

Pathology of shell disease in the blue crab, *Callinectes sapidus* Rathbun, (Decapoda: Portunidae)

E J Noga¹, R Smolowitz² and L H Khoo^{1,*}

¹ Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

² Marine Biological Laboratory, Woods Hole, MA, USA

Abstract

Blue crabs affected with shell disease displayed a wide array of pathologies in response to this very common affliction. Grossly, shell disease lesions most commonly presented as variably sized brown to black foci. Such lesions ranged from very small (1 mm²) to locally extensive (up to 200 mm² in area). The larger of the melanized lesions sometimes appeared ulcerated. The most severe lesions observed (Pamlico River shell disease-PRSD) resulted in loss of up to 25% of the entire carapace. A diverse bacterial flora consisting of aeromonads, vibrios and five other genera were isolated as the predominant organisms from shell disease lesions. Fungi were rarely observed in larger lesions. Protozoa and algae were also rarely observed on the surface of some lesions. Histologically, lesions ranged from mild erosion of the epicuticle and outermost layers of the calcified endocuticle to more extensive endocuticle erosion with accompanying inflammation. In the most severe cases, there was total loss of the endocuticle and epidermis, with pseudomembrane formation, intense haemocyte infiltration, and involvement of adjacent viscera. There was no apparent relationship between the size of gross lesions and their histological severity (as defined by the extent of tissue damage), suggesting that gross examination of shell disease lesions is not a reliable method for assessing the damage to affected blue crabs.

Correspondence E. J. Noga, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA (e-mail: ed_noga@ncsu.edu)

* Present address: Delta Research and Extension Center, PO Box 197, Stoneville, MS 38776, USA.

Introduction

Shell disease is one of the most common problems affecting freshwater and marine crustaceans (Sindermann 1989). It has been reported in many feral populations of crustaceans (Sindermann 1989), although the prevalence has usually been very low. However, high prevalence of shell disease has been associated with stressful environments, such as intensive aquaculture (Sindermann 1990), impounded populations (Prince, Bayer & Loughlin 1993), or polluted natural environments (e.g. the 106-mile dumpsite of the New York Bight; Ziskowski, Spallone, Kapareiko, Robohm, Calabrese & Pereira 1996). Shell disease can also be experimentally induced by exposure of crustaceans to sewage sludge (Young & Pearce 1975). Exposure to pesticides (Weis, Cohen & Kwiatkowski 1987) or heavy metals (Nimmo, Lightner & Bahner 1977) also produces shell disease-like lesions, suggesting that this syndrome may be a useful biomarker of environmental stress (Sindermann 1989, 1990).

Shell disease is characterized by various types of erosive lesions on the shell (Johnson 1983; Sindermann & Lightner 1988). The classical and most common form of shell disease is known as 'brown spot' or 'black spot', which consists of various-sized foci of hyperpigmentation (Rosen 1970). In recent years, blue crabs in the Albemarle-Pamlico estuary, North Carolina have been observed with a severe form of shell disease where up to one fourth of the carapace may be missing (McKenna, Jansen & Pully 1988). These severe lesions, which we have termed 'Pamlico River shell disease' (PRSD), have

also been observed in crabs from the St Johns River, Florida, Biscayne Bay, Florida, and Chesapeake Bay estuaries, as well as the Houston Ship Channel, Texas (Engel & Noga 1989; Noga, Engel, Arroll, McKenna & Davidian 1994).

While there have been numerous reports of the occurrence of shell disease in blue crabs (Rosen 1967) and other crustaceans, there are few comprehensive descriptions of the pathology of these lesions. The purpose of this paper is to describe the pathology of spontaneous shell disease in blue crabs and determine the relationship between the appearance of gross lesions and their histopathology.

Materials and methods

Necropsy and histological processing

Mature, intermolt blue crabs (at least 120 mm carapace width) were collected from the Albemarle-Pamlico estuary in 1993, 1995 and 1997. In addition, some lesions were studied in blue crabs that were held in closed system aquaria for varying periods of time. All tissues were collected immediately after euthanization. Lesions were measured, photographed, and immediately fixed in 10% neutral buffered formalin in 15% sea water. Tissues were then decalcified in EDTA (pH 6) and 5- μ m sections were stained with haematoxylin and eosin (HE). Selected sections were stained with Gomori methenamine silver with HE counterstain (GMS–HE) or Brown and Brenn Gram's stain. All slides were read blindly (i.e. no knowledge of source or size of lesion).

Microbiology

Bacteria from shell disease lesions or areas of normal shell were isolated as described previously (Noga *et al.* 1994) by scraping a small area with a 1- μ L disposable plastic loop. This sample was lightly touched to a small area of a plate having trypticase soy agar (TSA) with 5% defibrinated sheep blood (in preliminary studies, we found that this medium appeared to yield a slightly higher number of colonies compared to TSA supplemented with NaCl). The sample was then spread on the plate using a sterile swab (Mini-tip culturette, Marion Scientific, Marion Laboratories, Kansas City, MO, USA). Cultures were incubated

at room temperature. Predominant colonies were picked, purified by restreaking three times, and identified using API identification systems (API 20E, API 20NE strips, with salt supplementation as needed; Analytab Products, Plainview, New York, NY, USA), as well as standard tube tests for confirmation of some reactions. Attempts to culture fungi from selected lesions were performed using peptone-yeast extract-glucose-seawater (PYGS) agar (Hatai 1989). Pieces of large, ulcerated lesions were aseptically placed onto agar plates and incubated at room temperature for 14 days. Plates were examined daily for fungal growth.

Results

Anatomic pathology

Shell disease lesions in both captured and aquarium-reared blue crabs exhibited a similar continuum of damage and severity, ranging from pinpoint, black-pigmented foci to very extensive lesions with significant shell defects (Figs 1–5). After examining 78 lesions, we divided them into four categories, based upon microscopic appearance. A separate group of lesions comprising crabs having massive loss of the shell (Pamlico River shell disease, Figs 6 & 7) were described separately.

Grade 1 (very mild lesions)

These six lesions ranged from 1 \times 1 to 2 \times 2 mm, brown to black, pinpoint foci. Histologically, they were characterized as erosions that extended through the epicuticle and exocuticle and usually into the outer calcified endocuticle. The lesions were often melanized. Bacteria and debris were sometimes seen on the eroded surface. The underlying epithelium was normal to moderately hypertrophic, with mild numbers of granulocytes migrating into the epithelial layer and between the calcified endocuticle and the epithelium.

Grade 2 (mild lesions)

These 25 lesions ranged from 1–8 \times 1–20 mm, brown to black areas (Figs 8 & 9). Some were as small as 1 mm in diameter. The largest lesions appeared to arise from the coalescence of individual lesions. Histologically, lesions were shallow erosions with loss of the epicuticle, exocuticle and

outer calcified endocuticle. Bacteria were sometimes visible at the surface; algae were less common. The periphery of the lesions had variable cuticular necrosis and melanization. Melanization occurred in one of three forms (often in combination in any one lesion). First, melanization radiated

from the edges of the lesion into adjacent cuticle from 5–50 μm . Melanization also radiated as linear extensions between lamellae of the endocuticular layers parallel to the epithelium for up to 100 μm in some cases. Finally, melanization also occurred in distinct vertical columns, which corre-



Figure 1 Ventral carapace of a clinically normal blue crab (bar = 1 cm).

Figure 2 Diffuse brown melanization on the ventral carapace, which is characteristic of mainly grade 2 lesions. The more discrete black foci range from grades 2 to 4 (bar = 1 cm).

Figure 3 Linear black foci (arrow) on a chela (grade 3) (bar = 1 cm).

Figure 4 Moderate ulceration on a chela (grade 3–4) (bar = 1 cm).

Figure 5 Severe ulceration on a walking leg (grade 3–4) (bar = 1 cm).



Figure 6 Pamlico River shell disease on the dorsal carapace (arrow) (grade 3) (bar = 1 cm).

Figure 7 Pamlico River shell disease with loss of one quarter of the carapace on the right side (bar = 1 cm).

sponded to the shell structure itself (Stevenson 1985). In some cases, melanized, multifocal erosions into, and occasionally through, the exocuticle, appeared to follow the setal ducts.

A thin layer of untransformed haemocytes was often present between the melanized, calcified en-

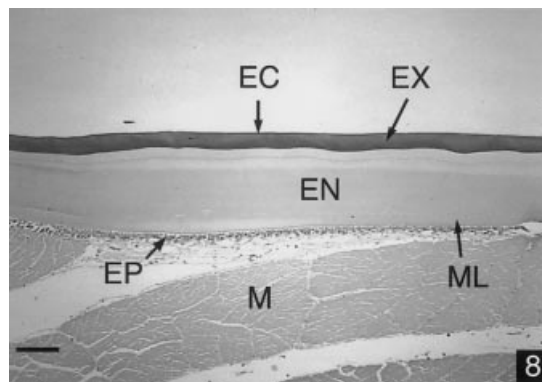


Figure 8 Normal carapace. EC = epicuticle, EX = exocuticle, EN = endocuticle, EP = epidermis, ML = membranous layer, M = muscle (H&E, bar = 100 μ m).

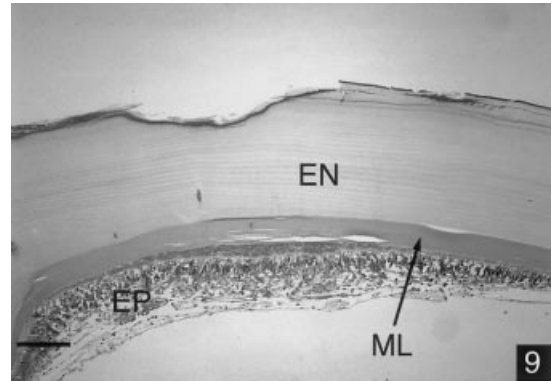


Figure 9 Mild (grade 2) lesion with erosion into calcified endocuticle, hyperplasia of the epidermis with moderate haemocyte infiltration, and proliferation of the membranous layer. EN = endocuticle, EP = epidermis, ML = membranous layer (H&E, bar = 100 μ m).

docuticle and the variously (mildly to greatly) thickened and often irregular uncalcified endocuticle/membranous layer. The epithelium was moderately to severely hyperplastic with some pinpoint (5–20 μ m in diameter) foci of melanization within it. There were moderate numbers of granular haemocytes within the epithelium and the underlying dermis.

In some cases, epithelial hyperplasia was associated with supraepithelial bullae filled with finely granular, eosinophilic matrix (presumably proteinaceous fluid) which may be an unpolymerized portion of the membranous layer or alternately an area of premature separation of the epithelium from the uncalcified cuticle. A layer of expanded granulocytes about one to six cells thick was often present between the epithelium and the uncalcified endocuticle or occasionally between the calcified and uncalcified endocuticle.

One lesion had a multifocal, chronic, fibrosing myositis with loss of muscle fibres in the bundle, possibly as a result of bacteria or rickettsia. Haemocytes often formed small accumulations of 2–10 melanized cells, indicating a chronic response. In some cases, a pseudomembrane (about five cells thick) of haemocytes overlaid the exposed calcified endocuticle, suggestive of older inactive lesions.

Grade 3 (moderate lesions)

These 33 lesions were 1–10 \times 1–20 mm, round, oblong, or irregularly-shaped, brown to black foci. Some were as small as 1 mm in diameter. They

were characterized by chronic erosion of the epicuticle, exocuticle and often all of the calcified endocuticle, exposing the surface of the underlying uncalcified endocuticle. The periphery of the lesions was often melanized as described in grade 2. Bacteria, protozoa (especially ciliates) and/or debris were often seen at the surface of the erosion (Fig. 10). Fungal hyphae of variable width (1.0–5.0 μm) were seen in one lesion. Overlying haemocytes sometime formed an early pseudomembrane (Fig. 11).

The uncalcified endocuticle was usually exposed and eroded superficially at the deepest part of the erosion. New layers of uncalcified endocuticle/membranous layer had been deposited by the epithelium between the older uncalcified endocuticle and the epithelium during the erosive process. This resulted in thickening and mild parallel lamination of the uncalcified endocuticle layer, sometimes up to 50 μm in depth. Haemocytes and necrotic debris accumulated at the surface of the lesions and occasionally extended laterally between the border

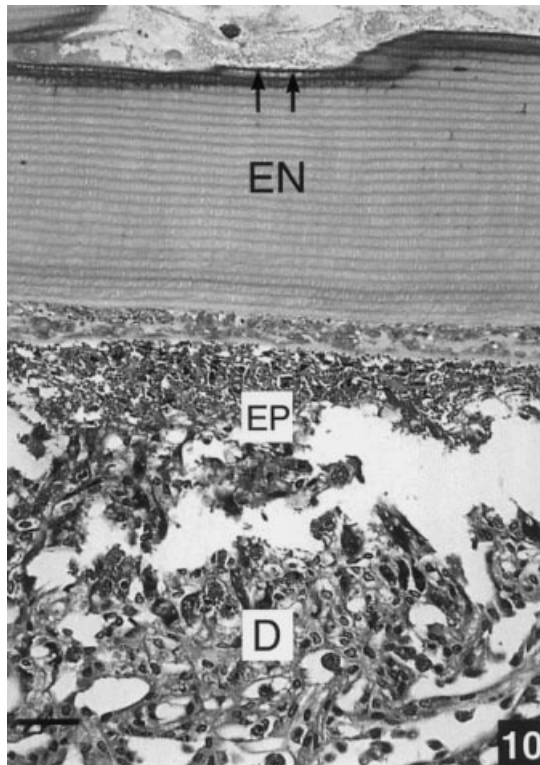


Figure 10 Grade 3 lesion with erosion extending into the calcified endocuticle (EN). Many bacteria and rare protists are at the surface of the erosion (arrows). Inflammation in the dermis (D) extending into the epidermis (EP) (H&E, bar = 25 μm).

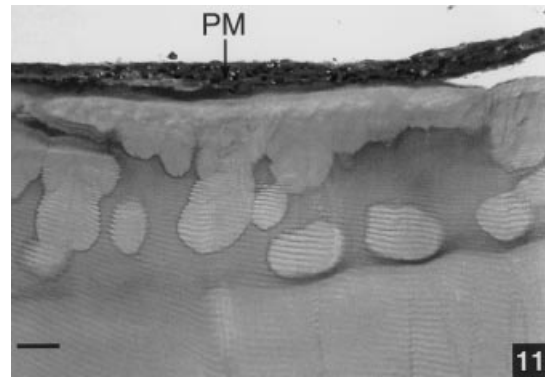


Figure 11 Grade 2 lesion with early pseudomembrane (PM) overlying a scalloped cuticular surface (epithelium not visible in this view) (H&E, bar = 25 μm).

of the uncalcified and calcified endocuticle at the edges of the lesions, occasionally resulting in production of well-formed pseudomembranes of transformed haemocytes (Fig. 11). In addition, haemocytes and debris accumulated (occasionally producing well-formed pseudomembranes) between layers of the older and newer uncalcified cuticle/membranous layer. These accumulations could be 30–40 μm deep and could extend beyond the lateral edges of the erosions between the parallel lamellae of the cuticle.

The underlying epithelium was usually moderately hyperplastic/hypertrophic, but was occasionally thin or severely hyperplastic. Haemocytes were more numerous in the epithelium and underlying connective tissue, compared with grade 2 lesions. Rarely, haemocytes appeared to be toxic (i.e. vacuolated and pyknotic). A few round foci of melanization were seen within the epithelium and just beneath it. These foci occasionally appeared to be associated with tegmental glands or ducts.

Grade 4 (severe lesions)

These 10 lesions, ranging up to 10 \times 20 mm were round, oblong, or irregularly shaped, brown to black foci. Some were as small as 3 mm in diameter. They were histologically characterized by loss of epicuticle and exocuticle, and either loss or severe fragmentation and necrosis of calcified endocuticle. Uncalcified endocuticle/membranous layer was usually thin and in severe cases was also eroded. The epithelium underlying such lesions was attenuated (cuboidal to squamous), or so decreased in number that pseudopodia-like exten-

sions of the cells stretched over and between the haemocytes and up to the pseudomembrane, or were not present at all (ulcerated). Intact epithelium on either side of the erosion was hypertrophic/hyperplastic and in some cases had produced a greatly thickened uncalcified endocuticle/membranous layer.

In deeply eroded areas, haemocytes and debris accumulated and separated the uncalcified endocuticle/membranous layer from the underlying epithelium. In many such locations, there was pseudomembrane formation (3–150 cells thick) by haemocytes (Fig. 12). Pseudomembrane melanization was common. Large numbers of primarily granular haemocytes sometimes accumulated in the pseudomembrane. In severe cases, when the epithelium could not be identified (ulcerated), the intact pseudomembrane usually replaced the epithelium. The most severe lesions showed extensive ulceration of the cuticle and epithelium, as well as loss of an intact pseudomembrane.

There was mild to moderate inflammation in areas with intact epithelium. In more severe erosions, the dermis/hypodermis had severe inflammation with haemocyte transformation and fibroblast proliferation. In some severe lesions, the epithelium and intra-epithelial haemocytes were vacuolated and melanized, suggesting a bacterial infection or toxin in the dermis. Additionally, the dermis occasionally contained melanized foci of haemocytic aggregation (as contrasted with the brown melanization of the tegmental glands). In the most severe cases, where ulceration was present, there was an underlying, intense, haemocytic inflammation in the dermis and hypodermis associated with

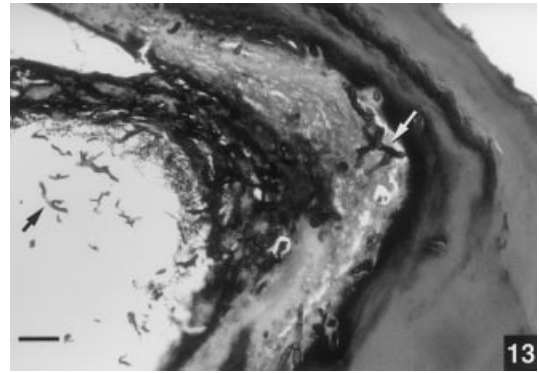


Figure 13 Fungal infection. Numerous variably wide hyphae (arrows) extending from the surface of the shell into the cuticle (GMS-H&E, bar = 100 μ m).

(depending upon adjacent structures) a necrotizing myositis, vasculitis or neuritis. In addition, haemocyte fibroblast transformation was also noted with attempts to encapsulate a small (10–25 μ m in diameter) focus (an internal form of pseudomembrane formation). In one animal with an ulcer, a displaced, necrotic portion of cuticle in the hypodermis contained tetrads of bacteria and was surrounded by transformed haemocytes in an attempt by the inflammatory process to wall off or encapsulate the abnormally placed cuticle (similar to mammalian granulomas). Other animals showed similar displacement of cuticle and the associated inflammatory reaction of walling off, demonstrating that at least some of the dermal (and deeper inflammation) was associated with a foreign-body-like reaction (Jones & Hunt 1983) to the displaced cuticle. In one case, septate, fungal hyphae of variable width (1.0–5.0 μ m) extended from the surface

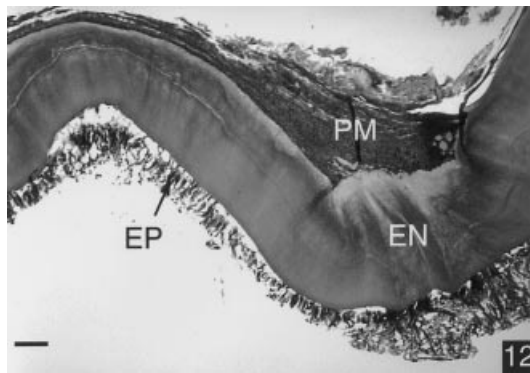


Figure 12 Grade 3 lesion with severe pseudomembrane (PM) formation over uncalcified endocuticle (EN). EP = epidermis (H&E, bar = 100 μ m).

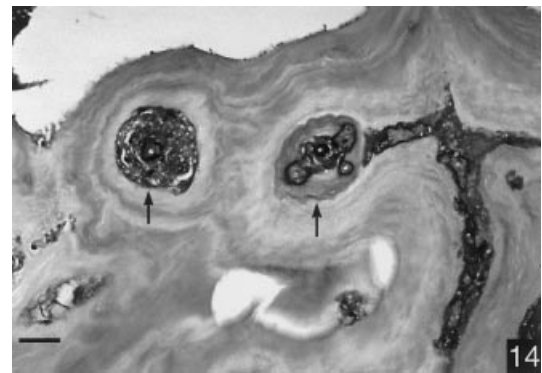


Figure 14 Fungal infection. Necrotic pseudomembrane is associated with an encapsulation reaction (arrows) to fungal hyphae. Grade 4 lesion (H&E, bar = 100 μ m).

(Fig. 13) into the dermis and elicited a melanized, encapsulation reaction (Fig. 14).

Pamlico River shell disease (PRSD)

These 17 lesions, mainly obtained from crabs collected in the Pamlico River but also from animals in other areas such as Albemarle Sound, were grossly characterized by the severe loss of much of the shell. This resulted in large open defects that ranged from 50 mm² in area to loss of nearly 25% of the entire shell (Figs 6 & 7).

The main features which distinguished these lesions from other shell disease lesions were their large size and grossly obvious, very deep penetrating nature. They were also much less pigmented than other lesions. Most were histologically similar to the moderate to severe lesions observed in both captured and aquarium-maintained animals. However, there were two consistent findings: all PRSD lesions appeared to have a pseudomembrane at the surface of the erosion that extended through the calcified endocuticle to the uncalcified endocuticle. In contrast, in grade 3 or grade 4 lesions, the beginning of a pseudomembrane and/or inflammation was noted in this area, but the calcified endocuticle was usually still present over the pseudomembrane and was still somewhat intact. In addition, there was occasional mixing of pseudomembrane with necrotic endocuticle. Variably wide fungal hyphae were seen in two lesions.

Culture results

The predominant bacteria isolated from shell disease lesions were extremely varied, as were those isolated from clinically normal shell (Table 1). Attempts to culture fungi from two lesions were unsuccessful. The rarity of this fungal infection precluded culture from more lesions.

Relationship between lesion size and histological severity

To determine if there was a relationship between the size (surface area) of the shell disease lesions and their histological severity, we performed a Pearson product-moment correlation analysis (Zar 1996) using SAS version 7.0 (SAS Institute, Cary, NC, USA). Assuming that lesion severity was a continuous variable between the minimum and maximum values, we found a very low correlation

Table 1 Bacteria isolated from blue crab carapace

Species	Anatomic source
<i>Achromobacter xyloxidans</i>	Shell disease lesion
<i>Acinetobacter calcoaceticus</i> v. <i>anitratus</i>	Shell disease lesion
<i>Aeromonas punctata</i>	Shell disease lesion
<i>Aeromonas sobria</i>	Shell disease lesion
<i>Plesiomonas shigelloides</i> (2)	Shell disease lesion
<i>Pseudomonas acidovorans</i>	Shell disease lesion
<i>Pseudomonas alkaligenes</i>	Shell disease lesion
<i>Pseudomonas putrefaciens</i>	Shell disease lesion
<i>Pseudomonas</i> sp.	Shell disease lesion
<i>Pseudomonas testosteroni</i>	Shell disease lesion
<i>Serratia</i> sp.	Shell disease lesion
<i>Vibrio alginolyticus</i>	Shell disease lesion
<i>Vibrio minimus</i>	Shell disease lesion
<i>Vibrio parahaemolyticus</i> (2)	Shell disease lesion
<i>Vibrio vulnificus</i>	Shell disease lesion
<i>Achromobacter xyloxidans</i>	Normal shell
<i>Acinetobacter calcoaceticus</i> v. <i>anitratus</i>	Normal shell
<i>Acinetobacter calcoaceticus</i> v. <i>haem.</i>	Normal shell
<i>Acinetobacter calcoaceticus</i> v. <i>lwoffi</i> (2)	Normal shell
<i>Aeromonas hydrophila</i> (3)	Normal shell
<i>Aeromonas punctata</i>	Normal shell
<i>Escherichia coli</i>	Normal shell
<i>Plesiomonas shigelloides</i>	Normal shell
<i>Pseudomonas alkaligenes</i> (2)	Normal shell
<i>Pseudomonas cepacia</i> (2)	Normal shell
<i>Pseudomonas putrefaciens</i> (2)	Normal shell
<i>Pseudomonas vesiculans</i>	Normal shell
<i>Vibrio minimus</i>	Normal shell

Values in parentheses represent bacteria that were isolated as a predominant organism from more than one sample.

($r = 0.102$, $P = 0.449$). Transforming the area using a log scale did not substantially increase the correlation ($P = 0.107$, Fig. 15). Thus, there was no significant relationship between lesion area and lesion grade.

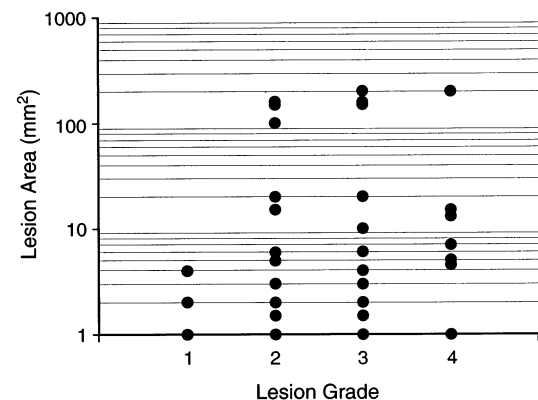


Figure 15 Relationship between lesion grade and surface area of lesions. Lesions with an intermediate grade (e.g. grade 2 and 3, 3 and 4, etc.) were not included in the analysis; $n = 49$ lesions.

Discussion

One of the most interesting findings of our study was that gross appearance of shell disease lesions did not closely reflect the severity of tissue damage or host response. For example, small, 1 mm², black foci could range from grade 1 (mild lesion) to grade 4 (severe). Conversely, relatively large lesions (e.g. 10 × 15 mm) could be as mild as grade 2. Similarly, while PRSD lesions were highly damaging by definition, all having large shell defects, many of these lesions were only grade 3 in histological severity and in some cases appeared to be in a very benign, healing phase. Thus, diagnosis of lesion severity based solely upon gross observation is highly inaccurate.

Virtually all surveys of shell disease in monitoring studies have relied entirely upon gross evaluation (Sandifer & Eldridge 1974; Sindermann 1989; Ziskowski *et al.* 1996). Our studies suggest that while gross examination might be used as a crude indicator of shell disease prevalence, it cannot be used to reliably indicate histologic lesion severity. The latter is important because lesion severity probably relates to the potential harm that such damage could inflict on the host.

Cuticular lesions revealed certain host responses in all grades examined, including cuticular erosion, uncalcified endocuticle/membranous layer hyperplasia, melanization, inflammatory cell infiltration and epithelial hyperplasia/hypertrophy. All of these responses were increasingly severe with grade 3 and grade 4 lesions. Melanization was least prominent in PRSD.

In more advanced lesions, pseudomembrane formation was common. Formation of a pseudomembrane is considered to be an attempt to protect the cuticular epithelium from ulceration (Smolowitz, Bullis & Abt 1992). In other crustaceans, the transformed cells responsible for pseudomembrane formation appear to originate from an influx of semigranulocytes or agranulocytes (Johannson & Söderhall 1989). Blue crab haemocytes in the haemolymph have been subdivided into three major morphological types; namely, hyaline, small granular and large granular haemocytes (Clare & Lumb 1994). However, inflammation in blue crabs has not been well studied and, thus, the definition of histological haemocyte equivalents requires further study.

In PRSD, the extension of the pseudomembrane through the calcified endocuticle and mixing of

pseudomembrane with necrotic endocuticle might indicate a more severe effect on the calcified endocuticle than the uncalcified endocuticle. However, it might also reflect other factors such as water temperature, age of the lesions, or other factors. The most prominent proliferation of the membranous layer was seen in grade 3 and to a lesser extent in grades 2 and 4. The membranous layer is laid down during stage C₃ of cuticular deposition in the moult cycle (Aiken 1980). It should be noted that while the membranous layer is often distinctly separated histologically from the overlying uncalcified endocuticle, it is not unusual for the junction between the uncalcified endocuticle and the membranous layer to be histologically undetectable. Thus, the terms 'uncalcified endocuticle/membranous layer' as well as membranous layer are used here, when appropriate.

Foci of melanization were occasionally seen to be associated with setal ducts and/or tegmental glands and probably reflected the susceptibility of tegmental glands and setal cells to infection. Other melanized encapsulations in the epithelium may have been related to stimulation of inflammatory cells by a bacteraemia or toxic products (Johannson & Söderhall 1989). Total loss of epithelium (ulcer formation) resulted in the most severe and penetrating inflammatory response, probably reflecting the critical importance of this tissue as a barrier to infection.

We separated PRSD from the other lesion grades because, while grades 1 to 4 appeared to form a continuum of a response, there was no obvious link between PRSD and these other lesion types. PRSD lesions were uniformly large, relatively unpigmented, and with extensive chronic inflammation, suggesting a slowly developing lesion. However, no small lesions which might be suspected to be an earlier stage were ever observed, suggesting that the initial changes leading to this lesion may occur very rapidly and/or may not be grossly evident on affected individuals. Thus, there is no clear link between the mild (presumably early) lesions that we observed and PRSD.

We are not certain which lesions may be eliminated via moulting, but the relatively minor damage seen with grade 1 and probably grade 2 lesions suggest that it is likely that they can probably heal in this manner. Grade 3 and 4 lesions often had inflammatory adhesions and, thus, it might be difficult for crabs to eliminate such lesions; although this would be more likely with grade 3

lesions. It is highly unlikely that crabs with PRSD would ever survive a moult. We held crabs with PRSD in the laboratory but did not observe any moulting. These crabs were also weaker than normal crabs and had poor survival rates compared with other crabs. Most importantly, the amount of adhesions and fibrosis associated with PRSD lesions would make it highly unlikely that they could ever moult. Adhesions are known to prevent crustaceans from moulting (Fisher 1988; Sindermann 1989).

While grade 3 and 4 lesions appeared to have more severe microbial infections, bacteria were histologically visible in some examples of all grades. However, histological examination is an insensitive measure of the presence of bacterial infections and typically underestimates its prevalence. Even healthy blue crab carapace has a heavy bacterial flora (Noga *et al.* 1994), suggesting that the mere presence of bacteria is not solely responsible for lesion development. This is also suggested by the diverse species of bacteria isolated from these lesions (Table 1, Noga *et al.* 1998). Fungal infections were rarely seen in all but grade 1 lesions, suggesting that they were secondary to the initial damage. To our knowledge, fungal or oomycete infections have never been observed in adult blue crabs. *Lagenidium callinectes*, an oomycete, infects blue crab eggs and larvae (Noga, Sawyer & Rodón-Naveira 1998). The width and the variability in width of hyphae in our lesions suggested that they might be an oomycete. Because of the rarity of this fungal infection, its culture and identification will require intensive sampling.

Shell disease in other crustaceans has been associated with many infectious agents (Sindermann 1989). While fungi have been occasionally involved, the great majority of cases have been linked to various bacteria, primarily vibrios (Sindermann 1989; Prince *et al.* 1993; Hameed 1994; Abraham & Manley 1995; Aguado & Bashirullah 1996). Previously, only bacteria have been associated with shell disease in blue crabs (Cook & Lofton 1973; Noga *et al.* 1994) and we did not find any histological evidence for the consistent presence of any other type of pathogen in what we presume to be the earliest lesions. It has been assumed that some form of damage to or weakening of the outer carapace (i.e. epicuticle), such as mechanical trauma, nutritional deficiency, or chemical damage, is needed for development of shell disease. This damage is hypothesized to allow the colonization

of shell degrading (i.e. chitinoclastic, lipolytic) bacteria which can feed on the deeper layers of the shell. However, this hypothesis does not explain several aspects of shell disease pathogenesis, including the presence of these same bacteria on healthy crab shell or the difficulty in reproducing shell disease with these isolates (Noga 1991).

A potent broad-spectrum, peptide antibiotic exists in blue crab haemolymph and can inhibit many shell-dwelling bacteria (Noga, Arroll & Fan 1996; Khoo, Robinette & Noga 1999). This antibiotic activity is reduced in crabs with PRSD. In addition, clinically normal crabs from environmentally 'stressed' areas have lower antibiotic activity than crabs from 'healthy' sites in the Albemarle-Pamlico estuary (Noga *et al.* 1994). These data suggest that the haemolymph may be an important source of chemicals for defending against shell disease. Invading shell disease pathogens are clearly exposed to haemolymph once shell integrity has been breached and lesions reach the epithelium. However, even intact or eroded shell might be protected by haemolymph activity.

The possible relationship between haemolymph antibacterial activity and shell disease has also been suggested by studies showing that exposure of crustaceans to cadmium induces shell disease lesions (Nimmo *et al.* 1977) and also causes a significant depression of circulating haemocytes (Victor 1993). Blue crabs from the Pamlico River have significantly higher tissue levels of cadmium, as well as other metals, compared with other sites in the Albemarle-Pamlico estuary (Weinstein, West & Bray 1992). Smith *et al.* (1995) found that shrimp, *Crangon crangon*, experimentally exposed to harbour dredge spoils had lower haemocyte counts. Exposure to dredge spoils has also been associated with development of shell disease (Gopalan & Young 1975). Haemocytes have been identified as the main source of antibacterial activity in several crustaceans (Chisholm & Smith 1995), including blue crabs (Noga *et al.* 1996).

While shell disease is one the most common diseases of crustaceans, there have been relatively few studies examining its pathogenesis. The previously most comprehensive study of shell disease pathology (Smolowitz *et al.* 1992) described progressive lesions in two intermoult substage groups of American lobster, *Homarus americanus*. Lesions in both groups were divisible into grades that demonstrated characteristic protective mechanisms. Shell disease in American lobsters most often ap-

peared to originate in the setal pits and then spread outward (Smolowitz *et al.* 1992). This was not evident in blue crabs examined as part of our study. Melanized cuticular foci consistent with a gross diagnosis of shell disease have also been observed in penaeid shrimp infected with Taura syndrome virus (Lightner, Redman, Hasson & Pantoja 1995). These melanized erosions were associated with a significant haemocyte infiltrate. It is important to recognize that shell disease in various crustaceans often appears grossly similar, although the pathogenesis of the response and the aetiological agents associated with and/or responsible for it can vary significantly.

Acknowledgements

The information in this document has been funded in part by the US Environmental Protection Agency under assistance agreement number 821584010 to North Carolina State University, lead by Dr Edward Noga. It has been subjected to the Agency's administrative review and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Funding was also provided by Grants NA46-RG-0087 and NA86-RG-0036 from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, to the North Carolina Sea Grant College Program and the College of Veterinary Medicine. We thank L. Henry of the North Carolina Division of Marine Fisheries and E. Henries (Carolina Seafood Co., Inc.) for providing samples, C. Lemons (NCSU) for excellent assistance with bacteriology and M. Nasution (NCSU) for assistance with statistical analysis.

References

- Abraham T.J. & Manley R. (1995) Luminous and non-luminous *Vibrio harveyi* associated with shell disease in cultured *Penaeus indicus*. *Journal of Aquaculture in the Tropics* **10**, 273–276.
- Aguado N. & Bashirullah A.K.M. (1996) Shell diseases in wild penaeid shrimps in eastern region of Venezuela. *Journal of Aquaculture and Aquatic Sciences* **8**, 3–6.
- Aiken D.E. (1980) Molting and growth. In: *The Biology and Management of Lobsters* (ed. by J.S. Cobb & B.F. Phillips), vol. 1, pp. 107–120. Academic Press, New York, NY.
- Chisholm J.R.S. & Smith V.J. (1995) Comparison of antibacterial activity in the hemocytes of different crustacean species. *Comparative Biochemistry and Physiology* **110A**, 39–45.
- Clare A.S. & Lumb G. (1994) Identification of haemocytes and their role in clotting in the blue crab, *Callinectes sapidus*. *Marine Biology* **118**, 601–610.
- Cook D.W. & Lofton S.R. (1973) Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab (*Callinectes sapidus*). *Journal of Wildlife Diseases* **9**, 154–159.
- Engel D.W. & Noga E.J. (1989) Shell disease in blue crabs of the Pamlico River. *Environs* **12**, 3–5.
- Fisher W.S. (1988) Shell disease of lobsters. *Developments in Aquaculture and Fisheries Science* **7**, 236–239.
- Gopalan U.K. & Young J.S. (1975) Incidence of shell disease in shrimps of the New York Bight. *Marine Pollution Bulletin* **6**, 149–153.
- Hameed A.S.S. (1994) Experimental transmission and histopathology of brown spot disease in shrimp (*Penaeus indicus*) and lobster (*Panulirus homarus*). *Journal of Aquaculture in the Tropics* **9**, 311–321.
- Hatai K. (1989) Fungal pathogens/parasites of aquatic animals. In: *Methods for the Microbiological Examination of Fish and Shellfish* (ed. by B. Austin & D.A. Austin), pp. 240–272. John Wiley and Sons, New York, NY.
- Johannson M.W. & Söderhall K. (1989) Cellular immunity in crustaceans and the proPO system. *Parasitology Today* **5**, 171–176.
- Johnson P.T. (1983) Diseases caused by viruses, bacteria, rickettsia, and fungi. In: *The Biology of Crustacea* (ed. by A.J. Provenzano), 6, pp. 1–78. Academic Press, New York, NY.
- Jones T.C. & Hunt R.D. (1983) *Veterinary Pathology*, 3rd edn. Lea and Febiger, Philadelphia, PE.
- Khoo L., Robinette D. & Noga E.J. (1999) Isolation of callinectin, an antibacterial peptide from blue crab (*Callinectes sapidus*) hemocytes. *Marine Biotechnology* **1**, 44–51.
- Lightner D.V., Redman R.M., Hasson K.W. & Pantoja C.R. (1995) Taura syndrome in *Penaeus vannamei* (Crustacea: Decapoda): gross signs, histopathology and ultrastructure. *Diseases of Aquatic Organisms* **21**, 53–59.
- McKenna S., Jansen M. & Pully M. (1988) Shell disease of blue crabs, *Callinectes sapidus*, in the Pamlico River, North Carolina. North Carolina Division of Marine Fisheries Special Scientific Report No. 51, Division of Marine Fisheries, Washington, NC, USA.
- Nimmo D.W.R., Lightner D.V. & Bahner L.H. (1977) Effects of cadmium on the shrimps, *Penaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris*. In: *Physiological Responses of Marine Biota to Pollutants* (ed. by F.J. Vernberg, A. Calabrese, F.P. Thurnberg & W.B. Vernberg), pp. 131–183. Academic Press, New York, NY.
- Noga E.J. (1991) Closing remarks. In: *Symposium on Shell Disease in Crustaceans* (ed. by C. J. Sindermann). *Journal of Shellfish Research* **10**, 505–506.
- Noga E.J., Arroll T.W. & Fan Z. (1996) Specificity and some physicochemical characteristics of the antibacterial activity from blue crab *Callinectes sapidus*. *Fish and Shellfish Immunology* **6**, 403–412.

- Noga E.J., Engel D.W., Arroll T.W., McKenna S. & Davidian M. (1994) Low serum antibacterial activity coincides with increased prevalence of shell disease in blue crabs *Callinectes sapidus*. *Diseases of Aquatic Organisms* **19**, 121–128.
- Noga E.J., Sawyer T.K. & Rodón-Naveira M. (1998) Disease processes and health assessment in blue crab fishery management. *Journal of Shellfish Research* **17**, 567–577.
- Prince D.L., Bayer R.C. & Loughlin M. (1993) Etiology and microscopy of shell disease in impounded American lobsters, *Homarus americanus*. *Bulletin of the Aquaculture Association of Canada* **93–94**, 87–89.
- Rosen B. (1967) Shell disease of the blue crab, *Callinectes sapidus*. *Journal of Invertebrate Pathology* **9**, 348–353.
- Rosen B. (1970) Shell disease of aquatic crustaceans. In: *A Symposium on Diseases of Fishes and Shellfishes*, pp. 409–415. Special Publication No. 5, American Fisheries Society, Washington, DC.
- Sandifer P.A. & Eldridge P.J. (1974) Observations on the incidence of shell disease in South Carolina blue crabs, *Callinectes sapidus*. In: *Proceedings of the Gulf Coast Regional Symposium on Diseases of Aquatic Animals* (ed. by R.L. Amorski, M.A. Hood & R.R. Miller), pp. 161–184. Publication No. LSU-SG-74-05, Louisiana State University, Baton Rouge, LA.
- Sindermann C.J. (1989) The shell disease syndrome in marine crustaceans. *NOAA Technical Memorandum MFS-F/NEC-64*.
- Sindermann C.J. (1990) *Principal Diseases of Marine Fish and Shellfish*, vol. 2. Academic Press, New York, NY, 521 p.
- Sindermann C.J. & Lightner D.F. (1988) *Disease Diagnosis and Control in North American Marine Aquaculture*, 2nd edn. Elsevier, New York, NY.
- Smith V.J., Swindlehurst R.J., Johnston P.A. & Vethaak A.D. (1995) Disturbance of host defence capability in the common shrimp, *Crangon crangon*, by exposure to harbour dredge spoils. *Aquatic Toxicology* **32**, 43–58.
- Smolowitz R.M., Bullis R.A. & Abt D.A. (1992) Pathologic cuticular changes of winter impoundment shell disease preceding and during intermolt in the American lobster, *Homarus americanus*. *Biological Bulletin* **183**, 99–112.
- Stevenson J.R. (1985) Dynamics of the integument. In: *The Biology of Crustacea* (ed. by D.E. Bliss & L.H. Mantel), vol. 9, pp. 1–42. Academic Press, New York, NY.
- Victor B. (1993) Responses of hemocytes and gill tissues to sublethal cadmium chloride poisoning in the crab *Parathelphusa hydrodromous* (Herbst). *Archives of Environmental Contamination and Toxicology* **24**, 432–439.
- Weinstein J.E., West T.L. & Bray J.T. (1992) Shell disease and metal content of blue crabs, *Callinectes sapidus*, from the Albemarle-Pamlico estuarine system. *Archives of Environmental Contamination and Toxicology* **23**, 355–362.
- Weis J.S., Cohen R. & Kwiatkowski J.K. (1987) Effects of diflubenzuron on limb regeneration and molting in the fiddler crab, *Uca pugnator*. *Aquatic Toxicology* **10**, 279–290.
- Young J.S. & Pearce J.B. (1975) Shell disease in crabs and lobsters from the New York Bight. *Marine Pollution Bulletin* **6**, 101–105.
- Zar J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey, 918 p.
- Ziskowski J., Spallone R., Kapareiko D., Robohm R., Calabrese A. & Pereira J. (1996) Shell disease in American lobster (*Homarus americanus*) in the offshore, northwest-Atlantic region around the 106-mile sewage-sludge disposal site. *Journal of Marine Environmental Engineering* **3**, 247–271.