

Black Mat Syndrome, an Invasive Mycotic Disease of the Tanner Crab, *Chionoecetes bairdi*

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Received December 18, 1978

Black mat syndrome, caused by an encrusting fungus on the exterior of the carapace of tanner crabs, has been known for many years. Although it is a nuisance when processing crabs for the commercial market, it has been thought to be restricted to the external surfaces of the crab and, therefore, nonpathogenic. In the present study, 11 tanner crabs from the Kodiak area of Alaska with and 9 without grossly recognizable masses of the fungus on the carapace were necropsied and examined histologically utilizing special stains selective for fungi. In all individuals with the syndrome, hyphae of the fungus, previously identified as *Phoma fimetari*, penetrated the carapace and virtually replaced the underlying epidermis. In the more advanced cases, the eyestalk was invaded and the epidermis destroyed, and hyphae extended into the eyestalk musculature and nervous tissue. To date, infections of the connective tissue sheaths surrounding the esophagus, stomach, heart, hemopoietic tissue, thoracic ganglion, antennal gland, and ovary have also been observed. None of the crabs without the syndrome contained internal hyphae. Although data on the lethality of the disease are not yet available, the ease with which the hyphae penetrate the chitinous exoskeleton, their extensive proliferation in the epidermis, and their ability to invade deep tissues causing obvious pathological effects, are highly suggestive that it is a virulent, probably fatal, disease that may have a significant impact on tanner crab population dynamics.

KEY WORDS: Marine mycosis; black mat syndrome; histopathology; tanner crab; *Phoma fimetari*; *Chionoecetes bairdi*.

INTRODUCTION

Chionoecetes bairdi is abundant in the Gulf of Alaska and the southeastern Bering Sea and has been the target for an important fishery since the late 1960s. The U.S. fishery has grown from 6800 pounds valued at \$680 to the fishermen in 1961 to over 94 million pounds worth more than \$50 million in 1977 (Anonymous, 1978). Biologists have traditionally used the common name "tanner crab" for *C. bairdi* and the other species in the genus, *C. tanneri* and *C. opilio*. When commercial utilization began, the industry first used the trade name "queen crab" for the product, but now prefers the name "snow crab" for both *C. bairdi* and *C. opilio*; the latter constitutes a minor portion of the catch. We prefer the more biologically traditional name, tanner crab.

Fishery biologists and commercial fishermen have been aware of an encrusting

fungus on the carapace of *C. bairdi* for many years, calling it "black mat disease" or, more correctly, "black mat syndrome." Because of the black pigment in the hyphae and fruiting bodies, it is a nuisance when fragments of the fungus are broken off and contaminate the meat during processing. Areas where crabs are known to have a high prevalence of fungal infection are sometimes avoided by crab fishermen.

It has long been thought that the fungus is restricted to the external surface of the carapace. In the only published study of the syndrome, Van Hyning and Scarborough (1973) identified the fungus as *Phoma fimetari*, noted that they never observed it in any of the internal tissues, and stated that it apparently does not have a deleterious effect on the crab. They did, however, postulate that "in an unusually extreme case, the eyes and mouth parts of females that have undergone their terminal molt could

be damaged and early death could result." A related species, *P. herbarum*, has been shown to invade visceral organs of hatchery-reared salmonid fishes (Ross et al., 1975). Van Hyning and Scarborough noted that the fungus appears to be both species specific and area specific. According to these authors, king crabs and Dungeness crabs in localities occupied by tanner crabs with heavy fungal infections are unaffected and the fungus has not been reported on either *C. opilio* or *C. tanneri* from the Pacific Northwest, Japan, or the Canadian Atlantic.

We recently had the opportunity to study a number of tanner crabs, both with and without fungus encrustations, from the Kodiak area of Alaska. Our primary purpose was to determine, by the use of special stains selective for fungi, whether the fungus is, indeed, present only on the surface of the carapace or if hyphae of the fungus penetrate the exoskeleton and invade underlying soft tissue.

MATERIALS AND METHODS

Crabs were collected by crab pot, try net, and otter trawl in Kalsin Bay, Kodiak Island, and in Izhut Bay, Afognak Island. Individuals selected for necropsy were rapidly bled by removal of all legs; portions of the carapace were removed to expose the underlying soft parts; and small random samples of a number of organs (gill, hepatopancreas, ovary or testis and vas deferens, epidermis, heart, bladder, hemopoietic tissue, cardiac stomach, esophagus, anterior and posterior caecum, pyloric stomach, midgut and ampulla, antennal gland, mandibular organ, brain, and thoracic ganglion) were excised and fixed in Helly's or Davidson's fixative. The entire eyestalk was also removed as were affected portions of the carapace of some crabs with the underlying epidermis still attached, fixed in Helly's, and subsequently decalcified in Davidson's.

Upon arrival at the National Marine Fisheries Service laboratory in Mukilteo, Washington, the tissues were dehydrated

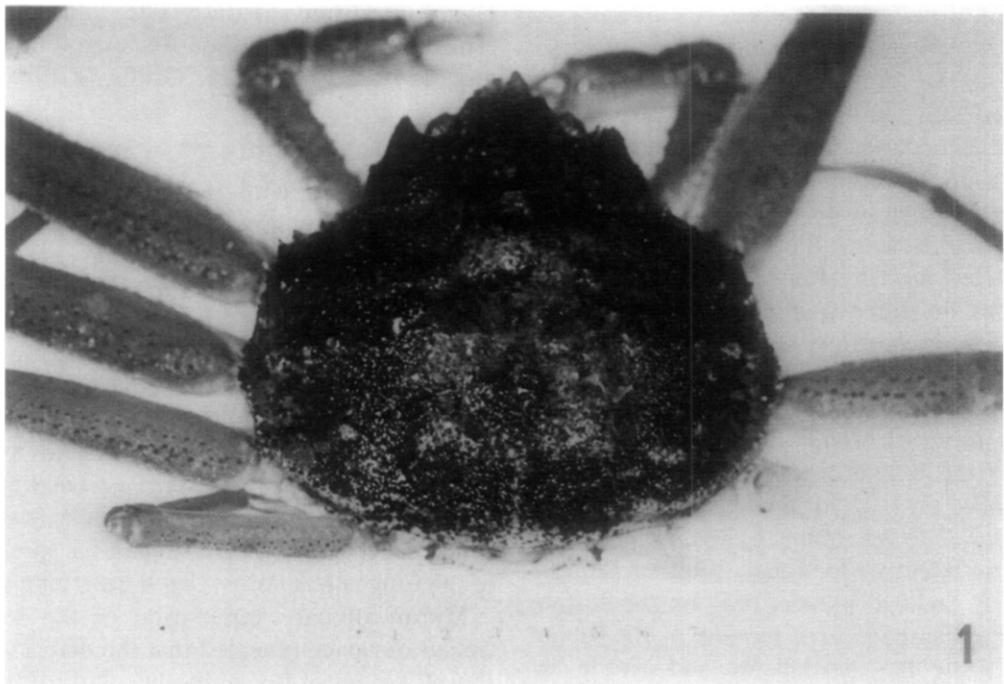
and blocked by standard methods, sectioned at 6–10 μm , and stained with Harris-modified hematoxylin and eosin (H&E), Grocott's method for fungi (GMS), and, in some instances, Gridley's fungus stain.

RESULTS

Tanner crabs with the syndrome were easily recognized grossly by the black mass of encrusting fungus (Fig. 1) on the external surface of the carapace. The encrustations appeared to begin as small discrete black spots on the dorsal cephalothorax and then to enlarge and coalesce to cover much of the dorsal aspect of the cephalothorax, eventually spreading to the appendages and the ventral side of the crab. Encrustations of the eyestalk apparently begin early in the syndrome but the exact sequential spread of the fungus has not yet been determined.

Microscopical examination of the sectioned carapace revealed that the mats consist of a mass of fungal hyphae and fruiting bodies (Fig. 2). In H&E preparations, the surface-encrusting fungi were well stained but even in heavy infections, hyphae in internal tissues were seldom apparent. Also, internal hyphae were nonpigmented and, therefore, not grossly recognizable. Gridley's fungus stain preparations were not particularly helpful, but were of some value in studying the fruiting bodies on the surface.

However, GMS preparations clearly revealed that fungal hyphae readily perforated the thick chitinous carapace, invaded the underlying epidermis, and proliferated laterally, replacing virtually all epidermal tissues (Fig. 3). The chitinous covering of the eyestalk was also penetrated by hyphae (Fig. 4) which proliferated by elongation and branching to essentially destroy the entire eye stalk epidermis. As growth of the fungus continued, eyestalk muscles were invaded with resultant swelling, disorganization, and destruction (Fig. 5). Hemal spaces and nervous elements of the eye were also invaded (Fig. 6), but no frank pathological effects were observed.



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FIG. 1. Gross appearance of tanner crab (*Chionoecetes bairdi*) with heavy encrustation of black mat syndrome. $\times 23$.

FIG. 2. Cross section of hyphae and fruiting bodies on surface of carapace. GMS. $\times 100$.

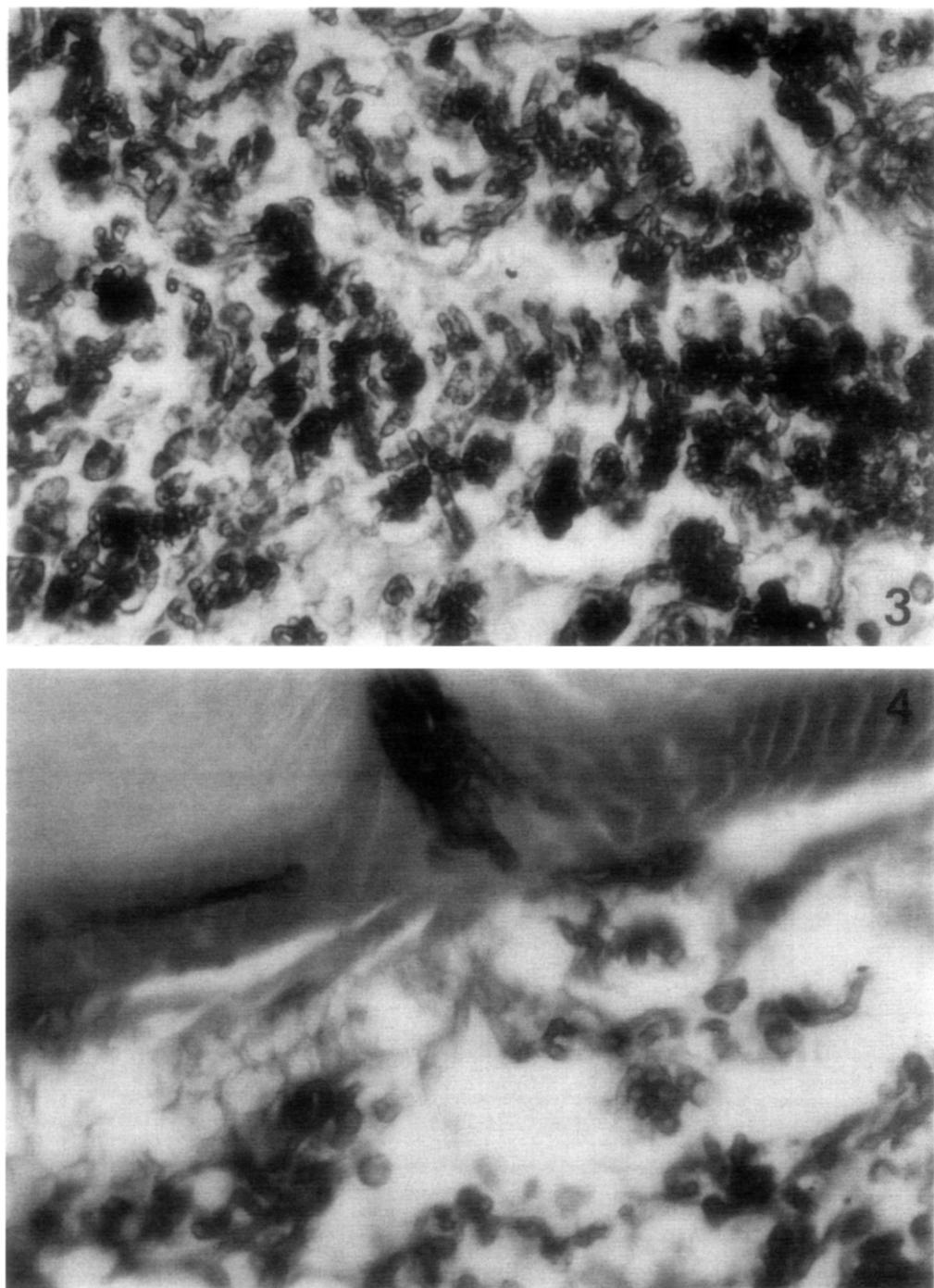


FIG. 3. Hyphae in epidermis of cephalothorax. Note that most of the epidermal tissue is replaced by masses of fungal hyphae. GMS. $\times 620$.

FIG. 4. Hyphae penetrating the chitinous covering of cystalk and proliferating in epidermis. GMS. $\times 620$.

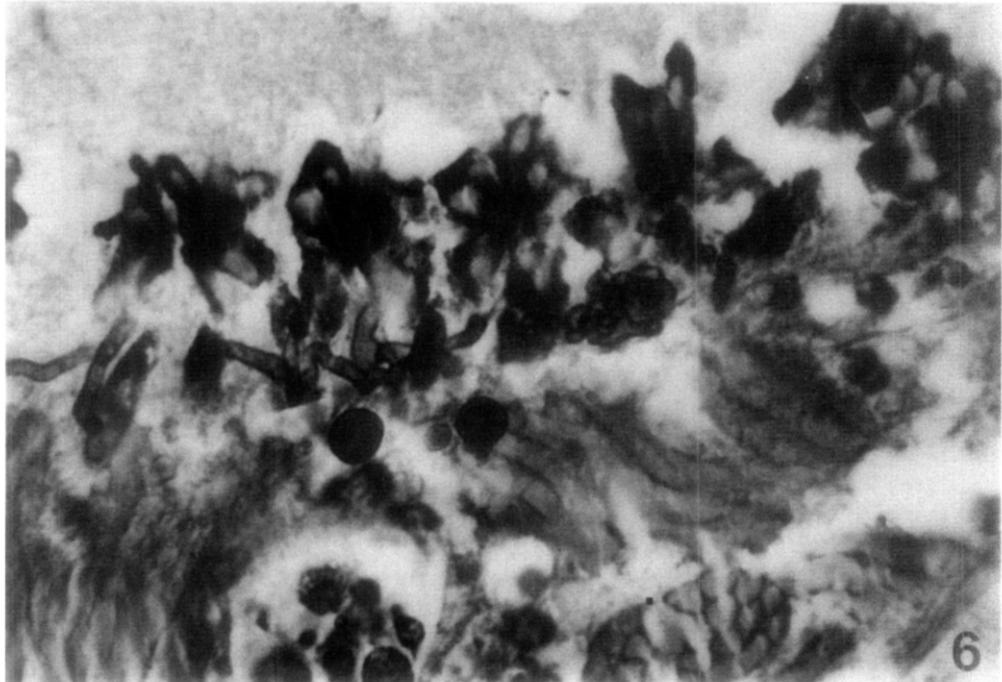
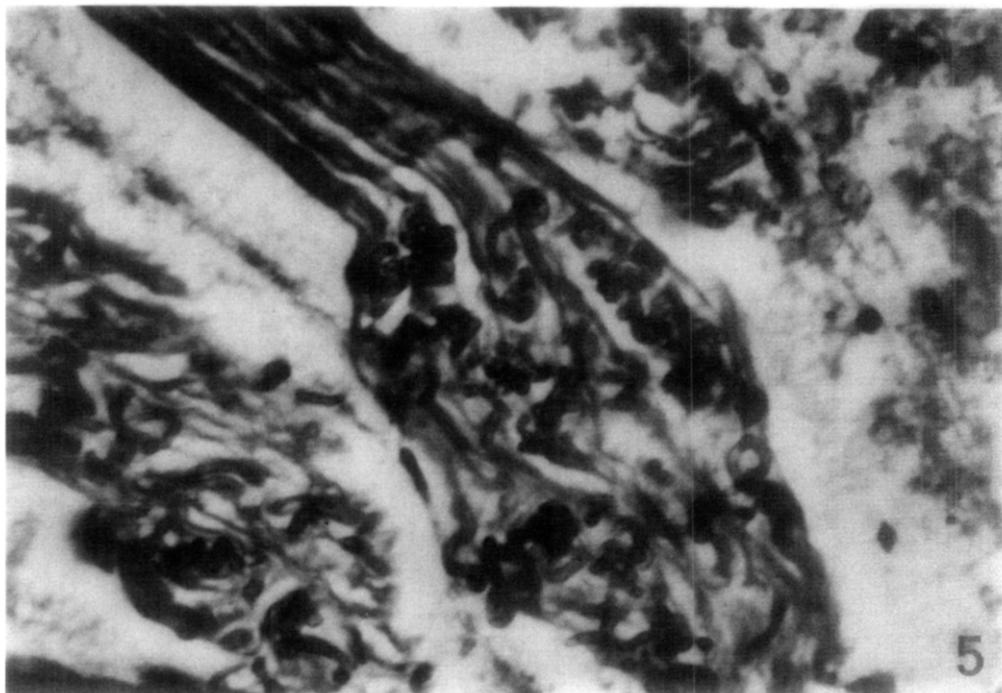


FIG. 5. Invasion of eyestalk musculature and proliferation by hyphae. Note the swelling and disorganization of the invaded portion of the muscle bundle in the center and the destruction of the muscle bundle at left. GMS. $\times 620$.

FIG. 6. Invasion of basement membrane of the eye. Note the rhabdomeres just above the hyphae. GMS. $\times 620$.

In the 11 cases studied to date, deep internal tissues associated with a number of vital organs were invaded (Table 1), including the connective tissue sheaths of the esophagus, stomach, heart, thoracic ganglion, hemopoietic tissue, ovary, hepatopancreas, and antennal gland. By contrast, careful study of GMS preparations of tissues from the nine crabs without grossly detectable black encrustations on the exoskeleton revealed no internal hyphae.

In both the surface encrustations and internal tissues, the hyphae were sparingly septate and sparingly branched. They grew tortuously and, especially in internal tissues, frequently consisted of tight bundles. In addition to being nonpigmented, hyphae in internal infections were about half the diameter of those on the surface of the exoskeleton (approximately 2.3 to 2.6 μm compared to 5.2 to 5.5 μm). Fruiting bodies were confined to the surface mats and no evidence of reproduction other than proliferative growth and branching of the hyphae was observed in internal tissues.

DISCUSSION

It seems evident that the invasive nature of this fungus disease has not been recog-

nized previously because of the nonpigmented condition of the tissue-invading hyphae and the lack of recognizable gross pathology. Indeed, the inadequacy of H&E and even Gridley's fungus stain in demonstrating the hyphae in tissue sections could have prevented such discovery in routine histological studies.

Van Hyning and Scarborough (1973) cultured the fungus and identified it as *Phoma fimeiti*, a soilborne fungus originally described from sheep dung (Dorenbosch, 1970). Its identification was confirmed by the Central Bureau for Schimmel Cultures, Baarn, Netherlands. We have not yet cultured the organism and can neither positively confirm nor refute its identification. There are, however, a number of factors that raise questions as to the probability of *Phoma fimeiti* being the cause of the disease: (1) its presence in marine waters with little adjacent land mass; (2) the reported host specificity; and (3) the fruiting bodies and spores in sections of the carapace show little similarity in the figures and description of *Phoma fimeiti* provided by Dorenbosch (1970).

The diminution in size of the internal hyphae is interesting, but not unique. Mohamed et al. (1978), for example, re-

TABLE 1
INTERNAL INFECTIONS OF TANNER CRABS WITH EXTERNAL ENCRUSTACEANS

Case No.	Degree of encrustation	Cephalothorax epidermis	Eye stalk epidermis	Eye stalk musculature	Eye stalk nerve tissue	Other organs, connective tissue
78-61	Moderate	+	+	+	+	-
78-63	Moderate	+	+	+	+	Antennal gland
78-66	Moderate	+	+	+	+	Antennal gland
78-67	Moderate	+	+	-	-	-
78-68	Light	+	+	-	-	-
78-71	Heavy	+	+	-	-	-
78-76	Heavy	+	+	-	?	Esophagus
78-77	Moderate	+	+	+	?	Esophagus, heart
78-81	Heavy	+	+	+	+	Thoracic ganglion, hemopoietic tissue
78-82	Heavy	+	+	+	+	Hepatopancreas, ovary
78-83	Very light	+	?	?	?	Ovary

^a + = Present; - = absent; ? = eye stalk not taken at necropsy or too fragmented for diagnosis.

ported that the hyphae of *Nomuraea rileyi* varied in size according to the organ of larvae of *Heliothis zea* in which they occurred.

Based on the presence of internal infections in all crabs we have examined with the syndrome and the absence internally in all cases without recognizable encrustations, it is probable that the mycosis develops by some stage of the fungus first settling on the exoskeleton and then, while expanding laterally, sending hyphae through the exoskeleton to infect and proliferate in internal tissues. The possibility remains, however, that the infective stage first penetrates, then proliferates in internal tissue and subsequently repenetrates the exoskeleton to produce the external fruiting bodies and, ultimately, spores in the manner common to many fungal parasites of insects and crustaceans, e.g., *Aphanomyces* in crayfish, *Lagenidium* in larval shrimp.

Speculation on the pathogenicity and, particularly, the potential lethality of this mycosis is possibly premature at this time. However, the ease with which it penetrates the exoskeleton, its extensive proliferation and known invasion of many internal tissues, and its demonstrated pathogenic effect on such tissues encourages the assumption that it is a serious and perhaps virulent disease. It is unlikely that crabs with all or much of the epidermis destroyed can successfully molt. We also believe there is a high probability that function of the eye would eventually be impaired or lost.

Because of the absence of gross lesions, portions of internal organs removed during necropsy for subsequent histological study were small random samples of the organ. Therefore, the demonstration of the fungus in a number of organ systems is suggestive of a systemic infection rather than the result of serendipitously selecting several foci of infections.

Nevertheless, we have not observed invasion of the functional portions of any organ, other than the epidermis and the

eye. It may be that the hyphae invade only relatively loose connective tissue deep in the cephalothorax, but, in view of the growth pattern in the exoskeleton and eyestalk, this seems unlikely. It appears more likely that we have not yet examined terminal cases in which the mycosis is truly systemic. Because of fishery regulations tanner crabs in the commercial fishery are taken only by crab pots. Moribund crabs would be unlikely to enter the pots and those reaching the moribund state within the pot would probably be consumed by their fellow prisoners. Likewise, dead or moribund crabs on the open bottom would be quickly consumed by the numerous predators and scavengers present in the highly diversified, rich biomass in which Alaskan tanner crabs occur.

It is obvious that much more research on this disease is needed, both in the field and, especially, in the laboratory. Certain areas in the Kodiak Island and Shumagin Island regions have high incidences of grossly recognizable black mat syndrome, as Van Hyning and Scarborough (1973) and Donaldson and Dick (1977) have noted (ranging from 7 to 75%). If, as we think possible, additional research shows it to be a fatal disease, it may prove to be a significant factor in tanner crab population dynamics.

ACKNOWLEDGMENTS

This study was supported by the Bureau of Land Management through interagency agreement with the National Oceanic and Atmospheric Administration, under which a multiyear program responding to needs of petroleum development of the Alaskan Continental Shelf is managed by the Outer Continental Shelf Environmental Assessment Program (OCSEAP) Office.

We would like to thank the following individuals for their contributions in the preparation of the manuscript: Dr. Bruce B. McCain provided the original photograph of the encrusted tanner crab; Mr. Steven E. Hughes and Dr. Michael C. Mix critically read the manuscript and contributed valuable suggestions for its improvement; and Mrs. Coleen M. Annis prepared the manuscript.

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