A histopathological survey of shore crab (*Carcinus maenas*) and brown shrimp (*Crangon crangon*) from six estuaries in the United Kingdom

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Abstract

Invertebrates show considerable potential as sentinel organisms for the monitoring of the health status of aquatic systems. They are generally small, abundant, relatively sessile, and may readily bioaccumulate toxins. Cascade-like stress responses can occur following acute or chronic exposures to contaminated environments and as such, the overall health status of individuals within those environments, both in terms of histopathological lesions and the presence of infecting organisms, may ultimately reflect the general health status of these sites. The current study provides baseline multi-organ histopathological data for two common crustacean species, the shore crab (*Carcinus maenas*) and the brown shrimp (*Crangon crangon*) collected from six UK estuarine sites. Changes in the metabolic condition of crustaceans from these sites (measured in terms of connective tissue storage cell status) were interpreted in relation to other health measures (including parasite load and the presence of microbial pathogens). The relative ease at which a holistic assessment of health can be made using histopathology and the suitability of these species as environmental sentinels provide support for the inclusion of crustaceans as indicators of aquatic environmental health. Studies linking disease status to burdens of industrial contamination in these environments are now required.
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1. Introduction

Stress has been implicated in the development of disease in aquatic animals, both in aquaculture (Houghton and Matthews, 1986) and in natural conditions (Ross et al., 1996). In such cases, induction of the 'stress cascade' can allow latent infections to manifest as disease via generalised host immunosuppression induced by the prevailing environmental conditions (Johnson, 1983). Under adverse conditions, the relatively primitive invertebrate immune system (of phagocytosis, encapsulation, and initiation of the prophenoloxidase system) may be compromised so that opportunistic pathogens, both from the ambient environment and those living on or within the host are allowed to proliferate, with pathological consequences. When baseline information is gathered on the prevalence of these diseases under a range of scenarios, the data may then be used as a marker to discriminate stressful aquatic environments.

Fish diseases and pathologies, with a broad range of aetiologies, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure (Matthiessen et al., 1993). Benthic invertebrates too show considerable potential as sentinel marker species in ecosystem health monitoring programs since they are small, common, relatively sessile, and tend to bioaccumulate toxicants present in their environment. In addition, the biochemical, physiological, and histological characteristics of several

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common species are sufficiently well known to discriminate exposed from non-exposed individuals (Viarengo, 1993). However, whilst studies on finfish have shown that histopathology is a sensitive indicator of individual and population health status, and results from numerous controlled laboratory exposures of shellfish (crustaceans and molluscs) to toxicants have shown that histopathological changes also occur in the organ and tissue systems of these animals, relatively few field studies have included shellfish histopathology in the suite of monitoring tools employed. Most studies of this type have centred on the use of the common mussel (*Mytilus edulis*) (see Lowe et al., 1981; Moore et al., 1987; Wedderburn et al., 2000). Studies on crustacean health status have focussed on the response of individual organ systems to laboratory exposure to a range of contaminants (Bhavan and Geraldine, 2000; Couch, 1977; Couch and Nimmo, 1974; Doughtie and Rao, 1983, 1984; Lightner et al., 1982; Sarojini et al., 1993; Soegianto et al., 1999a,b; Victor, 1993, 1994). However, whilst studies of this type undoubtedly provide an invaluable insight into the cellular response to pollutants, with an increasing emphasis on the effect of stress at the population-level, more holistic approaches are likely to be required to aggregate the combined effect on multiple organ systems. Relatively, few studies have applied such an approach to wild crustacean populations, despite their pivotal role within food chains (Bang, 1980; Couch, 1978; Overstreet, 1988; Sindermann, 1979).

The current study reports baseline prevalence data for a range of parasites and pathologies present in two common crustacean species (*Carcinus maenas* and *Crangon crangon*) found in UK estuaries. To the best of our knowledge, this is the first attempt to collect multiple organ histopathology data for these species from indigenous field sites. It has demonstrated the relative ease at which disease status can be assessed in these species and provides insights into how invertebrate disease may be used as a high-level indicator of ecosystem health.

2. Materials and methods

Shore crabs (*Carcinus maenas*) were captured during the autumn of 2002 by standard beam trawling from the Alde, Mersey, Tyne, Forth, and Clyde estuaries and from Southampton Water (Table 1). Approximately 30 crabs from each site were euthanised and fixed by direct injection with Davidson's seawater fixative (Hopwood, 1996). The carapace of euthanised crabs was carefully removed before placing the remaining carcass into the same solution for 24h before transfer to 70% industrial methylated spirit for transport and storage. Following fixation, the hepatopancreas, heart, gonad, gill, midgut, and muscle were removed and processed for histological

Table 1
Staging of *Crangon crangon* bacilliform virus (CcBV) infection severity

Stage 0 Absent	• Hepatopancreatocytes and midgut epithelial cells normal
	• No sign of aberrant nuclei or epithelial sloughing into lumen
Stage 1 Scattered	• Few aberrant nuclei (eosinophilic, enlarged, and peripheral chromatin)
	• Most hepatopancreatic tubules not affected
	• No epithelial sloughing into lumen
Stage 2 Frequent	• Frequent aberrant nuclei present in numerous hepatopancreatic tubules
	• Some separation of infected cells from their neighbours
	• Epithelial sloughing is infrequent
Stage 3 Abundant	• Majority of hepatopancreatic tubules contain numerous aberrant nuclei
	• Separation of large numbers of infected cells from their neighbour cells
	• Some epithelial sloughing of infected cells into lumen
	• Some tubules appear degenerate
Stage 4 Severe	• Majority to all hepatopancreatic tubules contain cells with numerous aberrant nuclei
	• Separation and apparent apoptosis of large numbers of infected cells
	• Large numbers of epithelial cells are sloughed into lumen
	• Tubules appear degenerate, often involving epithelial cells of the midgut

examination using standard protocols. Thin sections (3–5 μm) were obtained using a rotary microtome and were stained with haematoxylin and eosin (HE). Stained sections were analysed by standard light microscopy and digital images were captured using the LuciaG Screen Measurement System (Nikon, UK).

An index on the relative abundance of glycogen-containing reserve inclusion (RI) cells in histological sections of the connective tissues of crabs were devised based upon their presence in the connective tissues of the hepatopancreas. The index ranged from Stage 0 (RI cells absent) through Stage 1 (RI cells present but scarce), Stage 2 (RI cells scattered), Stage 3 (RI cells frequent) to Stage 4 (RI cells abundant and constituting the majority of connective tissue volume).

Brown shrimp (*Crangon crangon*) was captured using the same standard beam trawling methodology as mentioned above. Up to 35 brown shrimp (*Crangon crangon*) from the Alde, Thames, Mersey, and Clyde estuaries were euthanised and fixed in the same way as for *C. maenas*. Euthanised shrimp were immediately placed into the same solution for 24h before transfer to 70% industrial methylated spirit for transport and storage. For processing, whole shrimp were sectioned longitudinally and the cut surface was embedded outermost in the block for ease of sectioning. Whole shrimp sections were stained with

HE and selectively re-sectioned for staining with the Farley–Feulgen (FF) and Periodic acid-Schiff (PAS) stains. Stained sections were analysed by standard light microscopy and digital images were captured as above.

An index describing the pathological manifestation of a previously described virus infection, *Crangon crangon* bacilliform virus (CcBV) (Stentiford et al., 2004) was also constructed and applied to all shrimps sampled during the current study. The details of the index are given in Table 1. For ultrastructural confirmation of CcBV, the hepatopancreas was removed from 20 shrimp captured from the Clyde estuary and prepared for electron microscopy using the method previously described by Stentiford et al. (2004). Thick sections were stained with toluidine blue for viewing with a light microscope to identify suitable target areas. Ultrathin sections (70–90 nm) of these areas, mounted on uncoated copper grids and stained with uranyl acetate and Reynolds' lead citrate were examined using a JEOL 1210 transmission electron microscope.

3. Data analysis

Data pertaining to the index of the relative abundance of RI cells in *C. maenas* and the pathological manifestation of CcBV infection in *C. crangon* were tested for normality. Normally distributed data were analysed using one way analysis of variance (ANOVA) while the Mann–Whitney test was applied to non-normally distributed data. Significance differences between sites were considered to be at $p < 0.05$.

4. Results

4.1. Carcinus maenas

Thirty crabs were sampled from all sites apart from the Mersey ($n = 12$). Recently moulted crabs and those approaching imminent moult were not sampled. The sex ratio of crabs (determined by histology) differed between sites, with the Mersey (1.2:1), Alde (1.3:1), and Clyde (1.33:1) showing male bias and the Forth (0.36:1) and Southampton water (0.44:1) showing a strong female bias. A number of pathologies were observed in crabs from various estuarine sites. The prevalence of these pathologies in each organ sampled is given in Table 2.

The RI cell index for crabs captured from the various sampling sites is given in Table 2. A representative example of connective tissue RI cells is given in Fig. 1. The mean RI cell score was highest in crabs from the Alde estuary (1.7) and Southampton Water (1.63), and was lowest in crabs from the Mersey (0.75) and Tyne (0.97) estuaries. The mean RI cell score in crabs from the Mersey, Tyne, and Clyde estuaries was significantly lower than in crabs from the Alde estuary (all $p < 0.05$).

Secretory packets were seen within the vacuoles of the Blassenzellen (B)-cells of the hepatopancreas of crabs from the Tyne, Forth, and Clyde estuaries (Table 2). In a number of cases, it appeared that these vacuoles, or even whole cells, were being ejected into the lumen of the hepatopancreatic tubules (Fig. 2). No secretory packets were observed in crabs captured from the Mersey or Alde estuaries or from Southampton Water. General pathologies recorded in crabs from all sites included the presence of haemocytic aggregates and melanised nodules in the gills, heart, hepatopancreas, and gonad (Table 2, Fig. 3). The presence of these pathologies showed no clear site bias.

A number of parasites were observed infecting crabs from several sites. Of particular note was the high prevalence of *Sacculina carcini* infecting crabs from Southampton Water and the Forth estuary. No crabs from the Mersey, Alde, and Tyne estuaries were infected, while only a very low prevalence was observed in crabs from the Clyde estuary (Table 2). Rootlets of the parasite could be seen forming a systemic network in infected crabs, often displacing the tubules of the hepatopancreas (Fig. 4). In infected female crabs, invasion of the gonad led to atresia of the oocytes and to an apparent arrest of their development in the previtellogenic phase (Fig. 5). Several crabs appeared to mount an immune response to the invasive rootlets, manifested as haemocytic encapsulation and in some cases, melanisation (Fig. 6). It is noteworthy that the sites with the highest *S. carcini* prevalence (Southampton Water, Forth estuary) also showed a sex ratio with a strong female bias. The mean RI cell index was also lowest at sites with the highest *S. carcini* infection prevalence (see Table 2).

Another parasite of note in crabs captured from the Alde, Forth, and Clyde estuaries and from Southampton water was the digenean trematode *Microphallus primas*. Infection prevalence was highest in crabs from Southampton Water and from the Alde estuary, with lower prevalence in the Forth and Clyde estuaries (Table 2). The most common sites of infection was the hepatopancreas (Fig. 7) and the gill (Fig. 8). Infection did not appear to elicit an acute host immune reaction, though apparent 'pearl' formation did occur around metacercaria, outside of which, whorls of haemocytes were occasionally observed. Within the gill, encysted metacercaria appeared to form blockage of the secondary gill filaments and in some cases led to haemocyte stasis within the lamellae.

Fouling of the gills by filamentous bacteria (Fig. 9) and by stalked ciliates (Fig. 10) was observed in crabs captured from all sites. Bacterial fouling was most prevalent in crabs from Southampton Water and the Tyne estuary, while the prevalence of ciliates was highest in crabs from Southampton Water and the Tyne and Clyde estuaries. However, since detailed information on moult status (i.e., the particular stage of the intermoult) was not collected, care should be exercised when interpreting external fouling data of this kind.

Table 2
Health index parameters recorded from organs of *Carcinus maenas*

Hepatopancreas	Mean RI cell score	Secretory packets	Epithelial virus	*Sacculina carcini*	*Microphallus primas*	Microsporidian[a]
Mersey	0.32	0	0	0	0	0
Southampton	1.63	0	0	93.3	63.3	20
Alde	1.7	0	0	0	63.3	26.7
Tyne	0.97	13.3	3.3	0	0	0
Forth	1.23	10	0	73.3	20	0
Clyde	0.92	7.1	10.7	3.6	17.9	3.6
Gill	Haemocyte aggregates	Melanised nodules	Stalked ciliates	Other fouling organisms[b]	*Microphallus primas*	*Sacculina carcini*
Mersey	41.7	8.3	25	25	0	0
Southampton	73.3	10	60	93.3	66.7	3.3
Alde	73.3	0	46.7	40	60	0
Tyne	26.7	16.7	76.7	66.7	0	0
Forth	36.7	13.3	16.7	3.3	3.3	3.3
Clyde	36.7	6.7	73.3	6.7	0	0
Heart	Haemocyte aggregates	Melanised nodules	Myocardial necrosis	*Sacculina carcini*	*Microphallus primas*	*Hematodinium perezi*[c]
Mersey	45.5	0	9.1	0	0	0
Southampton	25.9	7.4	0	3.7	0	3.7
Alde	46.4	7.1	0	0	0	0
Tyne	56.7	10	3.3	0	0	0
Forth	28	16	0	0	0	0
Clyde	58.3	12.5	0	0	0	0
Gonad	Mature oocytes (F)	Mature spermatozoa (M)	Oocyte atresia (F)	Haemocyte aggregates	Melanised nodules	*Sacculina carcini*
Mersey	80	66.7	0	0	0	0
Southampton	33.3	100	44.4	46.1	15.4	61.5
Alde	100	84.6	10	0	0	0
Tyne	92.9	84.6	35.7	0	0	0
Forth	78.6	80	28.6	5	0	42.1
Clyde	77.8	91.6	33.3	0	0	0

All numbers refer to percentage prevalence of the parameter in a particular organ of crabs from a given site.

[a] Unidentified microsporidian infection of hepatopancreas.

[b] Mixed population of fouling epibionts, dominated by filamentous bacteria.

[c] Putative *Hematodinium perezi* infection based on original description of the parasite in *Carcinus maenas* (Chatton, 1931). (F) In female crabs,

A pathology similar to that previously associated with infection by the bacilliform viruses was observed in the hepatopancreatic epithelium of crabs from the Clyde (10.7%) and Tyne (3.3%) estuaries. The infection did not appear pathogenic in those crabs observed and since material was not collected for electron microscopy, no inferences can be made on the exact aetiology of this condition or of any similarity to the *C. crangon* virus recorded in shrimp from the Clyde, Mersey, Alde, and Thames estuaries (see below) (Fig. 11). Finally, one of the crabs collected from the Southampton Water site harboured an infection putatively identified as *Hematodinium perezi*. Uninucleate parasite cells with distinct nuclei formed small aggregates in the sinuses of the myocardium (Fig. 12).

4.2. Crangon crangon

The most significant pathology noted in *C. crangon* was that caused by infection by the intranuclear bacilliform virus, CcBV (for pathological and ultrastructural description, see Stentiford et al., 2004). Despite no obvious signs of external disease, high prevalences of infection were exhibited in shrimp from the Alde (73%), Thames (70%), Mersey (94%), and Clyde (96%) estuaries. The relative severity of CcBV infection was scored using a scale based upon the relative numbers of infected epithelial cells and the pathological manifestation of the disease (Table 1). Compared with scores of shrimp from the Alde, the mean CcBV infection severity was significantly higher in shrimp from the Mersey and Thames estuaries (both $p < 0.05$). While the mean infection severity in shrimp from the Clyde estuary was higher than that observed in shrimp from the Alde estuary, the difference was marginally not significant ($p = 0.058$) (Fig. 13). These results suggest that while infection prevalence can differ between estuarine sites, the severity of the disease state can also differ.
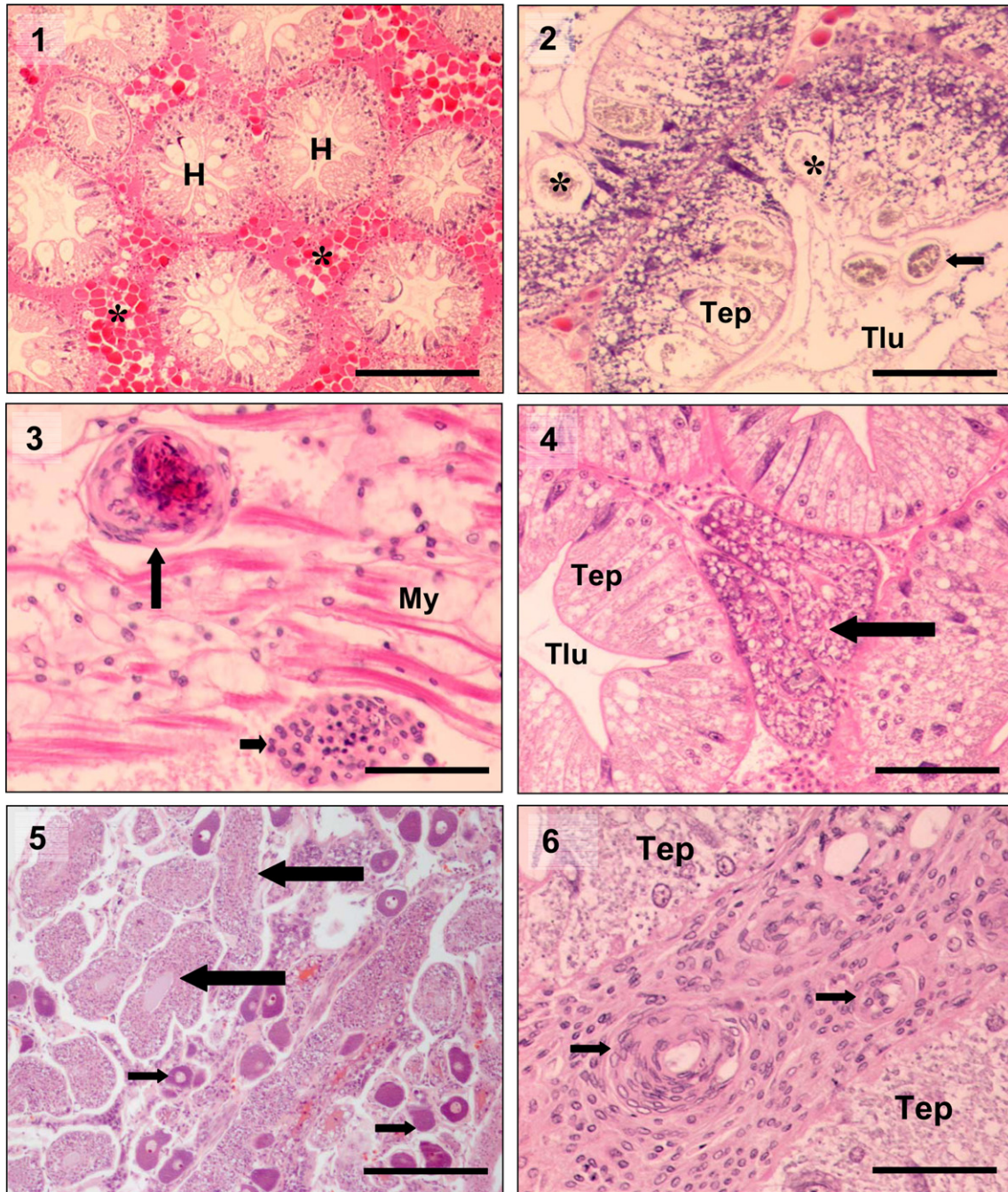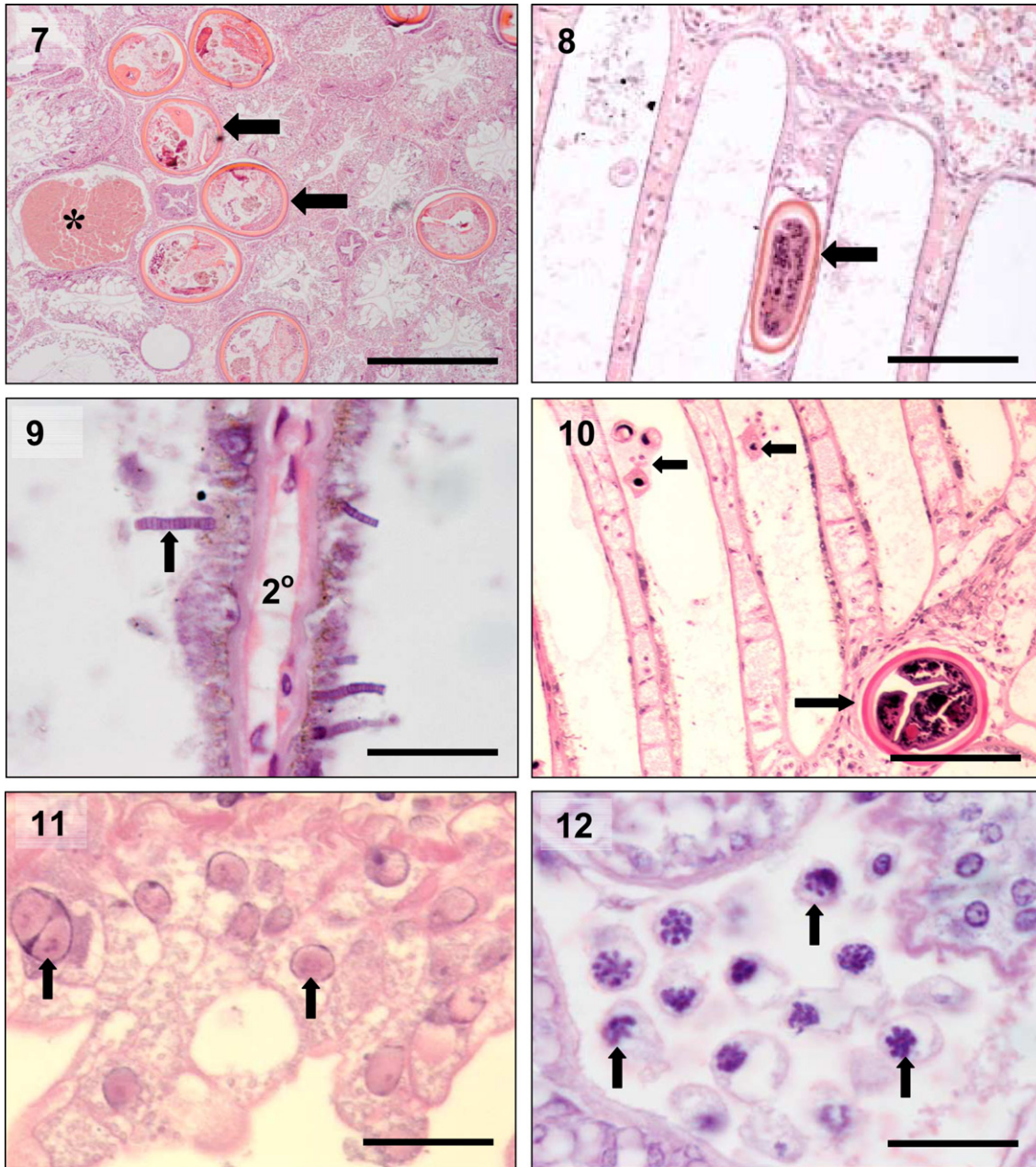
Figs. 1–6. (1) Hepatopancreas of *Carcinus maenas*. Normal hepatopancreatic tubules (H) are separated by connective tissues rich in reserve inclusion (RI) cells (asterisk). H&E, bar = 250 μm. (2) Hepatopancreas of *Carcinus maenas*. Tubule epithelium containing blister-like cells (Tep). Frequently, the vacuole within these cells contained 'secretory packets' (asterisk). These were occasionally exported to the tubule lumen (Tlu) (arrow). H&E, bar = 100 μm. (3) Heart of *Carcinus maenas*. Haemocytic aggregate (short arrow) and melanising granuloma-like lesion (long arrow) in the myocardium (My). H&E, bar = 50 μm. (4) Hepatopancreas of *Carcinus maenas*. Tubules containing normal epithelial cells (Tep) and lumens (Tlu) are surrounded by rootlets of the parasite *Sacculina carcini* (arrow). Note the complete replacement of normal connective tissues and RI cells by the parasite. H&E, bar = 100 μm. (5) Ovary of *Carcinus maenas*. Pre-vitellogenic oocytes (short arrows) are interspersed with rootlets of *Sacculina carcini* (long arrows). The parasite caused a massive invasion of the ovary of infected crabs, with oocytes apparently restricted to an immature status. H&E, bar = 250 μm. (6) Hepatopancreas of *Carcinus maenas*. Remnant *Sacculina carcini* infection showing massive infiltration by host haemocytes and apparent granuloma-like formations (arrows). Normal tubule epithelial cells bound the lesion (Tep). H&E, bar = 250 μm.

In addition to the CcBV infection in shrimp, 26.7% of shrimp captured from the Clyde estuary were co-infected by a previously undescribed yeast-like organism. Yeast cells could be observed free in the haemosinuses of infected shrimp and occasionally within the haemocytes and associated with encapsulation

Figs. 7–12. (7) Hepatopancreas of *Carcinus maenas*. Metacercarial cysts of *Microphallus primas* (arrows) displace normal hepatopancreatic tubules. An unidentified microsporidian parasite is also shown forming a large xenoma-like cyst (asterisk). H&E, bar = 100 μm. (8) Gill of *Carcinus maenas*. Metacercarial cyst of *Microphallus primas* (arrow) lodged within the secondary lamellae. H&E, bar = 100 μm. (9) Gill of *Carcinus maenas*. Fouling of the secondary lamellae (2°) by filamentous bacteria and other unidentified microbial epibionts (arrow). H&E, bar = 25 μm. (10) Gill of *Carcinus maenas*. Fouling of secondary lamellae by stalked ciliates (short arrows). Ciliates were often present with filamentous bacteria and other fouling microbial epibionts (see Fig. 9). A metacercarial cyst of *Microphallus primas* is seen in the primary lamellae (long arrow). H&E, bar = 100 μm. (11) Hepatopancreas of *Carcinus maenas*. Epithelial cells of a hepatopancreatic tubule containing enlarged nuclei with peripheral chromatin (arrows). H&E, bar = 25 μm. (12) Hepatopancreas of *Carcinus maenas*. Putative *Hematodinium perezi* infection of the haemal sinus. Uninucleate parasites containing characteristic nuclear profiles (arrows). H&E, bar = 25 μm.

responses (Figs. 14 and 15). The haemopoeitic centres were disrupted by yeast cells (Fig. 16), while muscle tissue appeared to be undergoing proteolysis due to yeast infection (Fig. 17). The yeast infection was not detected in shrimp from any of the other estuarine sites and was not detected in *C. maenas* captured from the Clyde estuary during the same sampling trip.

5. Discussion

This study has provided baseline data on a range of pathologies and pathogens present in two species of wild crustacean (*Carcinus maenas* and *Crangon crangon*) from several estuarine sites in the United Kingdom. The relative ease at which disease markers in these species
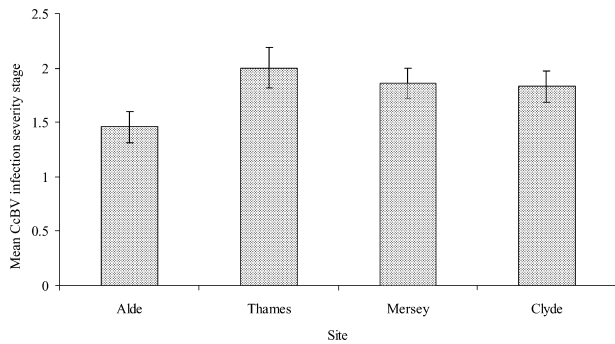
Fig. 13. Mean CcBV infection severity stage (±SE) in *Crangon crangon* collected from various estuarine sites. Infection severity staging is based on the criteria given in Table 1.

can be recorded using histopathology is encouraging and may be further applied to dedicated studies of diszease in relation to prevailing environmental conditions at such sites (e.g., the presence of contaminants).

5.1. Carcinus maenas

A number of pathologies were recorded in the organs and tissues of crabs from all sites. Interestingly, the sex ratio differed considerably between sites, with the Mersey, Alde, and Clyde showing a male bias, the Tyne showing a relatively even ratio and the Forth estuary and Southampton Water showing a strong female bias. In coincidence with these data were the high prevalence of the parasitic Rhizocephalan barnacle *Sacculina carcini* in crabs from the Forth and Southampton sites (with almost complete absence of infection in crabs from the other sites sampled). Infection prevalence was highest (over 90%) at the Southampton site, this considerably higher than that reported in previous studies at other sites (Werner, 2001). It has been suggested that infected hosts are more lethargic and are less easily caught by passive fishing methods such as traps, thereby underestimating true prevalence in the field (Crothers, 1968; Werner, 2001). In the current study, active capture by trawling may have provided a more realistic estimate of *S. carcini* prevalence at the sites where it is endemic.

Sacculina carcini can modify a range of physiological, biochemical, endocrinological, and behavioural traits in its host (Werner, 2001). Significantly, the secondary sexual characteristics of male crabs may also be altered (Høeg, 1995) by destruction of the androgenic gland by the parasite (Veillet and Graf, 1958). The manifestation
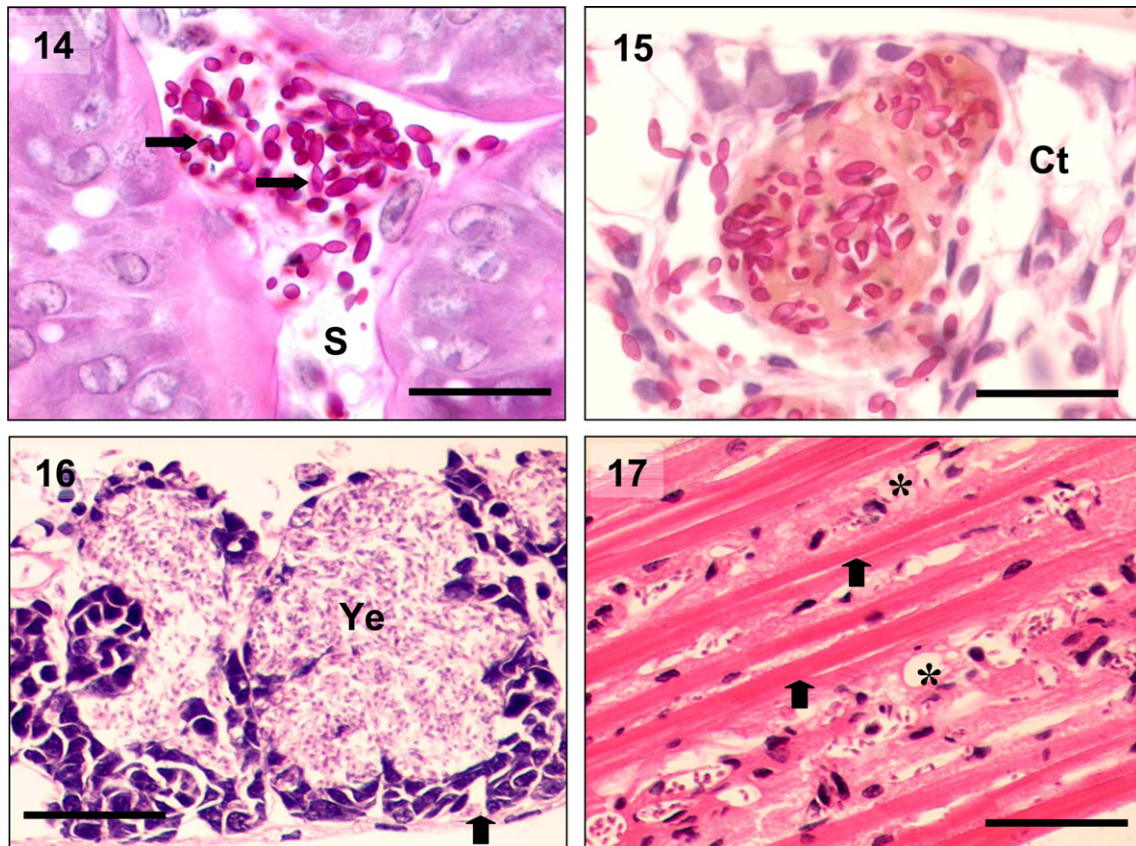
Figs. 14–17. (14) Hepatopancreas of *Crangon crangon*. Unidentified yeast-like organism (arrows) in the haemal sinus (S). PAS, bar = 25 μm. (15) Connective tissues (Ct) of *Crangon crangon*. Large granuloma-like lesion associated with yeast-like organism. PAS, bar = 25 μm. (16) Haemopoeitic tissue of *Crangon crangon*. Yeast-like organisms (Ye), apparently contained within the bounding membrane of the tissue, replace large regions of the tissue. H&E, bar = 100 μm. (17) Skeletal muscle of *Crangon crangon*. Yeast-like cells are seen between intact muscle fibres (arrows). Remnants of muscle fibres, apparently necrotic are interspersed between (asterisk). H&E, bar = 100 μm.

of this pathology leads to feminisation of males, externally visible by a widening of the abdominal pouch, and retarded development of other male-oriented features (such as claw size) (Høeg, 1995). Retardation of testicular growth and hermaphrodite sexual gland generation, with ovarian tissue appearing in the testis of previously infected hosts, has also been reported (Reinhard, 1956). It is not inconceivable than that the female biased sex ratios observed at the Southampton and Forth sites may, at least in part, be due to current and previous infection by this parasite. We can make no inference as to the cause of the male biased sex ratios at the Mersey, Alde, and Clyde sites though from the current study, it is recommended that any attempts to link external sex ratio or morphometric data to prevailing environmental conditions (e.g., contamination) should be carried out in conjunction with histological assessment of *S. carcini* prevalence in the population.

In addition to possible effects on the sex ratio of crabs captured from the various sites, *S. carcini* infection also appeared to alter the metabolic status of hosts in the form of reduced RI cell abundance. The function of RI cells are likely associated with the synthesis and storage of haemocyanin and other products such as glycogen; these reserves being utilised during stressful periods such as during moulting, disease or hibernation, and during normal reproduction (Johnson, 1980). Historical studies have stated that RI cells are common in well-fed *C. maenas* while absent in starving crabs (Cuénot, 1893). In light of this information, during the current study, we made an attempt to grade the RI cells status in crabs captured from the different estuarine sites. Interestingly, the RI cell score for the Alde estuary was significantly higher than that seen in crabs from the Mersey, Tyne, and Clyde estuaries. Significant differences between Southampton water and the Mersey, Tyne, and Clyde estuaries appeared to be precluded by the high prevalence of *S. carcini* at the Southampton site (which caused a reduction in connective tissue structure and an apparent loss of RI cells). The RI cell score may provide a useful tool for grading overall condition in *C. maenas* populations in estuaries. However, it should be noted that accurate moult staging and additional histopathological data (e.g., of parasitic infection prevalence) is required in order to provide confidence for interpretation of data of this type.

Previous studies on rhizocephalan infections of crabs have suggested that the internal rootlets of the parasite somehow avoid attack by the host immune system, possibly by generation of a glycoprotein-rich cuticle that prevents recognition of the parasite as non-self (Walker, 2001). However, in the current study, we report that in several cases, *S. carcini* rootlets were vulnerable to the host immune response and were often encapsulated with aggregates of flattened haemocytes and occasionally, within melanised nodules that corresponded to granu-

loma (Sparks, 1980). Whether this response is sufficient to regress the infection and to starve the externa of the parasite is not known.

Another parasite, *Microphallus primas*, was also found in high prevalence in populations of *C. maenas* captured from Southampton Water and the Alde estuary. A lower prevalence of infection was also observed in crabs from the Forth and Clyde sites. Adult *M. primas* are found as parasites of the digestive tract of several species of marine birds (Dawes, 1968; James et al., 1976) while the metacercarial cysts of the parasite infect *C. maenas*. The remaining larval stages occur within molluscan hosts such as *Hydrobia ulvae* (Saville and Irwin, 1991). In this study, metacercarial stages of the parasite were most commonly found encysted within the haemal sinuses of the hepatopancreas and gill, though occasionally, cysts were also detected within the muscle and the heart. Upon dissection, heavily infected crabs could be recognised by the presence of small melanised foci throughout the hepatopancreas. At microscopic level, these foci consisted of metacercarial stages of the parasite within an eosinophilic capsule, presumably of host origin (Martorelli and Schuldt, 1990). Infection by *Microphallus* spp. in other crab species has been reported to lead to structural damage to the hepatopancreas, necrosis of tubules and an alteration in the concentration of physiological storage products (Robaldo et al., 1999). In the current study, necrosis of tubules was not observed, though displacement of tubules were seen to occur in heavy infections. Apart from generation of the capsule, the encysted *M. primas* did not appear to elicit an acute haemocytic encapsulation response in the host, though on occasion, encysted parasites were surrounded by a thin layer of flattened haemocytes.

It is of note that no *M. primas*-infected crabs were detected at either the Mersey or Tyne sites and that infection prevalence was low at both the Forth and Clyde sites. Similar patterns of reductions in digenean infection prevalence have also been recorded in fish exposed to industrial pollution (Burn, 1980). In relation to such findings, MacKenzie et al. (1995) suggested that unequivocally linking environmental stressors such as pollution with parasite prevalence in fish is highly complex and thus problematic without consideration of various biotic and abiotic factors and the capability for migration of hosts. However, it is noteworthy that in the current study, the use of relatively sessile benthic invertebrates circumvents at least the issue of host migration. As such, the study of parasite communities as a means of assessing the impact of industrial contamination of aquatic ecosystems may be particularly relevant when applied to invertebrate hosts.

Fouling organisms (filamentous bacteria, ciliates) were detected on the secondary gill lamellae of *C. maenas* captured at all sites. The prevalence of ciliates was

highest in crabs from the Tyne and Clyde estuaries, and from Southampton Water, while filamentous bacterial fouling was most common in crabs from the Tyne estuary and from Southampton water. The occurrence of microbial epibionts on marine crustaceans has been documented in numerous studies (for review see Carman and Dobbs, 1997). In fish, it has been suggested that increased burdens of gill ciliates may be due to immunosuppression of the host, coupled with changes in the structure of the gill caused by pollutants (Khan, 1990; Yeomans et al., 1997). While the ecological significance of similar infestations in crustaceans is not well understood, there is evidence that epibiont load is correlated to intermoult duration, with longer intermoult periods or even terminal moult status expected to accumulate the greatest loads (Carman and Dobbs, 1997). As such, it is likely that epibiont loading such as that described in this study may be used to indicate not only prevailing microbiotic conditions within the aquatic environment but also the existence of normal moulting cycles within the crabs inhabiting these environments.

A low prevalence and intensity of a putative viral infection of the hepatopancreatic epithelial cells was recorded in *C. maenas* captured from the Tyne and Clyde estuaries. The histopathological manifestation was similar to that described for the intranuclear bacilliform virus described in *Crangon crangon* from the Clyde estuary in a previous study (Stentiford et al., 2004) and in the Mersey, Alde, and Thames estuaries in the current study. Finally, one crab captured from Southampton Water was found to harbour an infection by the dinoflagellate parasite *Hematodinium perezi*. This is the first report of this parasite in shore crabs from European waters since the original description by Chatton and Poisson (1931). It's rediscovery from close to the type location heralds' future opportunity for a full description and genetic sequence analysis of *H. perezi* in comparison to *Hematodinium* sp. isolates described from other crustacean hosts.

5.2. Crangon crangon

The ubiquity and relative abundance of the brown shrimp (*Crangon crangon*) affords it considerable potential for use as an environmental sentinel. The most significant pathologies noted in *C. crangon* during the current study was the intranuclear bacilliform virus (IBV) previously described infecting the hepatopancreatic epithelial cells (Stentiford et al., 2004). In the current study, the infected host range was extended from the Clyde to the Thames, Mersey, and Alde estuaries. The lesions observed in the epithelium of the hepatopancreas and midgut of *C. crangon* were identical to those previously described by Stentiford et al. (2004) and are typical of those caused by the bacilliform viruses infecting other crustacean hosts. The pathological manifestation of CcBV was marked by eventual degeneration of the stor-

age (R) epithelial cells of the hepatopancreatic tubules and of the midgut. In severe cases, large areas of the hepatopancreas appeared necrotic, with loss of tubular structure and organ integrity.

Although in several cases, the hepatopancreas of CcBV-infected shrimp appeared to be in a state of severe degeneration, no haemocytic encapsulation response (as noted by Johnson (1984) for baculovirus infections) was recorded. In addition to the possible effect of apoptosis in prevention of inflammatory reactions in virus-infected shrimp (Stentiford et al., 2004), the lack of host immune response to this pathology may also suggest that the immune system of shrimps captured from the various estuarine sites may be compromised. Previous studies on the defence capability of *C. crangon* have shown that exposure to contaminated harbour dredge spoils led to a reduced total haemocyte count and blood cell phenoloxidase activity (Smith et al., 1995). In such a way, it is conceivable that the difference in infection prevalence and infection intensity (derived from the severity index) may be caused at least partly by differences in environmental stressors present between the sites. Such interpretation is reinforced by previous studies that have indicated that environmental stressors can enhance the prevalence and severity of baculovirus infections. Crowding or exposure to sublethal amounts of PCBs increases the prevalence of *Baculovirus penaeii* in penaeid shrimps (Couch, 1974; Couch and Courtney, 1977), while poor environmental conditions, shell disease, general bacterial infections, and carapace fouling have been associated with an increased prevalence of Monodon baculovirus (MBV) in *Penaeus monodon* (Lightner and Redman, 1981). That stress has been found to exacerbate viral prevalence and disease in marine invertebrates is further reinforced by studies of vertebrate populations where it has been stated that '…the most important factor in transforming an infection into a disease is stress' (Overstreet, 1978). In such a way, latent infections may transform to patent disease (Sindermann, 1979) and even cause epizootics (Lightner and Redman, 1981). Johnson (1984) even states that studies on the effects of stress on viral disease in marine invertebrates will prove invaluable to studies of the effects of pollutants or other man-made stressors on natural populations and in aquaculture. The CcBV model in *Crangon crangon* may provide such an example.

A number of studies have reported the occurrence of secondary bacterial infections in invertebrates, particularly for virus-infected crustaceans under aquaculture conditions. In such cases, secondary infections are generally attributed to immunosuppression induced by the primary pathogen (Johnson, 1983). In the current study, a co-infection by a yeast-like organism was discovered in virus-infected *C. crangon* from the Clyde estuary. It is of note that while haemocytic encapsulation responses were not observed against virus-infected cells (see above), large encapsulation responses, were associ-

ated with the yeast-like cells, some of which were also observed within the cytoplasm of free haemocytes. Granuloma-like lesions, similar to those described by Sparks (1980) were also present. Numerous studies have shown that the prophenoloxidase system in crustaceans can be initiated by microbial cell wall components, such as β1,3-glucans in fungi and lipopolysaccharides and peptidoglycans in Gram-negative and Gram-positive bacteria, respectively (Thörnqvist & Söderhäll, 1997). It appears likely then that the immune system of virally infected *C. crangon* may still be able to respond to secondary pathogens, such as the yeast-like organisms, that express suitable stimulatory molecules in their cell walls. However, the mere presence of yeast-like cells in the haemolymph of shrimp may also suggest that the immune response is not sufficient to contain the proliferating yeast cells. The ubiquity of *C. crangon* in estuarine and coastal locations and the potential for epizootic viral and yeast infections in this species, may provide an ideal disease model for future studies on the effect of stress and pollution on the prevalence, pathogenesis, and severity of disease.

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