CO-INFECTION WITH A *Hematodinium*-LIKE DINOFLAGELLATE AND A YEAST-LIKE ORGANISM IN EDIBLE CRABS (*Cancer pagurus*): A CONSEQUENCE OF IMMUNOSUPPRESION?

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Figure 1: Crabs are captured in pots baited with fish. After hauling, the pots are re-baited and deployed back onto the seabed.

Figure 2: The edible crab (*Cancer pagurus*)

The European edible crab (*Cancer pagurus*) supports a large and valuable fishery in UK waters (27, 000t; £32m in 1999). This species lives on rocky and muddy substrates around much of the European coastline and is usually captured in pots baited with fish. About half of all crabs landed in British ports are transported by road to service the continental European market for this species. The muscle of the claws, and the hepatopancreas and ovary in the main body cavity, are harvested as the commercial meat product.

Discussion

Crustaceans (and other invertebrates) lack the specific immunoglobulins that higher vertebrates use for disease protection. As such, their 'immune' response towards pathogens centres on the circulating haemocytes and their production of the prophenoloxidase (proPO) activating system - a biochemical pathway comprising a cascade of serine proteases and other factors. This cascade is also thought to liberate other antibacterial, antifungal and lytic substances, to participate in nodule formation (melanisation) and to mediate cell-cell co-operation. Yeast infections have often been associated with the immunosuppressive effects of stress and disease in animals and humans. Yeast infections have also been associated with the stressful conditions that can accompany the culture of aquatic species (such as prawns). In the current study, we have shown that crabs that are infected with the dinoflagellate parasite *Hematodinium* sp. can also harbour a secondary infection by a yeast-like organism. This is the first record of a yeast infection in crabs. It is known that the haemocyte count of crabs infected with *Hematodinium* sp. and other similar parasites is severely reduced. Under such conditions, and with the relative absence of an efficient proPO cascade system in place, it is unlikely that the remaining cells can phagocytose and lyse the proliferating forms of the yeast at a rate sufficient to prevent disease manifestation. As such, the pathology associated with the yeast infection in edible crabs is severe, with a massive proliferation of free stages in the blood and a blockage of haemal sinuses within the major organs and tissues. It is likely that co-infections of this type exacerbate the pathological progression of the primary disease (in this case *Hematodinium* sp.) and may thus be at least partially responsible for the significant mortalities assigned to the primary infection. We propose that further studies on secondary and opportunistic infections of wild and cultured crustaceans species are required and that invertebrate models may be particularly useful in studying the pathological progression of diseases in animals held under stressful conditions.

Figure 3: Hepatopancreas of velvet crab infected with *Hematodinium* sp. Tubules are surrounded by haemolymph containing masses of parasitic cells (H). Tubule lumen (Tlu); Tubule epithelium (Tep). Scale bar = 100 µm (H&E).

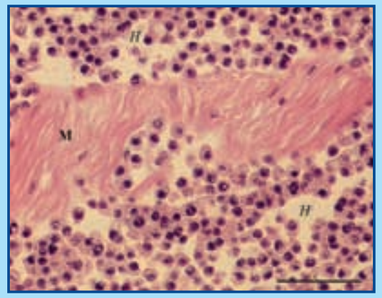

Figure 4: Claw muscle of edible crab infected with *Hematodinium* sp. Note the islands of unattached claw muscle (M) surrounded by large numbers of parasitic cells (*H*). Scale bar = 50 µm (H&E).

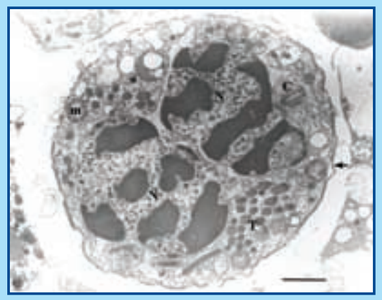

Figure 5: Bi-nucleate *Hematodinium* sp. cell in the haemolymph of edible crab. Note the condensed chromatin in the paired nuclei (N), the presence of trichocysts (T), mitochondria (M), centriole (C) and alveolar membrane (short arrow). Scale bar = 1 µm (TEM).

During the winter, edible crabs in the English Channel can harbour an infection by a *Hematodinium*-like dinoflagellate. This infection is responsible for a disease that has been termed 'Pink Crab Disease' (PCD) in this species (Stentiford *et al*., 2002). Velvet crabs (*Necora puber*), another commercially exploited crab species can also harbour *Hematodinium* sp. infections. The histopathological manifestation of the infection in both species is severe, with a replacement of normal blood cells by parasites, and destruction of the muscle and hepatopancreas. The parasite causes severe physiological and biochemical disruption in the crab and probably contributes significantly to natural mortality in the field.

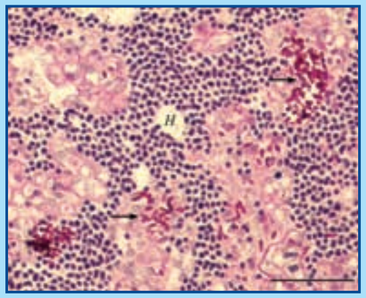

Figure 6: Hepatopancreatic sinus in velvet crab. Note the presence of large numbers of *Hematodinium* sp. cells (*H*) and budding yeast cells contained within haemocytes, and free within blood vessels and sinuses. Scale bar = 100 µm (PAS).

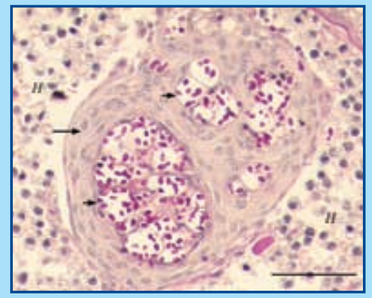

Figure 7: Hepatopancreatic sinus in edible crab. Note the presence of an inflammatory encapsulation response (long arrows) surrounding haemocytes infected with yeast-like cells (short arrows), some of which are budding. Large numbers of *Hematodinium* sp. cells (*H*) surround the lesion. Scale bar = 50 µm (PAS).

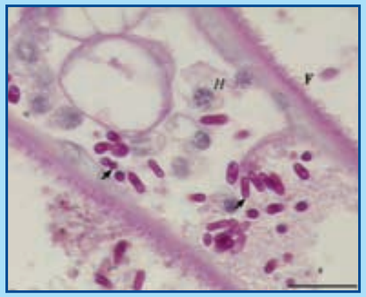

Figure 8: Gill lamella of edible crab. Yeast-like cells (short arrows) and *Hematodinium* sp. cells (*H*) are present in the haemolymph. Filamentous bacterial growth can be seen at the lamella-water interface (F). Scale bar = 20 µm (PAS).

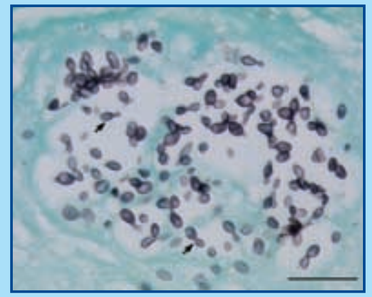

Figure 9: Hepatopancreatic sinus in edible crab. Cell wall polysaccharides are converted by oxidation to dialdehydes and are thus detected with the hexamine silver stain. Budding cells are indicated (short arrows). Scale bar = 20 µm (Grocott-Gomori methenamine silver stain).

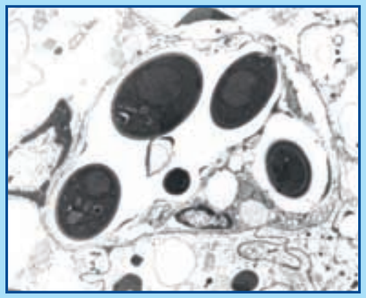

Figure 10: Haemocyte of edible crab. Cells were often seen to contain yeast-like organisms within their cytoplasm. Scale bar = 1 µm (TEM).

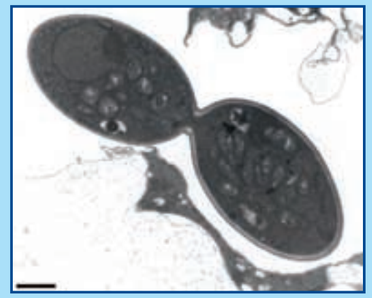

Figure 11: Budding yeast-like organism in the haemolymph of edible crab. Scale bar = 1 µm (TEM).

In the current study, a number of edible and velvet crabs with *Hematodinium* sp. infections also harboured a secondary infection of by a yeast-like organism. The yeast stained positively with PAS and silver stains. This is the first description of a yeast infection in crabs. Haemocytes were seen to contain budding yeast cells, while free forms of the yeast (also budding) were observed in the haemolymph and tissue interstices. Blockage of tissue sinuses, particularly in the hepatopancreas was a common histopathological consequence of this infection.

Reference

Stentiford, G.D., Green, M., Bateman, K., Small, H.J., Neil, D.M. and Feist, S.W. (2002). Infection by a *Hematodinium*-like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab *Cancer pagurus*. Journal of Invertebrate Pathology (submitted).

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