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Pathology of *Hematodinium* infections in snow crabs (*Chionoecetes opilio*) from Newfoundland, Canada

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Abstract

Bitter crab disease (BCD) of snow crabs, *Chionoecetes opilio*, is caused by a parasitic dinoflagellate, *Hematodinium* sp. The disease has shown an alarming increase in prevalence in the commercial fishery in eastern and northeastern areas of Newfoundland and Labrador since it was first recorded there in the early 1990s. We documented histopathological alterations to the tissues in snow crabs with heavy infections of *Hematodinium* sp. and during sporulation of the parasite. Pressure necrosis was evident in the spongy connective tissues of the hepatopancreas and the blood vessels in most organs. In heavy infections, little remained of the spongy connective tissues around the hepatopancreas. Damage to the gills varied; in some cases it was severe, particularly during sporulation, involving apparent thinning of the cuticle, loss of epithelial cells, and fusion of the membranous layers of adjacent gill lamellae. Affected lamellae exhibited varying degrees of distention with a loss of trabecular cells, hemocyte infiltrations, and swelling or "clubbing" along the distal margins. Large numbers of zoospores were located along the distal margins of affected lamellae suggesting that sporulation may cause a lysis or bursting of the thin lamellar cuticle, releasing spores. Pressure necrosis, due to the build up of high densities of parasites, was the primary histopathological alteration in most tissues. *Hematodinium* infections in the snow crab are chronic, long-term infections that end in host death, during sporulation of the parasite.

Keywords: Bitter crab disease; Bitter crab syndrome; Sporulation; Gills; Hepatopancreas; Fishery; Disease; Crab

1. Introduction

Snow crabs, *Chionoecetes opilio*, are highly prized for their delicately flavored meat, and have become one of Newfoundland's largest commercial fisheries. In 2005, the fishery landed 44,000 metric tonnes (mt) of snow crab, valued at CAN $140 million (DFO, 2006). In comparison, the snow crab fishery in 2003 had landings in excess of 58,000 mt worth nearly CAN $264 million (DFO, 2006). Changes in value of the fishery are largely due to market fluctuations, such as demand and price, and biotic forces such as highly synchronous molting events which result in a predomination of recently molted, low-yield male crabs, consequently resulting in premature closing of the fishery. In the last decade, bitter crab disease (BCD) has proliferated in the northern bays of Newfoundland, causing at least three documented outbreaks (Shields et al., 2005; Shields et al., in review) and may have contributed to the decline in landings.

In snow and tanner crabs (*C. opilio* and *Chionoecetes bairdi*), BCD is caused by *Hematodinium* sp., an endoparasitic dinoflagellate (Meyers et al., 1987). Bitter crab disease was first found in snow crabs from Newfoundland in 1990, at low levels (0.037% prevalence) (Taylor and Khan, 1995). Since then the prevalence of BCD has rapidly increased. In 2000, it was found in nearly 25% of females sampled from Conception Bay, Newfoundland, and an epizootic in 2005 affected approximately 25% of legal-sized males surveyed

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(Shields et al., in review). The disease is most prevalent in the northern bays of Newfoundland (Taylor and Khan, 1995; Pestal et al., 2003; Shields et al., 2005), although it also occurs at low prevalence offshore (Dawe, 2002). The disease may have a profound effect on the snow crab industry, including the unmarketability of infected legal-sized crabs, and the mortality imposed on pre-recruits to the fishery (Shields et al., 2005).

Snow crabs obtain infections in the spring, during molting, and infections take approximately 10–15 months to develop (Shields et al., 2005). Macroscopically diagnostic infections appear to develop over the course of 2–4 months (Fall season) with mortalities occurring 3–6 months later, few infected hosts survive more than a year (Shields et al, 2005). BCD is generally a disease of juvenile males and immature females, as they molt more frequently, and of males that have recently molted to sexual maturity (Shields et al., 2005; Shields et al., in review). Epizootics of *Hematodinium* have been linked to synchronized molting events of snow crabs (Shields et al., in review). Cannibalism may be a mode of transmission of the disease (Meyers et al., 1987; Dawe, 2002), but there is at present little evidence for this mode of transmission in snow crabs (Shields et al., 2005).

In crab and Norway lobster (*Nephrops norvegicus*) hosts, the rapid proliferation of the parasite in the hemolymph, and its high metabolic requirements during growth, decrease the protein and carbohydrate reserves of the host leading to host morbidity and eventually mortality (Stentiford et al., 2000, 2001; Shields et al., 2003; Stentiford and Shields, 2005). The depletion of energy reserves coupled with changes in the oxygen-binding characteristics of host hemocyanin, results in lethargy of heavily infected Norway lobsters (Taylor et al., 1996). Additionally, tissues of infected animals likely experience hypoxia, with resultant ischemia, because of tissue destruction caused by numerous parasites (Stentiford and Shields, 2005). Death probably results from massive tissue disruption, caused by sporulation of the parasite, or through depletion of the host's metabolic reserves.

Several studies have described aspects of the histopathology of *Hematodinium* infections in various crustacean hosts. The pathology includes occlusion of hemal spaces by the parasite (Meyers et al., 1987; Field et al., 1992; Hudson and Shields, 1994; Messick, 1994; Field and Appleton, 1995; Stentiford et al., 2002; Sheppard et al., 2003), effects on respiratory function and gill structure (Field et al., 1992; Hudson and Shields, 1994; Field and Appleton, 1995; Taylor et al., 1996; Sheppard et al., 2003), and damage to muscle fibers (Meyers et al., 1987; Field et al., 1992; Hudson and Shields, 1994; Messick, 1994; Stentiford et al., 2002; Sheppard et al., 2003). However, the effects of *Hematodinium* infections on the tissues of the snow crab and pathological changes during sporulation events have not been specifically addressed.

In this study, we documented the histopathological alterations to the tissues in heavy infections and alterations to the gills during sporulation of *Hematodinium* sp. in the snow crab. The intensity of infection in relation to the life history of the parasite was also examined.

2. Materials & methods

2.1. Collection of animals

Crabs were collected from Conception Bay on the northeast coast of Newfoundland (see Fig. 1 in Shields et al., 2005). The bay is partially enclosed and has a range of bottom types; sand and gravel at the mouth, and thick mud in the deep inner basin. Collections were made during the Department of Fisheries and Oceans (DFO) annual stock assessment surveys with the CCGS Shamook. Crabs were sampled as in Pestal et al. (2003) and Shields et al. (2005). Snow crabs examined in this study were taken from DFO surveys in Conception Bay during October 1998 ($n = 28$), February 2005 ($n = 200$), and October 2005 ($n = 25$) research cruises. Crabs were examined for sex, carapace width (CW), shell condition, maturity status, color change of carapace, and other macroscopic indications of BCD. Shell condition was categorized as in Taylor et al. (1989) to determine the molt status of the animal. BCD infections were diagnosed by a pronounced color change of the carapace giving the crabs a pink, cooked appearance (see Fig. 1 in Pestal et al., 2003). Heavily infected crabs also exhibit other gross signs of BCD such as lethargic behavior, the presence of a solid white ventrum, milky or chalky colored hemolymph, and cream-colored hearts and gills (Shields et al., 2005).

2.2. Histopathological examination

After physical parameters were recorded, the hepatopancreas, heart, and gills were removed and preserved in either Bouin's solution or Safe-Fix (Fisher). After 48 to ~168 h, tissues were transferred into 70% EtOH and shipped to the Virginia Institute of Marine Science (VIMS). Gill tissues were decalcified using the formic acid–sodium citrate method (Luna, 1968). All tissues were processed using paraffin histological techniques and stained with Mayer's hematoxylin and eosin (Luna, 1968). Prepared sections were examined for the presence of *Hematodinium* sp. with an Olympus BX51 compound microscope and photographs were taken using a Nikon DXM1200 digital camera with aid of the ACT-1 computer program (Nikon).

2.3. Histological examination

Sections of gill, heart, and hepatopancreas were examined for the presence of *Hematodinium*. When present, the life history stage and the relative intensity of the parasite were noted. The life history stages of the parasites in the tissues were categorized as single cells, filamentous trophonts, clump colonies, and plasmodial sheets (cf.

arachnoid sporonts, see Appleton and Vickerman, 1998). The intensity of infection was rated as follows: no parasite cells present, light infection (1–5 parasites per microscopic field at 200×), moderate infection (5–20 parasites per field), or advanced infection (20+ parasites per field). Relative intensity of the parasite in the tissue was used as the key indicator for the severity of infections.

3. Results

3.1. Prevalences and Intensities of Infection

October 1998 data: Crabs from the DFO survey were specifically selected based on their infection status, infected or uninfected, using the aforementioned macroscopic indications of BCD. Histologically, 24 of 28 (86%) crabs were infected with *Hematodinium*. The plasmodial sheet form, clump colony form, and the single cell form of the parasite were present in the tissues (Fig. 1). Most infections were heavy except for one crab that had a light infection of clump colonies in the heart.

February 2005 data: Crabs were randomly sampled by selecting every 10th crab measured in the DFO survey. Histologically, 24 out of 200 animals (12%) were infected with *Hematodinium* sp. Males comprised 7% (10/136) of the total number of infected animals, while female crabs had a slightly higher prevalence of infection of 22% (14/64). All infected crabs had advanced infections. No crabs from this collection had light or moderate infections suggesting a possible synchronization of the BCD infections with season. In addition, most of the infected tissues had the plasmodial sheet form of the parasite, not single cell or clump colony forms.

October 2005 data: For histological samples, crabs from the survey were selected as infected, uninfected, or as borderline infected based on their macroscopic characteristics. Histologically, 16 of 25 (64%) crabs were infected. All infected crabs were in advanced stages of infection. The infected tissues had both the single cell form and the plasmodial sheet form of the parasite.

3.2. Histopathology

3.2.1. Heart

In most heavy infections, tissues in the myocardium were devoid of spongy connective tissue, presumably as a result of pressure necrosis. There was a reduction or a complete loss of hemocytes, resulting in hemoctyopenia. Reserve inclusion (RI) cells were absent or effete, without inclusions. Fixed phagocytes were present and enlarged. The heart muscle had lost its normal dense appearance and the bands of muscle fibers had the appearance of separating from one another (Fig. 2a and b).

3.2.2. Hepatopancreas

The hepatopancreas was greatly altered by the presence of the *Hematodinium* parasite. Spongy connective tissue was apparently lost, presumably from pressure necrosis

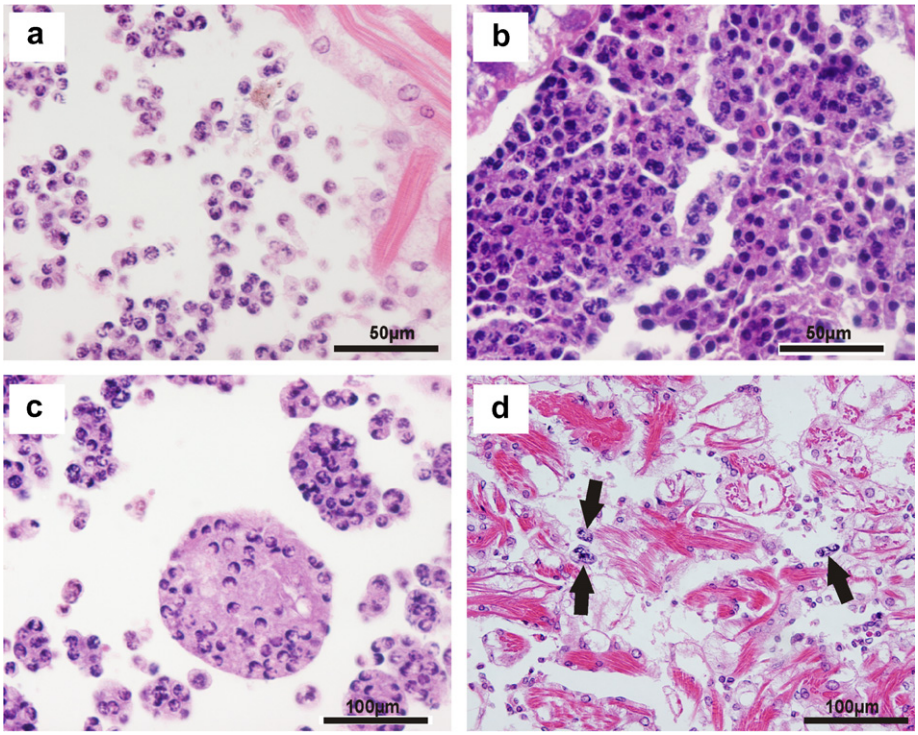

Fig. 1. Representative life history stages of *Hematodinium* sp. found in the tissues of *C. opilio*. (a) Single and small multi-nucleate stages in the heart. (b) Sheet-like form, or presumptive arachnoid sporont, in the hepatopancreas. (c) Presumptive clump colonies in the heart. (d) Light infection of multinucleated filamentous trophonts (arrows) in heart tissue.

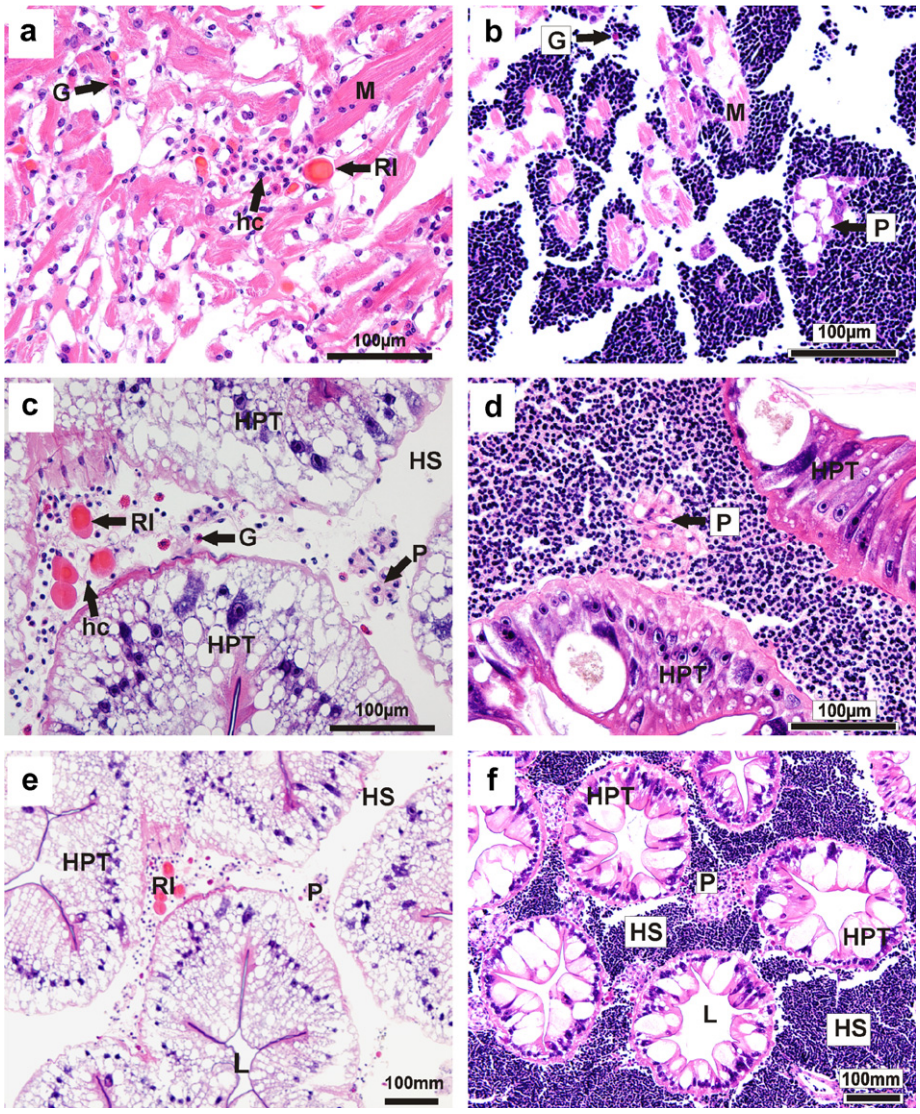

Fig. 2. Comparative sections of tissues from uninfected and *Hematodinium*-infected *C. opilio*. (a) Heart of uninfected crab with large reserve inclusion cells (RI) and healthy muscle tissue. (b) Heart from heavily infected crab showing gross changes to the muscles, loss of arterioles and activated fixed phagocytes. (c) Hemal sinus in the hepatopancreas of an uninfected crab with robust RI cells (RI), abundant hemocytes (hc) and fixed phagocytes (P). (d) Hemal sinus of the hepatopancreas of an infected crab. Much of the spongy connective tissue surrounding the sinus has been obliterated and the fixed phagocytes (P) are activated. (e) Hepatopancreas of an uninfected crab exhibiting the normal architecture of the hepatopancreatic tubules (HPT). (f) Hepatopancreas of a heavily infected crab exhibiting dilation of the hemal sinuses in the infected tissues and edematous changes to the lumen (L) of the tubule. Key: G, granulocytes; hc, hemocyte; RI, reserve inclusion cells; M, muscle; P, fixed phagocytes; HPT, hepatopancreatic tubule; HS, hemal sinus; L, lumen.

induced by the massive proliferation of the parasite. Large numbers of parasite cells filled the extremely dilated arterioles. RI cells were effete or without inclusions, and hemocytes were rare. In addition, the remaining fixed phagocytes were enlarged and activated. No granulomas or other evidence of bacterial infection was observed. The epithelial margin of the hepatopancreatic tubules appeared distorted, possibly due to pressure necrosis (Fig. 2c and d). The lumen of the tubules was enlarged and edematous, although there were no parasites within the lumen proper of the tubules (Fig. 2e and f).

3.2.3. Gills

In the gill stems of infected crabs the most noticeable histopathological changes included a loss of spongy connective tissue, a reduction or loss of podocytes, and a reduction or complete loss of RI cells. There was a noticeable metaplastic change in the epithelial cell layer (Fig. 3a and b). The epithelium was reduced or compacted, changing from its original thick, columnar cell shape to a compressed, squamous shape (Fig. 3c and d). As a result of the high density infections of the parasite, the gill lamellae exhibited apparent pressure necrosis of the connective tissue, a loss of the thin epithelial tissue layer, and the loss or necrosis of the trabecular cells (Fig. 3e and f). No granulomas or other evidence of bacterial infection was observed.

Sporulation of the parasite caused a more severe alteration to the gills. The immediacy of sporulation was identified by a large number of small uninucleate stages in the

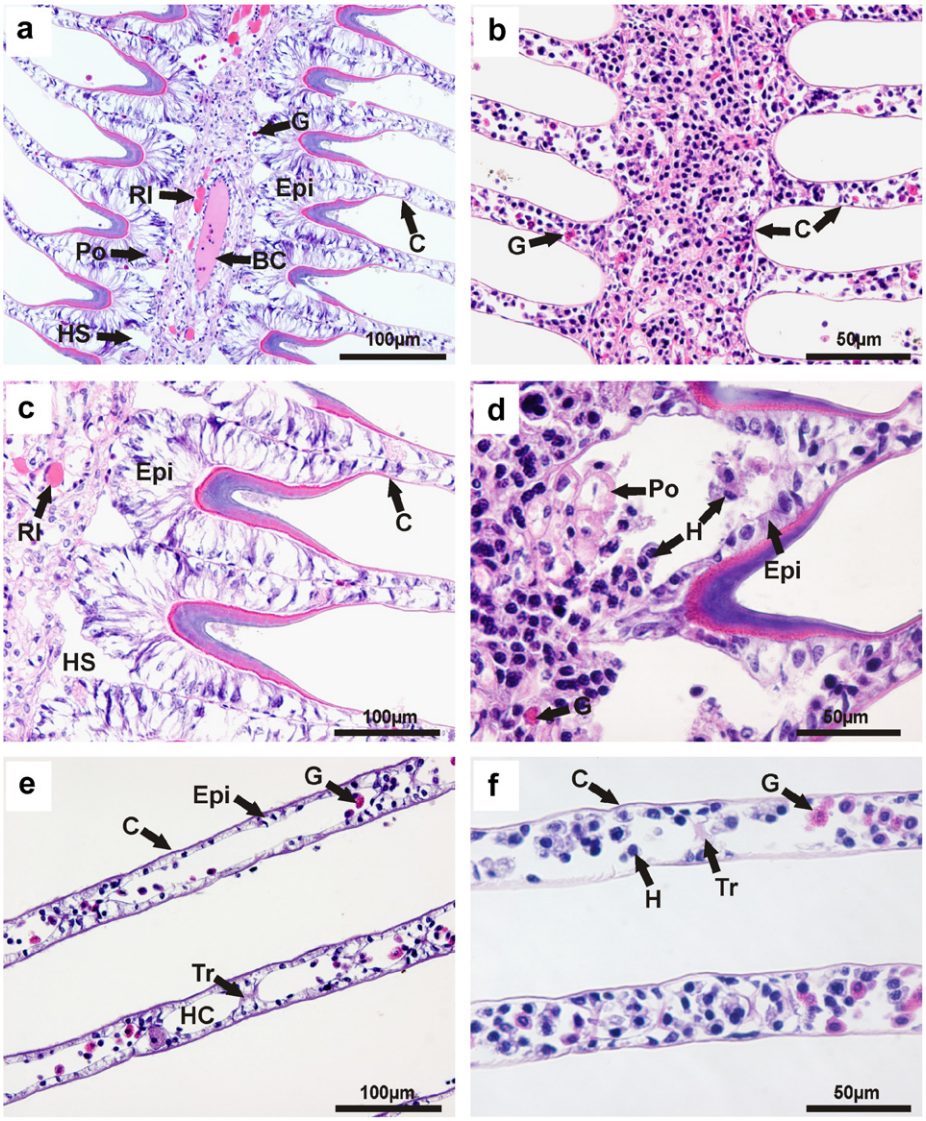

Fig. 3. Comparative sections of gill tissues from uninfected and *Hematodinium*-infected *C. opilio*. (a, c, and e) Gill tissues from uninfected crabs. (b, d, and f) Gill tissues from a heavily infected crab. (a) Longitudinal section through the gill stem of an uninfected crab exhibiting large numbers of reserve inclusion cells (RI) and the normal organization of epithelial and connective tissues. (b) Longitudinal section through the gill stem of an infected crab. Few hemocytes and connective tissue cells are visible and parasites fill the hemal sinuses and interstitial spaces. The cuticle at the base of the lamellae appears thinner. (c) Detail of gill stem from an uninfected crab showing the normal columnar epithelial cells at the base of each lamella. (d) Detail of gill stem from infected crab showing metaplastic change to squamous epithelial cells at the base of each lamella and enlarged, activated podocytes (Po). (e) Gill lamellae from an uninfected crab with normal trabecular cells (Tr) and thin, squamous epithelial tissue (Epi) underlying the cuticle. (f) Gill lamellae from an infected crab. Note the large number of parasite cells in the hemal sinuses and the apparent erosion of the epithelial tissue. Key: G, granulocytes; Epi, epithelium; RI, reserve inclusion cells; BC, blood channel; HS, hemal space; PO, podocytes; C, cuticle; Tr, trabecular cells; HC, hemal channel; H, *Hematodinium* cells.

hemolymph or from visual examination of crabs leaking a cloud of spores. Immediately prior to and during sporulation there was marked swelling or distension of the distal tips of the gill lamellae. In cross-section, distal regions of the lamellae changed from their normal pyriform shape to a more rounded, club-like appearance (Fig. 4a and b). High densities of the parasite accumulated within the affected lamellae, particularly along their distal margins (Fig. 4c). Moreover, sporulation induced fusion of lamellae with changes to the underlying connective tissues. This fusion was observed in 16 of 64 crabs (25%) with infections undergoing sporulation. The pressure-induced necrosis, alteration of the cuticular structure of the lamellae, and change in the underlying soft tissues appeared to be related to this fusion, particularly near the distal ends of the lamellae. Spores were apparently released during the final lysis of the cuticle layer between adjacent lamellae, and possibly at the tips of the distended lamellae (Fig. 4d, e and f).

4. Discussion

Infections of *Hematodinium* sp. lead to high densities of parasites in the hemolymph, causing a systemic infection of all major organs. Pressure necrosis and erosion of soft

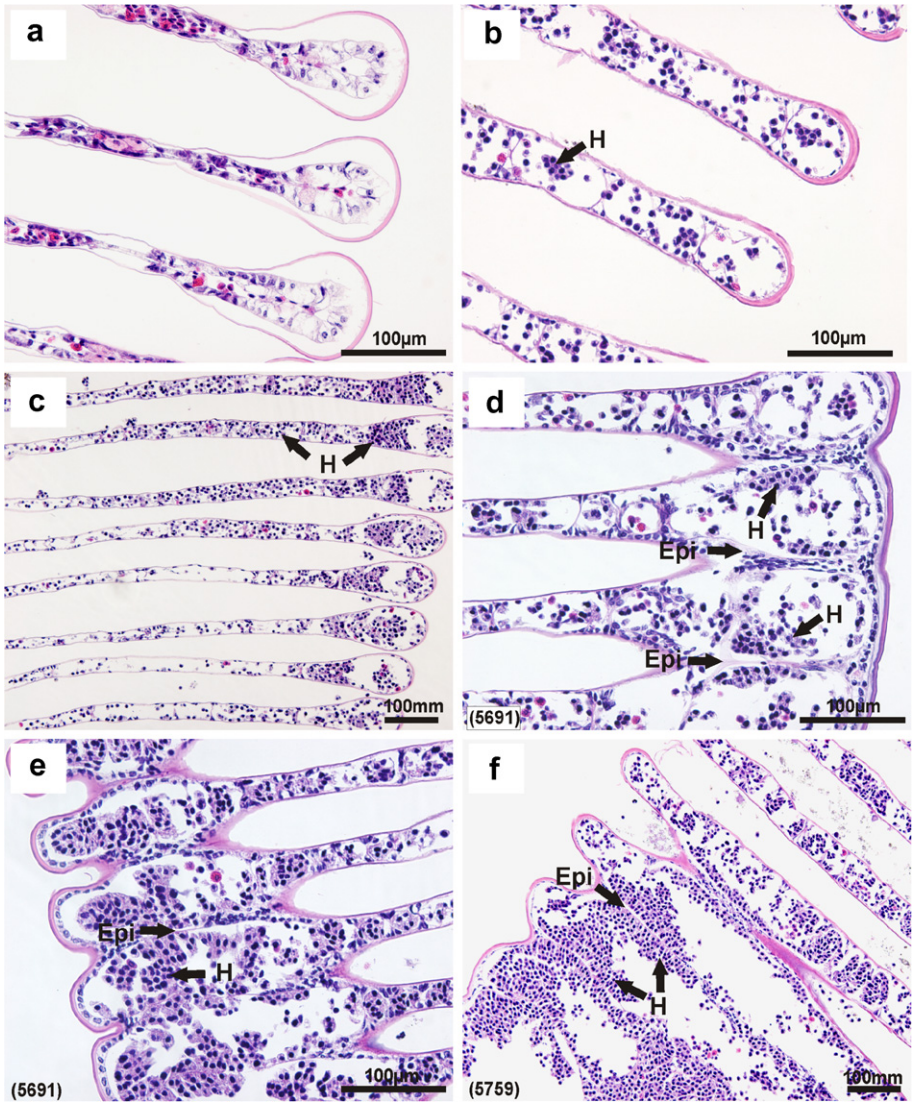

Fig. 4. Changes in gill structure as a result of heavy infections. (a) Distal tips of gill lamellae from an uninfected crab. The space between the cuticle and the epithelium is an artifact of fixation. (b) Swollen, club-shaped distal tips of gill lamellae from an infected crab. Note the enlarged hemal sinuses. (c) Gill lamellae of an infected crab with accumulations of the parasite (H). Note the widening of the lamellae as well as the distention of the distal end. (d, e, and f) Cuticular fusion in the distal tips of two heavily diseased crabs. Note the remnants of the epithelial layers (Epi) between individual lamellae and the large numbers of parasites in the lamellae. Key: H, *Hematodinium*; Epi, epithelium.

tissue layers are hallmarks of late stages of infection. The most notable histopathological affect of BCD in snow crabs is the alteration of the gill architecture just prior to, or during sporulation. Large numbers of the parasite congregated along the distal margins of gill lamellae causing loss of internal structural support and distention in the distal region of individual lamellae. Heavy sporulation of the parasite apparently caused lysis of the thin cuticle layer of the gill leading to an unusual fusion of adjacent lamellae, which may reflect continued suppurations of the cuticle and underlying tissues.

These severe changes in the gill structure may have a direct effect on the respiratory function of diseased snow crabs, similar to that observed in other *Hematodinium*-infected crustaceans. Field and Appleton (1995) proposed that massive sporulation of the parasite in the gills of Norway lobster, which caused an occlusion of hemal spaces, may lead to blockage of gill filaments and consequently interfere with respiratory exchange and metabolism. Field et al. (1992) reported heavy *Hematodinium* infections in the Norway lobster that resulted in distention of the gills, disruption of gas transport, and tissue anoxia. Taylor et al. (1996) studied the effects of the parasite on respiratory physiology in Norway lobsters. They hypothesized that the tremendous numbers of parasitic cells in the gills may cause blockage of the hemal sinuses, and showed that the oxygen-carrying capacity in heavily infected lobsters was reduced by 50% (Taylor et al., 1996). Unlike infections in blue crabs, *Callinectes sapidus*, where sporulation may not be necessarily fatal (Shields and Squyars, 2000), sporulation in Norway lobsters and tanner crabs is fatal to both species (Meyers et al., 1987; Appleton and Vickerman,

1998). Sporulation is likely fatal in snow crabs, as well. The gross changes in gill structure of snow crabs infected with BCD indicate significant respiratory and physiological stress that may be similar to that for Norway lobsters.

The histopathological changes in the heart indicate that, as in Taylor et al. (1996), the parasite causes loss of hemocytes, pressure necrosis in the spongy connective tissues, and loss of RI cells. A primary function of hemocytes is to respond to disease agents and injury (Johnson, 1980). The resulting hemoctyopenia caused by advanced *Hematodinium* infections affects the normal immune response of the host through the impairment of clotting ability and the inability to fight off secondary infections (Meyers et al., 1987; Stentiford et al., 2002; Stentiford and Shields, 2005). Bacteria were not observed in the tissues that we examined. BCD infections also caused a loss of reserve inclusion (RI) cells. RI cells store glycogen, which is later used in the formation of new cuticle during the molting process. A reduction in glycogen may lead to disruption of chitin deposition and consequently the altered coloration of infected crabs giving them a pink or cooked appearance (Stentiford and Shields, 2005). Reduction in glycogen may also reflect the alteration in health status of host due to environmental stress or pathogens (Stentiford and Feist, 2005).

In heavy infections there was a change in the musculature of the heart. Muscles lost their normal dense appearance and muscle fibers separated from one another (Fig. 2b) possibly due to loss in water content; this is similar to the "islands of unattached muscle" found in *Hematodinium*-infected edible crab, *Cancer pagurus* (Stentiford et al., 2002). This muscle degeneration also occurs in tanner crabs, *Chionoecetes bairdi* (Meyers et al., 1987), Australian swimming crabs, *Portunus pelagicus* (Hudson and Shields, 1994), and blue crabs, *Callinectes sapidus* (Messick, 1994).

Physiological changes resulting from parasitic infection or disease usually manifest in the hepatopancreas of crustaceans, making this organ a useful indicator of the prevalence of an infection (Johnson, 1980; Shields et al., 2003; Stentiford and Shields, 2005; Stentiford and Feist, 2005). In our study, the hepatopancreas was greatly altered by the presence of the *Hematodinium* parasite. As in the Norway lobster (Field and Appleton, 1995), fixed phagocytes in infected snow crabs appeared activated and enlarged. These cells are highly phagocytic (Factor et al., 2005) and their activation is likely due to the presence of the parasites, secondary invaders, or cellular debris arising from infection. Similar to the heart, spongy connective tissues and RI cells in the hepatopancreas were absent, due to the proliferation of the parasite. In addition, the hemal arterioles were extremely dilated and filled with numerous plasmodial cells. Similarly, Field and Appleton (1995) observed that the hemal sinuses between the hepatopancreatic tubules increased in size with the severity of infection in the Norway lobster. This dilation in the hemal sinuses likely results from massive proliferation of the parasite in the hemolymph. Edematous changes to the tissue were also apparent. Increased vacuolation and lysis of the tubules has been reported in several *Hematodinium*-infected crustacean species (Field et al., 1992; Messick, 1994; Stentiford et al. 2002). In other heavily parasitized crustacean species, the hepatopancreatic tubules often degenerate, and parasites are frequently found within the lumen of the tubules (Field and Appleton, 1995; Meyers et al., 1987; Stentiford et al., 2002). However, we did not find parasites within the lumen of the tubules.

Various life history stages of the parasite were observed in the infected tissues of the snow crab. Multinucleate plasmodial stages occurred as several distinct forms; sheet-like plasmodia, multinucleate rounded plasmodia, and clump colonies (Fig. 1). We speculate that the host becomes infected with an early uninucleate-stage parasite which then progresses through a series of multinucleate forms similar to those described by Appleton and Vickerman (1998). Late stages of infection in the snow crab appear to have a predilection for the hepatopancreas. The massive sheet-like plasmodium grows within the hemal sinuses of the hepatopancreas; it does not occur in other organs. It may represent the arachnoid sporont of Appleton and Vickerman (1998) because it is large, multicellular and entwined within the organ. The smaller, rounded, multinucleate plasmodial stage occurs in the hemolymph. This stage can be relatively large (>100 μm) and may be able to block arterioles, causing ischemia. This stage could represent either the filamentous trophonts or the sporoblasts of Appleton and Vickerman (1998). However, the filamentous trophonts are often present in early infections of *Hematodinium* in the blue crab (Shields and Squyars, 2000) and possibly the snow crab (J. Shields, unpubl. data). Therefore, the small multinucleate stages in our study, which were all from late stages of the infection, could represent sporoblasts. The smaller uninucleate forms occur in the hemolymph. They may be sporonts because late stages of infection normally have vast numbers of sporonts or developing dinospores (Meyers et al., 1987; Appleton and Vickerman, 1998).

With some exceptions, infections appeared to be synchronized, with all crabs examined in the different temporal periods having approximately the same level and type of infection. Most infected crabs were in an advanced state of infection during both October and February collections, with more sheet-like forms in the October collections and higher prevalence of single spores in the February collections. Snow crabs apparently become infected during molting or in post-molt condition and overt infections usually take 2–4 months to develop (Shields et al., 2005). Because transmission occurs during crab molting (Shields et al., 2005) and since molting occurs in the spring (Hébert et al., 2002), the increase in presumptive sporoblasts in the winter appears to be timed to the most active period of molting. However, sporulation has also been observed in October (this study) indicating that the parasite's life cycle may not be fully synchronized with the molting period of the host.

In conclusion, heavy infections of BCD lead to significant histopathological alterations to circulatory and respiratory tissues of the host crab. These alterations culminate in a surprising change to the gill architecture during sporulation. Fusion of the lamellae and metaplastic changes to the underlying connective tissues may contribute to the respiratory disruption that occurs during these infections.

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