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SHELL DISEASE IN THE AMERICAN LOBSTER, *HOMARUS AMERICANUS:* A SYNTHESIS OF RESEARCH FROM THE NEW ENGLAND LOBSTER RESEARCH INITIATIVE: LOBSTER SHELL DISEASE

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ABSTRACT The goal of this synthesis is to highlight some of the major findings of the New England Lobster Research Initiative (NELRI), provide a context for these findings based on previous research, discuss the potential impacts of this important emerging disease on the dwindling lobster populations in southern New England (SNE), and provide suggestions on avenues for future research. Most of the research funded in this initiative focused on epizootic shell disease (ESD), the emerging syndrome severely impacting lobster populations primarily in coastal waters in Rhode Island, southern Massachusetts, and eastern Long Island Sound (ELIS), but some new information about other forms of shell disease in lobsters is included. We also discuss how these novel findings on lobster shell disease should be used to inform management of lobster populations.

KEY WORDS: American lobster, *Homarus americanus*, epizootic shell disease, enzootic shell disease.

FACTORS DETERMINING THE EMERGENCE AND EXPANSION OF EPIZOOTIC SHELL DISEASE

Several forms of shell disease that can be distinguished by their characteristic clinical signs and prevalence have been described in American lobsters, *Homarus americanus* Milne-Edwards, 1837, including impoundment shell disease (ISD), burn-spot or rust-spot shell disease, Epizootic Shell Disease (ESD), and the enzootic form of ESD (Smolowitz et al. 2005). The goal of this synthesis is to highlight some of the major findings of the New England Lobster Research Initiative (NELRI), focused on ESD.

Although lobsters with similar lesions to ESD have been observed in wild American lobsters (*Homarus americanus*, Milne Edwards) from a variety of locations, including Maine (Chistoserdov et al. 2005), Canada (Comeau & Benhalima 2009), and even Norway (Van der Meeren 2008), epizootics have been centered in SNE, including inshore waters of Rhode Island, Buzzards Bay in Massachusetts, and ELIS. Prevalence in other areas is less than 5% (with the rare exception of Kittery, ME, where an outbreak was reported in 2003 to 2004). The temporal and regional patterns of prevalence of the disease in SNE are consistent with the epizootic starting somewhere in the inshore areas of Rhode Island and Connecticut during the late 1990s (Castro & Angell 2000). If the factors that determined the emergence and influence the current distribution of this disease were elucidated, this could allow researchers to predict the chances of ESD extending to other areas in the lobster's range.

Interestingly, ESD was not the only emerging disease affecting lobsters in SNE during the 1990s. From 1999 to 2001, the lobster population in central and western Long Island Sound (WLIS) experienced a significant mortality event thought to be triggered by stressful environmental conditions (sustained above-average water temperatures, hypoxia, release of sulfide and ammonium from sediments, pesticides), leading to infection with the parasitic amoebae *Neoparamoeba pemaquidensis* (reviewed by Pearce & Balcom (2005)). Similar to what has been observed for ESD in Rhode Island, this mortality event in WLIS immediately followed a period of record landings and lobster densities (Castro et al. 2006), suggesting that lobster populations at the time may have exceeded the carrying capacity of the ecosystem, forcing lobsters to live in less than optimal habitats. This situation, probably combined with other stressful environmental conditions, could have led to increased susceptibility to pathogens already present in the ecosystem, facilitating the initiation of epizootics. Furthermore, the relatively higher prevalence of other idiopathic conditions in SNE lobsters, including calcinosis (Dove et al. 2004), necrotizing hepatopancreatitis and nonspecific granulomas (Shields et al. 2012a), and idiopathic blindness (Maniscalco & Shields 2006, Shields et al. 2012a), suggests that these conditions are indicative of a stressful environment (Shields et al. 2012a). The research summarized here attempts to describe the factors that may contribute to the etiology of ESD.

THE ROLE OF MICROBES IN LOBSTER SHELL DISEASE

Although there is agreement among researchers that the characteristic lesions in shell disease are caused by bacteria that invade the carapace of the lobster from the surface of the shell (Fisher 1977, Malloy 1978, Chistoserdov et al. 2005, Getchell 1989, Smolowitz et al. 1992, Smolowitz et al. 2005, Quinn et al. 2009), the identification of a single bacterial species as a causative agent of the different forms of shell disease remains elusive. It is also unclear whether the different forms of shell disease that have been described in lobsters (reviewed in Tlusty et al. (2007)) are caused by the same pathogen. This is a result of the inherent difficulties in studying what is basically the equivalent of a cutaneous disease (with open lesions prone to contamination by secondary invaders) in the complex soup of microbes that constitute the marine environment. In addition, there are the well-known limitations of the stringent criteria included in the original set of the Koch's postulates, which, for example, cannot be fulfilled for nonculturable pathogens or for infectious diseases that have a strong unknown host and/or environmental component (Fredericks & Relman 1996).

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Most of the data indicate that shell disease is caused by an opportunistic pathogen or pathogens that take advantage of a compromised shell.

Three different culture-independent techniques—denaturing gradient gel electrophoresis (DGGE (Chistoserdov et al. 2012)), terminal restriction fragment length polymorphism (tRFLP (Bell et al. 2012)), and multitag pyrosequencing (Meres et al. 2012)—described the presence of a diverse microbial community in the shells of lobsters that changes in abundance and composition in shell disease lesions. Chistoserdov et al. (2005, 2012) have identified 2 bacterial species belonging to the Bacteroidetes— *Aquimarina 'homaria' and 'Thalassobius'* sp.—that are consistently associated with shell disease lesions of lobsters collected from several geographical locations, including Rhode Island; ELIS; Buzzards Bay, MA; and Kittery, ME. Moreover, *A. 'homaria'* and *'Thalassobius'* sp. were also present in the lesions of lobsters with impoundment shell disease (ISD) from the Maine Aquarium, and *A. 'homaria'* was also detected on lesions from lobsters with enzootic shell disease (EnSD) from Rhode Island (Chistoserdov et al. 2012). These 2 bacteria are also present in shells of some lobsters with no signs of disease (about 40%), but in less abundance (Chistoserdov et al. 2012). Hatchery-reared lobsters with shells that had been compromised by abrasion of the cuticle and exposed to filters impregnated with either *A. 'homaria,' 'Thalassobius'* sp., or both, developed lesions similar to those in wild lobsters with shell disease. These experiments strongly suggest the involvement of *A. 'homaria'* and *'Thalassobius'* sp. on lesion development in lobsters with compromised shells (Quinn et al. 2012). Although lesions developed faster and were more severe in lobsters exposed to the challenge bacterial candidates, lesions colonized by *A. 'homaria'* were also seen in lobsters with abraded shells exposed to filters impregnated with sterile seawater, as well as a few lobsters with shells that had not been abraded nor exposed to filters, showing that these bacteria can colonize lesions easily even when not applied directly to the shell, and may be able to cause lesions in the absence of shell abrasion (Quinn et al. 2012). These experiments provide support to the hypothesis that *A. 'homaria'* is a causative agent of ESD in lobsters.

Other research describes the presence of a diverse community of bacteria in lesions from lobsters with shell disease (Bell et al. 2012, Meres et al. 2012), with *Aquimarina* being only one of several bacterial genera (including *Jannaschia, Hirschia,* and *Oceanicola* to name a few) that contribute moderately to discriminate between shells with and without lesions (Meres et al. 2012). Based on their findings, Meres et al. (2012) hypothesize that shell disease in lobsters is caused by a dysbiotic shift in the microbial community of the shell, allowing opportunistic bacteria that normally reside on the shell to invade and cause lesions.

Lobsters with shell disease also show the presence of a diverse eukaryotic community of epibionts in lesions, including the bacterivorous nematode *Geomonhystera disjuncta,* barnacles, stramenopiles, and bryozoans. The diversity and lack of consistency in the presence of these eukaryotic organisms in shell disease lesions suggest that they are most probably secondary invaders of lesions (Quinn et al. 2009), although they may contribute significantly to lesion progression and degeneration of the epi- and exocuticle (Smolowitz et al. 2005).

How do these microbes contribute to lesion formation? Shell disease in other crustaceans has been associated with chitinolytic bacteria (reviewed in Tlusty et al. (2007)). However, lesions in ESD in lobsters, as observed in histological sections of affected shell, are characterized by the presence of leftover pillars composed of chitin, suggesting that bacteria are targeting components other than chitin, such as the protein matrix (Smolowitz et al. 2005). Consistent with histological observations, there was no significant difference on the potentials for chitinase activity between samples from lobsters with and without shell disease lesions, whereas there were significant differences in proteinase and cellulase activities, suggesting that bacteria in shell disease lesions may target proteins and polysaccharides preferentially (Bell et al. 2012).

THE ROLE OF A COMPROMISED HOST

There is consensus among researchers that shell disease is a manifestation of a "metabolic disturbance" leading to increased susceptibility to environmental bacteria (Sindermann 1991, Tlusty et al. 2007). Pathogens causing shell disease may take advantage of transient breaches in the cuticle as a result of physical or chemical damage to initiate lesions before the shell is repaired, or may exploit constitutive deficiencies in the structure of the shell and immune responses resulting from genetic, developmental, hormonal, or environmental factors.

The shell of crustaceans is normally an extremely efficient protective barrier to microbial infection. In addition to providing a strong physical barrier to infection through the combination of an outermost wax layer covering the epicuticle followed by a calcified endocuticle composed of chitin and an organic matrix (Aiken & Waddy 1980, Neville 1975), lobster shells demonstrate a series of dynamic immune responses to bacterial infection. Kunkel et al. (2012) describe the differences in the chemical signature of the inorganic component of the lobster shell, with a dense cuticle layer of calcite on the plane of the shell and relatively harder apatite covering the more vulnerable dermal and neural gland canals. They also provide an interesting model for the role of ion fluxes released from the calcite and amorphous calcium carbonate layers in the exo- and endocuticle as a potential chemical barrier to infection through the creation of an unstirred layer of high alkalinity in the surface of the shell. This ionic flux increases upon shell injury (Kunkel et al. 2012).

Other constitutive components of the innate immune system in the shell of lobsters are antimicrobial peptides (Mars 2010). Inducible responses of lobsters to shell damage and bacterial infection include melanization of the edges of shell lesions, with involvement of the prophenoloxidase system, and production of an inflammatory membrane underlying the affected carapace. These responses have been observed in both wild lobsters with ESD (Smolowitz et al. 2005) as well as in hatchery-reared lobsters challenged experimentally with *A. 'homaria'* and *'Thalassobius'* sp. (Quinn et al. 2012). Shell disease also induces immune responses in lobster hemolymph, including production of molecules with antimicrobial activity, phagocytosis, and respiratory burst by hemocytes, and further involvement of the prophenoloxidase system (Homerding et al. 2012).

ESD has been shown to induce changes in the molting behavior of lobsters, an effective strategy to get rid of shells affected by the disease before the cuticle is severely compromised (i.e., Smolowitz et al. 2005, Castro et al. 2006). The new cuticle is formed internal to the affected cuticle (Smolowitz et al. 2005). Berried females with severe lesions and eggs still attached have been observed to molt, even if it resulted in loss of the eggs (Laufer et al. 2005, Castro et al. 2006). This behavior may be mediated by ecdysteroids (Laufer et al. 2005). Intermolt female lobsters, however, do not appear to avoid males with shell disease (Rycroft et al. 2012), avoidance being a behavioral strategy described in the more social Caribbean spiny lobsters (*Panulirus argus,* Latreille) that prevents transmission of *P. argus* virus 1 in the laboratory (Behringer et al. 2006).

In this issue of the *Journal of Shellfish Research*, some interesting evidence is presented suggesting that differences in prevalence of the disease between geographical locations may be the result of genetic or developmental factors. Homerding et al. (2012) describe differences in the immune responses between lobsters from ELIS and lobsters from WLIS and Maine. Based on these results and previous research showing that lobsters from WLIS can be differentiated genetically from lobsters from other locations using microsatellite markers (Crivello et al. 2005), Homerding et al. (2012) hypothesize that differences in immune parameters between lobsters in ELIS and WLIS may be a consequence of strong selective pressure on WLIS lobsters resulting from environmental and fishing pressures, coupled with the massive lobster die-off in 1999 (Crivello et al. 2005). Interestingly, differences in population structure has been suggested within Narragansett Bay, RI, and between Narragansett Bay and offshore populations in Rhode Island (Atema, pers. comm.).

Other work has shown the importance of physiological and molt status on the susceptibility to shell disease. Patterns of gene expression in lobsters with shell disease indicate a potential systemic disruption of endocrine signaling and energetic metabolism (Tarrant et al. 2012). Low total protein in the hemolymph, an indication of poor physiological condition, as well as being in the intermolt stage have been identified as risk factors for development of ISD (Theriault et al. 2008). There is also evidence showing that sex may be a risk factor for shell disease-induced mortality, based on the higher prevalence of shell disease in ovigerous female lobsters in the wild and the decrease in the female-to-male ratio observed starting around 1998 in Rhode Island lobster populations, coincidental with the sharp increase in the prevalence of ESD (Castro et al. 2012). Interestingly, and despite the fact that females were shown to have a higher cumulative incidence of ISD than males in an experiment performed with lobsters in Nova Scotia, Canada, gender was not a significant predictor for development of this form of shell disease (Theriault et al. 2008).

THE ROLE OF ENVIRONMENTAL FACTORS

It has also been hypothesized that lobsters from SNE may be more susceptible to shell disease as a result of exposure to anthropogenic pollutants that can impact the health of crustaceans negatively. The pesticide methoprene leads to immune suppression in adult lobsters (DeGuise et al. 2004, DeGuise et al. 2005), causes high mortality in postlarval lobsters, inhibits protein synthesis and gene expression in the hepatopancreas of adult lobsters (Walker et al. 2005a, Walker et al. 2005b), bioaccumulates in the eyestalk, and leads to morphological changes in cells in the hepatopancreas that may affect the synthesis and incorporation of chitoproteins into adult postmolt shells (Walker et al. 2010). Alkylphenols, a class of pollutants derived from the manufacturing of many commercial products such as plastics and paints, have also drawn increasing attention in recent years because of their widespread use and the large amounts released to the marine environment. Representatives of the alkylphenols are toxic to lobster larvae and postlarvae, causing significant mortality and delaying each molt by 2–3.5 days (Laufer et al. 2012a). Alkylphenols have been shown to have juvenile hormone activity, the hormone that regulates metamorphosis and molting in crustaceans (Biggers & Laufer 2004). These pollutants can be incorporated into lobster cuticle, inhibiting the cross-linking of tyrosine in the new cuticle, probably preventing protein and chitin cross-linking during hardening, and leading to a weaker cuticle (Laufer et al. 2012b).

Although alkylphenol contamination is persistent and widespread in New England (Biggers & Laufer 2004, Jacobs et al. 2012), a clear relationship between the distribution, prevalence, and intensity of contamination of selected alkylphenols in the hemolymph of lobsters from different geographical locations and the presence of ESD has not been observed (Jacobs et al. 2012). Jacobs et al. (2012) point out that levels of alkylphenols in hemolymph only indicate recent exposure, and that it may be more relevant to determine the levels of these pollutants in shell or to measure exposure during development, which could have an impact on shell structure. Evidence linking the presence of shell disease with the magnitude or patterns of metal accumulation in the hemolymph of lobsters has not been found, but patterns were present between animals from Maine and Rhode Island (LeBlanc & Prince 2012).

The development of an experimental challenge model using bacteria isolated from lesions has proved particularly useful in investigating the role of physical damage (i.e., abrasion, described earlier), nutrition, and temperature on the development of shell disease in an aquarium system (Quinn et al. 2012, Tlusty & Metzler 2012). The emergence of ESD in SNE during the 1990s has been attributed to several years of warmer than average water temperatures (Glenn & Pugh 2006), leading investigators to speculate that the distribution of ESD may be limited by temperature. Evidence supporting that temperatures more than 20°C may impact the immunocompetency of lobsters includes the fact that wild lobsters collected in the summer showed higher bacterial loads in the hemolymph, a sign of systemic infection (Homerding et al. 2012). This is consistent with studies showing decreased immunocompetency in lobsters maintained at 23°C for 1 wk (Dove et al. 2005), decreased phagocytic activity at 20°C (Paterson & Stewart 1974), and increased mortality in lobsters maintained at 20°C for up to 1,021 days (Tlusty & Metzler 2012). Interestingly, shell disease (as determined by the extent and severity of the lesions, as well as how fast they developed) was generally greater in lobsters maintained at 15°C for up to 1,021 days than at 10°C and 20°C, even after correcting for duration of molt cycle (which is shorter at warmer temperatures). In addition to higher bacterial loads in lesions, lobsters maintained at 15°C also show a thinner outside calcite layer (Tlusty & Metzler 2012). Tlusty and Metzler (2012) concluded

that the effect of temperature on the dynamics of shell disease is mediated by a balance of complex effects on the host's physiology and the pathogen's growth, and that progression of the disease at warmer temperatures may be controlled by an array of factors, such as shorter molting cycles and lower bacterial proliferation in lesions. This research also confirms that ESD can occur at a wide range of temperatures (from 10–20°C), and that other environmental factors may contribute to determining the current range of the disease.

Poor nutrition could be an important factor leading to a compromised shell (Prince et al. 1995, Tlusty et al. 2008, Myers & Tlusty 2009). In nature, baitfish comprise a significant component of the diet of lobsters from heavily fished areas, as opposed to a varied diet composed of crustaceans and molluscs (see references in Bethoney et al. (2011)). Hatchery-reared lobsters fed a diet composed exclusively of fish for a year experienced significantly higher rates of shell disease (laboratory strain) and mortality than lobsters fed mixed diets (Tlusty et al. 2008). No relationship was found, however, between nitrogen isotope ratios ($\delta^{15}N$ values, used to determine differences in the diet of animals) and severity of ESD in lobsters from a highly fished area, suggesting that a diet comprised mostly of fish may not be a risk factor for development of shell disease in wild lobsters (Bethoney et al. 2011). Failure to find a correlation between diet and shell disease in wild lobsters indicates that many lobsters eat a variety of items in their diets (Bethoney et al. 2011), suggesting a lack of sensitivity of the nitrogen isotope ratios in detecting relevant differences in the quality of the diet in these lobsters.

IMPACTS OF SHELL DISEASE AT THE INDIVIDUAL LEVEL

In addition to the unsightly nature of the lesions in lobsters with ESD, which affects the commercial value of the lobsters, this disease leads to changes in the molting behavior of lobsters (Castro et al. 2006)—a behavior that may be mediated by temporal changes in ecdysone levels (Laufer et al. 2005). Although this behavior may help lobsters molt shells that have been compromised by the disease, it may also have a detrimental effect on reproduction and growth (Castro et al. 2006, Stevens 2009). ESD leads to significant changes in gene expression in the tissues of female lobsters, similar to changes observed in premolt healthy lobsters, suggesting that shell disease induces a hormonal state similar to premolt (Tarrant et al. 2010, Tarrant et al. 2012). Animals with ESD had significant changes in the expression in muscle and hepatopancreas of ecdysteroid receptor and CYP45, indicative of disruptions in endocrine signaling in affected lobsters. Lobsters with ESD also show decreased expression in thoracic muscle of arginine kinase, a phosphotransferase involved in energy metabolism that could be indicative of an energetic drain in lobster muscle in diseased lobsters (Tarrant et al. 2010, Tarrant et al. 2012).

Shell disease can lead to mortality in lobsters kept in captivity, but there does not appear to be a direct relationship between the severity of shell disease lesions and mortality (Quinn et al. 2012, Tlusty et al. 2008), suggesting that not all these deaths can be attributed directly to shell disease. In lobsters collected from the wild, systemic immune parameters like hemocyte phagocytic activity and oxidative burst are affected by ESD (Homerding et al. 2012) and may contribute to increased susceptibility to secondary infections acquired through other mechanisms. The presence of *Vibrio*–like bacteria in the hemolymph of lobsters collected from a site in Rhode Island with high prevalence of ESD was generally associated with injuries other than shell disease lesions (Shields et al. 2012a). Lobsters from this site also showed a high prevalence of 2 pathological conditions derived from inflammatory responses to pathogens in the tissues of lobsters—necrotizing hepatopancreatitis and granulomas—which were not associated with presence of shell disease (Shields et al. 2012a). Although similar pathological conditions have been described previously in lobsters with shell disease collected in Canada (Comeau & Benhalima 2009), Comeau & Benhalima (2009) concluded that shell disease was not the direct cause of the pathological conditions based on the lack of evidence linking damage of the cuticle in shell-diseased lobsters with these pathological conditions. More research needs to be done to determine whether pathological changes described in lobsters from populations affected with ESD are a consequence of the disease (leading to secondary infections) or a reflection of a generally compromised host (leading to shell disease and other conditions).

IMPACTS OF SHELL DISEASE AT THE POPULATION LEVEL

For many years, lobsters were thought to be nearly disease free. In stressful conditions such as shallow, crowded impoundments, 2 diseases—gaffkemia and ISD—sometimes reached epidemic proportions, but in the wild, diseased individuals were rare. During the late 1970s, a dramatic change in distribution and landings began, creating an enormous expansion of the population and the fishery. Throughout the Gulf of Maine, consecutive years of record landings occurred. Last year, 2011, appears to be another record year. The SNE region was on a similar trajectory until the late 1990s, when a population crash decimated the fishery and landings fell to near baseline levels of the 1980s (Howell 2012). The SNE stock has been declared to be in larval recruitment failure by the Atlantic States Marine Fisheries Commission (2010), and draconian management measures have been discussed, but not implemented. The cause of the crash is unknown, but candidates include stress induced by higher temperatures, overfishing, and a disease unknown until 1996. In this section, we explore some of the population and management consequences of ESD on the SNE population.

The emergence of ESD coincided with record landings in the SNE fishery during the mid 1990s, and a sharp decline in landings and population indices coincident with a high prevalence of ESD from 1997 until this writing. In Narragansett Bay, the most severely affected sector of the population were ovigerous females (both legal and sublegal, with a prevalence between 50% and 80% since 1998), followed by sublegal males and nonovigerous sublegal females (10–30%), and legal males (<10%) (Castro & Somers 2012, Howell 2012), indicating that the disease does not affect all sectors of the population evenly. Annual loss of individuals from the population in LIS may be in the range of 0.28, nearly double the estimate of deaths used customarily by managers for stock assessment models (Howell 2012). As prevalence rates of ESD are 10–40% in Rhode Island waters, it is fair to deduce that shell disease contributes substantially to increased mortality in the wild.

What are the population-level consequences of ESD? Increased mortality appears to have led to population decline. But, because death rates are not evenly distributed across all components of the population, consequences of ESD are not limited to a smaller population size. Differential mortality between the sexes, based on the higher prevalence of the disease observed in ovigerous females, should change the sex ratio toward favoring males. Indeed, there was a sudden shift in the female-to-male ratio around 1997 (Castro et al. 2012). Laufer et al. (2005) reported that ESD individuals have higher ecdysone titers than healthy lobsters, possibly causing early molting and a shorter interval between molts. This may be the mechanism by which growth rate slows when a lobster has ESD. Slowing the growth rate of individuals results in lower productivity of the population. Ovigerous females with ESD have been observed to molt before hatching their eggs, losing the entire clutch (Castro et al. 2006, Laufer et al. 2005). The high mortality of mature females, coupled with the loss of an entire clutch of eggs, argues for a lower egg production rate than would be found in a population without shell disease. If there is a significant correlation between spawning stock size and larvae (young of the year (YoY)), then a decrease in egg production would result in a decrease in pelagic postlarvae at the surface or settled YoY on the bottom. In fact, Wahle et al. (2009) have documented a major decline of YoY in inshore waters of SNE during the past decade. This time series of YoY abundance indices played a role in the decision of the Atlantic States Marine Fisheries Commission (American Lobster Technical Committee 2010) to declare the SNE lobster population recruitment overfished.

Understanding the complex pattern of mortality in the SNE lobster population is further complicated by the open nature of the population. Lobster life history characteristics include a pelagic larval phase lasting 15–30 days. Shelf and coastal currents along with directional swimming by the pelagic postlarva suggest that eggs hatched in one location may subsidize downstream populations. However important this may be, ecological and fishery models usually assume a closed population. Recruitment to a downstream population may allow the recipient population to continue in the face of heavy fishing (or disease) mortality, as Fogarty (1998) suggested for the inshore SNE stock. Accounting for the metapopulation structure in describing the dynamics of the SNE population requires more information than is available at this time; but, the possibility of metapopulation structure should be acknowledged, as done by Wahle et al. (2009) in their effort to distinguish between the effects of larval supply and mortality resulting from ESD on the dynamics of lobster populations in SNE. A nearly 20-y time series of annual larval settlement indices that spanned the emergence of ESD allowed Wahle et al. (2009) to explore the impacts of larval supply, disease prevalence, predation, and temperature on the ability of their model to predict numbers in the cohort 3 y after settlement. Results indicated that the supply of larvae to the population account for a remarkably large proportion of the variance in numbers of lobsters about to enter the fishery (prerecruits) 3 y after settlement. Neither predator biomass nor water temperature had any effect on the model's prediction. Larval supply appeared to drive the system until 1997, when shell disease became epizootic. After 1997, the settlement index alone failed to explain the numbers of prerecruits. Adding the terms for predation and temperature improved the fit of the regression only slightly. However, including a term for ESD prevalence improved explanatory power to more than 60%, and including the interaction term brought the explanatory power to 80%. Several potential lessons emerge from this modeling exercise, and are noted briefly here.

Data Sources

Wahle et al. (2009) were fortunate to have access to a long time series of settlement indexes, a fishery independent index of prerecruit abundance and predator abundance. Long-term data sets such as these are extraordinarily useful, but are seldom gathered. In this case, they allowed the evaluation of impacts of an emerging disease from the very first year of its appearance.

Differential Susceptibility

Early wisdom derived from several very large data sets over several years of trap sampling, at-sea sampling on commercial vessels, and standardized fishery-independent trawl surveys (Castro & Angell 2000) showed a positive correlation between lobster size and disease prevalence, suggesting that larger lobsters are more susceptible to the disease because they molt less frequently than smaller ones. More recent data (Castro & Somers 2012), however, call into question the assumption that large size is an important predisposing factor for ESD. Discovery of ESD-infected lobsters as small as 28 mm gives strong evidence that small size is not a refuge. The argument against size being an important factor is strengthened by data in the same report showing sublegal males having a considerably higher prevalence than larger, legal-size males (Castro & Somers 2012). To determine accurately the impact of ESD on lobster populations, it is important to determine the factors (hormonal or environmental) leading to increased prevalence in ovigerous females and sublegal males. Regardless, there may be ecological and evolutionary consequences if the mortality resulting from disease is applied differentially to selected sectors of the lobster population (different from the previous natural mortality distribution). For instance, a higher mortality rate in ovigerous females may lead to earlier (smaller size at) maturity, and other features of a semelparous life history.

Density Dependence

The significant term for the interaction of disease and settlement suggests that disease prevalence is higher among cohorts that settle at higher densities (Wahle et al. 2009). Some reports from the NELRI emphasize that transmission in the laboratory is difficult, but possible, to achieve. This does not appear to be the case in nature, where evidence of ESD can appear and spread quickly. Knowing how the disease is initiated and propagated is critical to developing interventions that can reduce disease-related mortality. Lobsters are caught in passive fishing gear through attraction behavior, and Karnofsky and Price (1989) provide convincing evidence that lobsters congregate in and near baited traps. They seem to enter and depart the trap nearly at will, and fights are common. Traps increase the effective density of lobsters. If transmission is density dependent, traps may be part of the model. Understanding transmission of the disease remains a high priority for research.

MANAGEMENT OPTIONS

In general, the primary tool in a fishery manager's kit is manipulation of fishing mortality (F) by size limits, reproductive status, or directly by closed seasons, quotas, or protected areas. Little is known about how to manage a wild stock affected by disease, but a few have been tested by modeling (e.g., McCallum et al. (2005), see also the review in Castro et al.(2012)). If prevalence is density dependent, then reducing the pathogen load to below threshold may control the disease, unless other hosts exist that can sustain the bacterial abundance.

Another strategy for managing ESD in lobsters may be just to wait it out. In SNE, the lobster population crashed and is still declining, probably in large part as a result of ESD. When the population reaches stable, lower stock size, it may be below threshold, or small enough so infected individuals are few and transmission is rare, as is the case with the lobster bacterial disease gaffkemia.

Careful monitoring of environmental conditions and potential links to the distribution, severity, and prevalence are required as the disease continues to affect the SNE lobster population. If ESD was to spread northward throughout the rich lobster grounds of the Gulf of Maine, where catches continue to increase to new records almost annually, there would be serious economic consequences. The lessons from the SNE epizootic show that when ESD is established in a population, it can cause dramatic impacts. Taking rapid and decisive action as soon as increases in ESD prevalence are seen in populations not previously affected by the disease is necessary to avoid establishment of the disease in new areas. In preparation for potential epizootics, emergency action plans should be prepared and approved so a rapid response is possible. However it is done, a rapid response (<1 y) to the threat of ESD could mean a great deal to the fishery and those who depend on it.

CONCLUSIONS AND FUTURE PERSPECTIVES

The research described in this issue confirms what has been named as the ''host susceptibility'' hypothesis (Tlusty et al. 2007), which states that ESD is an emerging disease caused by one or several opportunistic bacterial pathogens that take advantage of a host that is susceptible either through physical damage to the shell or other factors leading to stress and immunosuppression. The research of the NELRI has also pointed to many avenues of future work, and contributed many of the tools necessary to perform this research, including but not limited to microbial sequences of candidate pathogens, a collection of differentially expressed sequence tags, optimized assays to measure pollutants in lobsters tissues, assays to characterize immunity and physiological responses, identification of some potential biomarkers of disease, a model for bacterial challenge experiments in lobsters, and conceptual models of the disease. This initiative also involved the ''whole animal approach,'' in which sharing of information and common sets of samples between independently funded researchers, led to the ''100 Lobsters'' project, well described in Shields et al. (2012b).

Further efforts should be dedicated to understand which members of the microbial consortium are involved in the initiation and progression of lesions, and which may be opportunistic pathogens or secondary invaders. The sequence information gathered in this research will be extremely useful in the design of probes for *in situ* hybridization, allowing the determination of which bacterial species are consistently present at the leading edge of the lesion during the initial stages of the disease. Identification of the major player or players in lesion initiation and progression would greatly facilitate the study of the epizootiology of the disease.

Identification of the pathogen or pathogens, combined with the challenge model developed by Quinn et al. (2012), will also allow determining the role of genetics on disease susceptibility through direct evaluation of the impact of bacterial challenge on disease incidence and progression in lobsters from different geographical locations. Further research should also be done to confirm and expand promising results from the population genetics and odor recognition studies showing possible population structure within Narragansett Bay and between ELIS and WLIS (Atema, pers. comm.).

Although no direct link has been shown between the distribution and magnitude of some pollutants of anthropogenic origin (alkylphenols (Jacobs et al. 2012) and metals (LeBlanc & Prince 2012), the high prevalence of several idiopathic conditions in lobsters from a single location in Rhode Island are indicative of degraded environmental conditions. More epidemiological research should be dedicated to determining the prevalence of other pathological conditions in SNE, and to investigating the potential role of other pollutants, including pesticides, on lobster health. These studies should also consider the possibility that lobsters are acting as the proverbial ''canary in the coal mine,'' and investigate whether other benthic species are also being impacted by environmental degradation or changes in environmental conditions.

Further research should also focus on the role of changing environmental conditions on lobster shell disease, especially of those parameters that could have an impact on the chemical composition of the shell, the immune responses of lobsters, molting behavior, and the microbial community (i.e., pH, availability of organic and inorganic nutrients). Models studying the impact of temperature on shell disease should consider the impact of environmental factors (i.e., temperature, hypoxia, pH, pollutants) on lobster physiology (including molting cycle, shell thickness, growth, and response to xenobiotics), the impact of these factors on the growth and expression of virulence factors (i.e., proteases and lipases) by the pathogen, as well as any potential feedback loops possibly caused by the impact of the disease on lobster immunity, physiology, reproduction, and mortality (Castro et al. 2012).

Management must be more responsive to disease. Higher than normal abundance levels of a host combined with and increasing disease prevalence levels should be used as warning signs of an epizootic, triggering immediate action. In the lobster population, ''appropriate action'' might mean culling the most susceptible individuals. This would mean capturing diseased individuals (including ovigerous females) and removing them from the population. This strategy would also favor development of disease resistance in the population, but only if there is no significant contribution of susceptible larvae from other populations.

Last, but not least, further research should be done on the impact of shell disease on lobster populations and the SNE

ecosystem, including how shell disease affects other (non-commercial) crustaceans. This knowledge is critical for the adequate management of an important resource.

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