Exoskeletal lesions in the male snow crab

Chionoecetes opilio (Brachyura: Majidae) in the southern Gulf of St. Lawrence

Khadra Benhalima, Mikio Moriyasu, Elmer Wade, and Marcel Hébert

Abstract: During population surveys of the snow crab, Chionoecetes opilio, conducted in the southern Gulf of St. Lawrence, the prevalence of males with multiple exoskeletal lesions was recorded. Histological, histopathological, and scanning electron microscope observations revealed that the progressive lesions of the chitinous exoskeleton, usually accompanied by blackening of the necrotic region, were caused by Gram-positive bacilli. Between 1993 and 1995, an average of 2.1% of males larger than 60 mm carapace width (CW) in the southern Gulf of St. Lawrence were infected by bacteria. Infected animals were found to be concentrated in an area with a high density of male crabs in the southern Gulf of St. Lawrence. Adolescent males of <60 mm CW bore a heavily infected exoskeleton less frequently than adult males, and no significant difference in the rate of molting failure was observed between infected and uninfected groups of adolescent males. There is no evidence that the incidence of bacterial lesions is increasing in the southern Gulf of St. Lawrence.

Résumé: Les relevés au chalut de la population du crabe des neiges (Chionoecetes opilio) dans le sud du golfe du Saint-Laurent ont mis en lumière la présence de lésions multiples sur la carapace des animaux. Les observations histologiques et histopathologiques et l’examen au microscope à balayage ont révélé que ces lésions progressives de la partie chitineuse de l’exosquelette étaient causées par des bactéries Gram positif et qu’il y avait noircissement des zones nécrosées. Entre 1993 et 1995, en moyenne de 2,1 % des mâles de taille supérieure à 60 mm (largeur de la carapace, LC) dans le sud du golfe du Saint-Laurent étaient infectés. Les animaux infectés étaient concentrés surtout dans les régions du golfe où la population de mâles était très dense. Les mâles adolescents de taille supérieure à 60 mm (LC) portaient moins de lésions graves que les adultes et aucune différence significative du taux de mortalité lors de la mue n’a été enregistrée entre les mâles adolescents infectés et sains. Rien ne nous permet de croire que la fréquence des infections bactériennes de l’exosquelette soit à la hausse dans le sud du golfe du Saint-Laurent.

Introduction

The snow crab Chionoecetes opilio is the most important commercially exploited crab species in eastern Canada. Recently this species has been exported mainly as frozen cooked or raw products with shell to Japan. The snow crab industry has reported a high prevalence of shell disease in commercial-size males (295 mm carapace width (CW)) in 1992, which could significantly lower the commercial value.

In natural populations, shell disease is frequently reported in various species of commercially important crustaceans, including brachyuran crabs: the blue crab, Callinectes sapidus (Rosen 1967, 1970; Overstreet and Cook 1972; Cook and Lofton 1973; Sandifer and Eldridge 1974; Iversen and Beardsley 1976); Dungeness crab, Cancer magister (Baross et al. 1978); shore crab, C. pagurus (Gordon 1966; Bakke 1973; Leglise 1976; Ayre and Edwards 1982); rock crab, C. irroratus (Sawyer 1982); Jonah crab, C. borealis (Haeffner 1977); stone crab, Menippe mercenaria (Iversen and Beardsley 1976); red crab, Chaceon quinquedens (Bullis et al. 1988); and golden crab, C. fenneri (Wennner et al. 1987). Although mortality of adult crabs attributed directly to shell disease is believed to be infrequent (Rosen 1970; Fisher et al. 1978), this phenomenon, if widespread, can affect the commercial value of exploited species (Edwards 1979). In addition, some authors have used measures of exoskeletal erosion and blackened gills as indicators of pollution (Sawyer et al. 1983; Young and Pearse 1975).

In the genus Chionoecetes, two types of shell disease are reported from the eastern Pacific: (1) “black mat syndrome” caused by the ascomycete fungus Trichomaris invadens (van Hyning and Scarborough 1973; Sparks and Hibbits 1979; Hibbits et al. 1981; Sparks 1982) and (2) “black spot” (“brown spot,” “rust spot,” or “burn spot”) caused by chitinolytic bacteria, mainly Vibrio spp. and Pseudomonas spp., but also luminescent Photobacterium spp. and Maraxella spp. (Baross et al. 1978). However, little information is available on shell disease in the snow crab in eastern Canada (Bower et al. 1994). This paper reports the prevalence of shell disease in male snow crabs over a 3-year period in the southern Gulf of St. Lawrence, and the results of microscopic and microbiological analyses of the exoskeletal lesions.

Materials and methods

Sample collection

A large-scale trawl survey has been conducted in the southern Gulf of St. Lawrence since 1989, and the prevalence of shell disease in male crabs was noted during the 1993–1995 surveys. The following measurements were collected from all crabs caught: carapace width
Fig. 1. Macroscopic and histological details of the exoskeleton of a snow crab (Chionoecetes opilio) infected by chitinolytic bacteria. (A) Early stage of infection, showing lesions on the exoskeleton as small brown circles with a white center (→). (B) Intermediate stage of infection, showing darkened spots (→). (C) Late stage of infection, showing the eroded and blackened center (→). (D) Light photomicrograph of a transverse section of the cuticle at the early stage of infection, showing the very thin epicuticular layer with perforations. (E) Light photomicrograph of a transverse section of the cuticle at the intermediate stage of infection; the epicuticular and exocuticular layers are damaged by the infection and the epithelial layer appears to be invaginated (→). (F) Light photomicrograph of a transverse section of the cuticle at the late stage of infection, showing severely blackened tissues. All cuticular layers are congested with infected tissue. A wide space separates the cuticle from the underlying epithelium (→) containing dense material and droplets. En, endocuticle; Ep, epicuticle; Epi, epithelium; Ex, exocuticle; Inf, infection; L, lesion; ML, membranous layer; Ct, connective tissue.

Table 1. Criteria for determining shell disease stages in the snow crab Chionoecetes opilio in the southern Gulf of St. Lawrence.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Degree of infection</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>Uninfected</td>
<td>Imperceptible</td>
<td>No visible spots</td>
</tr>
<tr>
<td>I</td>
<td>Slight</td>
<td>Small light patches</td>
</tr>
<tr>
<td>II</td>
<td>Moderate</td>
<td>Small patches forming a crater with a blackened center</td>
</tr>
<tr>
<td>III</td>
<td>Severe</td>
<td>Blackening necrosis present, infected areas cracked and (or) softened</td>
</tr>
</tbody>
</table>

(CW, mm), chela height (CH, mm), carapace rigidity, measured with a durometer (Foyle et al. 1989), carapace condition and the degree of infection (uninfected or slightly, moderately, or severely infected; Table 1). The terminology used for the maturity status of male crabs follows Sainte-Marie et al. (1995). Contingency table analysis was conducted to compare the rates of infection between adolescent and adult males in the same year. Effects were deemed significant at the P < 0.05 level. To elucidate the geographic distribution of infected animals larger than 60 mm CW in the southern Gulf of St. Lawrence, the data were compiled and analyzed by a geostatistical technique, kriging (Matheron 1970; Clarke 1979; Conan 1985; Moriyasu et al. 1998).

Male snow crabs were collected between July and October 1994 using a Bay of Biscay “Bigouden” Nephrops otter trawl (Conan et al. 1994) in the southern Gulf of St. Lawrence at depths ranging from 50 to 100 m. In total, 4005 male crabs ranging from 60 to 134 mm CW were obtained. Five specimens per degree of infection (a total of 20) were brought to the laboratory for histological, histopathological, and scanning electron microscope (SEM) observations.

Molting observations
During the molting season of adolescent males, between January and April, the rate of molting failure was observed in 79 infected adolescent males (61–125 mm CW) and 122 uninfected adolescent males (58–127 mm CW). Each premolting adolescent male was placed in a 60 × 60 × 45 cm compartment until the old carapace was completely shed. Natural seawater was continuously pumped from Baie des Chaleurs into the reservoir tank and then passed through a sand-filter tank, charcoal filter, and ultraviolet sterilizer before distribution to each aquarium. During the observation period, the water temperature varied between 0 and 3°C and salinity between 28.5 and 30 ppm. Crabs were fed smelt (Osmerus mordax) and pink shrimp (Pandalus borealis) twice a week. Crabs were kept in dim light and all observations were made under red light to minimize disturbance of molting activity. If an individual died during the process of molting, this was considered molting failure. Contingency analysis was conducted to compare the rates of molting failure between infected and uninfected groups. Effects were deemed significant at the P < 0.05 level.

Histological and histopathological observations
For light microscopy observations, an approximately 1 cm² section of infected exoskeleton were excised using sterile techniques and preserved in Bouin’s fixative solution for 48 h. Fixed tissue was dehydrated in a series of graded ethanol solutions (50, 70, 95, and 100%), cleared in xylene, then embedded using a paraffin wax vacuum chamber. The cuticle samples were not decalcified, therefore a longer embedding time (12–24 h) was required to allow complete penetration of paraffin wax into the sample tissue. Serial sections (5 μm thick) were mounted on glass slides and stained with modified Masson’s trichrome, a modification of Goldener (Martajo and Martajo-Pierson 1967), then dehydrated with ethanol and cleared with xylene. For microbiological studies, Gram’s stain was applied to fresh smears of the necrotic areas.

SEM observations
SEM observations were made on infected samples of exoskeleton excised from the animals by means of sterile techniques. Small pieces (1 cm²) were fixed for 1 h at 4°C in 5% cold glutaraldehyde (with 0.1 M cacodylate buffer at pH 7.2) and rinsed in cold 0.175 M cacodylate buffer at pH 7.2. Specimens were postfixed with 2% osmium tetroxide in 0.31 M cacodylate buffer at pH 7.2 for 1 h, then rinsed in the buffer described above. Following dehydration (10 min) through a graded ethanol series, the specimens were placed in a Polaron CPD750 critical-point dryer and infiltrated with liquid carbon dioxide for 1 h before being dried by passing through the critical point. The sections were mounted on scanning electron microscope stubs using silver paint as an adhesive. The stubs were coated under vacuum with a 10–15 nm thick layer of gold–palladium. The samples were viewed with a Model JEOL 1500 SEM at 15 kV acceleration voltage.

Results
Histological observations
In general, multiple lesions of the exoskeleton were most frequently located on the walking appendages. As the infection progressed, severe burn spots appeared on the exoskeleton, resulting in extensive damage to the infected areas. The infected areas were soft and easily broken.

In macroscopic and microscopic observations, the early stage of the infection (stage I; Table 1) was recognized as numerous punctiform necrotic spots (light brown) on the surface of the exoskeleton (Fig. 1A). The necrotic areas stained very densely with Masson’s trichrome and showed penetration of bacteria through the thin epicuticular layer (Fig. 1D). At the beginning of the infection, the epicuticle, usually smooth and continuous, was affected by a small perforation, and the center of the spots became friable (Fig. 1D). At the moderate–infection stage (stage II; Table 1), brownish necrotic lesions with a circular depressed center (red) were observed on the exoskeleton (Fig. 1B). The degradation progressed slowly through each cuticular layer, resulting in a dense necrotic lesion (Fig. 1E). The columnar epithelial cells below the infected cuticle became eroded and formed a depression...
Fig. 2. SEM observations of the exoskeleton of a snow crab (Chionoecetes opilio) infected by chitinolytic bacteria. (A) Scanning electron micrograph of the cuticular surface of a walking leg covered with debris and a cuticular lesion (stage I). (B) Scanning electron micrograph revealing the centrally aggregated crater of the cuticular lesion (stage II). (C) Scanning electron micrograph showing the crater of the cuticular lesion (stage III). (D) Scanning electron micrograph of the cuticular lesion, showing a morphologically homogeneous population of bacteria. (E) Scanning electron micrograph showing the vertical distribution of the bacterial colony around the crater area. (F) Scanning electron micrograph of the cuticular lesion, showing bacterial encrustation. B, bacteria; CL, cuticular lesions; Cr, crater; D, debris.

Table 2. Numbers of samples and lightly infected (stages I and II) and severely infected (stage III) male snow crabs (Chionoecetes opilio) observed during bottom-trawl surveys from 1993 to 1995 in the southern Gulf of St. Lawrence.

<table>
<thead>
<tr>
<th></th>
<th>1993</th>
<th>1994</th>
<th>1995</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lightly infected</td>
<td>90</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>Severely infected</td>
<td>17</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Uninfected</td>
<td>3638</td>
<td>4196</td>
<td>2894</td>
</tr>
<tr>
<td>Total no. examined</td>
<td>3745</td>
<td>4234</td>
<td>2906</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lightly infected</td>
<td>170</td>
<td>65</td>
<td>34</td>
</tr>
<tr>
<td>Severely infected</td>
<td>39</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Uninfected</td>
<td>4488</td>
<td>4805</td>
<td>3270</td>
</tr>
<tr>
<td>Total no. examined</td>
<td>4697</td>
<td>4884</td>
<td>3315</td>
</tr>
</tbody>
</table>

Note: For an explanation of stages see Table 1.

Discussion

Causative bacteria and infection

Five genera (Pseudomonas, Vibrio including Beneckea, Aeromonas, Spirillum, and Flavobacterium) of chitinolytic bacteria were reported in crustaceans with shell disease by Cook and Lofton (1973), Mallory (1978), and Lightner (1988). Hess (1937) reported shell disease in American lobsters caused by chitin-destroying Gram-negative bacilli similar to Bacillus chitinovorans Types II and XIV of Benton (1935). Baross et al. (1978) reported exoskeletal lesions in Tanner crabs (C. tanneri) caused by the luminous chitin-digesting bacteria Photobacterium sp., Maraxella sp., and a species similar to Vibrio anguillarum. Although the shell disease of snow crab reported here is caused by chitin-destroying Gram-positive bacilli, further identification of the species was not possible.

The uppermost layer, the epicuticle, is made up of lipoproteins rather than chitin, which offers some protection against chitin-destroying bacteria (Young 1989). However, physical damage to the epicuticle, such as that caused by the frequent discarding of soft-shelled crabs by commercial fishermen, may facilitate the entry of chitin-destroying bacteria into inner layers. Hess (1937), Sawyer and Taylor (1949), and Rosen (1967) reported that introduction of bacteria was unsuccessful if the epicuticle was left intact, therefore careful handling by fishers is suggested when returning undersized soft-shelled snow crabs to the sea, to reduce unnecessary cuticular damage. In addition, chitinolytic bacteria are most numerous in the topmost layers of mud (Zobell and Rittenberg 1938), therefore bottom-dwelling species that burrow into bottom sediment, such as the snow crab, are much more susceptible to infection by chitinolytic bacteria. Contrary to the observations of Hess (1937), the infection is not entirely external and can reach living tissues. However, this seems to occur only in terminal-molt crabs with old carapace corresponding to a carapace age of 5–6 years (Sainte-Marie and Dufour 1994). Mortality of snow crabs that was due directly to shell disease seemed to be...
Fig. 3. Geographic distribution of population density contours of infected male snow crabs (*Chionoecetes opilio*) larger than 60 mm CW in the southern Gulf of St. Lawrence between 1993 and 1995, calculated by means of kriging based on the bottom-trawl surveys.
ininfrequent, especially since the prevalence of infection in the survey was low.

In snow crabs, chitin destruction seemed to be much slower, more than 1 year for severe chitin infection, than is observed in American lobsters (Sindermann and Rosenfield 1967). Juvenile and adolescent crabs usually molt at least once a year, thus they may be able to shed an infected carapace before the infection becomes invasive, as has been observed in other crustaceans (McLeese 1965; Rosen 1970). The majority of newly molted crabs were free of clinical signs of shell disease, as was reported for *C. tanneri* (Baross et al. 1978). Terminally molted snow crabs (adult), however, show a higher prevalence of the infection because they do not shed the infected carapace. The same phenomenon was reported by Young (1989) for the larger red crab, which has a longer intermolt period (probably longer than 1 year).

**Prevalence of infection and distribution of infected male snow crabs**
The average percentage (2.1%) of infected males is much lower than that reported for red crabs, where it can be 86–92% (Young 1989). Baross et al. (1978) reported that the prevalence of infection in female Tanner crabs (76%) was higher than in males (29%).

The percentage of infected animals decreased between 1993 and 1995. Because the total abundance of adolescent and adult males in the southern Gulf of St. Lawrence has also been decreasing annually since 1993 (Anonymous 1997), the prevalence of infection may correspond to the density of male crabs in this area. High population densities encourage the spread of these infections (McLeese and Wilder 1964; Rosen 1970), especially for species with an aggregate distribution, such as snow crabs. Adolescent males appeared not to be severely affected by the infection, whereas terminally molted males seemed to be susceptible to severe infection.

The decrease in the prevalence of infection between 1993 and 1995, parallel to the decrease in male crab abundance, leads to the suggestion that the temporary increase in shell disease in the southern Gulf of St. Lawrence was associated with high crab abundance. This phenomenon, therefore, is not a significant threat to the snow crab industry in this area.

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