high throughput analysis of differential

gene regulation. Clones from MDIBL EST libraries are available to investigators upon request.

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A comparison of bacterial diversity and abundance on healthy and shell diseased American lobster

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The exact etiology of shell disease in the American lobster remains elusive, but is assumed to be bacterial based the abundance of bacteria in the lesions (reviewed by Stewart, 1980 and Getchell, 1989). Other infectious agents such as viruses are unlikely to cause the disease due to the lack of tissue in the exoskeleton, and protozoa or fungi have not been implicated due to their low numbers in the lesions (Hsu and Smolwitz, 2003). Many of the bacteria that have been isolated from lesions are ubiquitous to the marine environment, including *Vibrio*, *Pseudomonas*, and *Aeromonas*, and several produce chitinase, an enzyme that can break down the chitin in the lobster shell (Malloy 1978, Stewart 1980, Getchell, 1989).

Identifying the bacteria responsible for causing shell disease is complicated for many reasons. First, the disease is on the outside surface, so any bacteria from the water column can settle in the lesion. Therefore the presence of an organism in the lesion does not mean it caused the disease. The constant exposure of the infection to the environment may also result in high bacterial diversity on the shell, making it hard to isolate the pathogen(s) from the large number of background organisms. Second, the disease may not be caused by one pathogen, but by a group of unrelated organisms, which all have the ability to degrade some component of the shell. It is also possible that a succession of microorganisms is responsible for different stages of the disease (Smolowitz *et al*., 1992). Third, if the disease is an opportunistic infection caused by normal microbiota resulting from a weakened immune system, then identifying and understanding the reason for small changes in the shell chemistry that allow for the infection to occur will be essential. Fourth, there are assumed to be several forms of shell disease in the American lobster caused by different pathogens. Lastly, the definitive proof of identifying the causative agent of any microbial disease is based on testing Koch's Postulates. Koch's Postulates is difficult to prove with this disease because the infection is so slow (on the order of months) and the risk of contamination high. Clearly, we are still in the infancy of understanding what microbe(s) are causing this disease.

With a better understanding of what bacteria are normally found on the carapace of a healthy lobster, it would be easier to establish if the microbes within the lesions are part of the normal microbiota or the result of a new, emerging pathogen(s). The only study that has looked at the normal microbiota of lobster was done with the Spiny Lobster (*Panulirus argus*; Porter *et al*., 2001). The goal of our research was to examine the bacteria found on healthy wild lobsters as compared to those found in lesions of diseased lobsters. The diseased lobsters we used for this study were maintained in captivity and monitored for disease progression over time (up to two years). We cultured bacteria from the lesions of these diseased animals and from freshly collected, healthy, wild lobsters and identified 82 of our isolates based on a partial sequence of the 16S rDNA gene. We also used electron microscopy to examine the distribution of bacteria in the lesion as compared a healthy lobster shell.

We have been monitoring shell disease in individual lobsters held captive in open-flow tanks over time. Our longest held lobster lived for two years. The lobsters were all caught locally, off the coast of New Hampshire and in the Piscataqua River. Disease progression was extremely slow, on the order of months. Individual animals showed differences in the rate of disease progression, even when maintained in the same tank. Several of the diseased animals molted and the new shell had no signs of infection or scarring.

The normal microbiota on the shells of healthy lobsters was compared with bacteria isolated from the lesions of diseased animals. The bacteria were grown on Salt Water Complete medium and 82 isolates (44 from diseased, 38 from healthy lobsters) were characterized with respect to 1) colony morphology (visual), 2) cell morphology (phase contrast microscopy), 3) motility (phase contrast microscopy), 4) lipase activity (Spirit Blue Agar), and 5) chitinase activity (chitin agar). Approximate one-third of the isolates produced chitinase, and one-fifth produced lipase. The organisms were then identified based on a partial sequence of their 16S rDNA gene, and they fell within 12 different genera. The total number of genera found on healthy and diseased animals was the same, and the relative abundance of the four most common genera was similar. *Pseudoalteromons* was the most prevalent genus in our collection, and other genera included common marine organisms such as *Cytophaga/Flavobacteriaceae*, *Vibrio*, *Oceanospirillium*, *and Colwellia*. These results suggest the bacterial diversity of bacteria on healthy and diseased lobsters is similar.

We used scanning electron microscopy to compare how the surface of a healthy shell differed from that of a lesion, and to examine the distribution of bacteria and other microorganisms on the shell (Fig. 1). On healthy shells the distribution of bacteria was patchy, often with higher numbers where the shell surface was irregular, such as around pores. The bacterial distribution in diseased lesion was also patchy, but the overall abundance was much higher as compared to outside the lesion or on healthy animals. Several different bacterial morphologies were observed; suggesting bacterial diversity is high in the lesions. Structurally, parts of the lesions looked like clumps of helical strings oriented vertically, which we assumed to be chitin. This would suggest that the protein and lipid matrix between the chitin is degraded before the chitin. At the edge of the lesions we also consistently saw one type of protozoan loricate (tube), suggesting this protozoan may be one of the early successional members of the community.

In summary, the shell disease affecting lobsters collected off the New Hampshire coast is a chronic disease that takes months for disease progression to occur. The types of bacteria isolated off wild, healthy lobsters were similar in diversity to the bacteria isolated from lesions. This suggests that the disease may be an opportunistic infection caused by normal microbiota, although this is a small sample size to reveal the high diversity that is likely present on an animal surface exposed to the environment. Bacteria distribution on the shell was patchy even within the lesion, but there were significantly higher numbers of bacteria seen within the lesions than outside. Several different bacterial cell morphologies were evident in the lesions, suggesting substantial bacterial diversity. Protozoa were also found on the shell, the most interesting of which were protozoan loricates seen at the edge of many lesions, suggesting they may be part of the succession of organisms that invade the compromised shell.

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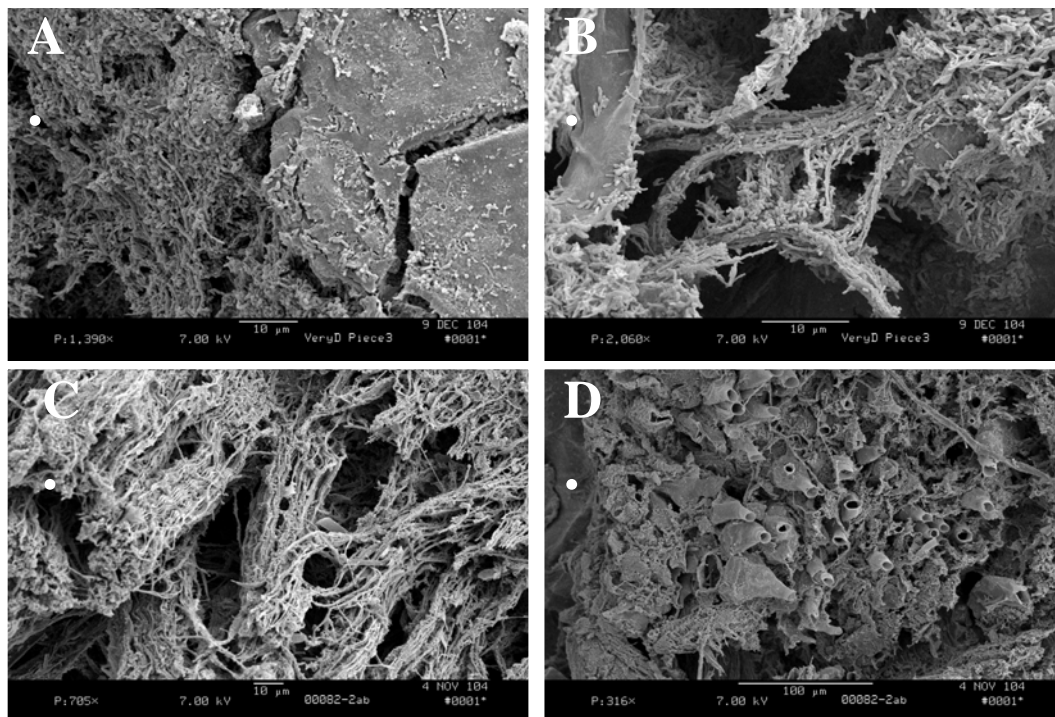
Figure 1: Electron micrographs of diseased lesions. A. The edge of a lesion showing that the abundance of bacteria within the lesion is much greater than outside the lesion. B. A close-up of bacteria within the lesion. C. Vertical strings of helices within a lesion. D. Protozoan loricates seen at the edge of the lesion.

Discussion on Causes of Disease

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Q.1. *Are the bacteria present at the leading edge of the lesions a specific species/strain or can similar species/strains cause the same lesions?*

In most shell disease investigations, bacteria are found in all the lesions at the leading wound edges (at the interface between normal and eroded carapace), and are usually the only organisms present deep in small vertical excavations in the shell. Histologically and anatomically, these vertical excavations are most consistent with normal shell pores of the carapace. *Flavobacteriacea* have been consistently isolated from shell disease affected lobsters in several anatomical locations, appearing to be the principle and primary group of bacterial pathogens involved in this disease. *Flavobacteriacea* strains and species cause severe ulcerative dermatitis in a variety of aquatic vertebrates and invertebrates. The pathogenesis and epidemiology of this group of bacteria has been documented in commercial aquaculture fish. Additionally, the group is ubiquitous at low levels in the aquatic environment. Various protozoa appear to expand the deep lesions laterally by helping to break down bacterially affected cuticle lattice, but these protozoa were not the primary organisms found at the leading edges of the initial lesions on histology, making them unlikely as the primary causative agent. Suggestions that bacteria are secondary to a yet undiscovered etiologic agent also seems unlikely, since evaluations of animals collected over several months time period with varying severity had no other organisms identified consistently in primary, deep, pore-like holes in the carapace. Further histopathologic investigations revealed a large number of secondary organisms identified histopathologically, with Labyrinthomorphid-like organisms often being noted in the lesions, but these organisms were always seen in very advanced lesions and seemed to enhance rather than precipitate carapace erosions. They were not primarily present in early lesions or erosions.

What causes a lesion to form? Is it different strains, or is it lobster susceptibility? There is a strong lobster innate immune response, which has been described at the histological level in the white paper (Smolowitz et al., this publication). To understand this disease, we need to consider potential pathogens in concert with host considerations and environmental conditions/changes (see Fig 1 in Tlusty, this publication). Different species of *Flavobacteriaceae* are seen on both shell diseased and healthy shelled lobsters. Photomicrographs revealed "hay-stacking" of bacteria at the lesions, which resembles similar images of *Flavo-* and *Flexibacteria* infections seen in marine fishes. The White Paper presentations in this volume (Chistoserdov et al., Smolowitz et al.) suggest that one species of *Flavobacteriaceae* may be responsible for this infectious disease because using PCR, one specific band length is always conserved in the denaturing gradient gel electrophoresis made from scraping infected animals. In addition, a caveat for understanding disease caused by *Alphaproteobacteria*, *Flavobacteria*, and *Psuedoalteromonas* is that they often cause disease under circumstances that increase the host's susceptibility to the pathogenic action of these bacteria. Unfortunately, other environmental and host factors causing lobster susceptibility to this disease caused by

these bacteria are not fully understood, making it difficult to fulfill Koch's postulates (laboratory based duplication of the infection, with re-isolation of the agent post-infection). Koch's postulates have been difficult to fulfill for other pathogenic strains or species of *Flavobacteria* and many other pathogens in fishes and other animals.

Consistently at a histological level, the lobster carapace is normal in appearance, and the innate immune response of the lobster to the carapace erosions seems appropriate. However, the community within biofilm is important, and there may be differences the biofilm of normal and shell diseased lobsters. Scanning electron microscopic studies show that normal carapace has very low to rarely moderate numbers of bacteria on the surface (around setae), while affected carapace is covered with layers of bacteria on the surface and in deep lesions of the affected carapace (Hsu and Smolowitz, 1993). The biofilm may be altered by temperature, community functions such as grazing etc., or by the molecular makeup of the carapace of the lobster thus resulting in a pathogenic biofilm that may be a direct cause of shell disease.

The biofilm dynamics and constituents need more investigation. There may be facultative or competitive interactions within the biofilm, and a succession scenario of the microbial community may result in shell disease infection. However, only 3 types of bacteria are prominent in the lesions (Chistoserdov et al., this publication), thus there are not a large number of different bacterial types as might be expected if the lesions were caused by bacterial succession. This again indicates it is likely only certain bacteria are capable of causing these lesions (Chistoserdov et al., this publication). However, when a lesion is initiated, other organisms will follow behind the organisms at the leading edge. This scenario is routinely identified histopathologically in other surface lesions on almost all animals in the aquatic environment, and often confounds our ability to identify the primary pathogens for disease.

The predator-prey dynamics within micro- and amoebic communities are unknown on lobster carapaces. The potential exists for remodeling of the bacterial population in the biofilm on the lobsters surface, resulting in higher proportions and/or numbers of *Flavobacteriacea* to be present on the carapace surface, than may occur normally. Biofilm community make-up could be a result of environmental or host changes, affecting scavenging microbes and metazoans that consume bacteria.

Is melanization around pore channels a late development in cuticle development, and are the pore channels and tegmental glands the avenues for attack? While space associated with the tegmental glands and setae appear important in the progression of impoundment shell disease, epizootic shell disease does not exhibit this characteristic. Epizootic shell disease starts as small vertical channels, which are often regularly spaced and histologically similar to the distribution of pore canals. Thus, pores provide a "potential space" that can give direct access into the carapace. Melanization consistently occurs around and within the deep erosions, as well as on the more generally eroded surface, and occurs at the same time and in step with the erosions noted both grossly and histologically. It does not follow secondarily. This is consistent carapace formation and melanization of the upper carapace layers as described by other authors. The hydroxyphenols and the phenolases are incorporated into upper layers of the carapace, when formed, and are only activated by erosions into the cuticles upper layers. (Neville, 1975). In the lower layers of the carapace (uncalcified endocuticle), the melanistic response relies on the hemocytic proteins brought to the inflamed location.

The external surface of the shell has a normal lipid layer that is renewed by the tegmental glands. Toward the end of the molt, those glands regress. The natural loss of the lipid layer may make the lobster more susceptible to bacterial invasion. The change in formation of the lipid layer, while the tegmental glands are still functioning, or a change in the biofilm, may make the bacteria more likely to invade the carapace after the tegmental gland secretions end and allow for invasions into the carapace.

Within the discussion of all the microorganisms on the surface of a lobster, it must be remembered that there is a host involved. Since the bacteria may be opportunistic, changes in the lobster itself may mediate response to bacteria or other pathogens. These bacterial species are ubiquitous in the oceans, but they may become pathogenic given changes in the environment and/or the host. Objective quantification of flora most likely is as important as subjective qualification in determining progression of shell disease in lobsters. Recent quantification of bacteria has been done using both SEM and culturing techniques (Chistoserdov et al, this publication and Hsu and Smolowitz, 2003), but the effort needs to continue.

Q.2. *Have changes in the environment increased the occurrence of pathogenic bacteria in the water and/or on the lobster surface?*

Anything that would increase the prevalence of *Flavobacteriacea* may have an associated increase in shell disease.

A question was raised if this disease was infectious, or, rather, a defect in host response, requiring a shift in focus to assess environmental effects on lobster immunity/shell formation. In Question 1, the difficulty with fulfilling Koch's postulates was discussed. The University of Connecticut laboratory has demonstrated effects of pesticides on lobster immunity, and that effects can be at concentrations 1000x lower than the lethal concentrations. It is likely that temperature affects lobster immunity (common in other marine organisms), but it has not been quantified at this point in time. Lobsters will exhibit differential gene regulation effects (for immune system related heat shock proteins) at different temperatures, with 19^0 or 20^0C as the temperature that the gene is turned on. Lobsters will exhibit changes in hemolymph serum chemistry after short-term exposure to elevated temperatures. Temperature may also influence shell mineralization and formation (at the molecular level), which may make a lobster more vulnerable to shell disease causing microorganisms. One important research component will be to compare how genetics, carapace formation proteins, mineralization, and the innate immune system affect carapace formation from different geographical populations of lobsters.

Another area of investigation is to assess the rate at which shell disease progresses in lobsters. It appears that the onset of shell disease can be quite rapid, with severe infections occurring in as short as one week (Castro et al. this volume). However, patterns of spread are confounded by lobster movement patterns and temperature. Lobster migration patterns have changed over the years, and there may be a difference in response for resident vs. transient populations. Likewise, temperatures have increased in the estuaries over the last 10-20 years. More temporal observations of the spread of shell disease within individuals are needed. A question was raised, whether other aquatic nuisance species may be carriers for the primary etiologic agent for lobster shell disease or predispose lobsters due to other competitive stresses for shared resources. Green crabs

were discusses as a potential causal link. However, anecdotal observations suggest that this species have been present for longer than the recent increased incidence of epizootic shell disease, and thus this is not a contributing factor. More research is needed to assess if the mega-faunal invaders are carrying associated bacteria or pathogens that can harm native biota.

Q.3. *What is the role of viral phages or plasmids in increasing the pathogenicity of shell disease bacteria?*

This is an area requiring greater research. There is not much specific information available, but the importance of microbial bacteriophages in the ocean is well known (Colwell and Grimes 2000). Most of this marine research has been on free-floating periplankton as opposed to "attached" communities of organisms. One important consideration is bacterial lysis, resulting in the release of nutrients and growth factors that may increase growth in a variety of microbes. This may be directly linked to environmental conditions. The growth of phages in the laboratory is greatly influenced by both nutrient media and temperature, and this could greatly affect how lobsters respond to shell disease. Generally, phages are species specific, but some vibrio strains will result in weaker infections in divergent species.

Q.4. *Are there other pressures (such as amoebic grazing, or inability of the weak/heat stressed lobsters to clean the dorsal carapace) that promote the growth of the bacteria?*

Lobsters do groom, and the most severe shell disease has been observed where lobsters have difficulty grooming (the triangular patch on the cranial portion of their dorsal carapace). Grooming may be influenced by stress or temperature. Grazing may be important in maintaining the microbial community within the biofilm as discussed in Question 1. One important factor for development of shell disease is the available time for the bacteria to grow. Shell disease is observed in all size classes of lobsters from larvae (Tlusty, this volume), through young of the year (R. Whale pers. comm.) to adults. However, shell disease is not seen equally across size classes. In young of the year sampling, few lobsters were observed with shell disease, and most were part of the larger size distributions, indicating that frequent molting may limit the severity or onset of the disease. In Massachusetts, sea sampling indicates that shell disease is observed more in larger animals, and in female animals. Female lobsters molt less frequently, and the females intermolt interval increases as compared with males of equal size or females from different areas. Thus, the frequency of shell disease is highest in females in southern New England. Very large market lobsters are typically not observed with shell disease, but few of these animals are observed in southern New England because they are typically captured. In Rhode Island, sea samplers observed most shell disease in larger animals, but, beginning in 2001, samplers began to observe symptoms in smaller animals (Castro et al., in this volume).

Q.5. *How do these lesions compare with lesions in other animals caused by similar species/strains bacteria?*

Lobsters experience different types of shell disease. Impoundment shell disease starts around the setae and the tegumental gland canals, and is very symmetrical before it spreads (Smolowitz et al 1992). The inflammatory response noted in impoundment shell

disease and the cause of impoundment shell disease (primarily bacteria) appear similar to epizootic shell disease, but the location of lesion occurrence, irregularity of spread of the erosions and deep invasion into cuticular pores forming "lattice pillars" is distinctive in epizootic shell disease as compared to other types of shell disease. Other crustaceans and invertebrates (e.g. sea urchins) exhibit shell disease (Sindermann 1989) and when compared to other phyla, electron micrographs of shell disease in lobsters are similar to those of Columnaris in fish. *Flavobacteriaceae* infections are not limited to cold marine species. Freshwater and tropical species can be infected with different species and strains of these bacteria. So, while the clinical signs are similar in the different areas, the numbers of bacterial types that compose the *Flavobacteriaceae* grouping (and that cause disease) are extremely diverse.

Shell disease of this type is not observed in spiny or slipper lobsters. One proposed explanation is that spiny and slipper lobsters harden their shell much faster than do American lobsters (days as opposed to weeks and months). The long exposure of soft shell may make the animals subject to the shell disease. There may also be differences in the rate of hardening in different lobster populations that may render some American lobsters less prone to shell disease. The rate of shell hardening is faster in smaller lobsters, which may explain the decreased prevalence of shell disease in this size class. To finish this session, the idea of lobster biology, particularly shell hardening was merged back into the environmental changes in that small environmental changes (temperature or presence of chemicals) may change the rate or ability of shell hardening.

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Chapter 3 Animal Responses

During the discussion period, the panel discussed the following questions, and the discussion was transcribed and is presented following the submitted papers.

1. Does status/quality/strain of animal influence the prevalence / susceptibility to shell disease?

2. Does the initial localization of epizootic shell disease on the dorsal carapace indicate focal structural carapace deficiency or method of exposure to the infectious agent?

3. Is the molecular makeup of the cuticle (i.e. protein matrix, phenolic components and mineral deposition) abnormal due to a changing environment or pollution?

4. Is there a metabolic cost associated with calcification of the cuticle and how does that effect the health of the infected lobsters?

5. What is the relative importance of active (e.g. mobilization of hemocytes and inflammatory shell deposition) vs. passive (melanization of outer layers or initial thickness of the cuticle) defense in development of shell disease?

Commentary on Shell Disease, which may or may not be an infectious disease

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Although shell disease is well recognized in many crustacean fisheries and aquaculture operations, the problem continues to be an enigma because there is no laboratory model which would allow detailed understanding of etiologies and pathogenesis. Sindermann (1991) and Noga (1991) earlier provided a conceptual approach and a summary, respectively, of potential mechanisms of cause-and-effect in shell disease. Recently Castro and Angell (2000) and Castro (personal communication) have documented the markedly increasing prevalence and severity of shell disease in lobsters from the southern New England and Long lobster fishery. This epidemic (epizootic) form (see Hsu and Smolowitz 2003) may be a significant mortality factor in the wild sector of the lobster fishery. The epidemic form is apparently different in origin and pathogenesis from the previously recognized impoundment form.

Bacterial biofilms could be important in the genesis of shell disease. A biofilm is a population of microorganisms concentrated at an interface (typically solid-liquid) and surrounded by an extracellular polymeric substance matrix ((Hall-Stoodley *et al*. 2004) (Figure 1). At ecdysis a new biofilm will develop on the lobster carapace; many internal and especially external factors probably can affect development of this biofilm, which can have a protective function. Perhaps the lesions of either impoundment or epidemic shell disease (or both) reflect an insult(s) to development of the biofilm, at either short or long periods after molting. Although biofilm formation may appear simple (see Figure 2), overall biofilms are structurally complex, dynamic ecosystems. In general biofilms are resistant to biocidal agents: the slime matrix could be protective; some cells in stationary phase dormancy could be resistant to antibiotics; and there could be subpopulations of resistant phenotypes in the bioflm (Hall-Stoodley *et al*. 2004). Recently Defoirdt *et al.* (2004) suggested that disruption of bacterial quorum sensing may be a useful strategy to attack bacterial infections in the aquatic environment. An excellent resource on biofilms is the Center for Biofilm Engineering at Montana State University (www.erc.montana.edu). C. O'Kelly and colleagues (personal communication) have suggested that interaction among protists, diatoms, bacteria; immunocompromised hosts (which is the physiological state of lobsters at ecdysis and immediately thereafter) and biofilms could lead to development of shell disease. An important confounding factor is the study of Biggers and Laufer (2004), which demonstrated the presence of alklyphenols in hemolymph and tissues of lobsters, and in marine sediments. These endocrine-disrupting agents, apparently derived from several industrial processes and the natural breakdown of plants, could adversely impact several physiological processes in lobsters, including molting. However, the significance of biofilms in shell disease has not yet been determined.

One of the significant challenges is determining whether diseases in the marine environment are increasing, decreasing or static in incidence (see reviews by Ward and Lafferty 2004, Harvell *et al.* 1999). There is little baseline information on the occurrence of marine diseases and few epidemiological studies have been conducted. Harvell *et al.*

(1999) suggested climate variability and human activities of various types reduce host resistance and facilitate pathogen transmission, leading to epidemics in oceans. Similarly Ward and Lafferty (2004) reinforced the suggestion that global warming and increased pollution of a multitude of types and from increasing numbers of sources can have very complex effects on disease. Application of epidemiological tools to the study of marine diseases is essential. Dohoo *et al*. (2003) suggested a naturalist paradigm could be a useful approach to multifactorial diseases, such as occur in crustacean fisheries. This fits with Stewart's (1993) proposition to utilize a holistic approach to the study of infectious diseases of Crustacea. Regarding the impact of disease on lobster populations, Stewart (1980) and Couch (1983) earlier suggested that all life stages, including eggs, larvae, juveniles and adults should be sampled. A tool, which could be useful in the assessment of shell disease, invasive species or other concerns with respect to the large-scale movement of fish, including lobsters, is risk analysis. Import risk analysis has four components: hazard identification, risk assessment, risk management and risk communication (MacDiarmid 2001) which require a diverse range of skills based on epidemiology, biostatistics and biology. There are many challenges associated with applying risk analysis in the aquatic environment, as reviewed by Hine (2001). The key is quality and quantity of information available and utilized in the analysis, and the manner in which the analysis is conducted. Transparency is essential to successful risk assessment (Wooldridge 2001). Input from users of a resource (i.e. lobster fishermen) is paramount, especially in the stages of risk management and communication. Overall, shell disease is an ongoing puzzle and requires an interdisciplinary, multifacetted approach to expedite and facilitate research. Acknowledgements Funding for the Canadian Lobster Health Project delivered by the AVC Lobster Science Centre represents a consortium of fishermen's organizations, private sector companies, First Nations, provincial and federal agencies, including the Atlantic Innovation Fund through the Atlantic Canada Opportunities Agency.

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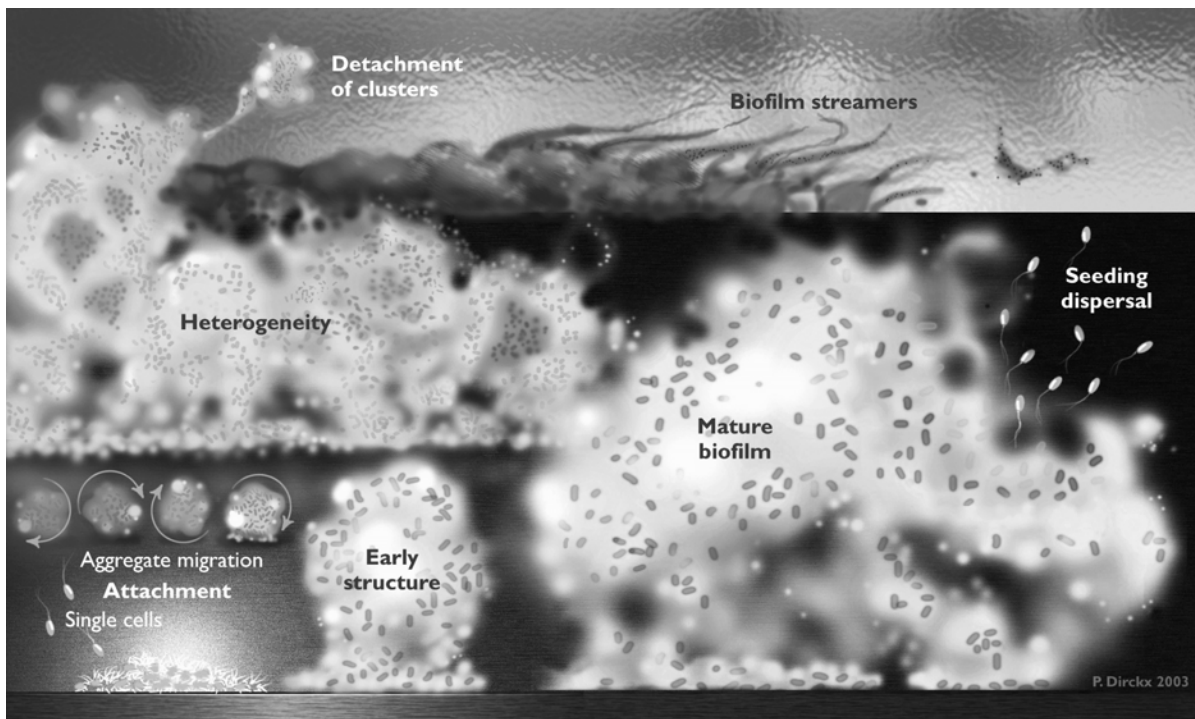
Figure 1: Complexity of biofilms. Image courtesy of P. Dirckx, Center for Biofilm Engineering, Montana State University.
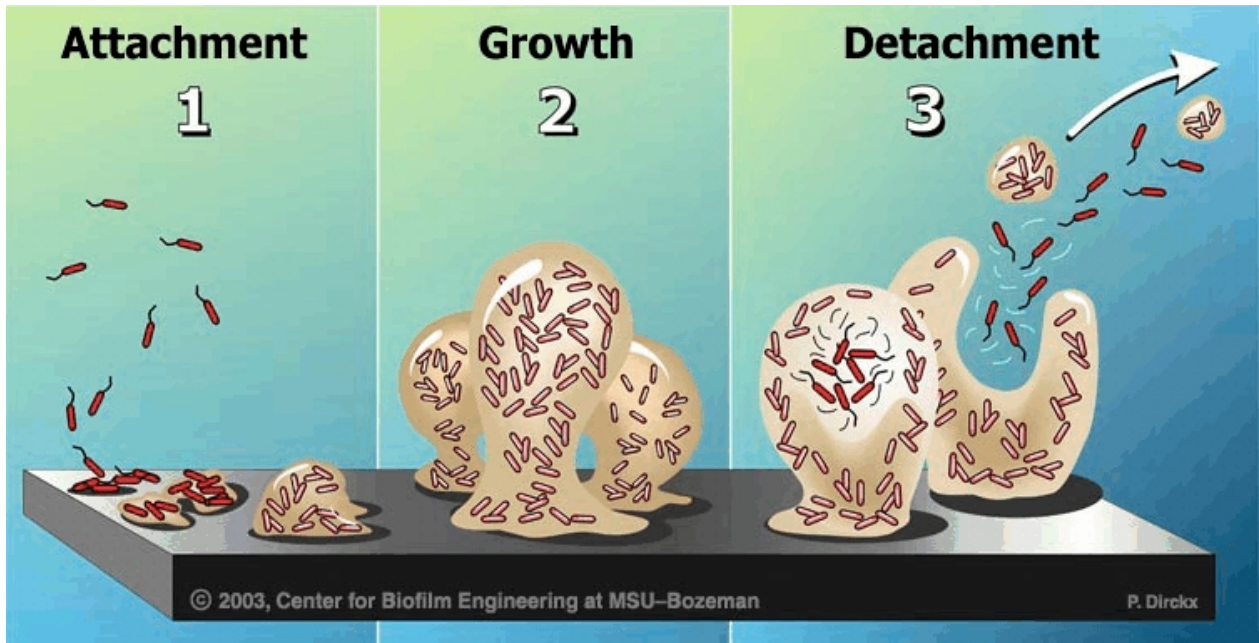
Figure 2 - Biofilm formation. Image courtesy of Center for Biofilm Engineering, Montana State University.

Are all lobsters created equal? Understanding the role of host susceptibility in the development of shell disease in *Homarus americanus*

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Background:

Prior to the recent shell disease outbreak in Southern New England coastal waters, shell disease in the American lobster has historically appeared most frequently in the tidal lobster pounds and lobster cars of Maine, New Brunswick, and Nova Scotia.   This form of shell disease was first observed in lobsters held in a tidal pound in Yarmouth, Nova Scotia in 1937 (Hess 1937). Since the initial documentation, shell disease has become one of the most economically important pathological conditions for the lobster storage industry.   Severely affected lobsters are physically disfigured by the disease to the point that they are not suitable for the lucrative live market. In addition, affected animals are frequently weak and experience elevated mortality during shipment. Estimates of annual losses to the lobster storage industry range from thousands to millions of dollars (Getchell 1991).

The Lobster Institute has investigated shell disease since the late 1980's. We have performed a variety of experiments to better understand the etiology and pathogenesis of the disease, including microscopic and microbiological examinations of the microbial flora of exoskeletal lesions, and disease model experiments to determine etiological agents. We have also attempted to understand the significance of environmental factors (lobster origin, holding and handling practices) and physiological factors (molt stage, sex, size, nutrition, and contaminant exposure) on disease development. Additionally, we have strived to develop and quantify general physiological indicators of health and disease status in lobsters (total and differential hemocyte counts, serum protein chemistry profiles, hepatosomatic indices).

Causes of Shell Disease: The Host Susceptibility Hypothesis

While bacteria are one of the primary agents of shell degradation (Malloy 1978; Smolowitz et al. 1992; Prince 1997; Prince 2002 Chistoserdov et al. this volume; Smolowitz et al. this volume), their presence alone is probably not sufficient enough to initiate shell disease in light of the following
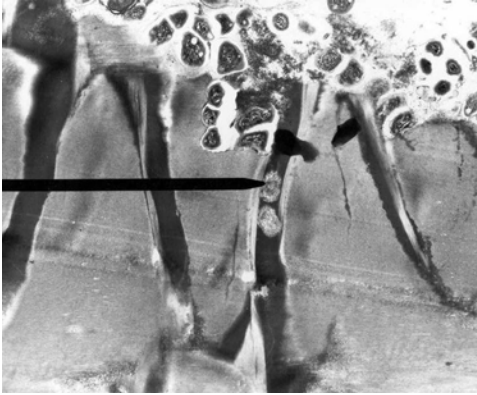
considerations: First, bacterial isolates appear to represent ubiquitous environmental strains (Smolowitz et al. 1992; Prince 1997; Prince 2002; Chistoserdov et al. this volume; Smolowitz et al. this volume), and have not been found exclusively within lesions (although higher bacterial numbers occur within lesions (Prince 2002; Chistoserdov et al this volume)). Second, the disease is extremely difficult to reproduce in both laboratory and in natural settings (Hess 1937; Prince 1997), and does not seem to be highly contagious, even in the high density environment presented by a lobster pound (Prince 1997). Finally, shell disease, with the exception of the current outbreak in Southern New England, has historically occurred at low levels (<2%) (Hess 1937; Prince 1997).

When elevated levels of shell disease have been reported, they are generally associated with environmental parameters that could be classified as sub-optimal or stressful to lobsters. The precise mechanism by which a lobster becomes susceptible to shell disease is presently unclear, but could presumably be due to due to suppression of both innate defenses, including biochemical and structural changes to the shell, and/or suppression of non-specific cellular and humoral defenses including phagocytosis, encapsulation, agglutination, coagulation, antimicrobial activity, and melanization. Inhibition of the processes of exoskeletal formation, maintenance and repair may be especially important to host susceptibility (Sindermann 1991).

Evidence of host susceptibility is perhaps best illustrated by shell disease development in lobster pounds. In an experiment to identify trends and potential risk factors associated with the development of shell disease in tidal lobster pounds, we identified lobster origin as the most significant predicator of risk (p<0.001). Lobsters from southwest Nova Scotia (LFD 34) were up to 11 times more likely to contract shell disease than those from three other Maine locations (Figure 1). It should also be noted that there were no significant differences in disease prevalence with respect to origin prior to the entering the pound, and although lobsters from all locations displayed some levels of shell disease at the conclusion of the pound cycle, the disease was largely confined to the lobsters of Nova Scotian origin.

The association of shell disease with southwest Nova Scotia long been recognized by those in the lobster industry (Hess 1937). Industry sources report that outbreaks of the disease are not only a problem in tidal pounds located in southwest Nova Scotia, but also in Maine and New Brunswick pounds that stock Nova Scotia lobsters for the winter pounding season. But exactly why does this profound association exist? Are there environmental conditions common to this region that contribute to disease development, especially with respect to lobster handling and husbandry following capture? Or are there inherent physiological differences, such as molt stage and nutritional status, among lobsters in this region that increase the likelihood of shell disease?
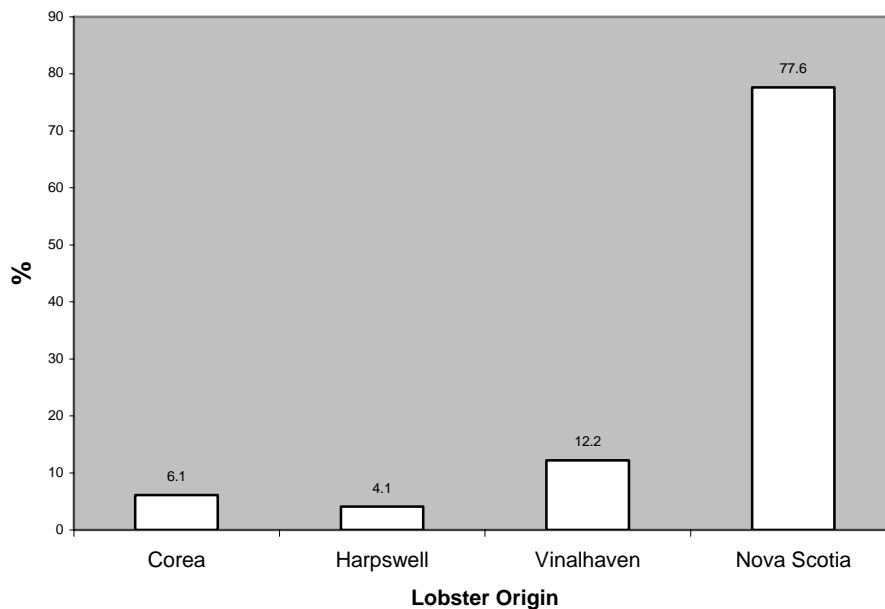
Figure 1: Frequency of shell disease in impounded lobsters by lobster origin.

Our evaluations of potential risk factors for the development of shell disease suggest several reasons why Nova Scotia lobsters may experience higher rates of shell disease: nutritional and/or metabolic disturbances, reduction in internal defense mechanisms, and exposure to toxic compounds in the environment. These factors may be independent or related. The following results present some of this evidence, although it should be noted that some observations may represent physiological states produced by the disease itself, rather than causative factors. It should also be noted that risk factors that apply to Nova Scotia lobsters and pounds may or may not be similar to those that promote the initiation of shell disease in the natural environment.

Evidence of Nutritional and Metabolic Disturbances:

We evaluated serum constituents for a group of lobsters before and after four months in a tidal pound (Table 1). Although shell disease was not initially present among the sample (as per visual inspection), it did develop in some animals during impoundment. When the potential effects of molt stage and lobster origin were eliminated, lobsters that developed shell disease had significantly lower initial mean values for hemolymph serum protein ($P<0.001$), glucose ($P<0.001$) and phosphorous ($P<0.001$) than did unaffected lobsters. Interestingly, there were no significant changes in serum constituents associated with shell disease ($P>0.05$). The reduced levels of protein, glucose and inorganic phosphorous measured in lobsters that acquired shell disease during the holding period may be indicative of a compromised, pre-disposing physiological state. The lower protein content of the hemolymph of affected lobsters implies lower levels of hemocyanin, coagulagen, enzymes, hormones, transport proteins, free amino acids, etc, and therefore an impaired ability to fight off infection, to repair the damages exoskeleton, and to transport nutrients and wastes.

We have also determined proximate, (% of dry weight), amino (% of total amino acids), and fatty acid (% total fatty acid) profiles of hemolymph, hepatopancreas, exoskeleton, and muscle tissue, and have revealed significant differences between healthy and shell-diseased lobsters. Affected lobsters displayed significantly lower levels of muscle carbohydrate, significantly different protein profiles for all tissues except the exoskeleton, and different fatty acid profiles for all tissues except the exoskeleton. Additionally, the exoskeleton of diseased animals had significantly lower levels of total carotenoids and ash, with significantly lower levels of total carotenoids seen areas of shell with and without lesions. Affected lobster also had lower hepatosomatic indices, with 35% less lipid and 266% higher levels of ash. The ash content of hemolymph was 35% higher in diseased lobsters, while the protein content was approximately 40% less. These results suggest that shell disease is associated with problems of nutrient intake and absorption, and the low hepatosomatic index is highly indicative of poor health. Necropsies of shell diseased lobsters from both pounds and the wild have revealed this phenomenon, as well as unusual coloring of the hepatopancreas. Furthermore, the elevated levels of ash in the hepatopancreas and hemolymph of affected lobsters may indicate impairment in the transport/deposition of nutrients to the exoskeleton, or withdrawl of these nutrients from the shell.

Evidence of nutritional/ metabolic disturbances is also suggested by our observations of a reduction in shell disease through the administration of an experimental diet (Fig. 2). When a pelleted diet containing higher protein and fat content than the traditional salted fish diet was administered to lobsters in a Nova Scotia pound, the prevalence of shell disease was observed in 7.73% of lobsters on the pellets, and in 10.39% of lobsters that received the traditional diet. Mortality rates were also lower for lobsters given the experimental diet (4.11% vs. 6.47%).
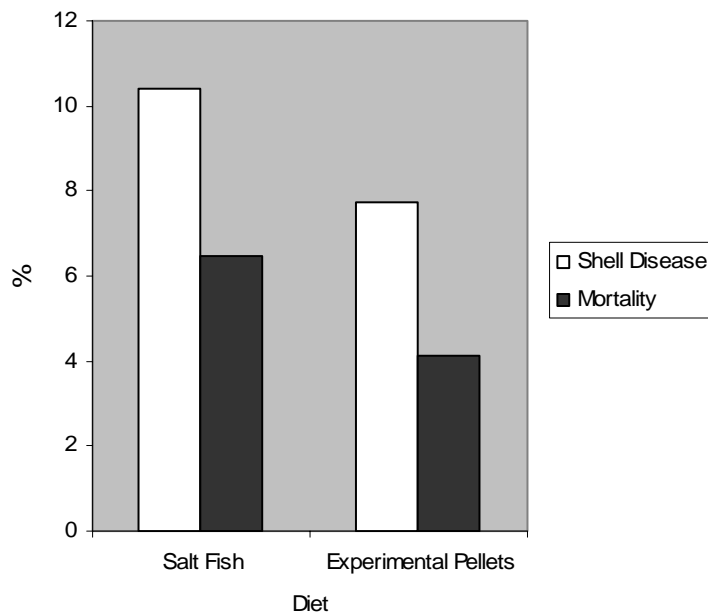
Figure 2: Shell disease and mortality rates for impounded lobsters with respect to diet. Prevalence and mortality are expressed as percent of total weight of lobsters recovered from pound.

Evidence of Reduction of Internal Defenses:

We have measured total and differential counts of circulating hemocytes for Nova Scotian lobsters with shell disease (Table 2). Hematocrit and percentages of hyaline cells, small granular hemocytes and large granular hemocytes were compared to values obtained from clinically normal impounded lobsters. Total counts of circulating hemocytes were significantly lower in diseased lobsters (p<0.001). Differential hemocyte counts demonstrated that diseased lobsters displayed greater

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proportions of small granular cells hemocytes, and reduced proportions of hyaline cells and large granular hemocytes. The observed differences in differential counts were not statistically independent of disease status (p<0.001). The reduced number of total hemocytes and percentage of large granulocytes observed in lobsters with shell disease would constitute a depression in the normal defense system. In particular, the loss of granular hemocytes would result in a decline in defense against foreign particles in crustaceans (Hose and Martin 1995), wound healing (Vacca and Fingerman 1983), reduced phenoloxidase activity and overall suppression of the prophenoloxidase system, which when released, stimulate a variety of responses including phagocytosis, encapsulation, hemocytosis, and melanization (Soderhall 1982).

Evidence of Exposure to Toxic Compounds in the Environment:

More recently, we have begun to assess the significance of contaminant loads as a factor in the development of shell disease. We have sampled tissues from Nova Scotia and Long Island Sound lobsters for metals, and Long Island lobsters for PCB's, PAH's pesticides, and dioxins. Our preliminary results indicate that both Nova Scotia and Long
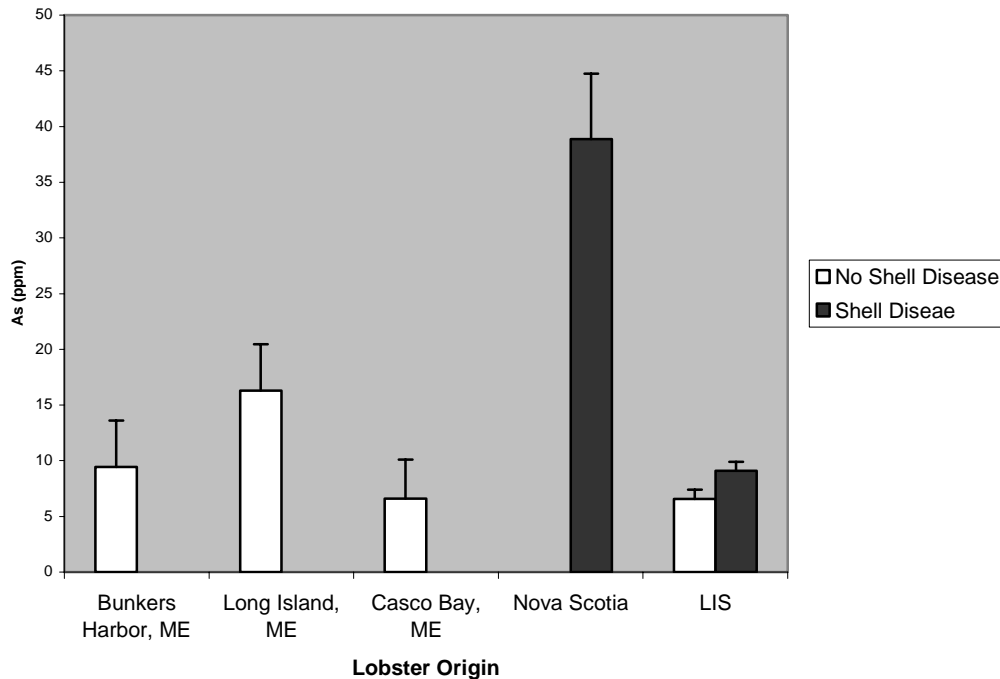

Figure 3: Arsenic levels in lobster hepatopancreas (wet) with respect to origin and shell disease status.
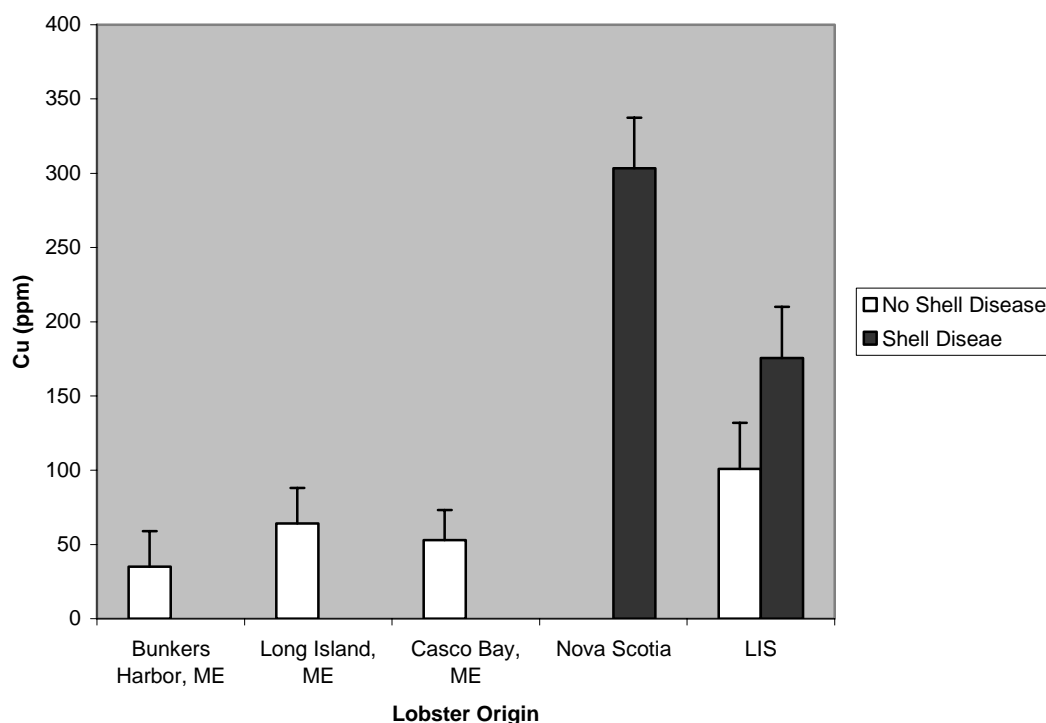
Figure 4: Copper levels in lobster hepatopancreas (wet) with respect to origin and shell disease status.

Island Sound lobsters with shell disease display higher levels of metals than control animals from the Maine locations (Figures 3-5). Affected Long Island Sound lobsters also displayed greater levels of arochlor 1260, and organochlorine pesticides than unaffected lobsters, although the sample size was too small to verify these differences statistically. The exact meaning of these contaminant loads is unclear, as the values for of analytes for both the unaffected and affected groups were within the ranges established for this species for "normal" lobsters (Mercaldo-Allen and Kuropat 1994) Yet, it is difficult to ignore the role that elevated levels of contaminants measured in shell diseased lobsters might have in disease development, especially if these compounds are persistent or slow to depurate.

Additional Risk Factors:

  Molt stage and molt frequency may be factors in shell disease risk, and should always be accounted for when measuring any physiological variable. In our observations of lobster pounds we find that lobsters from Nova Scotia are typically hard-shell, intermolt animals, whereas lobsters from Maine are more likely to be post-molt stage. However, we have been unable to establish a statistically significant relationship between molt stage and shell disease for lobsters in lobster pounds because the effect of this factor could not be separated from lobster origin due its uniformity within the Nova Scotian group. We have not evaluated this factor for lobsters in the wild.
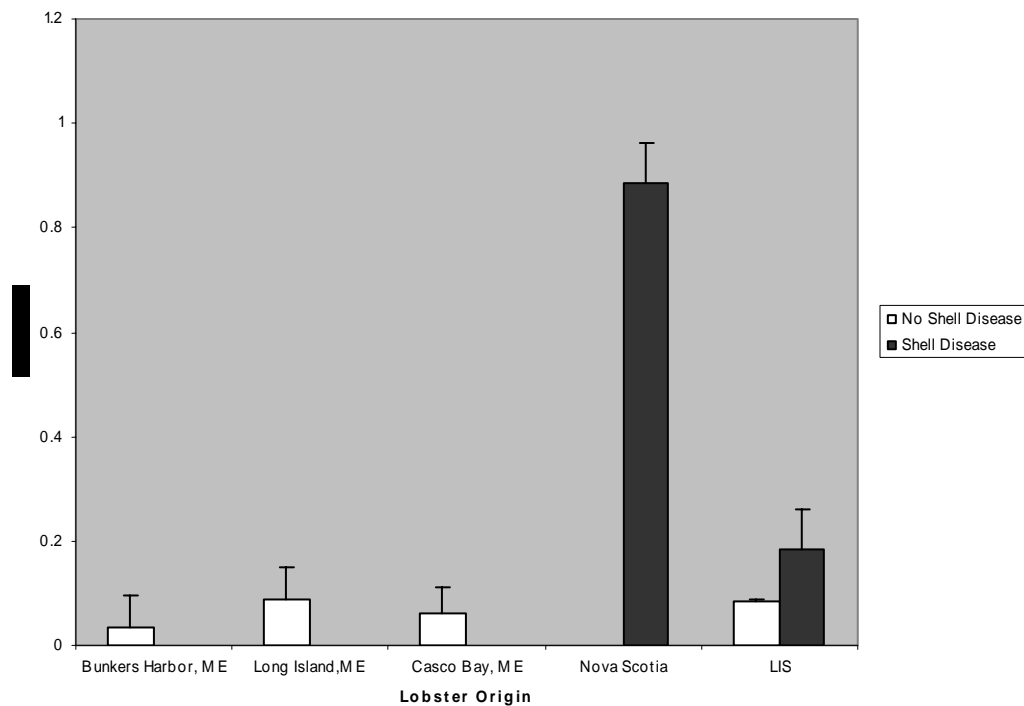
Figure 5: Mercury levels in lobster hepatopancreas (wet) with respect to origin and shell disease status.

Temperature may also present a significant shell disease risk, especially given that shell disease appears to be associated with extreme low (Nova Scotia) and high (Southern New England) temperatures. We have not evaluated this factor in either setting.

Injuries to the exoskeleton have frequently been cited as risk factors for shell disease.   In lobster pounds, injuries caused by handling and shipping to lobsters do not seem to contribute to shell disease development. In addition, holding lobsters in floating crates for less than 4 days does not appear to be a significant shell disease factor. We cannot assess the importance of injuries in the development of shell disease in the wild.

Future Directions: Evaluating Lobsters at Risk for Shell Disease:

Risk evaluation for shell disease is unquestionably hindered by the incomplete description of the etiology of the disease, and by lack of a reproducible disease model. Nevertheless, there are a number of potential directions in which future research might proceed:

1. Continued development of physiological indicators of health. These may include expanded serum chemistry profiles, total and differential hemocyte counts, assays of immunocompetence including phagocytosis indices, estimation of hemolymph clotting time, and phenoloxidase activity. Evaluating hormonal titers may also prove especially useful. Obviously, the development of these health indicators will be extremely time-consuming due the fact that they vary considerably throughout the lobsters' molt cycle and range.

2. Development of rapid, inexpensive means to measure stress. From a lobster dealer's perspective, a simple test that is indicative of lobster risk would be a

highly beneficial means to evaluate lobster vigor and minimize potential losses. We have evaluated the use of a simple glucometer in this capacity, but an ELISA-type test, possibly for crustacean hyperglycemic hormone, may be more appropriate.

3. Application of spatial data to identify if disease clustering exists, as well as provide surveillance. Human and veterinary medicine have been greatly advanced through the use of spatial analyses of diseases. In diseases such as shell disease that appear to have multifactorial origins, the use of GIS can provide a means to explore etiological hypotheses, and determine if lobsters from certain areas are much more at risk for shell disease..

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TABLE 1. Means +/- standard error of hemolymph serum total protein, glucose, inorganic phosphorous, and ammonia of impounded lobsters with respect to shell disease.

Serum Constituent	Disease Status	n	Initial	Final	Difference
Protein (g/dl)	No Disease	31	3.18 ± 0.30	3.47 ± 0.13	0.29 ± 0.12
	Disease	12	2.88 ± 0.39	2.97 ± 0.31	0.09 ± 0.20
Glucose (mg/dl)	No Disease	31	25.8 ± 1.7	33.0 ± 1.5	7.27 ± 1.1
	Disease	12	20.7 ± 2.7	26.0 ± 2.2	5.26 ± 1.9
Phosphorous (mg/dl)	No Disease	31	0.55 ± 0.09	0.50 ± 0.11	-0.05 ± 0.97
	Disease	12	0.13 ± 0.12	0.28 ± 0.14	0.14 ± 0.11
Ammonia (umol/l)	No Disease	31	602.3 ± 36.3	344.3 ± 28.8	-258.0 ± 40.1
	Disease	12	576.4 ± 71.0	276.0 ± 42.6	-300.4 ± 72.2

TABLE 2. Comparison of total and differential hemocyte counts in lobsters with and without shell disease.

Lobster Status	Total Hemocyte Count[1] (hemocytes/mm^3 hemolymph)	Differential Hemocyte Counts[2]		
		Hyaline Cells (%)	Small Granular Cells (%)	Large Granular Cells (%)
Non-Diseased	1.18×10^4 $\pm 5.27 \times 10^3$ $(6.42 \times 10^3 -$ $1.68 \times 10^4; 22)$	41.7 ± 1.5 (30-55; 24)	45.4 ± 1.3 (35-59; 24)	12.3 ± 1.0 (5-21; 24)
Diseased	8.61×10^3 $\pm 5.35 \times 10^2$ $(2.57 \times 10^2 -$ $1.41 \times 10^4; 24)$	39.0 ± 1.7 (25-52; 20)	53.8 ± 1.5 (44-71; 20)	6.2 ± 0.8 (1-14; 20)

Values are mean $\pm$ standard error (range; number of lobsters sampled).

[1] Duplicate counts were made on hemolymph collected from each lobster.
[2] Percentage of each type of hemocyte in 100 cells.

New *in vivo* methods to measure shell formation and possible implications for the study of shell disease.

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The presentation, prevalence and severity of a disease state in an animal is the synergistic interaction of the pathogen, the animal and the environment. There three components can be represented as intersecting circles where the intersection is the disease event (Snieszko 1973, Fig 1). While all three factors have to occur in a state that is permissive to the final disease state, any change in a single factor will alter the final prevalence or severity of the disease state.
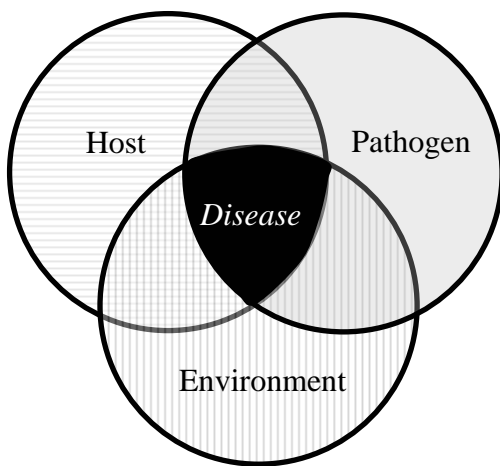
Figure 1. Snieszko's circles demonstrating the interconnectedness between the host, pathogen and environment on the presentation, prevalence and severity of a disease state.

The work being conducted in the Lobster Aquaculture Program at the New England Aquarium is beginning to address shell disease in American lobsters by focusing on the host, as well as host- environment interactions. Specifically, the program goal is to assess nutrition and growth in larval and juvenile lobsters to understand the relationship to shell deposition and mineralization. Since shell disease is a direct attack on the integrity of the cuticle, understanding the process of formation and repair will better elucidate the factors that affect a lobster's susceptibility to shell disease (SD). Currently, it is unknown if 1) lobsters with thicker cuticles are less subject to presentation of SD (or if bacteria attack locations with relatively thinner or weaker cuticle within a lobster); 2) lobsters experiencing SD increase melanization efforts or inflammatory cuticle deposition at the site of attack 3) the occurrence of SD affects new cuticle developing below the wound. This work will ultimately be integrated into host-pathogen studies, after the bacterial species and communities have been established.

One difficulty with examining cuticle formation in crustaceans is that most methods are destructive to the shell as well as the lobster. Thus, to advance the understanding of individual lobster's responses to bacterial invasion, a model system to examine cuticle formation *in vivo* is necessary. One potential solution to this is the rediscovery of the "white" lobster (Fig. 2). Early work on the juvenile diets observed that American lobsters fed a diet low in carotenoid pigments were pale in coloration to the point of being colorless or white (D'Abramo et al 1983).

Figure 2. A dietary white American lobster (right) next to a normal colored sibling grown at the New England Aquarium.

Work at the Aquarium experimented with feeding lobsters low cost feeds developed for Panaeid shrimp (Fiore and Tlusty 2005, Tlusty et al. submitted), and again observed the development of white lobsters. While in the past researchers did not take advantage of this dietary color anomaly, we began to investigate using the white lobsters to understand pigment deposition in the cuticle (Tlusty 2005, Tlusty and Hyland 2005).
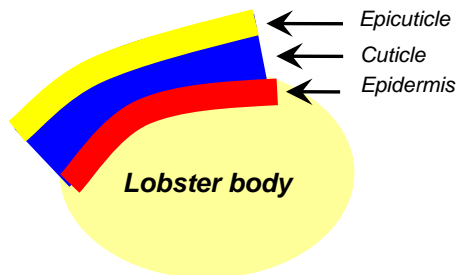

Figure 3. The distribution of the different forms of astaxanthin (the three colors) in a lobster shell

Astaxanthin is the primary pigment responsible for a lobster's color. This pigment occurs in an esterified state in the epidermis where it is a red color. In order to cross the membrane boundary into the cuticle, proteins bind to the astaxanthin in a complex known as crustacyanin. A result of the protein binding is that the astaxanthin is twisted and a bathochromatic color shift causes crustacyanin to have a purple (the β form) to blue hue (the α form). Multiple crustacyanin molecules finally stack like plates in the epicuticle to form crustochrin. Here the astaxanthin in crustochrin is hypsochromatically shifted to a yellow hue (references to studies on structure of crustacyanin and crustochrin can be found in Tlusty 2005, and Tlusty and Hyland 2005).

The differential color of astaxanthin depending on location within the lobster's shell (Fig. 3) has the makings of an excellent model system in which to examine the mineralization of lobster cuticle *in vivo*. Practically, any factor that delays the transport of astaxanthin into the cuticle, such as an increase in time to convert canthaxanthin to astaxanthin, will influence the distribution of astaxanthin within a lobster, and this will be observed as deviation from a normal pattern of color addition (Fig 4, Tlusty and Hyland 2005). Color is also likely to correlate to overall cuticle structure. In the laboratory experiments, while the color of the carapace and tail (uropods) were often the same color, occasionally they did differ. If they did differ, it was always that the carapace was bluish (thick cuticle) and the tail was reddish (thin cuticle). The condition of a blue uropod and red carapace was never observed. Furthermore, in adults, lobsters are counter shaded
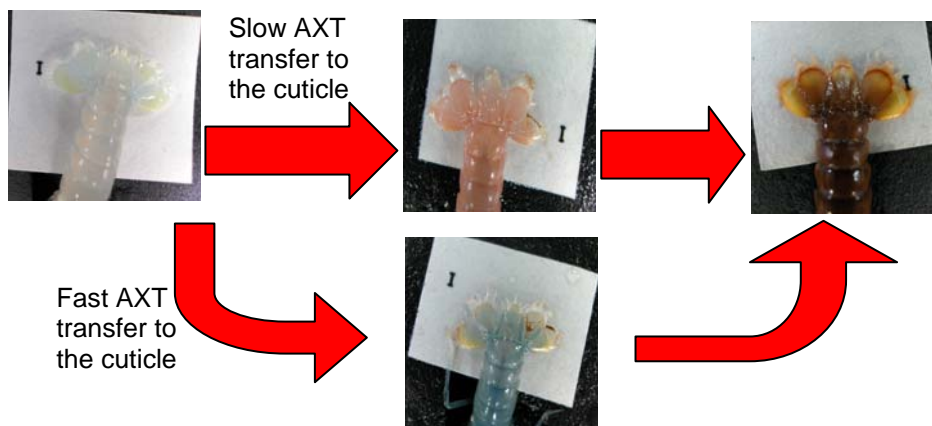
Figure 4. Changes in the rate at which astaxanthin (AXT) is transferred to the cuticle will impact the color transitions as white lobsters shift to a wild color. The upper middle lobster is a red color, while the lower middle lobster is a blue color.
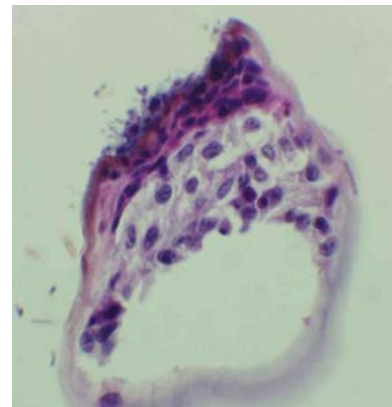
Figure 5. A gastrolith in a juvenile American lobster fluorescing under UV light as a result of feeding the lobster oxytetracycline.

(dark on the back (dorso-medial), lighter on the lateral and ventral sides, Tlusty 2005) which may very likely be due to the overall cuticle structure. Because color likely relates to shell structure, and does help measure chemical deposition within the shell, this system provides a model to assess how changes in a lobster's physiological status (e.g. nutrition, presence of infectious agents), or the environment (e.g. temperature, pH) influences its mineralization of the cuticle.

One assumption when using astaxanthin as a model for shell mineralization is that the pigment is being distributed within the lobster's body similarly to calcium. To assess if this is the case, work is also being conducted to mark calcium *in vivo*. While a variety of calcium markers are available, oxytetracycline was selected for initial tests because of it ease of use, low cost, and because it fluoresces under UV light when bound to calcium. Preliminary work with this marker has had limited success in marking calcium in living lobsters (Fig. 5). Frozen adult *Artemia* soaked in Oxytetracycline were fed to pre-molt juvenile lobsters. Under these conditions, calcium being deposited in the gastrolith fluoresced indicating that this can be used to assess calcium *in vivo*.

The work in the Lobster Aquaculture Program at the New England Aquarium has been focused on creating a model system to better understand shell formation and mineralization, and to examine this process in living animals. While shell disease is most often observed in adult lobsters, this program has been working with juveniles for a number of reasons. First, juvenile lobsters can (although not all do) exhibit the different types of shell disease when held in a hatchery setting, and epizootic shell disease has been observed in as young as stage III larvae (Fig. 6). Second, juvenile lobsters can be held at densities of up to 300 / m^2 for animals up to stage IX allowing for effective use of space while providing statistically appropriate sample sizes. Third, the quick intermolt interval of juveniles allows for multiple molt cycles to be tracked providing temporal information about the disease and disease responses. A study covering four molt periods would take four years in adults, while only six months in a year old lobster. Finally, careful management of larvae and juveniles can allow for experiments to be conducted on siblings, minimizing genetic influences on responses to pathogens and the environment. It is acknowledged that not all aspects of shell

Figure 6. A cross section of a walking leg of a stage III lobster larvae exhibiting shell disease and showing inflammatory responses that are the same as those noted in adult lobsters with shell erosions (melanization of the eroded cuticle surface and pseudomembrane formation).

disease in larval and juvenile lobsters will be equivalent to that in adults. Given the quick inter-molt interval, the severity of shell disease in juveniles will likely not reach the same levels as that in adults. However, the benefits of examining shell disease in this age class of animals will provide valuable for creating the model system.

As this work proceeds, environmental variables will be incorporated to better understand how shell growth can be impacted. The ongoing research within this program includes:

- Assessing how color changes through the molt cycle including:
  - Tracking single animals through the molt cycle
  - Assessing how astaxanthin moves into the cuticle at different points in the cycle
- Determining the impact bait has on health and shell quality and strength
- Determining relationship between cuticle strength and thickness and how color may be used as an indicator of both.
- Determining if shell disease impacts shell quality in the subsequent shell of post molt animals.
- Determining if pollutants effect shell formation and mineralization
- Determining high temperatures effect on cuticle formation and mineralization

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Shell Disease in the American Lobster and its Possible Relations to Alkylphenols

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Shell Disease:

Shell disease in the American lobster, *Homarus americanus*, is on the increase in Long Island Sound (LIS), Connecticut, Rhode Island, Massachusetts inshore waters and is beginning to appear in Maine waters. The disease disfigures the shell and in severe cases leads to the demise of the organism. In 1998, commercial landings of lobsters were 3.7 million pounds in Connecticut waters of LIS. Landings declined 70% between 1998 and 2002 to only 1.1 million pounds and declined even more by 2003 when 0.6 million pounds were harvested (Millstone Environmental Laboratory, 2004).

We are making several interesting findings relating to the biology of shell disease (SD). Lobsters with shell disease seem to molt more frequently than unaffected lobsters (Laufer et al., 2003, 2005). In 7 of 10 months throughout the year for which we have data for comparison, the SD lobsters have more of the molting hormone, ecdysone, in their hemolymph or blood than unaffected ones. Ecdysone concentrations in hemolymph are determined by radioimmunoassay (RIA) as described by Chang (1984), Laufer et al. (2005) using a polyclonal antibody against ecdysones. The average ecdysone concentration for 210 unaffected lobsters was 57 ± 16 ng/ml, while 76 SD lobsters had 89 ± 32 ng/ml in their blood (statistically highly significant by 2 way analysis of variance, P=0.002). The lowest level of ecdysone in unaffected lobsters was in July, this is the time of year this population (average weight 375 g) usually molts. Following molting the frequency of SD appears to have decreased. We consider that molting is a defense of the lobster to shed its carapace and to fend off shell disease.

Unaffected lobsters do not molt while they are berried or ovigerous, carrying eggs. They carry eggs which may take as much as eleven months until they hatch and are released as larvae from the mother's pleopods. SD lobsters have been reported to be molting. Our examination of SD ovigerous lobsters revealed ecdysone levels averaging as high as 165 ± 53 ng/ml (N=5), while unaffected ovigerous lobsters have about 13 ± 4 ng/ml (N=7) (statistically highly significant by the student's t-test, P<0.005). These finding supports the idea that molting is a lobster's defense against SD even at the cost of a brood of embryos, which if shed with the carapace at a molt would be lost (Laufer et al., 2005).

To determine whether molting is induced by shell damage or a defense against infection, we determined the effect of partial shell abrasion on the molting process. We used the Louisiana crayfish, *Procambarus clarkii* as an experimental model for lobsters and used eyestalk ablation as a positive control to induce molting. Untreated crayfish were used as negative controls. The eyestalk removal induces molting since the site of molt inhibiting hormone synthesis has been removed. These positive controls and the

shell abraded animals showed a parallel increase in ecdysone in their blood resulting in molting in the de-eyestalked animals by day 39 followed by molting in the abraded animals by day 50, while the untreated crayfish did not molt during the experiment. This experiment suggests that molting in crustaceans may be induced by damage to the intact carapace, and supports the idea that molting in SD lobsters is a defense against shell damage.

Alkylphenols:

In a search for bioactive compounds affecting lobsters, we started to look for compounds such as methoprene, an insecticide used in LIS to control West Nile virus carried by mosquitoes. We had developed a sensitive bioassay for the detection of compounds with juvenile hormone (JH) activity (Biggers and Laufer, 1992, 1996, 1999, 2004). Methoprene is an analogue of insect JH and works by preventing the metamorphosis of mosquito larvae into pupae and adults. While we did not detect methoprene in lobsters, we did find bioactive compounds in lobster blood, in some tissue samples and in ocean sediments (Biggers and Laufer, 2004). These compounds were identified by gas chromatography-mass spectrometry (GC/MS) to be alkylphenols. They were found in varying concentrations in the hemolymph of lobsters ranging from µg quantities to undetectable levels. The compounds which were found are #1: 2-t-butyl-4-(dimethylbenzyl) phenol in amounts as high as 1.15 µg/ml in blood and up to 21.6 µg/g in sediment; compound #2: 2,6-bis(t-butyl)-4-(dimethylbenzyl) phenol occurred up to 13 µg/ml in blood and 4.7 µg/g in sediment; compound #3: 2,4-bis-(dimethylbenzyl) phenol occurred in blood up to19.8 µg/ml and in sediment at 24.99 µg/g ; and compound #4: 2,4-bis-(dimethlybenzyl)-6-t-butylphenol achieved in blood and sediment up to 70.7 µg/ml and 125.6 µg/g, respectively. The detection limit was 0.3 ng/ml with the method used (Biggers and Laufer, 2004).

Alkylphenols are of major interest for several reasons. They are first and foremost known to be vertebrate estrogenic endocrine disruptors (Biggers and Laufer, 2004). They are in the marine environment as a result of anthropogenic activity. They are difficult to remove from the marine environment and tend to persist. We found alkylphenols to be endocrine disruptors in an invertebrate bioassay on *Capitella capitata* where they promote larval metamorphosis, behaving like compounds with JH bioactivity (Biggers and Laufer, 1992, 1996, 1999, 2004). Most significant for the present context, is that we found them to be present in lobster hemolymph in 42% of SD animals compared to 23% of unaffected lobsters. Furthermore, these compounds, #1: 2-t-butyl-4-(dimethylbenzyl) phenol; #2: 2,6-bis(t-butyl)-4-(dimethylbenzyl)phenol; #3: 2,4-bis-(dimethylbenzyl) phenol and #4: 2,4-bis-(dimethlybenzyl)-6-t-butylphenol, exist in higher concentrations in SD animals than in unaffected ones (Laufer et al.,2005a). These results were analyzed statistically by Mann-Whitney test and found to be statistically significant for compound # 1, approaching significance for compound #2, approaching high significance for compound #3, and highly significant for compound #4. Thus alkylphenols are implicated as possibly playing a role in lobster shell disease.

What the role of alkylphenols in SD may be, is presently not known, but is the subject of our current research. We have preliminary evidence that the structure of the shell is substantially weakened in shell diseased animals, and it is likely that alkylphenols

can interfere in shell formation and shell hardening, making the shell more susceptible to bacterial invasion and destruction.

In our quest to find how pervasive the presence of alkylphenols are, we had the opportunity to examine 15 offshore lobsters. Only one of these had detectable levels of alkylphenols in its hemolymph, while an examination of embryos carried by five of these offshore lobsters revealed that three of five batches of embryos (60%) were contaminated with alkylphenols. Our interpretation of these results is that the temperature of the offshore waters from which the lobsters were captured, were too low to permit egg maturation and reproduction. Therefore, the mothers had to have been inshore in order to mature their ovaries and to reproduce. This is where the mothers became contaminated and passed the contamination on to their broods. The 15 adults become decontaminated in the cleaner offshore environment, but the embryos continued to be contaminated because of their relatively impervious shell (Laufer et al., 2005b).

Our finding alkylphenols in higher concentrations and in higher frequency in shell-diseased lobsters suggest that these chemical contaminants may contribute to the occurrence of the disease, possibly by interfering in shell formation making the lobster more susceptible to microbial invasion and shell destruction. The finding of contaminated embryos at a higher frequency than the occurrence of alkylphenols in the mothers' blood suggests that lobsters, by being in clean water for a period of time, may be cleared of chemical contamination. Thus the remediation of alkylphenols contamination in lobsters is possible.

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Electron microprobe measurement of mineralization of American lobster, *Homarus americanus,* cuticle: Proof of concept

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Introduction:

Previously the histological study of lobster cuticle has been done primarily in the de-mineralized state. Tissue is fixed with an aqueous fixative, the minerals are leached out with an acidic bath and the resultant de-mineralized tissue embedded for traditional sectioning (Smolowitz et al., 1992). This project aims to provide a proof of concept that minerals can be measured *in situ* if the cuticle is treated for the electron microprobe as an aqueous soluble specimen. This could allow qualitative and quantitative analysis of the cuticle's mineral components as has been done for the land crustacean *Porcellio* (Ziegler, 2002) potentially to the micron level. This would provide a way to determine to what extent mineral content is a factor in the vulnerability of lobsters to shell disease.

The regular pattern of arthropod cuticle has been an important structural feature and subject of analysis. While the biochemical basis of some of the mineral features of crustacean cuticle has been recently studied (Ziegler, 2002; Becker et al., 2003), the fine structural basis is almost entirely lacking. This report outlines the progress we have made in preparing the lobster cuticle for electron microprobe analysis of mineral content.

Methods:
Sampling:

American lobsters (*Homarus americanus*) with epizootic shell disease lesions were collected for sampling by bottom trawl on the NOAA Ship Albatross IV at two randomly picked stations at the mouth of Naragansett Bay on the Spring 2004 Groundfish Bottom Survey AL0403. The six selected animals included one with no apparent shell disease lesions. Digital photos were acquired of the lesions at sea briefly after capture and preliminary diagnosis, fig 1. They were maintained in a circulating seawater tank until reaching port when they were packed in wet seaweed in a cooler for transport to UMass Amherst and fixation after 48 hours.

A second sample of lobsters was obtained at the NE Aquarium lobster rearing facility. The lobster samples included: (1) One nutritionally based blue lobster with shell disease (not epizootic) plus recently shed cuticle. (2) Two fourth stage swimming larvae (3) Two third stage swimming larvae and (4) One-second stage swimming larva. These were transported to UMass Amherst in a cooler and fixed within 24 hours.
Fixation:

Small (< 1 cm sq) sectors of the cuticle including lesion and non-lesion surface were plunge frozen by plunging into liquid propane cooled to –185°C with liquid nitrogen. The frozen tissue was then transferred into dry acetone at –86°C. The tissue was held at –80°C for 36 hours while the acetone substitutes for solid water, and then allowed to come to room temperature over a 12-hour period after which the acetone was replaced with fresh anhydrous acetone.

Embedding:

Embedding for treatment as a geological specimen required experimentation since little prior experience was available for this material. Sample 1 was embedded using Spur's resin. Sample 2 was embedded using Epo-Thin (Buehler) resin. Samples were equilibrated in 50% resin/acetone mixture on a rocking table for 15 minutes before embedding in pure resin under room temperature vacuum.

Embedded cuticle was sawed out of the initial blocks with a jewelers saw and reoriented for grinding and polishing in 25 mm diameter molds which fit the microscope carriage in groups of 4. Cuticle from several specimens was reoriented and embedded in parallel in order to allow comparison under the same microscopic conditions.

Grinding and Polishing:

The plastic embedded material was next treated like an aqueous soluble specimen for microprobe analysis. Initial coarse grinding with 180 grit discs on an 8" diameter grinding-wheel allowed approaching the correct cross-sectional level in the specimens. The grit level was changed to 320 and then 600 grit (Buehler) using non-aqueous polishing oil as a lubricant. At this point the specimens were polished further with Metadi II diamond pastes on low to high nap lapping cloths (Buehler Trident and Whitefelt) using an oil lubricant. Washing and rinsing of blocks between grinding phases was done with anhydrous isopropanol and acetone to inhibit ion displacement.

Light Microscope Analysis:

The blocks of embedded tissue were photographed with a 24-bit 2.1 megapixel digital camera (Kodak MDS 290) under various magnifications of a stereomicroscope (Parco) or Leitz Ortholux polarizing microscope under epi-illumination. A digital image record provides orientation of cuticular structure relative to shell disease lesion features on and below the polished surface. The shell disease severity scale devised for demineralized specimens (Smolowitz et al., 1992) was adapted (e.g. immune cell criteria are in general not visible although the epidermal layer can be discerned).

Electron Microprobe Analysis:

The analysis of geological and biological samples is being pursued using technology that has been described in detail for other subjects (Goldstein et al, 2003; Ziegler, 2002). Our specimen preparation needs to approach as good a polish as possible in order to achieve the highest resolution of analysis possible. The Spur's resin specimens were able to be polished using 3 μm diamond paste, but no further. The EpoThin-embedded-specimens can be further polished with 1 μand 0.25 μdiamond polish and may soon be examinable with the microprobe. For quantitative analysis by X-ray backscatter, the highest degree of flatness and polish are necessary. That has not been achieved as yet.

Preliminary Results:

Gross Specimen Observations:

Sample 1 from the mouth of Narragansett Bay was made up of individuals with mild to severe shell disease lesions centered in frequency on the anterior dorsal midline of the carapace (fig 1a). Sample 2 consisted of one shell diseased adult blue lobster (nutritionally generated) with its exuvium reared in the NE Aquarium lobster rearing facility (fig 1b). In addition, 4th instar stage larvae (fig 1c) from the New England Aquarium rearing facility were dissected in seawater and freeze substituted.

Figure 1. American lobster, Homarus americanus, specimen sources for curticle analysis. a) 1 of 6 shell diseased lobsters from RI coastal waters. b) Single blue lobster from NE Aquarium. c) Pair of 4th instar larvae.
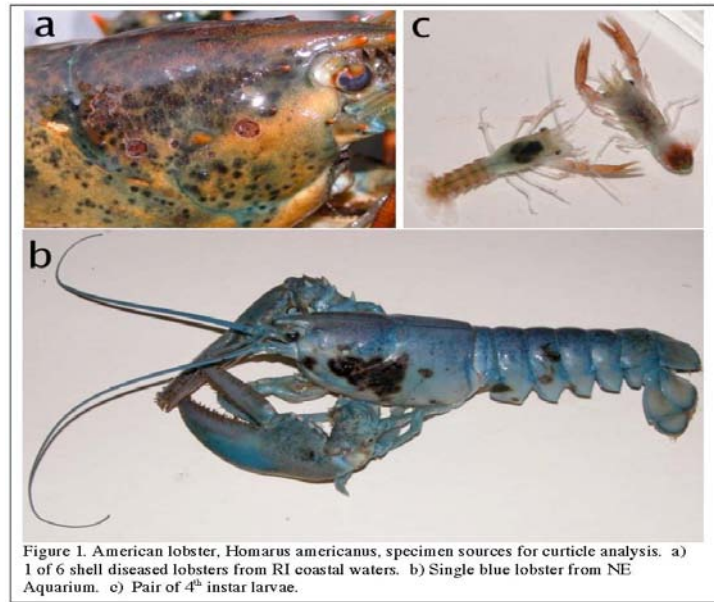
Light Microscope Observations and Organization *en bloc*:

Fixed and plastic embedded cuticle specimens (fig 2a,b) were realigned and remounted in groups to provide experimental contrasts (fig 2c), which could be ground and polished together. They were observed in a stereomicroscope to view the general anatomy of a lesion on the cuticle as well as the gross structure of the surrounding cuticle at a certain level of grinding and polishing (fig 2d). The pairs would provide contrasts within the same block providing internal controls for the quantitative phase. Digital images of the cuticle at the level of the light microscope was taken of each specimen at stages of the polishing process in order to be able to relate the lesion geometry to the surrounding cuticle.

Light Microscopy:

The block faces were observed in the stereo light microscope to identify cuticle features in control and shell-diseased lobsters. Lobster VI, with a Grade III erosion of the cuticle, below the focal plane, is viewed at several levels of magnification (fig 3). Epicuticular lined dermal gland and sensory nerve canals may be critical features around which to measure mineralization. They occur at a less than one per epidermal cell since they correspond 1 to 1 with dermal glands and sensory structures. Epidermal cell features are not visible in this mode of viewing but can be seen using a compound microscope with a reduced iris diaphragm to increase refraction.

The examination of *en bloc* polished lesion-associated mineralization patterns may help us understand the lesion process. Fig 4 illustrates a Grade IV(+) erosion with associated melanization of adjacent cuticle. Higher resolution examination of the polished cuticle under polarized light reveals pore canals that contain cellular extensions that wind through the cuticle at a higher density than dermal glands and sensory canals (Fig 5) and end at the interface of the exo- and endocuticle.

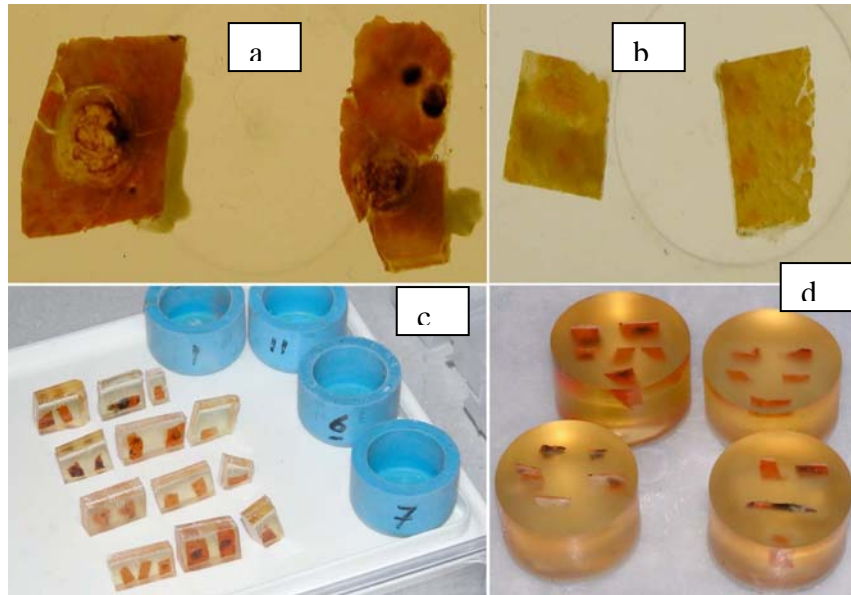Strategy To Identify Initial Infection Conditions:

Figure 2: Plastic embedded cuticle. Cuticle squares from lesioned (a) or control, unlesioned (b), lobsters I-VI were embedded in plastic. Cuticle squares were cut out of the blocks, realigned and re-imbedded in contrast group blocks 1-4. Blocs were ground and polished to a level of interest (d) for light microscopy and ion probe microscopy.

Using the above *en bloc* approach to studying the mineral content of the lobster cuticle:

1) Examine normal healthy lobster mineralization pattern.
2) Examine lobsters with Grade I – V lesions, studying the neighborhood of the spreading lesions to see if the advancing front exhibits abnormal mineralization levels associated with cuticle features such as dermal glands, sensory channels and pore canals.
3) Examine the Ca:Mg:P ratios for the cuticle regions to determine the major players in the mineralization process. A high Ca but low Mg and P content would suggest $CaCO_3$ as the most likely form of calcium.
4) Measure Na, K, Mg and Ca levels in the endocuticle to see if the earliest lesions show an encroachment of the seawater pattern of minerals adjacent to regions such as the dermal glands or pore canals.
5) Is the cuticle of lobsters from shell-diseased regions as dense with minerals as healthy lobsters from shell-disease-free regions.
6) Why is melanization is not able to prevent the encroachment of a lesion.

Discussion:
A strategy to identify the initial departure from healthy mineralization of the lobster cuticle is proposed. This involves freeze-substitution fixation of the cuticle, which immobilizes all molecules and ions combined with embedding in plastic and preparing the cuticle to be observed via electron microprobe microscopy. The ultimate objective is to be able to prepare the tissue for X-ray backscatter analysis of the mineral content. The resolution of this technique depends on the degree of refinement in specimen preparation that can be achieved.
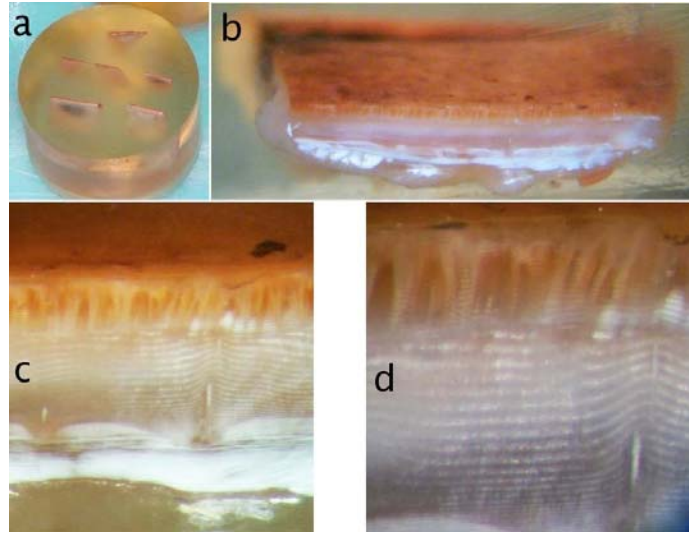
Figure 3: Lobster VI. a) block 7 specimens. b) single cuticle specimen VI epidermis near a Grade III erosion. c) polished cuticle face with 2 epicuticular lined canals. d) single epicuticular lined canal. Cuticular layers:e[ocitoc;e (arrowheads epi), endocuticle (brackets end).
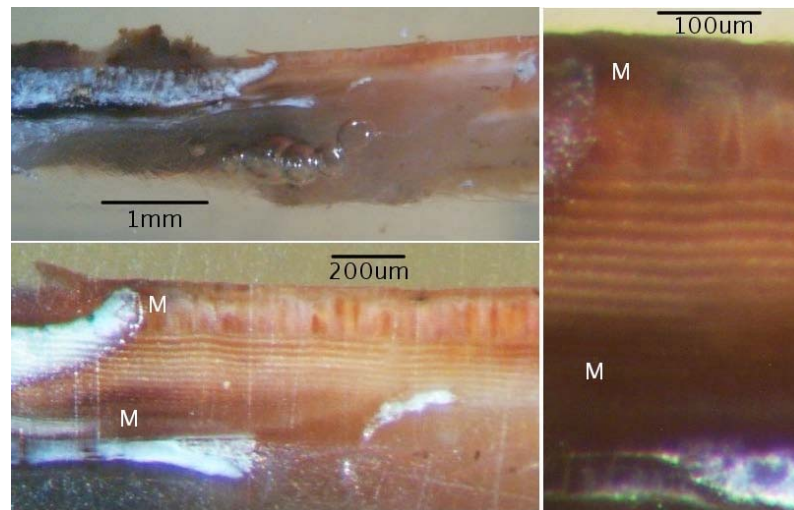
Figure 4: Three images of Grade IV(+) erosion of lobster cuticle at increasing magnification. Melanization (M) of cuticular regions in the neighborhood of an advanced shell disease lesion. Hyper-melanization is seen adjacent to the epidermis in the newly deposited endocuticle and adjacent to the cuticular surface in the exocuticular layer.

Currently, the plastic embedded specimens are well disposed for examining the cuticle surrounding shell disease lesions. Examining the *en bloc* cuticle with a stereomicroscope and polarizing light microscope allows details including dermal gland canals, sensory canals and pore canals to be identified. Previously the pore canals in the lobster had not been observed routinely possibly due to the rigors of acid extraction that needed to be used for observing sections of demineralized cuticle. The density of the pore canals (~8-10 per 20 µm) is consistent with approximately 100 pore canals arising from each epidermal cell. In addition, other features of light microscopic sectioning diagnosis of

Figure 5. Visualizing pore canals. **A.** Cuticular layers visible via reflected light microscopy of *en bloc* polished surface. **B.** Surface of 'A' viewed in epi-illuminated polarized light. **C.** Detail of the view between crossed-polarizers indicates that the structures revealed are the quasi-helical reflective lipid and ester-wax filled channels (>) of the pore canal system (Locke, 1964).

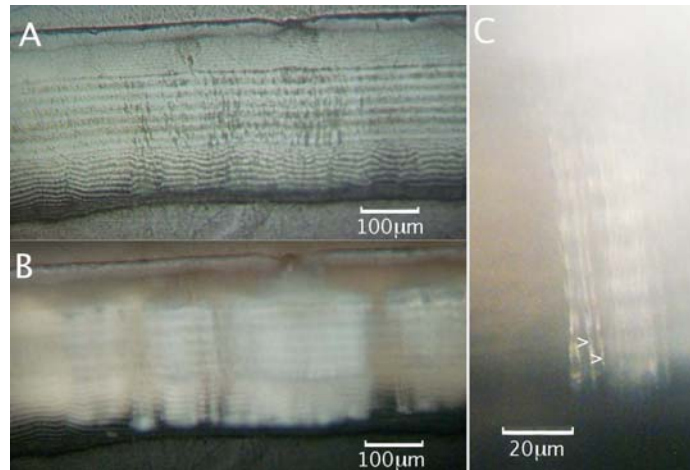the stages of shell disease lesioning (Smolowitz et al., 1992) such a super-melanization and surface incursions of microbial growth can be observed and recorded *en bloc*. Moreover, a compound microscope is capable of imaging details of cuticle structure below the polished surface by optical sectioning either with transmitted light, reflected light or polarized reflective light. The freeze substitution approach has provided an improved way of viewing aspects of the undisturbed morphology of the cuticle and hopefully the mineral composition in relation to its morphology.

Quantitative analytical work on the plastic embedded cuticle of this study is in its early stages of development. X-ray backscatter analysis is the ultimate objective using an EM Microprobe facility. Prior work with the minimally calcified cuticle and calcium storage deposits of terrestrial isopods, *Porcellio sp.,* was able to achieve limited area mineral analysis. Substantial variation in the type of deposits between species and between deposit locations was observed (Ziegler, 2003). The substantially more heavily mineralized cuticle and hypothetically finer structural detail being targeted in the lobster required modifying the protocols applied. Change to the geologist's embedding medium of choice for irregular surfaces, EpoThin resin, has allowed lap polishing the *en bloc* cuticle down to the 0.25 µm diamond polish required for high resolution. This approach should allow quantitative estimation of the insoluble and immobilized aqueous soluble mineral content in small sub-regions of the specimen. This high resolution is needed to allow statistics within a region (n-pixels square) to be calculated. This can lead to a 2-D contour map of the mineral content of a cross-section of the specimen. The resolution of such a contour map depends on the degree of flatness one can achieve in polishing the plastic embedded specimen. Another option for studying the lesions is a microfocus X-ray CT-scanner, which is available to use at neighboring Amherst College. This approach produces a 3-D estimation of the mineral content of the structure, which can be interpreted as a series of slices or as a 3-D contour map. The current problem remains how to calibrate the maps produced so that one could compare experimental or lesioned specimens *vs* control or normal contours.

Early signs of vulnerability of lobster cuticle have included accumulations of immune cells near the base of dermal gland or sensory neuron canals (Smolowitz et al., 1992) in Grade I shell diseased lobsters. It will be interesting to see if abnormalities of localized or general mineralization of the cuticle precede, are concomitant with or lag the earliest signs of shell disease lesions. It is also useful that features of the pore canal system are able to be routinely observed at the light microscope level using polarized light on the *en bloc* observed cuticle. That means that all of the important potential avenues of attack through the lobster cuticle (dermal gland canals, sensory neuron canals and pore canals) can be observed at the tissue preparation level.   Both the dermal gland canals and the sensory neuron canals are similarly epicuticle lined structures. This reduces the question of mechanism of microbial attack to a few surface types. All surfaces are protected by an epicuticular layer. The pore canals end blindly at the exo-endocuticular border as viewed by polarized light in intermolt cuticle but it is clear from other arthropods that they function as channels for providing wax, other hydrocarbon based molecules and perhaps other small molecules to the developing cuticle layers. This ability to recognize the fine structures of the cuticle will be of immense help in choosing sample cuticle surfaces to be examined by electron microprobe.

Other participants in the Lobster Shell Disease Workshop have noted that the American lobster's mineralization of the cuticle is slower than in some other decapod species which produce a hard cuticle shortly after molting and are also relatively free of shell disease. These observations make an understanding of the timing and pattern of normal mineralization of the cuticle a desirable goal.   Our freeze substitution protocol is designed to provide that information in the near future.

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Shell disease in American lobsters, Homarus americanus: Disease or malfunction of the calcification process followed by opportunistic infection?

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Shell disease has been defined as a syndrome that manifests itself as a progressive chitinolysis of the exoskeleton of aquatic and marine crustaceans, followed by necrosis of the underlying breached tissues (Rosen 1970). It was first observed in 1937 in impounded lobsters (Hess 1937), but the disease seen in impounded animals is now considered separate from that seen in wild lobsters and seems to be the result of different bacteria agents (R Smolowitz, pers. comm..). However, in both cases, lesions began as small dark pits (melanized) in the exoskeleton, indicating that the epicuticle has been breached (Getchell 1989). Thus far, only Malloy (1978) has been able to transmit the impoundment shell disease experimentally by abrading the epicuticle and directly applying *Vibrio* sp. to the otherwise healthy lobsters. However, this transmission appeared to be successful only when lobsters were held in degraded or overcrowded conditions, which presumably subjected them to severe stresses (Getchell 1989). Other attempts to transmit the disease have not been successful (Hess 1937; Prince 1997; Christoserdov et al. this volume). Thus, the important question seems to be, why are some lobsters susceptible to the disease, while others are not?

As both healthy and diseased lobsters may be found in the same localities, the answer may lie in differences in how these lobsters are laying down their exoskeleton prior to molting in the previous season. Sinderman (1991) suggested that shell disease might be an indication of a metabolic disturbance that prevented the lobster from depositing chitin appropriately. We suggest that it may not be chitin that is inappropriately deposited, but a disruption of the deposition of carbonic anhydrase, an enzyme that seems critical for cuticle formation in crabs (Roer & Dillaman 1984), barnacles (Coslow 1959), hermit crabs (Chockalingham 1971), as well as shell formation in mollusks (Simkiss & Wilbur 1989), crayfish (Horne et al. 2003), and avian eggs (Krampitz et al. 1974; Benesch 1984). The level of epidermal carbonic anhydrase increases five times in early postmolt stages (A_1 to B_2), when the exocuticle is calcifying and when new endocuticular lamellae are being formed and calcified (blue crabs, *Calinectes sapidus*: Henry & Kormanik 1985). Metabolic disturbances could arise via a number of environmental stressors, including, but not limited to, pollutants, changes in thermal regimes, anoxia, and poor nutrition (Sinderman 1991). Poor nutrition appears to increase the incidence of impoundment shell disease in juvenile lobsters (Fisher et al. 1978), by affecting the quality of the epicuticle. However, it may also have similar consequences for the quality of the underlying layers of the exoskeleton. If lobsters survive their initial infection, they can successfully molt out of the disease (McLeese & Wilder 1964)—again, this suggests that initial infections may be the result of temporary metabolic disturbances that can be overcome.

The lobster exoskeleton, or cuticle, consists of four layers: the membraneous layer, the endocuticle, the exocuticle, and the epicuticle (Travis 1963). The epiculticle and exocuticle are formed under the old exoskeleton prior to molting--they are the primary shell following molting until the endocuticle is secreted 24-48 hrs post-molt (Aiken 1980; Waddy et al. 1995). The epicuticle is composed of lipids, proteins, and calcium salts, but is lacking in chitin. The exocuticle is a matrix of chitin and protein. Both the epicuticle and exocuticle are hardened fairly soon (within 8 hrs) by tanning and calcification, after the lobster establishes its new postmolt volume (Travis 1960; Aiken 1980). The mineralization of these two outermost layers occurs at the same time that secretion of the endoskeletal lamellae occurs. All of these three endoskeletal layers are calcified (they all react histochemically for calcium), but the innermost lamellae are thin, flexible, and lack the vertical striations seen in the outermost layers, as well as the exocuticle and epicuticle layers (Aiken 1980). The final innermost layer or the exoskeleton, the membraneous layer, is thin, lacks calcium but contains chitin, and appears to break down to an amorphous mass during premolt presumably to make it simpler for the epidermis to lay down a new epicuticular and exocuticular layer prior to molting. By intermolt, the membraneous layer has reformed into a dense band; this band is complete at about 55 days postmolt and can be as thick as the exocuticular layer (Aiken 1980).

All of the exoskeletal layers are easily seen with light microscopy; and all but the membraneous layer give a positive response to histochemical tests for calcium (Aiken 1980). Electron microprobe microscopy (as per Kunkel et al. this volume), as well as standard biochemical techniques employing extraction of carbonic anhydrase from the exoskeleton with subsequent spectrophotometry (as per Horne et al. 2003), can be used to determine the mineral content within the exoskeleton, or perhaps even within individual layers of the exoskeleton. Moreover, microscopic examination should be used to determine whether pore canals, ducts and/or nerve channels might serve as conduits for bacteria within the exoskeleton. These tools should be used to examine not only healthy and shell diseased portions of a lobster's shell, but also to compare between the healthy portions of diseased and non-disease animals. They may also provide information on the developing epicuticular and exocuticular layers formed in premolt lobsters to determine if these layers have defects present.

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Environmental contaminants: A potential contributing factor to lobster shell disease in the American lobster (*Homarus americanus*).

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Research beginning in the Wise Laboratory of Environmental and Genetic Toxicology at the University of Southern Maine, and in collaboration with the New England Aquarium, seeks to address the potential contributing role of environmental contaminants to lobster shell disease in American lobsters and also as potential reproductive threats to the species. Specifically, the research is aimed at assessing the potential toxic effects of four classes of environmental contaminants on lobster health and development. The classes of contaminants include: metals (mercury, chromium, lead, arsenic), Brominated Flame Retardants (BFR), pesticides (e.g. vinclozolin) and phthalate esters (PHE).

Each of these classes of contaminants is known to be present in high levels in the waters of the Northeast where lobsters reside. Two of these classes (BFR, and PHE) have been increasing dramatically in marine species in recent years due to their common use in household items, but their potential health effects are uncertain particularly in marine species.

The other two classes (metals and pesticides) are known to induce potential toxic effects including molecular, cellular, physiological and behavioral changes that could be plausible contributing factors to lobster shell disease. For example, metals such as lead are known to mimic calcium and thus may alter calcification and mineralization of the shell. Metals and pesticides are known to induce subtle behavioral changes and thus may alter the frequency and/or duration of shell cleaning leading to longer retention times of bacteria on the shell.

Current hypotheses under consideration and in planning for investigation include:

> *Hypothesis #1: Environmental contaminants alter the mineralization/calcification of the lobster cuticle rendering the animal more susceptible to bacterial attack.*
>
> *Hypothesis #2: Environmental contaminants cause neurobehavioral toxicity leading to reduced cleaning-related behaviors rendering the animal more susceptible to bacterial attack.*
>
> *Hypothesis #3: Environmental contaminants disrupt endocrine cycling in lobsters making them more vulnerable to bacterial attack.*
>
> *Hypothesis #4: Environmental Contaminants cause gill damage decreasing oxygenation in the animals and increasing their stress making them more vulnerable to bacterial attack.*

Hypothesis #5: Environmental Contaminants cause genetic damage in the reproductive organs of lobsters, decreasing reproductive fitness

Our approach will be to use both *in vitro* and *in vivo* approaches. We will use cell lines to investigate cellular and molecular toxicity of these contaminants on neural, reproductive and gill cells; considering effects on calcium and mineral levels, canthaxanthin/astaxanthin ratios, hormonal levels and cycling, cellular lifespan and integrity of genetic material. Currently, these cell lines are in development in the Wise Laboratory.

We will also use a whole animal approach to study effects on larval and juvenile lobsters. We will use the New England Aquarium's model for canthaxanthin/astaxanthin and calcium mobilization in white lobsters to see if contaminants affect the composition of the cuticle. We will use behavioral studies to assess whether contaminants affect the frequency and duration of lobster cleaning behaviors after exposure of contaminants.

To guide our experimentation and to link the *in vitro* and *in vivo* work, we will assess contaminant loads in lobsters by measuring accumulation in the antennae, brain, reproductive organs and cuticle. We will face some challenges in assessing load as some lobsters will already have been exposed, thus we will need a measurement of load pre- and post- treatment. We will compare in vivo contaminant loads with *in vitro* intracellular levels to better determine if the effects seen in one are plausible in the other. Key *in vitro* results will be confirmed *in vivo*. For example, we will determine the amount of toxicant required to damage gill cells *in vitro* and if we find that this amount compares to *in vivo* loads, we will examine the gills for damage.

At a later time, this work will ultimately be integrated into contaminant-pathogen studies, after key bacterial species and communities have been determined.

Interactions among lobster diet, the environment, and lobster health: linking dietary changes and environmental pollutants to the incidence of shell disease

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Environmental processes can influence an organism's susceptibility to disease by modifying an organism's physical stress level. For instance, anoxic conditions in estuaries physically stress local fish and mobile invertebrates. In addition to the environment, biological factors such as population density and resource quality can affect disease dynamics. As population size increases, population density coupled with reduced resource availability can promote increased disease prevalence. Physical and biological processes also typically interact to influence disease dynamics. When anoxia occurs in estuaries, fish and invertebrates species typically aggregate on structured habitats adjacent to anoxic areas (Lenihan et al. 2001), so that separating out which factors contribute to population stress can be difficult. Thus, one of the challenges facing ecologists studying disease dynamics involves separating out how these contributing factors interact to promote diseases.

Diseases are quite common among fish and shellfish species in coastal habitats, and can result in the collapse of important fisheries. Thus, understanding how environmental and biological factors collectively influence disease dynamics of fisheries species is critical for coastal managers. Unfortunately, causes of disease are often not well understood, and our ability to counteract their negative impacts on fisheries species can be difficult. For example, in the southeastern United States, parasitic protozoan diseases have rendered attempts to restore native oyster populations largely ineffective (Lenihan et al. 1999) in spite of extensive amounts of research on oyster diseases. In addition to negatively impacting fisheries, diseases can alter the abundance and behavior of ecologically important species, which in turn can dramatically modify community structure and ecosystem dynamics. In Jamaica, the collapse of urchin populations suffering from disease resulted in coral reef mortality levels approaching 90% in the region in the eighties (Hughes 1994). These communities are still recovering today, indicating that diseases can have long lasting impacts on affected populations as well as local ecosystems (Hughes 1996, Hughes and Connell 1999).

In the northeastern United States, relatively little is known about which environmental and biological factors contribute most heavily to shell disease in lobster populations even though it has been linked to the collapse of landings throughout southern New England (French et al. 2001). Given that the vast majority of the lobster fishery historically is landed in coastal Maine, increasing our understanding of lobster shell disease and its risks to Maine's lobster populations is critical.

Lobster shell disease results in necrotic lesions on the lobster's shell that eventually render lobsters unmarketable. The disease is though to be caused by chitin-digesting bacteria and fungi (Hess 1937, Getchell 1989). While the incidence of disease is quite low in coastal Maine, nearshore coastal areas off of Rhode Island and Massachusetts have peaked at levels between 60 and 75% in the last five years (Castro and Angell 2000, Landers et al. 2001). Of even greater concern is that they found

greatest infections levels among egg-bearing females. Scientists have speculated that warmer water temperatures (Hood and Meyers 1974), increased population density (Castro and Angell 2000), an insufficient diet (Fisher et al. 1976), and coastal pollution (Getchell 1989) promote shell disease in crustaceans such as lobsters. However, investigations have yet to effectively demonstrate a causal relationship between these factors and shell disease of the American lobster in the field (Castro and Angell 2000). Therefore, comparison of the diet and contaminant levels of lobsters with shell disease vs. those without across broad geographic ranges will help identify whether these factors promote the disease.

Our research group has been investigating the influence of lobster fishing activities on the diet of lobsters in the Gulf of Maine using a chemical tracer to measure how much lobster tissue has been biosynthesized from consuming herring bait. Stable isotope ratios of nitrogen are effective natural tracers for the flow of organic matter in ecosystems (Frye and Sherr 1984, Owens 1987, Peterson and Howarth 1987, Robinson 2001). Of the nitrogen atoms on earth, approximately 99.6337% are the "normal" ^{14}N; the remaining 0.3663% is a heavier ^{15}N form. Bio and geochemical processes alter the ratio of these two isotopes, and the relative abundance of the heavier isotope is expressed as δ^{15}N on parts per thousand (‰) scale. In trophic interactions, the δ^{15}N of the consumer tends to be isotopically heavier (enriched in ^{15}N) than its food source by an average of 3.4 ± 1.1 ‰ (Minagawa and Wada 1984). Our previous research indicated that δ^{15}N value for herring ranged from 2.3 to 5.5‰ greater than the values of the three most common elements of the lobster diet (Grabowski et al. 2003). Frye (1988) also determined that δ^{15}N signature of herring differed from that of typical lobster prey (crabs, mussels, and brittle stars) by 3.2-4.3‰. These δ^{15}N differences propagate up the food chain and are reflected in lobster tissue.

We intend to utilize this technique to chemically fingerprint the tissues of diseased vs. non-diseased lobsters throughout the Gulf of Maine to identify how the disease affects the diet of lobsters. Given that concerns have been raised about whether herring bait activities influence disease dynamics, this approach will allow us to determine whether diseased lobsters consume relatively more herring bait. Furthermore, by determining how the disease affects the diet composition of lobsters, we aim to determine possible consequences of the disease on community structure.

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Discussion on Animal Responses

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Q.1.*Does status/quality/strain of animal influence the prevalence/susceptibility to shell disease?*

       The discussion highlighted the need to understand the any potential "strain" differences between lobster populations that might predispose those lobsters to shell disease. It is agreed that females appear more vulnerable due to the increased time that the carapace is retained while incubating eggs. It is not know how repeated trapping affects the occurrence of shell disease.

Q.2. *Does the initial localization of epizootic shell disease on the dorsal carapace indicate focal structural carapace deficiency or method of exposure to the infectious agent?*

       While the carapace appears normal in histological sections of unaffected areas on affected animals, much work needs to be done on the molecular components of the carapace and any associated molecular abnormalities. Information is needed in order to understand the effects of food quality/quantity on carapace formation; temperature effects on carapace formation, amount and type of phenolic compound and phenol oxidase incorporation into the upper layers of the carapace and how lack of those components may affect the innate immune systems ability to response in deeper layers of the carapace. It was suggested that alkylphenols may be accumulating in lobsters and may be paying a roll in the disease. However, it was also noted that alkylphenols have been around for a while and have not caused disease before 1998 when epizootic disease started. It was noted that alkylphenolic amounts are increasing in the environment and while levels that accumulate in animals are low, they may still be significant.

       We also need to investigate the roll that biofilm quality/quantity (including the occurrence of the Flavobacteriaceae clad of bacteria in that film) pays in the occurrence of the disease. Questions concerning the effects of temperature on carapace formation were raised. There is need to understand how pigment is deposited in the carapace and how perturbations in xanthine pigment (thought to protective abilities) inclusion in the carapace may predispose to the disease. What is the roll that pollutant associated alkylphenols pay in carapace formation and phenolic (melanization) reactions in the carapace.

Q.3. *Is there a metabolic cost associated with calcification of the cuticle and how does that effect the health of the infected lobsters?*

       Questions were raised concerning the potential for increased molting in affected animals due to increased ecdysone levels. While increased molting may be occurring in females with eggs, there was no data concerning molting in males and non-egged females with the disease. However several people noted that the molting period for lobsters in New England is changing from early July to a more extended period of molting with new molts seen even in December in one area. Bob Glenn noted that he has seen brown-eyed eggs on a female lobster indicating a delayed molt. Another participant verified that high settlement had occurred in January indicating a late hatch date.

Q.4. *Is there a metabolic cost associated with calcification of the cuticle and how does that effect the health of the infected lobsters?*

We need to develop a better understanding of how cuticular mineralization occurs. Are abnormalities present in the affected animals and, if present, are these abnormalities promoting the occurrence of epizootic shell disease. Specifically, are proteins responsible for transferring calcium to the lobsters cuticle abnormal or do environmental factors such as increased CO_2 in the sea waters ultimately result in inhibition of mineralization?

Q.5. *What is the relative importance of active (e.g. mobilization of hemocytes and inflammatory shell deposition) vs. passive (melanization of outer layers or initial thickness of the cuticle) defense in development of shell disease?*

Do low protein levels in the hemolymph predispose to shell disease development in animals in southern New England? More work needs to be done at the molecular level to understand communication channels between the lobsters body and the layers of the carapace. What is the role of pore canals and is neural tissue present in the carapace in areas other than the mechanoreceptor canals. Is the innate immune system working properly in animals that develop disease and how does active cellular inflammation relate to the protective inflammatory mechanisms in place in the carapace (ie. phenoloxidase / melanization reaction and inflammatory cuticle formation).

Chapter 4 Population Responses

During the discussion period, the panel discussed the following questions, and the discussion was transcribed and is presented following the submitted papers.

1. **What information on ecosystems health is required to improve surveillance for lobster health?**

2. **What is the quantitative snapshot of the epidemiology and geography of lobster diseases?**

3. **How do ocean currents influence the spread of bacterial diseases in lobsters?**

4. **Will levels of atmospheric carbon dioxide change significantly to influence the metabolic costs of calcification?**

5. **What are short and long term predictions of ocean temperature, and will this significantly effect bacteria / lobster interactions?**

Prevalence and severity of shell disease in American lobster (*Homarus americanus*) from eastern Long Island Sound, Connecticut.

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Population characteristics of lobsters inhabiting the nearshore coastal waters of eastern Long Island Sound have been monitored continuously since 1975, and with consistent methodology since 1978, using unvented wire research pots (Keser *et al.* 1983; DNC 2004). Since 1984, we have qualitatively assessed external damage to the carapace and abdomen and noted the presence of shell disease (chitinoclasia). Shell disease is characterized as a deterioration of the exoskeleton by chitinoclastic microorganisms (Rosen 1970; Sinderman 1970). Gross signs of the disease are similar in all crustacean species; the exoskeleton is pitted and marred with necrotic lesions and, although the disease is not immediately fatal to lobsters, death may occur (Fisher et al. 1978). The unsightly appearance of the lobster shell can greatly affect marketability.

Since we began monitoring shell disease, 170,000 lobsters have been examined. Shell disease was uncommon from 1984 to 1997; only a few lobsters (n=7, <0.1%) were found with signs of the disease (Table 1). The outbreak of shell disease in our population began in the fall of 1998, when 6% of the lobsters caught in September and October had signs of minor shell disease. The outbreak of shell disease coincided with record high lobster abundance in our area, as well as in other areas of Long Island Sound and southern New England. Following an increase in the incidence of shell disease in 1999 (9%; Table 1), lobster biologists from southern New England and New York developed standardized procedures in spring 2000, for monitoring the extent of shell disease in wild populations. The following index was established based on the percent shell coverage of disease symptoms (e.g., pitting, erosion, lesions) on the total surface area of the lobster: 0=no shell disease symptoms, 1=symptoms on 1-10% of shell surface, 2=symptoms on 11-50% of shell surface, and 3=symptoms on >50% of shell surface (NY/CT Sea Grant 2000).

The prevalence of shell disease increased again in 2000 to 16% and we began to observe a number of lobsters (1,363) that had mottled and scarred shells indicative of a previous shell disease infection. These observations and results from our tag and recapture studies indicated that lobsters infected with shell disease successfully molted. Some of these recently molted individuals became reinfected with shell disease and new pitting and lesions appeared to reoccur within the scared and mottled areas of the exoskeleton previously infected. Shell disease prevalence and severity reached peak levels in 2001, when 22% of the total catch had initial or reoccurring symptoms of the disease (Table 1). The percentage of the total catch observed with shell disease symptoms remained at 22% during 2002 and 2003. Last year however, the prevalence and severity of shell disease declined. During 2004, 15% of the total catch had signs of shell disease with the majority of individuals having <10% of the shell surface eroded. Since 2001, minor to severe shell disease has been observed in coastal lobster populations from eastern Long Island Sound to the Gulf of Maine, but the prevalence of shell disease in central and western LIS and in offshore canyon areas has never exceeded 5% (Landers et al. 2001).

Table 1. Summary of shell disease prevalence and severity[a] for American lobster collected in eastern Long Island Sound, Connecticut during the period 1984-2004.

Year	Number examined	Initial occurrence of disease			Scarring from disease	Second occurrence of disease			Total[b] diseased	% [b]
		<10%	11-50%	>50%		<10%	11-50%	>50%		
84-97	115,751	7	0	0	0	0	0	0	7	0.1
1998	10,991	157	0	6	0	0	0	0	163	1.5
1999	11,216	917	87	0	0	0	0	0	1,004	9.0
2000	8,707	282	221	435	1,363	90	113	286	1,427	16.4
2001	7,268	338	196	275	2,201	233	135	383	1,560	21.5
2002	4,829	261	151	139	1,425	159	91	278	1,079	22.3
2003	5,578	382	253	259	1,668	223	69	50	1,236	22.2
2004	5,001	363	191	91	657	84	15	1	745	14.9

[a] Severity index used was 0=no shell disease symptoms, 1=symptoms on 1-10% of shell surface, 2=symptoms on 11-50% of shell surface, and 3=symptoms on >50% of shell surface (NY/CT Sea Grant 2000).
[b] Total diseased and percentage diseased does not include individuals observed with scarring from previous infections.

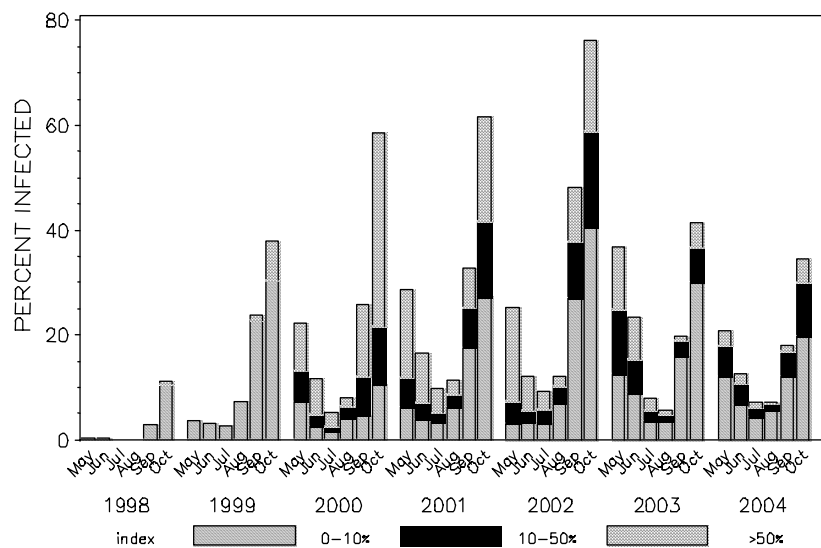During the past five years, the incidence of shell disease (all severity indices) closely corresponded to the molt cycle observed from May through October. Prior to the molt in May 20% to 40% of the catch had shell disease (Figure 1). Following the major molt in July, only about 5% of the catch was afflicted. The severity and percentage of diseased lobsters increased in August and September, reaching a peak in October when between 40% and 80% of the catch had symptoms of shell disease. Male and female lobsters of all sizes have been observed with shell disease, although larger sized individuals and egg-bearing females had higher incidence and severity of shell disease symptoms.

Figure 1. Monthly percentage and severity of shell diseased lobsters collected in eastern Long Island Sound, Connecticut from 1998 to 2004.

Between 50% and 60% of the egg-bearing females had shell disease during 2000 and 2001; the percentage increased to nearly 80% in 2002, but dropped to 66% in 2003 and 51% in 2004 (Figure 2). In comparison, between 11% and 23% of the non-berried females and males showed symptoms of shell disease from 2000 to 2004. In nearshore waters of Rhode Island over 50% of the egg-bearing females observed in the trap fishery were infected with shell disease in 1999 (Castro and Angell 2000). This is most likely

due to the molt cycle of large lobsters and egg-bearing females. Smaller lobsters molt more frequently (1-2 times/yr or more) and shed their shells before severe shell disease symptoms occur. Large lobsters and egg-bearing females experience more shell deterioration because they may only molt every 2 years.
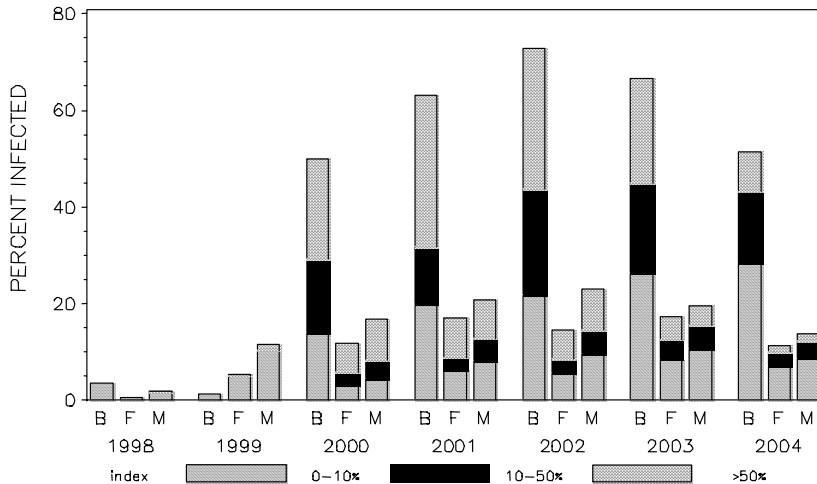

Figure 2. Percentage and severity of shell disease on male (M), female (F), and egg-bearing female (B) lobsters collected in eastern Long Island Sound, Connecticut from 1998 to 2004.

In the period since we have seen extensive evidence of shell disease (i.e., since 2000), we have also observed a 50% decline in lobster catch-per-unit-effort in our monitoring studies. More dramatic declines have been noted in other areas of Long Island Sound and southern New England. Since 1998 when a record 3.7 million pounds were landed in Connecticut waters, landings have declined by 80%; only 0.7 million pounds were landed in 2004. The sound-wide decline in lobster abundance was attributed to a significant lobster mortality event in western Long Island Sound in 1998-1999, although the outbreak and proliferation of shell disease in eastern Long Island Sound could also be a contributing factor for the recent decline in lobster abundance.

A number of isolated outbreaks of shell disease have been reported in lobster populations along the New England coast in the past century. However, these reports were limited to impounded lobsters in the Gulf of Maine during the 1930s (Hess 1937; Taylor 1948) and to infrequent occurrences in wild lobsters in the 1980s along Massachusetts coastal waters and the New York Bight (Sinderman et al. 1989; Estrella 1991). The present epizootic is clearly different from any other cases reported for American lobster. The cause of the outbreak is unknown; it may be due to water quality degradation along the coast or to natural environmental factors such as warmer seawater temperature. Average bottom water temperature measured from May through October as part of our lobster monitoring studies has significantly increased in the past 26 years (slope=0.031°C/year; $p < 0.05$); however, no significant correlation was found between temperature and the incidence of shell diseased lobsters. It is surprising that the prevalence of shell disease was low in central and western LIS, two areas known to be more polluted with domestic sewage and industrial contaminants than areas to the east. The etiology of shell disease may have been enhanced by increased transmission due to crowding as a result of the sharply higher abundance of lobsters in the late 1990s. The fact that shell disease prevalence and severity declined

substantially during 2004 may be early indication that the disease is subsiding. However, the prevalence and severity of shell disease in egg-bearing female lobsters remain high (>50%) and the effect of the disease on lobster recruitment in southern New England may be significant, if egg-bearing females suffer higher natural mortality due to shell disease.

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Can lobster movements contribute to the spread of shell disease?

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Currently the mechanisms involved in the spread of shell disease are not well understood. It is possible that lobsters serve as vectors and therefore it is important to understand both the temporal and spatial patterns of lobster movements. During the past 40 years a considerable amount of movement data have been obtained from tag/recapture studies and some general patterns have emerged. More recent studies using ultrasonic telemetry have both confirmed the tag/recapture findings and provided new insight into the small-scale movements of lobsters in coastal waters. This paper and presentation will consist of a brief overview of the most relevant findings obtained from previous investigations, followed by a summary of the movement patterns that have emerged from ultrasonic telemetry investigations in NH estuarine and coastal waters. When possible, data will be used to speculate about whether lobster movements can influence the spread of shell disease.

Every previous review of lobster movements has reached the conclusion that, while inshore lobsters are very mobile, their movements are fairly localized (reviewed by Krouse, 1980; Cooper and Uzmann, 1980; Haakonsen and Anoruo, 1994; Lawton and Lavalli, 1995). In general, the majority of the lobsters for which data are available moved < 10 km during their time at large, except during seasonal migrations in the spring and fall. This is clearly demonstrated in one of the most extensive ongoing tag/recapture studies based along the coast of Connecticut, near the Millford Power Plant. In that area 92% of the > 20,000 lobsters tagged have been recaptured within 5 kms of their release location (Don Landers, personal communication). In contrast, approximately 10% of the lobsters in most previous studies moved much greater distances, at rates of up to 3 kms/day. In some cases, such as around Cape Cod and near Nova Scotia, an even larger percentage of the lobsters moved long distances (Fogarty *et al*., 1980; Cambell and Stasko, 1985). While the most mobile inshore lobsters tend to be larger, only one study has demonstrated a significant relationship between the size of lobsters and the distance they move, at least in the size range typically studied (~50 mm CL to 100 mm CL). Campbell and Stasko (1985) found that immature lobsters did not travel as far as mature (>95 mm CL) lobsters. It should be noted that some of the animals tracked in Massachusetts and Nova Scotia waters were probably offshore lobsters that had migrated inshore. Thus, these findings should be considered more of the exception than the rule.

During the last 3 years, as part of a collaborative project funded by the Northeast Consortium, we have accumulated a considerable amount of data that have enabled us to compare the characteristics of the lobsters in the northern, middle and southern part of the offshore lobster fishery. In terms of size structure and the size at maturity for female lobsters, it appears as if the northern lobsters have different characteristics than those in the middle and southern areas. These differences could be a result of fishing pressure, but we believe they are a result of the range of water temperatures experienced by these lobsters and their migratory patterns. It is well documented that ~20% of the offshore lobsters from the middle and southern offshore canyons move considerable distances inshore in the summer and then back offshore in the late fall (Cooper, 1971; Cooper and Uzmann, 1971). In contrast, in the northern areas, offshore lobsters move up to shoal

areas, like Browns and Georges Bank (Pezzack *et al*., 1992), probably to gain the same thermal advantages that lobsters in other regions acquire by moving inshore. Therefore, if migrating lobsters serve as vectors for the spread of shell disease, it seems more likely that offshore animals in the south and middle regions would have a higher incidence infection than offshore lobsters in the north. However, based on our sampling of > 27,000 offshore lobsters, shell disease is still very limited in all offshore regions and it is not significantly higher in the south. Only 29 (0.11%) of the lobsters examined had shell disease and of these 29 lobsters, more than half were minimally infected (stage 1 and 2). However, our dataset does not include regions in the very southern portion of the range, and these are the areas where the highest incidence of the disease would be expected. In 1999 Castro and Angell (2000) reported that 0.8% of the lobsters sampled from Hudson Canyon had shell disease, which suggests that the incidence might be higher in offshore regions adjacent to the inshore areas where shell disease is most common.

In contrast to the inshore-offshore movements of very large lobsters in southern New England most lobsters < 100 mm CL restrict their activity to a fairly small range. Moreover, the ones that do move moderate distances typically travel along paths close to the coastline, often in the south or southeast direction (or southwest in Maine). Some of the larger lobsters tagged near Cape Cod may be the exception to this rule, moving north on some occasions, but as mentioned previously these could be offshore lobsters that are tagged while they are inshore (Fogarty *et al.,* 1980; Estrella and Morrissey, 1997). Given these tendencies, and the fact that most shell disease is found south of Cape Cod, and it would appear as if spread of the disease to northern waters by way of lobster vectors would be limited.

Recently, we have been conducting field studies using ultrasonic telemetry as well as laboratory experiments to determine the factors that influence their movements. The results from laboratory studies (Crossin *et al*., 1998; Jury and Watson, 2000) support previous suggestions that lobster movements are strongly influenced by temperature. They avoid both low and high temperatures and they behaviorally thermoregulate, preferring temperatures that are slightly higher than ambient. However, these laboratory data do not explain all lobster movements as demonstrated by the recent studies by Diane Cowan and her colleagues (personal communication).

Our ultrasonic telemetry studies support previous findings and shed some new light on local movements. First, our data support the view that lobsters are very active but remain in the same general area for days at a time. For example, the 45 lobsters we tracked in 2002 and 2003 moved an average of 845 m/day; yet they never moved more than 5km from their origin. Second, while lobsters as a whole tend to be nocturnal, they also move a considerable amount during the day and it is difficult to predict, on any given day, the time period when they will be most active. Third, although tag/recapture or manual telemetry tracking studies suggest that lobsters maintain the same shelter or home range for several days at a time, our high resolution, fixed array, tracking data indicate that lobsters are more nomadic and they typically change their home range every 1-2 days; even though they remain in the same "neighborhood". Finally, sporadically, lobsters will move longer distances, for unknown reasons, and take up residence in a new "neighborhood".

While these data reveal more about the details of daily lobster activities, the overall pattern of movement is consistent with previous reports. Most lobsters are residents, transients or migratory and the factors causing the transition from one state of activity to the next are poorly understood. It is only the migratory lobsters that are likely to serve as vectors for the spread of shell disease, and this is a much greater concern in the southern range of the population than in the north. Finally, it would be of interest to understand more about the relationship between molt stage, reproductive stage and migrations. For example, if lobsters tend to move the greatest distances following a molt when shell disease is limited, this would further reduce the likelihood of shell disease spreading from lobster movements. In contrast, shell disease typically occurs with a higher incidence in berried females and if they have a tendency to move the greatest distances they could play a major role in the spread of shell disease.

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Lobster movements and vulnerability to environmental stressors: Size matters

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Genetic variation is crucial to the persistence of healthy populations because the resulting diversity tends to protect biological systems from environmental stressors (Endler 1977; Statkin 1985). It is widely accepted that lobsters maintain genetic diversity via migratory movements and larval dispersal (NMFS 1996, ASMFC 2000). However, genetic variability can be compromised in populations that have become fragmented or isolated. This eventually reduces the ability of a population to adapt to environmental changes whether anthropogenic or natural. Investigating the possibility that lobster gene flow has been compromised due to the restriction of migrations and/or dispersal may help us to understand why disease epidemics have occurred in certain locations and not others.

Large, sexually mature female lobsters in Canadian waters show a variety of migratory behavior defined by Pezzack and Duggan (1986) as: (1) ground keepers, that do not migrate, (2) seasonal migrators, that move from deep to shallow waters to thermoregulate for optimal egg development during brooding; and (3) long-distance migrators. Campbell (1986) observed that most sexually mature female lobsters from Grand Manan Island were groundkeepers, while a few migrated hundreds of kilometers annually.

Body size of breeding lobsters may have potential implications on the genetic diversity of a population, possibly playing a role in population fragmentation or isolation. Relying too heavily on small brooders for local egg production would restrict gene flow under two conditions: (1) if small brooders fail to migrate – resulting in local larval hatching, and (2) if their larvae fail to disperse due to larval retention via local currents. Close examination of lobster population die-off events in effected coastal areas may yield evidence indicating that local egg production was disproportionately dependent on small lobsters – perhaps a factor in the population's demise.

The Lobster Conservancy designed a study to compare movements and temperature profiles for small versus large ovigerous lobsters in Muscongus Bay, Maine. We chose the delineation of 50% maturity (93 mm CL for Gulf of Maine) to separate small from large brooders. We predicted that small, inexperienced ovigerous lobsters would travel short distances, remain inside Muscongus Bay and brood at cold temperatures while large brooders would travel longer distances and brood at warmer temperatures in the deeper waters outside the bay.

Testing the prediction that small ovigerous lobsters do not move requires being able to detect animals that are inactive and therefore unlikely to be captured in traps. To track lobster positions remotely and record temperature conditions experienced by brooding lobsters we attached acoustic transmitters to the carapace and temperature loggers to chelipeds of 191 recently spawned lobsters captured in commercial traps (Fig. 1).

Figure 1: Lobster with blue identification tag, acoustic transmitter attached to carapace, and temperature data logger to cheliped.

We performed sea sampling trips aboard lobster boats to establish baseline data on size distribution and sex ratio of lobsters in Muscongus Bay. On sea sampling trips we captured 3,375 female lobsters (ovigerous and non-ovigerous) in 1,190 traps. Two factors prevented us from tagging the targeted 150 lobsters from each size class within Muscongus Bay. Firstly, only 79 ovigerous lobsters of the smaller size class were captured on tagging trips (3% of those captured; Fig. 2). The remaining females were non-ovigerous. Secondly, few large ovigerous lobsters were captured in the bay – we had to fish the mouth and outside of the bay to encounter sufficient numbers of large brooders (Fig. 3). Of the 3,375 females sampled, only 301 (<1%) were at or above the size at 50% maturity (Fig. 2). We tagged 112 (37%) of them.
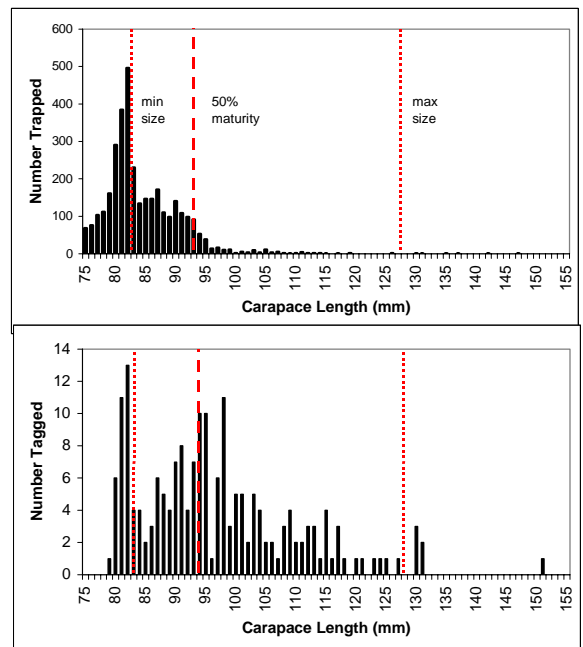

Figure 2: Length frequency histograms of female lobsters trapped (top) and tagged (bottom) in Muscongus Bay. Dotted lines indicate min. and max. legal size, dashed line is size at 50% maturity for Gulf of Maine.
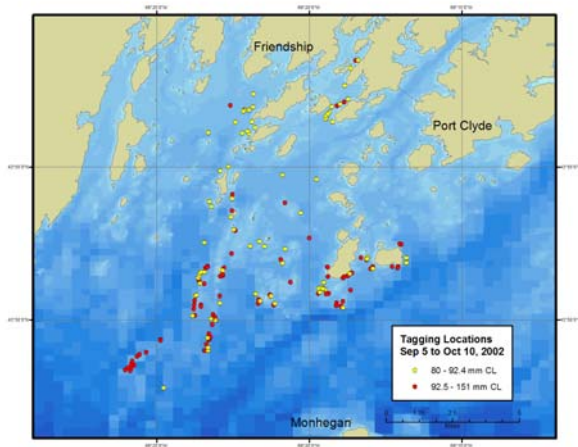

Figure 3. Tagging locations for small (yellow) and large (red) ovigerous lobsters.

Lobsters were tracked using three methods: (1) hydrophone detection of acoustic transmitters, (2) traditional recaptures in lobster traps from Maine to Massachusetts, and (3) SCUBA dive recaptures by hand using an underwater dive receiver. 78% of lobsters tagged were relocated at least once. 46% were
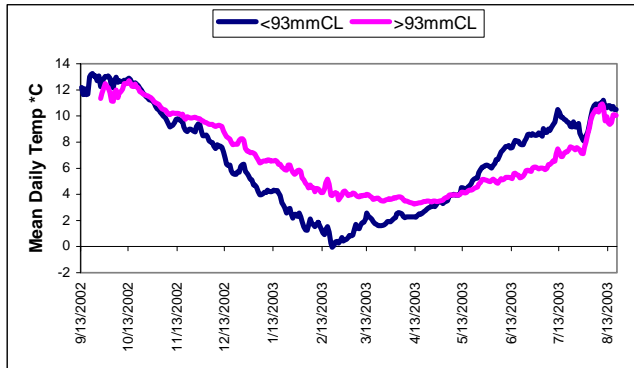
Figure 4. Mean daily temperature for ovigerous lobsters less than (blue) and greater than or equal to (pink) the size at 50% maturity for the Gulf of Maine.

recaptured. Individual lobsters were captured up to 6 times and detected via hydrophone up to 27 times over a one-year period. Temperature data were analyzed from 30 recaptured lobsters that carried temperature loggers for at least 224 and up to 358 days. Water temperatures experienced by small brooders were colder on average from Nov- Apr, and warmer from mid-May – Jul than for large brooders (Fig. 4). In addition, small ovigerous lobsters experienced greater extremes as well as more dramatic fluctuations in water temperature than large brooders that experienced more moderate temperatures throughout the year (Fig. 4). All brooding lobsters were at temperatures below 5°C for at least 2 weeks. This is of interest because laboratory observations show that "ovarian development usually requires temperatures below 8°C and perhaps below 5°C in December and January" (Waddy and Aiken 1992).

A vast majority of relocated lobsters traveled less than 20 km. Large ovigerous lobsters traveled up to 240 km and tended to travel greater distances than small brooders (Fig. 5). The overall direction of travel was south-southwest (Fig. 6).
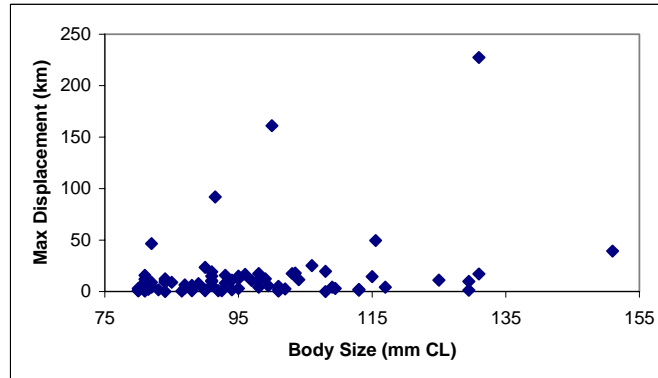

Figure 5: Maximum displacement of recaptured lobsters.

These data reveal that small and large ovigerous lobsters behave differently and experience different environmental conditions throughout the year such as temperature. Although small females were far more abundant, very few carried eggs (<3%; Fig. 2). Small ovigerous lobsters tended to be found inside the bay (Fig. 3), tended to remain inside the bay, and brooded at low temperatures (Fig. 4). In contrast, although large females represented less than 1% of all females captured, they were far more likely to be found carrying eggs (Fig. 2). Large brooders were found at greater distances from shore (Fig. 3), and although most brooded and hatched eggs near where they spawned, some traveled great distances (Fig. 5). Large ovigerous lobsters experienced warmer water temperatures while brooding regardless of how far they traveled.
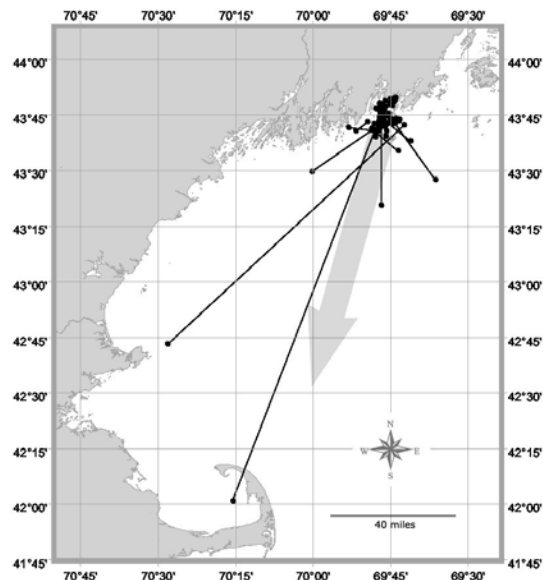
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Figure 6: Recapture locations of lobsters tagged at Muscongus Bay Sep-Oct 2002. Dense cluster of black dots appears near the tag locations at the top of the map. Lines connect point of origin to points of recapture. Gray arrow depicts mean direction of travel.

In spite of a yearlong brooding period and several weeks long larval life it appears that local recruitment is an important component in the American lobster breeding pattern. The results of The Lobster Conservancy's tagging study show that most ovigerous lobsters (especially of the small size class) brooded and hatched their eggs near spawning grounds. This suggests local recruitment. Local recruitment may translate into restricted gene flow in the presence of confined larval dispersal. Genetic diversity makes stocks stronger, especially in the face of adversity. It follows that a lack of large, migrating brooders may result in restricted gene flow that could in turn contribute to the collapse of a local fishery by making the genetically isolated population more susceptible to environmental stressors. It may be prudent to safeguard against potential problems associated with over dependency on local recruitment by encouraging greater potential for larval dispersal. Our results suggest that having more large lobsters in the population may lead to a greater degree of migration and dispersal.

More information on local current patterns would help to strengthen our assertions concerning how lobster populations can become fragmented or isolated. The ultimate test to demonstrate the relative importance of local recruitment would be to determine whether postlarvae settling locally are primarily the offspring of female lobsters spawning, brooding and hatching in local waters.

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Status of shell disease in Long Island Sound

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The CT DEP has been collecting shell disease data for American lobster (Homarus americanus) in Long Island Sound (LIS) since 1992. These data are collected during routine commercial sea-sampling trips aboard the vessels of cooperating commercial lobstermen and during research trawls conducted by the research vessel *John Dempsey*. While examining lobsters care is taken to identify wounds caused by mechanical action so as not to incorrectly identify them as shell disease. If erosion or deterioration of the exoskeleton at the site of mechanical damage is present then shell disease by chitinoclastic microorganisms is noted.

In June 2000, a workshop was held at Millstone Environmental Laboratory (see DRS 2003) to develop a uniform protocol for assessing the severity and proportion of lobsters affected with shell disease syndrome. The participants agreed that with an established index it would be possible to compare relative lobster health among several jurisdictions and it would also be possible to get a more complete coastal picture of the prevalence, severity and progression of shell disease along the range of the lobster resource. The index established during this workshop is applied by taking into account the percent coverage of shell disease on the total surface area of the lobster. The categories were designed to be broad in scope to aid in reducing subjectivity and are: 0 = no disease, 1 = 1 – 10 % of the shell surface, 2 = 11 – 50 % and 3 = > 50%. In instances where it is difficult to distinguish between two indices the severity (depth) of the shell erosion is taken into consideration when assigning the index.

Incidence of Shell Disease in the Commercial Catch:
Currently, sea sampling of the commercial lobster fishery is scheduled in proportion to the seasonal magnitude of landings. Sampling is equally divided among the three basins of the Sound: east, central and west (Figure 1), for a total of at least 24 trips annually. During the years immediately following the 1999 die off (2000-2002) more trips were taken in an effort to better characterize the composition of the fishery (maximum annual total = 78 trips). Prior to the die-off 15-20 trips were taken annually, however too few trips were taken in the central basin to characterize the smaller fishery there in comparison to the east and west.

Data recorded during these trips include: carapace length (measured to the nearest 1.0 mm except for lobsters 82.0-82.9 mm which are measured to the nearest 0.1 mm to distinguish minimum legal size); sex; shell hardness; relative fullness of egg mass (<1/4 complement, 1/4, 2/4, 3/4, full); developmental stage of eggs (green, brown, tan); damage observations to determine cull rates and incidence of damage to claws, carapace, abdomen, and walking legs; incidence of shell fouling organisms; incidence and extent of shell disease (0, 1-10%, 11-50%, >50% of body covered).
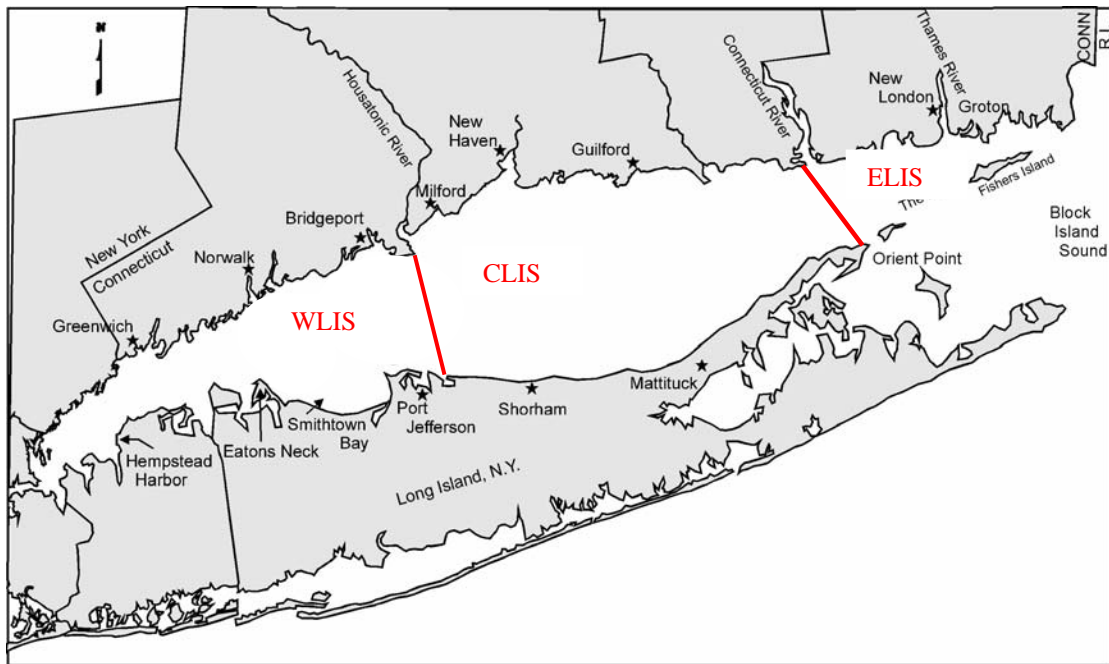
Figure 1: Long Island Sound (LIS) areas recorded for shell disease data. *(ELIS = Eastern LIS; CLIS = Central LIS; WLIS = Western LIS)*

Up until 1998, most shell disease occurrences were small lesions, or necrotic spots, sometimes noted as "burn-spots" on the carapace or tail. These LIS occurrences typically had a low prevalence rate in the observed commercial catch (Table 1, Figure 2). However, observations after 1998 from commercial sea-sampling indicated that perhaps a different type of shell disease, characterized by an extensive deterioration, erosion, or pitting of the exoskeleton, was present in the LIS lobster population. This higher prevalence of shell disease in Connecticut waters started from the near-shore Rhode Island and Block Island Sound waters and progressed into Fishers Island Sound in the late fall of 1998 and winter of 1999 (Gottschall et al. 2000).

Eastern Long Island Sound (ELIS) has typically been the site of the highest percentages of animals afflicted with shell disease (Figure 2). Percentages of shell disease in western and central LIS have ranged from 0.02% to 2.2% from 1992 through September 2004 (Table 1). Since 1992, the incidence observed in commercial sea-sampling trips in ELIS has been rising from 1-3% observed from 1992-1999, to a record high of 11.7% in 2002. Occurrence rates in 2003 and 2004 were slightly lower (9.3 and 10.3% respectively). This decline could be attributed to cooler seawater temperatures in 2003 and 2004. These results corroborate the correlation found between disease frequency and water temperature from data gathered by the Millstone Environmental Laboratory (DRS 2003).

When data from each basin are examined separately (Figure 3), the dramatic rise in disease occurrence in the eastern basin is obvious while occurrence remains at low levels in the western basin (>4% of western samples, Figure 3). Data collected from research trawl catches in the western and central Sound from 1993-2004 show a similar pattern.

Table 1: Percent of shell diseased lobster in the observed commercial catch, January-December 1992-2003, and January-September 2004. (*=no commercial sampling data available) *ELIS=eastern LIS, CLIS=central LIS, WLIS=western LIS.*

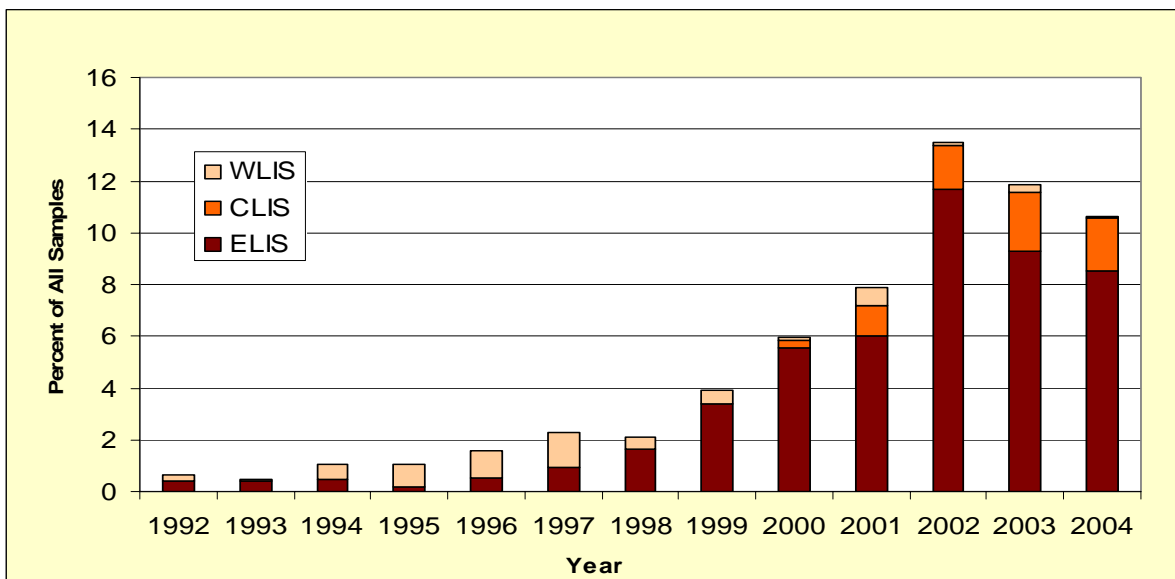| Year	ELIS	CLIS	WLIS
1992	0.42	*	0.22
1993	0.39	*	0.08
1994	0.45	*	0.58
1995	0.17	*	0.86
1996	0.51	*	1.05
1997	0.92	*	1.37
1998	1.63	*	0.49
1999	3.41	*	0.53
2000	5.52	0.31	0.10
2001	6.00	1.18	0.68
2002	11.69	1.69	0.11
2003	9.30	2.24	0.30
2004	8.53	2.06	0.02

Figure 2**:** Frequency of shell diseased lobsters in the total observed commercial catch by basin of Long Island Sound (LIS), January-December 1992-2003, January-September 2004. *WLIS=western LIS, CLIS=central LIS, E=eastern LIS*
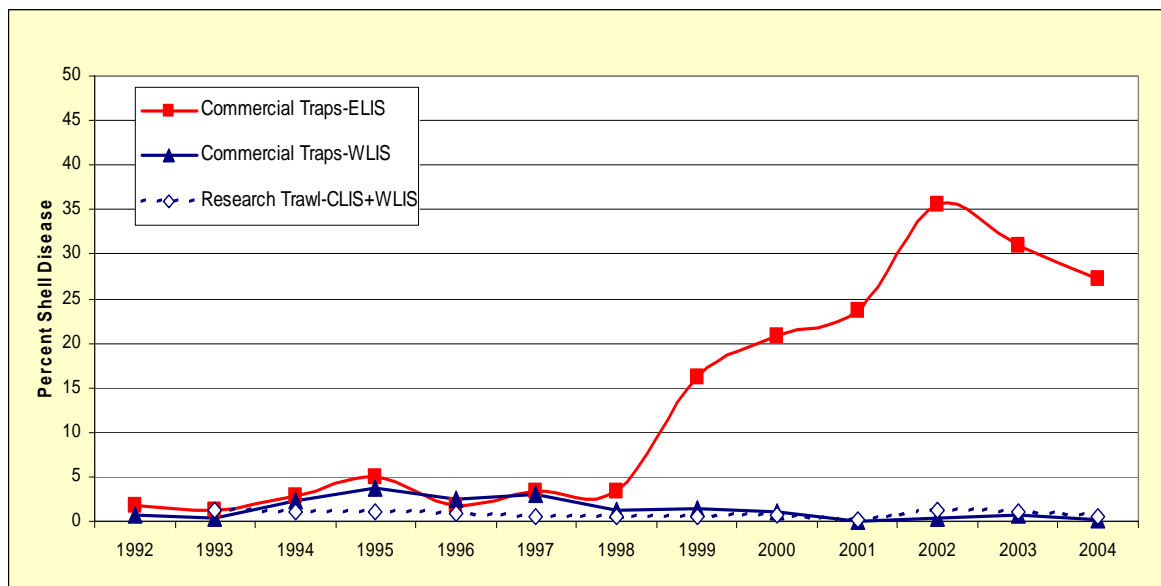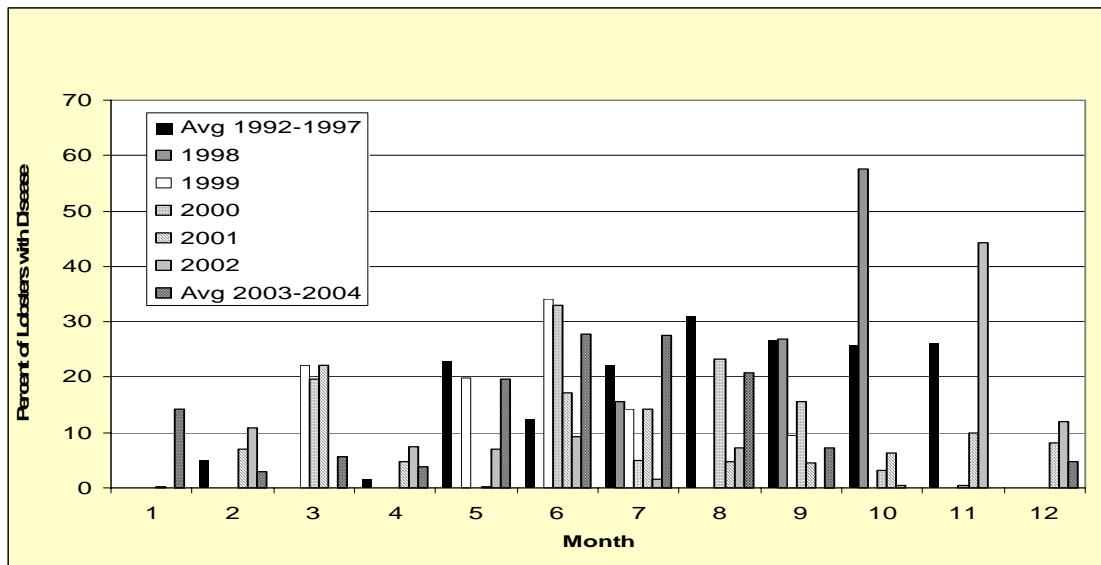
Figure 3: Frequency of shell diseased lobsters in the observed commercial catch in eastern LIS compared to western LIS and observed research trawl catch (central, western LIS), January-December 1992-2003, and January-September 2004.

Seasonality of Disease Occurrence:

Timing of field sampling trips to monitor shell disease is critical because incidence of shell disease decreases over the molting period as a higher percentage of lobsters gain their new shell. The disease then appears to increase in the post-molt months with increasing water temperature. The two molting periods in Long Island Sound typically occur during the summer (July – August) and fall (October-November). Monthly occurrence of shell disease has followed a predictable pattern around these two molt periods. Disease levels peak between May and August and again during October and November prior to the molt and decline following these months as animals successfully shed their old shells.

In the eastern basin, the seasonality of the disease has been consistent over the time period 1992-2004, but was most dramatic in recent years (Figure 4). Average shell disease occurrence is less than 25% January to April, but increases to as high as 57% in the warmer months before each molt. In late spring and fall, soon after most lobsters have molted, the incidence was less than 3.5%. Central and western LIS monthly incidence never exceeded 3.5% in any month, but had highest levels recorded in the summer months preceding the molt.

Figure 4. Frequency of shell diseased lobsters in the observed commercial catch in Eastern Long Island Sound by month, 1992-2003. January-September 2004 is averaged with 2003 data.

Size and Gender Prevalence in Disease Occurrence:

When the eastern basin catches are examined by gender and eggbearing status, it becomes clear that eggbearing females represent the majority of diseased animals (Figure 5). In the highest year, 2002, when 87% of all observed eggbearing females in eastern basin samples were diseased. Occurrence in non-eggbearing females and males also increased after 1997, but only to 10-20% of eastern lobsters. Since 2001, when documentation of disease severity was standardized, the bulk of legal sized lobsters observed with severe shell disease (scale 3, >50% coverage) were again eggbearing females (Figure 6). There is little difference between sublegal (Figure 7) and legal size classes: both classes of eggbearing females had the highest occurrence of severe disease.
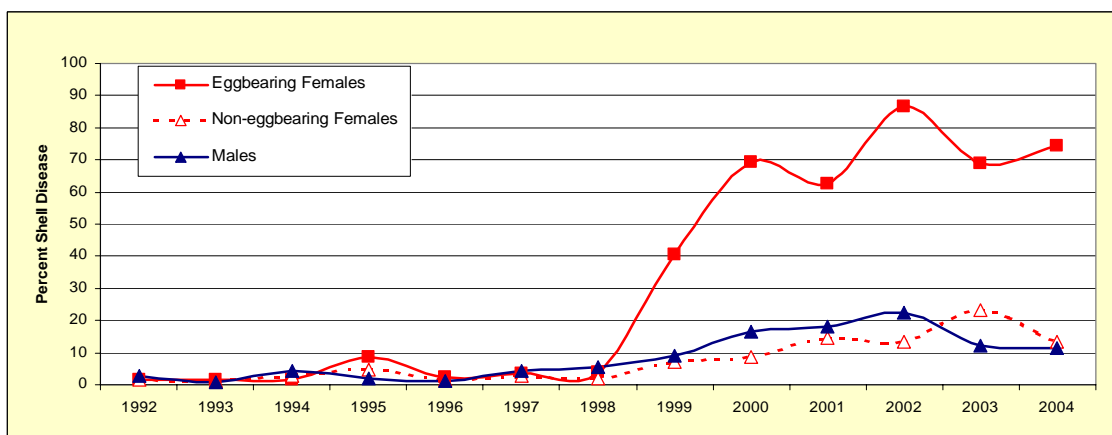
Figure 5: Shell disease occurrence in eastern basin observed commercial samples for eggbearing females, non-eggbearing females, and males 1992-2004.
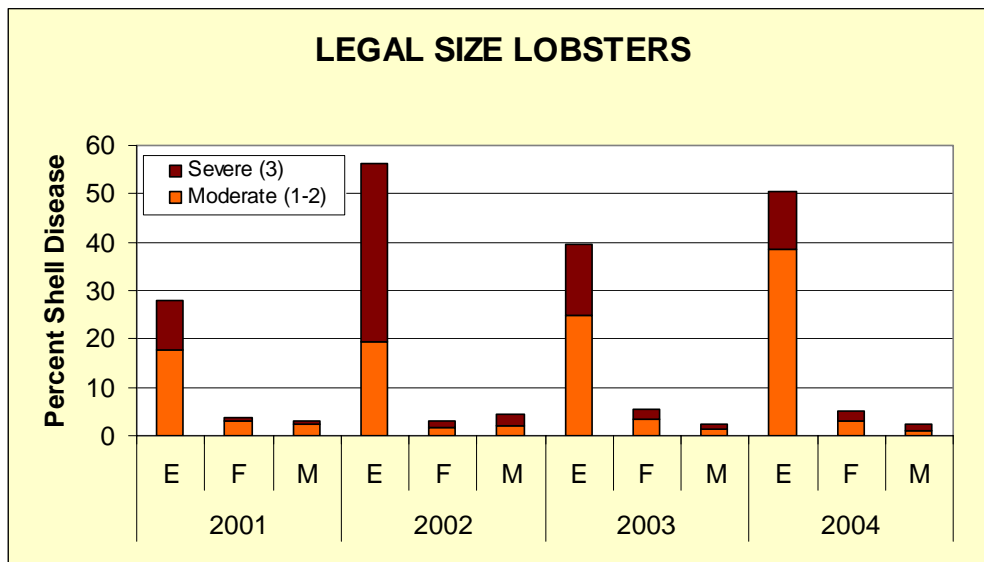
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Figure 6: Percent and severity of shell diseased lobsters in the observed commercial catch of legal sized animals, >82.5mm carapace length. (E=egg-bearing female; F=non egg-bearing female, M=male), 2001-2004.
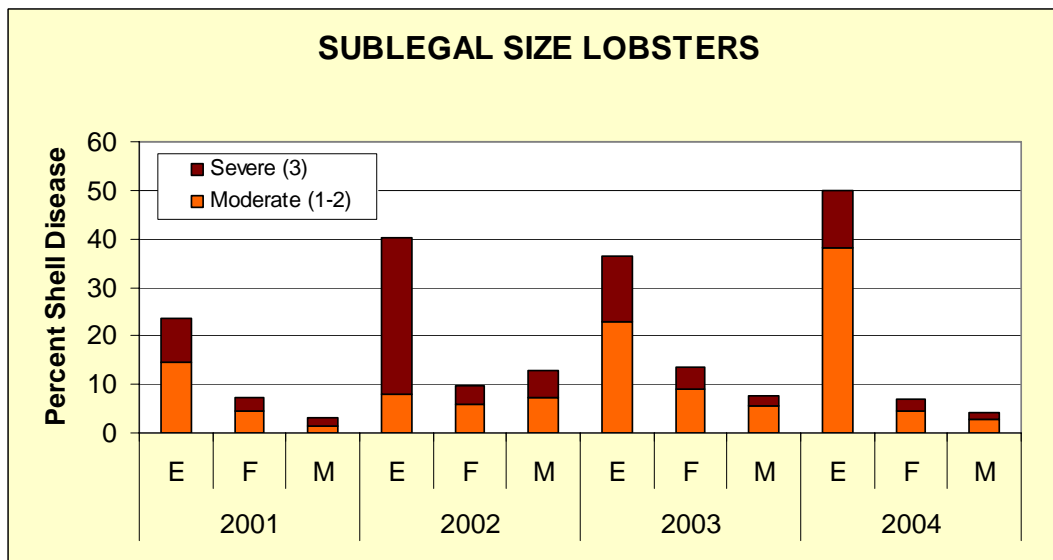

Figure 7: Percent and severity of shell diseased lobsters in the observed commercial catch of sublegal sized animals, <82.6mm carapace length. (E=egg-bearing female; F=non egg-bearing female, M=male), 2001-2004.

Progression and Retention of Shell Disease based on Tagging Results:

Lobsters were collected for tagging in otter trawl catches made during CT DEP Long Island Sound Trawl Survey in spring (April-June) and fall (September-October) and from commercial fishing vessels during routine sea sampling trips throughout the fishing season. Beginning in 2002, tagging was suspended during the months of July-September due to high mortality associated with high water temperatures (Simpson et al. 2003). Prior to tagging, each lobster's carapace length (CL), sex, egg color and complement, shell damage and shell disease was recorded. A target sample size was established at 500-1,000 lobsters for each of three size groups (legal >82.6 mm CL, recruits 72 to <82.6 mm CL, and pre-recruit 60 to <72

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mm CL) and two sexes plus eggbearing females. Lobsters were tagged using Floy® T-Bar anchor tags (#FD-94) inserted into the dorsal muscle under the carapace edge to the right of center. To prevent injury and improve tag retention, soft and pre-molt lobsters were not tagged. All lobsters were released as close as possible to the capture location. Waypoints were recorded on a handheld GPS.

Recapture information was obtained from commercial and recreational lobstermen and from the DEP Long Island Sound Trawl Survey. A two-tiered tag recapture reward system was used to enhance and evaluate recapture rates, distinguished by tag color. The standard tag was orange and carried a reward of $5 and a high value tag was white and carried a $100 reward. This reward was given for the information returned (not the tag); we asked the fishermen to return both legal and sublegal tagged lobsters to the water to maximize information from multiple recapture of individual lobsters. Information requested from fishermen included tag number, date and location of recapture, sex, size (sublegal/legal or gauge size), and presence/absence of shell disease. All license holders in Connecticut and New York were mailed two notices describing the tagging study, including a postage-paid form to record recapture information. To collect size at recapture, Connecticut commercial license holders were provided with a length gauge and instructions on use. This gauge measured size in one-centimeter intervals between 5 and 14 cm.

Tag return data were examined for changes in the occurrence of shell disease during days at large. Return records for 2,647 lobsters contained enough information to examine the retention and acquisition rates of shell disease (Table 2). The average duration between release and recapture for these animals was 148 days.   Of the 2,647 returns, 392 lobsters were tagged with and 2,255 were tagged without shell disease.

For those lobsters tagged without shell disease, 186 of 2,255 animals, or 8.2%, were recaptured with shell disease (Table 2). These recaptures included 26 legal, 152 recruit, and 8 pre-recruit size lobsters. This disease acquisition rate was calculated over a 41month period (August 2001-December 2004). However, interim calculations for each year gave a similar percentage acquisition. This acquisition rate should be considered a minimum value since fishers are more likely to forget to report shell condition ('false no') than to report it erroneously ('false yes'). For those lobsters tagged with shell disease, 244 of 392 animals, or 62.2% were recaptured still showing the disease. These included 58 legal, 185 recruit, and 1 pre-recruit size lobsters. Again, this retention rate was calculated over a 41month period (August 2001-December 2004) but interim calculations for each year gave a similar percentage. This disease retention rate should also be considered a minimum estimate for the same reasons as above.

Table 2: Presence and absence of shell disease in tagged lobsters, August 2001-March 2004. For lobsters with multiple recaptures, only the last observation is included.

Disease at time of Release	Disease at time of Recapture	
	No	Yes
No (n=2,255)	91.8% (n= 2,069)	**8.2%** (n=186)
Yes (n=392)	37.8% (n=148)	**62.2%** (n=244)

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Movement Patterns of Diseased Lobsters:

Shell diseased lobsters were tagged in all three basins of the Sound, although numbers released were much higher in the east (Figure 8). Those with severe levels of shell disease (scale 3, >50% coverage) appeared to be mixed at random among those with less severe levels of the disease. The movement patterns of lobsters recaptured with shell disease were not different from those that did not have shell disease (Figure 9). Total kilometers moved per day at large, as well as directionality, were indistinguishable for both groups. Analysis of movement patterns by gender and egg-bearing status also showed no statistical difference between those with and without the disease. Since recapture information from fishers was not complete enough to consistently determine severity of the disease at the time of recapture, movement by level of severity could not be examined.

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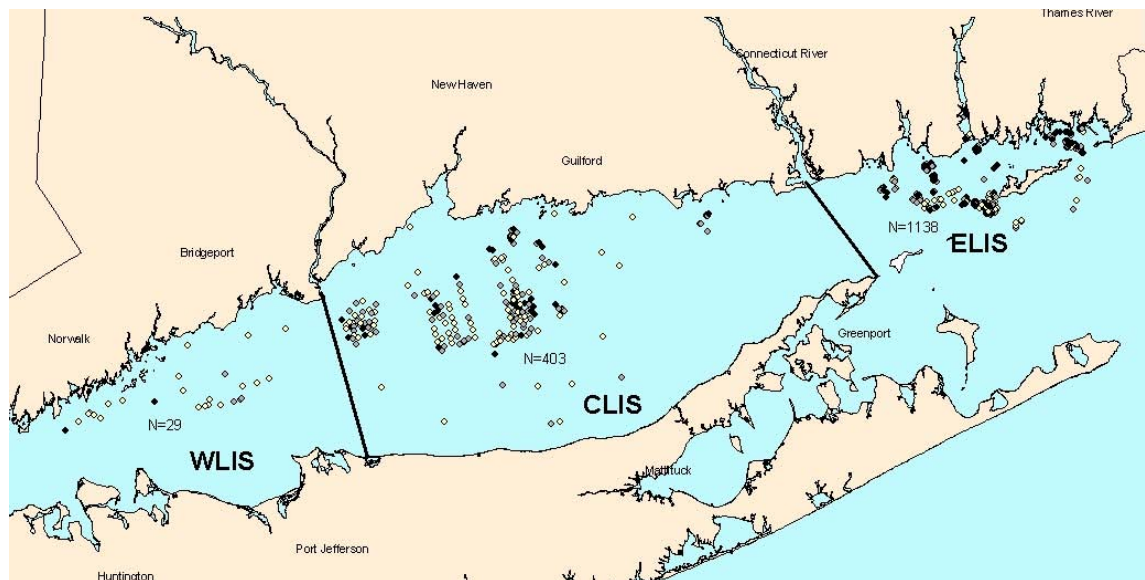
Figure 8: Number of lobsters tagged with shell disease in Long Island Sound by basin. Severity of shell disease is indicated by color (light=SDS index 1, medium= SDS index 2, dark=SDS index 3).
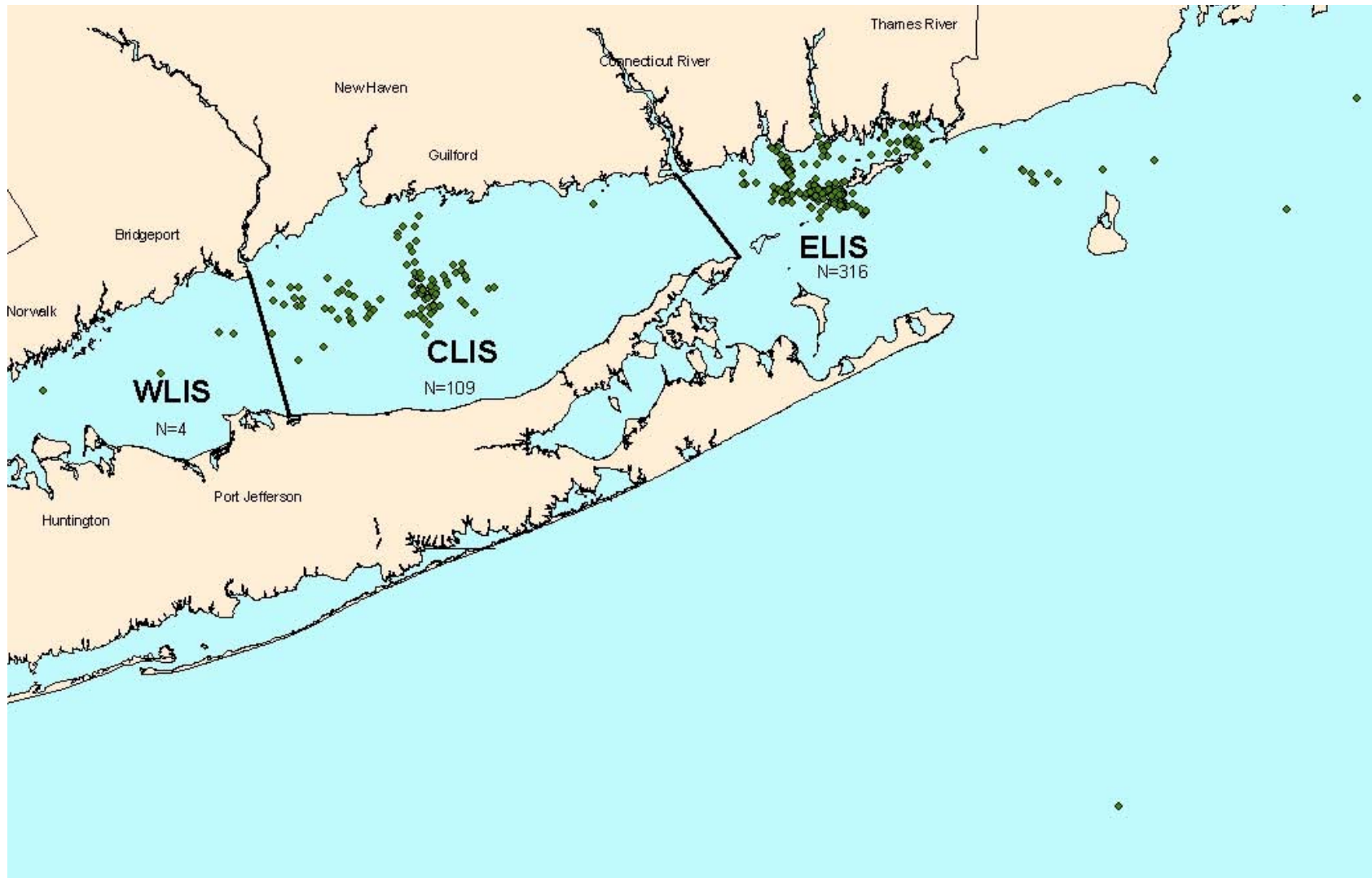
Figure 9: Number of tagged lobsters recaptured in Long Island Sound with shell disease (by basin).

Reduced recruitment of inshore lobster in Rhode Island in association with an outbreak of shell disease and management implications

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Introduction:

It is generally accepted that the contemporary inshore fishery for American lobster (*Homarus americanus*) is recruitment based (Ennis 1986, ASMFC 2000). While lobster can attain considerable size as evidenced by specimens to 42 pounds (Wolf 1978), the average weight in the Rhode Island inshore fishery is only about 1.2 pounds (Angell and Olszewski 2004). This is well below the 3 pound average that existed in 1841 during expansion of the US inshore fishery Goode (1887). Sea sample data collected by the Rhode Island Division of Fish and Wildlife (RIDFW) on board inshore lobster vessels in 2001-2003 indicate that 88% of the marketable catch was within one molt of legal size. Excessive exploitation is the most likely cause of truncation to the inshore size distribution. Both peer reviewed stock assessments concluded that the lobster resource was over fished throughout its range (ASMFC 1997, 2000). Over fishing was also identified as the cause of decreasing mean size during expansion of the offshore fishery (Skud 1969). Over fished status refers to exploitation rates in excess of that needed to maximize yield per recruit, also known as growth over fishing. No evidence existed that over fishing had reduced recruitment to the stock (ASMFC 2000).

The heavy dependence on new recruits makes the inshore fishery vulnerable to disruptions in larval supply and/or reductions in survival rate of pre-recruit lobster. A larval subsidy from offshore to inshore stocks is likely (Katz et al. 1994) would impart resilience to the inshore population when operational (Fogarty 1998). Still, changes in pre-recruit survival rates could impact recruitment to the fishable stock regardless of larval source. In 1996, National Marine Fisheries Service (NMFS) scientists used a hypothetical stock-recruitment model to show that small changes in survival rate could lead to recruitment and fishery failure (ASMFC 1996). The lobster population along the North Atlantic coast underwent a large increase in abundance during the 1990's (Drinkwater et al. 1996). Both fishery landings and catches in fishery independent surveys increased. Drinkwater et al. (1996) considered whether a sustained rise in water temperatures could explain the recent recruitment burst although other hypothesis had been advanced such as reduced predation and increases in minimum legal and escape vent size (Fogarty 1995). Although Wahle at al. (2004) predicted stability for the Rhode Island fishery on the basis of a larval settlement index, high abundance was not sustained and fishery landings have fallen to low levels (Figure 1). The sharp decline in abundance is confirmed by the University of Rhode Island Graduate School of Oceanography (URIGSO) trawl survey (Figure 2). The magnitude of the landings decline in an over capitalized industry has caused economic hardship. In response to the developing fishery crisis, the Atlantic States Marine Fisheries Commission (ASMFC), lead agency for US lobster management, initiated emergency management actions. They included an

acceleration of the schedule to increase minimum gauge size and an effort control program that remains under development in cooperation with local industry.

The decline in lobster abundance from 1997 to 2004 was coincident to an outbreak of shell disease in Rhode Island waters (Castro and Angell 2001). Shell disease in lobster is associated with chitinoclastic organisms (Sinderman et al. 1989) that opportunistically exploit vulnerable animals (Getchell 1989). Vulnerability apparently occurs when metabolic disturbance or trauma reduce the rate of chitin deposition below the rate of degradation by fouling microorganisms (Sindermann 1991). Heavy infections can result in death during molting (Martin and Hose 1995) and a pre-mature molt by berried females that results in loss of eggs (RIDFW- unpublished data). Occasional lobster specimens with incidental shell "rot" were noted by RIDFW trawl survey scientists from 1979 to 1995 (T. Lynch- RIDFW pers. comm.). There observations are consistent with the low incidence of shell disease observed by Wilk et al. (1997) from 1989 to 1991. RIDFW lobster biologists, conducting sea sampling on board commercial fishing vessels, began noticing increasing frequency and severity of the disease in the mid-1990s. A monitoring program was devised and begun in 1996. The incidence of inshore lobster afflicted with shell disease increased dramatically from 1996 to 1998 peaking in 2002 at 30% of animals examined (Figure 3). A similar increase in infected animals was observed at Millstone Nuclear Power Station on eastern Long Island Sound (DRS 2004). The coast wide assessments (ASMFC 1997, 2000) clearly show that growth over fishing was occurring when abundance began to trend downward. Moreover, Figures 1 and 2 indicate that trawl abundance (legal and sublegal) declined before fishery landings did. This is important because sustained landings in the face of declining abundance indicate high relative exploitation rates from 1998 to 2000. Although no direct evidence of large scale mortality is available, the synchrony of the trawl survey and shell disease data suggest the possibility that shell disease increased the natural mortality rate of lobster thereby reducing recruitment to the fishable stock. Continued heavy exploitation on the residual stock exacerbated the decline. This scenario would be consistent with one posed by NMFS scientists whereby a stock experiencing high productivity could temporarily sustain high exploitation rates until other factors reduced productivity leading to stock collapse (ASMFC 1996). Hilborn and Walters (1992) have cautioned on the implications of non-stationarity for scientists attempting to deduce production relationships from historical data. Fogarty and Gendron (1994) recently identified shifts in pre-recruit survival rate as a crucial determinant of the limiting rate of fishing or that rate where the likelihood of stock collapse is high.

Methods and Data Sources:

Testing a hypothesis that an increase in natural mortality rate reduced recruitment to a heavily exploited stock requires time series of abundance data for life stages separated by a sufficient time lag for the suspected mortality agent to operate. The intervening mortality rate should not be confounded by fishery removals and a quantitative measure of the mortality agent is needed. A candidate stock-recruitment model is needed to formally test the hypothesis if a nonlinear abundance effect is possible. Finally, a closed population is needed so that emigration does not bias the analysis.

The youngest life stage for which quantitative data exist for Rhode Island inshore lobster is new settlers. Egg bearing female lobster in the southern New England area hatch off eggs in the summer and the larvae follow with a 6-8 week planktonic life phase (Ennis 1995). After settlement to the bottom, the newly metamorphosed lobster can be sampled by divers using suction samplers (Wahle and Incze 1997). A standardized survey of this type has been conducted at stations along the south shore of Rhode Island and the mouth of Narragansett Bay since 1990 as part of a New England-wide survey (Wahle et al. 2003). This area is known to be a significant nursery given the massive mortality of juvenile lobster observed during the North Cape oil spill of 1996 (Gibson et al. 1997a, French et al. 2003). Density estimates of settlers in the form of number per square meter are available since 1990. Settlement of Rhode Island lobster was high in 1990 and 1991 but declined thereafter (Figure 4) although not as dramatically as the trawl survey and catch (Figures 1-2). The 1995 and 1996 cohorts were particularly poor, the latter being associated with the North Cape event. The 1997 to 2004 year classes have fluctuated between low and medium levels. In mid-coast Maine as well as Rhode Island, Incze et al. (1997) found that settlement was correlated with larval supply while for the same regions Wahle et al. (2004) showed that the abundance of juvenile lobsters was correlated with the abundance of settlers in earlier years.

As noted above, the RIDFW conducts trawl surveys in the same area as the settler survey. The fall cruise of the seasonal survey is recognized as the most reliable measure of lobster abundance as water temperatures are less variable than during spring cruises. The fall index is used in regional stock assessments (ASMFC 2000). Three size groups are recognized for assessment purposes; legals, recruits, and pre-recruits. The last group is composed of lobsters smaller than 73 mm in carapace length. They will require at least two molts to reach legal size. Examination of the carapace length frequencies shows that the vast majority of lobsters sampled by the trawl are below the current minimum gauge size of 85.7 mm (Figure 5). It is clear from the left hand limb of the distribution, that the smallest lobsters are not fully recruited to the bottom trawl gear, consistent with their shelter restricted behavior (Lawton and Lavalli 1995). Lobsters from about 55 mm to 72 mm are sampled well and were considered the pre-recruit index for this study. Based on the growth model developed for Rhode Island lobster killed in the North Cape oil spill, this size interval corresponds to ages ranging from 2.5 to 3.4 years (Gibson et al. 1997b). Given the findings of Fogarty and Idoine (1986), Fogarty (1995), Ennis and Fogarty (1997), and Wahle et al. (2004) one would expect that trawl abundance of pre-recruits would be related in a non-linear manner to settler abundance with a lag of three years.

We examined the relationship between settler abundance in year t and pre-recruit abundance in year t+3 using power and Ricker stock-recruitment models. Although the Beverton-Holt asymptotic model is arguably most appropriate for lobster (Fogarty and Idoine 1986, Caddy 1986), Ricker's over compensatory curve has better statistical properties particularly when adding additional explanatory variables. In that case, the linearized version of the model has the form of a multiple regression equation the properties of which are better understood (Hilborn and Walters 1992). It is particularly useful in examining factors inducing nonstationarity in S-R relationship as was done by Walters et al. (1985). The behavior of alternative S-R curves at very high stock abundances is unimportant in this study although it may be relevant to resource management. Ricker's (1975) classic curve has the form:

$$R=\alpha \ S \ \exp(-\beta S) \quad\quad\quad\quad\quad (1)$$

where: $R=$ recruitment ($=$ pre-recruit index, in this case)
$S=$ spawning stock ($=$ settlement index, in this case)
$\alpha=$ maximum rate of recruitment
$\beta=$ coefficient of compensatory mortality.

The α parameter defines the slope at the origin or the maximum rate of recruitment. The β parameter defines the density dependent mortality rate per unit of spawning stock (or settlement, in this case). The curve rises from the origin to a maximum recruitment at a spawning stock level (settlement density) equal to $1/\beta$. A solution to the Ricker curve given a set of data can be found using the linearized version that assumes lognormal errors:

$$\ln(R/S)= \ln(\alpha)-\beta S+ \varepsilon \quad\quad\quad\quad\quad (2).$$

Eq.2 states that the overall mortality rate from settlement to recruitment is composed of a density independent factor and one dependent on abundance. When other factors such as disease outbreaks or changes in predator abundance are thought to influence mortality, they can be included easily in the model:

$$\ln(R/S)= \ln(\alpha)-\beta S-\gamma D + \varepsilon \qu\quad\quad\quad (3)$$

where: $D=$ index of disease severity
$\gamma=$ coefficient of disease related mortality.
$\varepsilon=$ lognormal error term.

In this study, pre-recruit trawl abundance was considered the measure of recruitment. Post-larval settler abundance cannot necessarily be considered to be proportional to the local spawning stock as in Fogarty and Idoine (1986) and Fogarty (1995) because of an offshore larval subsidy suspected to contribute to Rhode Island's inshore recruitment. Thus the settler-to-recruit relationship described here may not be assumed to equate to an as yet unknown linkage between spawners and recruits. As noted earlier, a three year lag was used to link recruitment with the appropriate year of spawning and settlement. The disease severity index was configured as the cumulative proportion of animals displaying shell disease over the three years from settlement to recruitment. Table 1 summarizes the settler and pre-recruit abundance indices as well as the shell disease data from sea sampling.

Results:
The abundance of pre-recruit lobster in the trawl survey was strongly correlated at lag 3 with the settlement index for the 1990-1996 cohorts (Figure 6). The best fitting model was a power curve with exponent less than 1.0. The regression was highly

significant (F=0.002) and explained 88% of the variation in pre-recruit abundance (Table 2). The exponent of the power curve was estimated at 0.49 with a standard error of 0.08. A 95% confidence bound on the exponent was 0.28-0.70, significantly less than 1.0 and suggestive of compensatory mortality (Slade 1977). However, when the 1997-2001 cohorts were added, the regression was rendered non-significant (F=0.55). The more recent cohorts clearly do not conform to the 1990-1996 pattern (Figure 7). A time plot of the logarithm of pre-recruit to settler ratio (eq.2) suggests an elevation of mortality rates beginning with the 1997 cohort (Figure 8). With regard to the population closure assumption, it is known that some inshore lobster migrate to offshore areas (DRS 2004). An increase in emigration as an explanation for nonconformance of the 1997-2001 cohorts therefore cannot be ruled out although a ready explanation for the abruptness of a change in dispersion rates is not at hand.

Regression results for fitting the Ricker curve to the 1990-2001 cohorts are found in Tables 3 and 4. The two-parameter, restricted version of the model was not significant (F=1.73, P=0.22) and explained only 15% of the variation in ln(R/S). Regression residuals trended strongly from positive to negative over time demonstrating the inadequacy of the model and the likelihood of a non-stationary relationship. Addition of the shell disease term greatly improved the fit of the generalized model. The three-parameter regression was highly significant (F=27.56, P<0.01) and explained 86% of the variation in ln(R/S). Despite the low sample size, all parameters were supported by highly significant t-statistics (P<0.01). Importantly, the precision on the traditional Ricker parameters (α,β) improved with inclusion of the shell disease variable. The shell disease parameter (γ) was estimated at 2.42 with a standard error of 0.35 in the 3-parameter model. A time plot of residuals indicated that the generalized model resolved the serial correlation problem (Figure 9). The relative influence of settlement density and shell disease on resulting recruitment can be examined by evaluating the compensatory and shell disease mortality terms in eq.3 at their highest observed levels. For compensation, the βS term calculates to 2.241. For shell disease, γD calculates to 1.211. This indicates that density dependent mortality is about twice the mortality rate associated with shell disease.

Discussion and Management Considerations:

The inshore lobster population in the Rhode Island area underwent a major fluctuation in abundance during the past two decades (Gibson 2003). Trawl catches and fishery landings rose steadily from 1979 to 1994 and supported a very lucrative fishery. Abundance in the trawl surveys began to decline in 1998 and reached very low levels by 2002. Fishery landings began declining in 2000 and remain low. The collapse of the population occurred coincident with an outbreak of shell disease. Sea sample data show that the incidence of shell disease rose from insignificant levels in 1996 to over 30% of the population by 2002. Incomplete data for 2004 indicate that incidence of the disease remains high.

Modeling of fishery independent abundance estimates using a Ricker stock-recruitment function showed that mortality rate from the settler to pre-recruit life stage had a density dependent component and one associated with shell disease. The Ricker curve, generalized to include shell disease, was a significant improvement over the standard model. Variance explained and precision of parameter estimates improved in the

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generalized version. Statistical significance of the shell disease parameter however does not prove that shell disease has increased natural mortality rate. Several other variables have also increased coincident to shell disease including water temperature and predator abundance. Changes in emigration or molting rates could also have occurred and may have influenced the number of pre-recruits per settler. There is little doubt however that lobster health has deteriorated and a mortality rate increase is likely for lobster below legal size, a possibility envisioned by Wahle at al. (2004). If so, recruitment has been reduced and both fishery landings and catch per unit effort have fallen.

The impact of an increase in pre-recruit mortality to fishery dynamics is demonstrated in Figure 10 using an approach similar to that in ASMFC (1996). Several Ricker curves are plotted corresponding to a range of shell disease intensity using the parameters estimated above. The series of curves display decreasing recruitment for a given settler density as the incidence of shell disease increases. Fishing mortality (F) is represented by a vector out of the origin which rotates counter clockwise as F increases. The intersection of an S-R curve and a mortality vector represents the equilibrium level of settler density and pre-recruit abundance for that level of F. By definition, stock collapse will occur when the slope of the F vector exceeds the slope at the origin of the S-R curve. At low F (vector A), all of the S-R curves are sustainable, that is compensatory ability remains. However, when F is very high (vector B), only the "no" and "light" shell disease scenarios retain any compensatory ability. This simple demonstration suggests that the Rhode Island inshore lobster population collapsed under the scenario envisioned by NMFS scientists in 1996. That is, a stock with temporary high productivity sustained high F until intervening factors reduced productivity leading to stock collapse. There may be a larval subsidy from offshore to inshore but stability in larval supply has apparently been overwhelmed by an increase in pre-recruit mortality rate.

The finding that pre-recruit mortality rate in lobster is non-linear with respect to abundance is not surprising. Both Fogarty and Iodine (1986) and Ennis and Fogarty (1997) provided examples of highly compensatory S-R curves. Both examples cover a substantial portion of the life history, either eggs or stage IV larvae to recruitment to the fishery. It is not clear from the studies where compensation occurred but Fogarty and Idoine ruled out early larval stages. Wahle at al. (2004) used nonlinear power curves to describe the relationship between settlers and age 1+ juvenile in Maine and Rhode Island. Both of his fitted regressions had exponents well below unity (0.36-0.55) and similar to that found in this study prior to the onset of shell disease (0.49). Since this study involves a longer time lag than in Wahle (2004), the similar exponents suggest that most compensation occurs in the early years of life. Similar results have been demonstrated for Western rock lobster off of Australia by Phillips (1986) and Caputi and Brown (1986). In the former study, compensatory S-R curves were fit to settlement and fishery recruitment and in the latter power curves with exponent less than 1.0 described the relationship between settlement and juvenile abundance. Caddy (1986) hypothesized that natural mortality rate (M) declined with body size in lobster in accordance with fractal surface theory and the biological need to secure shelters. His analysis considered a "musical chairs" process in which a growing organism faced a continuous shortage of appropriate size shelters. The high M rates at smaller sizes indicate that the shortfall is most acute for small lobster and is consistent with compensation at a relatively early age. Wahle (2003) refined these concepts by discretely treating the probability of acquiring shelter and the

probability of predation absent a shelter. Both were size related and over a likely range of scenarios, the probability of survival was lowest for intermediate size animals. The finding by Castro et al. (2001) that new cobble substrate attracted settler densities equal to that on natural cobble sites and the ability of settling larvae to "sample" bottom types (Cobb 1995) indicate a preference for structured habitat early on. Fogarty (1995) used a Paulik diagram to illustrate the major stage transitions but included only one density dependent phase, stage IV larvae to adult. However, the adult stage may be decades long for large animals (Cooper and Uzman 1980). The numerous life stage transitions between late larvae and large adult make it likely that a redundant set of compensatory mechanisms exist such that if one fails at an early age, a second may exert a stronger than otherwise effect later. Animals that pass through more stages and with more opportunities for compensatory should exhibit more stability in abundance (Rothschild 1986). The likelihood that metapopulation dynamics exist in American lobster may require that more complicated treatments of stock-recruitment be undertaken that include source-sink terms (Fogarty 1998).

Long term trawl data (Figure 2) and anecdotal information from industry indicate considerable abundance fluctuations in the local lobster population over decadal scales. Could this variability be a self-fulfilling prophecy of a long history of overexploitation that has compromised the number of life stages? Fogarty and Gendron (2004) have reviewed the implications of high fishing mortality rate on lobster and likened it to the imposition of semelparity on an iteroparous species with concomitant loss of reproductive opportunities to offset environmental variability. Fishing mortality rate on inshore lobster has likely been high for a long time (Anthony 1980, Fogarty 1995) and the clear reduction in mean size has already been reviewed above. The attenuation of population size structure, particularly in males, may have minimized the shortage of shelter problem theorized by Caddy (1986) and contributed to an increase in pre-recruit survival. An increase in average survival could result in more variable survival because of the reduction in stabilizing opportunities. The long term abundance data suggest several periods of high and low abundance (Figure 2). Calculation of the autocorrelation function for the URIGSO trawl data shows significant autocorrelation at short term lags (1-3 years) as would be expected for multi-age animals but also long-term autocorrelation at lags of 22-24 years (r>0.7). The interval between population highs and lows is therefore about 11-12 years. This is well below the fishable lifespan given an age at recruitment of 6-8 years and a longevity measured in decades (ASMFC 2000). If over fishing had been avoided from the inception of the fishery and size composition preserved; recruitment, abundance, and landings would likely have been more stable in the face of shifting environmental conditions.

It is also worth asking what should be the appropriate management response when reduced recruitment, exacerbated by environmental conditions, leads to a fishery collapse. While prudent management would require a precautionary approach in the face of stock fluctuations (FAO 1996), the appropriate management response in this case is not as obvious as it might seem. One position supported by the industry is to hold the environmental conditions responsible and refrain from management actions that further restrict the fishery. Alternatively, absent control over environmental factors, a fishery manager's interpretation of Figure 10 would advocate a rapid reduction of F until a sustainable intersection with the new S-R curve is reached. Logbook data collected by

RIDFW indicates that lobster fishing effort in the inshore areas has declined substantially since 1999 in response to poor fishery performance (Figure 11). This attrition may have reduced F although proportionality with nominal effort is not certain. The reduction in effort should be captured by the developing ASMFC effort control program so that the prospects for stock recovery can be maximized.

The appropriate course of action may also depend on the extent to which the population of shell diseased lobsters is self-recruiting or subsidized by recruits from outside the affected area. Over the past decade larval settlement in Rhode Island has remained relatively constant despite the dramatic decline in adult lobsters, suggesting the region's larval supply is largely insensitive to a decline in Rhode Island's nearshore broodstock. It is likely the effective breeding population for coastal southern New England extends well beyond the area with shell disease, and perhaps to the edge of the continental shelf (Katz et al. 1994). While this might bode well for the eventual recovery of Rhode Island's stock, significant uncertainties remain about the future of shell disease in the region. While some may argue that debating the causes of stock decline is counterproductive to the precautionary approach (Rosenberg 2003, Fogarty and Gendron 2004), it is through our very ability to separate natural from anthropogenic impacts in this case, that more sensible management solutions may be devised. What should be avoided, however, is the not uncommon syndrome that eventually reinforces over-fishing, in which management is delayed in response to pessimistic data (Rosenberg 2003).

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Table 1- Rhode Island Lobster Settlement and Pre-Recruit Abundance Indices Used in Fitting the Stock-Recruitment Model. The Proportion of Shell Diseased Lobster Observed in RIDFW Commercial Sea Sampling is Also Given.

Year	No. Settlers per sq. meter	Pre-recruits per tow	Number Examined	Proportion w/ Shell Disease /1
1990	1.30	4.77	1778	0.00
1991	1.50	7.14	30145	0.00
1992	0.60	5.18	15661	0.00
1993	0.50	11.74	15913	0.00
1994	1.30	11.59	16409	0.00
1995	0.30	7.80	23777	0.00
1996	0.20	9.17	25939	0.00
1997	1.00	9.97	19712	0.04
1998	0.60	5.79	19946	0.19
1999	1.00	3.75	21277	0.20
2000	0.34	2.38	17390	0.22
2001	0.75	4.21	14268	0.23
2002	0.26	0.90	12385	0.31
2003	0.79	2.02	14325	0.25
2004	0.40	1.53	7245	0.34

/1 Note: 2004 is a partial data year for shell disease

Table 2- Summary output for the Regression of log Pre-recruits on log Settlers for the 1990-1996 Cohorts

Regression Statistics	
Multiple R	0.938159
R Square	0.880142
Adjusted R	0.85617
Standard E	0.157292
Observatio	7

ANOVA

	df	SS	MS	F	ignificance F
Regressior	1	0.908387	0.908387	36.71599	0.001766
Residual	5	0.123705	0.024741		
Total	6	1.032092			

	Coefficients	andard Errc	t Stat	P-value	Lower 95%	Upper 95%
Intercept	2.297696	0.06948	33.07009	4.75E-07	2.119093	2.476299
X Variable	0.494038	0.081533	6.059372	0.001766	0.284452	0.703625

Table 3- Summary Output for Linearized Version of Ricker Stock-Recruitment Model

Regression Statistics	
Multiple R	0.383704
R Square	0.147228
Adjusted R	0.061951
Standard E	0.937092
Observatio	12

ANOVA

	df	SS	MS	F	ignificance F
Regressior	1	1.516086	1.516086	1.72647	0.218205
Residual	10	8.781423	0.878142		
Total	11	10.29751			

	Coefficients	andard Err	t Stat	P-value	Lower 95%	Upper 95%
ln(alpha)	2.575384	0.578049	4.455307	0.001225	1.287411	3.863357
Beta	-0.8578	0.652836	-1.31395	0.218205	-2.3124	0.596814

Table 4- Summary Output for Linearized Version of Ricker Stock-Recruitment Model Including a Mortality Term for Shell Disease.

Regression Statistics	
Multiple R	0.927163
R Square	0.859631
Adjusted R	0.828438
Standard E	0.400756
Observatio	12

ANOVA

	df	SS	MS	F	ignificance F
Regressior	2	8.852062	4.426031	27.55844	0.000145
Residual	9	1.445447	0.160605		
Total	11	10.29751			

	Coefficients	andard Err	t Stat	P-value	Lower 95%	Upper 95%
ln (alpha)	3.073055	0.257942	11.91376	8.19E-07	2.48955	3.656561
Gamma	-2.42261	0.358455	-6.75848	8.28E-05	-3.233494	-1.611729
Beta	-1.4938	0.294624	-5.07018	0.000672	-2.160282	-0.82731

Fig. 1- Lobster Abundance in the RIDFW Fall Trawl Survey In Narragansett Bay and RI Coastal Waters and RI Inshore Fishery Landings, 1979-2004
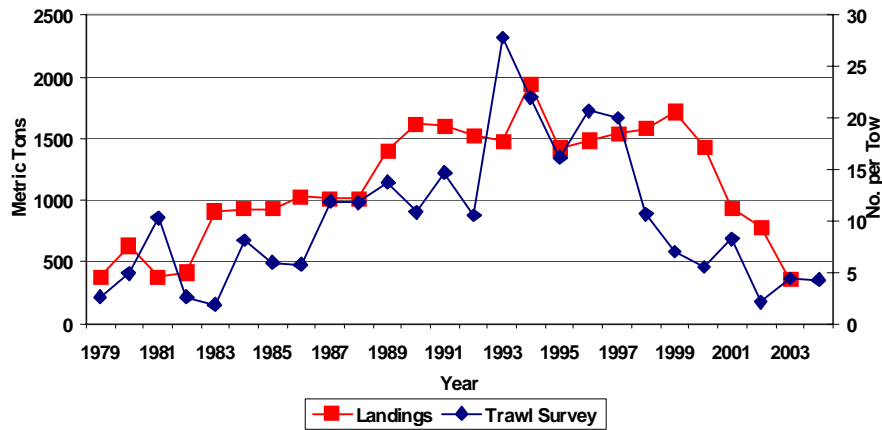
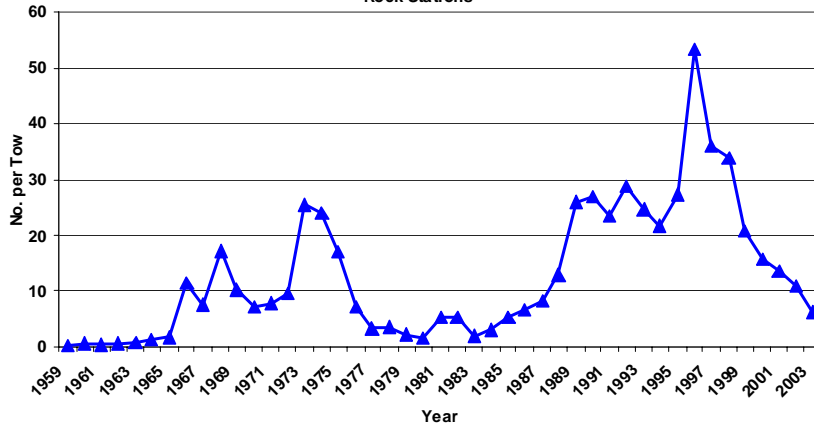Fig.2- Lobster Abundance in the URIGSO Trawl Survey at Fox Island and Whale Rock Stations

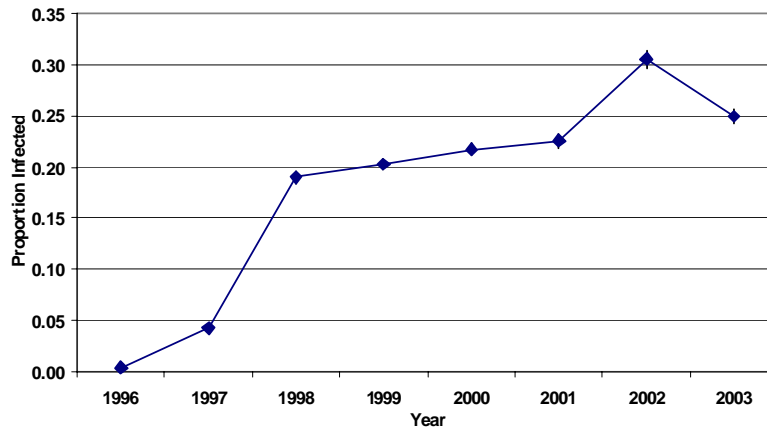Fig.3- Incidence of Shell Disease in Inshore Lobster from RIDFW Sea Sampling

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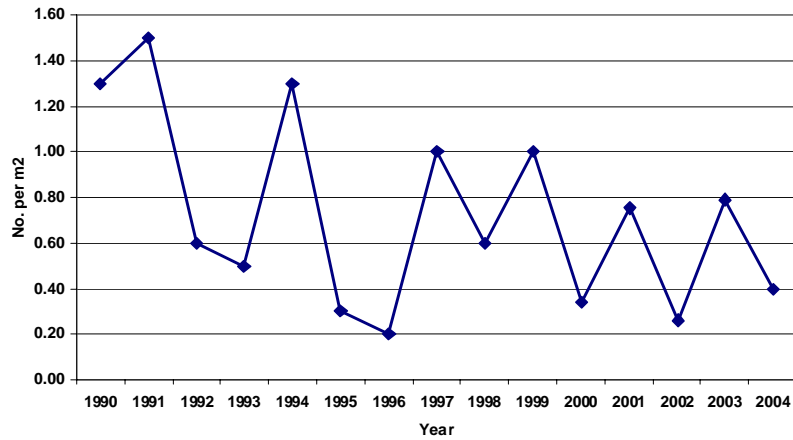**Fig.4- Abundance of Newly Settled YOY Lobster In Rhode Island**

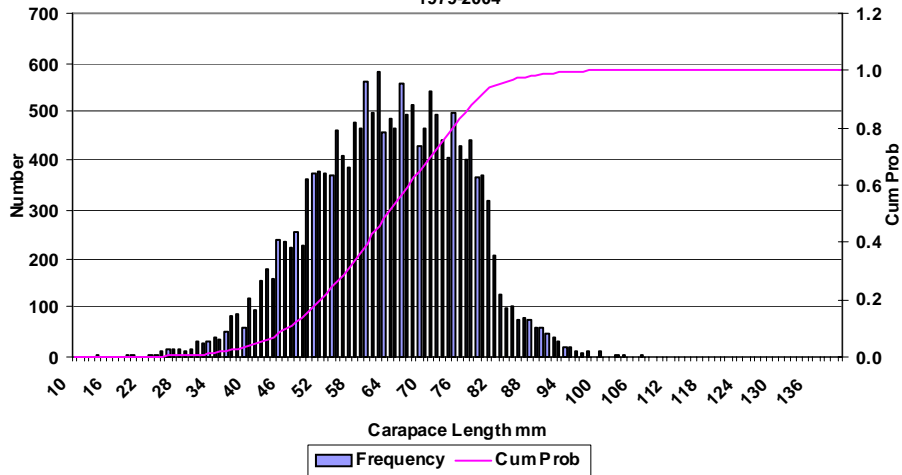**Fig.5- Length Frequency Distribution for Lobster in the RIDFW Seasonal Trawl Survey, 1979-2004**

Fig.6- Relationship Between Trawl Abundance of Pre-recruits and Settler Abundance with a Lag of Three Years for the 1990-1996 Cohorts

$$y = 9.9512x^{0.494}$$
$$R^2 = 0.8801$$
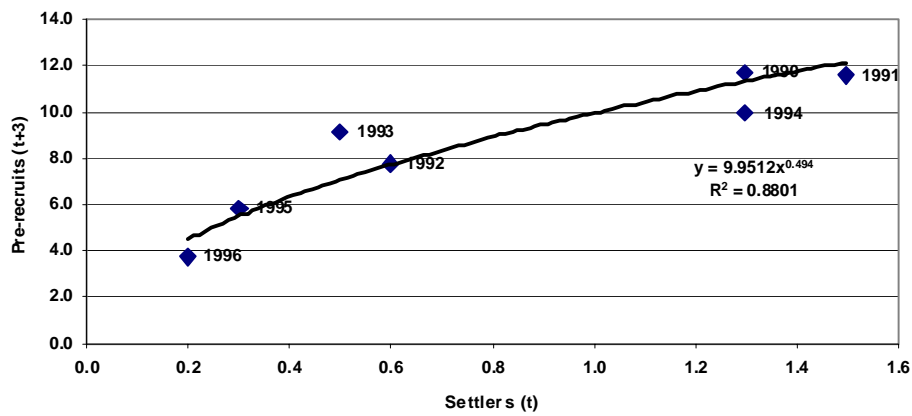
Fig.7- Relationship Between Trawl Abundance of Pre-recruits and Settler Abundance with a Lag of Three years for the 1990-2001 Cohorts

$y = 4.9529x^{0.2647}$
$R^2 = 0.0389$
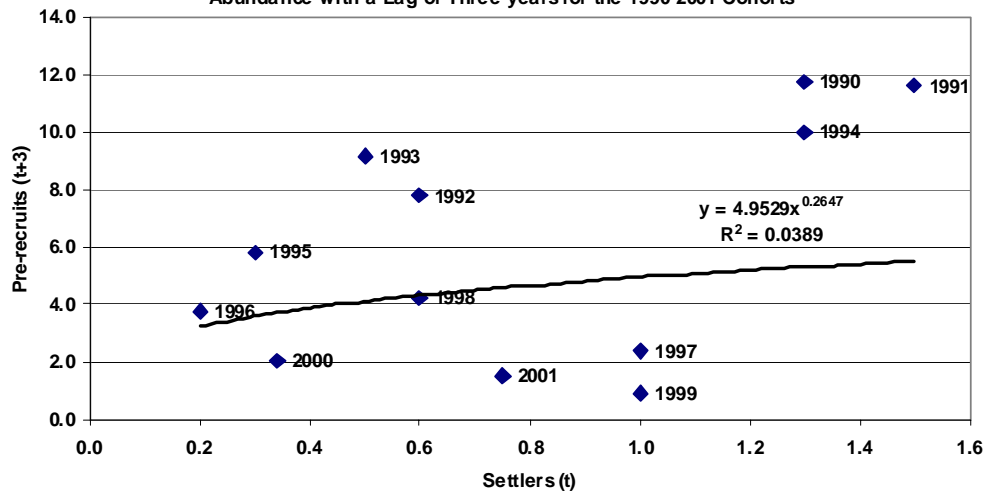
Fig.8- Relative Mortality Rate from Settler to Pre-recruit Life Stage
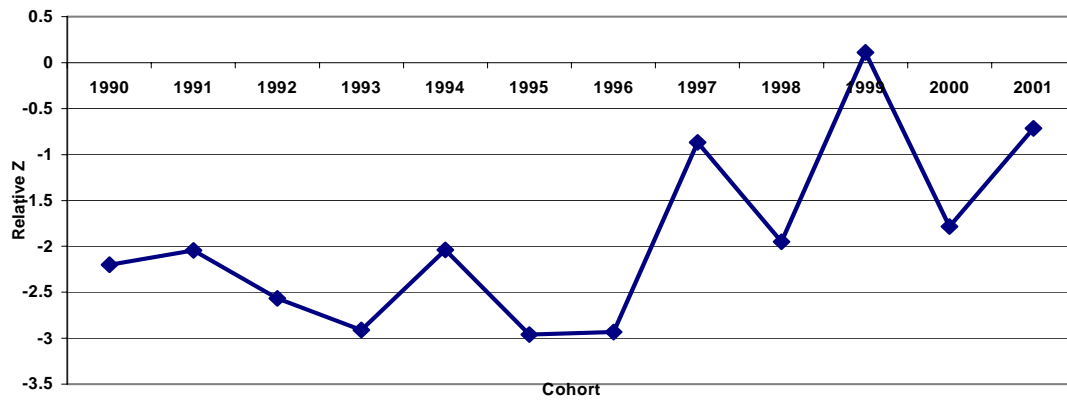
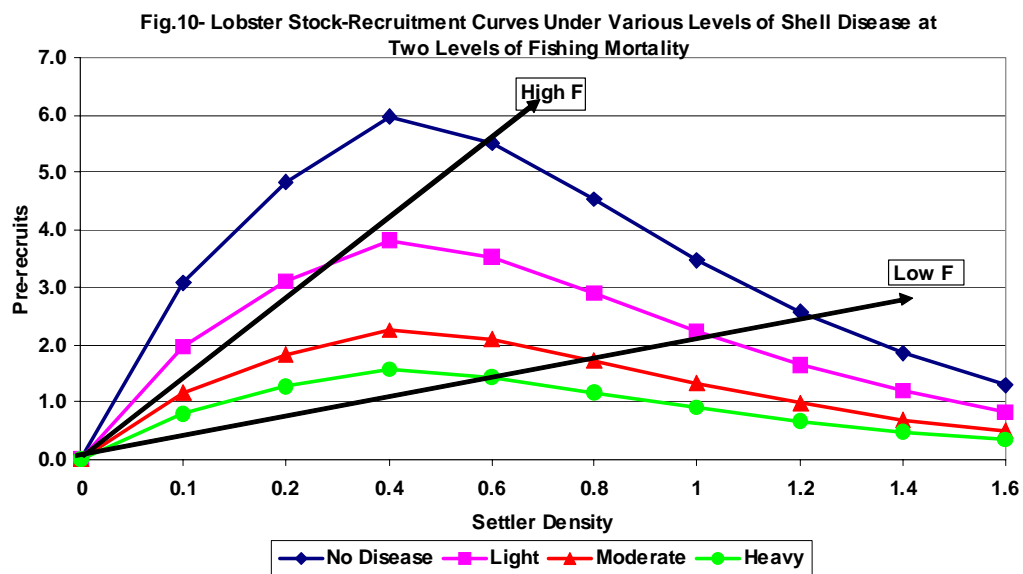Fig.9- Residual Plot for Ricker Stock-Rrecruitment Model Including Shell Disease

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Fig.10- Lobster Stock-Recruitment Curves Under Various Levels of Shell Disease at Two Levels of Fishing Mortality

Fig.11- Number of RI Inshore Lobster Pots Fished from DFW Logbooks and Computed Pot-Hauls from Landings and Mean Sea Sample CPUE

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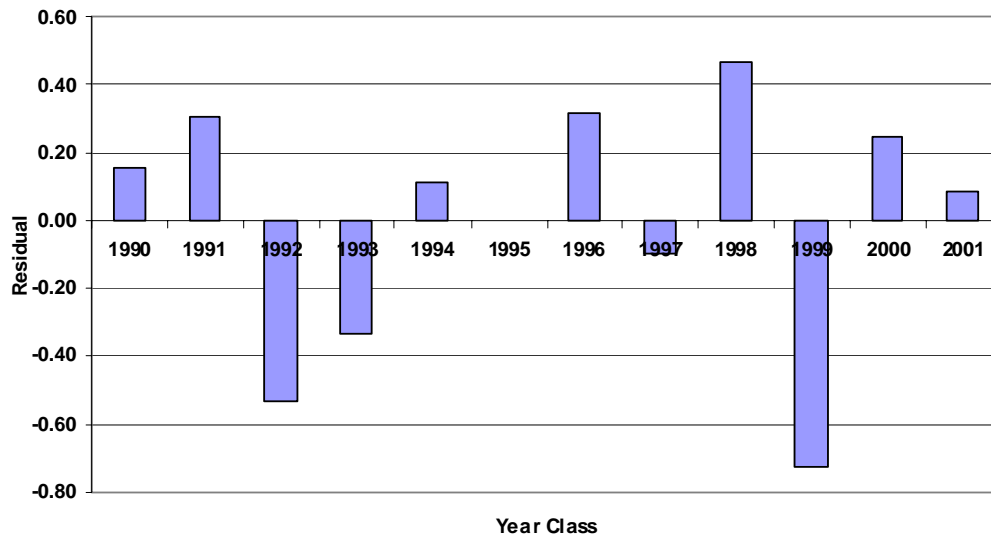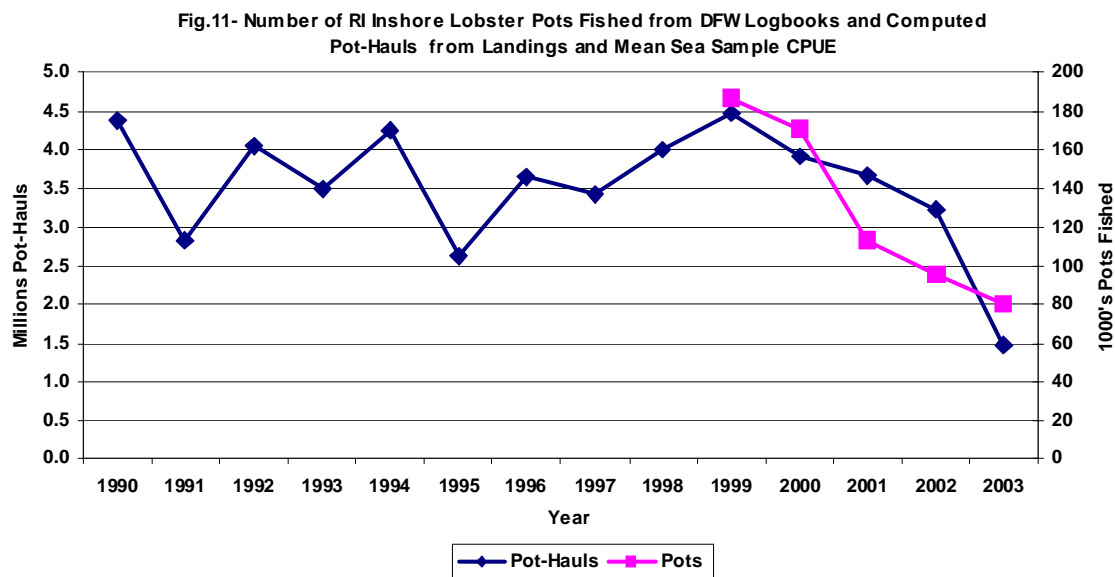
Short-term & seasonal change in transport and retention of biota in Massachusetts and Cape Cod Bays

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Abstract:

This study examines short-term and seasonal wind-driven currents, convergence of currents and Lagrangian drifting of water parcels in Massachusetts Bay in order to understand the roles of currents in determining transport, dispersion and retention of shellfish larvae. A Princeton Ocean Model is used with Mellor and Yamada's level ½ turbulence closure and forced by realistic wind fields and heat fluxes. A Lagrangian trajectory model is used to follow water parcels. The results indicate that the seasonal heating and wind pattern will lead to different pathways of current jets while the short-term wind forcing can dramatically change the seasonal flow pattern, especially coastal jets, in Massachusetts Bay. The transport of water parcels is complicated by the time-varying current fields and current convergence fields. Passive drifting particles (shellfish larvae) tend to be concentrated and retained in Cape Cod Bay and Stellwagen Bank.

Introduction:

Massachusetts and Cape Cod Bays (MBS) are of important areas for lobster and shellfish fisheries. During the larvae stages of Lobster and shellfish, the transport and dispersion of larvae are driven by currents, and later, larvae will settle to form spats. The success to form spats determines their adult population sizes and eventually determines their productivity. Hence, to understand the time-varying current fields is important in understanding the lobster and shellfish productivities, and in developing models for prediction and management.

Previous studies have indicated that the circulation in MB varies in response to short term and seasonal meteorological and boundary forcing (Bigelow 1927; Geyer et al. 1992; Lynch et al. 1996). The yearly-mean circulation in MBS is counterclockwise which is driven by both the intruding current off Cape Ann and baroclinic pressure gradients associated with freshwater runoff. Studies also indicate the circulation in MBS responds strongly to wind forcing (Jiang, et al. 2004), which leads to upwelling or downwelling along the coast.
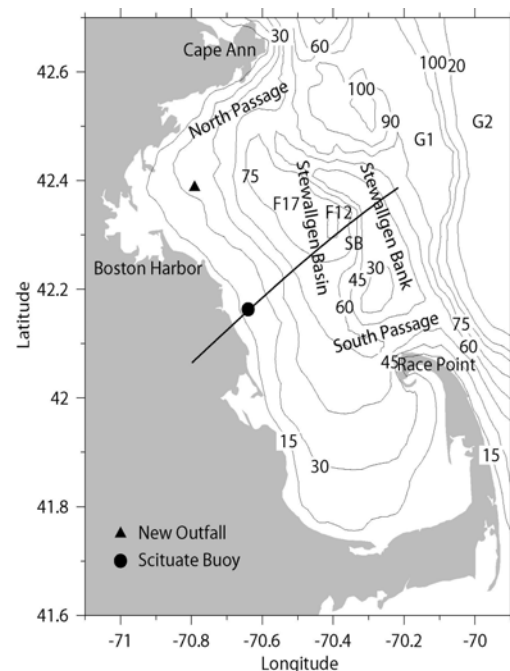

Figure 1: The bathymetry of MBS. The solid black line indicates the transect in Figure 6.

The bathymetry of MBS can be classified as a semi-enclosed embayment with an eastern open boundary connecting to the Gulf of Maine (GOM) (Fig. 1). The depth varies from the average 35 m to the deepest greater than 90 m in Stellwagen Basin while the bay is blocked by Stellwagen Bank of 20 m deep. The southern part of MBS is referred to as Cape Cod Bay (CCB) and is much shallow, and the northern part is referred to as Massachusetts Bay (MB).

The bathymetry significantly influences the circulation pattern in MBS. A branch of the West Maine Coastal Current (WMCC) splits off and intrudes into MBS through the North Passage off Cape Ann. The exit current from MBS is found in the South Passage off Provincetown. Stellwagen Bank significantly blocks the water exchange between MBS and GOM, especially the deep water (Jiang, et al. 2004). The wind driven currents interact with the topography and lead to convergence and divergence of currents.

In the time scale of 10s days when shellfish larvae disperse, currents in MBS vary responding to short-term wind forcing in days and to monthly-seasonal changes in 10s days. In a time-varying current field, the trajectories of passive water parcels are very different from the streamlines, which are significantly determined by residual currents and convergence. Passive particles tend to be concentrated in convergent current areas. It is important for understanding the transport, dispersion and retention of lobster and shellfish larvae to know where these convergent areas are and what those liable physical mechanisms are.

Models:

The hydrodynamic model used in this study is based on the Estuarine, Coastal and Ocean Model (ECOM-si) with Mellor and Yamada level 2.5 turbulence closure for the vertical mixing (Mellor and Yamada 1982; Blumberg and Mellor 1987; Blumberg 1991; Signell et al. 1996). The model is forced with hourly observed wind stresses and heat fluxes, daily fresh water discharges, M_2 tides, and low frequency surface slope at the open boundary for year 2000. The temporal variation of the low frequency surface slope is determined from current meter data, the geostrophic balance based on the monthly CTD survey data with the reference at 100 m, and empirical fitting to temperature, salinity and current data observed at the NOAA buoy (42°24'N, 70°40'W). The surface heat fluxes were estimated using the observed solar radiations, air temperature, air pressure and humidity at the same NOAA buoy based on the bulk formulation (Large and Pond, 1981). A detailed description and calibration of the model for MBS can be found in HydroQual and Signell (2001) and Jiang and Zhou (2004).

Figure 2: The spring (March-May) mean current field in MBS.

Results:

The seasonal mean circulation in the spring (March-May) 2000 is shown in Fig. 2, which is a counterclockwise circulation. The major driving force of this circulation includes the dominant northerly wind, the
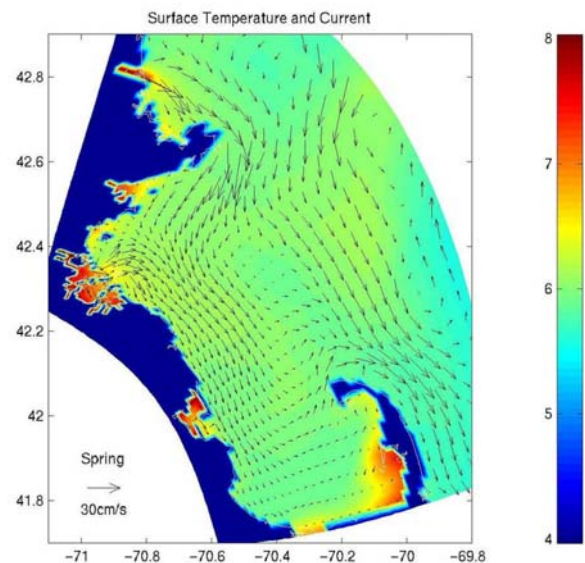
freshwater runoff and the surface slope off Cape Ann which representing the intruding WMCC (Geyer et al., 1992; Signell et al., 1996). It enters MB from the northeast along the shelf break, and splits into two branches off Cape Ann. The major one flows along the eastern flank of Stellwagen Bank, and the minor one intrudes into MB through the North Passage with a speed of approximately 30 cm s^{-1}. This intruding current joins coastal currents in the western side of MB, and flows southward often penetrating deeply into CCB. This spring circulation pattern also represents the seasonal circulation pattern in winter.

In summer (June-August) 2000, the WMCC flowed southward along the eastern flank of Stellwagen Bank with little intrusion into MB (Fig. 3). The magnitude of the flow

Figure 3: The summer (June-August) mean current field in MBS.

on the eastern flank of Stellwagen Bank was approximately 20 cm s^{-1}. The southward coastal current produced by the freshwater input from Boston Harbor, which typically flows southward along the west coast of MB, was pushed offshore away from the coast during the summer. The southward surface current was blocked by the warm CCB water, and exited from the South Passage. A weak northward coastal current of cold water was found flowing next to the coast, and joining the southward current off Scituate, which may represent the dominant southerly wind and its induced upwelling. The water temperature in CCB was approximately 2 to 3°C warmer than that of the rest of MB. The warm water in CCB produces a weak anticyclonic eddy.
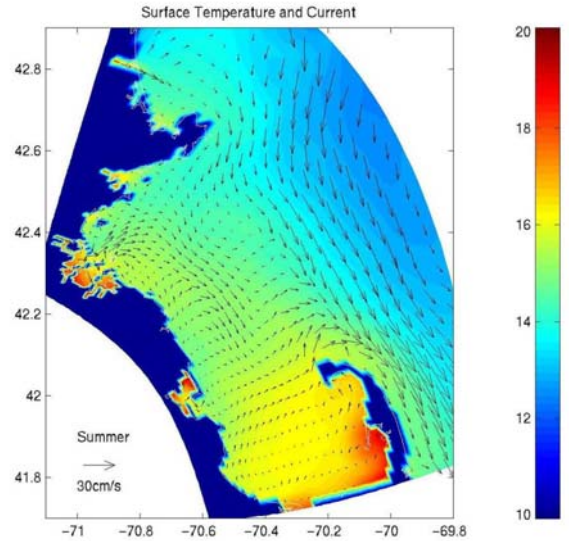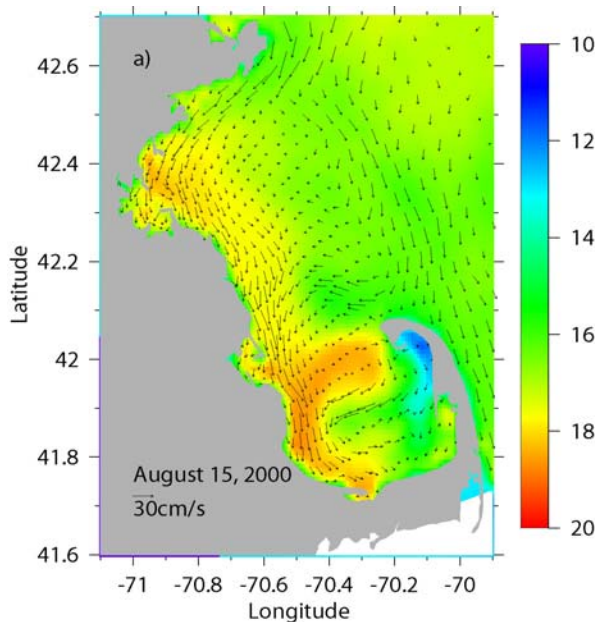

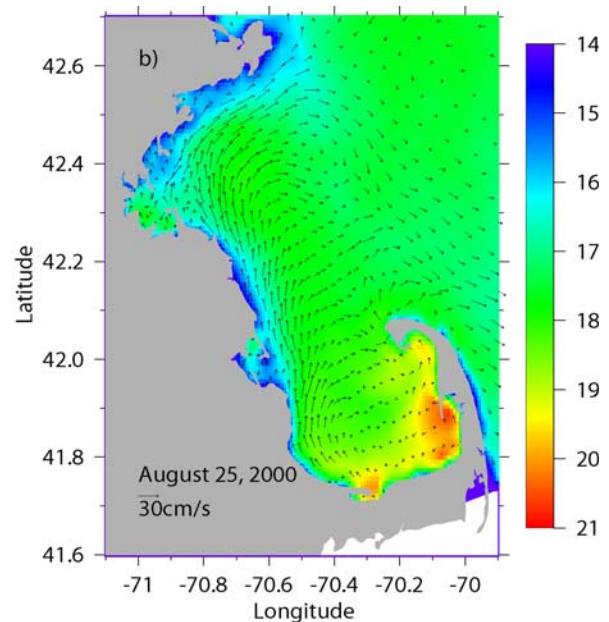Figure 4. The circulation pattern under the northerly wind in the summer.

Figure 5. The circulation pattern under the southerly wind in the summer.

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Within the summer, the wind events occur in a time scale of several days. In a northerly wind event, the onshore Ekman transport leads to downwelling and a southward coastal current which penetrates into CCB (Fig. 4). The warm water is piled up at the coast areas. The WMCC, which flows southward along the eastern flank of Stellwagen bank, splits, and a branch intrudes into MB from the South Passage. Because the water accumulates in MBS, the water exchange is limited (Jiang and Zhou 2004).

In a southerly wind event, the offshore Ekman transport leads to upwelling and a northward coastal current along the coastal upwelling front (Fig. 5). The circulation is clockwise. The exiting current off Cape Ann joins the southward WMCC. Under the southerly wind condition, the offshore Ekman transport is compensated by the onshore deep flow, which produces significant water exchange between MBS and GOM.

The vertical velocities associated with northerly wind are shown in Fig. 6 where a coastal convergent zone is driven by the onshore
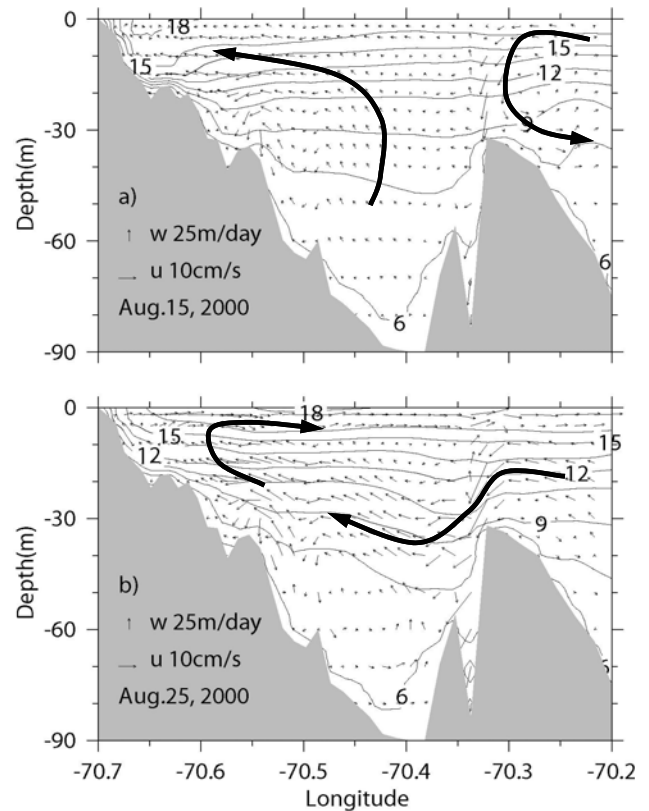

Figure 6: The circulation patterns along the transect indicated in Fig. 1 under the northerly wind condition in Fig. 4 (upper panel) and southerly wind condition in Fig. 5 (lower panel).

Ekman transport, and a divergent zone on the western flank and a convergent zone on the eastern flank of Stellwagen Bank are formed. The water exchange between MBS and GOM is limited. Under southerly wind, the offshore Ekman transport produces strong upwelling at the coast and a divergent zone (Fig. 6). The surface current forms a convergent zone on the western flank and a divergent zone on the eastern flank of Stellwagen Bank. The deep water overflows Stellwagen Bank into MBS.

Lagrangian passive particles are released at the surface in MB and CCB under dominantly northerly wind in March and dominantly southerly wind in June. The results under northerly wind show the particles are concentrated near the coast by onshore Ekman transport and advected southward into CCB by the coastal current. The water in CCB exits off Provincetown northward along the Stellwagen Bank, or eastward through the South Passage.

Under southerly wind, the offshore Ekman transport advects surface particles offshore and forms a convergent area west of Stellwagen Bank. Few particles are transported into CCB by some very short wind events. Particles exit MBS from the South Passage.

Discussion

The wind in winter and spring is dominantly northerly, which, together with the WMCC intruding current, drives an onshore Ekman transport (Fig. 2). The surface slope produced by the water accumulated near shore produces a southward coastal current, which penetrates into CCB deeply. Along with this coastal current, nutrients and biota are transported from MB into CCB where the current is sluggish, and nutrients and biota accumulate. In summer, the dominant southerly wind pushes the coastal current offshore, which flows eastward from Scituate and exits

in the South Passage (Fig. 3). A clockwise circulation in CCB forms a nearly enclosed system where the recycling of nutrients determines the productivity of ecosystem. Thus, the change of the circulation pattern from winter-spring to summer switches the ecosystem function from a new production and bio-accumulation system to a self contained recycling system.

The circulation pattern change also determines the transport and retention of shellfish larvae. In spring, larvae can be transported from the WMCC southward into CCB where they will be retained by sluggish currents. In summer, the enclosed clockwise circulation in CCB restricts any exchange between MB and CCB. However short-term wind events may periodically interrupt the seasonal mean circulation patterns (Figs. 4 and 5), alter exchange of water masses, nutrients and biota between MB and CCB, and produce transport of larvae into or out of CCB.

The water exchange between MB and GOM is strongly influenced by wind forcing. A northerly wind will produce a strong surface inflow into MB while a southerly wind will produce a strong surface outflow out of MB. During the northerly wind condition, the surface water is transported to the coast. Because it cannot sink simply due to its buoyancy, the water will be accumulated. The deep water exchange between MB and GOM is limited under the northerly wind condition. Under a southerly wind event, the offshore transport produces coastal upwelling and a deep onshore transport (Fig. 6), which enhances the deep water exchange between MB and GOM.

Distributions of passive particles (shellfish larvae) are sensitive to the convergence
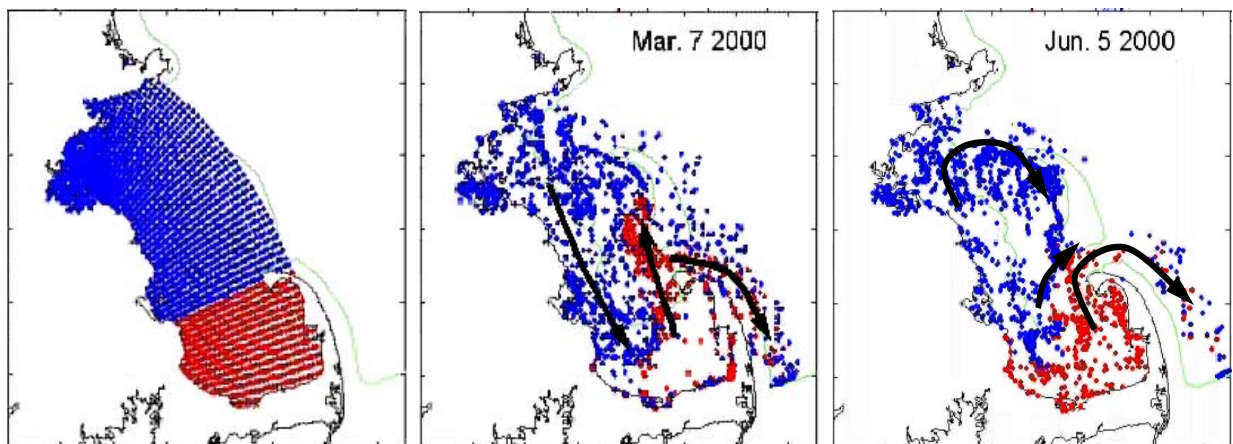

Figure 7: Locations of Lagrangian passive particles released at the surface. The red dots are released in CCB and blue dots are released in MB shown in the left panel on March 1 and June 1 2000. The middle panel shows the trajectories under the northerly wind, and the right panel shows the trajectories under the southerly wind. The black arrows illustrate the movements of particles.

and divergence of currents. In both northerly and southerly wind events, convergence will be formed on one side of Stellwagen Bank, which leads to high concentration of passive particles (Fig. 7). The sluggish currents in CCB can also lead to high larvae concentration. The migration of lobster and shellfish larvae is one of many important factors can further complicate the transport and retention of them. A Lagrangian model with larvae behavior is needed for further understanding how coupled temporal and spatial variations of current fields and larvae behavior determine their contribution to the transport and retention of larvae.

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Discussion of Population Responses

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Q.1. *What information on ecosystems health is required to improve surveillance for lobster health?*

Temperature was the primary factor discussed, yet it was acknowledged that there were inconsistencies in a direct relationship between temperature increase and the incidence/spread of lobster disease (e.g. outer Long Island (cooler Atlantic Ocean coast) has a higher incidence of shell disease than the warmer Long Island Sound; the cooler eastern Long Island Sound has a higher incidence of shell disease than the warmer western end of Long Island Sound). Other environmental variables do not show a consistent relationship with disease. It was noted that the degree of change in an environmental variable may be more important than the actual measured value (e.g. a relatively large percent change in temperature instead of the actual degree of temperature increase: or a large percentage shift in a bacterial species numbers within an microbial community rather than the presence/absence of particular microorganisms). Along these lines, someone mentioned that the greatest recent ocean temperature change lies in the area between Massachusetts and Rhode Island. Two observations were particularly highlighted: (1) the highest incidence of shell disease is in the southern extent of the lobster's natural range - organisms are generally more stressed at the extremes of their ranges; and (2) something about the Elizabeth Islands (the hottest hotspot) may be environmentally unique - but no one could speculate on just what that could be. These variabilities in the relationship of the disease to the environment indicate that the disease may be the result of the interaction of several factors and may not be directly related to only one factor (i.e. temperature or pollution). It suggests that multiple causative agents may have to be synergistically present for the disease to occur.

Q.2. *What is the quantitative snapshot of the epidemiology and geography of lobster diseases?*

This question was covered adequately in the presentations – the disease is generally a southern phenomenon, but with an apparent continuing invasion northward. The highest incidence appears to be in Buzzards Bay, around the Elizabeth Islands, with decreasing incidence to the north and south.

Throughout the workshop, the absence of shell disease in Western Long Island Sound was discussed. This is a conundrum, as WLIS is more polluted, and has higher temperatures than Eastern LIS. However, WLIS also experiences more hypoxia than ELIS, and this may prevent bacteria responsible for shell disease from successfully eroding lobster shells in WLIS. Alternatively, lobsters in WLIS may have been naturally selected for being able to survive the particular environmental parameters, and part of this selection process may have included an increased resistance to shell disease.

Q.3. *How do ocean currents influence the spread of bacterial disease in lobsters?*

It was acknowledged that ocean currents (with accompanying measurements of surface and bottom water temperature, salinity, depth, etc) need to be examined more closely. In particular, fine scale temporal and spatial patterns need to be analyzed.

Not only might ocean currents influence the spread of bacterial diseases in lobsters, but they clearly influence the distribution of animals through larval dispersion. Larval transport is an important factor to consider in a full assessment of lobster health. While circulation patterns have been determined for the Gulf of Maine, Georges Bank, and Brown's Bank, there appears to be a great deal of spatial and temporal variation in larval dispersal that is still unexplained. Larvae that are released inshore are more likely to be retained in the local area than larvae released offshore. If there is a female size component to where larvae are released (small females stay inshore), then fishing down lobster sizes may shift larval releases more inshore, which will lead to more local retention. If lobsters are retained locally, there may be more bacterial transfer of shell disease within populations. It was also generally acknowledged that molecular biological techniques need to be employed to determine the extent and the inter-breeding of lobster populations in the Northeast.

Q.4. *Will levels of atmospheric carbon dioxide change significantly to influence the metabolic costs of calcification?*

There is the perception that calcium is not be limiting to marine organisms that sequester calcium, since sea water has ample $CaCO_3$. However, it is the speciation of calcium in sea water that influences calcium uptake. It has been suggested that an increased concentration of carbon dioxide in sea water will decrease the aragonite saturation state by up to 30% due to a pH- induced shift in the speciation of dissolved inorganic carbon, a decrease in the carbonate, and an increase in calcium precipitation (Kleypas et al 1999). If that is the case, less Ca^{2+} and carbonate may be available for uptake and calcification processes.

While the possibility that increasing atmospheric CO_2 concentrations may effect pH-dependent calcium and carbonate speciation in temperate marine waters, no one was willing to speculate that this could effect shell calcification in lobster enough to encourage shell disease**.** One participant commented that the recent finding that WLIS lobsters exhibit calcinosis (calcium deposits around gills, Dove et al. 2004) suggests either an excess of calcium, or competitive interference (heavy-metal or viral) with calcium regulation (Verbost et al. 1989, Schoenmakers et al. 1992). Lobsters are adapted to widely fluctuating calcium levels and are equipped with mechanisms for rapid movement, conjugation and storage of large amounts of mineral calcium (Dove et al, 2004). However, shell mineralization responses to maximum and minimum calcium levels need to be better determined prior to dismissal of the hypothesis that carbon dioxide levels will significantly impact calcification.

Q.5. *What are short and long term predictions of ocean temperature, and will this significantly effect bacteria / lobster interactions?*

There are a number of ocean monitoring stations (NOAA and GMOOS buoys) throughout the lobster's range, which record water column data over a long term. These observing stations will be valuable in further research on oceanographic changes in lobster habitat. It was acknowledged that bottom water temperatures had changed, but the future extent/degree of this increase was not discussed. Any discussion of temperature was sidelined by the observation of low incidence of shell disease in WLIS (highest temperatures). Too little is known about lobster and bacterial responses in normal temperatures to speculate on how long term changes will affect the interaction. However, in addition to high temperatures, there was also a general discussion of temperature anomalies – both high and low. There have been cases where cold bottom water has remained inshore, delaying molts and shedding seasons. This may prolong the onset of shell disease, delay the "resetting of the clock" on individuals, and lead to long-term effects.

Overall, discussion within this session was brief given the extended time devoted to the presentations. The brevity of discussion presented here should not indicate a consensus, or a lack of importance of the interactions of oceanographic conditions and lobster biology and disease, but rather the vast knowledge necessary to fully integrate ocean physics and ecology into lobster biology. Significant integrative efforts by dedicated oceanographers will be needed to better understand this complex relationship.

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Verbost PM, Flik G, Pang PKT, Lock RAC, Bonga SEW (1989) Cadmium inhibition of the erythrocyte Ca2+ pump—a molecular interpretation. J Biol Chem 264:5613–5615

Chapter 5 Monitoring Programs and Management Implications

During the discussion period, the panel discussed the following questions, and the discussion was transcribed and is presented following the submitted papers.

1. **What information should be included in developing a lobster health database?**

2. **How can one enhance lobster health management by improving interaction at all levels of the fishing industry?**

3. **What management decisions are required to aid in lobster disease prevention?**

4. **What are the best predictors of outbreaks of shell disease?**

5. **Is bait a causative agent of lobster health problems, and if so, what mechanisms can be used to regulate bait usage in the lobster fishery?**

Observations on the chronology and distribution of lobster shell disease in Massachusetts coastal waters

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<u>Introduction</u>

An outbreak of shell disease in the American lobster (*Homarus americanus*) over the past several years has generated a great deal of attention and concern regarding the causes and spread of this disease. The Massachusetts Division of Marine Fisheries (*Marine Fisheries*) has observed shell disease in the catches of commercial lobstermen since the inception of our coastwide lobster trap sampling program in 1981**.** We characterized shell disease prevalence and severity in 1984, 1989, and instituted standardized shell disease monitoring in 2000. Examination of the data generated from both the historical and current shell disease sampling programs has led us to some preliminary conclusions regarding disease incidence, severity, and spatial distribution, which we present here along with suggestions for future work.

<u>Historical Perspective</u>

Shell disease is a common and widespread condition in marine and freshwater crustaceans. There have been a number of outbreaks in the northwest Atlantic in recent history, including the incidence of "black spot" on rock crabs (*Cancer borealis*) in the New York bight in the late 1970's (Sindermann et al., 1989), and localized outbreaks of "shell rot" among impounded lobsters (*Homarus americanus*) along the coast of Maine and Nova Scotia in the mid-1980's (Bullis, 1989). Locally, *Marine Fisheries* has observed epizootic shell disease on lobsters at low levels since the inception of our coastwide commercial lobster sea-sampling program in 1981. This program monitors commercial CPUE, size distribution, and other key biological parameters of American lobsters in seven regions spanning Massachusetts coastal waters (Figure1).

In 1984, *Marine Fisheries* summarized the regional prevalence of lobster shell disease from commercial catches in Massachusetts (Estrella, 1984). The overall incidence of the disease was moderate (12% coastwide), and typical symptoms consisted of minor pitting (Figure 2a). There was a notable difference in prevalence in northern
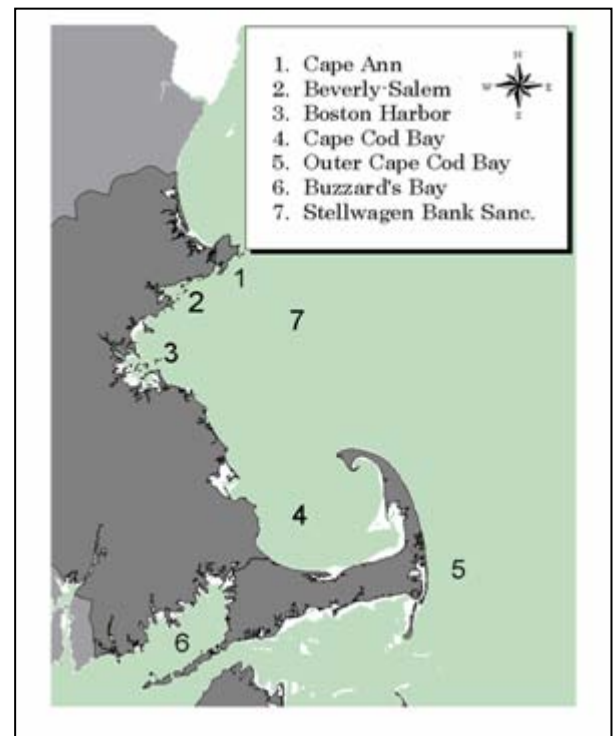

Figure 1: MADMF commercial trap sampling regions.

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verses southern regions within state waters, with observed incidence of shell disease highest in the Buzzards Bay region (Figure 3). Through 1989, shell disease prevalence was relatively low and primarily in the form of minor pitting and erosion.

Estrella (1991) developed a standardized sampling approach to allow for valid temporal and spatial comparisons, as well as trend analyses. This included incorporating a sub-sampling protocol into the sea sampling effort in which a minimum of 50 lobsters were sampled per trip. Disease presence and severity were recorded, as well as the anatomical location of symptoms.

Figure 2: Symptoms of epizootic shell disease in Massachusetts coastal waters. A) Minor pitting (arrows), typically observed in 1983. B) Moderate pitting and minor erosion (arrows), typical symptom observed in 1989.

In the 1989 study, overall prevalence was considerably higher compared to the 1984 study, at 33% coastwide. However, the magnitude of these data may not be comparable to previous data because of the increased screening scrutiny concomitant with the new sub-sampling protocol. Nonetheless, a comparison between the two studies of shell disease prevalence on a regional basis is
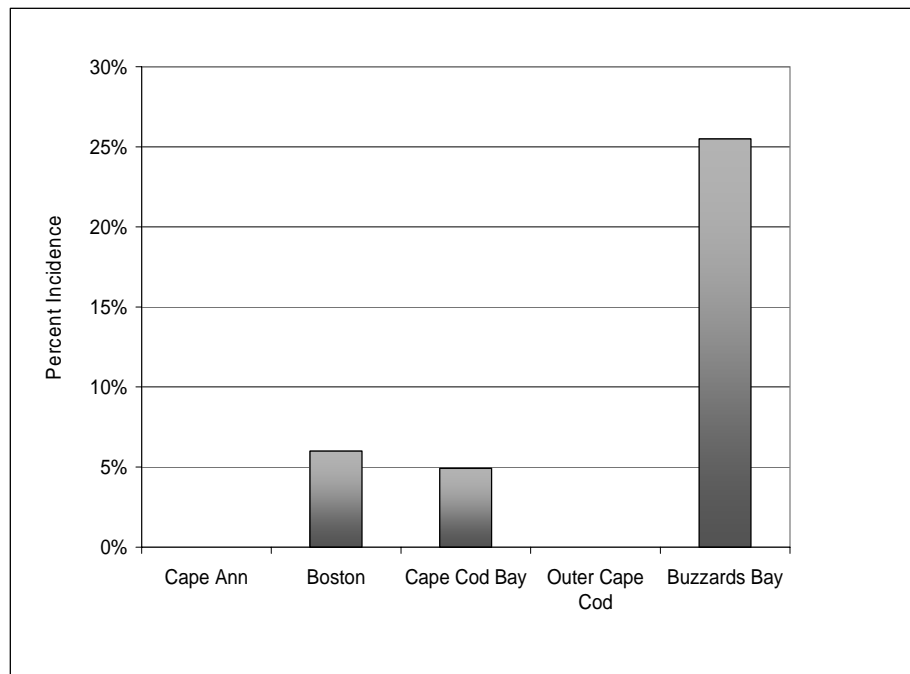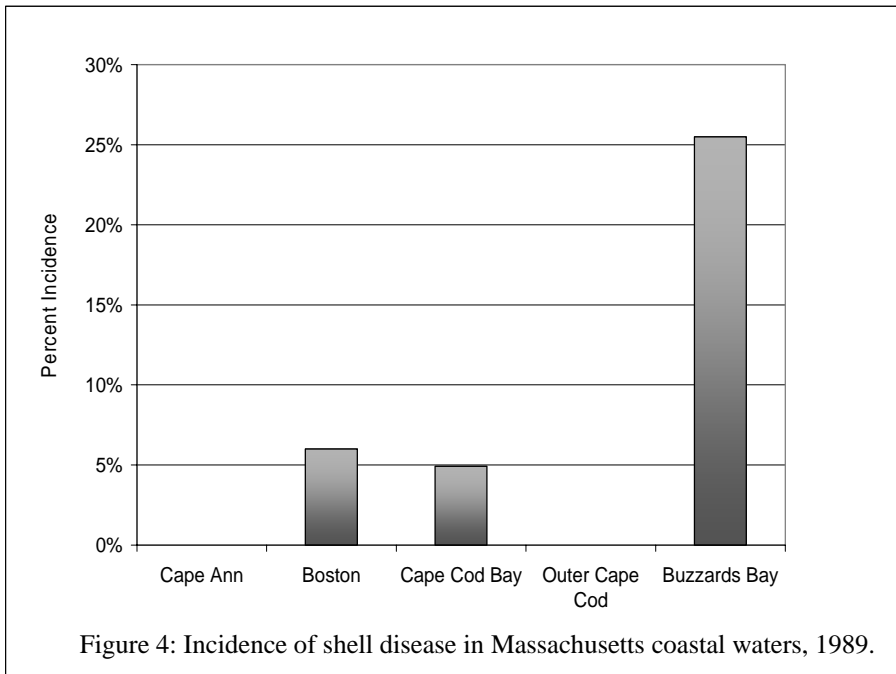

Figure 3: Incidence of shell disease in Massachusetts coastal waters, 1983.

Figure 4: Incidence of shell disease in Massachusetts coastal waters, 1989.

instructive. As in the 1984 study, there was a distinctive difference in prevalence between northern and southern regions (Figure 4). These data show a trend, as was suggested by the earlier data, of increasing prevalence north to south, with a notable dip in Outer Cape Cod. Disease symptoms ranged from moderate pitting to minor erosion (Figure 2b), and severe symptoms were never observed on juvenile lobsters. Large, hard shell female lobsters had the highest incidence of symptoms, suggesting a relationship with the time a shell is exposed to the environment and prevalence of symptoms. Accordingly, ovigerous females exhibited the highest prevalence, since the molting is delayed until after egg hatching, thus increasing its exposure to causative agents (Estrella, 1991).

Progression of 1997 to 2004 Outbreak

In the autumn of 1997, *Marine Fisheries* received numerous reports from commercial lobstermen indicating a high incidence of shell-diseased lobsters with severe symptoms in the lower portion of Buzzards Bay, around the Elizabeth Islands. Symptoms were radically different from those previously reported because they were no longer defined as localized pitting and erosion. Now the symptoms were characterized as pervasive erosion with extensive shell coverage (Figure 5), and some individuals were observed with 100% disease coverage of their shell.

Initially, the Massachusetts' reports were limited to the waters surrounding the Elizabeth Islands in the southeast portion of Buzzards Bay. However, starting in the summer of 1998 and extending through the 1999 season, lobster shell disease was observed throughout Buzzards Bay by *Marine Fisheries* staff during commercial lobster trap sampling trips. During those two years, respectively 24% and 21% of all lobsters observed had some degree of shell disease.

Figure 5: Symptoms (severe erosion) of shell disease typical of observations from 1998 to 2004.
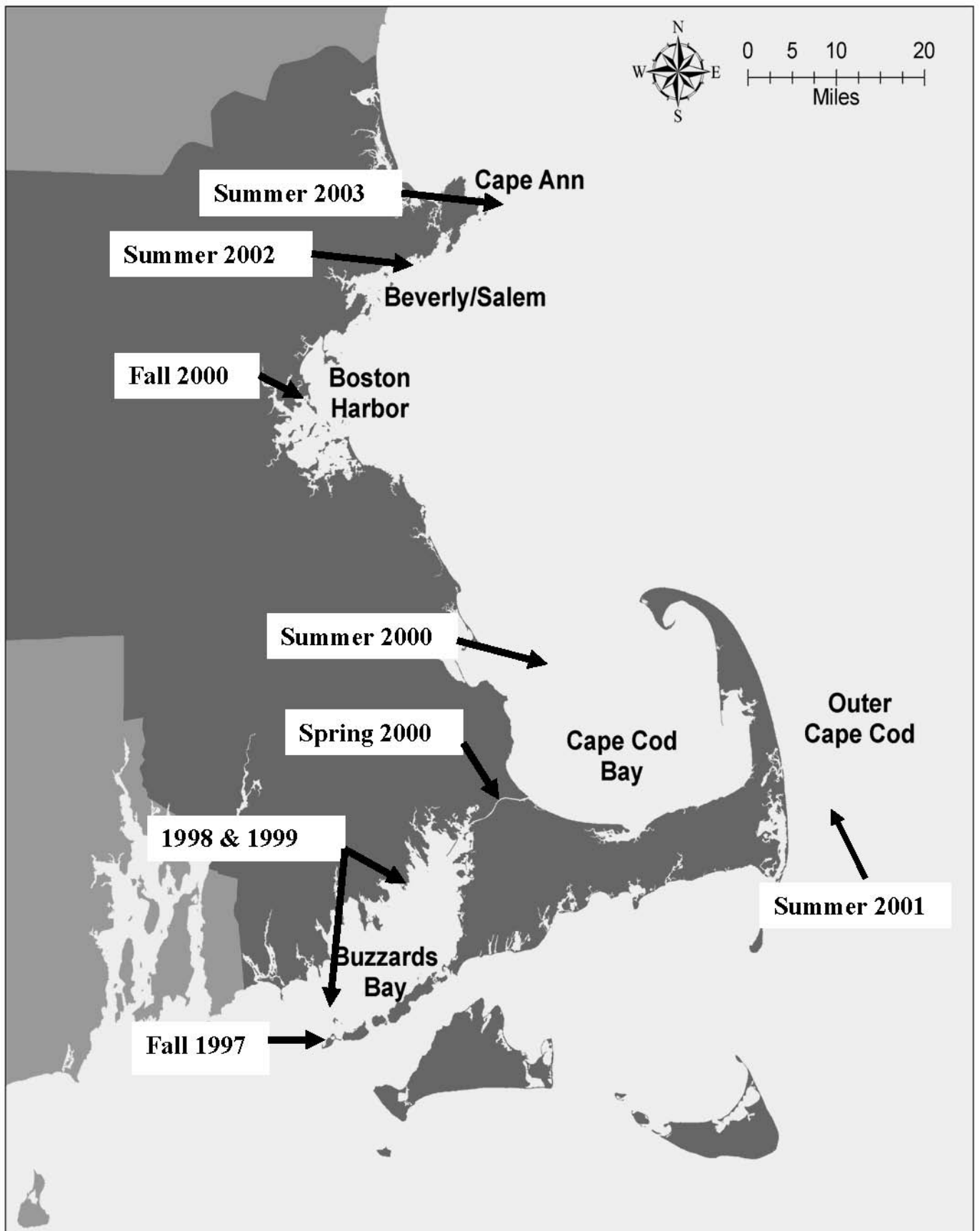
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Figure 6: Time line of the spread of epizootic shell disease in Massachusetts coastal waters.

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In the spring of 2000 we confirmed reports of shell-diseased lobsters in the Cape Cod Canal, and by that summer *Marine Fisheries* staff observed a moderate incidence of lobsters with severe shell disease symptoms in Cape Cod Bay, the southern-most portion of the Gulf of Maine. By the fall of 2000 shell disease was observed farther north in the Boston Harbor/Massachusetts Bay region. In the summer of 2001, lobster shell disease was observed to the east in the coastal waters off Outer Cape Cod at a very low prevalence. Shell disease was not observed in samples north of the Boston Harbor/Massachusetts Bay region during the current outbreak until the summer of 2002, where symptoms were observed in the Beverly/Salem region. In 2003 shell disease appeared in the Cape Ann region of Massachusetts, the northern-most region sampled. An expansion of *Marine Fisheries* commercial trap sampling to the Stellwagen Bank Sanctuary in 2003 revealed a moderate prevalence of shell disease. We are not sure if the disease was present prior to this observation because sampling was not implemented prior to this time.

Observations made by *Marine Fisheries* indicate a steady northward expansion of epizootic shell disease within the lobster population of Massachusetts coastal waters between 1997 and 2003 (Figure 6). During this period the disease spread from the southern end of Buzzards Bay (41° 11' 44.88" N, 70° 54' 16.2" W) to the coastal waters off of Cape Ann, (42° 42' 27" N, 70° 33' 30.96"W). The initial spread of the disease from southern New England waters to the southern portion of the Gulf of Maine most likely occurred via lobster migration through the Cape Cod Canal. This observation is supported by *Marine Fisheries* tagging studies conducted in the late 1970's and early 1980's which demonstrated a net easterly movement of lobsters from Buzzards Bay, through the Cape Cod Canal, and into Cape Cod Bay (B. Estrella and J. Fair, unpublished data).

While bacteria have been identified and implicated as the primary causative agents (Smolowitz et al., 2003), the reasons for the outbreak and its northward expansion have not been explained. An examination of our 2000 to 2004 shell disease monitoring data provides insight into some of the factors which determine the prevalence, severity, and spatial distribution of epizootic shell disease.

Biological Observations, 2000 - 2004

With growing reports of disease throughout all of southern New England waters, the ASMFC Lobster Technical Committee developed a standardized shell disease sampling protocol in the winter of 2000. Sampling entailed inspecting the shell of all lobsters from lobster traps chosen haphazardly throughout the course of a sampling trip, until a total of 50 lobsters were examined per trip. Each lobster was categorized by an index based on the percent coverage of shell disease symptoms on the total surface area of the shell. The categories are broad to help reduce the subjectivity in assigning an index. Four categories of shell coverage were defined: 0% = No shell disease symptoms; Low = 1-10%; Moderate = 11-50%; Severe = 51-100%. This protocol was integrated into the *Marine Fisheries* commercial lobster sea-sampling program for the 2000 season. From May 2000 to November 2004 a total of 21,089 lobsters were sampled for shell disease in Massachusetts waters.

Table 1: Percent incidence of shell disease by symptom category.

		2000	2001	2002	2003	2004	Series Mean
No Symptoms	*female*	94%	91%	87%	92%	97%	92%
	male	99%	96%	96%	96%	98%	97%
Low	*female*	4%	4%	6%	4%	2%	4%
	male	0%	2%	2%	2%	1%	1%
Moderate	*female*	2%	3%	4%	2%	1%	2%
	male	0%	1%	1%	1%	0%	1%
Severe	*female*	0%	2%	3%	2%	0%	1%
	male	1%	1%	2%	1%	1%	1%

The majority of all lobsters sampled did not have shell disease, with a time series mean of 97% and 92% of males and females respectively showing no symptoms. Of the lobsters that did have shell disease, most had minor symptoms, a few had moderate symptoms, and severe symptoms were relatively rare. The overall trends in severity of symptoms were similar among sexes across all years (Table 1).

The mean female sex ratio of lobsters with shell disease was 0.83, which was considerably higher than the female sex ratio of lobsters without shell disease, 0.66 (Table 2). This indicates that female lobsters were more likely to have shell disease symptoms than males. This heavily female-skewed sex ratio is most likely due to the reduced frequency of molting relative to sexual maturity and the egg incubation period. In fact, egg-bearing female lobsters were 7.5 times more likely to have shell disease than females without eggs. These trends support observations made by Estrella (1991), and may be related to the fact that females retain their shell for a much greater time period as compared to males, thus leaving their shell exposed to the effects of shell disease for a longer time period.

Table 2: Sex ratio of lobsters with and without shell disease (proportion female).

	2000	2001	2002	2003	2004	Time Series Mean
No Symptoms	0.66	0.70	0.67	0.63	0.65	0.66
All Symptoms Combined	0.91	0.86	0.87	0.77	0.76	0.83

When the presence of shell disease is examined in relation to lobster size, it is apparent that this disease is more common on larger lobsters than on smaller individuals. Disease prevalence was proportionally highest in lobsters one molt below minimum legal size (recruits) (Figure 7). Legal sized lobsters, in particular females, had the second highest relative incidence of disease symptoms. Interestingly, it was expected that legal size lobsters would have the highest prevalence of disease symptoms relative to their increased intermolt duration, which would increase their exposure to causative agents. However, this was not the case. This apparent contradiction in prevalence based on size class might be explained in a few ways.
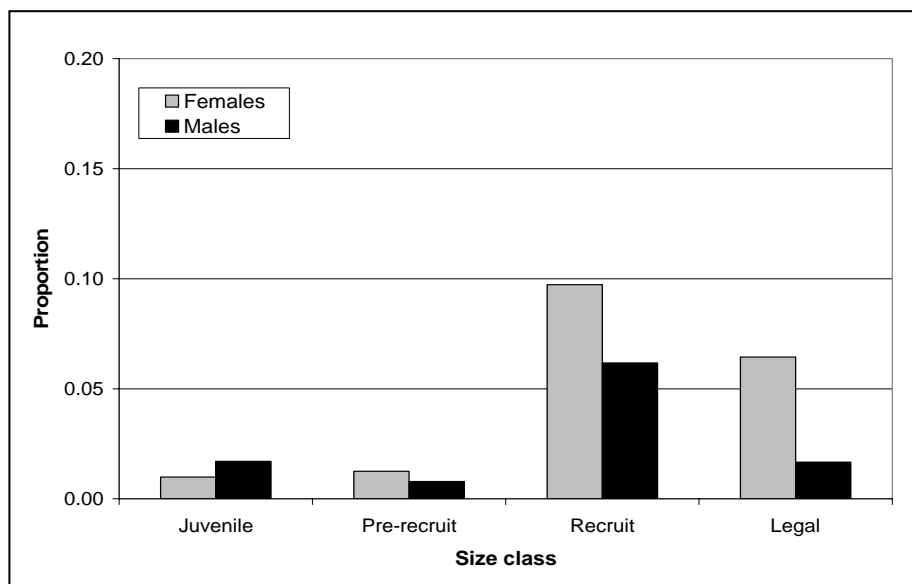
Figure 7: The proportion of diseased lobsters in four size classes. Size classes can be described as follows: Juveniles (30 to 58 mm), pre-recruits (59 to 70mm), recruits (71 to 82 mm) and legals (> 83 mm).

First, the recruit size class (71 to 82 mm CL) may be subject to damage and injury from other, larger lobsters and from moving in and out of traps. Handling by lobstermen on the surface as they are separated from the legal catch and returned to the water may also produce some body damage. It is possible that trap- and handling-related damage to their shells may provide a route for bacterial invasion in the form of scratches, culls, punctures, or other body damage. Symptoms of shell disease may then appear in and around that area of damaged shell.
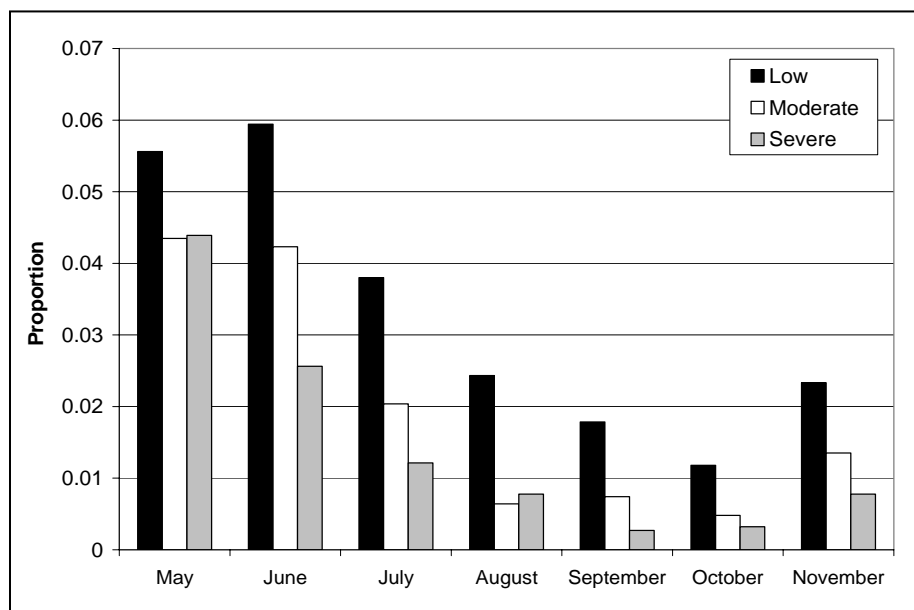

Figure 8: Average proportion of lobsters with disease symptoms annually, 2000 to 2004.

Second, it is possible that sub-legal lobsters are sampled more than once, since, unlike legal lobsters, they are returned to the water after observation. Thus there may be some artificial inflation of the incidence rates in the recruit size classes. An examination of recapture rates of marked individuals in this size class would clarify the degree to which the data are influenced.

Finally, the most likely explanation for this apparent contradiction in our disease-incidence-at-size data has to do with length of exposure to disease-causing agents. Disease incidence and severity are highest in May and June, before the start of the molt period (Figure 8). Fifty percent of all disease observations occurred in May and June, and 70% had occurred by the end of July. Once molting occurred the prevalence of shell disease declined. Lobsters are quickly removed from the population after reaching minimum legal size due to high commercial exploitation rates. Thus a higher proportion of legal sized lobsters, with new shells, are removed from the water before they have experienced lengthy exposure to the causative agents of shell disease. This decreased exposure time of legal sized lobsters may be reflected in the lower incidence of disease symptoms relative to the recruit size class observed in our data.
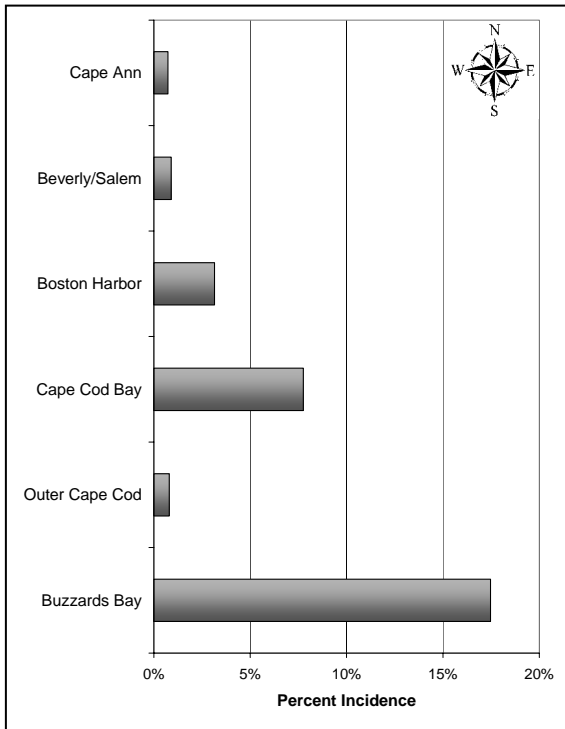

Figure 9: Mean incidence of shell disease in Massachusetts coastal waters, 2000 to 2004
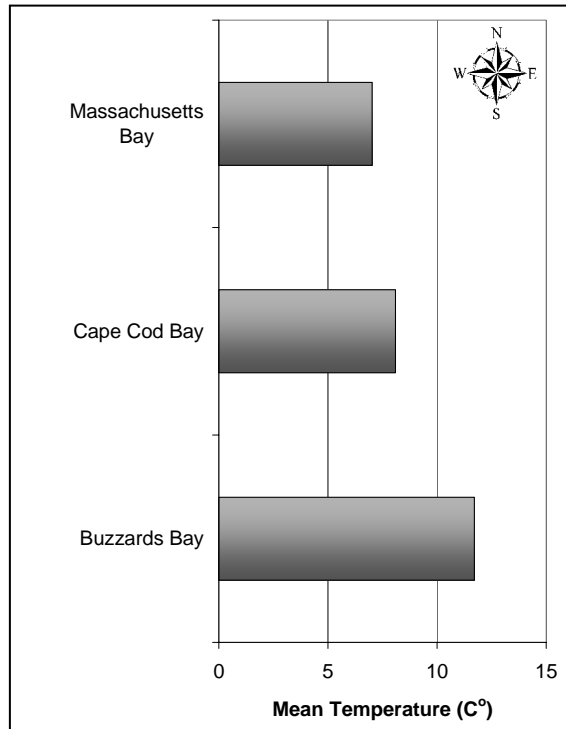
Figure 10: 2000 to 2003 mean bottom water temperature in three Massachusetts regions.

Spatial Patterns

Our data exhibit an increasing general trend in shell disease prevalence from north to south along the Massachusetts coastline (Figure 9). Starting in Cape Ann, our northern-most sampling region, shell disease prevalence was very low, increased slightly in each region heading in a southerly direction, dropped substantially in Outer Cape Cod, and increased sharply in our southern-most region Buzzards Bay. A comparison of shell disease prevalence data from 1983 and 1989 reveals a similar pattern (Figures 3 & 4). The similarity in patterns over time, in combination with identical suspected causative agents in the recent outbreak of shell disease in lobsters both north and south of Cape Cod (Smolowitz et al., 2003), suggest environmental influence on the observed spatial pattern. An examination of the regional annual mean bottom water temperature supports

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this hypothesis (Figure 10). Cape Cod serves as a distinct geographic barrier between the cold waters of the Gulf of Maine and the warmer water of southern New England. As such a north to south latitudinal gradient in bottom water temperature, similar to the pattern observed in shell disease prevalence along the Massachusetts coast, occurs. A bottom water temperature time series is not available for Outer Cape Cod, however this area is known to be a cold water environment dominated by mixing currents from the Gulf of Maine and Georges Bank.

The apparent influence of temperature on shell disease prevalence leads us to propose two mechanisms through which temperature may act independently or synergistically on disease prevalence.

Mechanism 1: It is possible that the cold water environments north and east of Cape Cod retard the growth of the bacteria suspected to cause shell disease. Conversely, the warmer waters south of Cape Cod may foster bacterial growth.

Mechanism 2: Temperature has a strong influence on the growth and reproductive cycles of lobsters (Waddy et al., 1995). These influences are manifested as clinal patterns in size at sexual maturity and growth rate. An examination of regional differences in size at 50% (L50) sexual maturity confirms this clinal pattern (Figure 11). Heading south along the Massachusetts coast the L50 declines, from a high of 90 mm in Cape Ann to a low of 76 mm in Buzzards Bay (Estrella and McKiernan, 1985). Upon reaching sexual maturity a female lobster's growth rate declines significantly in relation to its reproductive cycle (Aiken, 1980). As such, the average intermolt duration increases at the onset of sexual maturity. Examination of this parameter on a regional basis reveals notable differences in the average intermolt duration of legal sized female lobsters south of Cape Cod, and those north and east of Cape Cod (Table 3). The average legal sized female lobster in Southern New England waters retains its shell for approximately 175 more days than female lobsters in the Gulf of Maine (ASMFC, 2000).
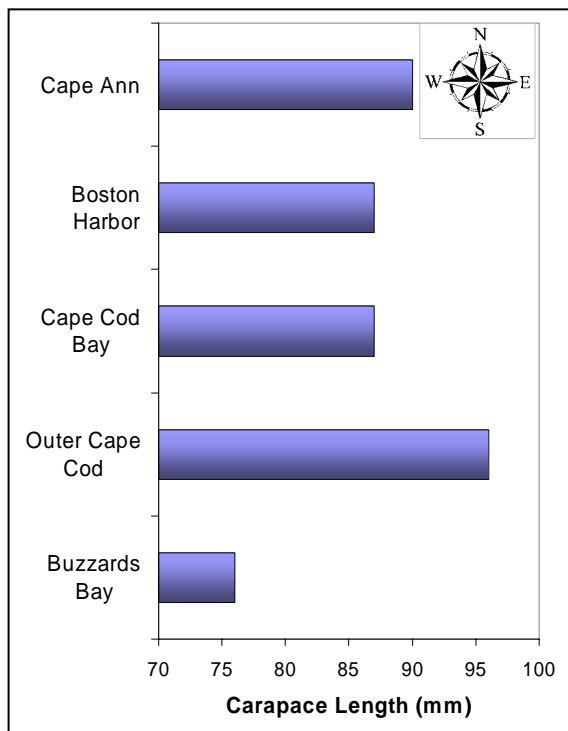

Figure 11: Size at 50% sexual maturity in five Massachusetts coastal regions

Table 3: Average intermolt duration (days) at minimum legal size

Region	Days
Gulf of Maine	394
Outer Cape Cod	376
Southern New England	575

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The notable similarities in the increasing north to south trend in water temperature, average intermolt duration, and incidence of shell disease suggest a functional relationship between the three parameters. They also support the hypothesis that shell disease prevalence is related to the time of exposure to causative agents, and likely explain the regional differences in shell disease prevalence observed along the Massachusetts coast.

Temporal Patterns

The prevalence of shell disease by Massachusetts coastal region from 2000 to 2004 is depicted in Figure 12. The incidence of shell disease in the three northern most regions, Cape Ann, Beverly/Salem, and Boston Harbor, remained below 5% throughout the entire 5 year period. Similarly, the incidence of shell disease in the Outer Cape Cod region remained extremely low from 2001, when it first appeared, to present. The Cape Cod Bay region had a moderate prevalence of shell disease in 2000 and 2001, and steadily declined to lower levels through 2004. In contrast to what was observed in regions north and east of Cape Cod, the Buzzards Bay region had a moderate incidence of shell disease in 2000, increased steadily to a high of 28% in 2003, and then dropped sharply to moderate levels in 2004.

Although bacteria have been implicated as the causative agents of epizootic shell disease, the reason for the recent outbreak remains unclear. It appears that the bacteria found on the leading edge of lobster shell erosions both north and south of Cape Cod are identical, and that these bacteria occur at background levels throughout the region (Smolowitz et al 2003). These findings suggest that the temporal patterns in shell disease prevalence are influenced by environmental parameters, not by the biota present. Two possible environmental parameters that may be related to shell disease prevalence are industrial/domestic pollution and temperature.

An examination of our regional prevalence data (Figure 12) suggests that pollution is not a primary factor related to shell disease prevalence. Boston Harbor, one of the country's most polluted urban embayments, has maintained a very low prevalence of shell disease throughout the time series. Conversely, the area around the Elizabeth Islands in the southern portion of Buzzards Bay is not adjacent to any major population or industrial centers, nor is it immediately down-current of such influences, yet this region has consistently had the highest prevalence of shell disease within Massachusetts coastal waters. This lack of correspondence between polluted areas and observed high disease incidence in our data seem to rule out pollution as the primary culprit.

We propose that there is a relationship between the temporal patterns in shell disease prevalence and water temperature. To examine this relationship we utilized the NOAA sea-surface temperature time series from Woods Hole, MA. This time series dates back to 1945 and provides a sufficient context of temperature over time against which recent changes in temperature may be examined. To gauge the cumulative effect of temperature on a lobster over the course of a year we calculated the number of days water temperature was above a certain threshold within each year. We chose 20 $^{\circ}$C as the threshold representative of the upper temperature range lobsters typically prefer (Aiken and Waddy, 1986). The number of days above 20 $^{\circ}$C was calculated for each year and then subtracted from the time series mean to develop an annual deviation from the average number of days above 20 $^{\circ}$C (Figure 13). The deviations from the mean
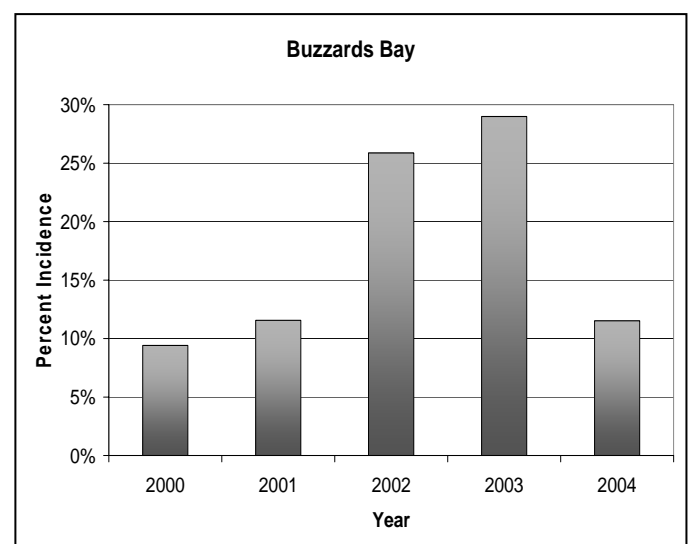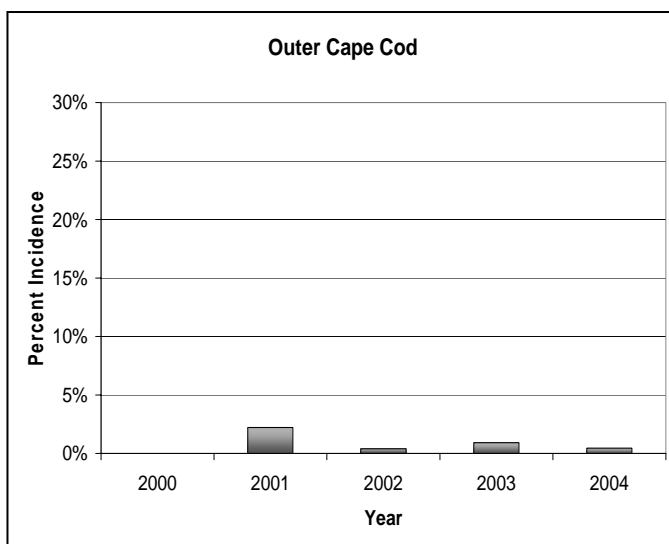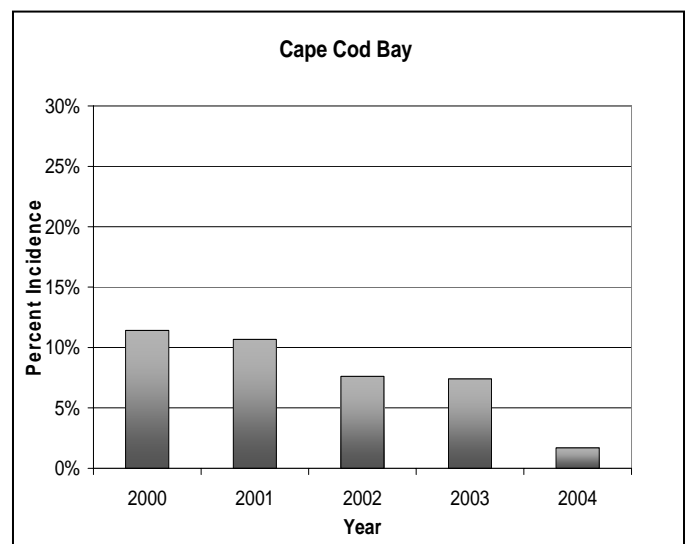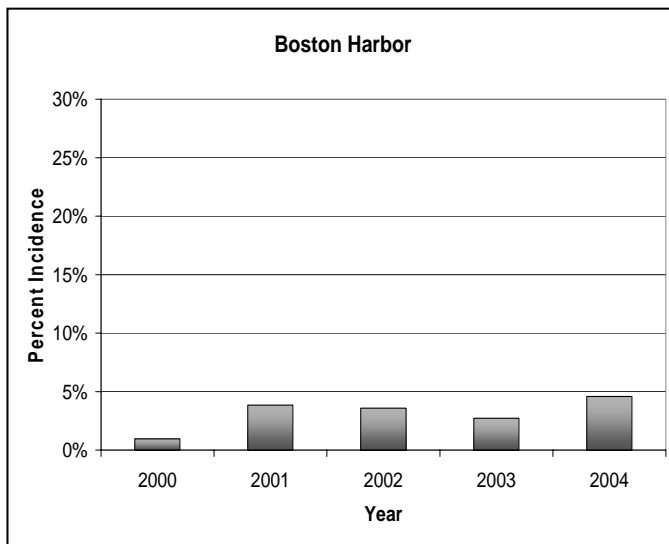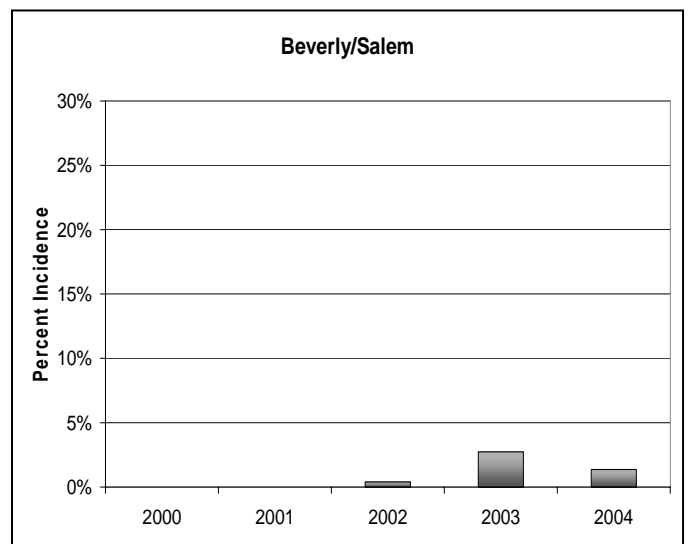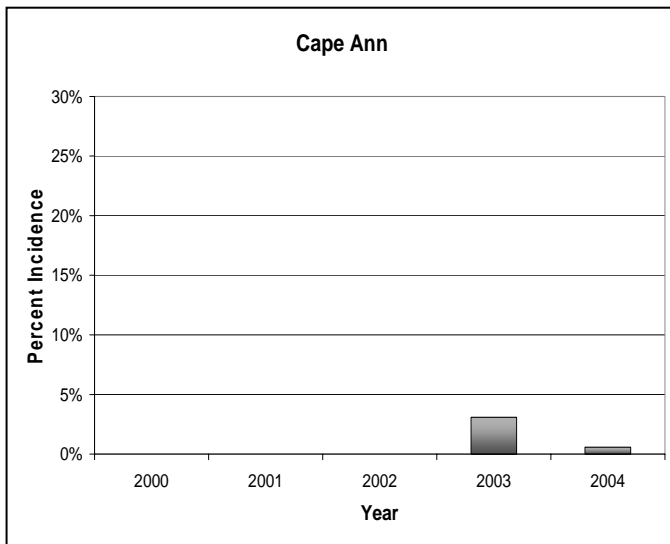
Figure 12: Prevalence of shell disease in six Massachusetts coastal regions, 2000 - 2004

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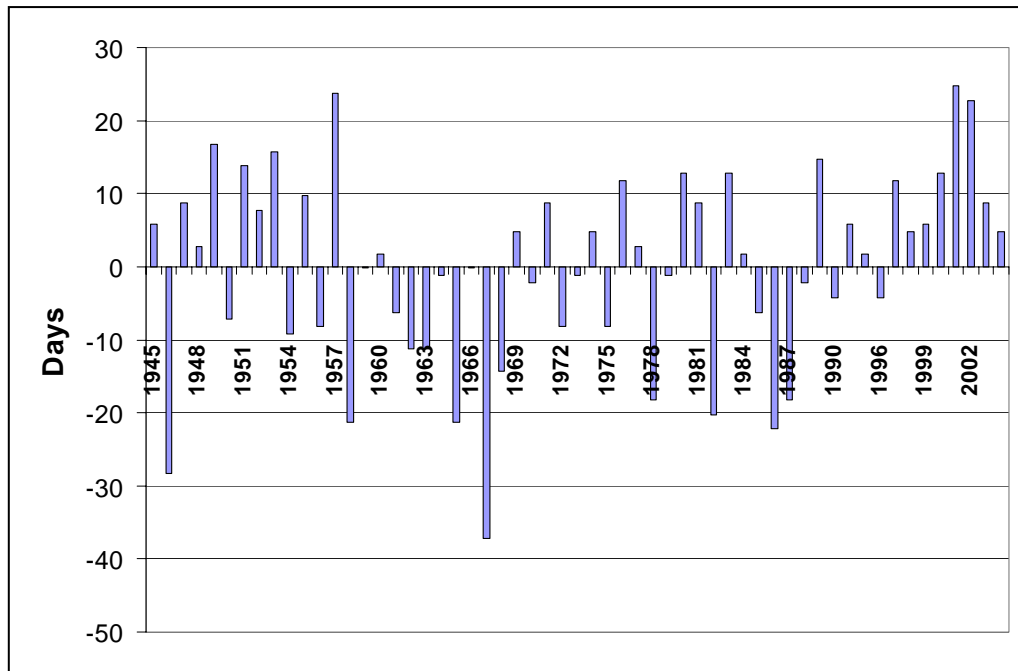Figure 13. Annual deviations from the time series mean number of days above 20 $^{\circ}$C

fluctuated from positive to negative over the course of the time series until 1997, when the number of days in a year above 20 $^{\circ}$C remained above the time series mean for the last eight consecutive years. At no other period in this time series has there been a consecutive string of positive deviations of this duration. Interestingly, this time period that corresponds to the shell disease outbreak in southern New England.
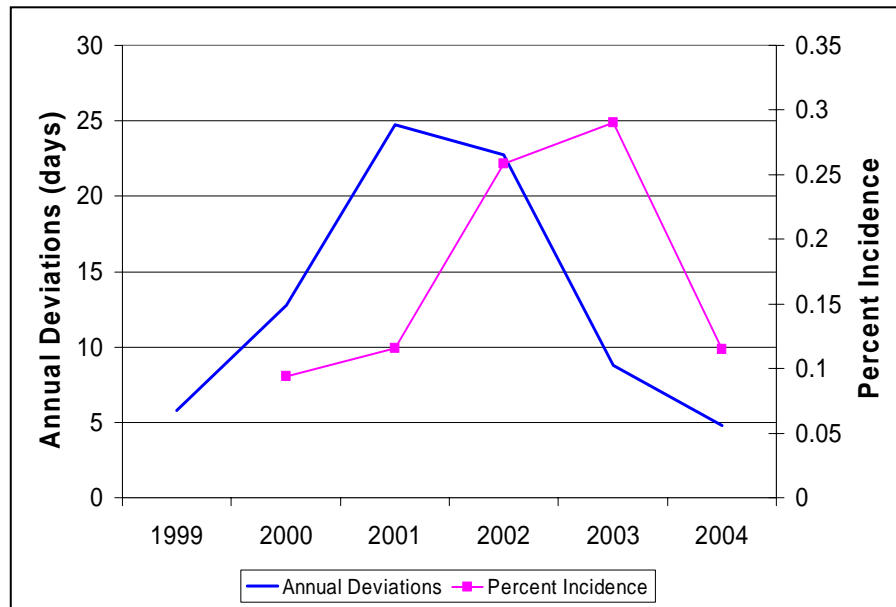

Figure 14. Annual deviations from the time series mean number of days above 20 $^{\circ}$C from sea-surface observations in Woods Hole, MA, and incidence of shell disease in Buzzards Bay

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A comparison of the Woods Hole sea-surface temperature time series and shell disease prevalence data from the adjacent Buzzards Bay region is presented in Figure 14. Both data series increase substantially and then decline over the time period 2000 to 2004; however there appears to be a delay of one year in the disease incidence data. We propose that these data are illustrating a lag in the relationship between water temperature and disease incidence, due to the correspondence of warm summer water temperatures and the development over time of disease symptoms. The visible symptoms of shell disease, pitting and erosion of the carapace, accumulate during the intermolt period. Recall that disease incidence is highest in May and June, before the major molt occurs, and lowest in August after most lobsters have molted into new, "clean" shells. If an increase in the number of days above 20 $^{\circ}$C is acting as an environmental stimulus for the development of shell disease, one would expect the summer water temperature in a given year to be related to the prevalence of shell disease in the following year, due to the molt cycle.

To examine the validity of this hypothesis we plotted the annual deviation from the mean number of days above 20°C in year (t), against the percent incidence of shell disease in the following year (t+1) for the period from 1999 through 2004 (Figure 15). There is a significant relationship ($R^2 = 0.91$, $p = 0.01$) between the yearly deviations from the historical average number of days above 20°C, and the prevalence of shell disease in the following year. These data should be interpreted cautiously because of the short time series for which we have overlapping temperature and shell disease observations. Nonetheless, they suggest that temporal trends in shell disease prevalence may be strongly related to temperature.
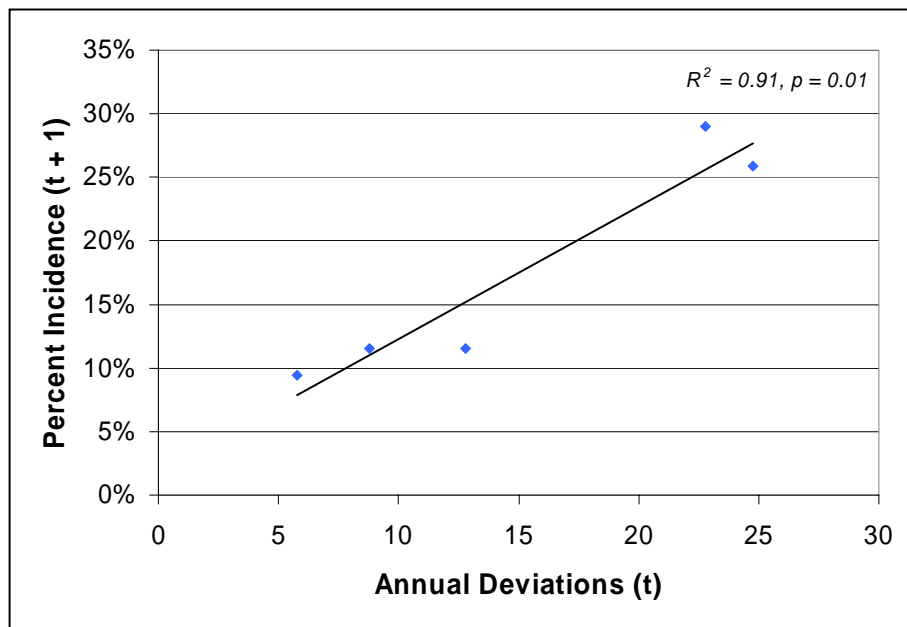

Figure 15. Relationship between annual deviations from the time series mean number of days above 20 $^{\circ}$C from sea-surface observations in Woods Hole, MA in year (t), and incidence of shell disease in Buzzards Bay in year (t+1).

Conclusions

In conclusion, while it is still unclear what is the precise mechanism behind infection, our data suggest that temperature and time of exposure are likely to be key factors in lobster shell disease. The prevalence of symptoms as they vary by size, gender, and geographical location appear to be linked to the influence temperature exerts on growth and sexual maturity. The increasing trend in shell disease in the waters of southern New England appears to be related to the length of exposure to a threshold temperature.

An argument has been presented linking warmer water temperatures to prevalence and spatial distribution of shell disease in Massachusetts waters. Further empirical testing should be conducted in order to verify these hypotheses. Possible avenues for further exploration into the causative mechanisms and effects of lobster shell disease include; mechanisms of infection, pathogenicity of bacteria, temperature as it relates to infection and symptom progression, and ramifications of the disease with regards to behavior and mortality.

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Observations of Shell Disease in Coastal Maine Waters: 2003 and 2004

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Shell disease is an unsightly condition characterized by erosion of the cuticle resulting from the establishment of mixed populations of chitonolitic bacteria (Smolowitz 1992). First documented in the wild (Estrella 1991) and in tidal impoundments (Smolowitz 1992) shell disease has traditionally considered a background condition of a normal lobster population where at times the occurrence of the disease has been observed to increase. The impounded form of shell disease is characterized by isolated pits originating on the dorsal surface of the carapace. Estrella (1991), reported an increase in disease prevalence with lobster size attributing this pattern to increased environmental exposure time by decreasing molt probabilities. Thus, hard-shell egg bearing female lobsters were observed to have the highest incidence of shell disease because molting is delayed until after hatching and is coincident with the 2-year ovarian cycle.

In the late 1990s, a dramatic increase in the presence of shell disease was first noted in southern New England. First documented in 1996 and 1997 with less than 5%

Lobster collected in Eastport Maine, with moderate incidence of shell disease. Photo courtesy of Chris Bartlett, Maine Sea Grant Extension.

of the population showing signs, a new form of rosette shell diease was described. Recent observations (2003) indicate the presence of the rosette shell disease has increased to 35-45% of the catch (Kathy Castro, personal communication March 2004).

In response to the increased occurrence of shell disease, lobster biologists from Massachusetts, Rhode Island, Connecticut and New York established a uniform protocol for assessing the severity and proportion of lobsters affected with shell disease syndrome in the field. It was thought a protocol would make it possible to compare an index of relative lobster health among several jurisdictions. An index was created to describe the percent coverage of shell disease on the total surface area of the lobsters. Four categories were identified to help reduce the subjectivity in assigning an index (0 = no shell disease, 1 = 1-10 %, 2= 11-50% and 3= >50% of the shells surface) (LIIS-LDW 2000, **http://www.seagrant.sunysb.edu/lilobsters/ShellDiseaseWorkshop/LISLI-ShellDiseaseWkshp.htm**).

In April 2003, the DMR adopted the shell disease index in all lobster sampling programs. This marked the first time where the incidence of shell disease has been systematically noted during DMR field observations. Previous observations were noted in the "comments" section for lobster found to be diseased, but these reports are likely un reliable as samplers were not specifically trained to look for shell disease prior to 2003. Additionally the DMR receives phone calls from harvesters who have caught diseased lobsters, these observations are not recorded in a manner, which can be queried.
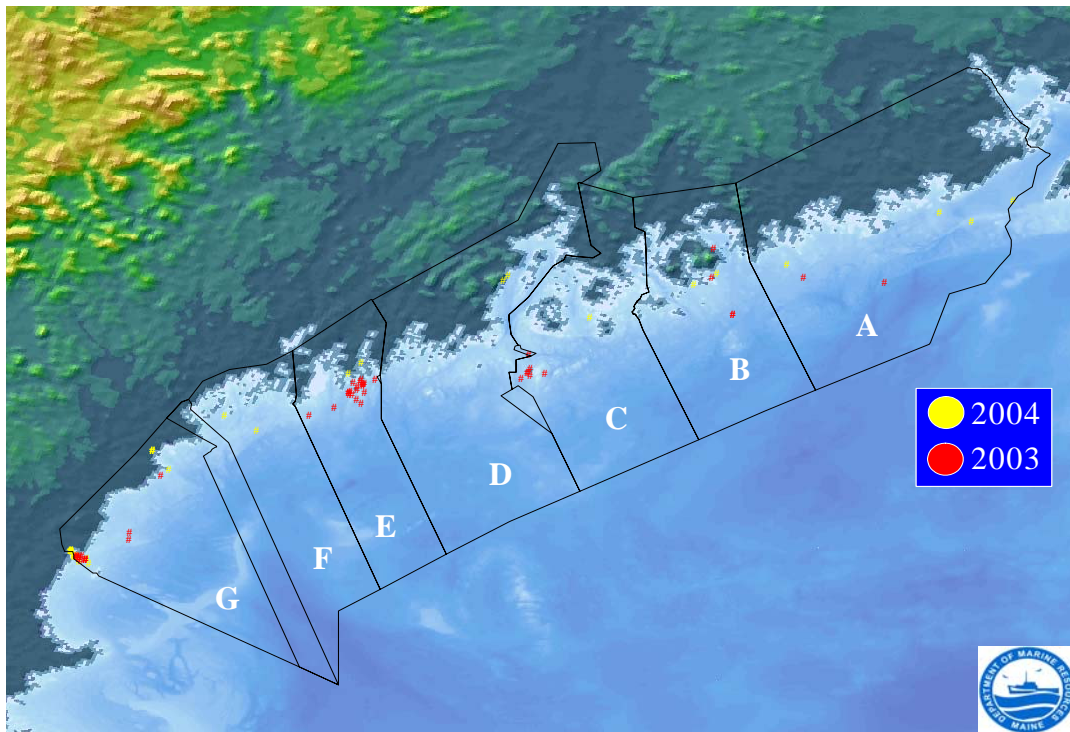

Figure 1: Initial observations of shell disease lobster during the 2003 and 2004 sampling seasons (May-November). Letters indicate Maine's Lobster Management Zones, contained within federal lobster management Area 1. In total, 93 lobster were observed as having shell disease, representing less than 0.01% of all lobsters sampled.

During the 2003 and 2004 sampling season, 93 lobsters were recorded as having shell disease, this represents less than 0.01% of lobsters measured during this period. More than 50% of shell disease observations were observed during one sea sampling trip in the June 2004 when 22 of 426 lobsters measured (5%) were scored as having shell disease (Figure 1).

In spite of the low incidence of shell disease, clear patterns confirm previous observations by Estrella (1991). The sex ratio was strongly biased towards female lobsters (82%), with 95% scored as hard-shell. The average size of lobster with shell disease was 87.5 mm with a minimum of 67 mm and a maximum of 137 mm (Figure 2). The severity of lobster shell disease increased with size, as 43% of lobsters scored with shell disease above 100 mm CL were classified as having lesions covering 10% or more of the carapace, while 34% of lobster less than 100 mm CL were similarly scored. Observations of harvester communicated to DMR, suggest that many of the less severely diseased lobster were able to molt successfully as some lobsters were held in storage crates when molting was observed and reports of shell disease decreased after the initiation of the summer molting season.
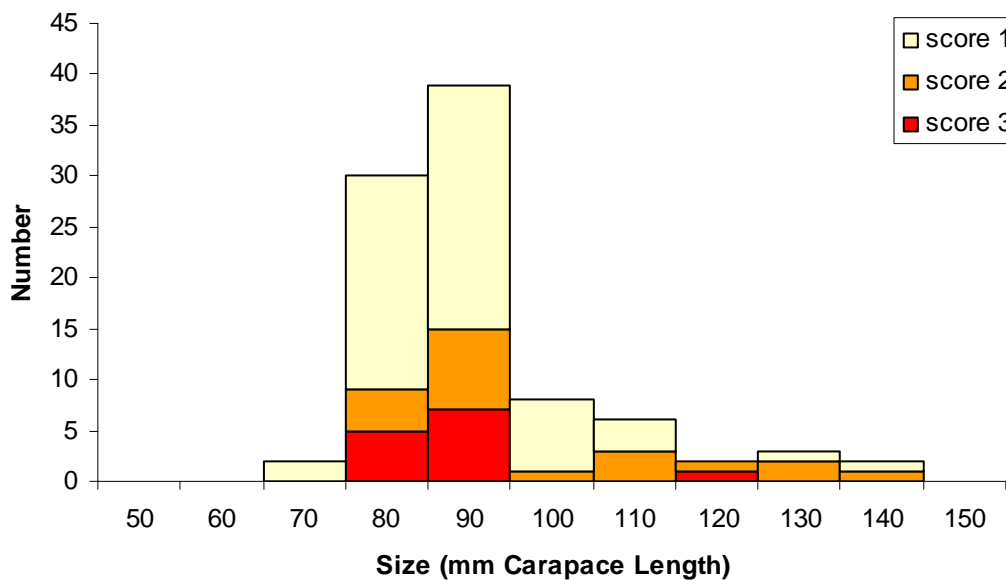

Figure 2: Number of shell disease lobster encountered during the 2003 and 2004 sampling season (n=93) Shell diseased lobsters are scored by four criteria that characterize the proportion of lobsters affected with shell disease (0= no signs, 1 = 1-10 %, 2= 11-50% and 3= >50% of the shells surface). Shell diseased lobsters were heavily biased toward hard-shell (95%) and female lobsters (82%).

Temperature increases in southern New England have been suggested as a possible mechanism for increased stress on lobsters and possibly facilitating the spread of shell lesions. Although not reaching the levels of temperature observed in southern New England, annual bottom water temperatures have been increasing in Boothbay Harbor Maine for the last 15 years. Between the periods of 1990-1994 and 2000-2004, summer bottom temperature have increased an average of 2.5'C (Figure 3).
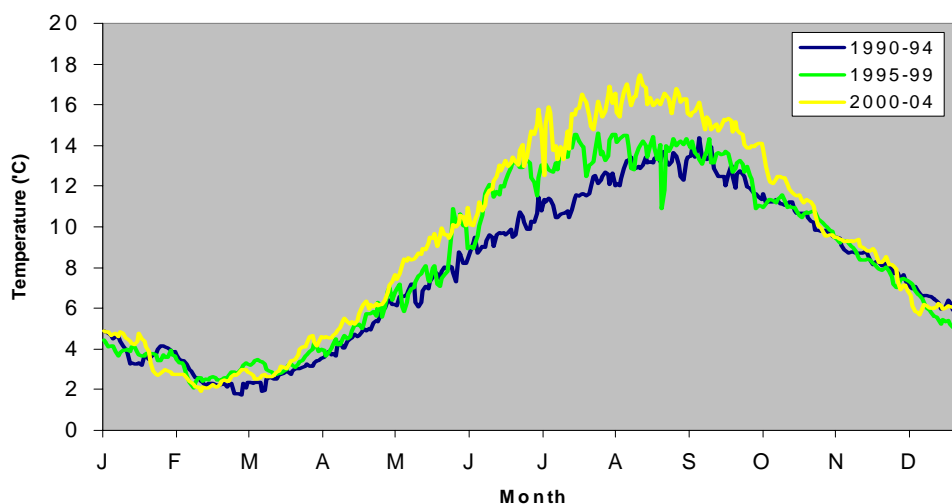
Figure 3: Average daily bottom temperature (5 m) by 5-year time periods, 1994-2004, for Boothbay Harbor, Maine.

The incidence of shell disease was low in 2003 and 2004 with less than 0.01% of measured lobsters showing signs of shell lesions in Maine waters. However, distinct patterns of lobsters primarily impacted by shell lesions such as being large, hardshell and female suggest segments of the lobster population are at a higher risk than others in Maine waters. Increased bottom temperatures indicates that the environment has changed in recent years and continued surveillance for shell disease and lobster health issues are warranted.

The DMR has worked closely with industry to maintain good communication and a constant visual on lobster health issues in Maine. In July 2003, DMR Marine patrol posted a "lobster health notice" at all lobster buying stations in Maine (Appendix 1). Reports of the incidence of shell disease in southern New England continue to be a problem for the Maine lobster fishery and have had ripple effects within the industry. In February 2005, an inaccurate report of the incidence of shell disease caused several panicked phone calls about the status of the $260,000,000 Maine Lobster Fishery. In response to this article, the DMR issued a clarifying press release (appendix 2), but "the damage had already been done". Future public relation efforts need to indicate an accurate representation of the occurrence of shell disease in all of New England, indicating where problems exist and where they do not at the present time.

References:

Estrella, Bruce T. (1991) Shell Disease in American lobster (Homarus americanus, H. Milne Edwards, 1837) from Massachusetts coastal waters with considerations for standardizing sampling. J. Shell. Res., 10:483-488.

Long Island Sound Lobster Disease Workshop. June 15, 2000 Millstone, CT. Meeting summary at: http://www.seagrant.sunysb.edu/lilobsters/ShellDiseaseWorkshop/LISLI-ShellDiseaseWkshp.htm.

Smolowitz, R.M., R.A. Bullis, and D. A. Abt (1992) Pathologic cuticular changes of winter impoundment shell disease preceding and during intermolt in the American lobster, *Homarus americanus*. Biol Boll. 183:99-112.

Appendix 1. Lobster Health Notice July 2003.

ATTENTION LOBSTERMEN:
Department of Marine Resources Lobster Health Notice

The Department of Marine Resources (DMR) is interested in learning about observations of diseased, weak or dead lobsters you may see in your lobster traps. Shell disease and weak lobster syndrome are caused by naturally occurring organisms (bacteria and amoebae) and may contribute to decreased survival in lobsters. These organisms do not pose a health risk to humans. The incidence of shell disease has increased dramatically in Rhode Island, southern Massachusetts and eastern Connecticut coastal waters, and may have contributed to a rapid decline in landings since 1999. Weak lobster syndrome has been implicated as one of many factors that may have contributed to the decline of lobster stocks in Long Island Sound since 1998.

Current DMR sampling programs have not identified significant concentrations of diseased, weak or dead lobsters in Maine. The DMR has received scattered reports of shell disease from Kittery to Winter Harbor this spring. **The DMR and industry are very interested in documenting the occurrence of these symptoms on the coast of Maine**. In June, the DMR Lobster Advisory Council approved funding of a study to document and characterize diseased lobsters in Maine's waters.

Researchers at the Bigelow Laboratory for Ocean Sciences will build on existing lobster health work in southern New England and Long Island Sound. Funding currently allows processing 5 diseased and 5 healthy lobsters each month. **Legal** lobsters can be transported directly to the DMR lab in Boothbay Harbor, or can be called in for pickup by DMR staff (contact # 207-633-9538). Each diseased lobster must have a "normal" control lobster from the same area for comparison. **The DMR is not asking fishermen to retain illegal lobsters. It is unlawful to possess illegal lobsters at any time. At this time, current research indicates that there is no compelling reason to remove diseased or weak lobsters from the population.**

What to do:

Immediately Contact:

Carl Wilson

633-9538

Carl.Wilson@maine.gov

Report:

- Size and condition of lobster
- Location, depth and substrate where captured

Transport

- Please isolate lobster in crate
- Retain one additional "normal" lobster from the same area for comparative analysis

DMR staff will work with you to arrange for collection and reimbursement of lobsters

SHELL DISEASE

Shell disease is ranked from 0 to 3:

0 – No shell disease symptoms

1 – Shell disease on 1 – 10% of the shells surface

2 – Shell disease on 11-50% of the shells surface

3 – Shell disease on >50% of the shells surface

From Maine January 2003

Many diseased lobsters can molt out of affected shell and show no signs of previous shell damage.
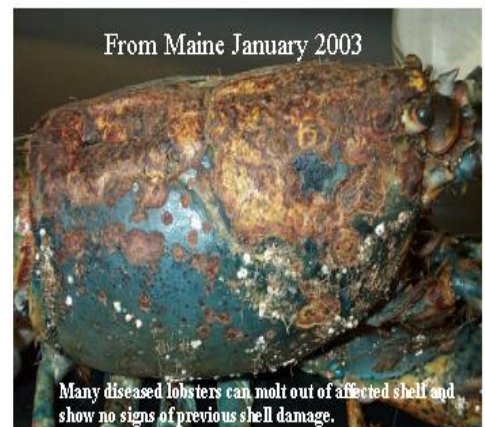
Appendix 2. Lobster Shell Disease
Press Release

PRESS RELEASE
Thursday 17, February 2005

SHELL DISEASE IN MAINE LOBSTERS

MAINE DEPARTMENT OF MARINE RESOURCES
BIGELOW LABORATORY FOR OCEAN SCIENCES

Some media reports this morning (Thursday 17, February 2005) have reported shell disease rates as high as 30% along the New England coast.

Carl Wilson (Maine Department of Marine Resources) and Charles O'Kelly (Bigelow Laboratory for Ocean Sciences) have been monitoring Maine lobsters for shell disease for several years, and especially since 2003. Epizootic shell disease (the form that occurs in wild-caught animals) has had a high incidence in lobsters from central Long Island Sound to Massachusetts Bay since the mid-1990s, and has affected both the marketability and, in more recent years, the populations of lobsters in southern New England.

At present, in waters throughout Maine, shell disease affects less than one-tenth of one percent of animals harvested by lobstermen and by scientists in trawl and trap surveys. The animals affected have been relatively inactive animals that have not molted in more than a year. This disease pattern differs significantly from that seen in Long Island Sound and Massachusetts Bay, where relatively active, recently molted animals frequently are affected. Wilson and O'Kelly believe that the present state of shell disease in Maine waters reflects the "background" condition, which has remained essentially unchanged for many years.

Research is needed to understand the risk factors associated with shell disease and other aspects of lobster health – an all-day symposium (Saturday 5, March 2005) at next month's Fishermen's Forum in Rockland is planned to address these issues, presenting the current state of knowledge and future needs. However, Wilson and O'Kelly believe that shell disease does not pose an immediate threat to the Maine lobster industry.

For further information please contact:
Carl Wilson
Maine Dept. of Marine Resources
207.633.9538
carl.wilson@maine.gov

Dr. Charles O'Kelly
Bigelow Laboratory for Ocean Sciences
 207.633.9656
cokelly@bigelow.org

Recent story on the status of shell disease in Maine
"Scientist Keep Wary Eye on Lobster Shell Disease"
February 2, 2005 Ellsworth American by Aaron Porter
http://www.ellsworthamerican.com/archive/2005/02-03-05/ea_news7_02-03-05.html

Maine Fisherman Forum Schedule: http://www.mainefishermensforum.org/

Management Implication of Lobster Shell Disease:
How do you manage what you don't understand?

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UMass/Boston, 100 Morrissey Blvd., Boston, MA 02125, John.Duff@umb.edu

Lobsters occupy a unique place in the marine economic and ecosystem of the northeast region of North America. The aggregate American lobster (H*omarus americanus*) fisheries of contribute tens of millions of dollars to regional economies and constitute tens of thousands of jobs.

For decades, efforts to manage the lobster fisheries have focused on the management of those who engage in harvesting the resource. Academics and policymakers saw the threat as an open access system that could ultimately lead to a tragedy of the commons over-exploitation result. Lawmakers and regulators designed capped effort allocation systems and management area designations as the remedy to the threat.

"Optimal" fisheries management, contend most, attempts to meet a three-fold objective of biological sustainability, economic efficiency, and social equity (Copes, 2000). Law, policy and management mechanisms created to strike a balance among these three objectives must often place a priority on one over the others. Today, the threat to the lobster fisheries manifests itself on the backs of the lobsters in the form of shell disease. That malady may strike at the heart of the sustainability objective and in so doing threaten the other objectives as well.

Unlike doctors, however, lawmakers and managers have not been called on to swear to "first, do no harm." As a result, legal and policy "remedies" to food supply, health and environmental problems may subject the threat to treatments that have for more dire consequences that the initial concern.

How does law respond to disease affecting food supply?

- Quarantine
- Moratorium
- Destruction
- Processing regulations
- Research and monitoring

However, when a disease appears and the causes and implications are not well understood, law and policymakers must either refrain from acting or act upon incomplete and/or inappropriate information.

The purpose of this brief paper are to identify some of the legal mechanisms available to lobster fishery managers and researchers in an effort to suggest the breadth of policy responses that may prove helpful as the disease, its causes and its consequences become better understood.

Researchers in this workshop have highlighted correlations between water temperature and disease prevalence. But the causative relationship and the *degree* to which it exists (assuming a causative relationship does exist) have not yet been shown.

As a result, the initial step in lobster disease management is the question formulation phase. Questions raised at the Workshop include:
- What information should be included in developing a lobster health database?
- How can one enhance lobster health management by improving interaction at all levels of the fishing industry?
- What management decisions are required to aid in lobster disease prevention?
- What are the best predictors of outbreaks of shell disease?
- Is bait a causative agent of lobster health problems, and if so, what mechanisms can be used to regulate bait usage in the lobster fishery.
- Can we attribute shell disease to harvesting techniques that either introduce or exacerbate the likelihood and spread of the disease?
- If so, do we afford more protection to those harvesters whose activities have protected the resource as opposed to those whose activities may have increased disease risk?

Having identified the disease concern and its currently identifiable range (New York to Maine), existing law, policy, and management mechanisms should be considered as either directly relevant tools to assess and manage the threat of the disease or as models for alternative law and policy tools. This consideration necessarily begins with a review of the institutions that exercise jurisdiction over the resource.

The American Lobster fishery includes a geographic range that straddles numerous state and federal, and insterstate fishery jurisdictions. As a result the fishery is managed via a tiered approach, i.e., management objectives, requirements and prohibitions are established by the Atlantic States Marine Fisheries Commission and are implemented by the various states. While laws and regulations related to the lobster fishery may vary from state to state, each state must mange their respective jurisdictions in a way that complies with the interstate fishery management plan (FMP). For example, minimum size and regional management zones are set in the FMP, while the allocation of effort employed in harvesting "catchable" lobster and statewide zones may be established by the respective states.

Accordingly management efforts devised to address lobster shell disease, in the form of obligations or prohibitions, may be more appropriate at the interstate FMP level in some circumstances and at the state level in other circumstances.

"Obligation" type measures included at the interstate FMP level include those related to, among other things:
- Permits and Licensing
- Escape Vents on Traps
- Maximum Trap Size
- Conservation Management Review
- Monitoring and Reporting

"Limitation" or "prohibition " type measures at the interstate FMP level include those related to, among other things
- Limits on the number of traps per vessel
- Area closures

"Obligation" type measures included at the state level include those referred to above, as well as those related to, among other things:

- Rules, etc., Relative to Sanitary Conditions Required for Certain Food Fish Processing or Distribution Establishments Escape Vents on Traps
- Maximum Trap Size
- Conservation Management Review
- Monitoring and Reporting
- Establishment of Research
- Policy investigations.
- Data collection.
- Cooperation with University researchers and industry.
- Reporting research results to state Legislature

"Limitation" or "prohibition " type measures at the state level include those noted above as well as those related to, among other things

- Prohibitions on taking egg-bearing females;
- Prohibitions on taking dead lobsters;
- Sanctions for mischaracterizing quality of catch;
- Sanctions for introducing adulterated food into commerce.

This universe of existing "obligation" and "prohibition" measures at the interstate FMP and individual state levels suggests that scientists, managers, and regulators concerned with the implications of lobster shell disease might easily fashion a set of notification, limitation, communication, monitoring and research provisions specifically aimed at collecting date on habitat, lobster disease appearance and prevalence, and research.

The legal framework exists today. Scientists and stakeholders must now determine the method of data collection and review necessary to fashion a set of appropriate measures than can be "plugged into" the interstate and state-level management regimes.

Reference:
Parzival Copes, The Need for Balance in Canada's Fisheries Policy, Discussion Paper #00-9, Simon Fraser University Department of Economics, Discussion paper Series (February 2002). Viewable at:
http://www.sfu.ca/economics/research/discussion/dp00-9.pdf

Lobster shell disease in southern New England: monitoring and research

Kathleen Castro, *University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences, Kingston, RI 02881;* Thomas Angell, *RI Dept of Environmental Management, Division of Marine Fisheries, 3 Fort Wetherill Road Jamestown, RI 02835;* and Barbara Somers, *University of Rhode Island, Department of Fisheries, Animal and Veterinary Sciences Kingston, RI 02881;* kcastro@uri.edu.

Describing the Outbreak:

Understanding marine disease and the timing and spread of outbreaks is important given the increasing anthropogenic and environmental stressors that are becoming more prevalent or noticeable (Harvell, et al, 1999; Ward and Lafferty, 2004). The University of Rhode Island and the RI Department of Environmental Management (RIDEM) have been monitoring this current epizootic event in Narragansett Bay since 1994. In 1996, lobsters began appearing with large areas of grotesque shell erosion, especially in the areas just behind the lobster's eyes. A year later the new disease increased alarmingly, appearing throughout Southern New England and Long Island Sound (Lobster management area 2) (Figure 1). Currently, lobsters with serious disease symptoms are being reported from Nova Scotia, Canada to Maryland (Castro and Angell, 2000).

The lobster fishery is a major industry in New England and the economic consequences of these affected populations are severe in the Southern New England fisheries management region. This area has also been the target of two major oil spills (1989 and 1996). The population of lobsters has shown a dramatic downturn since the early 1990's.
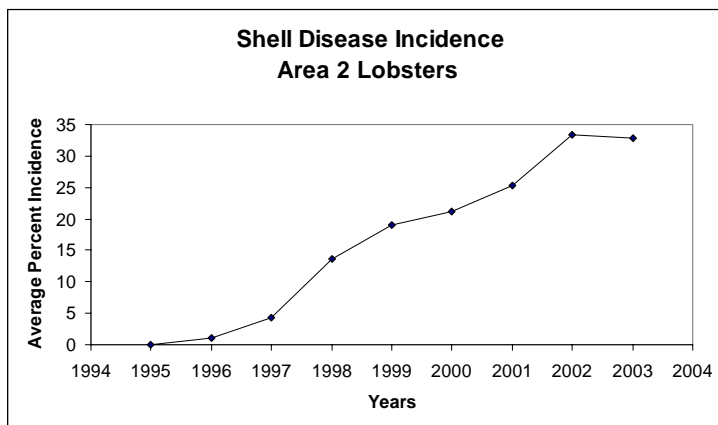

Figure 1: Incidence of shell disease in New England waters (Average of Southern Massachusetts, Rhode Island and Eastern Long Island Sound, Connecticut)

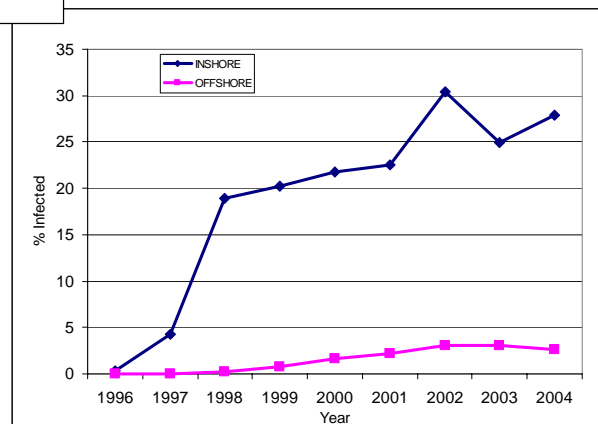Figure 2: Incidence of shell disease by area in Rhode Island (RI DEM).

Figure 3: Detailed area information on shell disease occurrence in RI waters (RI DEM)
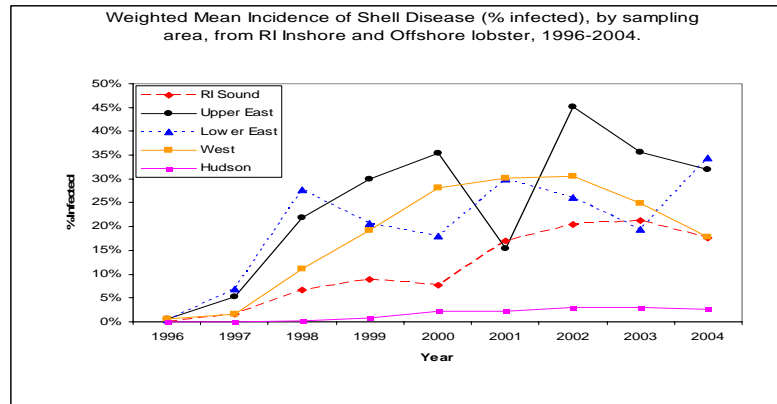
In Rhode Island the areas with the highest incidence rates are the inshore areas (Figure 2). Site-specific information in Rhode Island reveals different trends in incidence rates. East Passage of Narragansett Bay was the most affected (Figure 3). Ovigerous females continue to be the most affected (Figure 4 a, b and c). Males and non- ovigerous females are lower in incidence levels.

Figure 4: Incidence of shell disease by sex (RI DEM).

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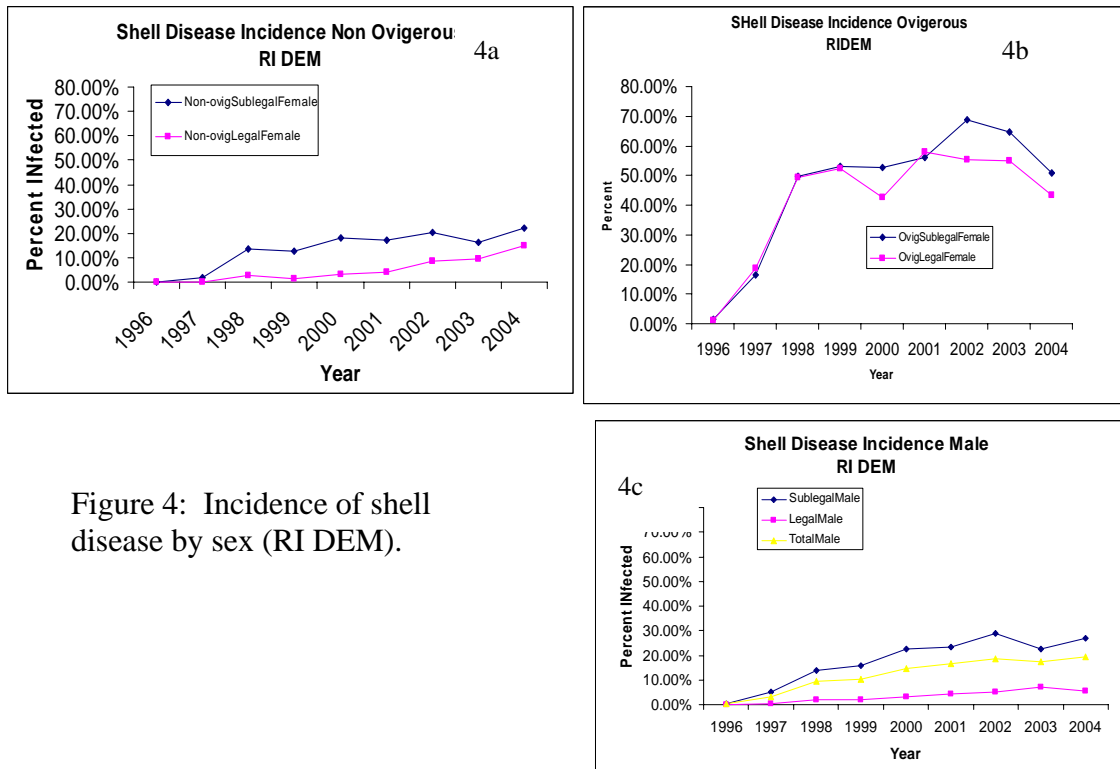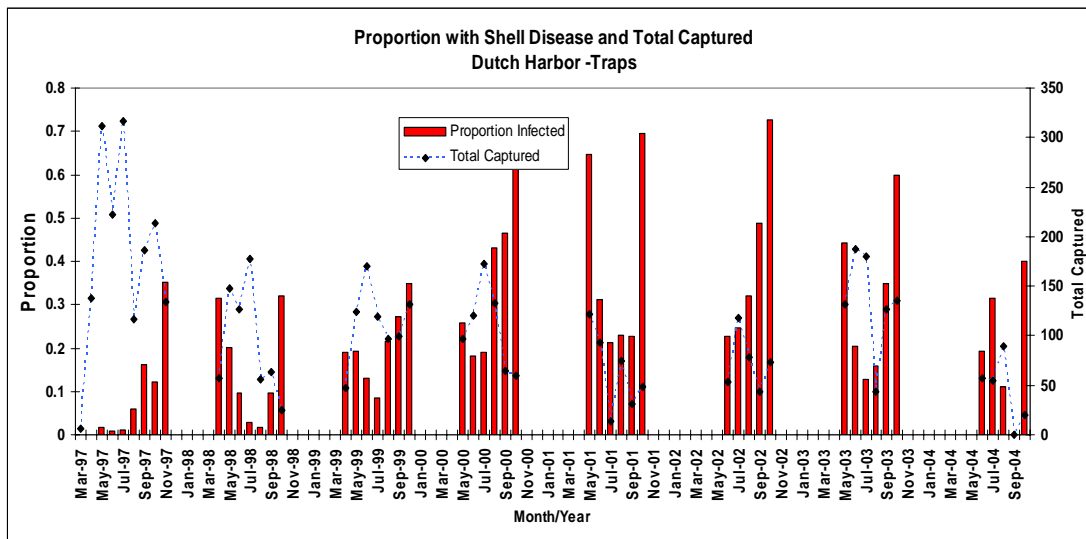Figure 5: Incidence by month (URI). Note: There is no sampling from October to April.

The incidence of disease shows a seasonal pattern. Each year the incidence of the disease decreases as the lobsters molt and then increase in the fall.

The size of lobsters being affected has shifted between years. In 1998, the larger lobsters were more affected. The disease pattern seems to have changed and smaller lobsters are now being infected (Figure 6).
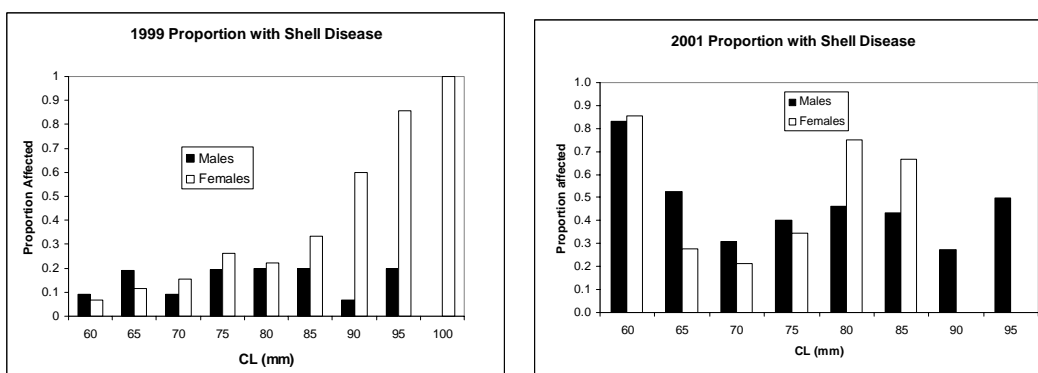
Figure 6: Change in incidence of shell disease by size from 1998-2001 (URI).

The severity of the disease was monitored using the regional scale for each sex for each year (Table 1).

Table 1: Regional shell disease scale

0 = no shell disease symptoms
Low = Shell disease symptoms on 1 – 10 % of the shells surface
Moderate = Shell disease symptoms on 11 – 50 % of the shells surface
Severe = Shell disease symptoms on > 50% of the shells surface

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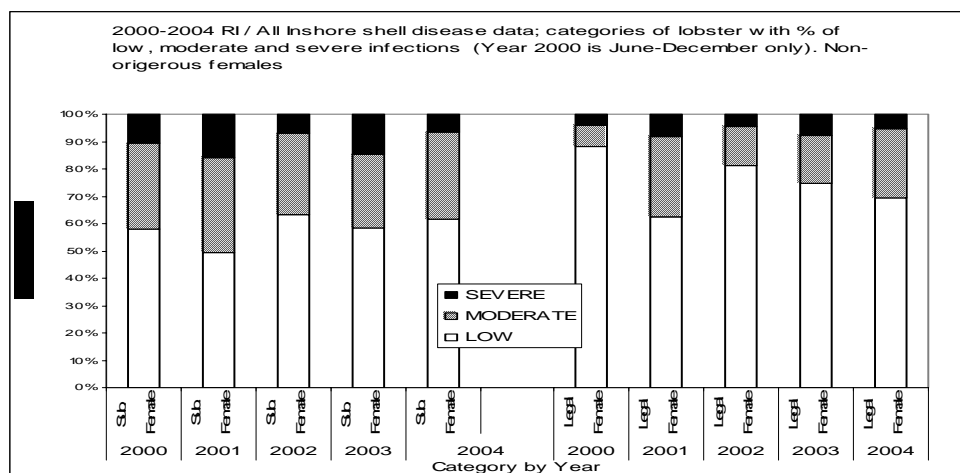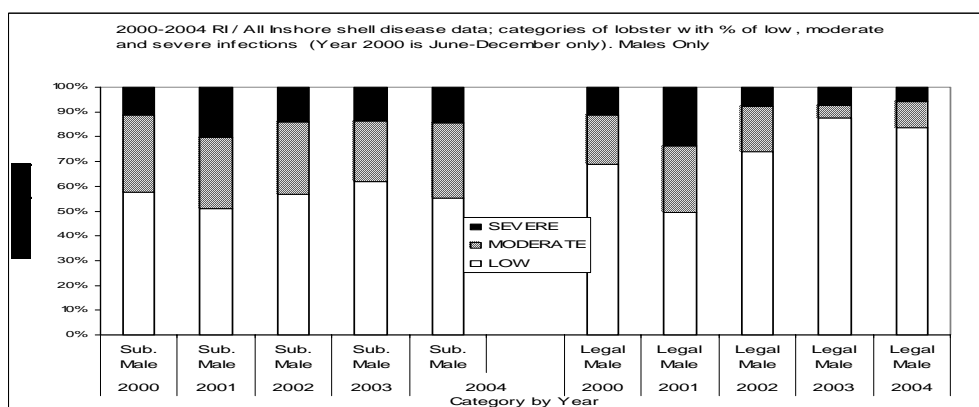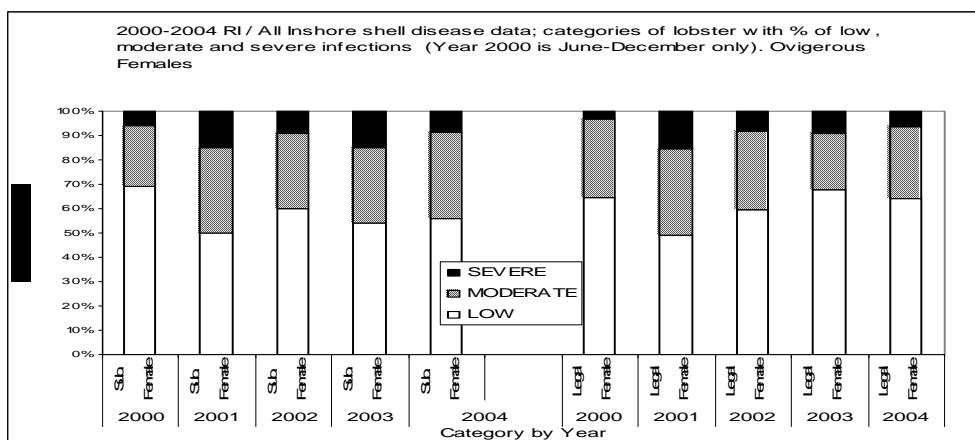
Figures 7: Severity indices for sex and year based on sea sampling (RI DEM)

Tag-Recapture Results:

From 1994-2004, lobsters were tagged with individually numbered Floy anchor tags on the artificial reefs in Dutch Harbor, RI. These were recaptured using small mesh traps in a weekly survey from May-September. Shell disease was noted in a more detailed form than that described for the regional research surveys (Table 2). The amount of shell disease on each body section of the lobster (dorsal carapace, claws and tail) was added together for a total of 300.

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Table 2: Shell disease scale used
for tag-recapture program (URI)

None = no shell disease
1 = 1-30
2 = 31-150
3 = 151-220
4 = 221-300
5 = scars

There were a total of 1931 recapture events: 700 were female and 1231 were male lobsters. Of these, 107 females and 242 male lobsters were recaptured multiple times allowing for a longer time series to evaluate shell disease progression.

Of the female multiple recapture events, 23 (of the 107) had shell disease during the time followed. Eight of these molted. Two were disease free when they molted and subsequently became diseased; six were shell diseased prior to the molt, five remained clean, and one re-contracted shell disease.

Of the male multiple recapture events, 78 (out of the 242) had shell disease during the time followed. Fourteen of these molted. Five were disease free when they molted and subsequently became diseased, nine were shell diseased prior to the molt, five remained clean, two re-contracted shell disease, and two were not seen again.

Timing for the occurrence of shell disease or a worsening event is shown in Figure 8. The shorter recapture periods illustrate how quickly shell disease can occur (Figure 9).
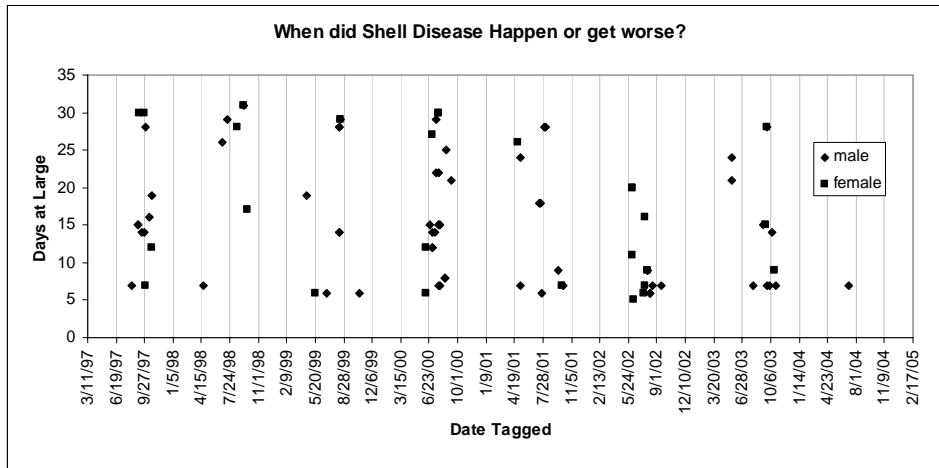

Figure 8. Days between recaptures and timing of appearance of shell disease for male and female lobsters.
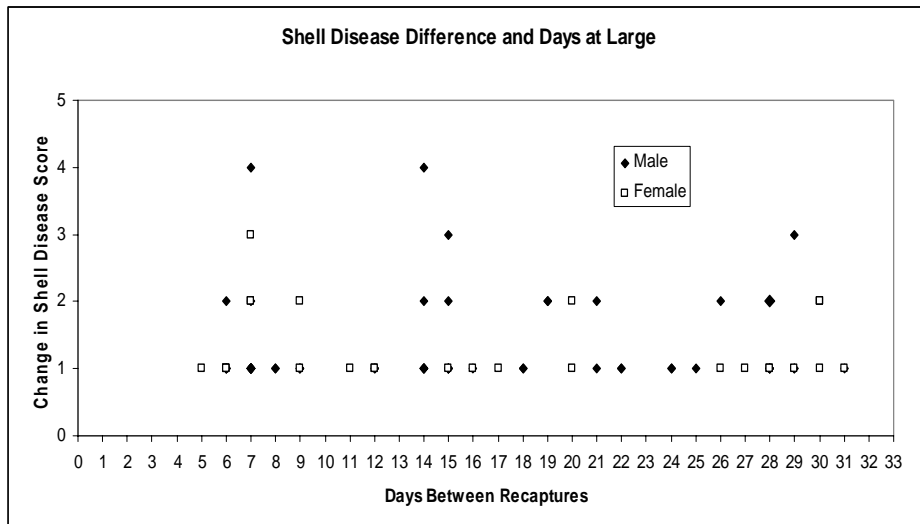
Figure 9: Difference in shell disease score as a function of days between recaptures.

Behavior - Time Budget Analysis (Preliminary Results):

There are several potential impact pathways where shell disease could be playing a critical role. The role of behavior is a critical one often overlooked.
Time budget analysis was used to evaluate general behavior of 20 shell diseased and 39 non-shell diseased lobsters. All lobsters were food deprived 12-24 hours prior to start of the experiment. Lobsters were observed under dark conditions using red lights by an observer using a stop watch. Individual lobsters were placed in a large circular tank containing rocks and a shelter in one corner. The lobster was allowed to acclimate for 15 minutes. The observation period started when a small food source was added into the opposite corner. The lobster was observed for 15 minutes, recording the time each behavior occurred. Behaviors were defined as:

- *Shelter*: Lobster was in physical contact with the cobble or with the actual shelter provided.
- *Explore*: Lobster left physical contact with cobble area.
- *Feed*: Lobster handled food provided. This does not imply consumption.

Data from each trial was analyzed for time spent in each behavior. Each behavior type (shelter use, exploring, or feeding) was summed for each 15-minute period for each lobster. Time budgets were constructed by summing the duration of discrete units of activity patterns and determining the proportion of time spent in each behavior. Proportions were transformed using the arcsin square root transformation. The transformed data was analyzed using an ANOVA and Tukey post hoc test. A contingency table analysis was used for the analysis of proportion data.

Results:

Preliminary results from the time budget analysis show a significant increase in the amount of time spent in shelter behavior for the shell diseased lobsters (Figure 10), (F=6.95, df=1, p=0.018). The Chi-square test of independence was significant (df=2, χ^2 = 43.03, p < 0.0001).
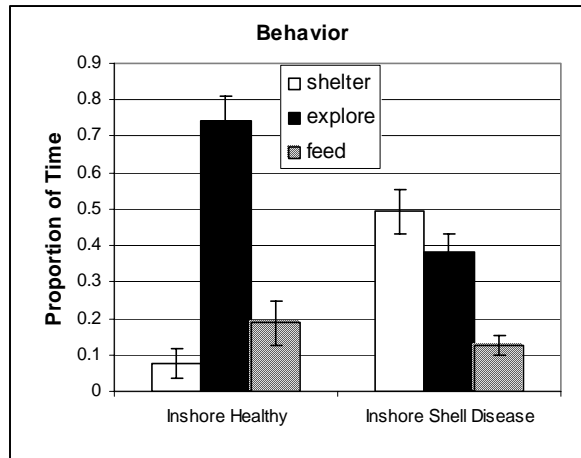
Figure 10: Proportion of time spent in each behavior for shell diseased and non-shell diseased lobsters (URI).

Natural Mortality:

The relationship between trawl abundance and settler abundance using a three year lag shows a change in relationship after 1997 (Gibson, RI DEM). Changes in natural mortality rates have been calculated using the change in ratio for unexploited age groups before and during the shell disease outbreak to estimate changes in natural mortality (Figure 11).
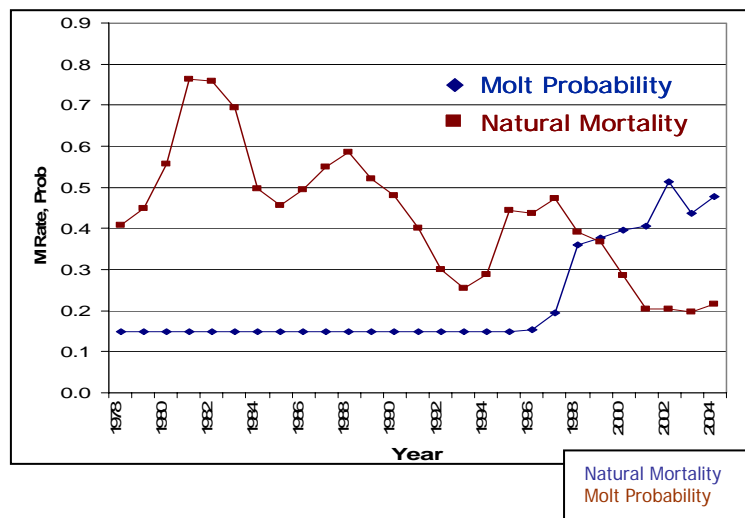
Figure 11: Predicted lobster natural mortality and molt probability for use in the Collie-Sissenwine Assessment Model (Data from RI DEM).

Discussion:

The research to date shows a major epizootic shell disease event that started in 1996-1997, beginning with high incidence rates reported for larger female ovigerous lobsters. Subsequently, smaller lobsters have been affected including males and non-ovigerous females. Severity seemed to be worse in 2001, but then appears to fluctuate without trend.

Since the disease is believed to be an external infection, the ability of the lobster to molt may alleviate many of the symptoms observed. However, tag return data and the proportion infected both point to re-infection occurring at some level over time.

Shell disease increases dramatically after the molt period in Narragansett Bay. During this time (fall) lobsters begin to show up with disease or increase in severity over a short time period. In extreme cases, a week period between recapture events showed several individuals progressing from no infection to severe. This does not coincide with higher temperatures in the Bay, but more with a fall overturn event (Figure 12).
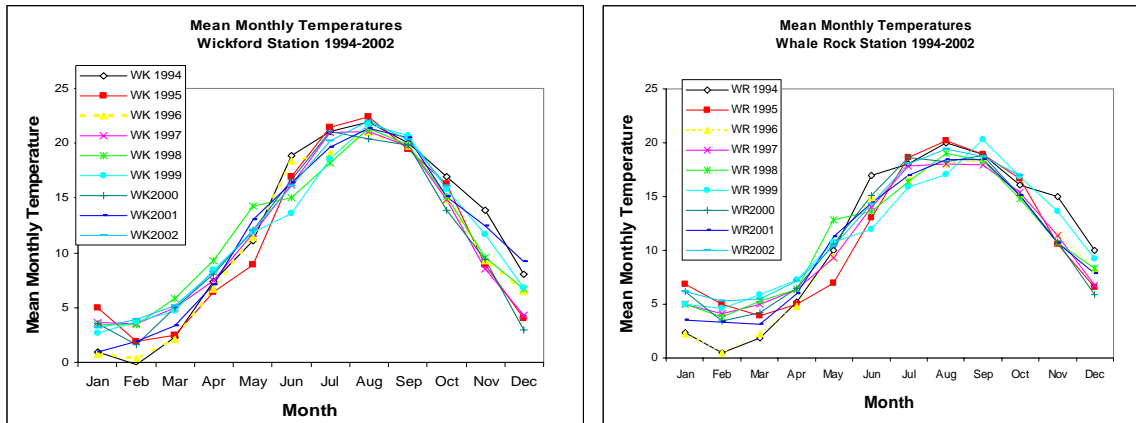
Figure 12: Monthly average bottom temperatures in the upper and lower Narragansett Bay, Rhode Island

Behavior data suggests that one of the consequences of shell disease may be a change in time spent sheltering and outcome of behavioral interactions between conspecifics (Cromarty and Kass-Simon, personal communication). This could ultimately affect molting, reproduction, feeding and mortality. Fishermen observations point to decreased feeding, lethargy, and delayed molting.

There are many unanswered questions about shell disease. This monitoring and preliminary research has just begun to elucidate many of the consequences for a shell diseased lobster.

References:

Castro, K.M. and T.E. Angell. 2000. Prevalence and progression of shell disease in American lobster *Homarus americanus* , from Rhode Island waters and the offshore canyons. Journal of Shellfish Research 19(2): 691-700.

Harvell, C.D. and 12 authors. 1999. Emerging Marine Diseases-Climate Links and Anthropogenic Factors. Science 285: 1505-1510.

Ward, J.R. and K.D. Lafferty. 2004. The Elusive Baseline of Marine Disease: Are Diseases in Ocean Ecosystems Increasing? PLoS Biology 2(4): 0542-0547.

Incidence of shell disease in American lobster (*Homarus americanus*) in New York waters

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Abstract:

The incidence of shell disease in lobsters has been recorded since June 2000 during sea-sampling trips on commercial lobster vessels in the Marine District of New York. The highest percentage of lobsters with shell disease occurred during sampling trips in the Atlantic Ocean off the south shore of Long Island. Lobsters sampled during the spring had the highest percentage of shell disease, which increased from 2002 (27%) to 2003 (41%). In the past incidence of lobsters with shell disease was associated with the sewage dumpsite in New York Bight. This study found lobsters in the most polluted areas had the lowest incidence of shell disease, and those with the highest incidence were found in the least polluted areas.

Introduction:

The American lobster has been one of New York's most important marine fisheries in terms of dockside value of the total annual harvest. Even with the recent declines in landings in Long Island Sound, American lobster still ranks number three in value for New York, at $4.4 million.

Over the last three decades, the New York State Department of Environmental Conservation has studied and described many aspects of its local lobster population and commercial lobster fishery. Much of this work has involved collecting data from commercial catch while at sea. Lobster landings and biocharacterization of these landings are necessary as part of lobster stock assessment through the Atlantic States Marine Fisheries Commission.

New York lobster landings decreased by 92% since the peak of 9.4 million pounds in 1996. This decrease was heavily influenced by a 94% decline in western and eastern Long Island Sound (Figure 1), where the majority of the landings occurred. The fishery in the east end (Block Island Sound) and in the Atlantic Ocean off the south shore of Long Island has shown somewhat different trends. The east end fishery has declined 88% since 1999 and the south shore has declined 81% since 1998. These are the two areas were lobstermen have caught lobsters with the highest incidence of shell disease.

Methods:

The commercial catch of lobster was measured by sea samplers who accompanied commercial fishers aboard their vessels. Sampling was designed to take place on a year- round basis, as long as cooperators were available and willing to take staff on their vessels. Sea sampling trips ranged from 6 to 12 trips annually from 1984-1991. This increased during 1992–1994, particularly in western LIS (33-57 trips annually). Sampling decreased to low levels from 1995-1998 (no sampling occurred during 1997), and increased again during 1999-2002 (21-37 trips annually); however, only six trips were made in 2003.

Long Island Sound and Vicinity Fishing Area Chart.

WLIS- western Long Island Sound

ELIS – eastern Long Island Sound

EE – east end

SS < 3 – south shore within 3 miles

SS > 3 – south shore further than 3 miles
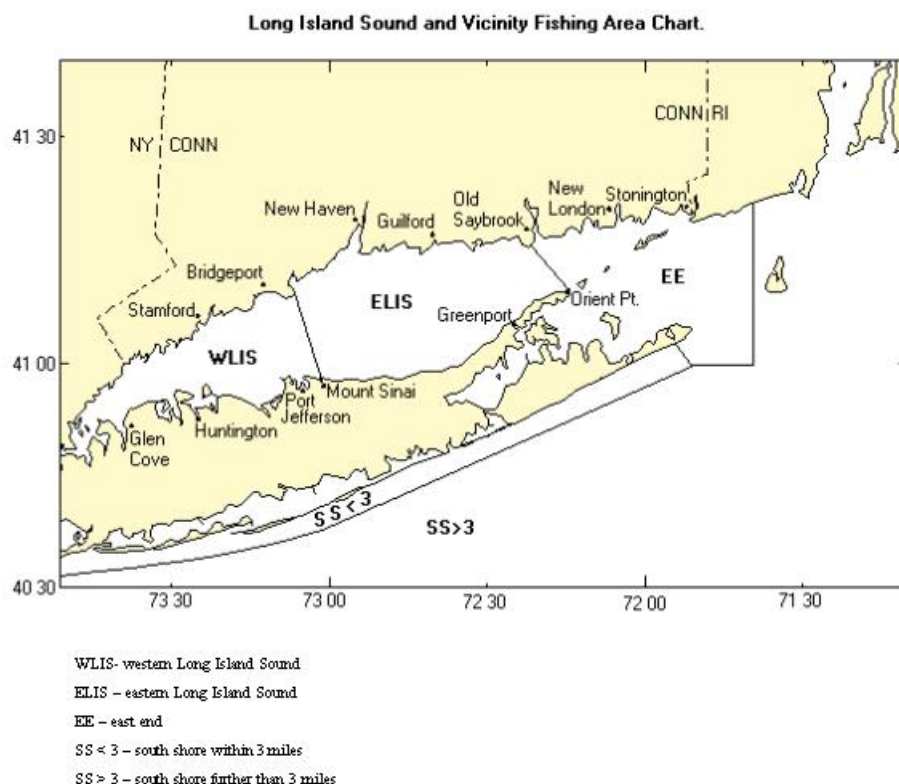
All lobsters were counted, and more detailed information was collected on either all or a subset of lobsters depending on the size of the catch. The detailed information included: size, sex, egg color and percent, shell condition (soft or hard), cull status, health and damage status, v-notch status, and whether the lobster was kept or released. Severity of shell disease, based on percent of shell affected, was been recorded since June 2000 (Table 1). In addition to the lobster information, staff also collected environmental data (weather, depth, and bottom water quality data (including temperature, salinity, and dissolved oxygen), set data (port, area fished, location of set, set days, ect.) and bycatch information.

Table 1. Shell Disease index used in NY monitoring program.

Shell Disease Index	
0	No shell disease symptoms
1	Symptoms on 1-10% of shell surface
2	Symptoms on 11-50% of shell surface
3	Symptoms on >50% of shell surface
4	New shell shows scars of a shell erosion

Results:

Tables 2 and 3 present the numbers and percent of lobsters sampled during 2000 – 2003 that showed incidence of shell disease (shell disease index 1-3 combined). Very few of the lobsters sampled in western Long Island Sound had shell disease, percentages were generally below 1%. Incidence of lobsters with shell disease was also low in eastern Long Island Sound, though the percentage did increase slightly in 2002 (no sampling in 2003). The percentage of lobsters with shell disease was highest in the east end for the two years sampled (11% and 14%); unfortunately the area wasn't sampled during 2002 and 2003. Lobsters sampled in the Atlantic Ocean off the south shore of Long Island have shown an increasing incidence of shell disease over time. Annual percentage ranged from 4% in 2000 to 21% in 2003. Shell disease occurrence was highest during the spring and increased from 27% in 2002 to 41% in 2003 (spring 2000 and 2001 were not sampled).

Table 2: Number of Lobster with Shell Disease

Year	Season	WLIS	ELIS	EE	SS	All Areas
2000	Summer	28	1	44	10	83
	Fall	11	5			16
	00 Total	**39**	**6**	**44**	**10**	**99**
2001	Winter	5	1			6
	Spring	1	3	281		285
	Summer	11	3	114	2	130
	Fall	6				6
	01 Total	**23**	**7**	**395**	**2**	**427**
2002	Winter	5	4			9
	Spring	8	35		31	74
	Summer	3	17		35	55
	Fall	5				5
	02 Total	**21**	**56**	**0**	**66**	**143**
2003	Winter					0
	Spring	19			102	121
	Summer				10	10
	Fall					0
	03 Total	**19**	**0**	**0**	**112**	**131**

Discussion:

The incidence of lobsters with shell disease seems to be increasing in lobsters caught in the Atlantic Ocean off the south shore of Long Island. The percent was highest in the spring, increasing from 27% during 2002 to 41% during 2003. The high occurrence in the spring is probably due to sampling before the summer molt. Trends in the east end cannot be determined due to lack of sampling during recent years.

Table 3: % of Lobsters with Shell Disease

YearSeason	WLIS	ELIS	EE	SS	All Areas
2000 Summer	0.00	0.00	0.11	0.04	0.01
Fall	0.00	0.01			0.00
00 Total	**0.00**	**0.00**	**0.11**	**0.04**	**0.01**
2001 Winter	0.00	0.00			0.00
Spring	0.00	0.00	0.23		0.04
Summer	0.00	0.01	0.07	0.13	0.01
Fall	0.00				0.00
01 Total	**0.00**	**0.00**	**0.14**	**0.13**	**0.02**
2002 Winter	0.00	0.01			0.00
Spring	0.00	0.02		0.27	0.02
Summer	0.00	0.03		0.09	0.02
Fall	0.03				0.03
02 Total	**0.00**	**0.02**		**0.14**	**0.01**
2003 Winter					
Spring	0.06			0.41	0.21
Summer	0.00			0.09	0.04
Fall	0.00			0.00	0.00
03 Total	**0.01**			**0.21**	**0.07**

In the past, incidence of shell disease in lobsters around New York has been associated with proximity to the sewage sludge dumpsite in New York Bight (Sindermann et al., 1989). The areas with the highest incidence of shell disease during this study were more open and unpolluted. Western Long Island Sound, which is the most heavily impacted area sampled, had very few lobsters infected with shell disease.

References:

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Discussion on Management Implications

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Q.1. *What information should be included in developing a lobster health database?*
 Many of the session presenters described data gathered through monitoring programs. These programs all gather the "basic" information including

- Location
- Prevalence
- Lobster condition
- Environmental conditions (e.g. temperature)

 Everyone agreed that this is critical baseline knowledge, and needs to be continued, and effort on these types of monitoring need to be increased.
 In addition to these data, the question of besides effort, can these monitoring programs be improved? Four man areas were discussed.

1. Discussion first centered on the "Prevalence" metric, which currently assessed how much of the shell surface is covered by lesions. The suggested was made to also assess the depth of ulceration, as this may be a better index of how shell disease is progressing within an individual lobster. It was pointed out that lobsters with deep lesions may not molt and may be more subject to mortality than lobsters with shallow lesions. It is also important to distinguish the different types of shell disease (e.g. epizootic compared to impoundment or burnt shell) in the monitoring.

2. The second factor discussed was a means to determine relative mortality. The work by Gibson and Wahle presented in session 3 brought to light the fact that mortality of shell diseased animals may be significant enough to uncouple the relationship between populations indices between pre-recruits and settlers. Thus any monitoring effort that could begin to assess mortality would be extremely beneficial.

3. Third, there was a significant amount of discussion about potentially new parameters, such as hemolymph sampling, that could be used to track lobster health. Sylvan DeGuise pointed out that there is no "magic bullet" and that more information needs to be gathered prior to determining which hemolymph parameter is best to measure. This discussion was initially discounted as being too time and money intensive. However, protein hemolymph is a parameter that can be measured relatively simply and inexpensively (required tools include syringe and a refractometer). Clearwater Lobsters, which holds lobsters in a flow through holding system prior to distribution, used hemolymph protein as an index of "storageability" for the lobsters arriving at their facility. This piece of evidence shifted the balance of thinking toward hemolymph monitoring, specifically that this parameter holds promise for being an indicator of health. There are many obstacles that have to be overcome prior to using hemolymph protein as an index of lobster health, including the two primary questions of a) does hemolymph protein vary between shell diseased and health animals, and b) is the variation

observed in protein hemolymph over the molting cycle (Mercaldo-Allen 1991) different in shell diseased vs. healthy lobsters, **c)** proof that hemolymph abnormalities are present in lobsters that develop epizootic shell disease (vs. impoundment shell disease) d) if hemolymph values can be tied to strains of lobsters?

4. Finally, discussion addressed the need to expand monitoring into the pre-recruit / settlement index survey work being conducted by the Lobster Conservancy and Whale's group. This would help to assess the natural mortality rates of shell disease lobsters.

Q.2. *How can one enhance lobster health management by improving interaction at all levels of the fishing industry?*

This question was well answered by John Duff of the University of Massachusetts at Boston. They key component to improving interaction at all levels of the fishing industry is to share information and coordinate efforts. The fishermen present at this workshop commented that they are pleased to see the scientists talking to one another, and will do all they can to assist in furthering the collaboration of scientists and fishermen. All present agreed that this meeting was a paramount first step to enhancing lobster health management.

Q.3. *What management decisions are required to aid in lobster disease prevention?*

This question was again addressed by John Duff, in that management decisions require:
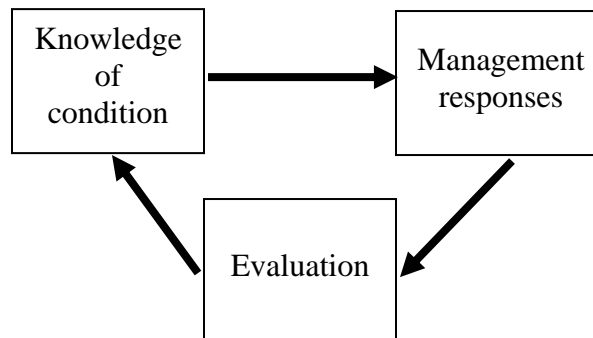
As discussed in Q1, questions still remain about the shell disease condition, and scientific understanding of shell disease is lacking. The primary concern raised during this discussion was the amount of shell disease induced mortality that populations experience. Furthermore, any knowledge of condition needs to account for the three different types of shell disease. These types are impoundment shell disease, burnt shell, and epizootic are three types of shell disease. According to Roxanna Smolowitz, these are different types of disease as opposed to being a difference in severity. There may be different causes for each type of shell disease.

As for management responses, bait usage (Q5) was discussed as one remedial response. There was also the discussion of determining some agent (e.g. an antibacterial) that could be proactively delivered. Possible modes of delivery included a incorporating the agent into feed, onto the trap so that the lobster carapace was "swiped" as the lobster

entered the trap, or a "dip" that lobstermen would administer prior to returning shorts to the sea. Oxytetracyline was discussed as one potential feed delivered anti-bacterial agent, however there would be difficulty in introducing this into the environmental in wild-fished animals (particularly in terms of HACCP protocols). There could be a closed area approach to management for the delivery of antibiotics to wild lobsters. This approach was deemed reactive as opposed to proactive, and that proactive (dips or swipes) may be a better approach. Yet, one difficulty with the proactive approach is that the reluctance by managers to fund proactive vs. reactive measures. The end consensus was that any proactive or reactive responses depend on the development of an understanding of the causative agent(s) and require controlled studies prior to implementation.

Evaluation issues were addressed in Q1.

Q.4. *What are the best predictors of outbreaks of shell disease?*

Temperature issues appear to be important in predicting the occurrence of epizootic shell disease given the potential relationship discussed by Bob Glenn between temperature in one year and the incidence of shell disease in the next year. Other potential environmental indicators were discussed, but temperature is the only factor for which a presumed relationship to incidence of shell disease has been established. The issue of the lack of shell disease in Western Long Island Sound, where temperatures are higher than that of Eastern Long Island Sound was raised as potential evidence that temperature was not an adequate predictor of incidence of shell disease, or that other factors such as unfavorable environment for the bacteria that cause shell disease may be occur in those areas. Questions were raised as to whether WLIS had undergone a natural selection event, if then the WLIS population was more resistant to either the effects of higher temperature or the causative agents of shell disease, compared to those lobsters in ELIS. This discussion lead to the suggestion that genetic markers for susceptibility to shell disease need to be established. Markers may prove to be a predictor of populations that may or may not be subject to outbreaks of shell disease.

There appears to be little correlation between pollution and anthropogenic inputs, and shell disease. Boston Harbor (more polluted) has less shell disease than Buzzard's Bay (less pollution). Western Long Island Sound (more polluted) has less shell disease than Eastern Long Island Sound (less pollution). However, the bacteria causing shell disease do reside in sediments and water columns, and thus certain environments may be more prone to causing shell disease than others.

Q.5. *Is bait a causative agent of lobster health problems, and if so, what mechanisms can be used to regulate bait usage in the lobster fishery?*

Bait is not considered a nutritionally complete diet for lobsters, and is essentially a "Wonder bread diet". While it was suggested that lobsters would be able to utilize it adequately in the short term, in the long term, it would result in significant health issues including increased susceptibility to shell disease. Additives to bait would be well received by the fishermen, but bait restrictions and limitations would not be. It was pointed out that Lavallee et al (1988) observed that lobsters caught on fresh mackerel were more likely to be graded as weak at the processing plant. Those authors suggested that mackerel, which are high in histidine, allowed for bacterial proliferation. If a relationship is observed between bait and lobster health, a solution may be as simple as a change in bait handling methods, as opposed to the adoption of different bait types.

Summary:

Are lobsters the canary in the coal mine? Could it be that shell disease actually indicates environmental problems at larger spatial scales? This session pointed out unlikely a "magic bullet" solution for shell disease is. Before this disease can be adequately managed, we will need to better understand the causative agents, effect**s** on lobsters, and ultimately agents that can be used to decrease its prevalence in lobsters. Once these tasks have been accomplished, the best proactive or reactive course to be taken can be determined**.** As with any management issue, it has to be remembered that lobster fisheries are linked to that the other fisheries. As management solutions increase the operating expense of lobster fishermen, those fishermen will seek work in other fisheries. Thus any solutions have to be supported by the fishermen, as well as being sound biologically and financially.

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Lavallee J, Hammell L, Spangler L, Cawthorn R, Baker AW. 1998. Survivability of Prince Edward Island Lobster: impact assessment of post-harvest health status, transport, and holding conditions. PEI AFRI Project file no 004LH01.
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Chapter 6 Priority Setting

Priority Setting For Continued Shell Disease Research

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The expertise of participants in this workshop encompassed many active and diverse lines of research including: directed study of epizootic shell disease, supportive research that that is necessary for understanding of shell disease, and important information gathering / monitoring efforts tracking diseases in lobsters fished along the northeastern U.S. coastline. What became obvious during this meeting was the lack of sufficient information about epizootic shell disease of American lobsters resulting in a lack of conclusions as to why it is occurring.

Discussions during this workshop did highlight that the disease results from the interaction of several factors and, importantly, in order to understand this disease, we need to conduct investigations of these factors. In many cases, we will need to develop a better understanding of normal processes in order to understand the disease on its pathogenesis. To further understand shell disease, we urgently need to develop a laboratory model of lobster carapace formation in which to study the onset and progression of the erosive process. The model could be used by many laboratories to standardize research procedures. It would allow for controlled laboratory experiments that would result in elucidation of the various factors influencing the onset of shell disease, as well as examining potential treatments and remedies.

Necessary Investigations:
Areas of investigation can be divided into 4 main categories.

I. Bacteria
   The *Flavobacteriaceae* clade of bacteria appears to be very important in the occurrence of the disease. However, to better understand how this clade of bacteria influences shell disease in American lobsters, the lobster science community needs to:
   1) Determine if all or only a few *Flavobacteriaceae* are important in the disease.
   2) Fulfill Koch's postulates:
      A. The microorganism must be detectable in the infected host at every stage of the disease.
      B. The microorganism must be isolated from the diseased host and grown in pure culture.
      C. When susceptible, healthy animals are infected with pathogens from the pure culture, the specific symptoms of the disease must occur.
      D. The microorganism must be re-isolated from the diseased animal and correspond to the original microorganism in pure culture.
         Ultimately, the routes of bacterial transfer need to be understood, and the relative contributions of transfer between animals compared to environmental transfer (through the sediment or water column) need to be understood.

3) Define to what extent the *Flavobacteriaceae* are present in the bio-film (the thin biologically active layer at the water-lobster interface) on the lobster and how this related to increased amounts of shell diseased.
4) Determine if shell disease is due to a change in occurrence or quality of the *Flavobacteriacae* on the animal's carapace.
5) Understand how environmental changes may affect the population of *Flavobacteriacea* in the environment.
6) Evaluate the relationships between the community of microorganisms, and how the predator-prey interactions on the lobster's carapace (i.e. amoebic grazing on bacteria on the carapace surface) influence the onset of shell disease.

II. Carapace Formation

Microscopically the carapace of a lobster affected with shell disease appears normal in areas distant from the diseased carapace. Additionally, histologically, the inflammatory responses of these lobsters to erosions appear appropriate and good indicating that many parts of the innate immune system appear to be functioning well. However, molecular changes may occur within the carapace as it is formed reducing its overall effectiveness in resisting the erosive bacteria. Additionally changes in the innate immune system may make the animal less able to react at a molecular level when erosions occur in the carapace. Specifically, the lobster scientific community needs to:
1) Understand if shell disease is promoted by a decrease in the quality of epicuticle production or maintenance (as epicuticle is produced first and is the first line of defense of the cuticle).
2) Evaluate the effect of nutrition on cuticle formation. Specifically we need to know what the role nutrients such as astaxanthin, proteins and calcium, have in shell formation and strength, and if shell formation can be compromised by fishing practices (through a change in nutrition via bait composition) or pollutants such as pesticides or alkyphenols (which act as endocrine disruptors, and may affect the melanizing abilities of the outer carapace or ability of the hemocytes to respond to erosions of the inner carapace at the molecular level).
3) Evaluate the level of calcification in affected and unaffected regions of the carapace of individual lobsters within and between populations.
4) Understand how signals from the diseased areas of the carapace are received in the underlying tissues and how these signals stimulate the inflammatory responses originating at the cuticle base.
5) Examine the population structure of lobsters to determine if some potential strains are more susceptible to shell disease than others.

III. Increased Temperatures

Increased temperatures have been correlated with the increase in shell disease in eastern Long Island Sound and the Rhode Island and the Massachusetts coast line. This area is the most southern extent of the American lobsters range. The American lobster scientific community needs to:

1) Know if increased temperatures are coincidentally associated with epizootic shell disease or if there is a causative relationship.
2) Determine the reason for an apparent 1 year lag between higher temperatures and higher levels of shell disease along the coast of MA.
3) Determine if the number of days of temperature over some threshold is important in shell disease occurrence.
4) Investigate temperature effects on new cuticle formation and the molt cycle.
5) Determine how increased temperatures affect lobster behavior, molting season, egg production and larval movement and settlement.

IV. Innate Immune Systems

Lobsters possess an innate immune system. Unlike vertebrates, this system does not have "memory." In order to understand how a deficient innate immune system might affect the occurrence of shell disease, the lobster scientific community needs to:
1) Determine if shell disease is promoted by a defective innate immune system and if a poorly functioning innate immune system affects the molecular composition of the shell as it is formed.
2) Evaluate protein levels (total protein, hemocyanin or molting cycle proteins) in the hemolymph and determine if altered levels can affect the susceptibility of individual lobsters to epizootic shell disease.
3) Investigate the role of the hemocytes (the innate immune system cells) using function tests or hemocyte quantification and qualification tests.

Actions:

Experiments in each of the above categories need to be conducted. The approach used to investigate these subjects will include both field and laboratory work. Progress would be greatly accelerated by the availability of a laboratory-based model of shell formation as well as a laboratory model of shell disease. A shell formation model would be used to establish normal values of lobster functioning under controlled conditions as well as to examine how varying various factors influence shell formation and the development of shell erosions. Such a model of shell disease could then be used to examine how environmental perturbations (including temperature changes, exposure to pollutants, and different bacterial communities) and changes in health or stress status of lobsters affect the progression of shell disease. Without a model system for understanding shell disease, progress advancing our scientific understanding of shell disease, and ultimately determining an appropriate managerial response, will be greatly limited.

In addition to laboratory investigations of shell disease, fishery observer based monitoring of the progression of the disease is essential, as these data are critical to fully understanding the status and spread of the disease. Along with the detailed spatial and temporal data on the incidence of the disease, environmental factors (salinity, oxygen, temperature) need to be simultaneously collected to be able to determine correlates and potentially contributing factors. Finally, in a larger context, it is critical to understand how shell disease impacts the natural mortality of lobsters. Past stock assessments assumed constant natural mortality rates. With increasing incidence of shell disease in the southern extent of the lobsters' range, the ASMFC Lobster Technical Committee is integrating time varying natural mortality into population models in the current on-going

lobster stock assessment. Modeling the relationship between shell disease and natural mortality has proved difficult in the absence of data on the rates of shell disease related mortality. These data are critical to assessing and managing the American lobster resource.

To initiate the necessary research described here, two objectives need to be implemented – first, the scientists need to organize into a consortium, coalition, or working group, and second, funding needs to be identified for work to be accomplished. Some delay in response by the scientific community is due to a lack of communication between the scientists. As a group, scientists were not aware of the breadth of work being conducted on shell disease. Individuals at this workshop represent a good cross-section of the scientific community, coming from numerous private and public institutions. The priorities set forth in this document represent a step toward the organization of an action group. It would be best to have this group formally organized to assure that goals and priorities are met and progress is made. The Lobster Institute formed the Lobster Health Coalition, with a successful first meeting. However, there has been little follow-up, as there was no money to assist the coalition with their goals.

Funding of research is a major factor in assuring that the progress made in this workshop carries forward. To initiate the recommended additional research described here, significant funding is needed, over and above any funding to continue the described research and monitoring of the disease. It is clear that the economic importance of lobsters in the northeast creates a priority for funding this initiative. Understanding lobster shell disease will not only prevent decrease in value of the resource, but may also assist in increasing its economic value. Support of lobster fishermen and the commercial lobstermen's associations is essential in any funding request, and there support was evident in the participation of lobster fishing associations and fishermen at this meeting. The private foundations, particularly Darden and Sudbury were very helpful in beginning this initiative. However, it is apparent from the amount of work that needs to be done that significant government funding will have to be recruited. It is abundantly clear that without significant funding, appropriate action to understand shell disease will not be initiated until a significant problem occurs in the fishery when it may be too late.

While shell disease is not the only disease impacting lobster health, it is a key disease to understand. It appears the prevalence and severity of the disease may be influenced by the pathogen (type, density, pathogenicity), internal lobster factors (shell quality, nutritional status), and the environment (ocean temperatures, current patterns, microbial communities). Only by concomitantly assessing these three areas will researchers fully understand how this and other diseases will affect lobster populations, and the management methods necessary to control the spread of this and other lobster diseases.

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