



# Infection by a *Hematodinium*-like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab *Cancer pagurus*

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## Abstract

The edible crab (*Cancer pagurus*) supports a large and valuable fishery in UK waters. Much of the catch is transported live to continental Europe in specially designed live-well ('vivier') vehicles. During the winter of 2000/2001, many trap-caught crabs from Guernsey, Channel Islands, UK, were reportedly moribund and pink in colour. These crabs generally died before and during vivier transportation. We provide histological, immunological, and molecular evidence that this condition is associated with infection by a *Hematodinium*-like dinoflagellate parasite similar to that previously reported in *C. pagurus* and to an infection causing seasonal mass mortalities of the Norway lobster (*Nephrops norvegicus*). Pathologically, every altered host bore the infection, which was characterised by very large numbers of plasmodial and vegetative stages in the haemolymph and depletion of reserve cells in the hepatopancreas. Due to the hyperpigmentation of the carapace and appendages, we have called this infection 'Pink Crab Disease' (PCD). Similar *Hematodinium* infections cause 'Bitter Crab Disease' in tanner and snow crabs, which has had a negative effect on their marketability. At present, little is known about the seasonality, transmission, and market impact of this infection in *C. pagurus*. © 2002 Elsevier Science (USA). All rights reserved.

**Keywords:** *Cancer pagurus*; Crab fishery; Dinoflagellate; *Hematodinium*; Histopathology; *Nephrops norvegicus*; Mortality; Parasite; PCD; Pink Crab Disease

## 1. Introduction

The edible crab (*Cancer pagurus*) is one of the most valuable shellfish species captured in European waters, with a large fishery existing in the waters surrounding the United Kingdom (landings of over 27,000 t, worth £32 m in 1999—UK Sea Fisheries Statistics, 1999). Of the crabs landed in the United Kingdom, some are processed and sold locally, while a significant quantity, around 14,000 t in 1999 (UK Sea Fisheries Statistics, 1999), are transported live to continental Europe in specially designed live-wells known as 'viviers.' To ensure sale, exported crabs must be alive and in good condition on arrival at market.

Crustaceans are often exposed to an array of stressors during and after capture, which include crowding, me-

chanical damage to the cuticle, and exposure to light, air, and heat (see Chang et al., 1999; Jussila et al., 1997; Morris and Airriess, 1998; Paterson and Spanoghe, 1997; Stentiford and Neil, 2000). Another important stressor is infection by pathogens (for review, see Thompson, 1983). Considerable post-capture mortalities in holding tank conditions have been reported from decapods following epizootic infections by viruses (Arcier et al., 1999), bacteria (Cheng and Chen, 1998; Stewart, 1980), and ciliates (Armstrong et al., 1981; Bang et al., 1972; Cawthorn, 1997). In other cases, large-scale mortalities have occurred due to unknown agents or to idiopathic phenomena (see Anderson et al., 1990; Lindqvist and Mikkola, 1978; Stentiford and Neil, 2000).

The parasitic dinoflagellates of marine crustaceans are known to inhabit the eggs, stomach, soft tissue, and haemal sinuses of their hosts (Shields, 1994). Infections by parasitic dinoflagellates of the genus *Hematodinium*

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have been reported in a number of commercially important crustacean hosts (Field et al., 1992; Hudson and Lester, 1994; Hudson and Shields, 1994; Maclean and Ruddell, 1978; Messick, 1994; Meyers et al., 1987; Newman and Johnson, 1975; Taylor and Khan, 1995; Wilhelm and Boulo, 1998; Wilhelm and Mialhe, 1996). Latrouite et al. (1988) have also reported an infection by a *Hematodinium*-like parasite in populations of *C. pagurus* taken from the English Channel, the Irish Sea, the Bay of Biscay, and the west coast of Scotland. In these cases, the haemolymph and muscle of affected crabs assumed a pink colouration, with the meat having an irregular texture and a bitter taste when cooked. Similar features of infection have been ascribed to *Hematodinium* infections in tanner crabs (*Chionoecetes bairdi* and *C. opilio*), where the condition, termed 'Bitter Crab Disease' renders the meat unmarketable (Meyers et al., 1987; Taylor and Khan, 1995).

During the autumn and winter 2000, creel-caught *C. pagurus* from the west coast of Cornwall and from the island of Guernsey displayed an altered colouration (pink hyperpigmentation) with a general morbidity. These crabs would usually die following handling, pounding, and subsequent vivier transportation. Signs of this condition were also noted in animals taken directly from creels. We provide histological, ultrastructural, and molecular evidences that PCD is associated with an infection by a *Hematodinium*-like dinoflagellate which is probably the same as that described by Latrouite et al. (1988) and provide recommendations for future monitoring and pathological studies.

## 2. Methods

*Cancer pagurus* were captured from the south coast of Guernsey (UK Channel Islands; 49°24'N, 2°32'–2°43'W) using conventional crab pots. Crabs were transported to the shore, following which tissues and organs were removed from crabs showing signs of PCD (morbid, pink hyperpigmented carapace) and from apparently healthy crabs. These were prepared for light and electron microscopy, immunological parasite detection, and molecular characterisation using standard methods.

### 2.1. Histopathology

Crabs were anaesthetised by chilling to 4°C. For histopathology, the hepatopancreas, claw muscle, gill, gonad, hindgut, and heart of infected and uninfected crabs were removed and placed immediately into Davidson's seawater fixative (see Hopwood, 1996). Fixation proceeded for 24 h before samples were transferred to 70% industrial methylated spirit (IMS).

For electron microscopy, small pieces of tissue were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) with 1.75% sodium chloride for 2 h at room temperature (21°C). Fixed tissue samples were rinsed in 0.1 M sodium cacodylate buffer with 1.75% sodium chloride (pH 7.4) and post-fixed in 1% osmium tetroxide, reduced with 1.75% potassium ferrocyanide in 0.1 M sodium cacodylate buffer for 1 h at 4°C. Specimens were washed in three changes of 0.1 M sodium cacodylate buffer and stained en bloc in 0.5% aqueous uranyl acetate for 1 h. Following dehydration through an acetone series, specimens were embedded in epoxy resin 812 (Agar Scientific-pre-mix kit 812). Semi-thin sections (1–2 µm) were stained with toluidine blue for viewing with a light microscope, suitable areas were identified and ultrathin sections (70–90 nm) of these areas were cut and mounted on uncoated copper grids. Sections were stained with uranyl acetate and Reynolds lead citrate (Reynolds, 1963) and were examined using a JEOL 1210 transmission electron microscope.

### 2.2. Immunological characterisation

A 70 mg sample of Davidson's-seawater-fixed *C. pagurus* hepatopancreas was macerated in 300 µl sample buffer (62.5 mM Tris-HCl pH 6.8, 12.5% glycerol, 1.25% β-mercaptoethanol) and heated at 95°C for 4 min. Lanes on a standard acrylamide gel (12.5%) were loaded with either 20 µl of whole sample or 20 µl of supernatant from a centrifuged sample (17,000g, 2 min). These were analysed for the presence of *Hematodinium* antigens using a polyclonal antibody raised against the *Nephrops norvegicus* isolate of *Hematodinium* (see Field and Appleton, 1996) applied by the Western blotting method of Stentiford et al. (2001c) with *Hematodinium*-infected *N. norvegicus* haemolymph run as a positive control (see Stentiford et al., 2001c). *Hematodinium*-positive samples generally appeared as multiple-bands or smears due to the reaction of numerous parasite proteins to the polyclonal antibody (as described by Stentiford et al., 2001c).

### 2.3. Molecular characterisation

Aliquots of 100 mg samples of fixed *C. pagurus* hepatopancreas from asymptomatic crabs and those showing the signs of PCD, were homogenised separately in 500 µl extraction buffer (50 mM Tris, 5 mM EDTA, 100 mM NaCl, and pH 8), 200 µl of 10% SDS, and 20 µl Proteinase-K (10 µg/ml) and incubated at 56°C for 24 h. DNA was purified by phenol/chloroform extraction, ethanol precipitated, and resuspended in sterile deionised water. PCR reactions were performed in 20 µl total reaction volume by adding 2 µl of 10X reaction buffer (final concentration 10 mM Tris-HCl, pH 9, 50 mM KCl, 0.1% Triton X-100), 1.2 µl MgCl<sub>2</sub> (final concentration 1.5 mM), 1 µl dNTP mix (final concentration

100  $\mu\text{M}$ ), 1  $\mu\text{l}$  each of forward and reverse primers (final concentration 0.5  $\text{pmol}\mu\text{l}^{-1}$ ), volume of target DNA to approximately 100 ng, 1 unit of *Taq* polymerase, and sterile deionised water to a final volume of 50  $\mu\text{l}$ . Reactions were overlaid with 10  $\mu\text{l}$  mineral oil.

Thermal cycling conditions were as follows: denaturation at 94  $^{\circ}\text{C}$  for 1 min; primer annealing at 52  $^{\circ}\text{C}$  for 1 min; chain extension at 70  $^{\circ}\text{C}$  for 3 min; repeated for 35 cycles with a final cycle incorporating a 7 min extension. Primer sequences were as described by Hudson and Adlard (1994). Amplification products were run on a 1.5% agarose gel, stained with ethidium bromide and viewed under a UV light source.

### 3. Results

#### 3.1. Gross clinical signs of PCD

Crabs showing clinical signs of PCD were moribund and were reported to die quickly following capture and during transit. Heavily infected crabs typically displayed hyperpigmentation (pink) of the carapace and discolouration (yellowing) of the arthrodial membranes and the genital pores. This yellowing was later found to be

due to the creamy consistency and colouration of the haemolymph caused by infection with large numbers of single, bi-, and multi-nucleate plasmodia. Internally, organs and tissues were friable, with creamy multicellular parasite deposits covering their outer surfaces.

#### 3.2. Histopathology

Histologically, during severe PCD, the haemal sinuses of the hepatopancreas was heavily dilated and filled with large numbers of plasmodial cells of the parasite which had condensed chromatin profiles (Fig. 1). The hepatopancreatic tubule cells of infected crabs were relatively devoid of lipid reserves and in a number of specimens, hepatopancreatic tubule cells appeared degenerate with plasmodial forms of the parasite observed within the lumen of the tubules (Fig. 2). Due to the severely dilated haemal sinuses, haemolymph vessels, and their associated fixed phagocytes were rarely observed.

Pathological changes to the muscles of the claw and the body cavity were extensive. In the claws of infected crabs, muscle tissue was almost completely replaced by large numbers of plasmodia, with only small islands of identifiable muscle fibres remaining (Fig. 3). Intense

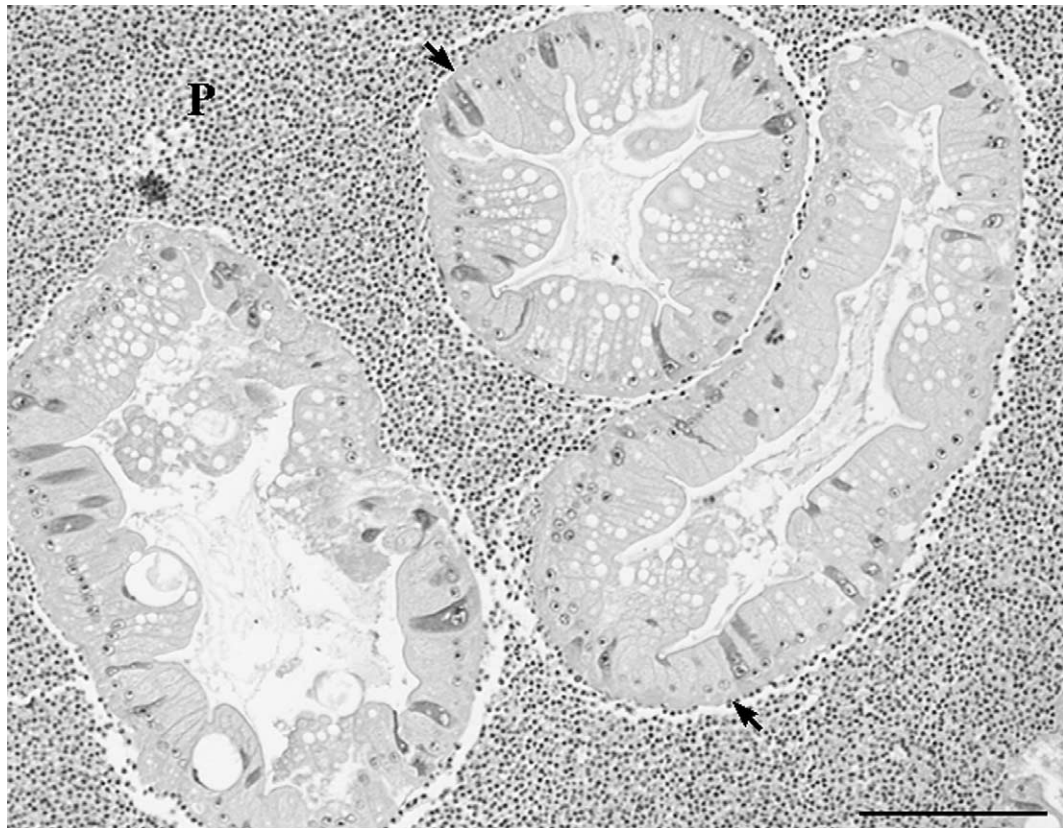


Fig. 1. Hepatopancreas of crab with PCD. Note the dilated haemal sinuses filled with masses of parasitic plasmodial cells (P). Vessels and associated fixed phagocytes were rarely observed and reserve cells were not observed in the inter-tubular connective matrix. Tubule cells were often seen to be devoid of lipid reserves (arrows). Haematoxylin and Eosin, 5  $\mu\text{m}$  section. Bar = 200  $\mu\text{m}$ .

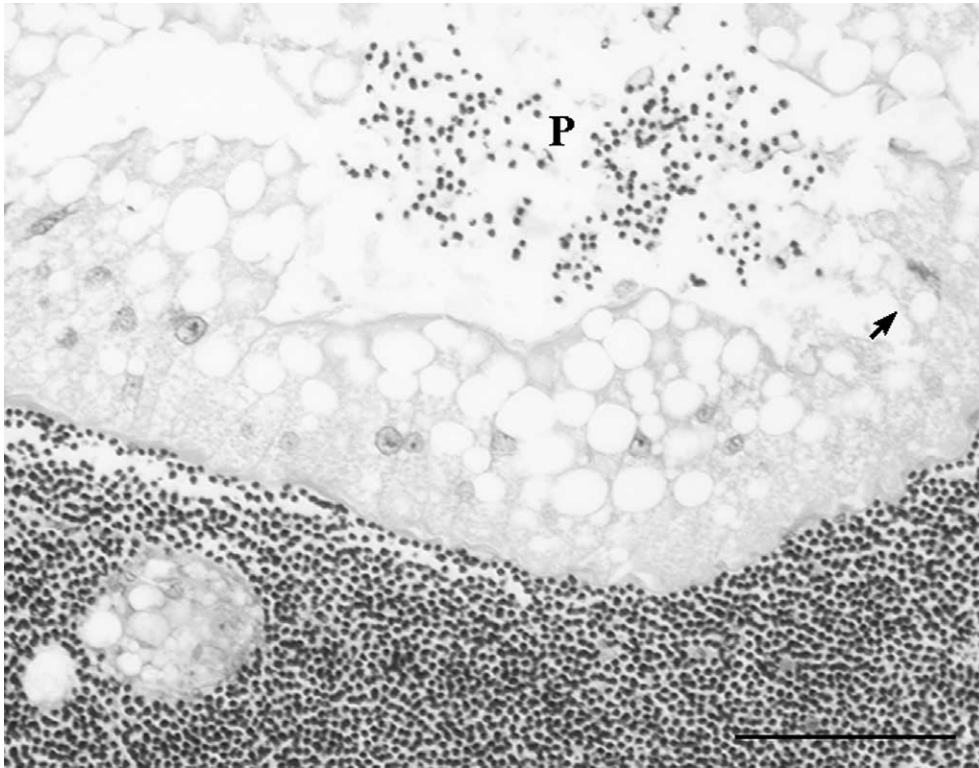


Fig. 2. Hepatopancreas of crab with PCD. Note the degraded nature of tubule cells (arrow) and the invasion of the tubule lumen by parasitic plasmodial cells (P). Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 100  $\mu$ m.

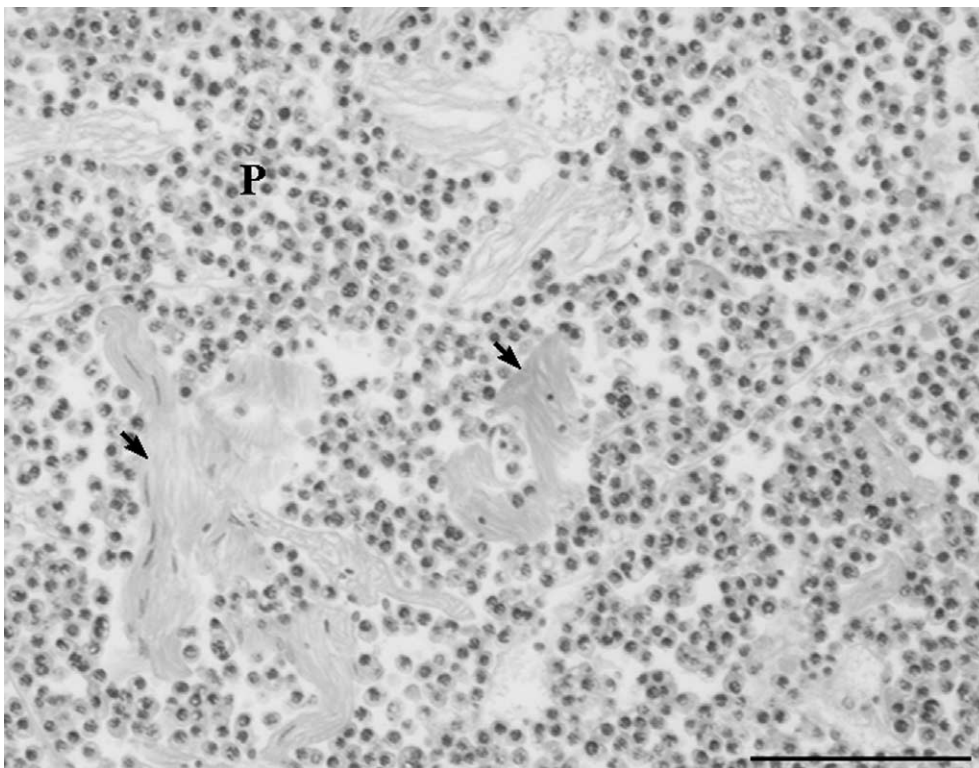


Fig. 3. Claw muscle of crab with PCD. Note the 'islands' of unattached muscle blocks (arrows) surrounded by masses of parasitic plasmodial cells (P). Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 100  $\mu$ m.

multi-focal inflammatory granulomas were commonly seen in the claw tissue of heavily infected crabs (Fig. 4). Similar encapsulation responses were observed in the myocardium and occasionally in the pericardium of the heart (Fig. 5) and in the connective material surrounding the gut (Fig. 6). Melanised nodules were rarely observed within the gill lamellae. Reserve (RI) cells were not observed in any crabs showing the signs of PCD. The ovary of female crabs showing the signs of PCD was heavily infiltrated by masses of parasitic plasmodial cells. Vitellogenic oocytes were not observed in any of the infected female crabs studied (Fig. 7).

### 3.3. Ultrastructure

Electron microscopy revealed that the haemolymph and tissues of crabs with PCD harboured a *Hematodinium*-like dinoflagellate parasite similar to that previously described in *N. norvegicus* (Field et al., 1992). Plasmodia typically had condensed chromatin profiles (up to 5 nuclei per plasmodia), abundant lipid droplets, membrane-bound trichocysts, and mitochondria, and a surrounding alveolar membrane. The centriolar apparatus was observed in a number of parasites (Fig. 8). Plasmodia were frequently found in close association with the outer surface of the hepatopancreatic tubules and with the muscle sarcolemma. Remnants of degen-

erated host tissue (such as atrophied mitochondria, myelin bodies, and membranous material) were often found surrounding plasmodia at the periphery of the remaining tissue (Figs. 9 and 10). The pathology of muscle breakdown was characterised by a severe disorganisation of filaments in the region of the Z-line (Fig. 11) followed by an increase in the sub-sarcolemmal space (see Fig. 10).

### 3.4. Immunological characterisation

Western blots of proteins extracted from the hepatopancreas of crabs with PCD were performed using a primary antibody raised against *Hematodinium* isolated from *N. norvegicus*. Tissue from infected crabs showed a clear positive reaction to this polyclonal anti-*Hematodinium* antibody, characteristically as a multi-band smear (Fig. 12).

### 3.5. Molecular characterisation

PCR amplification of the first internal transcribed spacer (ITS1) region of ribosomal DNA and flanking 3' end of the small subunit (SSU) was achieved using primer sequences previously used for the diagnosis of *Hematodinium* infections in other crustacean species (Hudson and Adlard, 1994). A single 680 bp amplifica-

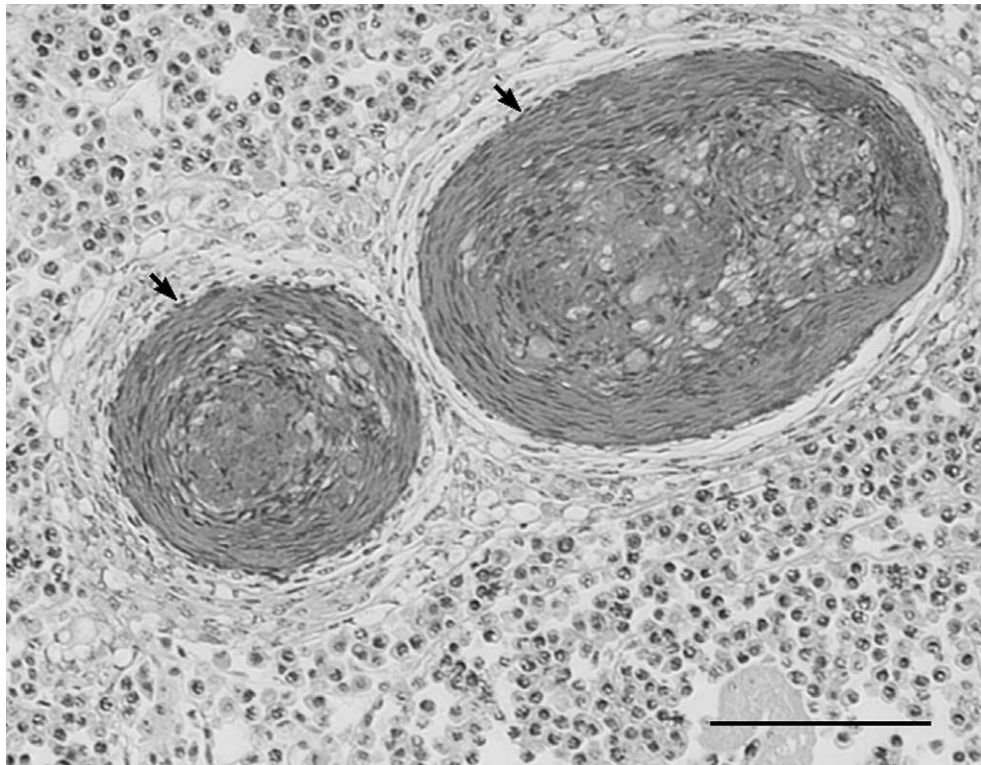


Fig. 4. Two large focal granulomas (arrows) in the claw muscle of a crab with PCD. Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 100  $\mu$ m.

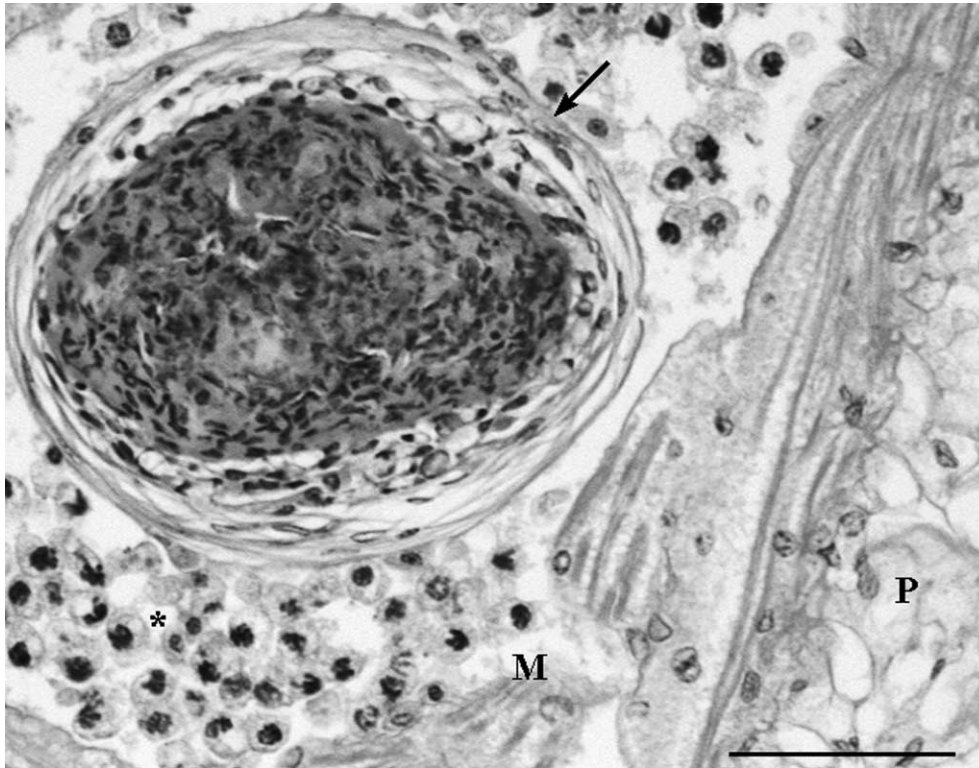


Fig. 5. Heart of crab with PCD. Note the pericardium (P) and the myocardium (M) containing a large melanised granuloma (arrow). In some crabs, multiple granulomas were observed throughout the myocardium and occasionally within the pericardium. Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 50  $\mu$ m.

tion product was produced in crabs showing the symptoms of PCD, while no reaction product was seen in crabs asymptomatic for PCD (Fig. 13).

#### 4. Discussion

##### 4.1. Aetiology

The histological, ultrastructural, and molecular data presented in this study have shown that PCD is caused by a parasitic dinoflagellate of the genus *Hematodinium* and formally confirms the presence of this parasite in the English Channel fishery for *C. pagurus*. Furthermore, application of *Hematodinium*-specific primers led to the appearance of a 680 bp PCR amplification product from the ribosomal DNA of this parasite, suggesting a strong similarity to the *Hematodinium* strains isolated from other crustacean species (see Hudson and Adlard, 1994). It is highly likely that this is also the same organism as previously reported to colonise the haemolymph of *C. pagurus* captured from various European locations (Latrouite et al., 1988) and similar to that reported from a number of other commercially important crustacean hosts (Field et al., 1992; Hudson and Lester, 1994; Hudson and Shields, 1994; Maclean and Ruddell, 1978; Messick, 1994; Meyers et al., 1987; Newman and

Johnson, 1975; Taylor and Khan, 1995; Wilhelm and Boulo, 1998). Whether this parasite is the same as the *Hematodinium*-like species thought to be responsible for commercially significant declines in populations of velvet swimming crab (*Necora puber*) from the English Channel remains to be shown (see Wilhelm and Mialhe, 1996).

Uni-cellular, bi-cellular, and multi-cellular (up to 5 nuclei) stages of the parasite were observed in the haemolymph and within the tissue interstices of crabs with PCD. In their description of the type species, *Hematodinium perezii*, in portunid crabs captured from French waters, Chatton and Poisson (1931) describe motile stages within the haemolymph (a similar finding to that of Newman and Johnson, 1975; Messick, 1994 and Shields and Squyars, 2000, in *C. sapidus*). In addition, Appleton and Vickerman (1997) have described motility in the *Hematodinium* sp. isolated and cultured from *N. norvegicus*, suggesting that such motile forms may represent the early trophont stages in all *Hematodinium* species. Observations of fresh haemolymph preparations of low-level *Hematodinium* infections are required if these findings are to be confirmed. In addition, further molecular and ultrastructural comparisons of *Hematodinium*-like sp. to the type species (*H. perezii*) are required to apply accurate taxonomic status to these isolates.



Fig. 6. Transverse section through the hindgut of crab with PCD. The gut lumen (L) contains food material and multiple large melanised granules were commonly seen in the connective tissue (C) (long arrow). Separation of gut epithelium (short arrow) from basement membrane is likely to be an artefact of tissue preparation. Haematoxylin and Eosin, 5  $\mu\text{m}$  section. Bar = 100  $\mu\text{m}$ .

Anecdotal reports suggest that PCD may show a seasonal epizootiology, with peak infection occurring during the winter and the spring, and with a latent infection or absence during the summer and early autumn (G.D. Stentiford, personal observation). Observations on the prevalence of *Hematodinium* infection in other crab species also suggest highly seasonal disease outbreaks, with peak infection occurring over a relatively narrow time period, followed by a longer period of undetectable or low level prevalences (see Messick and Shields, 2000; Shields, 1994). Studies on Scottish *N. norvegicus* populations have revealed similar features of *Hematodinium* infection epizootiology (Field et al., 1992, 1998; Stentiford et al., 2001b).

#### 4.2. Pathology

Crabs infected with PCD revealed significant alterations from the normal structure of muscle and hepatopancreatic tissue. The alteration in the lipid content of hepatopancreas tubule cells during PCD is consistent with a progressive parasite-induced physiological starvation. The absence of reserve (RI) cells in the connective tissue throughout crabs with PCD confirms this hypothesis. Similar effects of *Hematodinium* infection have pre-

viously been reported in *N. norvegicus* (Stentiford et al., 1999, 2000, 2001a) and in *Callinectes sapidus* (Whittington et al., 1997). Histology revealed that the tubule cells of the hepatopancreas were frequently degenerate, possibly explaining the presence of parasitic plasmodia within the lumen of tubules themselves. This feature of *Hematodinium* infection has also been reported in *N. norvegicus* (Field and Appleton, 1995). The significance of the presence of parasites within the tubule lumens is not presently known, though this may reflect a possible route of transmission (via the gut) to other hosts. In addition to their presence within the lumens of the tubules, parasites were also observed in close association with the myoepithelial layer surrounding these tubules. Whether these cells are attached to the tubule surface or whether their presence is an artefact of tissue preparation is difficult to elucidate. However, similar features of *Hematodinium* sp. infection have previously been reported in *N. norvegicus* (Field and Appleton, 1995). In this species, it has been suggested that the hepatopancreas and other tissues may represent the seat of latent *Hematodinium* infection (Field and Appleton, 1995, 1996; Stentiford et al., 2001c). Further studies of apparently uninfected *C. pagurus*, possibly out of the main infection period for this disease may allow such latent stages to be located.

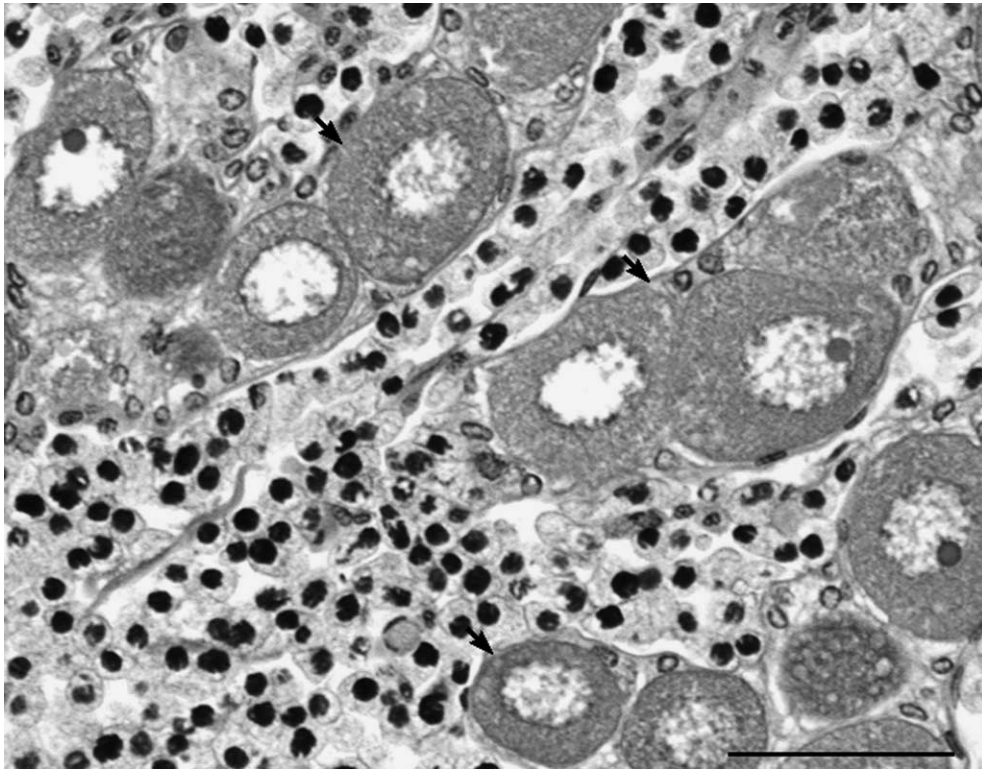


Fig. 7. Ovary of female crab with PCD. Note the presence of pre-vitellogenic oocytes (short arrows) and the presence of masses of parasitic plasmodial cells between the oocytes. Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 50  $\mu$ m.

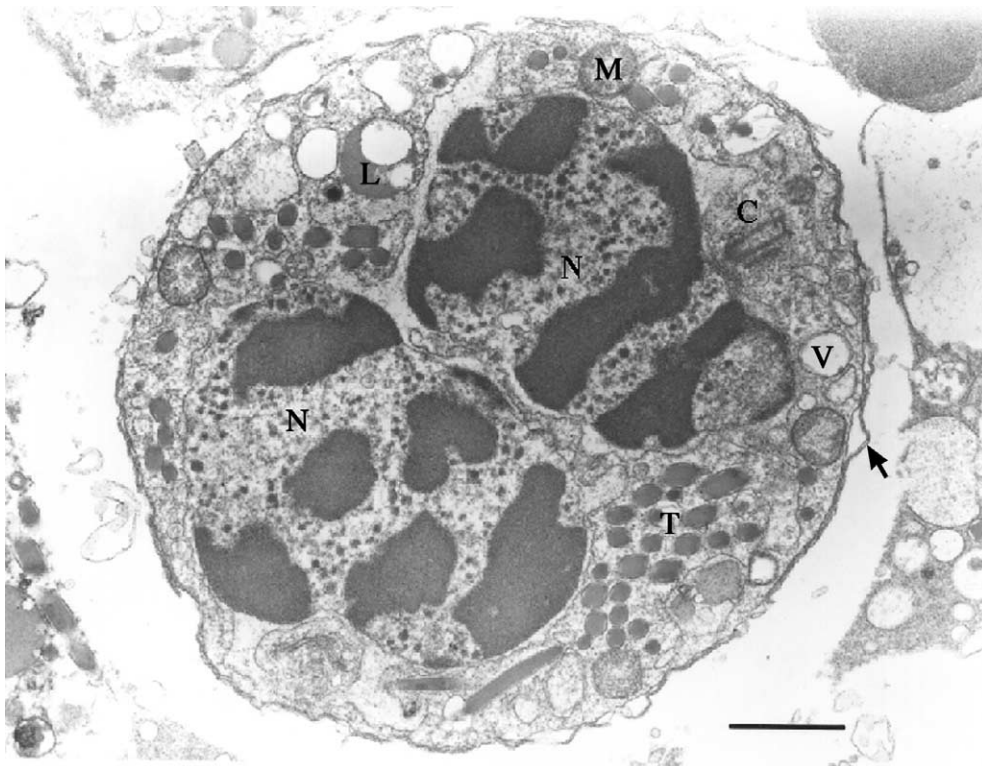


Fig. 8. Bi-nucleate parasitic plasmodium in the haemolymph of a crab with PCD. Note the presence of lipid droplets (L), trichocysts (T), mitochondria (M), vacuoles (V), centriole apparatus (C), and a surrounding alveolar membrane (arrow). Parasites typically contained between one and four nuclei (N). TEM, scale bar = 1  $\mu$ m.



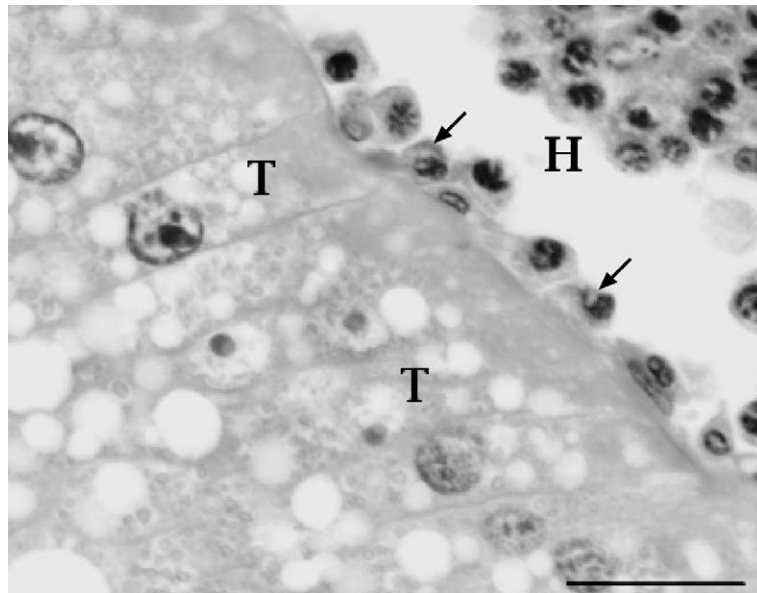


Fig. 9. Parasitic plasmodial cells at the periphery of a hepatopancreatic tubule. Note the close association of parasites (arrows) and tubule cells (T). Parasitic plasmodial cells filled the haemal spaces (H). Haematoxylin and Eosin, 5  $\mu$ m section. Bar = 50  $\mu$ m.

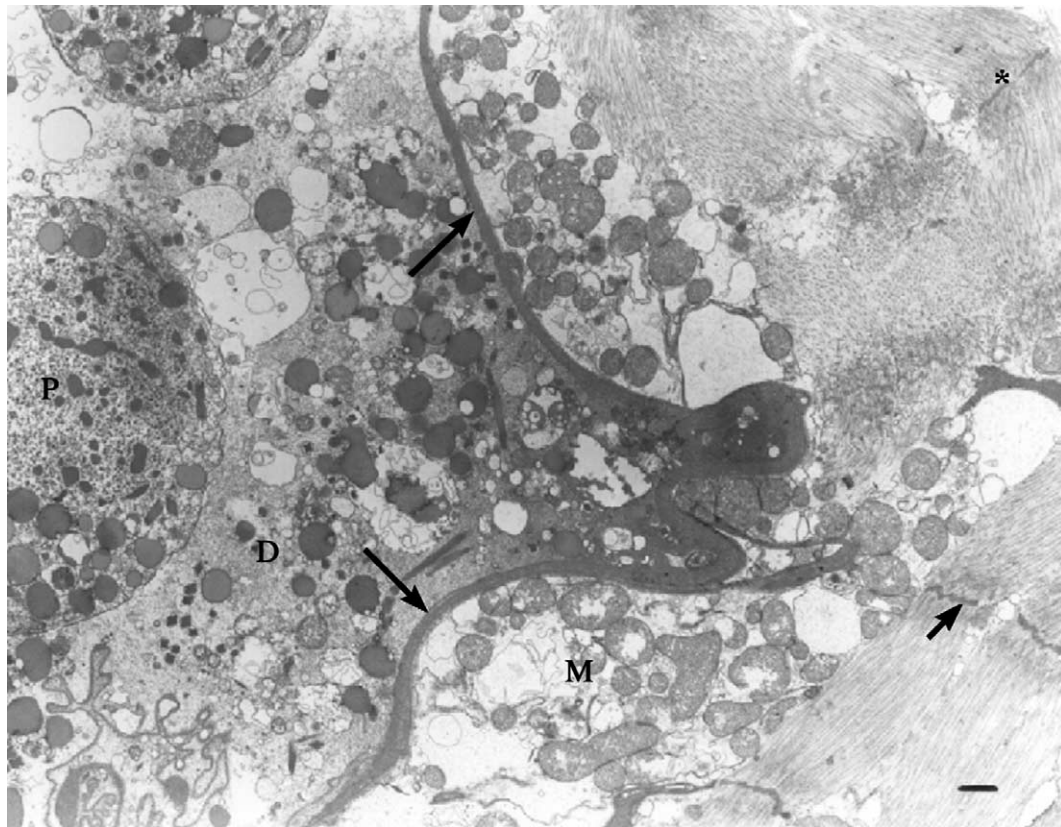


Fig. 10. Claw muscle of crab with PCD. Parasitic plasmodial cells (P) were often seen in close association with the sarcolemmal membrane (arrows) which was often well separated from the contractile muscle blocks. Note the presence of mitochondria at the muscle periphery (M) and the presence of Z-lines in various states of degeneration (arrow and asterisk). Cellular debris of host and parasite origin was commonly seen on the surface of the sarcolemma (D). TEM, scale bar = 1  $\mu$ m.

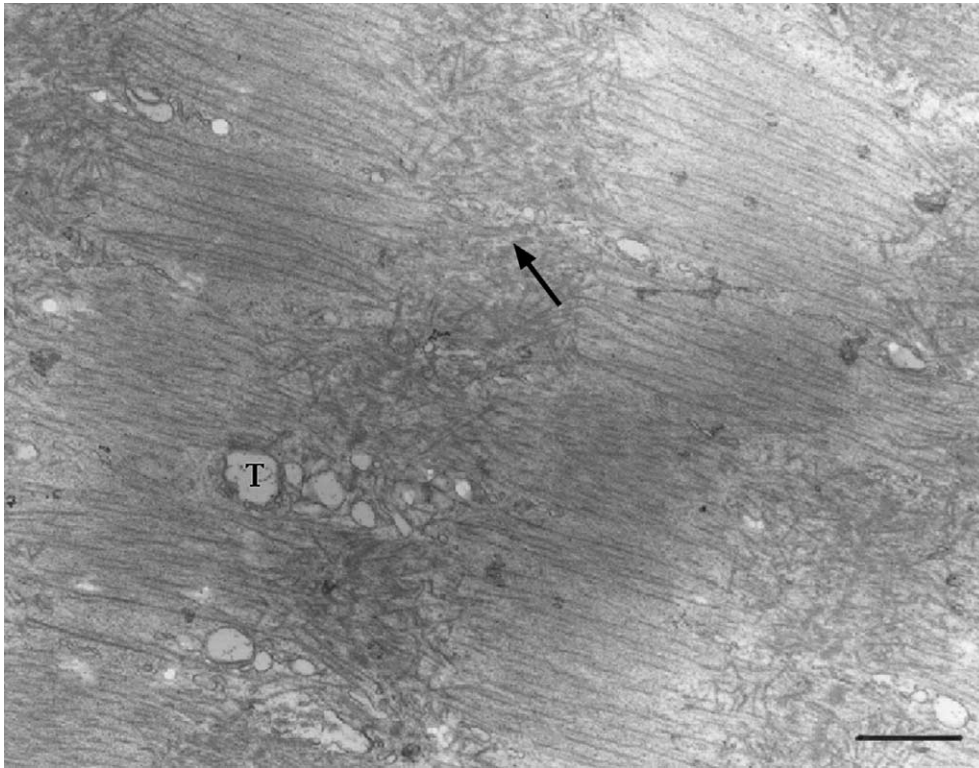


Fig. 11. Claw muscle of crab with PCD. Note the disorganisation of fibrils in the region of the Z-lines (arrow) and remnants of the tubular system (T). TEM, scale bar = 1  $\mu$ m.

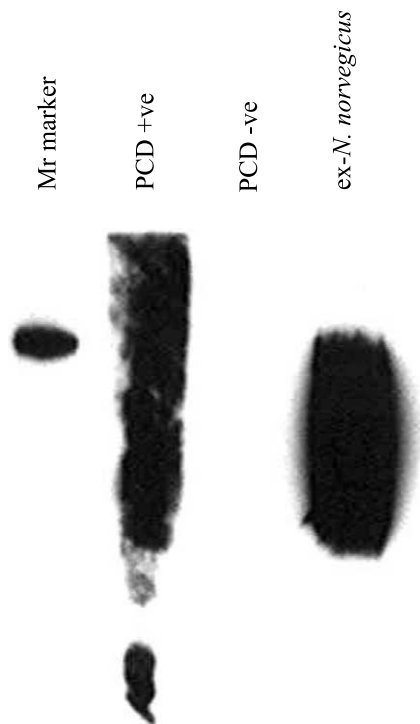


Fig. 12. Western blot of hepatopancreas from crab with PCD (*C. pagurus*) using anti-*Hematodinium* (ex-*Nephrops norvegicus*) polyclonal primary antibody. Multiple band or smear reactions were seen in PCD +ve crabs. No reaction was seen in PCD -ve crabs. In vitro cultured *Hematodinium* (ex-*N. norvegicus*) was used as a positive control.

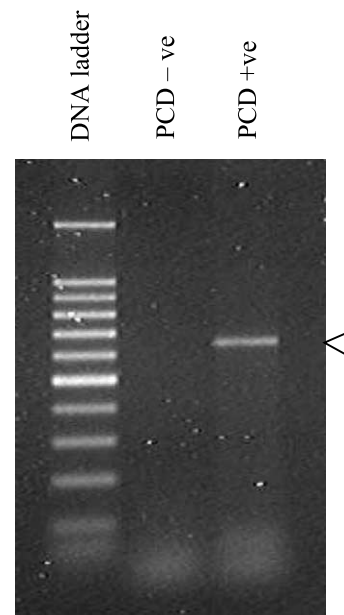


Fig. 13. Agarose gel showing 680 bp amplification product (arrowhead) from hepatopancreas of crab exhibiting the symptoms of PCD (PCD +ve). No amplification product was seen in uninfected crabs (PCD -ve). DNA marker also shown.

Muscle tissue, particularly that found within the claws, was almost completely destroyed in crabs with PCD, with 'islands' of apparently unattached muscle

tissue surrounded by masses of parasitic plasmodial cells. Granuloma-like foci of hyalinocytes were observed within the remaining blocks of claw muscle and also within the heart and surrounding the gut. Such foci have been described as aggregations of flattened hyaline cells encapsulating foreign material and which lead to the deposition of melanin either on the object or within the haemocyte matrix. In the case of parasite infection, the parasite is destroyed as the inner layers of these foci become necrotic (Smith and Söderhall, 1986). Such encapsulating lesions have previously been recorded in the gills and heart of *Hematodinium*-infected *N. norvegicus* (Field and Appleton, 1995; Field et al., 1992), their presence thought to indicate a previous microbial or parasitic infection. Whether these lesions relate to the original infection site of *Hematodinium* sp. has not been shown to date. Interestingly, these melanised encapsulation responses were rarely observed in the gill lamellae of crabs with PCD. As such, if these lesions do mark the infection route of *Hematodinium* sp., it is possible that this route differs between *C. pagurus* (via the gut) and *N. norvegicus* (via the gills). Further studies are required to elucidate the route of entry of this parasite to its respective hosts.

Ultrastructurally, the remaining intact muscle fibres showed an exaggerated separation of the sarcolemma from the contractile myofibrils at the fibre periphery. However, in contrast to the infection in *N. norvegicus*, where the Z-lines of the sarcomeres remain intact (Stentiford et al., 2000), the sarcomeres from the muscle of crabs with PCD showed severe disorganisation of filaments in the region of the Z-line. Loss of Z-line material is reported to occur in a number of pathological and physiological conditions (Kumudavalli Reddy et al., 1975) and apparently represents an early step in normal premoult atrophy in crustaceans (Mykles and Skinner, 1990a). The calcium-dependent proteases involved in premoult atrophy are localised in the sarcoplasm (Mykles and Skinner, 1990b) and it is conceivable that severe disruption of the muscle during PCD in *C. pagurus* may assist in the activation of these proteases. Alternatively, proteases of parasitic origin may be responsible for the differential breakdown of muscle within crab and lobster hosts. The reason for the difference in the breakdown characteristics of muscle in PCD and in *Hematodinium* infection of *N. norvegicus* warrants further investigation.

#### 4.3. Potential commercial impact

PCD has the potential for considerable commercial impact at several levels. *Hematodinium* is ultimately fatal to its *N. norvegicus* host (Stentiford et al., 2001b), with seasons of high infection prevalence at a particular site being linked to reductions in landings per unit effort in the following season (Field et al., 1998). Additionally,

*Hematodinium* infections of *C. bairdii* (Meyers et al., 1987), *C. opilio* (Taylor and Khan, 1995), *C. sapidus* (Messick and Shields, 2000; Shields and Squyars, 2000), and *N. puber* (Wilhelm and Mialhe, 1996) have been associated with large commercial losses. As little is known about the prevalence and seasonality of PCD in the field, no inference can be made as to its likely role as a mortality factor in the fishery. However, due to the severe pathology associated with PCD in *C. pagurus*, similar effects as those observed within the fisheries for *C. bairdii*, *C. opilio*, and *C. sapidus* may manifest themselves in populations of *C. pagurus* which harbour PCD. The current study has also shown that *Hematodinium* sp. infections are likely to disrupt the reproductive ability of infected crabs. Whilst the presence of infection per se may be expected to cause significant population effects (through increased natural mortality), at present, little is known about how sub-lethal levels of infection may impact upon the reproductive output of host species. Further research into the reproductive status of infected *C. pagurus*, coupled with monitoring of offshore and inshore sites and a retrospective analysis of landings data would facilitate study of the potential for such effects on commercial stocks.

In addition to their reduced survivability during holding and transportation, the quality and yield of meat from crabs infected with PCD is also of potential commercial significance. The severe pathology associated with the hepatopancreas ('brown meat') and the claw muscle ('white meat') is likely to cause considerable alteration in the yield, texture, and appearance of these tissues. Previous studies on the biochemical composition of *Hematodinium*-infected *N. norvegicus* tissues also suggests that disruptions in the normal carbohydrate and amino acid profiles of these tissues may be implicated in the 'bitter' taste of the meat that accompanies this and other *Hematodinium* infections (Meyers et al., 1987; Stentiford et al., 2001a,b). The cooking of *Hematodinium*-infected and uninfected tanner and snow crabs under batch conditions has been suggested to cause tainting of the whole batch (see Meyers et al., 1987). The batch preparation of uninfected *C. pagurus* with those infected with PCD may be expected to cause similar effects.

#### 4.4. Future studies

The mode of transmission of *Hematodinium* infections in the field is the subject of some conjecture. However, a number of studies have suggested that there is a significant risk of spread through in-transit culling and disassembly of the catch at sea (see Hudson and Shields, 1994; Love et al., 1993; Taylor and Khan, 1995). Anecdotal evidence suggests that the potential for the spread of PCD via these practices, and others that involve the use of crabs as bait for the capture of other

species, is significant. This is noteworthy when considering the suggested route of entry (via the gut) for the parasite causing PCD. Knowledge gained via studies on the transmissibility of the *Hematodinium* species causing PCD may be applied to improve current commercial capture and holding practices, which may to some extent be facilitating transmission of this parasite in the field.

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