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MiniReview

Shell disease in crustaceans – just chitin recycling gone wrong?

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Summary

The exoskeletons of aquatic crustaceans and other arthropods contain chitin, a biopolymer of β-(1,4)-linked *N*-acetylglucosamine together with associated proteins. Despite the vast amounts of chitin within such animals little is found in sediments and open water because microorganisms rapidly degrade this following its loss after moulting or upon the animals' death. Shell disease syndrome is a worldwide disease condition that affects a wide range of crustaceans. It comes about as a result of bacterial degradation of the exoskeleton leading to unsightly lesions and even death if the underlying tissues become infected. There are at least two potential forms of the disease; one that appears to centre around chitin degradation and an additional form termed 'epizootic' shell disease, in which chitin degradation is of less significance. This account reviews our current understanding of the causative agents of this syndrome, assesses the potential economic consequences of the disease, and critically examines whether it is associated with anthropogenic disturbances including pollution. Overall, despite extensive studies during the last few decades, the potential links between faecal, heavy metal and insecticide pollution and shell disease are still unclear.

Introduction

Next to cellulose, chitin, an insoluble biopolymer of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) is the most abundant biopolymer on the planet. Within the oceans, it forms a major component of the exoskeletons of crustaceans and is a major source of carbon and nitrogen for the microbial population. The annual production of chitin from the moult casts of marine arthropods has been estimated as 1.3×10^9 tons (Cauchie, 2002) yet only trace quantities of this substance can be detected in marine sediments and the reason for this is largely attributed to the efficiency of bacterial recycling (Keyhani and Roseman, 1999).

Under normal conditions and despite having an epibiotic microbial population, the shells of crustaceans are not significantly broken-down until they are shed by the animal during moulting or at death. However, in some instances, exoskeletal degradation occurs on the *living* crustacean resulting in a condition known as shell disease syndrome.

Shell disease is a worldwide phenomenon affecting numerous species of crustaceans from both natural and captive (aquaculture or impounded) populations. The very fact the disease has been recognized as a syndrome (Stewart, 1993) implies there is no single causative agent. There are probably at least two forms of shell disease. The 'classical' form has been widely reported in a range of crustaceans including lobsters, crabs and shrimp and is thought to occur as a result of chitin degradation in the cuticle (e.g. Smolowitz *et al*., 1992; Vogan *et al*., 1999). More recently, a new form of the disease has been identified in lobsters which differs from the classical form in that chitin degradation appears to be of secondary importance in the overall pathology of this condition (Smolowitz *et al*., 1992; 2005). Various bacteria and fungi are thought to be involved in the generation of the characteristic lesions of both forms of shell disease. The aetiological agents of lesion formation are likely to be specific for the different types of the disease, may vary according to the geographic location of the crustacean population, and perhaps may also be host specific. Elevated levels of shell disease have been reported in animals from polluted environments (e.g. Goplan and Young, 1975; Young and Pearce, 1975; Weinstein *et al*., 1992), suggesting that deleterious environmental situations may trigger or simply exacerbate the condition.

Although as already noted, shell disease is a global phenomenon, this brief review mainly focuses on two

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long-term surveys of this condition in crustacean populations off the New England coast and in the Gower Peninsula, South Wales, UK. It critically reviews the recent reports of the nature of the causative agents and examines the body of evidence that supports the commonly held view that anthropogenic disturbances play a critical role in disease progression.

Chitin degradation in aquatic environments

The complete process of chitin degradation, from the moment the shell is ecdysed from the crustacean, to the catabolism of the breakdown products by marine bacteria such as vibrios, has been reviewed by Keyhani and Roseman (1999) and involves four main stages, namely (i) sensing of chitin, (ii) attachment to exuviate (chitin), (iii) enzymatic degradation, and (iv) uptake and utilization of carbon and nitrogen-containing breakdown products. Bacteria exhibit chemotaxis along $(GlcNAc)_2$ and higher oligosaccharide gradients within sediments or probably in open water (Keyhani and Roseman, 1999). Recently, Li and Roseman (2004) have provided detailed insight into the molecular mechanisms that enable bacteria, such as *Vibrio furnissii* and *V. cholerae*, to adapt to the presence or absence of chitin in their environment. They found that $(GlcNAc)_n$ was the essential signal 'informing' these bacteria of the presence of chitin which resulted in the induced expression of a series of 'chitin catabolic cascade genes'. Once all the chitin in the environment had been completely utilized, this system was once more turned off.

Adhesion with the chitinous substrate may occur as a result of various proteins on the bacterial surface that are specific for chitin (e.g. Kirn *et al*., 2005; Suginta, 2007) or non-specific (via hydrophobic or ionic interactions) adhesion processes. These interactions ultimately lead to the formation of biofilms containing mixed microbial assemblages. Bacterial degradation of chitin has been extensively studied in a wide range of culturable aquatic bacteria including *Alteromonas* sp. (Orikoshi *et al*., 2005), *Salinivibrio costicola* (Aunpad and Panbangred, 2003), *Vibrio alginolyticus* (Suginta, 2007), *V. cholerae* (Li and Roseman, 2004; Meibom *et al*., 2004; Kirn *et al*., 2005), *V. harveyi* (Svitil *et al*., 1997) and *V. furnissii* (Bassler *et al*., 1991; Li and Roseman, 2004). The process involves a series of enzymatic steps involving chitinases (E.C. 3.2.1.14) and β-glucosaminidases that sequentially degrade the termini of the chitin polymer $(GlcNAc)_n$ into smaller, more soluble, oligosaccharide units [e.g. $(GlcNAc)_{3,2}$] ultimately resulting in the formation of acetate, ammonia and fructose 6-phosphate. Keyhani and Roseman (1999) suggested that bacteria produce a suite of chitinolytic enzymes with overlapping specificities where the expression of the associated genes may vary according to environmental conditions. For instance, in *Alteromonas* sp. four chitinases (ChiA, ChiB, ChiC and ChiD) are secreted in the presence of chitin, with one (ChiA) appearing to play a dominant role in chitin degradation (Orikoshi *et al*., 2005). While many of these chitinases are inducible, in some cases the genes that code for these enzymes are constitutively expressed and the presence of chitin or end-products of chitin digestion do not appear to alter the levels of some forms of this enzyme (Aunpad and Panbangred, 2003). The final stage in chitin degradation as defined by Keyhani and Roseman (1999) is the uptake and utilization of the resultant carbon and nitrogen-containing compounds [mainly $(GlcNAc)_2$] for further intracellular catabolism. These products are transported into the bacteria via chitoporins (Keyhani *et al*., 2000; Suginta, 2007).

Chitin found in the crustacean cuticle is not the only source of this polymer in the aquatic environment in that the faecal pellets from copepods, some fungi and algae all contain chitin (Kirchman and White, 1999). While the overall estimates for chitin production in aquatic environments differ, it may be as high as 10^{11} metric tons per year (Keyhani and Roseman, 1999) making this a major source of carbon and nitrogen. The degradation of chitin and associated protein within crustacean cuticles has been investigated by placing such animals in sealed flasks containing sediment from their surrounding environment (Stankiewicz *et al*., 1998). Such studies have shown the relatively rapid breakdown of both chitin and protein, such that after 8 weeks gross structural changes in the chitinous fibres were apparent. In an attempt to determine the overall importance of chitin in bacterial production in the natural environment, Kirchman and White (1999) measured the degradation of fungal-derived ^{14}C-chitin and the radiolabel's subsequent appearance in dissolved organic matter. They concluded that although the rates of dissolved organic matter release were low in comparison with overall bacterial production, a level of *c.* 10% of bacterial production could be supported by chitin. They pointed out, however, that as the zooplankton cuticle is a complex of chitin and protein rather than just pure chitin, their estimates might not reflect the true picture of microbial growth supported by such material. Whatever the relative importance of chitin in aquatic ecosystems, it is clearly of significance in the growth of heterotrophic bacteria in sediments and probably in open waters (Boyer, 1994; Aluwihare *et al*., 2005). In the case of vibrios, including *V. cholerae*, the ability of such organisms to bind to the cuticlar components of copepods via chitin-binding proteins, is probably of major significance in their survival in seawater (Huq *et al*., 1984) and interestingly mutants lacking this protein show reduced colonization of the human intestine (Kirn *et al*., 2005).

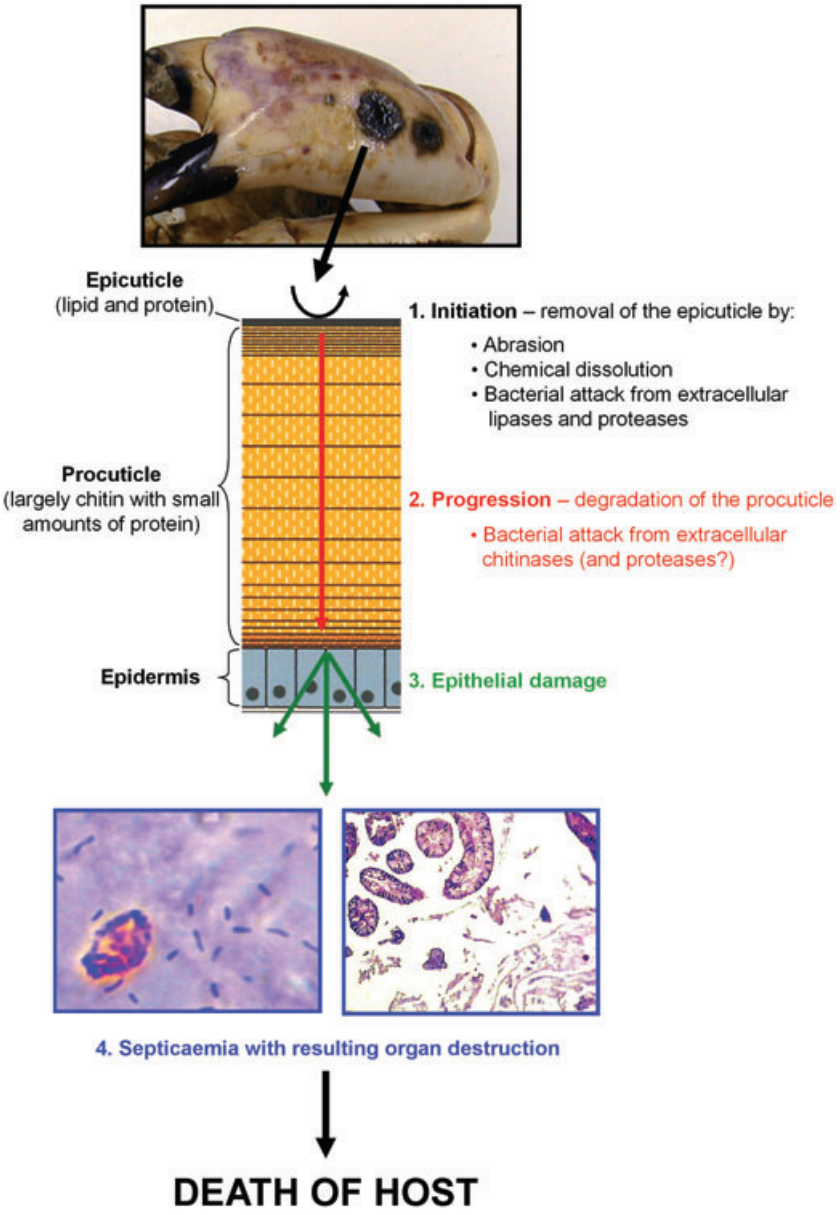

Fig. 1. Overview of the stages of shell disease as seen in edible crab (*Cancer pagurus*) populations. In early stages, the epicuticle is eroded leading to bacterial degradation of the procuticle. In severe cases the epidermis becomes breached and bacteria gain entry to the haemocoel causing septicaemia and organ damage as shown in the hepatopancreas. Depending on the nature of the bacteria in the blood and the overall health status of the crab, death may ensue. While superficial infections of the cuticle may not result in host mortality they do detract from the commercial value of the affected animals.

Shell disease syndrome – pathology

Shell disease is characterized by the external manifestation of black-spot lesions on the exoskeleton of crustaceans (Fig. 1). The black colouration of lesions is the end-result of the melanization reaction, a defence response triggered by cuticular damage (Lee and Söderhäll, 2002). In early stages of the 'classical' form of the disease, these lesions appear as small, shallow discoloured depressions which are thought to be the result of superficial damage to the outer cuticular layer, the epicuticle (Smolowitz *et al*., 1992). As the disease progresses, degradation extends into the lower layers of the cuticle, the procuticle, spreading outwards causing many of the smaller lesions to unite. Early stages of the disease may be lost at moult (Smolowitz *et al*., 1992) but if the lesion has penetrated into the soft underlying tissues, mortality can result either because of secondary infection through the ruptured cuticle (Vogan *et al*., 2001; Fig. 1) or by adhesion between the two moult shells at the lesion site leading to only partial withdrawal from the exuviate during moult (Smolowitz *et al*., 1992). Recent reports have identified a potential new form of shell disease christened 'epizootic' shell disease that affects lobsters from New England coastal waters (Smolowitz *et al*., 2005; Castro *et al*., 2006; Glenn and Pugh, 2006). This form has dis-

tinctive differences to the 'classical' form of the disease in terms of nature and location of lesions and it may result in higher levels of mortality in affected animals than that previously reported. While classical shell disease appears to largely involve chitin breakdown, this may be of less importance in the epizootic condition thought to be mainly associated with chitoprotein degradation (Smolowitz *et al*., 2005).

Most studies of 'classical' shell disease (i.e. the non-epizootic form) have attributed the disease progression to the extracellular chitinolytic activities of microorganisms in the lesions. However, removal of the outermost, non-chitin containing epicuticle, is required before chitin degradation can occur (Fig. 1). As reviewed by Vogan and colleagues (1999), destruction of the epicuticular layer may occur by proteolytic and lipolytic microbial activities, predatory or cannibalistic attacks, chemical attack or the abrasive action of sediment and/or articulated body parts. Penetration into the chitin-containing layers may also proceed thorough the setal pores (Smolowitz *et al*., 1992; Prince *et al*., 1993). Other studies have suggested that the microbes involved in lesion formation are opportunistic exploiting animals that are in a weakened physiological state (Noga *et al*., 1994).

Marine crustaceans are reported to have exoskeletons with approximately 70% of the organic fraction being chitin (Brimacombe and Webber, 1964). Hence, microbial chitinases are widely believed to be vital to shell disease lesion progression, at least in the classical form of the disease. Culturable marine microbial chitinoclasts include species within the genera *Vibrio, Photobacterium, Aeromonas, Alteromonas, Pseudoalteromonas, Clostridium, Cytophaga* and *Chromobacteria* as well as actinomycetes and fungi (Gooday, 1990; Vogan *et al*., 2002; Costa-Ramos and Rowley, 2004; Bhattacharya *et al*., 2007). Although there are reports of chitinolytic fungal infections of the crustacean cuticle (Noga *et al*., 2000), bacteria are the most commonly cultured microorganisms from shell disease lesions (e.g. Noga *et al*., 2000; Porter *et al*., 2001; Vogan *et al*., 2002; Chistoserdov *et al*., 2005). Rosen (1970) was the first scientist to suggest that the lesions act as microniches being occupied by several microbial groups whose interactive effects result in lesion progression and later Cipriani and colleagues (1980) proposed that extracellular lipases and proteases were also involved in cuticular degradation. These reports have lead others (e.g. Sindermann, 1990; Smolowitz *et al*., 1992) to conclude that the disease represents the collective effects of the extracellular activities of the microbial lesion communities, as illustrated in Fig. 2, with some microbes acting as active enzyme producers while others fulfil a scavenger or predatory role.

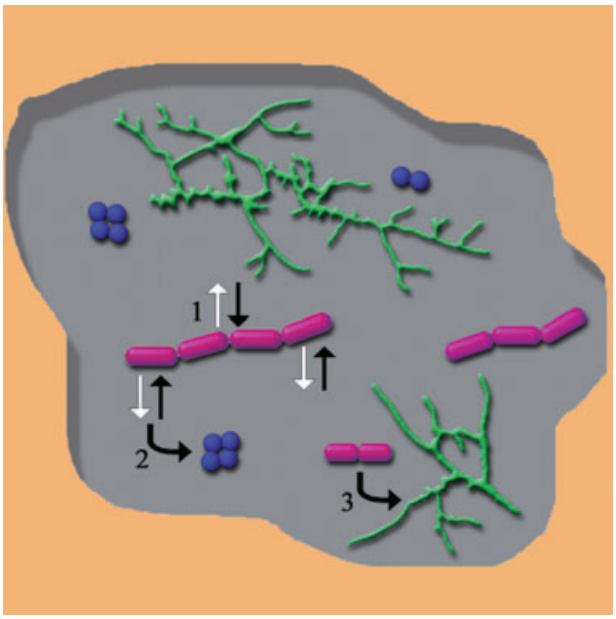

Fig. 2. Hypothetical and highly simplistic microbial lesion community structure. This consists of microorganisms that are active producers of extracellular enzymes such as chitinases (1); scavengers that do not produce such enzymes themselves but can utilize the breakdown products of others (2) and predators or grazers that breakdown other microbes or their extracellular enzymes (3). Gram-negative bacteria are red, Gram-positive bacteria are blue and fungi are in green. White arrows indicate extracellular enzyme production and black arrows indicate substrate utilization.

Bacterial isolation and identification of microbial communities in shell disease lesions

All studies to date on the bacterial agents associated with shell disease have relied on culture-dependent identification. For example, Noga and colleagues (2000) found a variety of pseudomonads, vibrios and aeromonads associated with the lesions of shell disease affected blue crabs, *Callinectes sapidus* but their identification was based on culture-based identification using phenotypic methodologies that are unlikely to give reliable identifications (e.g. Vandenberghe *et al*., 2003). Although the first to identify shell disease isolates using 16S rRNA sequencing, Porter and colleagues (2001) still relied on a preliminary culture step for their initial bacterial isolation. Similarly, Vogan and colleagues (2002) and Costa-Ramos and Rowley (2004) identified a number of culturable chitinolytic bacteria from lesions on edible crabs (*Cancer pagurus*) including *Pseudoalteromonas atlantica* and *Stenotrophomonas maltophilia*. Chistoserdov and colleagues (2005) showed by 16S rRNA sequencing of bacteria cultured from lesions from American lobsters (*Homarus americanus*) with epizootic shell disease, that this condition was associated with a number of bacterial genera including *Pseudoalteromonas gracilis* and a

member of the Flavobacterium. Of further interest was the finding that culturable chitinoclastic bacteria only accounted for 1% of the bacteria recovered from these lesions (Chistoserdov *et al*., 2005) perhaps strengthening the idea that the epizootic form of the disease may have only limited similarity to the classical form that is viewed to be mainly associated with chitin degradation.

As it is now widely recognized that pure culture techniques can seriously underestimate the total number and cause bias in group dominance of the actual marine microbial community (e.g. Eilers *et al*., 2000), the approaches undertaken to date are unlikely to show the true nature of the microbial communities in lesions from shell diseased animals. Furthermore, only concentrating on chitinolytic forms within these isolates (e.g. Vogan *et al*., 2002) is probably a mistake as other non-chitinolytic microorganisms in the lesions may be involved in proteolytic breakdown of the cuticle that will contribute to lesion development. Recent studies have indicated that the unculturable α-proteobacteria may be important as well as numerically dominant chitin degraders within the marine environment (e.g. Cottrell *et al.,* 2000) again warning of the potential dangers of relying on culture-based identification for evaluation of the relative importance of chitinolytic versus non-chitinolytic bacteria in lesion development (Chistoserdov *et al*., 2005). Overall, we would argue that the true dynamics of the epibiotic microbial community, and hence the aetiological agents of the several forms of shell disease, are still largely unknown.

By taking a non-culture-dependent approach to microbial enumeration and identification of the lesion microbiota, important questions that will yield a fundamental insight into shell disease will be answerable. These include: (i) Are the lesion communities the same in diseased animals collected from different geographic areas? (i.e. is shell disease a homogeneous disease in terms of its aetiology?) (ii) Are there different bacterial communities involved in classical and epizootic forms of shell disease? (iii) Are the microorganisms from lesions the same or distinct to those normally living on the integuments of non-diseased animals? (iv) What are the roles of non-chitinase producing microorganisms in lesion communities?

Cuticular degradation in shell disease observed in the edible crab, Cancer pagurus

Shell disease levels have been found to be abnormally high in populations of *C. pagurus* around Langland Bay, Gower Peninsula in South Wales, UK surveyed from 1997 to 2006 (Vogan *et al*., 1999; 2002; Powell and Rowley, 2005). This long-term survey has highlighted the importance of regional sediment characteristics in the induction and progression of shell disease in *C. pagurus*. The abrasive quality of sandy sediments is likely to be a significant factor in the removal of the outermost non-chitinous epicuticle (Vogan *et al*., 1999). The subsequent colonization of the chitinous procuticle is also enhanced by the sediments as they contain large numbers of chitinolytic microorganisms that possess efficient chemosensory responses to newly exposed chitin. For *C. pagurus*, in the warmer months, all these processes are aided by the animals' onshore behavioural response of sediment backburrowing leading to mechanical abrasion of the integument (Vogan *et al*., 1999).

The ultimate fate of a shell-diseased animal will largely depend on its age (Vogan *et al*., 1999) and the overall health status. As crustaceans age, the frequency of moult declines, microbes have longer to degrade the cuticle and the probability of pathogen entry into the main blood space (haemocoel) through the lesion site increases (Fig. 1; Vogan *et al*., 2001). Ultimately, it is the ability of the shell diseased animal to combat microbial invaders that enter its tissues following breakdown of the cuticle which is paramount to its survival. Hence the bacteria that predominate in the lesions may not necessarily be those that multiply in the blood resulting in organ damage and septicaemia. In the case of *C. pagurus*, several of the different species of chitinolytic bacteria isolated from lesions were also found to be pathogenic when injected into crabs (Vogan *et al*., 2002; Costa-Ramos and Rowley, 2004), and Vogan and colleagues (2001) found that the severity of shell disease was correlated with bacterial numbers found in blood.

Why are some species of crustaceans more susceptible to shell disease than others?

In certain situations the reasons for elevated levels of shell disease are intuitive. For example, some aquaculture and impoundment/holding systems contain high concentrations of animals thus increasing the chances of mechanical damage from contact and fighting resulting in subsequent cuticular disease. However, given that the levels of shell disease increase with the age of the animal in natural populations (e.g. Vogan *et al*., 1999), one would expect crustaceans that have undergone a terminal moult would exhibit high levels of the disease.

In the case of the Langland Bay study site already reviewed, the rock holes and crevices where edible crabs are found at low tide are frequently inhabited by European lobsters (*Homarus gammarus*), velvet swimming crabs (*Necora puber*) or even green shore crabs (*Carcinus maenas*). These three species all display negligible levels (< 1% infection rates) of the disease in comparison to *C. pagurus* living in the same environment (C.L. Vogan, unpubl. obs.). Whether this variation in species suscepti-

bility results from differences in behaviour, cuticular antimicrobial abilities, cuticular repair processes or epicuticular thickness remains to be determined.

What is the economic cost of shell disease?

The edible crab fishery in the UK alone is said to be worth in excess of £20 million per year, while worldwide crustacean production from fisheries and aquaculture exceeded 4×10^6 metric tons in 1998–99 (Wickins and Lee, 2002). Although the levels and severity of shell disease in edible crabs collected in UK coastal waters are highly variable, up to 50% of such crabs collected by baited pot reportedly show this condition and severely affected individuals have no market potential owing to the tainting of the muscle (crab meat) and the presence of unsightly lesions on the carapace (Vogan *et al*., 1999). Neither lobsters nor edible crabs are currently subject to traditional aquaculture because of the inability to raise individuals from larvae to marketable adults under captive conditions. Instead, such crustaceans are taken from the wild and held for relatively brief periods in pounds or 'viviers' until shipped to market. During these captive periods, shell disease is unlikely to be a major consequence to the overall health status of such shellfish. However, in the case of tail fan necrosis (an unusual form of shell disease) in the southern rock lobster, *Janus edwardsii*, such live holding of these animals prior to market is severely hampered by this condition (Musgrove *et al*., 2005). Whether shell disease is a cause or significant contributory factor in the reported declines in catches of lobsters off the New England coast or in edible crab fishery in parts of the UK is largely unknown.

The crustacean cuticle – a defence against shell disease

Much is known about the crustacean immune defence mechanisms that are activated should a microorganism breach the cuticle and enter the body cavity (haemocoel) of the animal (for reviews see Lee and Söderhäll, 2002; Bachère *et al*., 2004). However, comparatively little has been reported on the possible antimicrobial defences that may combat cuticular degradation. Noga and colleagues (1994) suggested that the crustacean cuticle may permit the transport of haemocoelic defensive molecules, such as antimicrobial peptides, to its surface via the tegumental gland. If this is the case, it would provide an explanation for how anthropogenic chemicals thought to be associated with shell disease that have also been shown to suppress the internal (haemocoelic) immune system, could directly influence lesion progression. Although a number of studies have shown that products of the melanin-producing prophenoloxidase cascade found in the cuticle have the ability to suppress the growth of fungi (Söderhäll and Ajaxon, 1982), their role in general microbicidal activity within the cuticle remains unproven. There is no doubt that melanin and associated products of the prophenoloxidase cascade are produced in response to cuticular damage, but the progressive nature of the cuticular lesions in shell disease itself suggests their ability to cause cuticular disinfection may be limited. Haug and colleagues (2002) have demonstrated that antimicrobial compounds can be extracted from the exoskeletons of *Pandalus borealis* (northern shrimp), *Pagurus bernhardus* (hermit crab), *Hyas araneus* (spider crab) and *Paralithodes camtschatica* (king crab). Their nature, diversity and site(s) of biosynthesis are unknown but it is tempting to speculate that they may to be related to the increasingly large family of antimicrobial peptides that have been found in the blood of crustaceans (Bachère *et al*., 2004). Finally, it is possible that chitin itself and some of its breakdown products could have antibacterial activity (Tsai and Hwang, 2004).

Overall, although there is some knowledge of the antimicrobial activities in the cuticle, this is very incomplete. Key questions still remain, for example, if anthropogenic disturbances in the marine environment really result in heightened levels of shell disease in crustaceans, is one potential mechanism for this general suppression in the biosynthesis of such antimicrobial factors in both the blood and cuticle?

Pollution: is the correlation with heightened shell disease a coincidence?

Since the 1970s, the occurrence of shell disease in decapod crustaceans has been linked with three forms of environmental pollution, namely sewage, metal ions and chemical insecticides. Young and Pearce (1975) were the first workers to show that lobsters under aquarium conditions exposed to dredge from sewage dumping grounds in the New York Bight had a high prevalence of shell disease-like lesions. More recently, the prevalence of shell disease in lobsters collected from the 106 mile site off the US eastern seaboard, which between 1986 and 1992 received *c.* 10 million tons of sewage/year from main cities including New York, has been reviewed (EPA, 1995). This detailed report concluded that although shell disease prevalances were higher in female lobsters in areas thought to be affected by sewage dumping, overall, the authors were unable to categorically conclude that such activities had caused the disease. One further long-term study has surveyed the prevalence and severity of shell disease in edible crab (*C. pagurus*) populations from Langland Bay, South Wales, UK between 1997 and 2007 before and post closure of a nearby raw sewage outfall (Vogan *et al*., 1999; Powell and Rowley, 2005). Crabs

sampled in 1997–1998 (before sewage outfall closure) revealed extremely high prevalence of shell disease in both males and females (Vogan *et al*., 1999). In 1999, the associated sewage outlet was closed resulting in rapid reduction in coliforms in that area and dramatic improvements in the quality of the bathing water (http://www.environment-agency.gov.uk). A further follow-up report of shell disease prevalence and severity in Langland Bay post closure in 2003 revealed no compelling change in disease prevalence (Powell and Rowley, 2005) and additional unpublished surveys at this location in 2005 and 2006 also failed to observe any significant changes in either the severity or prevalence of shell disease in this population. Although as discussed sewage is a suspect contributory factor in shell disease, the potential link has not been substantiated.

In the case of metal pollution, a thorough survey by Weinstein and colleagues (1992) compared shell disease and metal content of blue crabs (*Callinectes sapidus*) in the Albemarle-Pamlico estuaries in North Carolina. Healthy crabs from the control environment (Albemarle estuary) and shell disease affected crabs from an estuary contaminated by metal waste (Pamlico River estuary) were sampled. Higher concentrations of a variety of heavy and transition metals, in particular manganese, were found in sediment samples from the contaminated estuary and in organ samples of shell diseased affected crabs. The authors concluded that the accumulation of such metals might play a minor role in shell disease in this locality. As Hernroth and colleagues (2004) have found that manganese suppresses the immune system of the Norway lobster, *Nephrops norvegicus,* this may suggest a possible mechanism of its action in relation to shell disease. Preliminary studies on single bacterial isolates taken from shell disease lesions of *C. pagurus* and sediment culture supernatants from regions with high levels of shell disease, have revealed the potential of metal ions to directly enhance (Fe^{2+}, Mg^{2+}, Cu^{2+}) or inhibit (Hg^{2+}, Zn^{2+}) chitinase activity (C.L. Vogan, unpubl. obs.), perhaps suggesting that the putative links with shell disease and pollution might be due to regional enhancement of chitinase activity in the presence of specific metal ions. It should not be forgotten that chitin is a metal chelating polymer (Muzzarelli, 1977), but it is not known whether animals from contaminated areas have higher proportions of metal pollutant ions in their exoskeletons. If the contaminant metal ions serve to enhance chitinase activity of sediment-derived microorganisms it could have detrimental consequences for the animals. Overall, despite the small body of research, there is some evidence to suggest that certain metal ions could have a direct role in shell disease initiation and progression.

It is well established that insecticides affect cuticle formation in decapods and hormone analogues such as methoprene have also been found to inhibit chitin synthesis in the blue crab, *Callinectes sapidus* at part per billion concentrations (Horst and Walker, 1999). From 1997 onwards a sudden and devastating mortality event of lobsters, *Homarus americanus* occurred in the Long Island Sound, New England, USA. Lobsters were typically limp and some exhibited cuticlar lesions similar to those seen in shell disease. The high economic losses incurred led to a research initiative to investigate the cause of mortality (for overview see Pearce and Balcom, 2005). The cause was initially thought to be an accumulation of insecticides in coastal waters resulting from an attempt to use such chemicals to control the mosquito vector of the West Nile Virus, which occurred the summer before the lobster mortality event. Subsequently, trace concentrations of methoprene were found to increase moulting in lobster larvae and accumulate in the epidermal cells of adults, where they could potentially inhibit chitoprotein synthesis (Walker *et al*., 2005a,b). Other insecticides, such as malathion and resmethrin, have been shown to act as immune suppressants, by reducing the phagocytic ability of lobster haemocytes for up to 3 weeks after exposure (De Guise *et al*., 2004; 2005). However, others have found no apparent toxicity or immune suppressive activity in lobsters for insecticides at the concentrations found in waters in the Long Island Sound (Zulkosky *et al*., 2005).

This mass mortality event in lobsters was complicated by other factors including unusually high water temperatures (Glenn and Pugh, 2006) and outbreaks of other diseases, notably *Neoparamoeba* (Pearce and Balcom, 2005) and limp lobster syndrome caused by *Vibrio fluvialis* (Tall *et al*., 2003). Alkylphenols, compounds used in plastic manufacturing, have also been found in the local marine sediments and tissues of lobsters where they have endocrine disrupting effects (Biggers and Laufer, 2004). A current suggestion widely reported in the popular literature, is that these chemicals may modulate cuticular synthesis and hence adversely affect the interaction between shell disease microorganisms and the integument.

In conclusion, it would appear there is sufficient evidence to infer that the association between shell disease and metal ions or insecticides is probably not a simple coincidence. It is probable that these two groups of pollutants promote the degradation of the cuticle by opportunistic bacteria directly by reducing chitin/chitoprotein synthesis and carapace toughness, or indirectly by immunosuppression and physiological disruption. The putative link between shell disease and sewage pollution is not proven, partly owing to a lack of laboratory study and partly owing to the less discrete nature of the pollutant. It is also possible that sewage promotes existing lesion progression indirectly, by a general nutrient enrichment of the water and sediment, hence enhancing the abundance of the bacteria associated with shell disease.

Temperature change and shell disease

There is increasing interest in the relationship between climate change and diseases in the aquatic environment (e.g. Lafferty *et al*., 2004; Mydlarz *et al*., 2006; Bruno *et al*., 2007). In the case of shell disease in lobster populations in the New England region, the prevalence of this condition has been found to be positively correlated with periods of higher than average water temperatures (Glenn and Pugh, 2006). While these data are derived from a relatively brief timescale (2000–2004) they may indicate that temperature could be a major driver in observed changes in this condition. As pointed out by Glenn and Pugh (2006), lobsters held at temperatures higher than 20°C (a temperature that has been shown to be exceeded in these surface waters during summer months in the period studied) are subject to heightened physiological stress that can lead to immunosuppression and heightened microbial growth.

Conclusions

A key conclusion of this review is that a radical re-evaluation of the roles of chitinolytic versus non-chitinolytic bacteria in lesion progression in the different forms of shell disease is overdue. However, this can only take place after the true nature of the microbial communities in shell disease lesions has been systematically evaluated. Second, our appreciation of the nature of antibacterial activities of the crustacean cuticle is lacking and this will impede any future analysis aimed at firmly linking anthropogenic disturbances and general environmental change with outbreaks of shell disease. Finally, a simplistic model for shell disease based only on abnormal chitin recycling is unlikely to adequately reflect the complexity of this syndrome either at the level of microbial interaction within lesions or in different crustacean populations. Thus, shell disease cannot simply be viewed any longer as just chitin recycling gone wrong.

References

Aluwihare, L.I., Repeta, D.J., Pantoja, S., and Johnson, C.G. (2005) Two chemically distinct pools of organic nitrogen accumulate in the ocean. *Science* **308:** 1007–1010.

Aunpad, R., and Panbangred, W. (2003) Cloning and charcaterization of the constitively expressed chitinase C gene from the marine bacterium, *Salinivibrio costicola* strain 5SM-1. *J Biosci Bioeng* **96:** 529–536.

Bachère, E., Gueguen, Y., Gonzalez, M., de Lorgeril, J., Garnier, J., and Romestand, B. (2004) Insights into the anti-microbial defense of marine invertebrates: the penaeid shrimps and the oyster *Crassostrea gigas*. *Immunol Rev* **198:** 149–168.

Bassler, B.L., Yu, C., Lee, C.Y.C., and Roseman, S. (1991) Chitin utilization by marine bacteria. Degradation and catabolism of chitin oligosaccharides by *Vibrio furnissii*. *J Biol Chem* **266:** 24276–24286.

Bhattacharya, D., Nagpure, A., and Gupta, R.K. (2007) Bacterial chitinases: properties and potential. *Crit Rev Biotech* **27:** 21–28.

Biggers, W.J., and Laufer, H. (2004) Identification of juvenile hormone-active alkylphenols in the lobster *Homarus americanus* and in marine sediments. *Biol Bull Woods Hole* **206:** 13–24.

Boyer, J.N. (1994) Aerobic and anaerobic degradation and mineralization of ^{14}C-chitin by water column and sediment inocula of the New York river estuary, Virginia. *Appl Environ Microbiol* **60:** 174–179.

Brimacombe, J.S., and Webber, J.M. (1964) *Mucopolysaccharides.* Amsterdam, the Netherlands: Elsevier.

Bruno, J.F., Selig, E.R., Casey, K.S., Page, C.A., Willis, B.L., Harvell, C.D., *et al.* (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biol* **5:** 1220–1227.

Castro, K.M., Factor, J.R., Angell, T., and Landers, D.F., Jr (2006) The conceptual approach to lobster shell disease revisited. *J Crustacean Biol* **26:** 646–660.

Cauchie, H.M. (2002) Chitin production by arthropods in the hydrosphere. *Hydrobiologica* **470:** 63–96.

Chistoserdov, A.Y., Smolowitz, R., Mirasol, F., and Hsu, A. (2005) Culture-dependent characterization of the microbial community associated with epizootic shell disease lesions in American lobster, *Homarus americanus*. *J Shellfish Res* **24:** 741–747.

Cipriani, G.R., Wheeler, R.S., and Sizemore, R.K. (1980) Characterization of brown spot disease of Gulf coast shrimp. *J Invertebr Pathol* **36:** 255–263.

Costa-Ramos, C., and Rowley, A.F. (2004) Effects of extracellular products of *Pseudoalteromonas atlantica* on the edible crab, *Cancer pagurus*. *Appl Environ Microbiol* **70:** 729–735.

Cottrell, M.T., and Wood, D.N., Yu, L., and Kirchman, D.L. (2000) Selected chitinase genes in cultured and uncultured marine bacteria in the α- and γ-subclasses of the *Proteobacteria*. *Appl Environ Microbiol* **66:** 1195–1201.

De Guise, S., Maratea, J., and Perkins, C. (2004) Malathion immunotoxicity in the American lobster (*Homarus americanus*) upon experimental exposure. *Aquat Toxicol* **66:** 419–425.

De Guise, S., Maratea, J., Chang, E.S., and Perkins, C. (2005) Resmethrin immunotoxicity and endocrine disrupting effects in the American lobster (*Homarus americanus*) upon experimental exposure. *J Shellfish Res* **24:** 781–786.

Eilers, H., Pernthaler, J., Glockner, F.O., and Amann, R. (2000) Culturability and *in situ* abundance of pelagic bacteria from the North Sea. *Appl Environ Microbiol* **66:** 3044–3051.

Environmental Protection Agency (1995) *Monitoring, Research, and Surveillance of the 106-Mile Deepwater Municipal Dump Site and Environs*. Report to Congress, EPA-842-R-95–001.

Glenn, R.P., and Pugh, T.L. (2006) Epizootic shell disease in American lobster (*Homarus americanus*) in Massachusetts coastal waters: interactions of temperature, maturity, and intermolt duration. *J Crustacean Biol* **26:** 639–645.

Gooday, G.W. (1990) The ecology of chitin degradation. *Adv Microbial Ecol* **11:** 387–430.

Goplan, U.K., and Young, J.S. (1975) Incidence of shell disease in shrimp in the New York Bight. *Mar Pollut Bull* **6:** 149–153.

Haug, T., Kjuul, A.K., Stensvåg, K., Sandsdalen, E., and Styrvold, O.B. (2002) Antibacterial activity in four marine crustacean decapods. *Fish Shellfish Immunol* **12:** 371–385.

Hernroth, B., Baden, S.P., Holm, K., Andre, T., and Söderhäll, I. (2004) Manganese induced immune suppression of the lobster, *Nephrops norvegicus*. *Aquat Toxicol* **70:** 223–231.

Horst, M.N., and Walker, A.N. (1999) Effects of the pesticide methoprene on morphogenesis and shell formation in the blue crab *Callinectes sapidus*. *J Crustacean Biol* **19:** 699–707.

Huq, A., Small, E., West, P., and Colwell, R.R. (1984) The role of planktonic copepods in the survival and multiplication of *Vibrio cholerae* in the aquatic environment. In *Vibrios in the Environment.* Colwell, R.R. (ed.). New York, USA: Wiley, pp. 521–534.

Keyhani, N., Li, X.B., and Roseman, S. (2000) Chitin catabolism in the marine bacterium *Vibrio furnissii*. Identification and molecular cloning of a chitoporin. *J Biol Chem* **275:** 33068–33076.

Keyhani, N.O., and Roseman, S. (1999) Physiological aspects of chitin catabolism in marine bacteria. *Biochim Biophys Acta* **1473:** 108–122.

Kirchman, D.L., and White, J. (1999) Hydrolysis and mineralization of chitin in the Delaware estuary. *Aquat Microbiol Ecol* **18:** 187–196.

Kirn, T.J., Jude, B.A., and Taylor, R.K. (2005) A colonization factor links *Vibrio cholerae* environmental survival and human infection. *Nature* **438:** 863–866.

Lafferty, K.D., Porter, J.W., and Ford, S.E. (2004) Are diseases increasing in the ocean? *Annu Rev Ecol Evol Syst* **35:** 31–54.

Lee, S.Y., and Söderhäll, K. (2002) Early events in crustacean innate immunity. *Fish Shellfish Immunol* **12:** 421–438.

Li, X., and Roseman, S. (2004) The chitinolytic cascade in vibrios is regulated by chitin oligosaccharides and two-component chitin catabolism sensor/kinase. *Proc Natl Acad Sci USA* **101:** 627–631.

Meibom, K.L., Li, X.B., Nielsen, A.T., Wu, C.-Y., Roseman, S., and Schoolnik, G.K. (2004) The *Vibrio cholerae* chitin utilization program. *Proc Natl Acad Sci USA* **101:** 2524–2529.

Musgrove, R.J., Geddes, M.C., and Thomas, C. (2005) Causes of tail fan necrosis in the southern rock lobster, *Jasus edwardsii*. *NZ J Mar Freshw Res* **39:** 293–304.

Muzzarelli, R.A.A. (1977) *Chitin.* Oxford, UK: Pergamon Press.

Mydlarz, L.D., Jones, L.E., and Harvell, C.D. (2006) Innate immunity, environmental drivers, and disease ecology of marine and freshwater invertebrates. *Annu Rev Ecol Evol Syst* **37:** 251–288.

Noga, E.J., Engel, D.P., Arroll, T.W., McKenna, S., and Davidian, M. (1994) Low serum antibacterial activity with increased prevalence of shell disease in blue crabs, *Callinectes sapidus*. *Dis Aquat Organ* **19:** 121–128.

Noga, E.J., Smolowitz, R., and Khoo, L.H. (2000) Pathology of shell disease in the blue crab, *Callinectes sapidus* Rathbun, (Decapoda: Portunidae). *J Fish Dis* **23:** 389–399.

Orikoshi, H., Nakayama, S., Miyamoto, K., Hanato, C., Yasuda, M., Inamori, Y., and Tsujibo, H. (2005) Roles of four chitinases (ChiA, ChiB, ChiC, and ChiD) in the chitin degradation system of marine bacterium *Alteromonas* sp. Strain O-7. *Appl Environ Microbiol* **71:** 1811–1815.

Pearce, J., and Balcom, N. (2005) The 1999 Long Island Sound lobster mortality event: findings of the comprehensive research initiative. *J Shellfish Res* **24:** 691–697.

Porter, L., Butler, M., and Reeves, R.H. (2001) Normal bacterial flora of the spiny lobster *Panulirus argus* and its possible role in shell disease. *Mar Freshw Res* **52:** 1401–1405.

Powell, A., and Rowley, A.F. (2005) Unchanged prevalence of shell disease in the edible crab *Cancer pagurus* four years after decommissioning of a sewage outfall at Langland Bay, UK. *Dis Aqua Organ* **68:** 83–87.

Prince, D.L., Bayer, R.C., and Loughlin, M. (1993) Etiology and microscopy of shell disease in impounded American lobsters, *Homarus americanus*. *Bull Aqua Ass Can* **4:** 87–89.

Rosen, B. (1970) Shell disease in aquatic crustaceans. In *Symposium on Diseases of Fishes and Shellfishes*. Special Publication no. 5. Snieszko, S.F. (ed.). Washington, DC, USA: American Fisheries Society, pp. 409–415.

Sindermann, C.J. (1990) *Principal Diseases of Marine Fish and Shellfish*, Vol. 2. New York, USA: Academic Press.

Smolowitz, R., Chistoserdov, A.Y., and Hsu, A. (2005) A description of the pathology of epizootic shell disease in the American lobster, *Homarus americanus,* H. Milne Edwards 1837. *J Shellfish Res* **24:** 749–756.

Smolowitz, R.M., Bullis, R.A., and Abt, D.A. (1992) Pathological cuticular changes of winter impoundment shell disease preceding and during intermolt in the American lobster, *Homarus americanus*. *Biol Bull Woods Hole* **183:** 99–112.

Söderhäll, K., and Ajaxon, R. (1982) Effect of quinines and melanin on mycelial growth of *Aphanomyces* spp. & extracellular protease of *Aphanomyces astaci* a parasite of crayfish. *J Invertebr Pathol* **39:** 105–109.

Stankiewicz, B.A., Mastalerz, M., Hof, C.H.J., Bierstedt, A., Flannery, M.B., Briggs, D.E.G., and Evershed, R.P. (1998) Biodegradation of the chitin-protein complex in crustacean cuticle. *Org Geochem* **28:** 67–76.

Stewart, J.E. (1993) Infectious diseases of marine crustaceans. In *Pathobiology of Marine and Estuarine Organisms.* Couch, J.A., and Fournie, J.W. (eds). Boca Raton, FL, USA: CRC Press, pp. 319–342.

Suginta, W. (2007) Identification of chitin binding proteins and characterization of two chitinase isoforms from *Vibrio alginolyticus* 283. *Enzyme Microb Technol* **41:** 212–220.

Svitil, A.L., Chadhain, M.N., Moore, J.A., and Kirchman, D.L. (1997) Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. *Appl Environ Microbiol* **63:** 408–413.

Tall, B.D., Fall, S., Pereira, M.R., Ramos-Valle, M., Curtis, S.K., Kothary, M.H., *et al.* (2003) Characterization of *Vibrio fluvialis*-like strains implicated in limp lobster disease. *Appl Environ Microbiol* **69:** 7435–7446.

Tsai, G.J., and Hwang, S.-P. (2004) In vitro and in vivo

antibacterial activity of shrimp chitosan against some intestinal bacteria. *Fish Sci* **70:** 675–681.

Vandenberghe, J., Thompson, F.L., Gomez-Gil, B., and Swings, J. (2003) Phenotypic diversity amongst *Vibrio* isolates from marine aquaculture systems. *Aquaculture* **219:** 9–20.

Vogan, C.L., Llewellyn, P.J., and Rowley, A.F. (1999) Epidemiology and dynamics of shell disease in the edible crab *Cancer pagurus*: a preliminary study of Langland Bay, Swansea, UK. *Dis Aquat Organ* **35:** 81–87.

Vogan, C.L., Costa-Ramos, C., and Rowley, A.F. (2001) A histological study of shell disease syndrome in the edible crab *Cancer pagurus*. *Dis Aquat Organ* **47:** 209–217.

Vogan, C.L., Costa-Ramos, C., and Rowley, A.F. (2002) Shell disease syndrome in the edible crab *Cancer pagurus* – isolation, characterization and pathogenicity of chitinolytic bacteria. *Microbiology* **148:** 743–754.

Walker, A.N., Bush, P., Puritz, J., Wilson, T., Chang, E.S., Miller, T., *et al.* (2005a) Metabolic effects of acute exposure to methoprene in the American lobster, *Homarus americanus*. *J Shellfish Res* **24:** 787–794.

Walker, A.N., Bush, P., Puritz, J., Wilson, T., Chang, E.S., Miller, T., *et al.* (2005b) Bioaccumulation and metabolic effects of the endocrine disruptor methoprene in the lobster, *Homarus americanus*. *Integr Comp Biol* **45:** 118–126.

Weinstein, J.E., West, T.L., and Bray, J.T. (1992) Shell disease and metal content of blue crabs, *Callinectes sapidus*, from the Albemarle-Pamlico estuary system, North Carolina. *Arch Environ Contam Toxicol* **23:** 355–362.

Wickins, J.F., and Lee, D.O'C. (2002) *Crustacean Farming: Ranching and Culture*, 2nd edn. Oxford, UK: Blackwell Science.

Young, J.S., and Pearce, J.B. (1975) Shell disease from crabs and lobsters from New York Bight. *Mar Poll Bull* **6:** 101–105.

Zulkosky, A.M., Ruggieri, J.P., Terracciano, S.A., Brownawell, B.J., and McElroy, A.E. (2005) Acute toxicity of resmethrin, malathion and methoprene to larval and juvenile American lobsters (*Homarus americanus*) and analysis of pesticide levels in surface waters after Scourge™, Anvil™ and Altosid™ application. *J Shellfish Res* **24:** 795–804.