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OCCURRENCE OF SHELL DISEASE IN LOBSTERS, *HOMARUS GAMMARUS* (*L.*), IN THE SOUTHERN PART OF OSLOFJORD, NORWAY

By

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ABSTRACT

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Shell disease in a natural lobster population in Norway is described. The external signs were characterized by necrotic lesions of the exoskeleton, especially on the large chelae. Chitin-degrading bacteria were cultured from the necrotiv erosions.

INTRODUCTION

Exoskeleton lesions have frequently been observed on many different marine crustaceans, particularly on commercially, important neretic species such as the American lobster, *Homarus americanus* (Hess 1937; Rosen 1970; Young and Pearce 1975), the European lobster, *Homarus vulgaris* (Fisher 1977), the blue crab, *Callinectes sapidus* (Rosen 1967; Krantz, Colwell and Lovelace 1969; Cook and Lofton 1973), the king crab, *Paralithodes camtschatica* (Bright, Durham and Knudsen 1960), the tanner crab, *Chionoecetes tanneri* (Baross, Tester and Morita 1978), and various penaeid shrimps, *Penaeus spp.* (Cook and Lofton 1973). I n Norway this disease seems to be very frequent among the common edible crab, *Cancer pagurus*.

The gross signs of shell disease are similar in all species. The exoskeleton is pitted and marred with necrotic lesions, and although the disease is not immediately fatal, death may occur. Sawyer and Taylor (1949) reported that shell disease may also cause erosion of lobster gills, resulting in impaired gas exchange. The disease has been found to be contagious, especially when the lobsters are held in mass confinement. Lobsters may overcome minor cases of shell disease by molting (Mcleese 1965).

Most investigations have been carried out on adult lobsters, although larvae and post-larvae are also susceptible (Fisher, Rosemark, and Nilson 1976).

It is generally believed that chitin-digesting bacteria are the principal causative organisms of shell disease. Chitin-digesting *Vibrio spp.* (frequently called *Beneckea spp.)* have been successfully isolated from all marine crustacean exoskeleton lesions (HESS 1937; ROSEN 1967, 1970; COOK and LOFTON 1973, YOUNG and PEARCE 1975; MALLOY 1978). It is not precisely known what sequence of events leads to shell erosion; however, many investigators report that mechanical damage to the shell is the chief prerequisite to lesion formation (ROSEN 1970). High incidences of necrotic lesions have also been observed in lobsters and rock crabs collected in or near dumping grounds of sewage sludge (YOUNG and PEARCE 1975).

This paper reports the incidence of shell disease among adult European lobsters sampled over a four month period in 1979 in the area of the three small islands of Bolærne in the southern part of Norway.

In this work, believed to be first reported incidence of shell disease in a natural lobster population in Norway, the results of microscopic and microbiologial examinations of exoskeleton lesions are described.

MATERIAL AND METHODS

From August to November 1979 European lobsters *(Homarus gammarus (L.))* were collected by help of monofilament nets along the nearshore waters of the islands of Bolærne in he southern part of Oslofjord, Norway (Fig. 1). This area was selected as the area of study because our first cases of shell disease were received from this region. During the period 1959—1975 large quantities of sewage sludge were disposed two nautical miles north of these islands.

The lobsters were obtained alive and kept for a short time in wooden tanks three feet by six feet in seawater, before they were brought to the laboratory for examination.

Normal and diseased tissues were prepared for microscopic examination by fixation in 10% buffered formalin, decalified in 5 % nitric acid solution, embedded in paraffin, sectioned and stained with hemtoxylin and eosin (H & E).

Swabs of typical exoskeleton lesions were streaked on chitin agar (NEEDHAM 1978) which was incubated aerobically at 22°C for two weeks. Chitin utilization was indicated by clearing of the opaque medium around the colonies (LEAR 1963).

The salt requirement of isolates capable of utilizing chitin was determined on nutrient agar (Difco) with and without 3% NaCl. Other test wedia were made with 3% NaCl.

Cell shape and motility were determined on trypticase soy broth

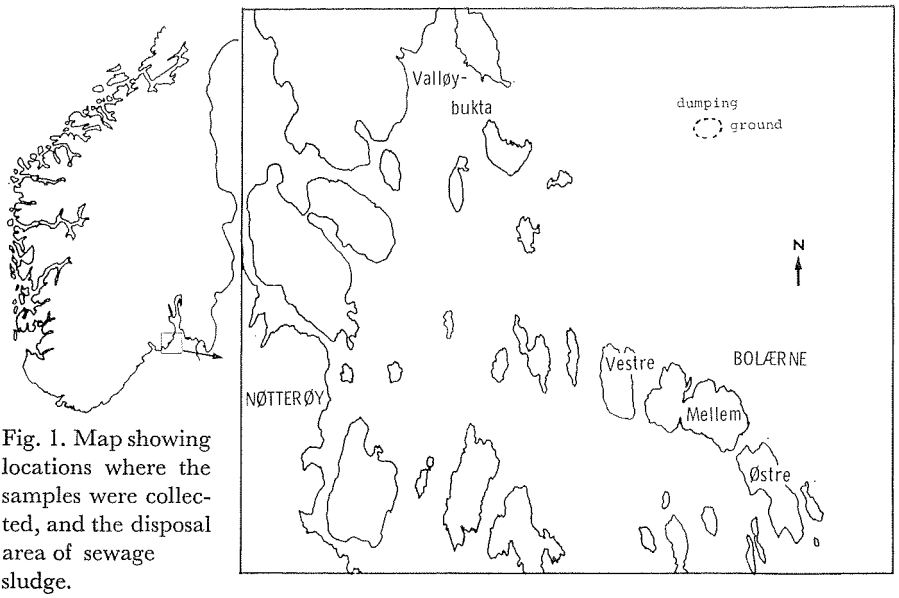

Fig. 1. Map showing locations where the samples were collected, and the disposal area of sewage sludge.

(Difco) cultures by phase contrast microscopy. Gram stains were performed on 24 hr trypticase soy agar (BBL) cultures. The type of flagellation was determined by electron microscopy of negatively stained preparations from 24 hr nutrient agar cultures.

The catalase and oxidase activity (SANDVIK 1972) was examined on nutrient agar.

Hemolytic activity was tested on blood agar containing 5% defibrinated goat blood.

For detecting indole production, chitinolytic isolates were grown on a broth containing 0.5% peptone, 0.1% yeast extract, 0.01% ferric phosphate and supplemented with 1% tryptone. For detection of nitrate reduction, this broth was used with 0.2% KNO_3 (MALLOY 1978).

Starch hydrolysis, gelantinase activity and casein hydrolysis were determined on nutrient agar containing 0.2% filter-sterilized starch, 3% gelatin or 30% skim milk, respectively (MALLOY 1978).

The medium of HUGH and LEIFSON (1953) was used to test the ability of the isolates to utilize glucose, sucrose and lactose.

Antibiotic sensitivity was tested on freshly-seeded trypticase soy agar plates with the following antibiotic discs: 10 I.U. Penicillin,* 100 μg Streptomycin,* 80 μg Tetracycline,* 100 μg Novobiocin,* and 0.1% vibriostat 0/129 (2,4 diamino — 6,7 di-isopropylpteridine).

An electrophoretic casein precipitation test (CPI-test) was performed

* A/S Rosco, 2630 Taastrup, Denmark.

as described by SANDVIK (1967), in order to reveal any relationship between extracellular proteinases of the isolates and those produced by *Vibrio anguillarum*.

Isolates capable of utilizing chitin were classified at the generic level with the schemees of SHEWAN *et al.* (1960), SKINNER and SHEWAN (1977), BAUMANN, HOBBS and HODGKISS (1971), and BERGEY'S MANUAL (1974).

RESULTS

From August to November 1979, 67 adult lobsters were examined. Four female and four male lobsters (12%) were affected and showed visible lesions on some part of the shell. Affected animals showed no clinical symptoms of disease, such as weakness or abnormal movements.

The external signs were characterized by medium to advanced necrotic lesions in the exoskeleton. In the early stages, the lesions appeared macroscopically as few to numerous punctiform dark brown to black crater-like erosions, especially on the ventral side of the large chelae (Fig. 2 a). These early stages were also present on the dorsal side of the large chelae and on the carapace and only one lobster showed typical small erosions scattered over the dorsal carapace. In later stages the marks joined to form large irregular areas with a deep necrotic center. Large necrotic erosions were especially found on the ventral side of the large chelae, where lesions up to 5 centimetres in diameter were seen (Fig. 2 b). All lesions were limited to the normal shell surface by darkly colored lines surrounding the necrotic areas. In these dry necroses, normal broken off material could be recognized.

Historically it could be seen that in the diseased areas all calcified layers of integument were attacked, and in severely eroded areas the calcified shell was completely dissolved. Penetration of the innermost layer of the shell (noncalcified endocuticle) was not observed, this dense tissue of the integument appearing to form a barrier to the diseased shell. The underlying muscle tissue was not attacked. In none of the affected animals were the gill or gill membranes injured. Microscopical examination of smears from the necrotic areas showed the presence of numerous motile and non-motile Gram negative rods.

Twelve chitinolytic isolates were obtained from different necrotic lesions. All isolates were relatively large, straight, Gram negative rods with polar flagella. On agar surface they grew with smooth, opaque, round, low convex, slightly cream coloured colonies, They all required NaCl supplement for growth. Concerning growth rate and biochemical properties there were some differences between the isolates. The general classification schemes divide the isolates into three groups: *Vibrio* spp.,

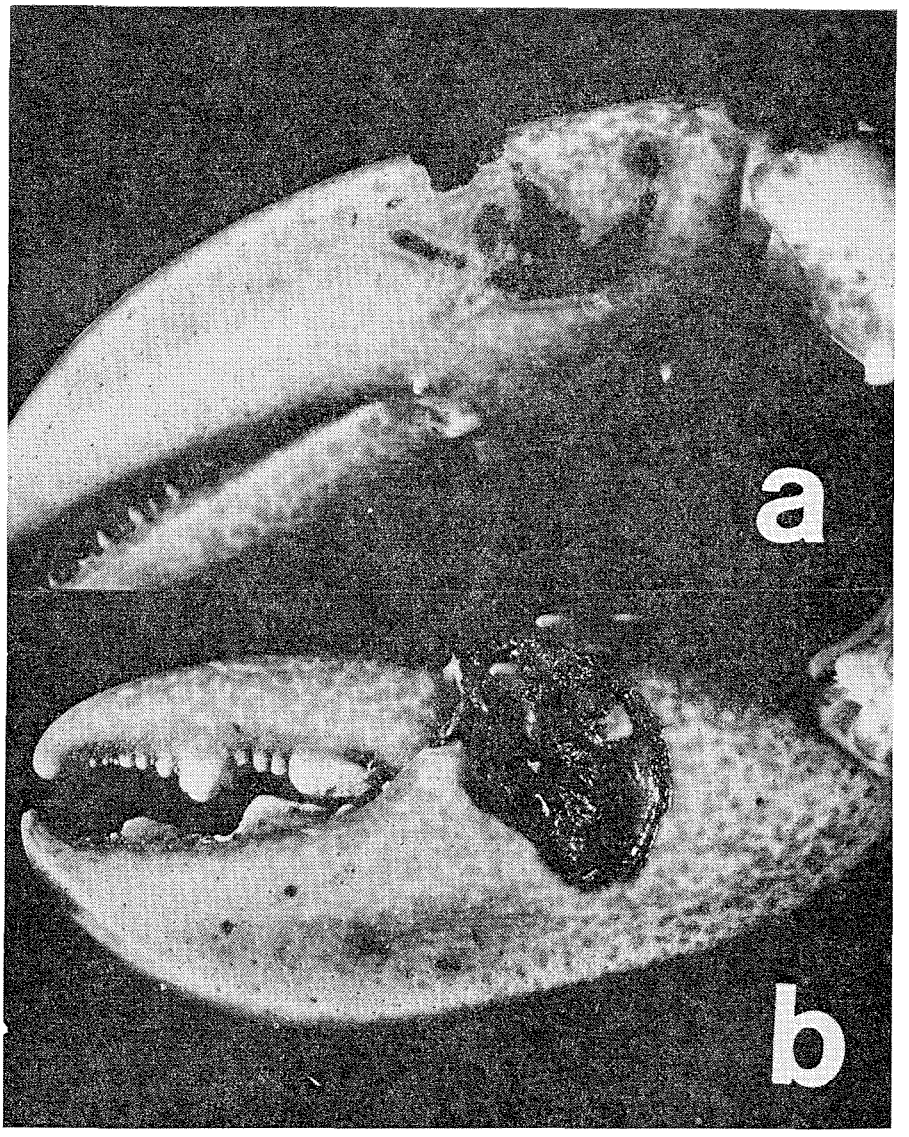

Fig. 2. Early stages (a) and later stages (b) of the shell disease on the ventral side of the large chelae.

Aeromonas-like bacteria, and *Pseudomonas*-like bacteria (Table 1). Two of the isolates (isolate nr. 1 and nr. 6) classified as *Vibrio* spp. gave positive CPI-reactions, showing an enzymo-serological relationship with *V. anguillarum*. In the other ten isolates the enzyme production was so weak that the test could not be performed.

In addition to the chitinoclasts, a varity of psychrophilic nonchitin digesters were isolated from lesions sampled. Fungi were not found.

Table 1. Properties of the chitinolytic isolates.

	Vibrio spp.						*Aeromonas*-like bacteria				*Pseudomonas*-like bacteria	
Isolate number	1	2	3	4	5	6	7	8	9	10	11	12
Gram reaction	—	—	—	—	—	—	—	—	—	—	—	—
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Polar flagella	+	+	+	+	+	+	+	+	+	+	+	+
Salt requirement	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	—
Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+
Hemolysis	+	+	+	+	—	+	+	+	—	—	—	—
Indole production	+	+	+	+	+	+	—	—	—	—	+	—
Nitrate reduction	+	+	+	+	+	+	+	+	+	—	+	+
Scarch hydrolysis	+	+	+	+	+	+	+	+	+	+	—	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	—
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	—
Ability to utilize Carbohydrates												
Glucose	F	F	F	F	F	F	F	F	F	F	0	0
Sucrose	+	+	—	—	+	—	+	+	+	+	—	—
Lactose	+	+	+	+	+	—	—	—	—	+	—	—
Antibiotic sensitivity												
Penicillin	+	+	+	+	+	+	+	—	+	+	—	—
Streptomycin	+	+	+	+	+	+	+	+	+	+	+	+
Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+
Novobiocin	+	+	+	+	+	+	+	+	+	—	+	+
Vibriostat o/129	+	+	+	+	+	+	—	—	—	—	+	+

+ = positive reaction
— = negative reaction
F = fermentative metabolism
0 = oxidative metabolism

DISCUSSION

The gross signs and microscopic findings of the shell erosions in the study corresponded well with documented descriptions of shell disease in lobster (Hess 1937; Rosen 1970; Young and Pearce 1975; Malloy 1978). In our material the highest incidence of necrotic erosions was found on the large chelae, whereas the prevalence of disease found by other workers (Hess 1937; Malloy 1978) seemed to be located especially on the carapace.

Eight of the 67 (12%) adult lobsters were attacked by shell disease.

Lobster shell disease appears to be quite rare in natural environments (HESS 1937). TAYLOR (1948) found 0.06% incidence of the diseace in a survey of Canadian lobster producing-centers. Compared to these observations, the frequency of the shell disease in the Bolærne area seems to be high. Relatively high incidences of shell disease have been reported from lobsters and rock crabs collected in or near dumping grounds of sewage sludge (YOUNG and PEARCE 1975). In the actual area the influence of pollution has not been documented, however only two nautical miles north of Bolærne large amounts of sewage sludge have been dumped from 1959—1975, and a connection between these disposals and the occurence of the disease cannot be excluded.

Twelve isolates of chitinolytic bacteria were collected for further examination from different necrotic erosions. Six of the isolates were found to belong to the genus *Vibrio*, four resembled *Aeromonas*, while two isolates fitted in the genus *Pseudomonas*, except that one of them showed negative catalase reaction ,and they were both sensitive to vibriostat. According to MALLOY (1978) vibriostate sensitivity does not exclude the diagnosis of *Pseudomonas*. In the taxonomic designations we have paid little attention to the origin of bacteria, or to their morphology.

There is considerable agreement among various investigators that the primary cause of shell disease is chitinoclastic bacteria which occur abundantly in the environment (HESS 1937; SAWYER and TAYLOR 1949; ROSEN 1967; BRIGHT 1960). MALLOY (1978) isolated chitin-degrading spescies of bacteria in the genera *Pseudomonas*, *Vibrio* and *Beneckea* from the lesions of lobsters with shell disease. He was able to reproduce the shell disease in experimental lobsters with a spescies of the genus *Vibrio (Beneckea)* when the integument had been damaged prior to inoculation. Until now no attempts to infect healthy lobsters with our isolates have been undertaken.

Further studies concerning development of the disease, mortalityrate and contagiousness of lobster shell disease in Norwegian waters are recommended.

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