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A new shell disease in the mud crab *Scylla serrata* from Port Curtis, Queensland (Australia)

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ABSTRACT: In 1994 a previously unreported rust spot shell disease was seen in mud crabs *Scylla serrata*—Forskal from Port Curtis, central Queensland, Australia. Of 673 crabs surveyed, 21.7% had shell lesions. Of these, 82.9% had rust spot lesions on the carapace. The majority of rust spot-affected crabs (78.8%) were female. Rust spot lesions were predominantly non-perforated (89.4%) and 54.8% were bilaterally symmetrical. There was also a gender difference in the areas of the carapace most commonly affected. The main histological features of the rust spot lesion included: a cavity in the upper endocuticle; indentation of the endocuticle below the cavity; remains of muscle attachment adhesive epithelium within the cavity; fibrous connective tissue between the damaged carapace and the attached muscle; and islands of endocuticle in this fibrous connective tissue. Histopathology of the internal organs failed to find evidence of an infectious or parasitic cause of the rust spot lesions. The cause(s) of the syndrome appear(s) to be non-infectious.

KEY WORDS: Shell disease · *Scylla serrata* · Cuticle · Crustacea · Decapoda · Mud crab · Carapace

INTRODUCTION

Commercial fishermen first noticed 'rust spot' shell lesions in the Portunid mud crab *Scylla serrata*—Forskal in Gladstone Harbour, Port Curtis, Queensland, Australia, in 1994. This previously unreported shell disease has the potential to damage the lucrative Queensland mud crab market worth approximately AUS $7 million yr^{-1} (Williams 2000). The irregularly shaped cuticular lesions commonly called 'rust spots' are confined mainly to the dorsal carapace and appear initially as well-circumscribed orange-coloured areas. Large proliferative calcified areas are present on the internal surface of the carapace adjacent to lesions. In advanced cases, the lesions may progress to penetrating wounds that expose the underlying soft tissues.

Shell disease has been reported in many crustaceans of economic importance (Sindermann 1989a), in association with a variety of environmental conditions (Noga 1991). The pathogenesis of shell disease is thought to be multifactorial and strongly influenced by mechanical damage to the epicuticular layer; chitinoclastic activities of invading bacteria (Cook & Lofton 1973, Baross et al. 1978, Malloy 1978) and fungi (Alderman 1981); and external factors, including water and soil contaminants, low dissolved oxygen and high nutrient loads (Young & Pearce 1975, Engel & Noga 1989, Sindermann 1989b). These processes have been reviewed by Sindermann (1989a).

The cuticle of normal intermoult crabs consists of an outer epicuticle, an exocuticle, an endocuticle and an epidermis (Johnson 1980). In previous reports of shell disease, the lesions frequently develop after a breach in the epicuticle has occurred and then progress to erosion or full cuticular ulceration (Sindermann 1989b). In contrast, we describe a shell disease of mud crabs in

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which the initial lesion appears in the endocuticle while the outer exocuticle and epicuticle remain intact. This communication records the prevalence of crabs with rust spot shell lesions and describes a grading system to document the type and pattern of carapace lesions. The histopathology of the rust spot shell lesions and of the internal organs of these crabs is also described.

MATERIALS AND METHODS

Crab survey. From October 1998 to April 1999, 673 crabs were collected from Gladstone Harbour on the Central Queensland coast (E 151° 15.00′, S 23° 50.00′) using baited standard mesh crab pots. Crabs were examined within 24 h of capture for the presence of cuticular lesions. Examination included sexing, measuring the carapace width (to the nearest 5 mm) using a standard plastic metric ruler and recording details of any carapace lesions. Although the crabs were not moult staged according to setal development (Aiken 1973), shell hardness was determined by sclerotization and loss of flexibility of the integument, as adapted by Heasman (1980). Previous examination of over 200 mud crabs had determined that rust spot lesions only occasionally involved the appendages, abdomen or ventral thorax, and so only dorsal carapace lesions were recorded in this survey. Lesions were graded according to size and colour and whether they had perforated the surface of the cuticle. The distribution of lesions was allocated to specific regions on the carapace (Fig. 1) to determine if there was any association between the area in which lesions occurred and the underlying soft tissues.

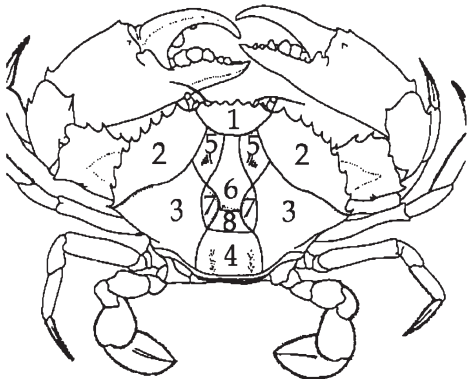


Fig. 1. *Scylla serrata.* The 8 areas of the carapace to which rust spot lesions were allocated

Classification of lesions. The grading system was as follows: Grade 1 = non-perforated cuticular lesion <5 mm; Grade 2 = non-perforated cuticular lesion ≥5 mm; Grade 3 = perforated cuticular lesion <5 mm; Grade 4 = perforated cuticular lesion ≥5 mm but <20 mm; Grade 5 = perforated cuticular lesion ≥20 mm. The Grade 1 and 2 lesions had a rust- or orange-coloured appearance similar to the colour of cooked crab shell (Fig. 2).

Histopathology. Sixty crabs with rust spot lesions were examined. Although animal ethics approval is not required at present for invertebrates, the crabs were always handled in a humane manner and were sedated in a cold room at 6°C for approximately 1 h prior to necropsy. Samples of the shell lesions, eyestalks, heart, gills, hepatopancreas, haematopoietic tissue, stomach, oesophagus, midgut, cerebral and thoracic ganglia, antennal gland, gonad, posterior midgut caecum, midgut ampulla and skeletal muscle were placed in fixative. The calcified tissues were placed in Davidson's solution and non-calcified tissues were placed in 10% formalin/seawater. The fixed and decalcified tissues were cut, blocked and stained with haematoxylin and eosin according to standard procedures. To establish whether the carapace lesions originated internally or externally, 5 µm serial sections of 8 non-perforated lesions (including small, medium and large lesions) were cut and every 15th section examined histologically. The tissues from a sample of 30 mud crabs without rust spot lesions and collected from throughout northern Australia were used as a reference.

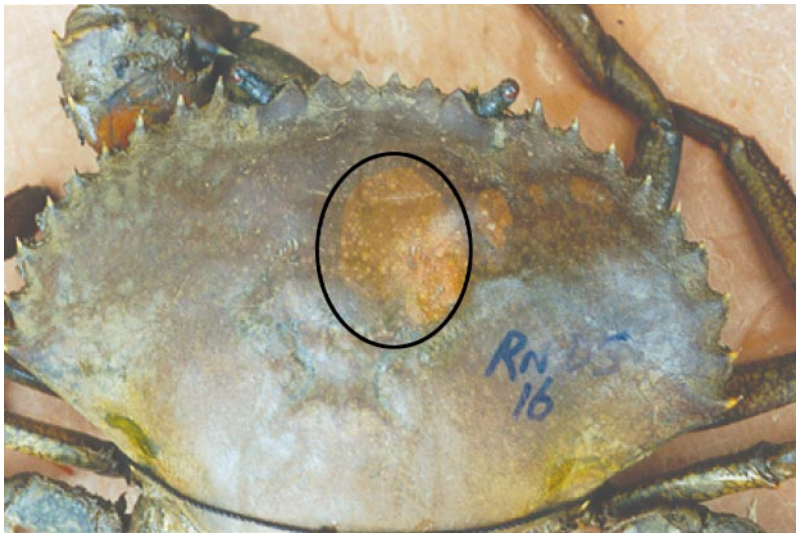


Fig. 2. *Scylla serrata.* Carapace of an adult female mud crab with typical Grade 2 rust spot lesions (circled). Carapace width is 180 mm

RESULTS

Survey of carapace lesions

Of the 673 crabs (size range 80 to 215 mm carapace width) examined, 146 (21.7%) had shell lesions. The average size of female crabs examined (174 mm) was significantly larger than males (153 mm) ($p < 0.01$, 2-sample *t*-test). The majority of crabs examined were semi-hard to hard shelled as determined by digital pressure on the thoracic sternites and subhepatic area. Of the crabs with shell lesions, 121 (82.9%) had rust spot lesions of the carapace. These crabs were predominantly female (78.8%) ($p < 0.01$, χ^2 test), and the majority of crabs (>69%) had more than one lesion. Of the rust spot lesions, 54.8% were bilaterally symmetrical but only 10.6% were perforated. The prevalence of rust spot lesions on each of the 8 areas of the carapace for both male and female crabs is presented in Figs. 3 & 4 respectively. The prevalence of rust spot lesions for male crabs ranged from 67.5% (area 7) to 0% (area 8), while that for female crabs ranged from 37.0% (area 5) to 0.9% (area 6); it differed significantly between sexes ($p < 0.01$, χ^2 test).

Histopathology of a typical carapace lesion

A majority of the features described in the following were seen in each rust spot lesion. An elongated cavity, parallel to the surface of the cuticle was present in the outer endocuticle adjacent to the exocuticle (Figs. 5 & 6). This cavity usually had a darkened edge and contained a pale-staining amorphous material. Within this cavity, the remains of tendinal cells (Johnson 1980) that facilitate attachment of the adjacent muscle bundle were occasionally present (Fig. 7). There were no foci of either bacteria or fungi. Proximal to the endocuticular cavity, the laminated endocuticle was usually indented (folded inwards) towards the muscle to which it had formerly attached. The muscle fibres adjacent to the attachment were replaced by fibrous connective tissue for a variable length from the point of attachment to the carapace. This fibrous tissue often contained foci of inflammatory cells. Islands of endocuticle-like material were often found within this connective tissue (Fig. 8).

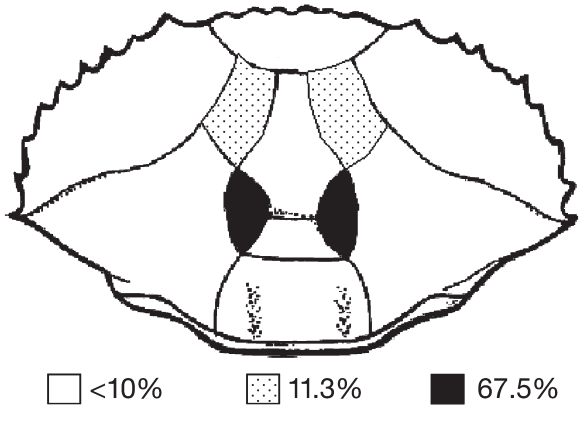


Fig. 3. *Scylla serrata.* Prevalence of rust spot lesions on each of the 8 areas of the carapace of male crabs

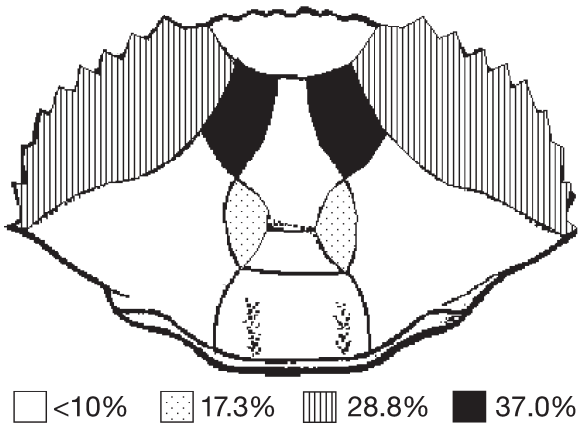


Fig. 4. *Scylla serrata.* Prevalence of rust spot lesions on each of the 8 areas of the carapace of female crabs

In addition to the above characteristics of a rust spot lesion, those in area 7 had some additional features (Fig. 9). The calcified bilateral internal cuticular partition, which separates the gill and heart chambers, was often damaged adjacent to its association with the internal surface of the dorsal carapace. The epidermis and adjacent membranous layer of the overlying carapace were often missing or had separated from the ventral surface of the carapace. The adjacent hypodermal tissue was frequently inflamed. An elongated cavity parallel to the surface of the carapace was frequently present in the endocuticle. The epidermal cells adjacent to the lesion were often hypertrophic. From an examination of a series of sections from 8 lesions from 4 crabs, there was no evidence that the rust spot lesions had originated from the exterior via an erosion of the epi- and exocuticular layers of the carapace.

Histopathology of internal organs

Of the 60 crabs with rust spot lesions chosen for histopathological examination, small granulomas or focal inflammation were seen in the following tissues (numbers of affected crabs in parenthesis): eyes (3), heart (19), gills (11), hepatopancreas (3), stomach (1), midgut/rectum (5), posterior caecum (2), antennal gland (1) and urinary bladder (2). Sloughed cells were

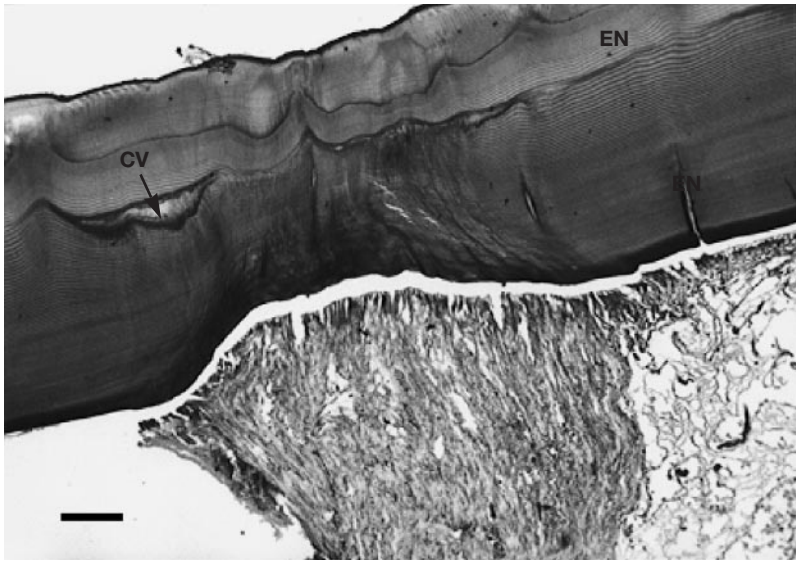
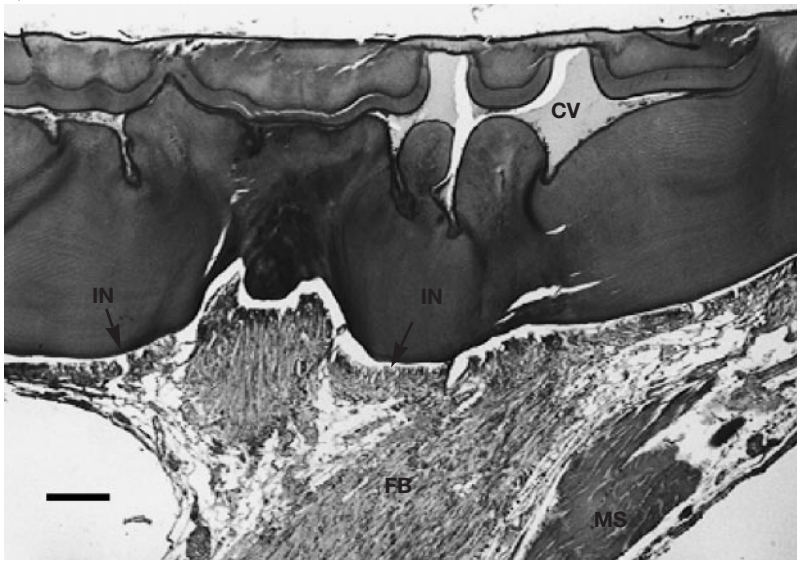
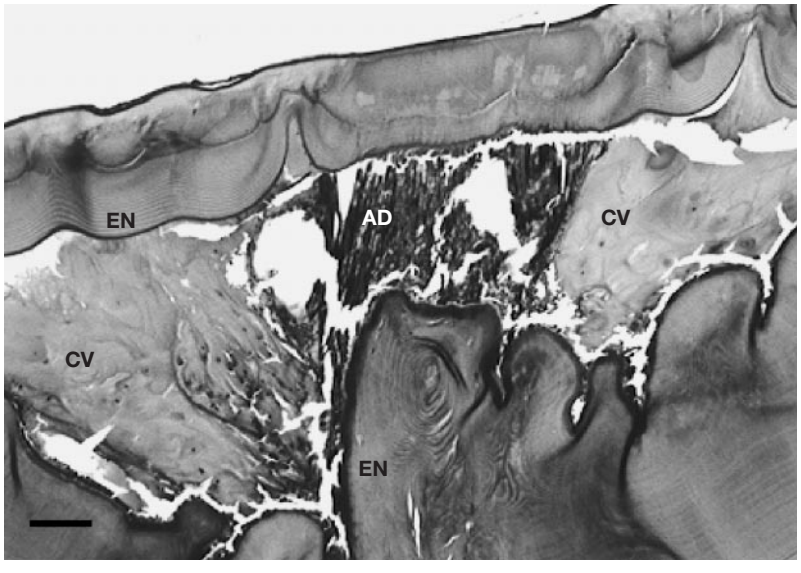


Fig. 5. *Scylla serrata.* A mild rust spot carapace lesion (area 2). There is a small cavity (CV) in the upper endocuticle (EN). Scale bar = 126 µm

Fig. 6. *Scylla serrata.* A severe rust spot carapace lesion (area 2). There is a large cavity (CV) in the upper endocuticle; the lower endocuticle is indented (IN). FB: fibrous connective tissue; MS: muscle tissue. Scale bar = 126 µm





seen in the lumen of the antennal gland (1); helminth parasites (intermediate stage of a tapeworm/fluke) were present in the connective tissue adjacent to the thoracic ganglion (11); baculovirus-like inclusions were present in the hepatopancreas (7); amorphous material was seen in the lumen of the antennal gland (19) and in the lumen of the urinary bladder (1). The reference group of 30 crabs had similar numbers of small granulomas, focal inflammation, baculovirus-like inclusions, helminth parasite infestations and amorphous material in the lumen of the tubules of the antennal gland to the rust spot crabs.

DISCUSSION

The pathology of non-perforated rust spot lesions in mud crabs harvested from Gladstone Harbour is restricted to the endocuticle and the adjacent muscle attachment. It is distinctly different to previously described shell disease

Fig. 7. *Scylla serrata.* A severe rust spot carapace lesion to show the remaining muscle adhesive epithelium (AD) present in the cavity (CV) in the upper endocuticle (EN). Scale bar = 126 µm

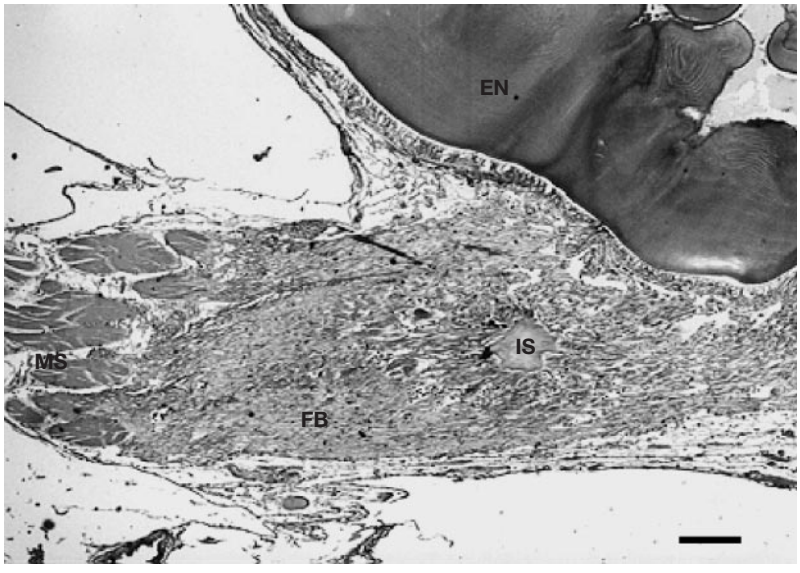


Fig. 8. *Scylla serrata.* An island of endocuticle (IS) is present in the fibrous connective tissue (FB) between the endocuticle (EN) and the attached muscle (MS) of a severe rust spot carapace lesion (area 2). Scale bar = 126 µm

(Rosen 1967, Baross et al. 1978, Morado et al. 1988, Comely & Ansell 1989, Smolowitz et al. 1992) in other Crustacea, where the initial action is an erosion of the exocuticle resulting in eventual exposure of the endocuticle to the external environment. Although some have suggested a possible systemic pathway for the initiation of shell disease (Comely & Ansell 1989, Smolowitz et al. 1992), a widely held view is that crustacean shell disease is caused by ubiquitous chitinolytic microorganisms acting alone or in concert (Sindermann 1989a). Others have suggested that an impaired host defence is an important contributing cause (Prince et al. 1993, Noga et al. 1994). Sequential sections of non-perforated rust spot lesions of mud crabs, however, have demonstrated that it is unlikely that chitinoclastic pathogens gain entry into the endocuticle via erosions of the epicuticle and exocuticle. Cuticular pores have also been suggested as portals of entry for bacteria and other micro-organisms in lobsters *Homarus americanus* (Prince et al. 1993). However, the lack of any foci of bacteria or fungi in our non-perforated rust spot lesions suggests a different aetiology. Furthermore, from an examination of the internal organs, the lack of any evidence of an infectious or parasitic agent being associated with the lesions suggests that the likely cause is non-infectious. Since the endocuticular layer is formed in the post moult phase of the moult cycle (Travis 1957), it would appear that the lesions occur due to a defect in the formation of this endocuticular layer rather than as a result of pathogenic cuticular erosion.

It is also noteworthy that 54.8% of the rust spot lesions were bilaterally symmetrical and were related to bilateral muscle insertions on the ventral surface of the carapace. Bilaterally symmetrical shell lesions have been reported by others. Bullis et al. (1988) suggested that symmetrical lesions in the deep-sea red crab *Geryon quinquedens* appeared to evolve as hyperpigmentation of the uniformly spaced microscopic sensory organelles located on the surface of the carapace. Borkowski & Bullis (1989) and Young (1991) observed symmetrical shell lesions in the crabs *Cancer borealis* and *Chaceon quinquedens* respectively but offered no explanation. Prince et al. (1993) suggested that invasion at the sites of setae and other sensory structures could explain the bilateral occurrence of shell lesions in the lobster *Homarus americanus*. However, the unique pathogenesis of our non-perforated rust spot lesions can suggest a reasonable explanation for this symmetry.

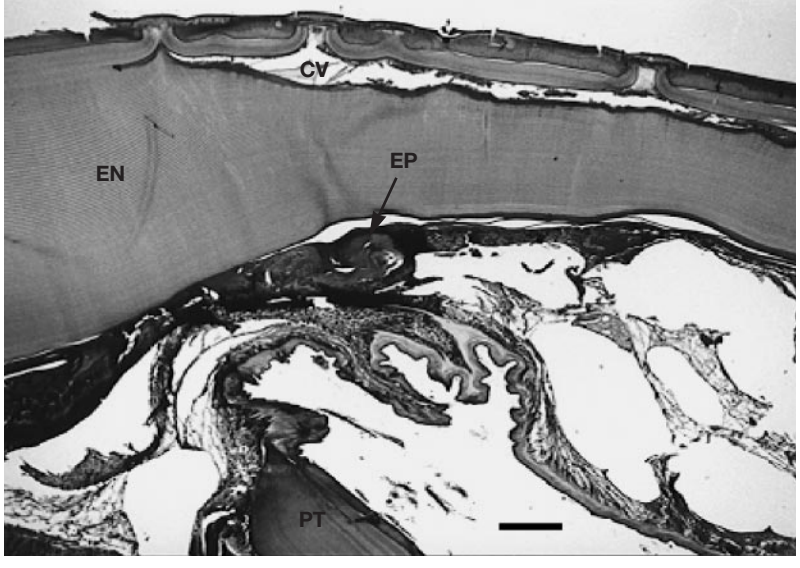


Fig. 9. *Scylla serrata.* A moderately sized cavity (CV) in the endocuticle (EN) of the carapace (area 7) above damaged epidermis (EP) and an internal cuticular partition (PT). Scale bar = 212 µm

We suggest the following hypothesis to explain the pathogenesis of rust spot lesions: At ecdysis, approximately 90% of the whole body calcium is lost with the exuviae (Scott-Fordsmand & Depledge 1997). After moulting when the endocuticle is being laid down, a rapid active uptake of calcium occurs from the environment (Greenaway 1985), allowing cuticular calcification to take place. Interference with this process of calcification could prevent the uncalcified endocuticle from attaining the necessary strength to support the forces exerted by the muscle insertions. Premature contraction of these muscles would cause either (1) the non-calcified endocuticle to rupture between the laminations which run parallel to the surface of the carapace or (2) the adhesive epithelium of the muscle attachment to tear away from the partially calcified endocuticle. These processes could produce a cavity in the endocuticle or one beneath the endocuticle, with some of the adhesive epithelial cells of the muscle attachment sometimes remaining in the latter cases. The dislocated epidermis would continue to produce more layers of endocuticle proximal to the cavity. Local repair mechanisms including inflammation and fibrous tissue replacement would occur in an attempt to heal the muscle adjacent to the endocuticle. Islands of endocuticle may be formed in this fibrous tissue from segments of torn and dislocated epidermis that have folded in on themselves and have continued to produce endocuticle. Once a cavity has formed in the endocuticle, or the epidermis has been pulled from the endocuticular surface, the pore canals containing cytoplasmic extensions of the epidermis, which extend into the overlying cuticular layers (Roer & Dillaman 1984), are severed. The cuticle exterior to the cavity 'dies' and assumes a rust-coloured appearance.

The high prevalence of rust spot lesions in females (78.8%) compared to that in males (21.2%) is of significance. Nevertheless, these differences must also be considered in conjunction with the prevalence patterns of carapace lesions for each sex, as these are also different. The lesions in area 7 differed histologically from the other areas. It is possible that the cause of lesions in this area also differs. Pressure on the epidermis of the dorsal carapace of area 7 from the impact of the internal cuticular partition beneath it could cause damage to this epidermis and subsequent rust spot lesions, again due to disruption of cytoplasmic extensions. In males, if area 7 lesions are disregarded because of a possible confounding effect of trauma in this area, 67.5% of rust spot lesions could be disregarded. This leaves only 1 carapace area (area 5) with a lesion prevalence of more than 10% in males. In contrast, if area 7 lesions are disregarded for the female crabs, areas 2 and 5 will still have an elevated prevalence of 28.8 and 37.0% respectively. This suggests that there may be at least 2 causes operating in the production of rust spot lesions: trauma together with 1 or more additional factors. The average size of female crabs examined was larger than that of males. These larger and therefore older female crabs (Heasman 1980) will have had longer exposure to the environment and various causal agents and this could be one explanation for the higher prevalence of lesions in this group. The stresses and demands of egg production in the post moult female could also account for gender difference in lesion prevalence. As the ovaries are internally concentrated beneath area 2, this could also account for the high prevalence of lesions in area 2 in females (28.8%) compared to males (5.0%). However, an understanding of the importance of this finding can only be made when the cause of the defects has been determined. Other researchers have reported gender differences in the prevalence of shell disease in crabs (Baross et al. 1978, Comely & Ansell 1989, Sawyer 1991). However, only Baross et al. (1978) have suggested a reason, namely that the increased prevalence in *Chionoecetes tanneri* adult females was due partly to females ceasing to moult after their pubertal moult. In contrast, however, the pubertal moult in the female mud crab is not necessarily the terminal moult (Heasman 1980). Further investigations into the prevalence of rust spot lesions in pre-adult and juvenile mud crabs may shed light on this phenomenon.

In conclusion, our findings indicate that rust spot lesions are part of a new type of shell disease with a unique histology compared to previously reported cases of shell disease. Although the epidemiology is still not understood, the possibility that the cause is environmental is the subject of further research.

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