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Observations on the Occurrence of *Hematodinium* sp. (Dinoflagellata: Syndinidae), the Causative Agent of Bitter Crab Disease in Newfoundland Snow Crab (*Chionoecetes opilio*)

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We describe the first occurrence in Atlantic Canada waters of "bitter crab disease" in the snow crab, *Chionoecetes opilio*. The condition is caused by a parasitic dinoflagellate, *Hematodinium* sp. Male crabs held in captivity harbored vegetative, prespore, and dinospore stages. Incidence of the disease was low (=<0.11%) off the eastern and northeastern coasts of Newfoundland in 1992–1993, except in Conception Bay, where 3.7% of 135 crabs examined were infected. The significance of *Hematodinium* sp. to snow crab populations in Newfoundland and to the commercial fishery is not yet clear, but it is prudent to monitor this parasite which has caused local depletion of Tanner crab populations in Alaska.

KEY WORDS: ***Hematodinium;* bitter crab disease; *Chionoecetes opilio;* snow crab; commercial fishery.**

INTRODUCTION

The snow crab (*Chionoecetes opilio*) has supported an intensive commercial fishery off the east coast of Newfoundland since 1968. Although snow crabs harbor assorted parasites (Brattey *et al.*, 1985), until recently, they have been virtually free of problems associated with naturally occurring pathogens or parasites. In comparison, the Alaskan fishery for the congener of *C. opilio,* the Tanner crab *Chionoecetes bairdi,* has been severely affected by a high incidence of bitter crab disease (BCD), a syndrome caused by a parasitic dinoflagellate, *Hematodinium* sp. found in the hemolymph of infected crabs (Meyers *et al.*, 1987).

Since 1985 *Hematodinium* sp. has become an increasingly important pathogen in Alaskan tanner crab populations (Meyers *et al.*, 1987; Eaton *et al.*, 1991; Love *et al.*, 1993). In its advanced stages, the disease is easily recognizable by the "cooked" appearance of the affected crabs. The ventral sides of the limbs are an opaque, bright, white color, rather than the normal translucent whitish-gray color. Dorsally, the carapace is slightly pinkish as opposed to the normal orange–tan color. Diseased crabs display signs of acute morbidity, such as drooping limbs and mouthparts. The hemolymph is milky white in color, rather than the normal translucent light gray. Meyers *et al.* (1987) report that the meat of infected crabs is chalky in texture and bitter to the taste, rendering blocks of frozen meat containing infected flesh unsuitable for market. The taste of the meat from crabs in the late stages of the disease is so distinctive that Meyers *et al.* (1990) consider it a diagnostic tool in determining the occurrence of the disease in Alaska.

We first discovered snow crabs similar in appearance to Alaskan crabs with BCD in Newfoundland Canada waters in 1990 during the annual assessment survey by the Department of Fisheries and Oceans (DFO) in Bonavista Bay on the east coast of Newfoundland (Fig. 1). Diseased crabs have subsequently been recovered by crab fishermen from other commercial crab fishing grounds off coastal Newfoundland and by DFO research staff conducting commercial sampling at processing plant holding facilities. The purpose of this paper is to report our observations on the occurrence and incidence of bitter crab disease in *C. opilio* in Newfoundland waters.

MATERIALS AND METHODS

Crabs from Newfoundland sampled during research cruises were captured exclusively by standard commercial crab traps set in long-line fleets of 12 and baited with short-finned squid (*Illex illecebrosus*) and Atlantic mackerel (*Scomber scombrus*). Sample sites occupied during the research cruises were selected randomly and stratified by depth. Because female crabs have a patchy distribution, are too small to be retained by traps using commercial-sized mesh, and are not fished commercially, we examined male crabs only. Carapace width was measured to the nearest 1.0 mm and shell condition was classified according to the criteria of Taylor *et al.* (1989). Crabs infected with bitter crab disease were discovered during this examination. Identification was based on the "cooked" appearance of

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the appendages and milky white hemolymph. The condition was subsequently confirmed by microscopic examination. In addition to screening for BCD during research cruises, research staff examined snow crab from live-holding facilities of processing plants as a part of their regular commercial sampling procedure.

Animals collected during research cruises and exhibiting the symptoms of BCD were held in running seawater cooled to 1°C (approximately 2°C above ambient). After a 2-day acclimation period, 3 cc of hemolymph was removed by syringe and 10–12 wet-mounts were made and examined by phase microscopy at 400× magnification. Detailed examination of hemolymph was accomplished by air-drying slides at room temperature, incubating them at 60°C for 30 min, and then staining them with Giemsa's stain (5 to 95 dilution with phosphate buffer, pH 7.6). For the preparation of transmission and scanning electron micrographs of *Hematodinium* sp. hemolymph was preserved in Karnovsky fixative (prepared with seawater) at 4°C for 24–48 hr. Samples were postfixed in 1% osmium tetroxide in sodium cacodylate buffer and stored in cacodylate buffer. Each sample was prepared for both transmission and scanning electron microscopy. Samples for transmission electron microscopy were stained *en bloc* with saturated aqueous uranyl acetate, dehydrated, and then infiltrated with spurr resin. Thin sections were stained with Reynold's lead citrate and examined in a Phillips 300 TEM using an accelerating voltage of 60 kV. Scanning electron microscopy samples were filtered onto an Anpore 0.2-μm membrane filter disc mounted on an aluminum stub, sputter coated with gold, and then viewed in an Hitachi S570 scanning electron microscope with an accelerating voltage of 20 kV. Images were recorded on Polaroid 665 positive/negative film.

After the dinoflagellate infection was confirmed the crabs were isolated in 60-liter tanks supplied with running seawater maintained at a constant temperature of 1.0°C. Samples of hemolymph were examined weekly to follow the course of the infection.

During 1992, a total of 38,121 male *C. opilio* was examined macroscopically for BCD in the three areas covered by annual research surveys (Fig. 1). An additional 135 animals were examined macroscopically and slides of hemolymph of 80 apparently healthy crabs were prepared during a 1-day survey in Conception Bay in April of 1993.

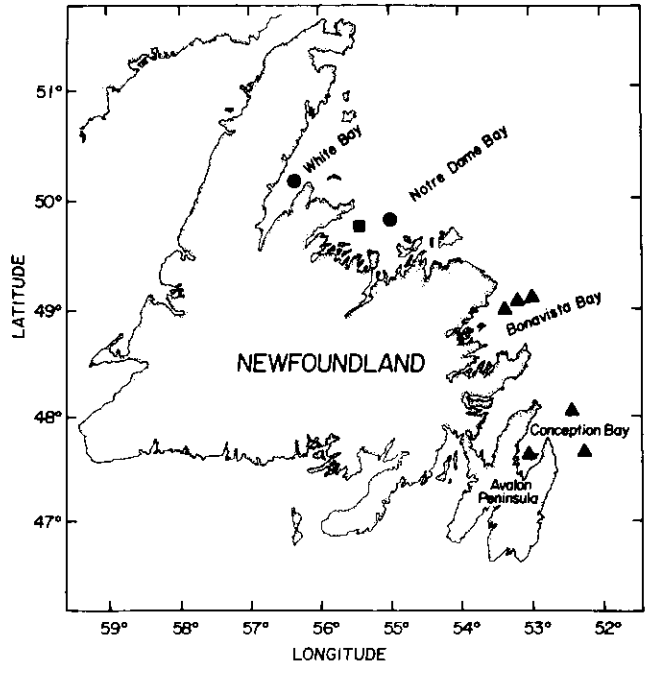

FIG. 1. Map of Newfoundland depicting areas of *Hematodinium* sp. incidence. Triangles represent areas where diseased snow crab *Chionoecetes opilio* samples were obtained during annual surveys; squares represent areas where infected specimens were encountered during commercial port sampling; and circles represent areas where anecdotal evidence suggests that commercial snow crab fishermen have encountered infected animals.

RESULTS AND DISCUSSION

Crabs infected with BCD were easily recognized during examination after capture by their opaque ventrum, extreme listlessness, and milky hemolymph. The incidence (%) of the disease based on macroscopic examination was low (Table 1) in Bonavista Bay and off the Avalon Peninsula, where 4 and 10 infected crabs were caught in each respective area. The distribution of BCD-infected crabs was extremely patchy. The 4 infected crabs from Bonavista Bay were recovered from 3 of 32 sample sites, while the 10 specimens from off the Avalon Peninsula came from 2 of 31 sites. Crabs examined for BCD ranged in size from 40- to 133-mm carapace width (CW) and included soft-shelled, new-hard, and old-hard individuals. Infected crabs ranged in size from 92- to 120-mm CW and had new-hard shells, indicating that they had molted within 2 years of capture (Taylor *et al.*, 1989). In Conception Bay no infected snow crabs were encountered during the 1992 survey despite its having been found in 1990 and 1991

TABLE 1

Summary of the Prevalence of Bitter Crab Disease (BCD) in Snow Crab, *Chionoecetes opilio,* from Newfoundland Coastal Waters

Date	Area	No. of males sampled	No. of males with BCD (%)
June 1992	NE Avalon	9,379	10 (0.001)
August 1992	Bonavista Bay	16,865	4 (0.0002)
November 1992	Conception Bay	11,877	0
April 1993	Conception Bay	135	5 (0.037)

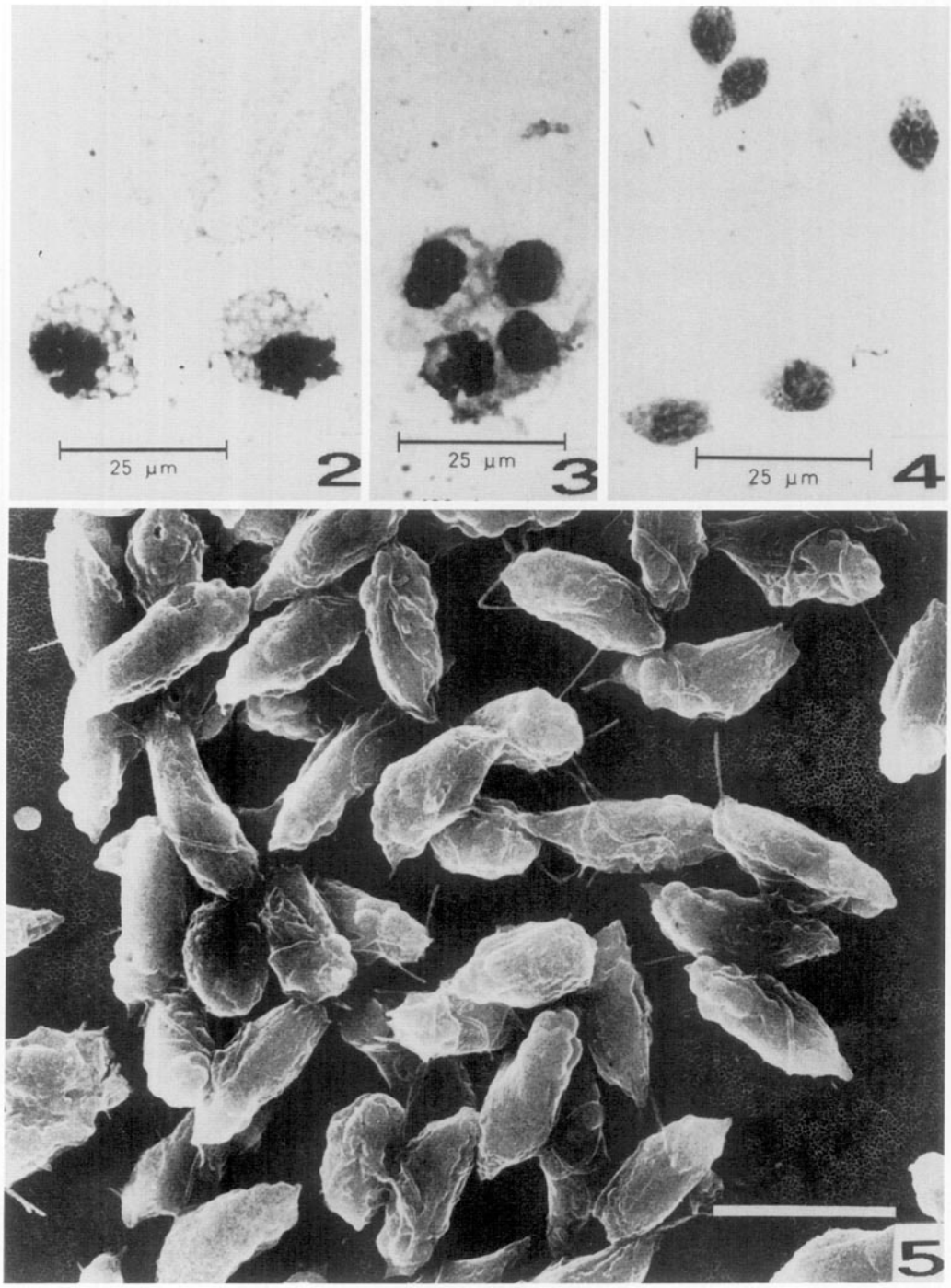

FIG. 2. Trophic stages of *Hematodinium* sp. from snow crab *C. opilio* taken off Newfoundland.
FIG. 3. Divisional (multinucleate) stage (prespore) of *Hematodinium* sp. from *C. opilio.*
FIG. 4. Dinospores of *Hematodinium* sp. from *C. opilio.*
FIG. 5. Scanning electron micrograph of dinospore of *Hematodinium* sp. from *C. opilio.*

(Taylor, unpublished data). However, 3.7% of 135 crabs examined in April 1993 in Conception Bay were infected. During this survey one of us (R.A.K.) cooked an infected crab and tasted the flesh. It had the characteristically bitter taste described by Meyers *et al.* (1987). None of the slides of hemolymph from apparently healthy crabs contained stages of the parasite.

Infected crabs sampled from off the Avalon Peninsula and kept in the aquaria at 1°C developed all three stages of *Hematodinium* sp. as described by Meyers *et al.* (1987), based on microscopic examination of wet-mounts of hemolymph. A vegetative, nonmotile, uninucleate stage was predominant in 5 of 7 (71%) infected crabs examined (Fig. 2). Unstained organisms

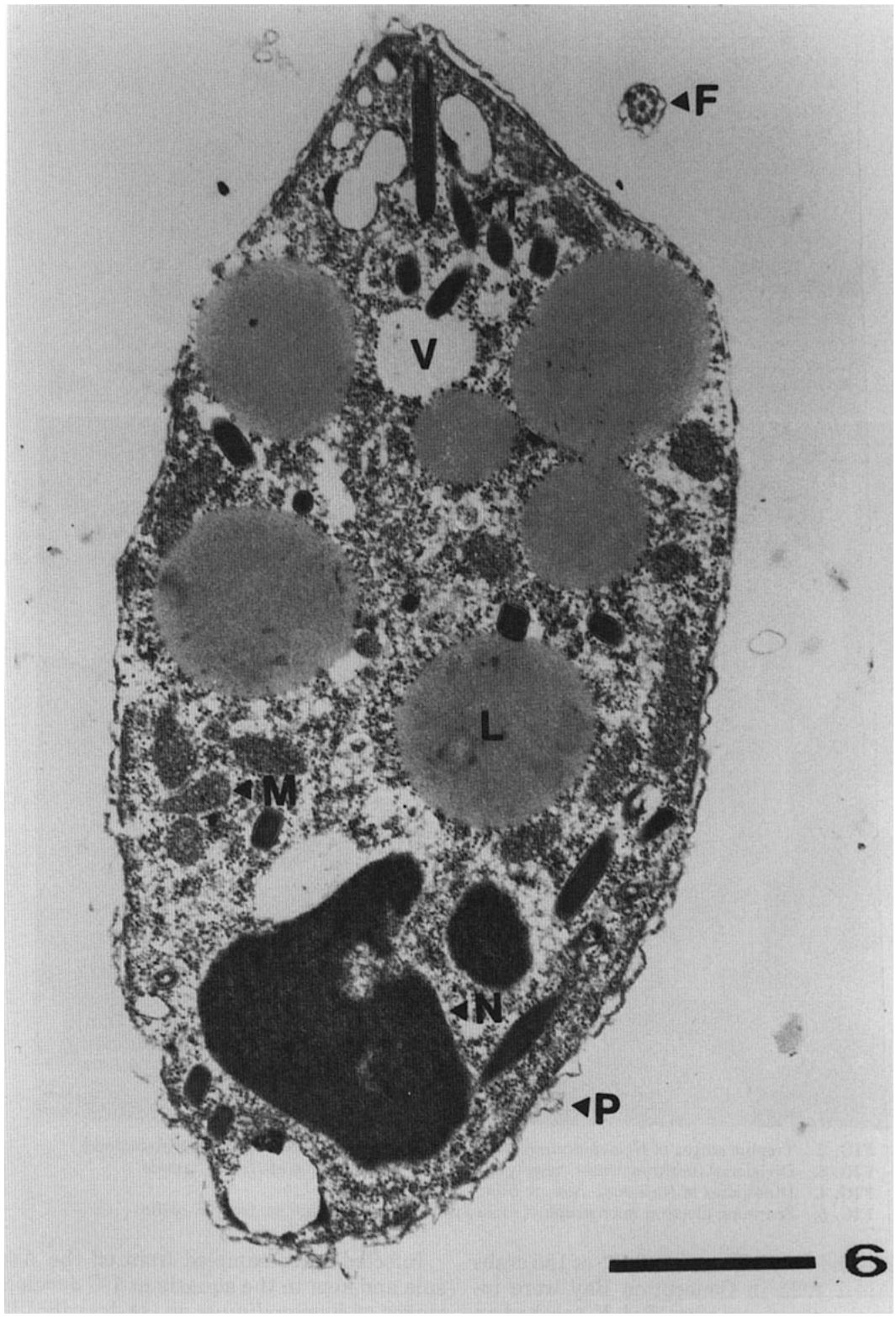

FIG. 6. Transmission electron micrograph of *Hematodinium* sp. dinospore from *C. opilio;* F, flagellum; T, trichocyst; V, vacuole; L, lipid body; M, mitochondrion; N, nucleus; P, pellicle. Scale bar, 1 μm. Magnification ×30,000.

contained a vesicular cytoplasm, but when stained with Giemsa's, an eccentric magenta-colored nucleus was observed in an amorphous and lightly stained cytoplasm. Microscopic examination of stained sections of crab tissues revealed that similar stages also occurred in the hemal spaces of the epidermis, gills, muscle, myocardium, and walls of the gut. Hemolymph of the sixth crab contained uni- and multinucleated (2–5) stages (Fig. 3) of smaller dimensions and appeared to be the prespore stages described by Meyers *et al.* (1987). This crab also harbored some dinospores (Figs. 4–6) characterized by their pyriform shape, granulation at one (ovoid) extremity, and possession of two flagella. The dinospores had one to several filament-like extensions of varying lengths. The seventh crab contained mostly dinospores with fewer prespore stages in its hemolymph. Among 7 crabs retained in captivity with the infection, transformation from the vegetative stage to the dinospore occurred in 5 to 9 days. Survival after this time did not exceed 3 days.

The dinoflagellate from Newfoundland snow crab is similar to the dinoflagellate infecting Alaskan *Chionoecetes* sp. and to *Hematodinium perezi* described by Chatton and Poisson (1931) from specimens of *Carcinus maenas* and *Portunus depurator* from Europe. Recently, Latrouite *et al.* (1988) also reported a *Hematodinium* sp. from European waters in the edible crab *Cancer pagurus,* and Wilhelm and Boulo (1988) observed a similar infection in *Liocarcinus puber* from Brittany, France. On the east coast of the United States, Newman and Johnson (1975) and more recently Messick (1994) reported *Hematodinium* sp. in the blue crab *Callinectes sapidus,* while MacLean and Ruddell (1978) found it infecting *Cancer irroratus, Cancer borealis,* and the lady crab, *Ovalipes ocellatus.* In addition, infections of *Hematodinium* sp. have been reported from *Portunus pelagicus* in eastern Australia (Shields, 1992).

Hematodinium sp. infections have had severe economic consequences in the *C. bairdi* fishery in southwest Alaska. Direct losses of income associated with discarding infected crabs at sea are estimated to be as high as $3 million; losses caused by the premature death of both prerecruit and legal-sized infected individuals have not been estimated, but are presumed to be extremely high. Losses on the fishing grounds off Kodiak Island, a relatively small contributor to the overall landings of tanner crab, are estimated to be about $1.5 million. These losses are occurring in a fishery where commercial abundance is already at an extremely low level (W. E. Donaldson, State of Alaska, Department of Fish and Game, Kodiak, AK, personal communication). The disease increased substantially in many areas of Alaska within 3–4 years of its occurrence being recognized and has become a serious economic problem. It is noteworthy that all specimens in this study were obtained by means of baited traps. Given the extreme listlessness of all infected crabs examined in this study it is conceivable that many infected animals are too weak to enter the traps. A trawl survey in areas where crabs are known to be infected with BCD might provide a more accurate indication of the proportion of the population (of all sizes) that is infected.

It would be prudent to maintain a monitoring program utilizing Newfoundland research surveys and dockside monitoring of commercial landings. Additionally, an effective educational program aimed at various sectors of the harvesting and processing industry should be implemented in order to avoid its spread through normal fishing activities such as intransit culling of the catch at sea. Such activities might have accidently facilitated the spread of the disease in Alaska (Meyers *et al.,* 1987, 1990).

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