Histopathological alterations associated with Perkinsus spp. infection in the softshell clam Mya arenaria

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**INTRODUCTION**

Significant losses of bivalves reported worldwide have been associated with infections by protozoa of the genus *Perkinsus*. For example, *Perkinsus marinus* causes deadly systemic infections in the eastern oyster (*Crassostrea virginica*) along the Atlantic and Gulf coasts of the United States (reviewed in Andrews, 1988). In tissues of infected oysters, division occurs by successive bipartition of *P. marinus* cells in which karyokinesis is followed by cytokinesis (Perkins, 1996). *Perkinsus atlanticus* causes high mortalities in the Portuguese clam (*Ruditapes decussatus*, Azevedo, 1989). The parasite proliferates primarily in gill tissues causing death of infected clams (Chagot *et al.*, 1987). *Perkinsus osleni* destroys the adductor muscles of the black-lipped abalone (*Haliotis ruber*) leading to their death (Lester & Davis, 1981). Conversely, *Perkinsus* species have been identified in over 60 other mollusks worldwide with no reported mortalities (Perkins, 1993). The softshell clam (*Mya arenaria*) is abundant from Maine to Virginia and is an important filter-feeder of the mesohaline portion of the Chesapeake Bay. Occasional occurrences of *Perkinsus* spp. have been reported in *M. arenaria* from as early as 1954 (Andrews, 1955). Epizootiological studies of 20 upper Chesapeake Bay sites by the Maryland Department of Natural Resources and the National Marine Fisheries Service between 1969-1989 also showed only rare occurrences of *Perkinsus* spp. infection in histologic sections of over 3500 softshell clams (Sara V. Otto, 1989).

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Oxford, Maryland, personal communication). Recent observations, however, indicate that the prevalence of *Perkinsus* spp. infection in *M. arenaria* is on the rise (McLaughlin et al., 1995; McLaughlin, 1996). Hence, the objective of this study was to describe the most common histopathological alterations associated with *Perkinsus* spp. infection in *M. arenaria*.

**MATERIALS AND METHODS**

**SAMPLING SITES AND CLAM COLLECTION**

Softshell clams were collected from four sites located in the mouth and lower Chester River, Maryland (Fig. 1) during 1995-1996 by hydraulic escalator dredge, kept on ice, and immediately transported to the Cooperative Oxford Laboratory, Oxford, Maryland. The clams averaged 66.1 ± 12.0 mm in length (ranging from 40 mm to 100 mm) and were held overnight in separate 76 l glass aquaria supplied with artificial seawater at temperatures between 10-16 °C and salinities adjusted to approximate those of the collection sites, i.e., between 2 and 14 ppt.

**HISTOPATHOLOGY**

A total of 240 clams was processed for microscopical examination following the conventional histologic procedures of Howard & Smith (1983). Transverse pieces of tissues containing gills and most of the internal organs were fixed in 1 % glutaraldehyde-4 % formaldehyde in seawater of half the ambient salinity (McDowell & Trump, 1976; Farley *et al.*, 1986). Paraffin-embedded sections (4-6 µm) were stained with Mayer's hematoxylin and eosin (MHE). Additional slides were stained with Periodic Acid-Schiff procedure (PAS) and malt diastase digestion (MPAS) to demonstrate glycogen and various mucosubstances. Fixation and staining of tissues were performed as described by Howard & Smith (1983) from ingredients purchased from Fisher Scientific, Fairlawn, New Jersey, and Poly-scientific, Bayshore, New York.

**RESULTS**

Examination of hematoxylin and eosin-stained tissue sections indicated a prevalence of *Perkinsus* spp. infection in 28 out of 240 (~ 12 %) softshell clams. In this study, ~ 71 % (20/28) of the clams were lightly infected with the parasites found predominately in gill tissue. Moderate infections were observed in ~ 21 % (6/28) of the clams examined and were characterized by increased parasitism in the gills and low to moderate numbers of parasites in other tissues. Only ~ 7 % (2/28) of the clams examined were considered to be heavily infected with most tissues being affected.

Gills appear to be the target tissue for *Perkinsus* spp. infections. In lightly infected clams, the protozoal cells were often seen between gill lamellae either free (Fig. 2.1) or within granulocytic hemocytes (Fig. 2.2). At sites where *Perkinsus* spp. cells were present, fusion between adjacent lamellae was often noticed. In some instances, hemocytes were observed encompassing a single protozoal cell (Fig. 2.2). Clusters of trophozoites were often seen in the gills embedded in amor-
Fig. 2. – *Perkinsus* spp. in the gills of the softshell clam stained with MHE. 2.1: Unencapsulated trophozoites (arrow). 2.2: Protozoal cells within (arrow) or surrounded by (arrowhead) granulocytes. 2.3: Degenerated trophozoites embedded in amorphous eosinophilic material and tissue debris (arrow). 2.4: A typical *Perkinsus* spp. cyst (arrow) showing centrally located protozoal cells and acellular outer wall, notice fusion of adjacent gill lamellae (arrow head).

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In advanced infections, *Perkinsus* spp. cells predominated the internal structure of gill lamellae (Fig. 4.1) and subepithelial connective tissue (Fig. 4.2). Large lesions were observed in the branchial connective tissues that consisted of encapsulated or free trophozoites, tissue debris, structureless amorphous acidophilic substrate, and hemocytes. Cyst formation was accompanied by loss of the underlying tissue structures including gill lamellae (Figs. 4.3 & 4.4). Cysts observed in the digestive gland were similar in size and morphology to those found in the gills (Fig. 5.1) and were distributed in the connective tissue underlying the mucosa or between the glandular tissue (Fig. 5.2). *Perkinsus* spp. cells were sometimes seen within the gut lumen. Figure 5.3 shows a large lesion in the digestive gland.

*Perkinsus* spp. cells and cysts were also noticed within the connective tissues of the gonads (Fig. 6.1). Multiple, hypercellular lesions were noticed in gonadal ducts (Fig. 6.2). These lesions varied in size and often were ruptured (Fig. 6.3). The lesions consisted of free and encysted trophozoal cells and granulocytic hemocytes within an eosinophilic matrix (Figs. 6.4 & 6.5). Similar lesions were occasionally noticed in the kidneys. The parasite was found in the intertubular connective tissue (Fig. 7.1) with obvious aggregation of granulocytic hemocytes (Fig. 7.2).

*Perkinsus* spp. cells were also noticed in muscle tissues and sinuses. Except for the presence of hemocytic infiltration, the structure of the adjacent muscles seems to be unaffected (Fig. 8).
It was apparent that the parasite propagated by schizogony in affected tissues. Schizonts containing up to four daughter cells were also noticed (Fig. 9). Sizes of the schizonts ranged from 6 \( \mu \text{m} \) to 12 \( \mu \text{m} \) and averaged 7.97 \( \pm \) 1.6 \( \mu \text{m} \) in diameter.

**DISCUSSION**

Protozoal cells in clam tissue sections exhibited the typical morphology and division patterns of *Perkinsus* spp. The average diameter of trophozoites was larger than those reported for *P. marinus* in stained tissue sections (Mackin, 1951). Diagnostic thioglycolate assays as described by Ray (1952, 1963) confirmed the presence of *Perkinsus* spp. in tissues of infected clams in this study (data not shown). Moreover, analysis of the small subunit ribosomal RNA gene confirmed the relationship of softshell clam *Perkinsus* spp. to other members of the genus Perkinsus (Kotob et al., 1998a, 1998b).

The pathological alterations in infected softshell clams are different from those described for eastern oysters infected with *P. marinus*, where the infection runs an aggressive systemic course (Mackin, 1951). Widespread tissue lysis often observed in oysters heavily infected with *P. marinus* was not observed in softshell clams infected with *Perkinsus* spp. This lysis is due to...
the production of extracellular serine proteases by 
P. marinus (La Peyre & Faisal, 1995; Faisal et al., in press). In the softshell clam, the parasite was observed mainly in the connective tissue, mostly without damaging epithelial lining, which is in accordance with other Perkinsus spp. including P. marinus (Perkins, 1976, 1993).

A clear tissue response has been provoked in M. arenaria due to the presence of Perkinsus spp. with the infiltrating granulocytic hemocytes emerging as a major player. The three-layered cyst, outer hemocytic, middle structureless, PAS-positive material, and centrally entrapped trophozoites, typically seen in infected softshell clams share striking similarities to those described in other venerid clams parasitized by P. atlanticus or unidentified Perkinsus spp. (Chagot et al., 1987; Comps & Chagot, 1987). The elegant studies of Montes et al. (1995, 1996) provided evidence that the structureless matrix of the cyst originates from secretory products released following the death of the granulocytic hemocytes. It is suspected that the cyst wall may function in blocking trophozoite dissemination (Navas et al., 1992, Montes et al., 1995, 1996); however, such a cellular reaction may also be suspected to disrupt normal functions of affected tissues such as the gills or block the gonad alveoli as seen in this study.

The gills appear to be the major site of infection by Perkinsus spp. in the softshell clam. In general, the infection seems to run a mild course with the host prevailing in encapsulating the invading parasites. In cases of moderate or heavy parasitism, however, substantial areas of the gills were occupied by Perkinsus spp. aggregates and hemocytes. These sometimes massive, parasitic aggregates may interfere with respiration and other physiologic processes. Similar involvement of gill tissue has been reported with P. atlanticus and other Perkinsus spp. infecting other bivalve molluscs such as R. decussatus (Azevedo, 1989, Rodriguez & Navas, 1995), Tapes semidecussatus (Azevedo, 1989, Montes et al., 1995; Sagrista et al., 1995), and Macoma balthica (Perkins, 1988).

In cases of heavier parasitism, free trophozoites, cysts of different sizes containing protozoal aggregates, and lesions have been observed in other vital organs such as the digestive gland, gonads, and kidneys. It is difficult to assess the impact of infection on functions of these organs; however, the presence of large lesions, particularly in the gonads, may adversely affect fertility and growth of affected clams. The low number of severely parasitized clams may either indicate few clams reach high parasite burdens or that the heavily infected clams die from Perkinsus spp. or from secondary invaders. The presence of Perkinsus spp. trophozoites in sections of the muscle of heavily parasi-
Fig. 6. – *Perkinsus* spp. in the gonads stained with MHE. 6.1: *Perkinsus* spp. cells (arrow) in the connective tissues of the gonads. 6.2: Multiple lesions (arrows) in and around gonadal alveoli. 6.3: A large lesion (A) that ruptured. 6.4 and 6.5: Larger magnifications of a lesion (A) showing protozoal cells and clusters (arrows).
tized softshell clams suggests that the protozoal cells could be disseminated to the entire body via hemolymph.

Isolation of *Perkinsus* spp. cells from infected softshell clams and *in vitro* propagation was successful (McLaughlin & Faisal, in press). Studies to determine species identification and host range of softshell clam *Perkinsus* spp. are being carried out.

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