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COMMUNICATIONS

Histological Analysis of an Outbreak of QPX Disease in Wild Hard Clams *Mercenaria mercenaria* in New York

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Abstract.—Over 600 hard clams *Mercenaria mercenaria* from a wild population in Raritan Bay, New York, were examined histologically to assess the extent and intensity of quahog parasite X (QPX) disease in the fishery area. The overall rate of positive samples was 5.8%. A further 5.1% of clams had inflamed lesions suggestive of QPX infection but no QPX organisms were observed. In over half of the QPX-positive cases, infections of the viscera were involved. The significant proportion of visceral infections and the appearance of the pathogen in the gonads of clams are previously unreported presentations of QPX disease. Visceral infections are of concern for fisheries because they involve significantly larger numbers of parasites and higher biomass than mantle-only infections.

Quahog parasite X (or quahog parasite unknown [QPX]) disease of the hard clam *Mercenaria mercenaria* is caused by a poorly known protistan parasite. Its DNA sequence analysis places the QPX parasite among the thraustochytrid stramenopiles (Stokes et al. 2002). Thraustochytrids are common protists in marine sediments and the water column (Santangelo et al. 2000; Raghukumar et al. 2001), but only a few other thraustochytrids are known as parasites of marine animals (Bower 1987a, 1987b, 1987c). Although QPX disease was first recorded on the Atlantic coast of Canada in the early 1960s (Drinnan and Henderson 1963), it did not become a major economic problem until its appearance in cultured clams at Prince Edward Island in 1989 (Whyte et al. 1994), Massachusetts in 1992 (Smolowitz et al. 1998), and Virginia in 1997 (Ragone-Calvo et al. 1998). Infected clams are characterized by the presence of blisters or pustules in the mantle and later by gaping and death.

During summer 2002, substantial mortalities of clams were observed in a fishery area of Raritan Bay off the south shore of Staten Island, New York. Preliminary analysis of a sample of 30 clams collected from the Raritan Bay site showed that the QPX organism was present. The primary aims of this study were to quantify the prevalence and intensity of QPX infections in clams at the Raritan Bay site and to understand the distribution of the disease within the fishery area.

Methodology

Clams were collected by raking from a boat in 5–7 m of water. Thirty clams were collected from each of 21 sites, except for two sites where 15 and 18 clams, respectively, were all that could be collected. Statistical analyses were made using Minitab for Windows (Minitab, Inc. 2000)

Clams were transported from Raritan Bay and stored in a cool room for 1–3 d until they could be shucked and fixed at our laboratory. Clams were shucked (by severing the adductor muscles) and then fixed in Davidson's fixative (alcohol : formalin : acetic acid mixture in the ratio 70 : 10 : 5) for a minimum of 24 h. After fixation, a transverse sectional slice of fixed clam tissue approximately 5 mm thick was taken from the middle part of the body for histology, ensuring that the following tissues would be represented in sections: mantle,

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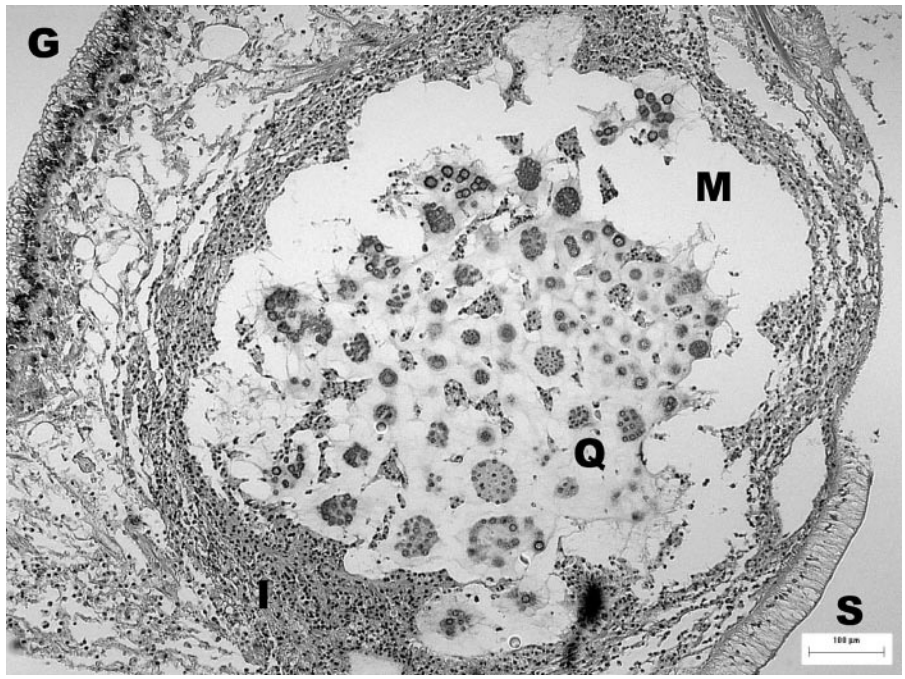

FIGURE 1.—Typical QPX lesion in the mantle of an infected clam. Abbreviations are as follows: G = gill side epithelium; S = shell side epithelium; Q = QPX organisms in mucus envelopes; M = mucus and shrinkage artifact; and I = granulomatous inflammatory cells. Hematoxylin and eosin stain was used; the scale bar = 100 μm.

gills, musculature, nerve, gonad, hepatopancreas, stomach–intestine, and (sometimes) kidney. Sections were made at 4 μm using a rotary microtome and were stained in hematoxylin and eosin, and mounted in Permount synthetic mounting medium. Slides were examined microscopically for QPX parasites. All images were captured using a Spot/Insight digital CCD camera (Diagnostic Instruments, Inc.). Images were postprocessed in Adobe Photoshop 7.0 (levels correction and unsharp mask only).

Results and Discussion

A total of 603 clams was examined. Of all the clams examined, 35 (5.8% overall) were determined by microscopic examination to be infected. There was no statistically significant difference detected in infection rates between male and female clams ($\chi^2 = 1.7$; $p = 0.42$). Although individual clam sizes were not recorded, none of the 16 largest clams (those requiring two slides to section) was QPX positive.

The typical QPX infection consisted of multifocal spherical to subspherical lesions wherein the center of each lesion was composed of mucous material with embedded thalli and zoosporangia, surrounded by concentric layers of granuloma-like hemocytic inflammation (Figures 1–4). Individual lesions associated with QPX showed a range of presentations. In extent, they were focal (Figures 1–3), multifocal, coalescing (Figure 4), or diffuse; in appearance, they varied from granuloma-like to fibrinomucous to purulomucous. At times, the mucous material characteristic of the typical QPX lesion was not observed; rather the hemocytes were observed to be in direct contact with parasite thalli and zoosporangia. Generalized hemocytic inflammation was also present in many cases; this phenomenon was restricted to the affected tissues in some cases and was apparently systemic in others. Multinucleated giant host phagocytes were observed in association with many lesions.

Throughout the tissues of infected clams QPX infections were recorded (Figures 1–4). The kidney was the only tissue not found to be infected in at least one case. However, kidney tissue was not observed in every section, so the significance of this observation is limited. In this study, mantle involvement occurred in 22 of 35 (62.8%) of cases and visceral involvement occurred in 20 of 35 (57.1%) cases. Visceral QPX lesions were more likely to involve a coalescing or diffuse appear-

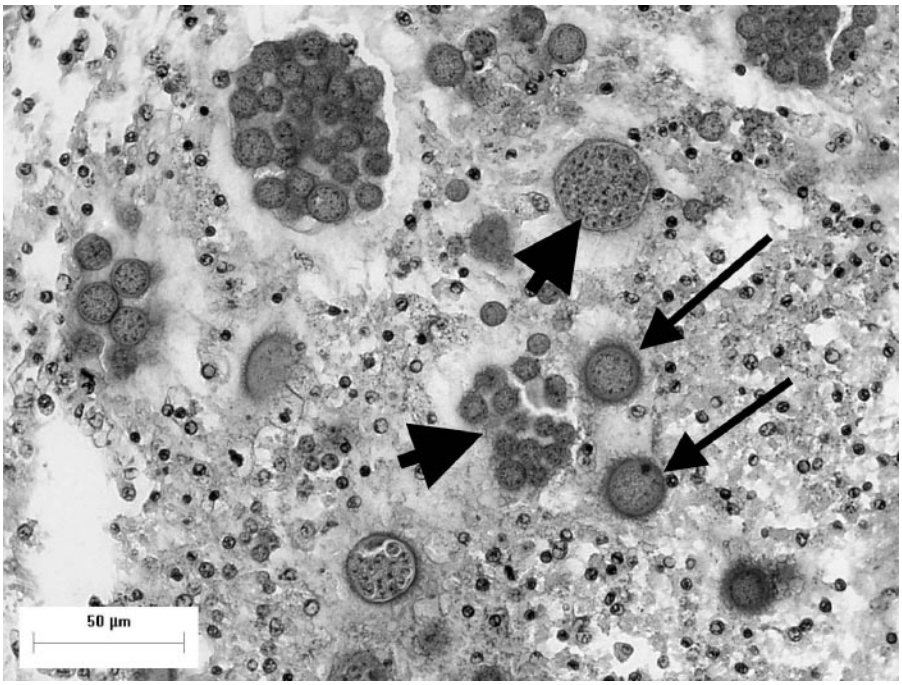

FIGURE 2.—Higher magnification of QPX. Thalli (long arrows) and zoosporangia (short arrows) are interspersed with clam inflammatory hemocytes. Hematoxylin and eosin stain was used.

ance than mantle lesions, which tended to be focal or multifocal. For the first time (to our knowledge), QPX organisms were observed in the gonads (Figures 3, 4).

In addition to the 35 clams in which QPX organisms were observed, a further 31 clams (5.1%) had diffuse, multifocal, or focal inflammation suggestive of QPX infections but without observable QPX organisms. While QPX organisms were not observed in these lesions, perhaps due to sectioning artifact, it seems likely that a significant portion of these clams was QPX positive.

This study is the largest survey of wild clams for QPX disease of which we are aware. The QPX organism has typically been associated with disease in cultured clam populations in the intertidal zone, more so than in wild subtidal clams. The QPX outbreak at Raritan Bay is unusual for several reasons. First, it is the first major outbreak of QPX that has caused large-scale mortalities in wild clams anywhere. Second, it is the first major outbreak of QPX disease in a fully subtidal clam population. Finally, it is the first time that visceral and gonadal infections with the QPX organism have been a major feature of the disease presentation. The involvement of the gonads and the significant amount of visceral QPX observed in this survey are cause for concern, and should be considered when assessing tolerable levels of infection prevalence in management decisions.

Attempts to construct even a crude predictive model of QPX epizootics are hampered by a lack of understanding of the basic aspects of QPX and clam biology. For QPX, this includes basic biology, particularly life cycles, other reservoir hosts, asexual proliferation, transmission efficiency, and longevity. Important fishery and ecological values for clams are also not sufficiently known for the Raritan Bay clam population, such as standing population density, recruitment rates, age structure, and the variation of these parameters over time and with abiotic factors. Nonetheless, the widespread presence of QPX throughout the study area and the emergence of visceral QPX as a major presentation of the disease indicate that a major QPX epizootic was occurring at the Raritan Bay fishery site at the time of sampling.

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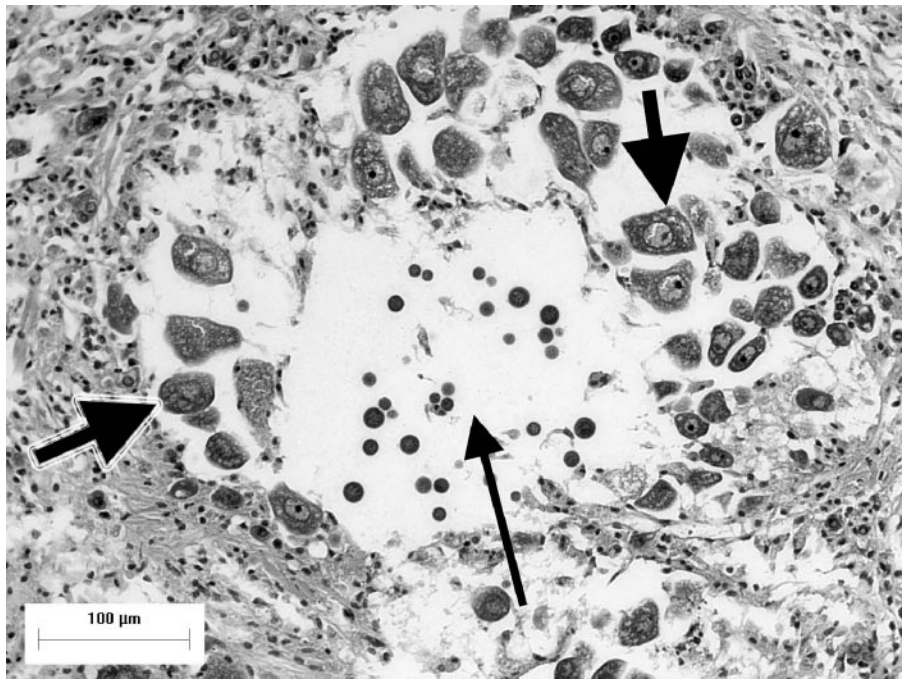

FIGURE 3.—QPX lesion within an ovarian follicle. A cluster of thalli (long arrow) appears among oocytes (short arrows). Hematoxylin and eosin stain was used.

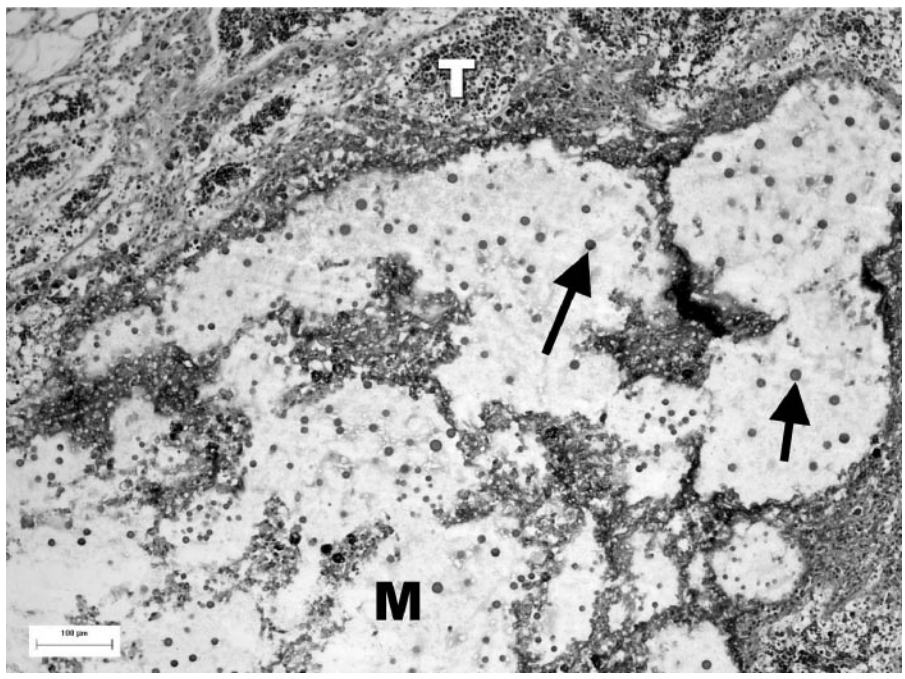

FIGURE 4.—Complex, coalescing visceral QPX lesion in the testes of a male clam. Abbreviations are as follows: T = testis; M = mucus-filled space. Arrows denote individual QPX thalli. Hematoxylin and eosin stain was used; the scale bar = 100 µm.

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