

NOTE

Cancer pagurus bacilliform virus (CpBV) infecting juvenile European edible crabs *C. pagurus* from UK waters

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ABSTRACT: This study provides the first description of an intranuclear bacilliform virus infecting the hepatopancreatic epithelial cells of juvenile European edible crab *Cancer pagurus* from the English Channel, UK. This is the first field report of a virus infecting a member of the *Cancer* genus and follows a report of a different virus infection detected in *C. pagurus* held under experimental conditions. We have named the virus *Cancer pagurus* bacilliform virus (CpBV). The morphology and size of the nucleocapsid and the complete virion align the virus most closely with intranuclear bacilliform viruses reported from other decapod species. The virus was only observed in juvenile *C. pagurus* with carapace widths ranging between 20 and 70 mm. Viral infection was not observed in crabs above the minimum landing size of 140 mm carapace width. Due to the importance of *C. pagurus* as a fisheries target, the increasing relative reliance on crustacean fisheries in Europe and the relative dearth of information on diseases of this species, it is pertinent to consider the effects that infections such as CpBV may have on mortality of juvenile crabs and on the sustainability of the fishery as a whole.

KEY WORDS: *Cancer pagurus* · Bacilliform virus · TEM · Histology · Intranuclear · Hepatopancreas · Epithelial cells

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INTRODUCTION

Viruses are the most abundant members of marine ecosystems and can be found infecting all types of marine organisms, from protists to molluscs and crustaceans to fish and mammals (Munn 2006). Despite their ubiquity, there are surprisingly few reports on their presence in wild marine animals. In this respect, crustaceans are no exception, with only limited examples of viral disease in species obtained from the wild (Johnson 1984, Shields & Behringer 2004, Stentiford et al. 2004).

Crustacean fisheries are key marine resources in Europe. In many countries (such as the UK) crustaceans are amongst the most valuable targets in marine fisheries, ranking above several finfish species in terms of production quantity and value. The European edible crab *Cancer pagurus* supports a large fishery in the UK, with 14 500 t landed during 2005, with a

first sale value of approximately €31.5 million. UK landings contributed 43% of the total European catch for this species (www.fao.org/fishery/statistics/global-production/1).

Several protistan pathogens have been described from English Channel stocks of *Cancer pagurus* (Stentiford et al. 2002, 2003, 2007). However, to date, only one virus, *C. pagurus* systemic bunya-like virus (CpSBV), has been described from the *Cancer* genus (Corbel et al. 2003). This virus was discovered in control crabs during white spot syndrome virus (WSSV) challenge trials with *C. pagurus*. Hemolymph from CpSBV-infected crabs contained numerous virions measuring 60 to 70 nm virions. Virions were also present within the cytoplasm, between cells and within cytoplasmic vesicles of a number of cell types in the gill, hepatopancreas, heart and connective tissues. However, the presence, prevalence and distribution of CpSBV in wild populations are currently unknown (Corbel et al. 2003).

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Lack of reports of viral infections in *Cancer* crabs may demonstrate reduced susceptibility or may be an artefact of sampling bias, with a focus on larger, mature crabs that may be less susceptible to infection (Johnson 1984). Size and age variations affect the pathogenicity of many infections; Pestal et al. (2003) showed that bitter crab disease (BCD) was more prevalent in juvenile snow crabs *Chionoecetes opilio* and may be related to frequency of moulting, as adults cease moulting and are therefore less susceptible to infection.

Edible crabs were sampled as part of a research contract studying population level effects of disease. Crabs of varying size were assessed for disease using histology and electron microscopy. Here we provide the first description of a virus, *Cancer pagurus* bacilloform virus (CpBV), in wild crabs of the *Cancer* genus. CpBV is morphologically similar to other intranuclear bacilliform viruses (IBVs) described from crabs, freshwater crayfish and from penaeid shrimps (Johnson & Lightner 1988, Huger & Krieg 1991, Stentiford et al. 2004).

MATERIALS AND METHODS

European edible crabs *Cancer pagurus* were sampled from the fishery in the South Kimmeridge Bay region of Dorset, UK (Fig. 1). Dispensation licences from the United Kingdom Marine Fisheries Agency (Council Regulation 850/98) allowed for the sampling of legally undersized (carapace width [CW] <140 mm) and legal sized (CW >140 mm) crabs, both from the fishery off South Kimmeridge Bay and from the shoreline directly inshore from the fishery site.

Cancer pagurus were captured in 2 sets of baited creels from the South Kimmeridge Bay region in April 2007. The first string of pots was laid between 50° 32.50' N, 002° 10.00' W and 50° 32.00' N, 002° 10.00' W; the second string of pots was laid between 50° 32.50' N, 002° 11.00' W and 50° 32.00' N, 002° 11.00' W. Similarly, juvenile *C. pagurus* (CW <75 mm) were collected from the shoreline, directly inshore from the fishery site (50° 36.28' N, 002° 08.30' W). Following transportation to the Centre for Environment, Fisheries and Aquaculture Science (Cefas) laboratory, crabs were assessed for external signs of damage and shell disease and processed for histology and electron microscopy.

We sampled 150 *Cancer pagurus* (60 with CW <100 mm, 50 with CW between

100 and 140 mm, and 40 with CW >140 mm). Crabs were anaesthetised by chilling on ice for 30 min before dissection. Hepatopancreas, gill, gonad, heart and body muscle was excised and placed immediately into Davidson's seawater fixative for 24 h, then transferred to 70% industrial methylated spirit. Samples were infiltrated with paraffin under vacuum using standard protocols. Sections were cut at a thickness of 3 to 5 µm, mounted onto glass slides, and stained with haematoxylin and eosin (H&E) or Feulgen. Stained sections were analysed by light microscopy (Nikon Eclipse E800); digital images and measurements were obtained using the Lucia™ Screen Measurement System (Nikon).

Small blocks (2 mm³) of hepatopancreas were fixed for electron microscopy 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) and post-fixed for 1 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. Specimens were washed in 3 changes of 0.1 M sodium cacodylate buffer before dehydration through a graded acetone series. Specimens were embedded in Agar 100 epoxy resin (Agar Scientific, Agar 100 pre-mix kit medium) and polymerised overnight at 60°C in an oven. Semi-thin (1 to 2 µm) sections were stained with Toluidine Blue for viewing with a light microscope to identify suitable target areas. Ultrathin sections (70 to 90 nm) of target areas were mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and Reynolds' lead citrate (Reynolds 1963). We examined grids using a JEOL JEM 1210

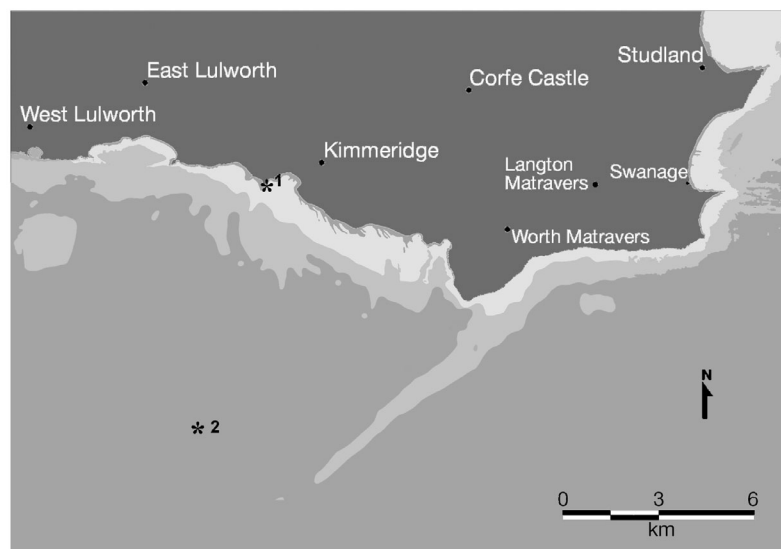


Fig. 1. South coast of the UK. Juvenile *Cancer pagurus* were collected from the South Kimmeridge Bay shoreline (1), directly inshore from the fishery site (2), where *C. pagurus* were captured using baited creels

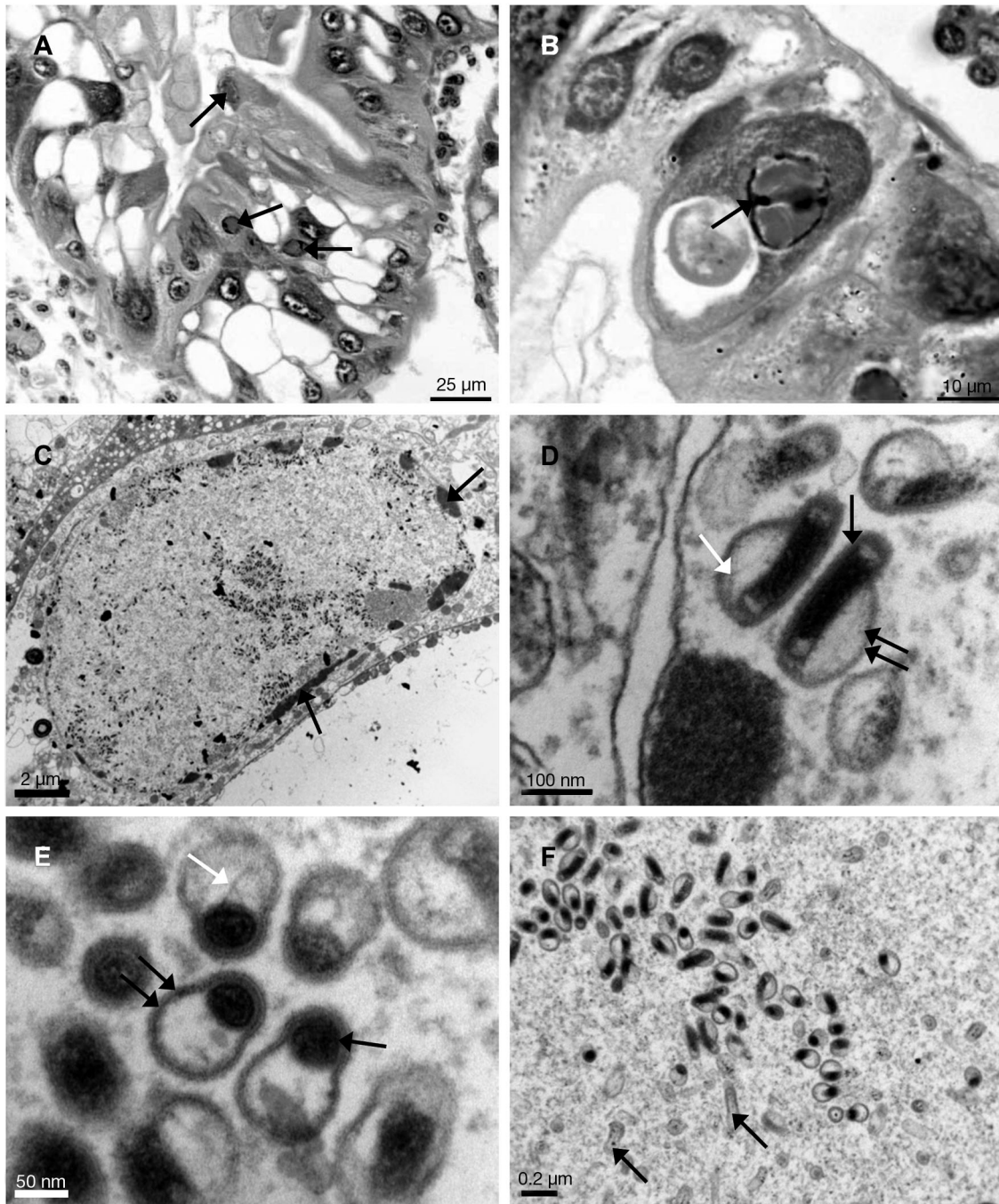


Fig. 2. *Cancer pagurus* infected with *C. pagurus* bacilliform virus (CpBV). (A) Hepatopancreas of CpBV-infected *C. pagurus*, reserve (R) and fibrillar (F) cells contain enlarged nuclei with marginal chromatin pattern and fine granular eosinophilic matrix (arrows). H&E stain. Scale bar = 25 μm . (B) F-cell within hepatopancreas tubule with enlarged nucleus with marginalized chromatin. Margination of the chromatin has formed septa (arrow) that has led to intra-nuclear compartmentalisation. A putative sub-nucleic lysosome is also present. H&E. Scale bar = 10 μm . (C) Enlarged nucleus from epithelial cell of hepatopancreatic tubule displaying marginalised chromatin (arrow) and containing rod-shaped bacilliform virions. Virions are present in transverse and longitudinal section. TEM. Scale bar = 2 μm . (D) Longitudinal section through bacilliform virus. Virions consist of a nucleocapsid (black arrow) surrounded by a trilaminar membrane with a lateral extension (double arrow) at one end. Tail-like appendage can be seen within the expansion (white arrow). TEM. Scale bar = 100 nm. (E) Transverse section through bacilliform virus. Electron-dense nucleocapsid (black arrow) is present on one side of the membranous lateral extension (double arrow). Tail-like appendage can be seen within the expansion (white arrow). TEM. Scale bar = 50 nm. (F) Fully formed virions present in transverse and longitudinal section. Free trilaminar envelopes can be seen within the viroplasm (arrows). TEM. Scale bar = 0.2 μm

transmission electron microscope and captured digital images using a Gatan Erlangshen ES500W camera and Gatan Digital Micrograph™ software.

RESULTS

A virus infection was found in 5% of crabs collected from the Kimmeridge Bay fishery and the shoreline. Infections were only recorded in crabs between 20 and 70 mm CW, with larger individuals and those above the legal CW (140 mm) apparently not infected. Previously described pathogens (*Hematodinium* spp., *Enterospora canceri*) and an undescribed *Paramarteilia* sp. were also discovered infecting crabs from the South Kimmeridge Bay fishery; these results will be presented elsewhere.

Infected crabs showed no obvious external signs of disease. Histological analysis revealed hypertrophied nuclei containing an eosinophilic, finely granular matrix within the epithelial cells of hepatopancreatic tubules (Fig. 2A). Affected nuclei also displayed marginated chromatin with strands of chromatin traversing the nucleoplasm, leading to apparent intranuclear compartmentalisation (Fig. 2B). Affected cells appeared singly or in clusters; cells with hypertrophied nuclei were consistently found to be reserve (R) or fibrillar (F) cells. The infection was not observed in regenerating epithelial (E) or blister (B) cells. Transmission electron microscopy (TEM) revealed rod-shaped virions within enlarged nuclei (Fig. 2C). Virions possessed an elongated electron-dense nucleocapsid with a relatively electron-lucent end. The nucleocapsid was surrounded by a trilaminar envelope, which expanded at one end to form a lateral protuberance (Fig. 2D,E). Within this expansion appeared a fine tail-like structure that emerged from the proximal end of the nucleocapsid (Fig. 2D,E). Virions in various stages of development were observed in the nuclei (Fig. 2F). The mean length of complete virions ($n = 30$) was 210.9 ± 2.6 nm, with a mean diameter of 60.3 ± 0.8 nm and a mean envelope width of 6 ± 0.2 nm. The mean length of the nucleocapsid was 181.6 ± 2.5 nm, with a mean diameter of 43.4 ± 0.4 nm.

DISCUSSION

We provide the first description of a virus infecting *Cancer pagurus* sampled directly from the wild. The IBV, CpBV, infects the R and F cells of the hepatopancreas of juvenile crabs, with infection apparently absent in crabs above CW 70 mm, including those captured in the fishery (above CW 140 mm). Pathology observed in the hepatopancreas is typical of previously

described IBV infecting other crustacean species (Johnson 1977, Pappalardo et al. 1986, Johnson & Lightner 1988, Groff et al. 1993, Edgerton et al. 1996, Stentiford et al. 2004).

Johnson (1984) noted the absence of described virus infections in the commercially significant *Cancer* genus and speculated that infections may be observed if field surveys included juvenile crabs or those below the legal landing size imposed by the fishery. This study confirms this hypothesis and highlights a need to consider age and size when carrying out disease surveys in wild crustacean hosts. Several pathogens of commercial and ecological significance have been described in *C. pagurus* from the English Channel fishery, including *Hematodinium* spp. (Stentiford et al. 2002), *Enterospora canceri* (Stentiford et al. 2007) and a *Paramarteilia* sp. (G. D. Stentiford unpubl. data). While the discovery of CpBV in exclusively juvenile *C. pagurus* from the same fishery may indicate an additional age-specific (and potentially 'silent') mortality driver, further work is required to assess the relative pathogenicity of the virus and its prevalence in juveniles throughout the season and across the geographic range of *C. pagurus*.

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