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HISTOLOGICAL ASSESSMENT OF THE LOBSTER (HOMARUS AMERICANUS) IN THE “100 LOBSTERS” PROJECT

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ABSTRACT The emergence of epizootic shell disease in the American lobster (Homarus americanus) has been devastating to the industry in the coastal waters of southern New England. A comprehensive assessment of the disease syndrome, known as the “100 Lobsters” Project, was initiated to examine health and physiological parameters among laboratories involved in the research on lobster shell disease. A histological study of the 100 lobsters was undertaken as part of that assessment. Tissues from 90 lobsters from Rhode Island and 19 lobsters from Maine were examined as a general health assessment of the 100 lobsters. Approximately half the lobsters from Rhode Island were selected because they had frank epizootic shell disease, whereas none of the lobsters from Maine exhibited the syndrome. In addition to epizootic shell disease, the histological findings revealed 3 other idiopathic syndromes—necrotizing hepatopancreatitis, idiopathic blindness, and nonspecific granulomas—in higher prevalences in lobsters from Rhode Island compared with those from Maine. Necrotizing hepatopancreatitis, a newly described disease syndrome in lobsters, was observed in 15% of the lobsters from Rhode Island. Idiopathic blindness was present in 54% of the lobsters from Rhode Island, and 16% of the animals from Maine. This is the first report of the syndrome in lobsters from Maine. None of the idiopathic syndromes was associated with epizootic shell disease. The detection of multiple disease syndromes such as epizootic shell disease, necrotizing hepatopancreatitis, and idiopathic blindness may be indicative of exposure to environmental stressors in Narragansett Bay, RI.

KEY WORDS: necrotizing hepatopancreatitis, idiopathic blindness, contaminants, epizootic shell disease, histology, American lobster, Homarus americanus

INTRODUCTION

Several health issues have recently affected the fisheries and management of the clawed lobster, Homarus americanus Milne Edwards, in the United States. Mortality events and high levels of morbidity have occurred in commercially important fishing areas. Some problems appear to be environmentally related, such as increased bottom temperatures during summers and the general effects of eutrophication (Pearce & Balcom 2005). Others are possibly the result of intoxication from anthropogenic substances (Zulkowsky et al. 2005), and yet others may result from newly emerging pathogens. In 1999, the pathogen Neoparamoeba pemaquidensis emerged in concert with environmental stressors to decimate the commercially important lobster population in western Long Island Sound (LIS) (Mullen et al. 2004, Pearce & Balcom 2005). In 2002, lobsters suffering from calcinosis, a physiological response to temperature stress, were reported from central LIS (Dove et al. 2004), and, at about the same time, ~50% of the lobsters in portions of western LIS were shown to have some form of blindness (Maniscalco & Shields 2006). Another emergent pathogen, Vibrio fluvialis, caused focal mortalities off Maine (Tall et al. 2003). From about 1996 to the present, epizootic shell disease (ESD) has sharply increased in lobsters from eastern LIS (Castro & Angell 2000, Castro et al. 2005, Landers 2005, Powell et al. 2005) and Buzzards Bay (Glenn & Pugh 2005), and the syndrome has apparently spread to lobsters from Cape Cod Bay (Glenn & Pugh 2005). It is extremely rare in the waters off Maine (Wilson 2005).

The newly emergent syndrome, known as ESD (Smolowitz et al. 2005a, Smolowitz et al. 2005b), is different from classic, or endemic, shell disease. Notably, the epizootic disease has not been shown to be transmitted horizontally to healthy lobsters in laboratory experiments (Chistoserdov et al. 2003, Quinn et al. 2012). It has also been associated with bacterial species other than Vibrio spp., such as members of the Flavobacteriaceae, particularly Aquimarina sp. (Chistoserdov et al. 2005a, Chistoserdov et al. 2005b, Chistoserdov et al. 2012). Further, the lesions form in a manner quite different from those in classic shell disease, causing deep pits to form early in the infection, followed by friable areas of shell (pillars of lattice crystal) that eventually coalesce into large, broad ulcers (Smolowitz et al. 2005a, Smolowitz et al. 2005b, Kunkel et al. 2012). In addition, the erosion of the shell appears to be the result of bacterial degradation of the interstitial matrix of the epicuticle (Smolowitz et al. 2005a, Smolowitz et al. 2005b). Lobsters with the disease syndrome have increased levels of proteases and lipases, presumably from bacterial degradation (Bell et al. 2012), as well as a dysbiotic microbial flora with imbalances in certain community members (Meres et al. 2012).

ESD appears to be reducing both the quantity and quality of commercial landings of lobster (Cobb & Castro 2006, Wahl et al. 2009). It has spread rapidly in the lobster population of eastern LIS and Cape Cod Bay (Castro & Angell 2000, Cobb & Castro 2006). In addition, the incidence of ESD has increased markedly, with focal outbreaks affecting 25–35% of lobster populations in eastern LIS (Castro et al. 2005, Glenn & Pugh 2005, Howell et al. 2005, Landers 2005). From 1997 to 2004, ESD has shown a north–south cline, wherein the disease is least prevalent in lobsters in the Gulf of Maine and most prevalent in lobsters off Rhode Island (20–30%) (Castro & Angell 2006). Interestingly, there is little occurrence of ESD in western LIS or off the New York Bight. Tagging studies have shown that lobsters with shell disease may rapidly contract the disease again shortly after molting out of it (Castro et al. 2005). It is not known whether these animals carried the agent with them or
whether they were more susceptible to the development of the disease.

The “100 Lobsters” Project was developed as a means to share insights, tissues, and samples among researchers from several institutions for shared research objectives (Shields et al. 2012). As part of the development of the demonstration project, we took various tissue samples from the 100 lobsters for histological assessment. Histological analysis of these tissues represented an opportunity to match histological data with data from a variety of other analyses. Thus, the objectives of this study were to analyze the histology of the animals in the 100 Lobsters Project to give an overview of the general state of each animal and to document any unusual conditions that might be associated with the newly emerging phenomenon of ESD from eastern LIS. A new disease syndrome, necrotizing hepatopancreatitis, was observed in animals from Rhode Island; therefore, a secondary objective was to describe the pathology of this syndrome and analyze its prevalence in relation to ESD and other conditions. An assessment of idiopathic blindness was also undertaken, and this idiopathic syndrome was highly prevalent in lobsters from Rhode Island waters.

**MATERIALS AND METHODS**

**Collection of Animals**

Lobsters were collected from Narragansett Bay, RI, in June, July, and October 2008. Animals from Narragansett Bay were collected by commercial lobstermen and the Rhode Island Department of Environmental Management (RIDEM) using unvented, baited traps. Lobsters from Maine were collected from off Mount Desert Rock, December 2008, by personnel from the Maine Department of Marine Resources using commercial gear as just noted. Shortly after capture, lobsters were shipped in Styrofoam coolers packed with blue-ice bricks to the Virginia Institute of Marine Science (VIMS) via overnight express delivery. On arrival, lobsters were sorted into 3 groups: those few that had died and were not fit for further processing, those that were moribund and required immediate dissection and processing, and those to be housed in a refrigerated aquarium (220 gal.) for short-term holding (<3 wk) at 10°C. Animals to be processed were examined for sex, carapace length (CL), shell condition, and injuries. Lobsters were rated as having no signs of shell disease, or intensities that were light, moderate, or heavy, as in Landers (2005) (Fig. 1). Prior to processing and dissection, lobsters were photographed with a digital camera (Olympus 3000) (Fig. 1).

For dissection and processing, hemolymph was drawn with a 27-G. syringe from the juncture of the basis and ischium of the 5th walking leg. The area was swabbed with 70% ethanol prior to bleeding. A few drops of hemolymph were dotted onto TCBS plates (thiosulfate citrate bile salts sucrose agar, specific for cultivation of *Vibrio* spp.) and incubated at room temperature for 24–48 h. Lobsters were then killed by injection of a 1-mL aliquot of ice-cold saturated KCl into the region of the ventral nerve ganglia, as in Battison et al. (2000). The ligaments around the carapace were then severed with a knife, the carapace was removed, and various tissues were dissected for later processing and analyses. For histology, samples (~1 cm² pieces) of the hepatopancreas, heart, epidermis underlying the dorsal carapace, gonad, gill, cuticle, and the entire left eye were excised, placed in cassettes, and fixed in Bouin’s solution (standard formulation; Fisher Scientific, Pittsburgh, PA). Other tissues were sampled occasionally as well, including the thoracic muscle, hematopoietic tissue, and antennal gland. After 48 h, the tissues were washed for at least 3 h in running tap water, then stored in 70% ethanol until further processing. Pieces of the gills were decalcified for 2–3 h using the formic acid–sodium citrate method (Luna 1968). Pieces of the shell and eye were decalcified overnight using the same method. After decalcification, each eye was bisected sagittally with a razor. All tissues were processed using paraffin histological techniques and stained with Mayer’s hematoxylin and eosin (Luna 1968). Tissues from a subset of animals were processed through the Leavander modification of Brown and Brenn’s stain for bacteria as well as Wheatley’s modification to Mallory’s trichrome stain (Humason 1979). Prepared sections were examined with an Olympus BX51 compound microscope, and photographs were taken using a Nikon DXM1200 digital camera with aid of the ACT-1 computer program (Nikon).
RESULTS

General Assessments

Lobsters from Rhode Island were collected for the study based on their sex and status of ESD (none, light, moderate, heavy). There were 57 female and 33 male lobsters, totaling 90 lobsters from Rhode Island. Of these, 23 females and 20 males had no signs of ESD (n = 43), and 34 females and 13 males had various intensities of shell disease (n = 47). Of the animals with ESD, 12 had light infections, 14 had moderate infections, and 21 had heavy infections. Lobsters from Maine were collected to match the relative sizes and sexes of animals from Rhode Island. There were 9 female and 10 male lobsters, totaling 19 animals from Maine; of these, none had signs of ESD.

From the TCBS plates, Vibrio-like bacteria were commonly isolated from the hemolymph of animals from Rhode Island and Maine (Table 1). However, lobsters from Maine had a significantly lower prevalence (6%) of Vibrio-like bacteria than those from Rhode Island (37%; chi-square = 7.110, df = 1, P = 0.008). Excluding the animals from Maine, lobsters with ESD (51%) had a higher prevalence of Vibrio-like bacteria in their hemolymph than those without shell disease (23%; chi-square = 7.387, df = 1, P = 0.007; Table 2). However, this pattern was dependent on whether the animals had sustained other injuries, such as limb loss (autotomy) or other types of lesions associated with fishing. That is, animals without injuries showed no differences in the prevalence of Vibrio-like bacteria whether they had ESD (36%) or not (25%; chi-square = 0.705, df = 1, P = 0.401, n = 19, 24), whereas animals with injuries and ESD (60%) had a significantly higher prevalence of Vibrio-like bacteria than their injured counterparts without shell disease (21%; chi-square = 7.204, df = 1, P = 0.007, n = 19, 28, respectively).

Histological Assessment and Idiopathic Conditions

Several idiopathic conditions were observed in the histological assessments of lobsters from Rhode Island (Tables 1 and 2). Idiopathic conditions are those with no specific or known causality. The most noteworthy finding was the occurrence of necrosis in the hepatopancreas, which was observed in 15% of the animals from Rhode Island. Focal necrosis of individual hepatopancreatic tubules occurred in 9% of the animals, and a severe, coalescing form, termed necrotizing hepatopancreatitis, was found in 6% of the animals (see description in next paragraph). The most common lesions observed in histology were idiopathic blindness arising from the loss of ommatidia in the eyes, followed by the presence of nonspecific, or idiopathic, granulomas in a variety of tissues. Some of the granulomas were present in large quantities or occurred in a number of different organs, a condition tentatively identified as early calcinosis. Localized, or focal, necrosis of ill filaments was observed in a few animals from both Rhode Island and Maine, but the condition was extensive on 1 gill podobranch in only 2 lobsters from Rhode Island (Table 1). For animals from Rhode Island, there were no differences in idiopathic conditions in lobsters with or without ESD, with the exception of Vibrio-like bacterial infections in the hemolymph (Table 2). There were no differences in the histology of the gonads (ovaries or testes) between lobsters with and without shell disease. Normal gonadal development was observed in all male and female lobsters (not shown).

TABLE 1.
Presence of idiopathic conditions in lobsters from Rhode Island and Maine.

| Condition                        | Rhode Island | Maine |
|----------------------------------|--------------|-------|
| No. of lobsters                  | 90           | 19    |
| Vibriosis                        | 38%*         | 6%    |
| Granulomas                       | 44%*         | 5%    |
| Possible early calcinosis        | 3%           | 0%    |
| Hepatopancreatitis (any form)    | 15%*         | 0%    |
| Focal necrosis                   | 9%           | 0%    |
| Coalescent necrosis (severe)      | 6%           | 0%    |
| Filament necrosis in gill         | 8%           | 5%    |
| Idiopathic lesions in eyes        | 54%*         | 16%   |
| Severity of eye lesions           | 21.5% ± 26.5%| 1.5% ± 4.7%|
| Acanthocephalan cystacanth       | 7%           | 0%    |

* Chi-square, P < 0.05.

TABLE 2.
Presence of idiopathic conditions in lobsters from Rhode Island with epizootic shell disease compared with those without the syndrome.

| Condition                        | No Shell Disease | Epizootic Shell Disease |
|----------------------------------|------------------|-------------------------|
| No. of lobsters                  | 43               | 47                      |
| Vibriosis in hemolymph           | 23%              | 51%*                    |
| Granulomas                       | 40%              | 49%                     |
| Possible early calcinosis        | 5%               | 9%                      |
| Hepatopancreatitis (any form)    | 16%              | 15%                     |
| Focal necrosis                   | 9%               | 9%                      |
| Coalescent necrosis (severe)      | 7%               | 6%                      |
| Idiopathic lesions in eyes        | 55%              | 53%                     |
| Severity of eye lesions           | 17.0% ± 20.4%    | 25.6% ± 30.6%           |

AFFECTED LOBSTERS HAD LIGHT, MODERATE OR HEAVY INFESTATIONS OF EPIZOOTIC SHELL DISEASE. * Chi-square, P < 0.05.

Necrotizing Hepatopancreas

The normal architecture of the hepatopancreas was observed in all lobsters. It consisted of healthy hepatopancreatic tubules supported by a thin network of spongy connective tissue cells, interlaced rarely with reserve inclusion cells, with primary arterioles and fixed phagocytes interspersed sparingly between the tubules (Fig. 1). In most animals, the reserve inclusion cells were not abundant, and in postmolt and some intermolt animals, the reserve inclusion cells were depleted, giving the appearance of their absence from the hepatopancreas. The B cells ("blasenzellen," a cell that secretes digestive enzymes) in the hepatopancreas were observed for vacuoles as a possible sign of feeding or metabolic state, but they were too variable for this purpose (Fig. 1C–F). The other cell types—F, E, and R cells—did not appear to be altered in histological samples.

In 15% of the animals from Rhode Island, portions of the hepatopancreatic tubules exhibited pathological necroses that were rated as focal lesions (9%) or coalescent lesions (6%), and...
were identified as necrotizing hepatopancreatitis. The focal condition consisted of the necrosis of an entire hepatopancreatic tubule (Fig. 2). The surrounding tissues showed signs of nodulation and encapsulation, and in some cases, the rescaffolding of adjacent spongy connective tissues. Infiltration of granulocytes, the primary cells involved in nodulation and encapsulation, was rarely observed in the focal condition. In animals with coalescent hepatopancreatitis, the condition was more extensive than the focal form, involving at least four or more tubules (Figs. 2–4). The affected tubules showed extensive nodulation and encapsulation of the necrotic area, with the involvement of the basement membranes, extensive infiltration of hemocytes, particularly granulocytes into the surrounding connective tissues, and proliferative rescaffolding of the spongy connective tissue adjacent to necrotic areas (Figs. 3 and 4). Apoptotic cells were present in areas with extensive rescaffolding of the spongy connective tissues (Fig. 3D). In some cases, tubules adjacent to those undergoing nodulation possessed epithelial cells that had clearly undergone metaplasia, with cuboidal epithelial cells replacing the normally squamous E and R cells (Fig. 4). Hepatopancreatic tubules adjacent to necrotic tubules occasionally possessed sloughed cells from the tubules (Fig. 4A and D).

**Idiopathic Granulomas**

Non-specific, or idiopathic, granulomas were present in significantly more lobsters from Rhode Island (44%) than in those from Maine (5%; chi-square = 10.263, df = 1, P = 0.001) (Fig. 4E, F). One animal from Maine and 6 animals from Rhode Island had granulomas present in large numbers in a number of different organs. The granulomas were focal in nature, never presenting as coalescent lesions. Calcification was not ruled out because histological stains for calcium were not done on these animals. Bacterial infections may be associated with the presence of granulomas in the tissues, but there was no association between the presence of *Vibrio*-like bacteria in the hemolymph and the presence or intensity of granulomas in the tissues of lobsters from Rhode Island (chi-square = 1.598, df = 1, P = 0.206), nor was there a difference when stratified by presence or absence of shell disease (chi-square = 0.804, df = 1, P = 0.370). In a few cases, the granulomas showed evidence of melanization by the host response (Fig. 4F). Bacteria were not observed in the tissues using the Leaver adaptation of the Brown and Brenn method (Humason 1979).

**Epizootic Shell Disease**

The pathology of animals infected with ESD was virtually identical to that published by Smolowitz et al. (2005a, 2005b). In the histology, deep, penetrating pits occurred on the cuticle of animals with the syndrome (Fig. 5). Within the pits were friable remnants of the cuticle that appeared as pillars of material in histological sections. Extensive melanization of the...
endocuticle was evident in the larger lesions. Lateral erosion of the endocuticle was not observed in the shell-disease lesions. In several histological sections, large areas of the endocuticle appeared to delaminate from their normal lamellar appearance, but this was likely an artifact of the processing of diseased tissues, which were more friable than healthy ones. The membranous layer of the cuticle was affected by nodulation and the infiltration of hemocytes into the adjacent epidermal area in some animals. Pseudomembranes underlying the affected cuticle were observed in 10 lobsters with severe ESD. In these animals, the medial portion of the membranous layer had moderate nodulation with pseudomembranes occurring between the affected cuticle and the underlying epidermis. The underlying musculature was not affected.

**Idiopathic Blindness**

Idiopathic lesions in the eye indicated the presence of a nonspecific blindness (hereafter called idiopathic blindness).
6 cases having a complete or nearly complete loss of the ommatidia, and 4 animals with light cases, less than 10% of the ommatidia affected.

The prevalence of idiopathic blindness was significantly higher in animals from Rhode Island (54%) than in those from Maine (16%). Moreover, the severity of idiopathic blindness was much higher and more variable in animals from Rhode Island (mean, 21.5% ± 26.5% of the lineal area; n = 89; see Magel et al. 2009, for lineal area) compared with those from Maine (mean, 1.5% ± 4.7% of the lineal area; n = 19) (untransformed t-test, t = 3.263, df = 106, P = 0.001). The severity data were calculated as a percentage of the lineal area of the cross-section of the eye; hence, the data were arcsin-square root-transformed to reduce the variance. Whereas the transformed data showed significant differences as above, they also had a very large variance structure (arcsin-square root-transformed data, t-test, t = 3.388, df = 106, P = 0.001). In the lobsters from Rhode Island, the prevalence of idiopathic blindness was not significantly different between animals with shell disease (53%) and those without (54%; chi-square = 0.022, df = 1, P = 0.882), indicating no relationship between ESD and idiopathic blindness.

DISCUSSION

The histological assessment of lobsters in the 100 Lobsters Project revealed several important findings. First, lobsters from Rhode Island have considerably more idiopathic disease syndromes and significantly greater intensities of disease than their counterparts from Maine. This finding highlights the importance of incorporating animals from a control site from outside the endemic area of disease, because the conditions that give rise to ESD likely lie along a continuum of exposures within eastern LIS and Narragansett Bay, RI. That is, lobsters from Rhode Island exhibit similar levels of several disease syndromes regardless of whether they have ESD. These disease syndromes are indicative of exposure to stressors or etiological agents that do not occur off Maine.

Second, at least 3 of the idiopathic syndromes in lobsters from Rhode Island can be quite severe. The prevalence of necrotizing hepatopancreatitis in lobsters is startlingly high, and requires further examination. Necrotizing hepatopancreatitis in clawed lobsters is a new discovery. Its etiology in lobsters is unknown, but a similar disease, also known as necrotizing hepatopancreatitis, occurs in penaeid shrimp, and is caused by a rickettsial agent (Freiler et al. 1992, Lightner & Redman 1994). The pathology in the lobsters showed no obvious signs of intracellular bacteria. The presence of necrotic hepatopancreatic tubules has been described in clawed lobsters from Canada (Comeau & Benhalima 2009), but the pathology was not described in sufficient detail to compare it with necrotizing hepatopancreas from the current study. The consequences of necrotizing hepatopancreatitis to lobster health are unknown, but the damage to the affected parts of the hepatopancreas is certainly a complete loss of function. However, the histological samples represented only a small portion of the hepatopancreas; thus, the extent of the disease in individuals was not fully assessed.

The second syndrome with severe effects is idiopathic blindness. Using physiological assessments, Magel et al. (2009) showed that animals with light to moderate cases of this syndrome (~20% affected areas) have nearly complete loss of vision in the affected eye. Given the prevalence of the syndrome in lobsters in the current study, and in those by Maniscalco and Shields (2006) and Magel et al. (2009), we estimate that nearly half of all adult lobsters in LIS have some form of blindness. The current study extends the range of idiopathic blindness eastward into Narragansett Bay, Block Island Sound, and potentially into the Gulf of Maine, albeit at low levels in the latter. The cause of the idiopathic blindness remains unknown. Maniscalco and Shields (2006) speculated that environmental contaminants released from sediments or multiple stressors during hypoxic events might be involved in blindness.

The third syndrome with severe effects is ESD. Several of the animals in the current study had severe ESD, with extensive erosion of the cuticle covering up to 50% of the body surface...
Multiple stressors and host susceptibility to ESD has been examined in a hypothetical model that provides a solid framework for the further exploration of causality and etiology (Tlusty et al. 2007).

Contaminants may not play as important a role in shell disease as some have suggested. Boston Harbor is a heavily contaminated embayment, yet there is a low prevalence of ESD in that region (Glenn & Pugh 2006), albeit endemic, or classic, shell disease is relatively common in lobsters harvested there (Estrella 1984). Interestingly, ESD does not occur in western LIS, an anthropogenically affected area that experiences high summer temperatures (>25°C) and environmental contaminants. These discrepancies indicate that the link between contaminants and shell disease is not consistent, or that focal contaminants through oil spills or other sources may be important to the etiology of the syndrome or that selection pressures have already operated on lobsters in that heavily impacted area. Regardless, the nature of the internal idiopathic syndromes observed from lobsters from Narragansett Bay, RI, is more like that observed in other animals exposed to contaminants (e.g., granulomatous lesions without microbial involvement) rather than to infectious agents. Indeed, some contaminants can cause subtle effects in marine animals, such as immunosuppression (Arkoosh et al. 1998) and reduced metabolic responses (Baldwin et al. 2009), that can appear as infectious etiologies in their hosts. Clearly, many factors, either alone or in combination, may lead to the development of ESD in American lobsters (Castro et al. 2006, Tlusty et al. 2007).

Idiopathic blindness represents another indicator of anthropogenic exposure in lobsters harvested from Rhode Island and eastern LIS. Because of the important role of the endocrine glands in the eyestalk of lobsters, and their role in several physiological processes such as molting and growth, we strongly suggest that this syndrome be included as part of a continued lobster health assessment management program so that this disease and its possible northward advancement into the Gulf of Maine can be monitored. Additional sampling from locations within Block Island Sound, central LIS, and elsewhere should be undertaken to determine the extent and range of the syndromes reported here. Clearly, more research is needed on lobster health to determine the factors and thresholds responsible for tipping the balance from normal host conditions to disease.

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