Occurrence of Hemic Neoplasia in Slipper Oyster, *Crassostrea iredalei* (Faustino, 1928), in Dagupan City, Philippines

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ABSTRACT

Cases of hemic neoplasia in the Philippine slipper oyster (*Crassostrea iredalei*) have been confirmed from a one-year histology-based disease survey conducted in Dagupan City. Samples were collected on a quarterly basis from the BFAR-NIFTDC demonstration farm and processed for histopathology. Slides were stained with hematoxylin and eosin (H & E) or Feulgen picromethyl blue (FPM) as required. Of 210 oysters examined, 3% showed the presence of hemic neoplasia. Histopathological features of the disease condition are described. This is the first documented case of this disease in slipper oysters in the Philippines.

INTRODUCTION

Hemic neoplasia (also termed disseminated neoplasia, hematopoietic neoplasia, sarcomatoid proliferative disease, disseminated sarcoma and leukemia), is a disease known to affect approximately 15 species of bivalves around the world (Peters, 1988; Brousseau and Baglivo, 1994). As the term implies, it affects the blood cells or hemocytes. The disease was first described in the blue mussel, *Mytilus edulis*, from Yaquina Bay, Oregon, USA by Farley (1969). It was reported for the first time in soft-shelled clams, *Mya arenaria*, sometime in the 1970's along the New England coast of the United States (Peters, 1988). Since then, hemic neoplasias have been confirmed to occur in other species of bivalves, with the notable exception of scallops from many different geographical locations (McGladdery, pers. comm.).

An important and common feature of the disease among bivalves is the gradual appearance of neoplastic hemocytes throughout the soft tissues and consequent disruption of normal hemocyte function. In soft-shell clams where neoplastic condition is well-studied, results showed that the disease is progressive and fatal in most cases (Farley *et al*., 1986), although a small number of cases indicate chronic or even remission-like states (Cooper *et al*., 1982). The disease was initially linked to organic pollutants, but researchers have had limited

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success in consistently correlating specific contaminant history of a specific site to prevalence of hemic neoplasia (Craig *et al*., 1989). The disease can be transmitted experimentally by injecting neoplastic cells into the hemolymph of non-diseased bivalves (Kent et al., 1991; Sunila, 1992; Leavitt *et al*., 1994). Suspicion of a viral etiology has been reinforced by demonstration of reverse transcriptase activity indicative of RNA viruses, such as retroviruses that have been implicated in vertebrate neoplasia conditions (Medina *et al*., 1993). However, a viral etiology has yet to be conclusively established. Inoculation experiments done in Atlantic Canada also indicate possible transmissibility but the exact etiology of hemic neoplasia still has to be identified and may be multifactorial (McGladdery *et al*., 1993).

In the Philippines, there have been no previous reports of hemic neoplasia in any bivalve species. This paper reports for the first time the occurrence of hemic neoplasia in slipper oysters, *Crassostrea iredalei* (Faustino, 1928).

MATERIALS AND METHODS

Slipper oysters, *Crassostrea iredalei,* were obtained from the BFAR-NIFTDC demonstration farm in the town of Binmaley and Dagupan City, Pangasinan Province. Soft tissues were fixed in 10% buffered formalin in filtered ambient seawater in the laboratory. Sections (5 μm) were cut and stained with hematoxylin and eosin. Some slides were also stained with Feulgen picromethlyl blue (FPM) to better demonstrate histopathological features of the nuclei of neoplastic hemocytes in slipper oysters. Microscopic analyses were done at the Gulf Fisheries Center, Department of Fisheries and Oceans, New Brunswick, Canada in October 2001.

RESULTS AND DISCUSSION

Early and advanced stages of hemic neoplasia were observed in some samples. Hemic neoplasia in *Crassostrea iredalei* is characterized by increased numbers of morphologically altered cells circulating in the hemolymph. Affected hemocytes have large, diffuse nuclei and a lower nucleoplasm to cytoplasm ratio. Early stages of the disease showed pockets of abnormal looking hemocytes without evidence of mitotic activity. Advanced stages of hemic neoplasia were characterized by extensive hemocyte infiltration throughout the connective tissues and other organs, as well as heavy diapedesis. No mitotic figures were observed in advanced stages (Figures 1-4).

The prevalence of hemic neoplasia from the sampling survey is low (3%; n = 210), which is consistent with "normal" levels of this condition in other bivalves (Morrison *et al*., 1993; McGladdery *et al*., 2001). BFAR technicians who monitor the demonstration farm, as well as oyster farmers in the vicinity, did not report any kind of mortality. However, their observations and the low prevalence of the disease is not an assurance that the area is not prone to outbreaks of hemic neoplasia or any other type of disease condition, since the oyster culture industry is undergoing significant development. As the oyster production intensifies, several diseases, which may currently be insignificant may become more serious. Knowledge of normal levels of the condition is an essential prerequisite for assessing whether or not disease profiles are changing as the industry intensifies. This is particularly important in light of oyster culture development in the Philippines, where there is little current knowledge on oyster diseases and limited facilities available for molluscan health examination and training of aquaculture technicians and farmers.

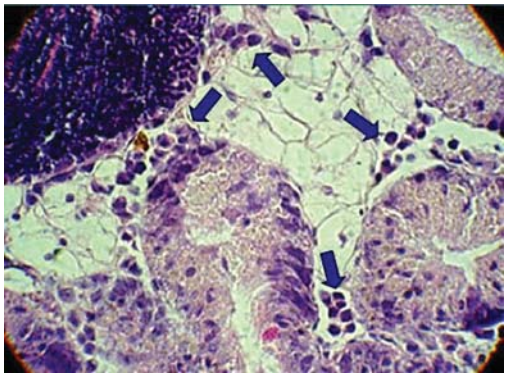

Figure 1. Early stage of hemic neoplasia in *C. iredalei.* Affected hemocytes (arrows) are highly basophilic. HHE stain (630x).

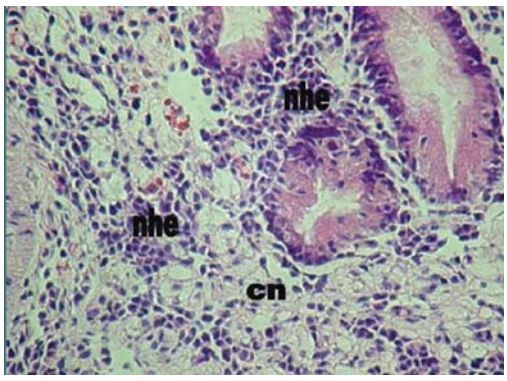

Figure 2. Advanced stage of hemic neoplasia in *C. iredalei.* Infiltration in the connective tissue (cn) of *C. iredalei* by neoplastic hemocytes (nhe). Affected hemocytes (nhe) have large diffuse nuclei and relatively little cytoplasm compared to normal hemocytes. No mitotic figures were observed. HHE stain (630x).

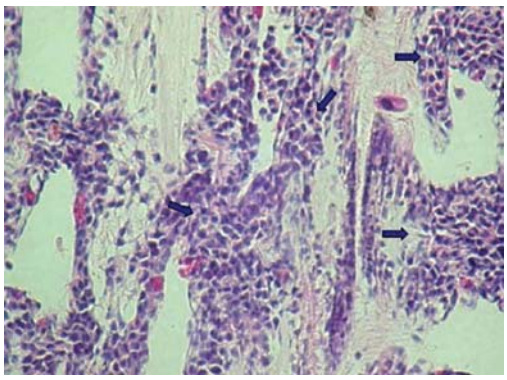

Figure 3. Massive aggregation of neoplastic hemocytes (arrows) disrupting the epithelium of the gills in *C. iredalei.* HHE stain (250x).

Figure 4. Neoplastic hemocytes in the connective tissue of *C. iredalei.* Neoplastic hemocytes have very little cytoplasm in relation to nucleoplasm with enlarged pleomorphic nuclei. nh = normal hemocyte; nhe = neoplastic hemocyte. FPM stain (1000x).

SUMMARY AND RECOMMENDATIONS

A preliminary survey of diseases in farmed oysters from around Dagupan City showed that hemic neoplasia is present in cultured slipper oysters, *Crassostrea iredalei.* This condition is known to be fatal to bivalves such as soft-shell clams, *Mya arenaria*, and blue mussels, *Mytilus edulis*, due to the invasion of normal tissue (such as gill, gonad and digestive gland) by non-functional blood cells. The energetic costs of hemocyte proliferation and displacement of functional hemocytes via diapedesis as well as neoplastic transformation, leads to weakening and eventual mortality. A few cases revealed small foci of neoplastic hemocytes, that may indicate that the condition in some slipper oysters is non-proliferative and may be relatively benign. Likewise, even heavily affected specimens showed no clear evidence of mitotic figures that would indicate a clearly proliferative condition. More studies are required to determine the potential of this condition to impact developing slipper oyster culture, or not. This should include transmission investigations to determine whether affected oysters can spread the condition to uninfected oysters. This is particularly important as high concentrations of single species are developed for culture purposes.

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