

Disease profiles differ between non-fished and fished populations of edible crab (*Cancer pagurus*) from a major commercial fishery

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Despite their significant contribution to global marine fisheries, relatively little information is available on the pathogen profile of commercially exploited decapod crustacean populations. Most of the information published relates to adult (fished) subpopulations, with almost nothing known about disease processes and mortality drivers in juveniles. The seasonal profile of pathogens in non-fished (prerecruit) and fished (recruit) subpopulations of *Cancer pagurus*, a major target fishery target in European waters, is investigated. Histopathology and ultrastructural assessment of tissues demonstrated a distinct pathogen profile in the two subpopulations, the apparent prevalence of specific pathogens varying with both season and life stage of the host. In some cases, highly prevalent pathogens in the prerecruit subpopulation were not observed in the recruit subpopulation. In this context, the discovery of a novel and highly prevalent haplosporidian-like parasite infecting the antennal gland and bladder of prerecruit life stages of *C. pagurus* is reported. Co-infections with pathogens described previously, such as *Hematodinium* sp. and *C. pagurus* bacilliform virus, were also observed. Disease assessments in the prerecruit subpopulation of commercial decapod fishery targets could perhaps be utilized to improve the estimation of cohort success and, therefore, forecasts of future recruitment to the fishery.

Keywords: *Cancer pagurus* bacilliform virus, edible crab, haplosporidian-like parasite, *Hematodinium* sp., juvenile, *Microphallus primas*.

Introduction

The European edible crab (*Cancer pagurus*) is a relatively large species that fulfils a predatory life style by consuming a variety of molluscan and crustacean prey, including smaller members of their own species (Lawton and Hughes, 1985; Lawton, 1989). Juvenile edible crabs settle in the intertidal zone between late summer and early autumn (Bennett, 1995), then remain there for ~3 years until they attain a carapace width of 60–70 mm (Regnault, 1994). Larger crabs move to increasingly subtidal areas with the subsequent growth rate varying with age and gender, but also possibly with ambient depth and geographic location (Bennett, 1979). Adult crabs support an important fishery in Europe, where they are captured using baited traps. As such, *C. pagurus* is considered a key fisheries resource within European waters, with catches exceeding 42 000 t and first sale values of £60 million in 2008 (www.fao.org/fishery/statistics; www.marinemanagement.org.uk). A significant proportion of this take (over 50%) is landed at ports in the UK (22 745 t, estimated value £30 million). The English Channel fishery supports a significant proportion of UK national landings for the species.

The fishery is currently managed on a national basis and utilizes a minimum landing size (MLS) that stipulates the minimum size of crabs that may be retained for market sale. The MLS differs between geographic regions owing to variations in growth rate and other life-history parameters within different subpopulations (Bennett, 1995). The MLS also essentially defines the size at which

crabs enter the fishable population. However, as growth in crustaceans is not continuous and only occurs following moulting, accurate determination of the age of individual animals (as is done for finfish) is not possible (Edwards, 1979). Early studies on this issue by Pearson (1908), and later Bennett (1974), concluded that it was extremely difficult, if not impossible, to distinguish specific year classes with significant overlap in size and moult increment even during the early stages. The issue is apparently compounded when attempting to determine the age of larger crustaceans, hence stifling the use of age-based stock assessment models as widely used in finfish stock assessment (Bennett, 1995). Attempts to address this using biochemical techniques based on the measurement of accumulation of neurolipofuscin in the nervous system of crustaceans have suggested that whereas edible crabs may recruit to the fishery at ~4 years of age, individual growth rate is highly variable (Sheehy and Prior, 2008).

Despite the inherent difficulties in assigning age classes to subsets of a crab population, it is feasible to consider those animals below the MLS (so-called prerecruits) as a separate subgroup to bigger animals (recruits). In effect, the size of subpopulations of animals below the MLS should be limited only by natural mortality (e.g. caused by disease or predation), whereas those above the MLS will be additionally limited by fishing mortality (removal from the population by fishers). In these terms, we can remove the necessity to understand mortality in specific age classes by instead considering factors that may limit the likelihood

of prerecruit populations being recruited to the fishery. By identifying, and potentially quantifying, mortality drivers (such as disease) in prerecruit subpopulations of crabs, it may then be possible to estimate prerecruit success for a given fishery within a given year. Although it is stressed that more work is needed to confirm the assumption that prerecruit crabs enter a fishery in the same area, such data could be used to pre-calculate the future supply of animals recruiting to a fishery.

In a recent review of the pathogen profile of *C. pagurus*, it was noted that most published information relates to infections in the recruit population (adults), with very little available data on the pathogen profile of prerecruit subpopulations (Stentiford, 2008). While providing valuable insight into pathogens that may cause direct or indirect losses to the fishery, in some cases, the prevalence of infection may be poorly estimated as a consequence of factors such as the differential catchability of infected animals or the inadvertent preselection of healthy or infected animals by fishers during sampling (Stentiford *et al.*, 2001; Stentiford and Shields, 2005). Some studies have, however, focused on the pathogens of juvenile *C. pagurus*, with examples ranging from viruses (Bateman and Stentiford, 2008), metazoans (Boschma, 1955), and parasitoids (Kuris *et al.*, 2002) appearing to show a specific preference for infecting younger age classes.

In an attempt to provide an overview of potential mortality drivers associated with pathogenic infections in prerecruit and recruit subpopulations of *C. pagurus*, we sampled crabs from the English Channel, UK. Prerecruit crabs were sampled from the shore and recruits (above MLS, > 14 cm) from commercial fishing vessels operating immediately offshore of the shore-collection sites. Samples were collected each month for 12 months and analysed using histology and electron microscopy for the detection and identification of pathogens. The pathogen profile and apparent prevalence of specific pathogens varied with season and, significantly, differed between prerecruit and recruit subpopulations. In some cases (e.g. a novel haplosporidian infection of the antennal gland and bladder), highly prevalent pathogens discovered in prerecruit subpopulations were not observed at all in the recruit subpopulation. As previously noted by Stentiford (2008), with increasing pressure on global commercial stocks of decapod crustaceans, there is a growing requirement to understand the factors that limit recruitment to the fishery and, further, to understand so-called silent mortality drivers in the prerecruit subpopulation. Disease assessments in shoreline (prerecruit) populations of *C. pagurus* are proposed as a means of forecasting likely future recruitment to the commercial fishery for this species and, as such, might be a useful tool for predicting long-term stability of the fishery.

Material and methods

European edible crabs (*C. pagurus*) above the MLS of 14 cm, hereafter referred to as recruits, were captured in baited traps from the commercial fishery in Weymouth Bay (50°34'N 02°22'W) each month between December 2003 and November 2004. Edible crabs below the MLS (hereafter referred to as prerecruits) were sampled from the shore of Weymouth Bay each month between April 2008 and March 2009. On arrival at the laboratory, the visual health of each crab was assessed before each was placed in running seawater in small tanks for up to 12 h, before processing for histology and electron microscopy. Recruit and prerecruit subpopulations were sampled during different years owing to availability under specific monitoring programmes carried out by the Cefas laboratory within those years.

Up to 30 recruit and prerecruit crabs were sampled each month from the fishery and shoreline sites, respectively. Crabs were anaesthetized by chilling on ice for 30 min before dissection. The hepatopancreas, gill, gonad, heart, antennal gland, epithelial tissue, body muscle, and claw muscle were removed from each crab for histology. Excised samples were placed immediately into Davidson's seawater fixative, and fixation was allowed to proceed for 24 h before samples were transferred to 70% industrial methylated spirit. Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at a thickness of 3–5 µm on a rotary microtome and mounted on glass slides before staining with haematoxylin and eosin (H&E), Giemsa, and Feulgen stains. Stained sections were analysed by light microscopy (Nikon Eclipse E800) digital imagery, and measurements were taken using the Lucia™ Screen Measurement System (Nikon, UK).

For electron microscopy, the hepatopancreas, antennal gland, epithelial tissue, and gonad were removed; small blocks of tissue (2 mm³) were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at room temperature. Fixed tissue samples were rinsed in 0.1 M sodium cacodylate buffer (pH 7.4), then post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. Specimens were washed in three changes of 0.1 M sodium cacodylate buffer before dehydration through a graded acetone series, then embedded in epoxy resin 812 (Agar Scientific-pre-mix kit 812) and polymerized overnight at 60°C in an oven. Semi-thin (1–2 µm) sections were stained with Toluidine Blue for viewing under a light microscope to identify suitable target areas. Ultra-thin sections (70–90 nm) of these areas were mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). Grids were examined using a JEOL JEM 1210 transmission electron microscope, and digital images were made with a Gatan Erlangshen ES500W camera, using Gatan Digital Micrograph™ software.

Results

A range of previously known and novel pathogens were detected in prerecruit and recruit subpopulations of *C. pagurus* throughout the course of the study. A summary of prevalence of specific pathogens by season and subpopulation type is listed in Table 1. Data are arranged according to the classification of the infection as either high prevalence (>10%) or low prevalence (<10%) in respective subsamples.

High-prevalence pathogens

Three pathogens predominated in the histopathological analyses carried out on recruit and prerecruit crab subpopulations. *Hematodinium* sp., which causes so-called pink crab disease (Stentiford *et al.*, 2002), was detected in both prerecruit and recruit subpopulations (Figure 1a and b), but apparent prevalence was higher in recruits (peaking in April, May, and June at 30, 47, and 27%, respectively; with a smaller peak over winter). The highest infection in prerecruits was in August (10%), but in contrast to the recruit subpopulation, did not appear as a distinctive peak, but rather as a low-level prevalence (<5%) in most months of the year. The two remaining high apparent prevalence pathogens appeared to be limited exclusively to prerecruits. Encysted metacercarial stages of *Microphallus primas* (Saville and Irwin, 2005) were observed at high apparent prevalence throughout the study period (Figure 1c), with infection peaking at 74% in

Table 1. Summary and percentage apparent prevalence of pathogens found infecting both prerecruit and recruit edible crabs over a 12-month sampling period.

Parameter	January	February	March	April	May	June	July	August	September	October	November	December
Average size (mm)												
Prerecruit	42	41	43	50	43	50	34	38	35	30	41	42
Recruit	157	161	164	162	167	171	170	178	163	164	175	157
Sex ratio (M/F)												
Prerecruit	33/67	42/58	32/68	33/67	64/36	56/44	50/50	48/52	43/57	63/37	42/58	48/52
Recruit	57/43	54/46	83/17	80/20	53/47	57/43	47/53	63/37	46/54	27/73	54/46	83/17
<i>Hematodinium</i> sp.												
Prerecruit	0	3	4	0	4	5	0	10	3	3	0	0
Recruit	13	3	0	30	47	27	7	3	8	13	8	17
<i>Paramarteilia canceri</i>												
Prerecruit	0	0	0	0	0	0	0	0	0	0	0	0
Recruit	0	3	3	0	0	0	0	0	0	3	0	0
<i>Enterosporea canceri</i>												
Prerecruit	0	6	4	0	0	3	0	0	0	0	0	0
Recruit	0	0	3	3	7	3	0	0	0	0	0	0
Haplosporidian												
Prerecruit	67	27	80	78	57	67	44	67	50	62	74	72
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
CpBV												
Prerecruit	8	7	4	0	11	5	6	7	3	0	4	3
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
Yeast												
Prerecruit	0	0	0	0	0	0	0	0	0	0	0	0
Recruit	3	7	0	0	0	0	0	0	0	0	0	0
<i>Fecampia erythrocephala</i>												
Prerecruit	0	0	0	0	4	0	0	0	0	0	0	0
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sacculina inflata</i>												
Prerecruit	0	0	0	0	4	0	0	0	0	6	0	0
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
<i>Microphallus primas</i>												
Prerecruit	44	17	40	50	29	41	50	33	50	50	74	55
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
Muscle microsporidian												
Prerecruit	0	0	4	0	0	0	0	0	0	0	0	0
Recruit	0	0	0	0	0	0	0	0	0	0	0	0
HP microsporidian												
Prerecruit	0	7	0	0	0	0	0	0	0	0	0	0
Recruit	0	0	0	0	0	0	0	0	0	0	0	0

the November subsample and present in at least 17% of prerecruit crabs sampled in each of the other months.

A novel and highly prevalent haplosporidian-like parasite was also discovered infecting the antennal gland and bladder of prerecruit crabs. Infection was observed grossly as a massively hypertrophied, discoloured, and somewhat gelatinous antennal gland and bladder (Figure 1d). Histology revealed that both the major lateral lobes of the antennal gland and bladder were massively proliferated in the regions next to the hepatopancreatic lobes, with uninucleate and multicellular parasitic plasmodial stages distributed throughout the epithelial cells of infected glandular tubules (Figure 1e). Electron microscopy confirmed the infection of these epithelial cells by a haplosporidian-like parasite, with unicellular stages developing to multicellular plasmodia (Figure 1f). Infection in prerecruits was highly prevalent (>27%) in each month of the survey, peaking at 80% in the subsample collected in March. As stated above, this pathogen was not observed in the recruit subpopulation of *C. pagurus* during the study period, nor in previous studies of recruit populations of *C. pagurus* carried out by the Cefas laboratory.

Low-prevalence pathogens

Many pathogens were observed at relatively low prevalence in subpopulations of recruit and prerecruit *C. pagurus*. Usually, the prevalence of these pathogens did not appear to indicate any obvious seasonality, although, in general, the pathogenic expression of disease caused by these infections was severe and apparently led to a significant negative effect on the fitness of individual host crabs.

Cancer pagurus bacilliform virus (CpBV; Bateman and Stentiford, 2008) was detected infecting the hepatopancreatic epithelia of juvenile crabs at low prevalence throughout the study, peaking at 11% in the May subsample. Infected nuclei were hypertrophied with marginalized chromatin and were found in the reserve (R) and fibrillar (F) cells of the hepatopancreas (Figure 2a). Electron microscopy confirmed the infection of these cells with CpBV via the appearance of intact virions within enlarged host cell nuclei. Virions were rod-shaped, measured $\sim 210 \times 60$ nm, and consisted of an electron dense nucleocapsid surrounded by a trilaminar membrane with a lateral extension at one end (Figure 2b). This infection was not

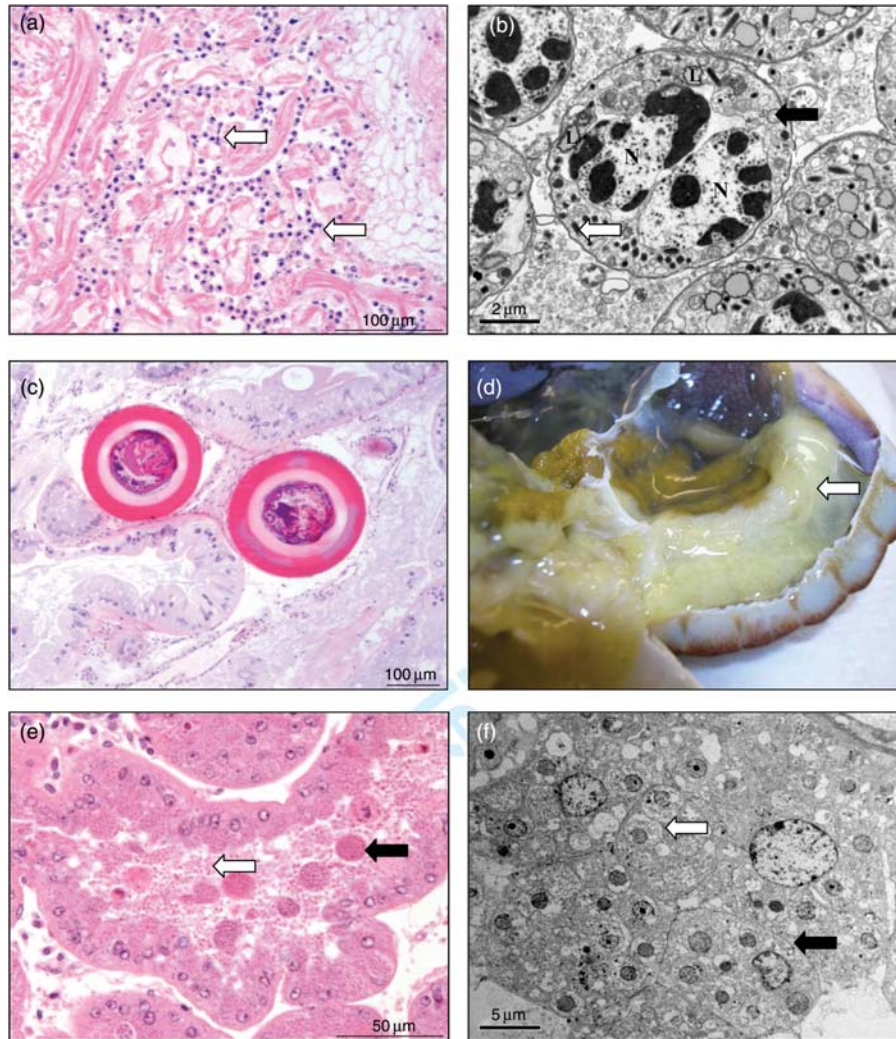


Figure 1. (a) Uninucleate trophont stages (arrows) of *Hematodinium* sp. within the heart muscle. This parasite infects both prerecruit and recruit edible crabs (H&E stain; scale bar 100 μ m). (b) Ultrastructure of a multinucleate plasmodia of *Hematodinium* sp. within a hepatopancreatic sinusoid. Parasites typically contain between one and four nuclei (N), trichocysts (white arrow), lipid droplets (L), and mitochondria (black arrow) (TEM; scale bar 2 μ m). (c) *Microphallus primas* metacercarial cysts within the hepatopancreas tissue of a prerecruit edible crab (H&E stain; scale bar 100 μ m). (d) Haplosporidian-infected antennal gland, which is enlarged and appears yellow (arrow) with a gelatinous texture. (e) Proliferation of the antennal gland, with tubules heavily infected with the haplosporidian-like parasite. Uninucleate stages (white arrow) and multinucleate (black arrow) plasmodia are evident (H&E stain; scale bar 50 μ m). (f) Ultrastructure of haplosporidian-like parasite within the antennal gland tubule. Uninucleate (white arrow) stages can be seen present within the multinucleate plasmodia (black arrow) (TEM; scale bar 5 μ m).

seen in any of the recruit subpopulation sampled during the study. Filamentous bacteria were observed on gills (data not shown) and considered to be epibionts, and no cases of true systemic bacterial infection were observed during the study.

Several low-prevalence infections by parasites of the Phylum Microsporidia were observed during the study. The musculature from one prerecruit crab sampled during March revealed a microsporidian infection in the skeletal musculature. There were no external signs of this infection, but histology revealed destruction of the muscle tissue structure, and small spores could be seen in the affected areas (image not shown). Although samples were not available for electron microscopy or nucleic-acid-based diagnostics from this specimen, this was likely to be an infection with the previously described *Ameson atlanticum* (Vivarès and Azevedo, 1988).

Microsporidian infections of the hepatopancreatic epithelia were also observed, in both prerecruit and recruit subpopulations of *C. pagurus*. A novel, previously undescribed, microsporidian was found infecting prerecruit crabs only. Although there were no external signs of infection, the cytoplasm of the hepatopancreatic epithelial cells contained large numbers of spore stages that were confirmed, via electron microscopy, to be a parasite of the Phylum Microsporidia (Figure 2c). Mature spores measured 1.7 μ m and consisted of a trilaminar wall, a unikaryotic nucleus, and an anchoring disc attached to a polar filament that underwent 8–9 turns (Figure 2d). These features, along with the differences in pathology from the previously described *Enterospora cancri* (Stentiford *et al.*, 2007), likely distinguish this pathogen as novel. Preliminary studies have shown that this microsporidian is phylogenetically similar to *Hepatospora eriocheir* (Stentiford

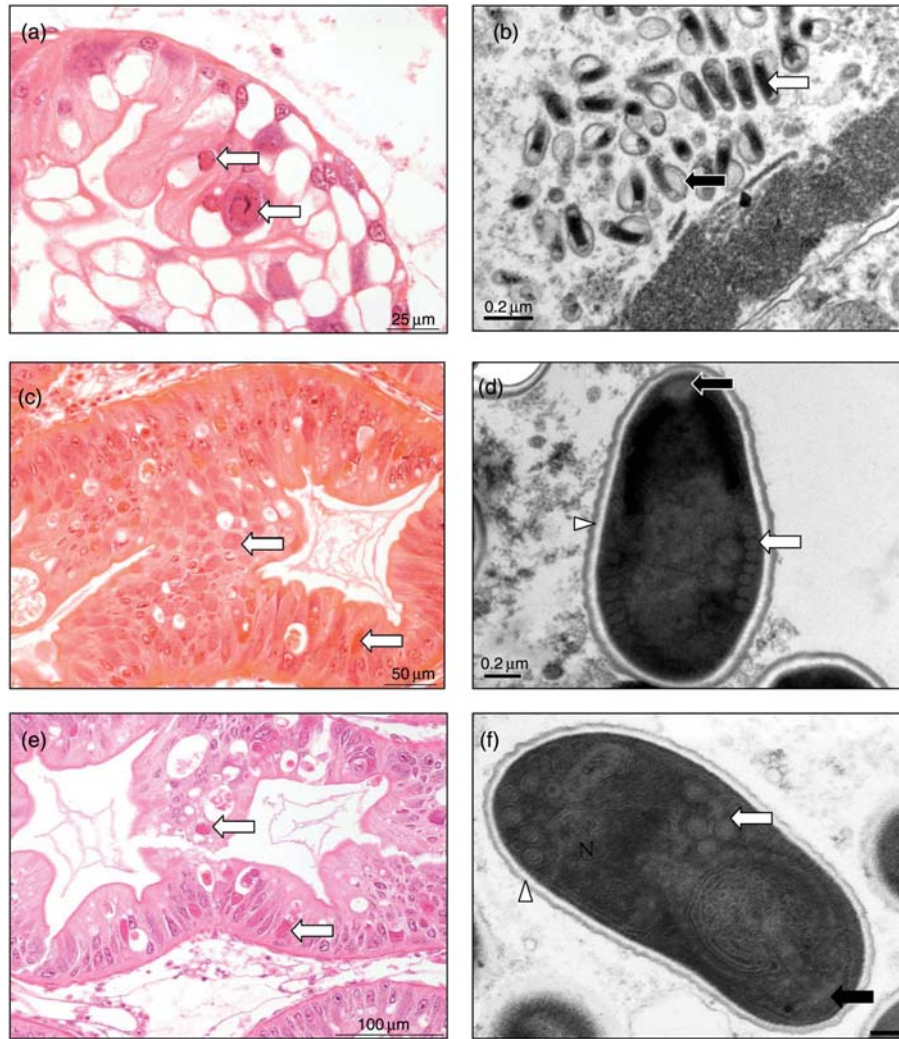


Figure 2. (a) CpBV infection within the hepatopancreatic tubule epithelial cells. Infected nucleus is enlarged with (arrowed) marginalized chromatin (H&E stain; scale bar 25 μm). (b) CpBV virus particles within an infected nucleus. Virions (present in both longitudinal and transverse section) are rod-shaped and consist of a nucleocapsid (white arrow) surrounded by a trilaminar membrane with a lateral extension at one end (black arrow) (TEM; scale bar 0.2 μm). (c) Unidentified microsporidian parasite infecting the hepatopancreas, tubule epithelial cells contain multiple eosinophilic inclusions (arrows) (H&E stain; scale bar 50 μm). (d) Unidentified microsporidian from the hepatopancreas, with spore measuring 1.7 μm consisting of a trilaminar membrane (arrow head), anchoring disc (black arrow) and possessing 8–9 turns of the polar filament (white arrow) (TEM; scale bar 0.2 μm). (e) Hepatopancreatic tubules infected with the microsporidian *E. canceri*. This microsporidian infected both prerecruit and recruit edible crabs. Infected nuclei (arrows) contained multiple spores (H&E stain; scale bar 100 μm). (f) Ultrastructure of mature *E. canceri* spore, with spores measuring 1.3 μm, consisting of a trilaminar wall (arrow head), nucleus (N), and anchoring disc (black arrow), and possessing 4–5 turns of the polar filament (white arrow) (TEM; scale bar 100 nm).

et al., 2011) found infecting an invasive population of Chinese mitten crabs (*Eriocheir sinensis*) in the River Thames, UK. Further work is now required to fully characterize this microsporidian parasite from *C. pagurus*.

The intranuclear microsporidian *E. canceri* was also observed at low prevalence between February and June in the prerecruit subpopulation of *C. pagurus* and between March and May in the recruit subpopulation. Again there were no external signs of infection, but histology revealed the presence of life stages of this microsporidian within the nuclei of hepatopancreatic epithelial cells (Figure 2e). Infection was confirmed via electron microscopy; mature spores 1.3 μm long and consisting of a trilaminar wall, a unikaryotic nucleus, and an anchoring disc attached to a polar filament that underwent 4–5 turns (Figure 2f). Infection prevalence

peaked at 7% in prerecruit crabs (May) and 6% in recruit crabs (February).

Four other low-prevalence pathogens were observed either exclusively in prerecruit or recruit subpopulations of *C. pagurus*. Prerecruit crabs infected with the turbellarian parasitoid *Fecampia erythrocephala* (Kuris *et al.*, 2002) could be grossly distinguished from uninfected crabs by their pale colouration compared with non-parasitized crabs (Figure 3a). This pathogen (Figure 3b) was rarely observed in prerecruit crabs during this study (just two occurrences, i.e. 4%, in the May subsample), and never observed in the recruit subpopulation. The parasitic barnacle *Sacculina inflata* (Boschma, 1955) was also observed infecting prerecruit crabs only, during May and October in the current study. Usually, the externa was observed beneath the

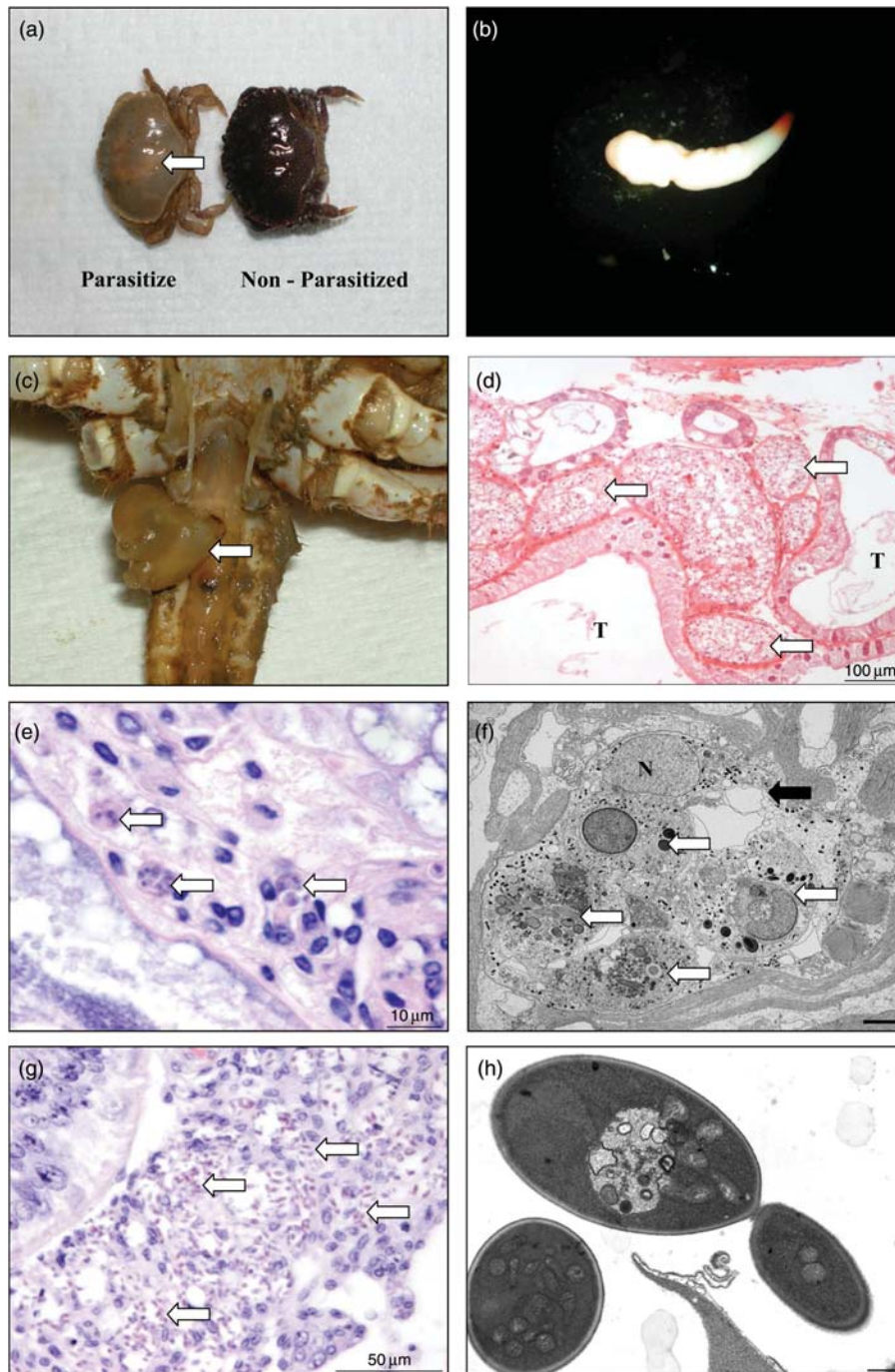


Figure 3. (a) *Fecampia erythrocephala*. Parasitized crabs are pale in colour relative to non-parasitized crabs, and the parasite can be seen within the cephalothorax (arrow). (b) A single *F. erythrocephala* dissected from an infected crab's cephalothorax; the organs of the infected crab were displaced and atrophied. (c) A male edible crab displaying the externa (arrow) of the parasite *S. inflata*. (d) Network of *S. inflata* rootlets (arrows) between hepatopancreatic tubules (T) (H&E stain; scale bar 100 μm). (e) *Paramarteilia canceri* (arrows) within the connective tissue of the hepatopancreas of recruit crabs (H&E stain; scale bar 10 μm). (f) Ultrastructure of a typical large multicellular stage of *P. canceri*. The primary cell (black arrow) contains a prominent nucleus (N) and four tertiary cells (white arrows) (TEM; scale bar 1 μm). (g) Yeast-like organisms (arrows) in the sinusoids between hepatopancreatic tubules of recruit crabs (H&E stain; scale bar 50 μm). (h) Ultrastructure of budding yeast-like cell in the haemolymph (TEM; scale bar 500 nm).

abdominal flap before dissection (Figure 3c). Systemic infection was confirmed with histology via a network of parasitic rootlets that penetrated all major organ and tissue systems, and was particularly evident in the haemal sinuses of the hepatopancreas (Figure 3d).

Pathogens exclusively infecting the recruit subpopulation of *C. pagurus* in the current study were *Paramarteilia canceri* (Feist *et al.*, 2009), and a yeast-like infection, of similar pathology to that previously described by our laboratory causing co-infections in *Hematodinium*-infected crabs (Stentiford *et al.*,

2003). *Paramarteilia canceri* was observed at very low prevalence (3%) in recruits sampled during February, March, and October. Histology (Figure 3e) and electron microscopy (Figure 3f) revealed the characteristic cell-within-cell arrangement of this type of pathogen. The yeast-like pathogen was present in the recruit subpopulation sampled in January (3%) and February (7%). This pathogen was once again only observed as a co-infection with *Hematodinium* sp. Histology (Figure 3g) and electron microscopy (Figure 3h) revealed the systemic nature of infection along with the characteristic budding that defines infection with yeast-like organisms.

Discussion

The results of this study have demonstrated significant variations in the pathogen profile of non-fished (prerecruit) and fished (recruit) subpopulations of the European edible crab (*C. pagurus*) from the English Channel fishery in UK waters. Although fished and non-fished animals were sampled in different years, the key finding of this study highlights the fact that the prerecruit crab population appears to be susceptible to a different and greater range of pathogens and parasites than does the recruit subpopulation. Some of these (such as the novel haplosporidian-like parasite) are highly prevalent in prerecruits, but apparently absent from recruits. Others (such as *Hematodinium* sp.) appear to be more prevalent in the recruit subpopulation. The difference in sampling time may affect the prevalence of a particular pathogen, but is unlikely to affect the pathogen profile of the two groups significantly. Although the presence or the absence of particular pathogens can sometimes be clearly related to the ecology of the host and the parasite, and differences in host diet [e.g. prerecruit edible crabs are more likely to be infected with the digenean trematode *M. primas* through the reliance on the presence of the first intermediate host (snail) and definitive host (bird) in the littoral zone; Saville and Irwin, 2005; Stentiford, 2008], the presence or the absence of other pathogens are not as easily explained.

In this context, a key finding in the current study was the prevalence of a novel and pathogenic haplosporidian-like parasite infecting the bladder of (exclusively) prerecruit crabs. The infection was visible upon dissection as a result of organ hypertrophy and in histology via the significant pathogenic alteration of the organ. The production and liberation of uninucleate and plasmodial stages of the parasite into the urine of host crabs provides the most likely transmission route for the parasite and explains its high prevalence (up to 80%) in the shore-sampled population. As this parasite has not been detected previously in recruit populations of *C. pagurus* (Stentiford, 2008), it may be a specific pathogen of juvenile life stages of this host.

To date, few haplosporidians have been described as pathogenic agents in crustacean hosts. *Haplosporidium cadomensis* (Marchand and Sprague, 1979), *H. louisiana* (Perkins, 1975), *Claustrosporidium gammari* (Larsson, 1987), and unclassified forms apparently lacking spores (Newman et al., 1976; Dyková et al., 1988) have been reported, although *H. cadomensis*, *H. louisiana*, and *Haplosporidium* sp. (Rosenfield et al., 1969) from crabs may be conspecific species (Perkins and van Banning, 1981). Spore-forming haplosporidians of crustaceans have been described infecting the decapod *Rhithropanopeus harrisi tridentatus* (Marchand and Sprague, 1979), and recently from freshwater amphipods of the genus *Diporeia* (Messick, 2009). Two other apparently asporous haplosporidian-like pathogens have also been described. First, an organism originally described as a dinoflagellate-like pathogen

was discovered from spot prawns (*Pandalus* spp.) on the coasts of Alaska (Meyers et al., 1994) and western Canada (Bower and Meyer, 2002), and second, an unclassified haplosporidian-like parasite from European shore crabs (*Carcinus maenas*) from UK waters, with features intermediate between the spot prawn parasite and the unclassified parasites of *P. vannamei* and *Callinectes sapidus* (Stentiford et al., 2004).

All these parasites are likely haplosporidians, differing in some features from those reported from molluscs. Most of the literature on this group is concentrated on infections of molluscs, because species such as *H. nelsoni* (Andrews and Frierman, 1974; Barber et al., 1997) and *Bonamia* spp. (Meuriot and Grizel, 1984; Grizel, 1985; Doonan et al., 1994) cause epizootics and are listed by the OIE (Office International des Epizooties, of the World Animal Health Organization, Paris) as internationally notifiable diseases. Although the morphological features that characterize the Haplosporidia are somewhat unclear, a recent review by Hine et al. (2009) compared morphological and molecular features of pathogens reported as haplosporidians. They identified ultrastructural features that may be important for the taxonomy of the group and proposed that the subset of asporous haplosporidians from crustaceans may be considered basal within the group. Further work is now required to characterize the phylogenetic position of the novel haplosporidian-like pathogen described in the current study from (specifically) prerecruit *C. pagurus*. The lack of infection in recruit populations suggest that prerecruit *C. pagurus* may act as intermediate hosts for this pathogen, with alternative hosts, e.g. mollusc species, necessary for completion of the parasite life cycle existing on the same shoreline as the prerecruits.

In addition to defining the relationship between this pathogen and that infecting molluscs and *C. maenas* (which shares a habitat on European coastlines), it will be important to consider the likely outcome of the disease in prerecruits (with regard to host fitness and mortality) and the basis for its apparent absence from the recruit stock. The potential for hosts to either recover from, or succumb to, the infection is a key issue affecting the supply of prerecruits into the fished population. Targeted investigation of this issue is clearly required.

Pathogens are increasingly being recognized as important components affecting general host life history, survival, population structure, and ecosystem functioning (Dobson and Hudson, 1992; Hudson et al., 1998; Marcogliese, 2004; Lafferty et al., 2006; Feist and Longshaw, 2008; Kuris et al., 2008). Notable recent examples include that of Longshaw et al. (2010), who demonstrated a significant role of parasitism in the growth and recruitment success of early life stages of cyprinid fish. Here, a negative relationship was reported between the growth of age-0 fish and the parasite *Myxobolus* spp. Rolbiecki (2006) showed that the abundance and composition of parasitic fauna changed with fish size (age), certain parasite taxa being found in specific length classes and not others. Stentiford et al. (2010) demonstrated that the age of onset of specific fish diseases (including those caused by parasites) is an important feature discriminating flatfish populations sampled from different offshore locations and furthermore that diseases observed in certain life stages are often not observed in others.

Differential susceptibility between age cohorts of the same host species has also been demonstrated in aquaculture scenarios, with early age classes being afflicted by different diseases from those observed in adults (Kiran et al., 2002; Bergmann et al., 2003).

However, although these studies provide evidence that pathogens are certainly present at differing prevalence and intensity in different host life stages, few studies have provided sufficient evidence to support the negative effects on growth, fecundity, and survival found in a density-dependent manner capable of leading to regulation of population size. Manipulation of the host–parasite relationship in an experimental setting can be used to predict and demonstrate regulation (Thompkins *et al.*, 2002; Møller, 2005). Although this is often logistically difficult when using ecologically or economically relevant host species (particularly vertebrates that can develop acquired immunity to a specific pathogen challenge), invertebrate systems have significant potential for studying life history and survival outcomes. Specifically for the current study, the relative survival of (for instance) juvenile crabs naturally infected with the highly prevalent haplosporidian-like parasite may be tested in a controlled laboratory setting, to provide important data for cohort-to-cohort stock assessment models.

In summary, the pathogen profile of prerecruit subpopulations of the European edible crab (*C. pagurus*) differs from that observed in recruit subpopulations from the same fishery. In several cases (e.g. *F. erythrocephala*, the haplosporidian parasite, and infection by digenean metacercariae), pathogens present in prerecruits were not observed in recruits. For *F. erythrocephala* and the haplosporidian-like pathogen, this may result in direct mortality to infected hosts, whereas for the digenean, infection mortality may be indirect through an increase in predation on infected crabs. Both may be considered as potentially significant drivers of mortality in the prerecruit population before entry into the UK commercial fishery. Interestingly, both *F. erythrocephala* and the haplosporidian, although potentially lethal, were present at very different prevalence. Kuris *et al.* (2002) showed that *F. erythrocephala* develop rapidly and kill their hosts in a few weeks. A low prevalence of these parasites may be significant, because infections are ephemeral and do not build up, so prevalence at a single point in time may underestimate the impact of these parasites.

Preliminary seasonal survey data of this type are important in understanding the potential for disease to cause so-called silent mortalities in commercially exploited populations of fish and shellfish, and with further refinement may contribute to improved modelling of natural mortality in fisheries stock assessment models (Kuris and Lafferty, 1992). The discovery of novel pathogens in a relatively well-studied species such as *C. pagurus* also highlights our basic lack of understanding of infection and disease processes in even our most important commercial species, as well as the need to collect basic health data from all life stages of important stocks if we are to manage them in a sustainable manner into the future.

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