Parasitization of juvenile edible crabs (*Cancer pagurus*) by the dinoflagellate, *Hematodinium* sp.: pathobiology, seasonality and its potential effects on commercial fisheries

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SUMMARY

This study reports on the prevalence and severity of infections caused by the parasitic dinoflagellate, *Hematodinium* in juvenile edible crabs (*Cancer pagurus*) found in 2 intertidal survey sites (Mumbles Head and Oxwich Bay) in the Bristol Channel, UK. Crabs were assessed for the presence and severity of *Hematodinium* infections by the histological examination of infected tissues. Such infections were found to exhibit a seasonal trend in the 2 study areas with high numbers of animals (*ca*. 30%) infected in the spring to summer but with low severity. Conversely, in November only *ca*. 10% of crabs were infected but these animals had large numbers of parasites in their haemolymph and other tissues. At this time, the carapace and underlying tissues of infected crabs had the chalky, pinkish-orange appearance that is characteristic of this disease. *Hematodinium*-infected crabs ranged in size from 12 to 74 mm carapace width. Overall, it is concluded that the high prevalence of infection of juvenile crabs in this area may have implications for the sustainability of the edible crab fishery in the Bristol Channel.

Key words: edible crab fisheries, disease ecology, decapod crustaceans.

INTRODUCTION

The edible or brown crab, *Cancer pagurus*, is an important species of fished shellfish in Northern Europe. For instance, 26 600 tonnes of this species were landed in the UK in 2010 worth an estimated £35·2 million (MMO, 2011). The distribution of juvenile and mature edible crabs differs. Juveniles are readily found in the intertidal–subtidal environments where they feed on of macroalgae and invertebrates, including bivalve and gastropod molluscs (Mascaró and Seed, 2001). Most foraging behaviour occurs at night to limit the chance of predation by larger crabs, octopi and fish (Burton and Burton, 2002) and at low tide they hide under boulders often in soft sediment to give additional protection. During this stage on shore their moult intervals are frequent especially during the summer months when growth rates are at their highest (Silva *et al*. 2014). After *ca*. 3 years in the intertidal zone, crabs migrate to deeper waters. They reach sexual maturity at *ca*. 110 mm carapace width (CW) for males and 120 mm CW for females (Fish and Fish, 1989). At this stage they live on the benthos in on shore and off shore waters but return to shallow water to reproduce.

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Edible crabs are subject to a wide range of infections from micro- and macro-parasites (Stentiford, 2008; Behringer, 2012) and most of our knowledge of these conditions in this species has come from studies on mature (recruit) animals above the minimum landing size (115–140 mm CW in the UK). Recent studies including those by Stentiford (2008) and Bateman *et al*. (2011) have, however, highlighted the importance of detailed surveys of such diseases in both juvenile (pre-recruit) and adult edible crabs. They have shown that juvenile animals are susceptible to a range of diseases that differ in prevalence and severity to their adult counterparts. Indeed, the wide variety of disease agents and their prevalence in juvenile crabs led Stentiford (2008) to conclude that such conditions could act as a bottleneck in the development of edible crabs to the detriment of the fishery. These studies reinforce the importance of assessing diseases in early life stages to give a more comprehensive picture of disease dynamics in animal populations.

One of the key diseases of crabs and several other species of decapod crustaceans is 'pink' or 'bitter crab' disease (Stentiford *et al*. 2002; Stentiford and Shields, 2005; Small, 2012). The cause of this condition is the dinoflagellate endoparasite, *Hematodinium* spp. (Stentiford and Shields, 2005; Morado, 2011). *Hematodinium*-related diseases are known to affect over 40 species of crustaceans mainly

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in the Northern Hemisphere (Morado *et al*. 2010), but 'outbreaks' have also been reported in Australia and China (Hudson and Shields, 1994; Gornik *et al*. 2013; Li *et al*. 2013). The disease is of significance to the fisheries in the areas affected, due to the observation that the condition is probably lethal in most infected crabs (Messick and Shields, 2000; Shields and Squyars, 2000; Shields *et al*. 2005; Stentiford and Shields, 2005). For example, in snow crabs (*Chionoecetes opilio*) animals were most susceptible to disease immediately after moulting, with death occurring some 3–4 months later (Shields *et al*. 2005). Although *Hematodinium* infects crustaceans of all life stages, most reports show that it is more prevalent in juveniles (e.g. Messick, 1994; Shields *et al*. 2005, 2007; Morado, 2011). For instance, Messick (1994) found that early juvenile blue crabs (*Callinectes sapidus*) in the size range 5–29 mm CW showed up to 100% parasitization while larger animals generally showed lower infection rates.

The main aim of the current work was to elucidate the potential significance of *Hematodinium* infections in pre-recruit edible crabs in the Bristol Channel, within the Western Channel and Celtic Sea region. This region is important to study as it supports a large crab fishery of economic importance to the area (Bannister, 2010).

MATERIALS AND METHODS

Animals and processing

Juvenile edible crabs (*C. pagurus*) were collected in the intertidal zone from 2 locations on the Gower Peninsula in South Wales, UK (namely Mumbles Head and Oxwich Bay) between February 2011 and January 2012. At every monthly time point, 30–52 animals were collected at each site (see Tables 1 and 2) and immediately transported back to a seawater aquarium at Swansea University. Small animals (up to *ca*. 25 mm CW) and soft post-moults were processed within 2–3 h of returning to the laboratory. Larger animals (in excess of 25 mm CW) were maintained in the aquarium and typically processed within 2–3 days of their arrival. All animals were sized, sexed, examined for limb loss, carapace damage and any other externally visible abnormalities. The larger crabs were bled aseptically and the blood examined for the presence of *Hematodinium* using phase contrast microscopy.

Histology

Small crabs were processed whole for histology. They were injected with Davidson's seawater fixative, limbs removed and the main body left in this solution for 24 h. Subsequently, they were bisected and placed in decalcifying solution (10% formalin, 55 g L^{-1} EDTA) for a further 5–7 days. Larger crabs were also injected with Davidson's seawater fixative. Portions of gill, hepatopancreas (together with associated connective tissue) and antennal gland were removed and fixed in Davidson's solution for 24 h. All samples were processed using routine methods and paraffin wax sections cut at *ca*. 7 μM. Sections were stained using Cole's haematoxylin and eosin and photographed on an Olympus BX41 microscope with an Olympus SC30 digital camera. Images were saved as tiff files and adjusted for contrast, brightness and colour balance. The severity of infection (i.e. parasite load) with *Hematodinium* was assessed using the criteria shown in the online Supplementary material.

Statistical analysis

Data were analysed using parametric tests and Prism v.5 (GraphPad Software, CA, USA) and SPSS software (IBM, UK). In some instances disease presence in terms of the number of infected and the number of uninfected individuals was analysed using binomial generalized linear models. All explanatory variables (size 'class', site and moult status) were fitted as categorical covariates. Assessment of main effects and interaction terms was by analysis of deviance.

RESULTS

Animals and survey sites

Four hundred and eighty-six juvenile edible crabs were collected from Oxwich Bay, Gower Peninsula between February 2011 and January 2012, and 456 crabs were collected from Mumbles Head, Swansea in the same period (Tables 1 and 2). The size of crabs examined ranged from 5 to 102 mm at Oxwich Bay (Table 1) and 9 to 84 mm at Mumbles Head (Table 2). There was no significant difference between the mean sizes of crabs between locations (G-test; $G = 2{\cdot}836$, D.F.=11, $P = 0{\cdot}99$). The sex ratio at Oxwich Bay varied significantly between months (G-test; $G = 26{\cdot}81$, $P < 0{\cdot}0001$) and there was also a difference in the sex ratio between months at Mumbles Head (G-test; $G = 21{\cdot}05$, $P < 0{\cdot}0001$). There was a significant difference in the sex ratio between the 2 locations (G-test; $G = 169{\cdot}5$, D.F. = 33, $P < 0{\cdot}0001$) but no significant difference in the total number of males and females collected between the 2 sites (Fishers exact test; $P = 0{\cdot}7414$). Figure 1 shows the temporal changes in the percentage of sexes of the crabs collected from Mumbles Head and Oxwich Bay between February 2011 and January 2012. Both sites showed similar trends with higher percentage of males in April–July and higher percentage of females between August and March.

The moulting profile of crabs in the 2 survey sites differed (Tables 1 and 2). For instance, at Mumbles Head the highest numbers of 'soft' (post-moult)

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Table 1. Features of juvenile edible crabs (*C. pagurus*) collected at Oxwich Bay, Gower Peninsula, between February 2011 and January 2012

		Carapace width (mm)		Moult stage (%)[a]		
Month	*N*	Mean (±S.D.)	Range	Pre	Post	Inter
February	30	30·7 (±8·8)	20–48	10	3	87
March	32	36·4 (±15·5)	16–83	6	6	88
April	52	41·6 (±16·8)	13–77	29	2	69
May	45	41·5 (±14·0)	14–69	20	20	60
June	40	39·9 (±24·3)	10–102	13	32	55
July	48	35·3 (±16·8)	9–89	8	13	79
August	45	35·8 (±18·4)	13–85	16	4	80
September	38	33·9 (±11·7)	14–58	26	8	66
October	48	28·7 (±12·0)	5–58	21	12	67
November	40	33·4 (±9·1)	14–60	15	10	75
December	33	31·8 (±11·8)	10–56	36	6	58
January	35	34·1 (±10·8)	19–59	14	14	72

[a] Moult stage determined by external examination, dissection and histology.

Table 2. Features of juvenile edible crabs (*C. pagurus*) collected at Mumbles Head, Gower Peninsula, between February 2011 and January 2012

		Carapace width (mm)		Moult stage (%)[a]		
Month	*N*	Mean (±S.D.)	Range	Pre	Post	Inter
February	37	30·2 (±12·8)	9–62	14	0	86
March	37	45·3 (±16·6)	11–76	8	3	89
April	36	45·0 (±15·9)	12–79	25	0	75
May	35	44·3 (±14·7)	19–81	31	23	46
June	40	41·7 (±18·3)	16–84	13	17	70
July	47	42·3 (±16·9)	13–70	0	17	83
August	35	37·7 (±17·9)	10–75	26	17	57
September	33	41·0 (±14·8)	16–75	27	6	67
October	43	35·3 (±13·1)	10–75	12	2	86
November	42	36·0 (±11·5)	12–63	3	2	95
December	34	27·3 (±12·8)	9–54	0	9	91
January	37	30·1 (±14·7)	12–67	3	5	92

[a] Moult stage determined by external examination, dissection and histology.

crabs (17–23%) were found between May and August (Table 2). The moulting profile differed at Oxwich Bay with significant numbers of soft crabs collected over a longer timescale from May to July and from October to January (Table 1).

Methods of disease detection

Although we observed that some individuals of *C. pagurus* with *Hematodinium* infections had a characteristic pinkish-orange tinge to the ventral carapace and claws (see Supplementary material – in online version only), this was only apparent in grossly affected crabs (i.e. severity Level 4 – see Supplementary material – in online version only for description of severity levels). Therefore, this method was not suitable to determine the prevalence of disease in the 2 populations. In those crabs that could be bled (usually over 25 mm CW), examination of live or fixed haemolymph preparations by phase contrast microscopy proved to be a reliable and rapid method for the assessment of the presence of *Hematodinium* in infections established at this site. The morphology of *Hematodinium* in the haemolymph was highly variable both in terms of size, shape and the number of nuclei per cell (see Fig. 2). In crabs judged by histology to have low severity infections (severity Levels 1 and 2; see Supplementary material – in online version only), the characteristic forms in the haemolymph were mainly unicellular. In contrast, in animals with higher infection severity (severity Levels 3 and 4; see Supplementary material – in online version only), the parasites in the haemolymph were morphologically more variable ranging from small uninuclear to larger multinucleate forms (Fig. 2A–C). These multinucleate forms of *Hematodinium* were sometimes seen in clumps of varying number (Fig. 2B). Crabs displaying high severity

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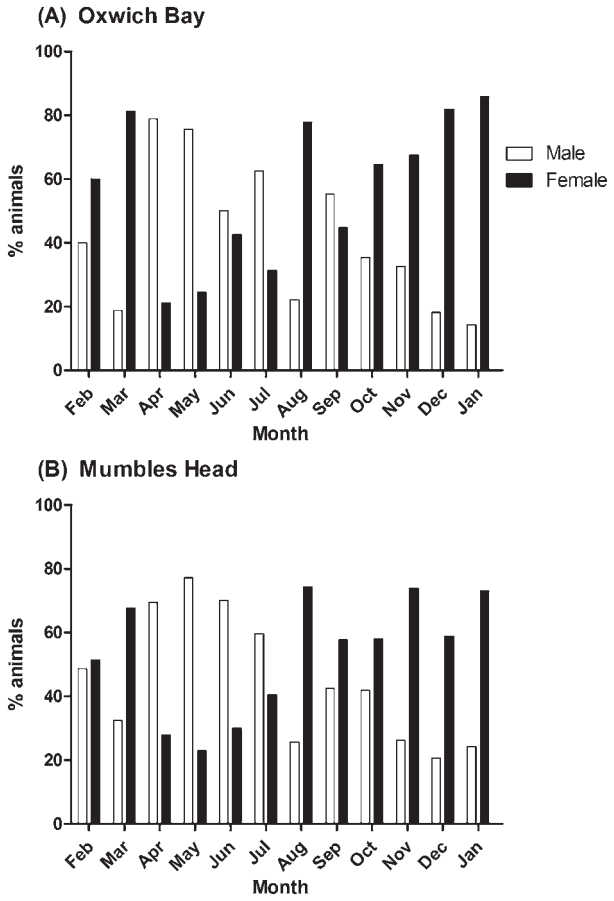

Fig. 1. Changes in percentage of male and female edible crabs sampled in the intertidal zone at (A) Oxwich Bay and (B) Mumbles Head between February 2011 and January 2012.

Hematodinium infections were more likely to display the multinucleate forms of the parasite often with a distinct granular appearance (Fig. 2C). Such animals also had very few circulating haemocytes (Fig. 2A). None of the forms of *Hematodinium* in the haemolymph were observed to have amoeboid movement. Furthermore, on no occasions were dinospores seen in the haemolymph of *Hematodinium*-infected crabs.

Because only animals of *ca.* >25 mm could be routinely bled, this method was not suitable for determining both prevalence and severity of infection across all crabs surveyed. Therefore, histological examination was used as the main method to assess these criteria in all animals. Small (< *ca.* 25 mm CW) specimens of crabs were sectioned whole, and it was therefore possible to examine the distribution of *Hematodinium* in all tissues and to assess whether their presence caused any pathological changes. In animals with low severity infections (i.e. Levels 1 and 2), various stages of the parasites were seen in the connective tissue spaces underlying the cuticle, in the interstitial space (haemal sinus) around the hepatopancreatic tubules, and in the heart in close association with cardiac muscle cells (not shown). Other tissues with a well-developed blood supply, such as the gills, muscle, haemopoietic tissue underlying the gastrointestinal tract and the antennal gland labyrinth, also contained *Hematodinium* (not shown). Uni- and multinucleate forms of the parasite were found in the gills in the haemal sinuses of the main branchial septa, in the lamellae and also in the outer lamellar sinuses at the tips of the gills (Fig. 3A and B). In infected crabs that contained elongated, apparently multinucleate *Hematodinium*, such stages were often seen wrapped around the edges of the lamellar sinuses (Fig. 3B) and free in the haemal sinuses of the branchial septa (not shown).

The distribution of *Hematodinium* in crabs with high severity infections (Levels 3 and 4) differed from those with lower severity infections. In these heavily infected animals, most parasites accumulated in enlarged (oedematous) intertubular spaces around the hepatopancreatic tubules (Fig. 3C) with lower densities in the gills, general connective tissues and heart (not shown). There was no histological evidence of any significant tissue damage or alteration as a direct result of the presence of *Hematodinium*, regardless of severity. For example, in the hepatopancreas despite the large numbers of parasites in the intertubular spaces, the epithelial cells of the tubule wall appeared to be structurally normal (not shown). Furthermore, the parasites showed no interaction with the host's blood cells despite being in direct contact and only in 1 of the 8 crabs with a high-intensity infection (i.e. Level 4) was there any host response in the form of capsules/nodules in the hepatopancreas. These structures did not contain any *Hematodinium* (not shown) implying that their presence was not directly elicited by the parasite's presence in the blood.

Temporal changes in the prevalence and severity of Hematodinium infections

A total of 942 crabs were sampled from both sites during the12-month period (Tables 1 and 2) and of these 923 were studied histologically to assess the prevalence and severity of infection by *Hematodinium*. All animals, regardless of size, were examined histologically and this was used to assign prevalence and severity of infection. Severity was assigned on a scale of 1–4. The highest level of severity (Level 4) was characterized by animals with large numbers of parasites in the connective tissue around the hepatopancreatic tubules and no, or few, remaining haemocytes (see Supplementary material – in online version only for examples and a description of these levels of infection).

Changes in the prevalence and severity of *Hematodinium* infection in juvenile edible crabs collected from Mumbles Head and Oxwich Bay are shown in Fig. 4. The prevalence of disease differed at

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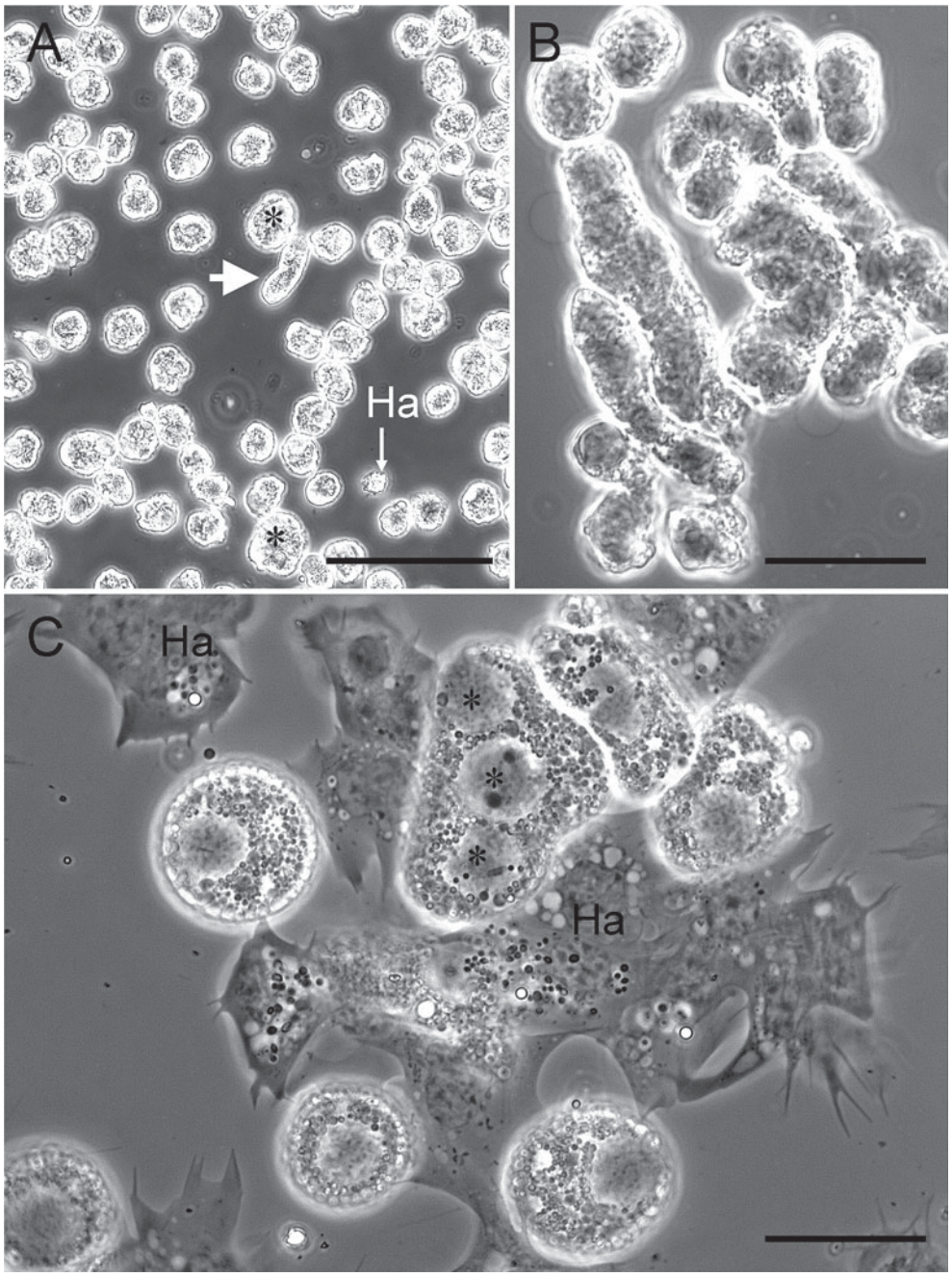

Fig. 2. Phase contrast micrographs showing the variable morphology of *Hematodinium* in the blood of infected crabs. (A) Formalin-fixed blood from a crab with *Hematodinium* (severity Level 4). Note the large numbers of parasites of variable morphology from small to large multinucleate (*) and elongate (arrow). Haemocyte (Ha). Scale bar = 50 μM. (B) Clump of *Hematodinium* showing variable morphology and phase refractile appearance. Scale bar = 20 μM. (C) Micrograph of living cells showing the spread appearance of the Ha in comparison with the rounded, phase-bright *Hematodinium*. Note the multinuclear form (*) and the refractile, granular nature of the parasite's cytoplasm. Scale bar = 20 μM.

the 2 sites with, for example, a maximum prevalence of 46% at Mumbles Head in January compared with only 6% at Oxwich Bay at the same time point (Fig. 4A and B). As shown in Fig. 4A, there was a decline in the prevalence of disease at Oxwich Bay during the year with high levels of infection in the spring to summer and lower levels of infection in the autumn months (September–November). Both sites showed similar temporal patterns of infection particularly in terms of severity. For instance, the severity of infection was the highest in November at both sampling sites. During this month's sampling at Mumbles Head, 7 of the 42 animals sampled were found to be infected by *Hematodinium*, with 5 at Level 4, 1 at Level 3 and 1 at Level 2 severity. In the Oxwich Bay sample for November, only 2 of the 40 crabs (5%) surveyed were infected by *Hematodinium* and both of these had Level 4 severity infections.

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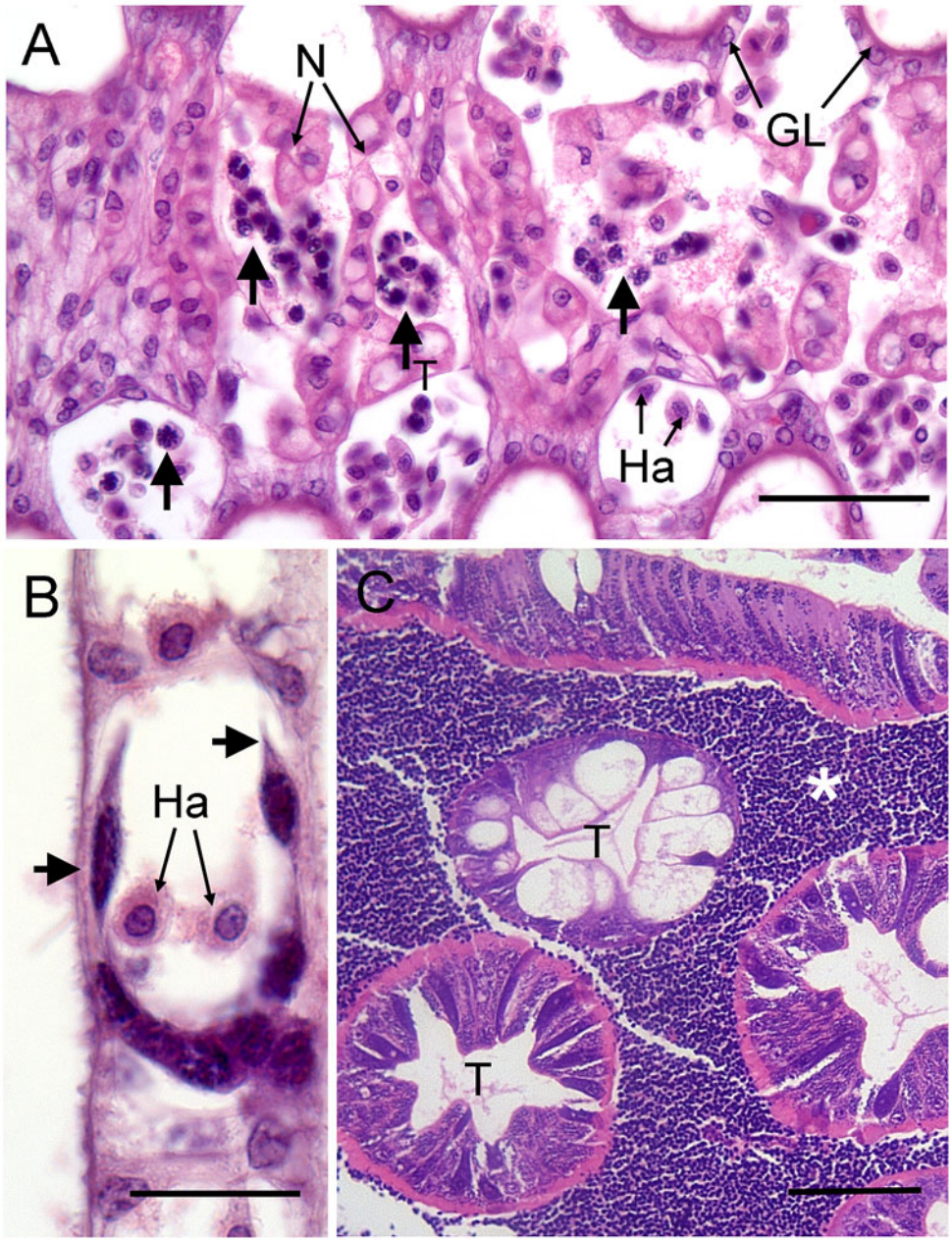

Fig. 3. Appearance of *Hematodinium* in the gills and hepatopancreas of edible crabs. (A) Micrograph showing the branchial septum of a gill which contains blood spaces with circulating haemocytes (Ha) and fixed nephrocytes (N). Note the characteristic nuclear arrangement in these forms of *Hematodinium* (unlabelled arrows). Gill lamellae (GL). Scale bar = 50 μM. (B) Elongate, multicellular form of *Hematodinium* in intimate association with the epithelial cells that form the blood space of a GL containing free Ha. Scale bar = 20 μM. (C) Low power micrograph of the hepatopancreas from a Level 4 severity infected crab. Note large numbers of *Hematodinium* in the swollen intertubular space (*) around the tubules (T). Scale bar = 100 μM.

The smallest crab infected with *Hematodinium* was 12 mm CW (collected from Mumbles Head with Level 1 severity infection) whereas the largest infected crab was 74 mm CW (collected from Oxwich Bay with a Level 3 severity infection). There was no relationship between the size of crabs with and without *Hematodinium* infections for Mumbles Head and Oxwich Bay or when all the data from the 2 survey sites were combined (data not shown; binary logistic regression; $P > 0{\cdot}05$). The distribution of infected animals in terms of size was also analysed by splitting them into arbitrary (10 mm CW) size 'classes' (Fig. 5A and B). The statistical interaction between size class (9 levels) and site (Mumbles Head, Oxwich Bay) was not significant (deviance = 5·79, D.F. = 8, $P = 0{\cdot}67$). However, size 'classes' did explain a significant amount of observed deviance, irrespective of site (deviance = 16·3, D.F. = 8, $P = 0{\cdot}038$). Averaged across size classes, Mumbles Head had significantly higher infection levels than Oxwich Bay (log odds

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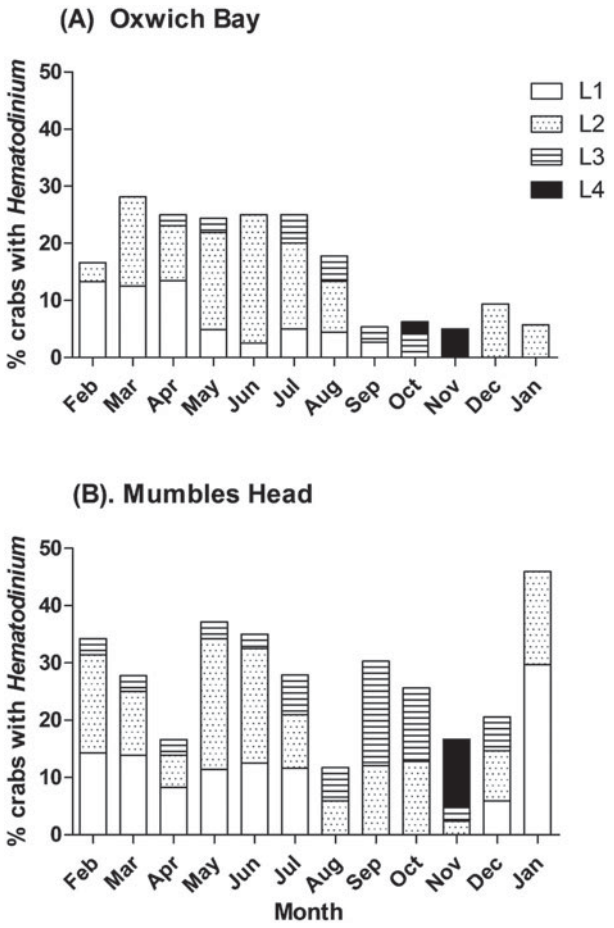

Fig. 4. Temporal changes in the prevalence of *Hematodinium* infections of edible crabs in (A) Oxwich Bay and (B) Mumbles Head, Gower. Both the prevalence and severity of infection were determined using histological analysis.

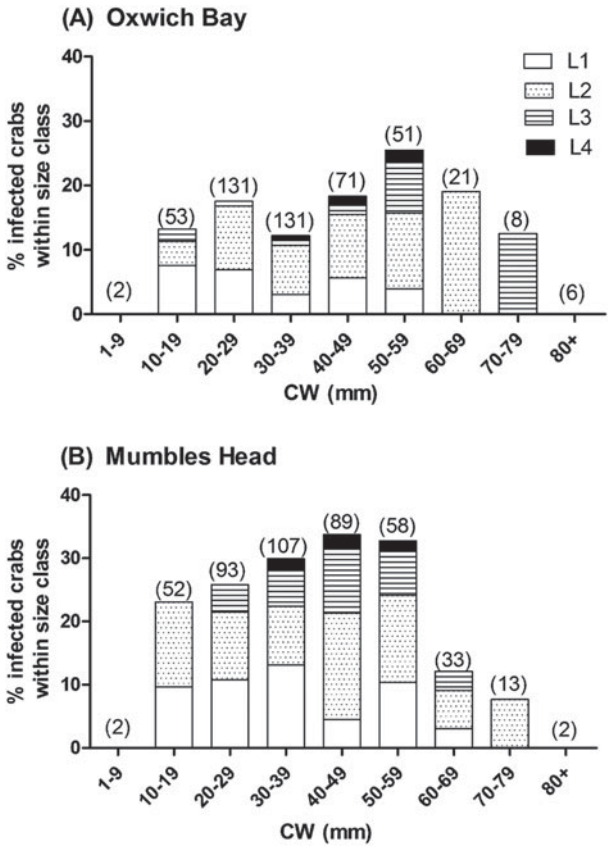

Fig. 5. Relationship between size of crabs, in terms of CW and the percentage of these animals infected by *Hematodinium* at the 2 sample sites. The values in parentheses are the total number of crabs (i.e. both infected and uninfected) in each size 'class'.

ratio [S.E.] = 0·653 [0·166], deviance = 15·8, D.F. = 1, $P < 0·001$). Interestingly, animals in the larger size 'classes' (60 mm+) were apparently less likely to be infected than the lower size 'class' of 50–59 mm. Although only a small number of animals of 80 mm+ were surveyed (6 at Oxwich Bay and 2 at Mumbles) these were all uninfected by *Hematodinium*.

Analysis of infection severity by crab size (in terms of CW) revealed that at Oxwich Bay there was a relationship between size and severity of infection (Fig. 6A; 1 way analysis of variance, $P = 0·02$). There was a significant difference in size between those crabs with infections graded as Levels 1 and 3 severity (Bonferroni multiple comparison post-tests; $P < 0·05$). There was no significant relationship between the severity of disease and crab size for animals surveyed at Mumbles Head where no significant differences were seen (Fig. 6B, $P > 0·05$). Examination of the percentage of *Hematodinium*-infected male and female crabs over the 12-month survey period showed no significant difference in prevalence of infection between the 2 sexes at both sites (data not shown). The overall percentage of intermoult animals that were infected with *Hematodinium* at Oxwich Bay was 18·9% compared with 9·6% in newly moulted (soft) animals. At Mumbles Head, 29·9% of intermoult animals were found to be infected compared with 21·1% of newly moulted individuals. The statistical interaction between moult status (intermoult and post-moult) and site (Mumbles Head, Oxwich Bay) was not significant (deviance = 0·25, D.F. = 1, $P = 0·62$). However, the overall effect of moult status did explain a significant amount of observed deviance, irrespective of site (deviance = 4·09, D.F. = 1, $P = 0·043$). Averaged across moult statuses, Mumbles Head had significantly higher infection levels than Oxwich Bay (log odds ratio [S.E.] = 0·626 [0·175], deviance = 13·2 and D.F. = 1, $P < 0·001$).

DISCUSSION

This present study has shown that the parasitic dinoflagellate *Hematodinium* is present in juvenile edible crabs in the intertidal zone in Gower, UK, with occurrences in both sample locations (Mumbles Head and Oxwich Bay). The sample sites showed a seasonal trend of infections, with high prevalence but low severity (i.e. low parasite load) of infection in

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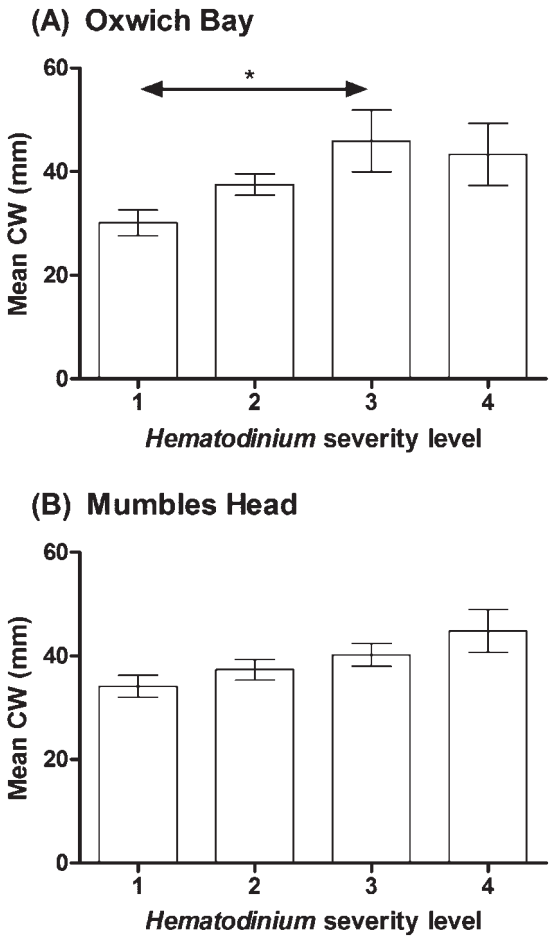

Fig. 6. Effect of size of crabs, in terms of CW, on the severity of *Hematodinium* infection. Mean values S.E.M. *$P<0{\cdot}05$ Bonferroni post-test.

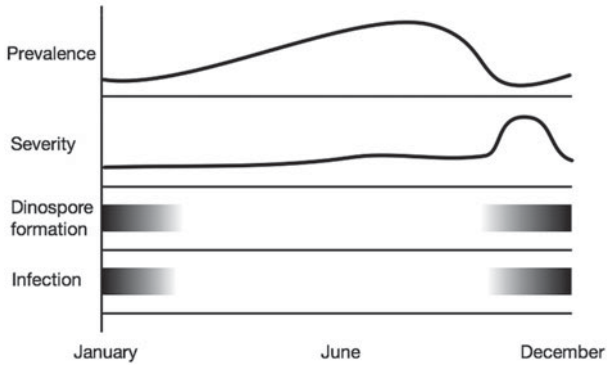

Fig. 7. Diagrammatic representation of putative temporal changes in *Hematodinium* infections in pre-recruit edible crabs in the Bristol Channel region based on the field data of the present study. The presence of dinospores is inferred from previously published reports (e.g. Stentiford and Shields, 2005).

spring to summer. Later in the year, in the autumn months, the number of crabs on shore found to harbour *Hematodinium* was much lower but these individuals all had high severity infections characterized by large numbers of parasites in the blood and other tissues. Other studies have also suggested a high prevalence of *Hematodinium* sp. infections in a variety of crustacean populations in spring months in areas close to our sample sites including Scotland (Field *et al*. 1992, 1998) and Ireland (Briggs and McAliskey, 2002). Based on our field-based data, we postulate that the infections increase in severity during the year with multiplication of the parasite in the tissues of the host (Fig. 7). By the late autumn, the small number of infected animals that are observed on shore all had high severity infections and these may ultimately have released dinospores to infect new hosts (Stentiford and Shields, 2005; Frischer *et al*. 2006), causing the low severity infections seen in our survey in the next spring (Fig. 7). While we favour an explanation that the small number of infected animals found in the autumn reflects death of the host as a result of *Hematodinium* infection, it is possible that infected animals migrate either off shore or higher up the shore, resulting in the apparent low prevalence of disease found in the crabs in this location.

It is unknown how long it takes from the onset of infection of edible crabs with *Hematodinium* to the subsequent death of the host presumably as a result of other secondary infections (Smith *et al*. 2013) or metabolic exhaustion leading to dinospore production (Stentiford and Shields, 2005). Indeed, we failed to find any crabs showing evidence of dinospore production in heavily infected individuals; however, it needs to be highlighted that only 8 of the 916 animals examined had Level 4 infections and therefore this may explain why we failed to observe this stage in parasite development. If our interpretation of the field data is correct, it takes up to 10 months from initiation of infection in the host, in the winter, to death in the late summer–early autumn (Fig. 7). A pilot study conducted under aquarium conditions with juvenile edible crabs suggests that it takes 2–3 months post-challenge for the parasite to establish infection in the blood of edible crabs (Smith and Rowley, unpublished observations). Death follows but only several months to as long as a year after first contact with the parasite. Hence the timescale of this pilot study, carried out under aquarium conditions, is similar to the predicted life cycle of the parasite shown in Fig. 7 based on our field data. Messick and Shields (2000) and Shields and Squyars (2000) performed similar infectivity experiments but with blue crabs (*C. sapidus*). Shields and Squyars (2000) found that *Hematodinium* was present in the haemolymph 2 weeks post-injection and mortalities occurred after around 40 days, whereas Messick and Shields (2000) found that mortality occurred after only 17 days. In similar experiments with snow crabs (*C. opilio*), 50% of experimentally infected animals were dead or moribund by 99 days post-challenge and on average it took 91 days for susceptible animals to die (Shields *et al*. 2005). In these 2 latter species of crabs it

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appears that the time taken to death following challenge is somewhat less than we found in our pilot studies with juvenile edible crabs. Differences in environmental temperature are likely to be of key importance in determining the development times for *Hematodinium* infections in their different hosts. For instance, blue crabs are in sub-tropical and temperate zones whereas snow crabs are from boreal areas with their lower water temperatures. Several other factors, however, may also affect the time taken from challenge to death in these different species of crabs including host susceptibility, variability in the strain and potential species of *Hematodinium* studied (Small, 2012), and differences in the infective dose employed.

The mode of transmission of *Hematodinium* is not well understood but thought to be via the production of dinospores that may gain entry to new hosts via the cuticle (Stentiford and Shields, 2005). Although some authors have postulated that cannibalism of infected hosts may be a mode of transmission (Walker *et al*. 2009), others have found no evidence for such a route (Li *et al*. 2011). Stentiford *et al*. (2001*a*) considered that the Norway lobster (*Nephrops norvegicus*) was more susceptible to infection when they had recently moulted, presumably as a result of the cuticle being soft and therefore more vulnerable to damage and infection from a range of pathogens and parasites (Smith *et al*. 2014). If it is assumed that transmission of disease is entirely as a result of dinospore production, and that this event occurs in edible crabs with high severity infections (i.e. Level 4), then this process is most likely to occur in the intertidal environment in late autumn to early winter (see Fig. 7). Therefore, we predict that the peak of disease transmission in juvenile edible crabs in the Bristol Channel occurs in mid-winter. However, at Mumbles Head only a small percentage of crabs found during this period were found to have recently moulted ranging from 0% in February to a maximum of only 9% in December. If crabs are most vulnerable to infection by *Hematodinium* post-moult, then it would be expected that the peak of dinospore production would be synchronized with times of high moulting frequency (as in *N. norvegicus* see Field *et al*. 1992, 1998) but at this site this largely occurs in the summer months from May to August (Table 1). At Oxwich Bay, the frequency of moulting is greater in the winter months with, for example, 14% of crabs collected in January being soft bodied. Although the timing of moulting and dinospore production at 1 site would appear to be sub-optimal for transmission, we cannot rule out the possibility of parasite transfer from other species of crustaceans, such as velvet swimmers and shore crabs, which are also hosts to this parasite (Morado, 2011) and co-inhabit with edible crabs at the sites surveyed. Furthermore, there is the possibility of a reservoir of infection in the plankton (e.g. Frischer *et al*. 2006; Li *et al*. 2010; Hamilton *et al*. 2011) and in other alternate hosts (Pagenkopp Lohan *et al*. 2012) and so direct transfer of disease from crab to crab may not be the only available mode of transmission.

A question unanswered in our survey relates to at what age do edible crabs become susceptible to infection by *Hematodinium*. If we assume that moulting leaves crabs more vulnerable to infection (see Stentiford and Shields, 2005 for review) then because the smaller crabs moult more regularly than larger animals, this would be expected to favour heightened susceptibility to infection. Our survey of infection at 2 sites found that crabs below the size of 12 mm CW were uninfected; however, the numbers of animals of this size examined was small therefore potentially limiting the value of this observation. Larger animals (i.e. those >60 mm CW) generally showed a lower prevalence of infection. One explanation of this is that crabs become infected when they are relatively small and the infection develops over several months during which time these animals moult and grow. Unfortunately, to our knowledge, there are no published studies that show the timescale of moulting and development in juvenile edible crabs of different size 'classes' especially in the Bristol Channel region and so it is impossible to estimate the time taken for edible crabs in the intertidal zone at our 2 survey sites to grow to a particular size. Furthermore, it is also possible that the growth profile of infected edible crabs could differ from their uninfected counterparts as there are both hormonal and metabolic changes following infection by *Hematodinium* (Stentiford *et al*. 2001*b*; Shields *et al*. 2003; Stentiford and Shields, 2005) likely to affect this. Preliminary aquarium-based studies have found that juvenile edible crabs infected with *Hematodinium* moult less frequently than uninfected animals of similar size range and it is noteworthy that the overall percentage of infected crabs was lower in animals that had just moulted compared with those in intermoult.

One potential problem of the design of this is study is that sampling in the same environment (tide height and location) on monthly intervals does not ensure that the same population of crabs exists from month to month leading to potential under or over-reporting on the prevalence of disease. Juvenile edible crabs, such as those in this study, live and forage in the intertidal–subtidal zones (Silva *et al*. 2014). Little is known about the potential migrations of juvenile crabs but our monthly survey of crabs from the low tide zone (*ca*. 1 m above Chart Datum) showed significant variation in the sex ratio from 1 season to another suggesting some sex-dependent migration either further up the shore or into the sub-tidal zone. The limited number of studies on migration patterns of edible crabs is largely restricted to adults in coastal waters where migration distances of up to *ca*. 100 km have been recorded (Bennett and Brown, 1986; Hunter *et al*. 2013). A recent survey of juvenile

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edible crabs from rocky shores in the south west of the UK using tag recapture approaches found between 10 and 20% recovery of animals *ca*. 30 days later on the same shore (Silva *et al*. 2014). Furthermore, the authors reported 'significant numbers' of crabs could be recovered for up to a year from the same location suggesting only limited migration. Overall, our observations and those of others suggest that the population of crabs in the intertidal zone is not static with potential migration probably caused by foraging behaviours which are gender specific. The feeding behaviour of crabs infected by *Hematodinium* is reduced especially in those animals with severe infections (Smith and Rowley, unpublished observations) and disease agents, in general, are also known to affect the behaviour of their hosts such that they are more or less likely to be collected in such surveys, especially those that rely on capture as a result of their feeding behaviour (e.g. potting in crabs and lobsters). This will undoubtedly lead to bias in datasets, which can result in under of over reporting of disease prevalence and intensity.

Hematodinium infections have become a problem to commercial crustacean fisheries in the Northern Hemisphere (e.g. Wilhelm and Miahle, 1996; Lee and Frischer, 2004; Siddeek *et al*. 2010; Mullowney *et al*. 2011; Morado *et al*. 2012; Small, 2012; Rowley *et al*. 2014). For instance, the decline in catches of velvet swimming crabs (*Necora puber*) in Brittany during the 1980s was attributed to their infection by *Hematodinium* where peak prevalence of infection was *ca*. 80% (Wilhelm and Miahle, 1996). In the present study, if an assumption is made that all crabs infected by *Hematodinium* eventually succumb to the infection (Small, 2012), then up to 45% of juvenile edible crabs in the Bristol Channel region may be lost from the fishery as a result of this disease alone. This may have a major effect on the fisheries studied where concerns have already been expressed over the sustainability of fisheries practice (Bannister, 2010). Changes in the practices of fishers to deal with *Hematodinium*-infected animals, such as stopping their use as bait in creels and appropriate disposal of grossly infected animals, should be considered if this disease condition is to be controlled. Recent reports of the potential of various environmental reservoirs of *Hematodinium* (e.g. Frischer *et al*. 2006; Hamilton *et al*. 2011) are of concern and any resulting strategy to minimize the spread of disease will need to take these into account. It must be stressed, however, that it is not known if the 'strain' of *Hematodinium* sp. infecting *C. pagurus* in this region is responsible for mortality of these animals while in the intertidal zone.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0031182014001255.

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REFERENCES

Bannister, R. C. A. (2010). On the management of brown crab fisheries. *Shellfish Association of Great Britain*.

Bateman, K. S., Hicks, R. J. and Stentiford, G. D. (2011). Disease profile differ between non-fished and fished populations of edible crab (*Cancer pagurus*) from a major commercial fishery. *ICES Journal of Marine Science* **68**, 2044–2052.

Behringer, D. C. (2012). Diseases of wild and cultured juvenile crustaceans: insights from below the minimum landing size. *Journal of Invertebrate Pathology* **110**, 225–233.

Bennett, D. B. and Brown, C. G. (1986). Crab (*Cancer pagurus*) migrations in the English Channel. *Journal of the Marine Biological Association of the United Kingdom* **63**, 371–398.

Briggs, R. P. and McAliskey, M. (2002). The prevalence of in *Nephrops norvegicus* from the western Irish Sea. *Journal of the Marine Biological Association of the United Kingdom* **82**, 427–433.

Burton, M. and Burton, R. (2002). Edible crab. In *International Wildlife Encyclopedia*, 3rd edn, pp. 471–472. Marshall Cavendish, New York, USA.

Field, R. H., Chapman, C. J., Taylor, A. C., Neil, D. M. and Vickerman, K. (1992). Infection of the Norway lobster *Nephrops norvegicus* by a *Hematodinium*-like species of dinoflagellate on the west coast of Scotland. *Diseases of Aquatic Organisms* **13**, 1–15.

Field, R. H., Hills, J. M., Atkinson, R. J. A., Magill, S. and Shanks, A. M. (1998). Distribution and seasonal prevalence of *Hematodinium* sp. infection of the Norway lobster (*Nephrops norvegicus*) around the west coast of Scotland. *ICES Journal of Marine Science* **55**, 846–858.

Fish, J. D. and Fish, S. (1989). *A Student's Guide to the Seashore*, 3rd edn. Unwin and Hyman, London.

Frischer, M. E., Lee, R. F., Sheppard, M. A., Mauer, A., Rambow, F., Neumann, M., Brofft, J. E., Wizenmann, T. and Danforth, J. M. (2006). Evidence of free-living life stage of the blue crab parasitic dinoflagellate, *Hematodinium* sp. *Harmful Algae* **5**, 548–557.

Gornik, S. G., Cranenburgh, A. and Waller, R. F. (2013). New host range for *Hematodinium* in Southern Australia and novel tools for sensitive detection of parasitic dinoflagellates. *PLoS ONE* **8**, e82774.

Hamilton, K. M., Tew, I. F., Atkinson, R. J. A. and Roberts, E. C. (2011). Occurrence of the parasite genus *Hematodinium* (Alveolata: Syndinea) in the water column. *Journal of Eukaryotic Microbiology* **58**, 446–451.

Hudson, D. A. and Shields, J. D. (1994). *Hematodinium australis* n. sp., a parasitic dinoflagellate of the sand crab *Portunus pelagicus* from Moreton Bay, Australia. *Diseases of Aquatic Organisms* **19**, 109–119.

Hunter, E., Eaton, D., Stewart, C., Lawler, A. and Smith, M. T. (2013). Edible crabs "go west": migrations and incubation cycle of *Cancer pagurus* revealed by electronic tags. *PLoS ONE* **8**, e63991.

Lee, R. F. and Frischer, M. E. (2004). The decline of the blue crab. *American Scientist* **92**, 548–553.

Li, C., Shields, J. D., Miller, T., Small, H. J., Pagenkopp, K. M. and Reece, K. S. (2010). Detection and quantification of the free-living stage of the parasitic dinoflagellate *Hematodinium* sp. in laboratory and environmental samples. *Harmful Algae* **9**, 515–521.

Li, C., Wheeler, K. N. and Shields, J. D. (2011). Lack of transmission of *Hematodinium* sp. in the blue crab *Callinectes sapidus* through cannibalism. *Diseases of Aquatic Organisms* **96**, 249–258.

Li, C., Song, S., Liu, Y. and Chen, T. (2013). *Hematodinium* infections in cultured Chinese swimming crab, *Portunus trituberculatus*, in northern China. *Aquaculture* **396**, 59–65.

Mascaró, M. and Seed, R. (2001). Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus* L. *Marine Biology* **139**, 1135–1145.

Messick, G. A. (1994). *Hematodinium perezi* infections in adult and juvenile blue crabs *Callinectes sapidus* from coastal bays of Maryland and Virginia, USA. *Diseases of Aquatic Organisms* **19**, 77–82.

Messick, G. A. and Shields, J. D. (2000). Epizootiology of the parasitic dinoflagellate *Hematodinium* sp. in the American blue crab *Callinectes sapidus*. *Diseases of Aquatic Organisms* **43**, 139–152.

Marine Management Organisation (MMO) (2011). The UK Fishing Industry in 2010: Landings, A National Statistics Publication.

Morado, J. F. (2011). Protistan diseases of commercially important crabs: a review. *Journal of Invertebrate Pathology* **106**, 27–53.

Morado, J. F., Dawe, E. G., Mullowney, D., Shavey, C. A., Lowe, V. C. and Cawthorn, R. J. (2010). Climate change and the worldwide emergence of Hematodinium-associated disease: is there evidence for a relationship? In *Biology and Management of Exploited Crab Populations under Climate Change* (ed. Kruse, G. H., Eckert, G. L., Foy, R. J., Lipcius, R. N., Sainte-Marie, B., Stram, D. L. and Woodby, D.), pp. 153–173. Alaska Sea Grant, University of Alaska Fairbanks, Fairbanks, AK, USA.

Morado, J. F., Siddeek, M. S. M., Mullowney, D. R. and Dawe, E. G. (2012). Protistan parasites as mortality drivers in cold water crab fisheries. *Journal of Invertebrate Pathology* **110**, 201–210.

Mullowney, D. R., Dawe, E. G., Morado, J. F. and Cawthorn, R. J. (2011). Sources of variability in prevalence and distribution of bitter crab disease in snow crab (*Chionoecetes opilio*) along the northeast coast of Newfoundland. *ICES Journal of Marine Science* **68**, 463–471.

Pagenkopp Lohan, K. M., Reece, K. S., Miller, T. L., Wheeler, K. N., Small, H. J. and Shields, J. D. (2012). The role of alternate hosts in the ecology and life history of *Hematodinium* sp. a parasitic dinoflagellate of the blue crab (*Callinectes sapidus*). *Journal of Parasitology* **98**, 73–84.

Rowley, A. F., Cross, M., Culloty, S. C., Lynch, S. A., Mackenzie, C. L., Morgan, E., O'Riordan, R. M., Robins, P. E., Smith, A. L., Thrupp, T. J., Vogan, C. L., Wootton, E. C. and Malham, S. K. (2014). The potential impact of climate change on the infectious diseases of the commercially important shellfish populations in the Irish Sea – a review. *ICES Journal of Marine Science* **71**, 741–759.

Shields, J. D. and Squyars, C. M. (2000). Mortality and hematology of blue crabs, *Callinectes sapidus*, experimentally infected with the parasitic dinoflagellate *Hematodinium perezi*. *Fisheries Bulletin* **98**, 139–152.

Shields, J. D., Scanlon, C. and Volety, A. (2003). Aspects of the pathophysiology of blue crabs, *Callinectes sapidus*, infected with the parasitic dinoflagellate *Hematodinium perezi*. *Bulletin of Marine Science* **72**, 519–535.

Shields, J. D., Taylor, D. M., Sutton, S. G., O'Keefe, P. G., Ings, D. W. and Pardy, A .L. (2005). Epidemiology of bitter crab disease (*Hematodinium* sp.) in snow crabs *Chionoectes opilio* from Newfoundland, Canada. *Diseases of Aquatic Organisms* **64**, 253–264.

Shields, J. D., Taylor, D. M., O'Keefe, P. G., Colbourne, E. and Hynick, E. (2007). Epidemiological determinants in outbreaks of bitter crab disease (*Hematodinium* sp.) in snow crabs, *Chionoecetes opilio* from Newfoundland, Canada. *Diseases of Aquatic Organisms* **77**, 61–72.

Siddeek, A. S. M., Zheng, J., Morado, J. F., Kruse, G. H. and Bechtol, W. R. (2010). Effect of bitter crab disease on rebuilding in Alaska Tanner crab stocks. *ICES Journal of Marine Science* **67**, 2027–2032.

Silva, A. C. F., Boaventura, D. M., Thompson, R. C. and Hawkins, S. J. (2014). Spatial and temporal patterns of subtidal and intertidal crab excursions. *Journal of Sea Research* **85**, 343–348.

Small, H. J. (2012). Advances in our understanding of the global diversity and distribution of *Hematodinium* spp. – significant pathogens of commercially exploited crustaceans. *Journal of Invertebrate Pathology* **110**, 234–246.

Smith, A. L., Hamilton, K. M., Hirschle, L., Wootton, E. C., Vogan, C. L., Pope, E. C., Eastwood, D. C. and Rowley, A. F. (2013). Characterization and molecular epidemiology of a fungal infection of edible crabs (*Cancer pagurus*) and interaction of the fungus with the dinoflagellate parasite *Hematodinium*. *Applied and Environmental Microbiology* **79**, 783–793.

Smith, A. L., Whitten, M. A. A., Hirschle, L., Pope, E. C., Wootton, E. C., Vogan, C. L. and Rowley, A. F. (2014). Bacterial septicaemia in prerecruit edible crabs, *Cancer pagurus* L. *Journal of Fish Diseases* **37**, 729–737. doi: 10.1111/jfd.12163.

Stentiford, G. D. (2008). Diseases of the European edible crab (*Cancer pagurus*): a review. *ICES Journal of Marine Science* **65**, 1578–1592.

Stentiford, G. D. and Shields, J. D. (2005). A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Diseases of Aquatic Organisms* **66**, 47–70.

Stentiford, G. D., Neil, D. M. and Atkinson, R. J. A. (2001*a*). The relationship of *Hematodinium* infections prevalence in a Scottish *Nephrops norvegicus* population to season, moulting and sex. *ICES Journal of Marine Science* **58**, 814–823.

Stentiford, G. D., Chang, E. S., Chang, S. A. and Neil, D. M. (2001*b*). Carbohydrate dynamics and the crustacean hyperglycaemic hormone (CHH): effects of parasitic infection in Norway lobsters (*Nephrops norvegicus*). *General and Comparative Endocrinology* **121**, 13–22.

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* **79**, 179–191.

Walker, A. N., Lee, R. F. and Frischer, M. E. (2009). Transmission of the parasitic dinoflagellate *Hematodinium* sp. infection in blue crabs *Callinectes sapidus* by cannibalism. *Diseases of Aquatic Organisms* **85**, 193–197.

Wilhelm, G. and Miahle, E. (1996). Dinoflagellate infection associated with the decline in *Necora puber* crab populations in France. *Diseases of Aquatic Organisms* **26**, 213–219.