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Dinoflagellate infection associated with the decline of *Necora puber* crab populations in France

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ABSTRACT: Since 1984, the fishery for the velvet swimming crab *Necora puber* in Mor-Braz (south Brittany, France) has declined significantly and the frequency of dying crabs found in fishery trawls and crabpots has increased. The etiology of these mortalities was investigated using histological examination of diseased crabs. A dinoflagellate parasite was found in association with pathogenic lesions of the hepatopancreas, gonads and muscles. Based on morphological features, the dinoflagellate was tentatively identified as *Hematodinium* sp. The presence of tissue pathology, fluctuations in crab mortality and the presence of the dinoflagellates led to the conclusion that these dinoflagellates may be the cause of the mass crab mortalities at Mor-Braz.

KEY WORDS: *Hematodinium* sp. · *Necora puber* · Dinoflagellate · Brittany

INTRODUCTION

In France, the principal fishery for the velvet swimming crab *Necora puber* is the Mor-Braz area of southern Brittany. Depending on the type of sea bed, crabs are caught using trawls or crabpots. Between 1984 and 1988 crab catches fell from 1100 t to 48 t, a decrease of 96%. Fishing effort over the same period only decreased 60%. The crab decline could not be explained by overfishing or abnormal climatic conditions. Unusual mortalities of crabs started appearing in 1986 in trawls and crabpots and during loading on boats; hence the possible role of an infectious disease was investigated.

Mass mortalities of crabs attributed to various types of infectious agents have been investigated over the last 28 yr, namely viruses (Bonami 1977, Johnson 1978, Sparks & Morado 1987, Mari & Bonami 1988); rickettsias (Pappalardo & Bonami 1980, Johnson 1984, Sparks et al. 1985), bacteria (Colwell et al. 1975, Leglise & Raguenes 1975, Johnson 1976) and protozoa (Couch 1967, 1983, Perkins 1975, Newman et al. 1976, Johnson 1977, Vivarès 1978, Armstrong et al. 1981). Particularly devastating epizootics have also been attributed to dinoflagellate infections, specifically of the blue crab *Callinectes sapidus* (Newman & Johnson 1975) and the Alaskan Tanner crab *Chionoecetes bairdi* (Meyers et al. 1987, 1990, Eaton et al. 1991).

As soon as mass mortalities were reported in June 1986 from the Mor-Braz crab fishery, specimens were collected and examined histologically. This led to the discovery of a dinoflagellate infection, sometimes in association with a haplosporidian-like protozoan parasite. The morphology of the dinoflagellate and crab histopathology were examined using light and electron microscopy. In addition, the epizootiology of the disease was monitored from 1986 to 1988 to determine whether or not there was any correlation with seasonality of infection, crab maturity stage or fishing techniques. Similar investigations were conducted at the same time on fishing grounds in Spain and northwestern France.

MATERIALS AND METHODS

Crabs. Adult *Necora puber* were collected with trawls or crabpots from several areas in France (Fig. 1) and in Galicia, Spain. The size and the frequency of

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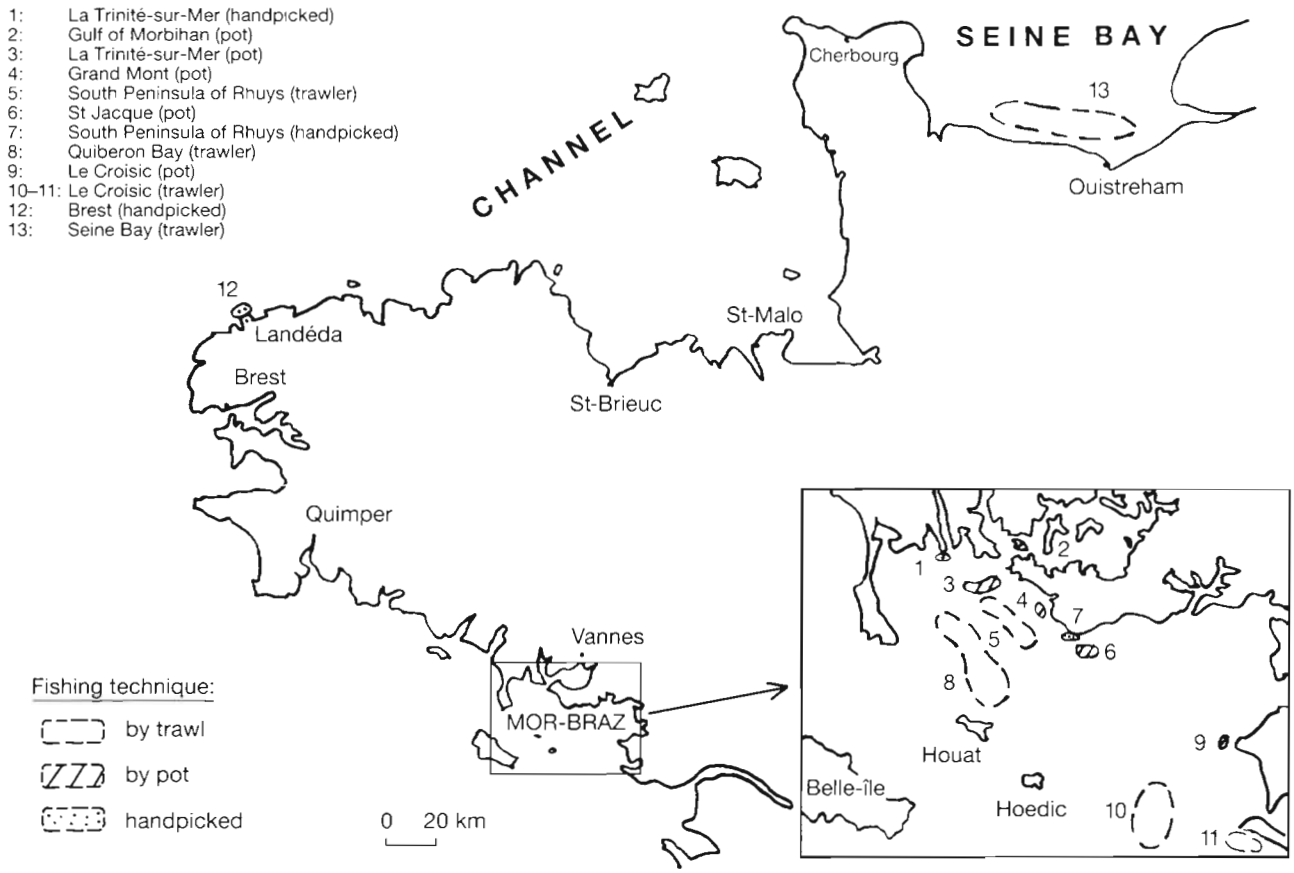


Fig. 1. Areas where *Necora puber* was collected in water around Brittany, France, from June 1986 to March 1988

sampling varied with geographic areas. The epizootiological survey was the most extensive in Mor-Braz (south Brittany) because of the local importance of crab fishing. Young crabs were collected by hand along the shores. Size, weight, sex and shell condition (newly molted or old shell) were noted for each crab.

Histology. Fresh hemolymph samples were withdrawn from the leg articulations and placed on a glass slide for examination using light phase microscopy.

Parasites were routinely detected in smears prepared from heart tissues which were air-dried and stained (using the Merck Hemacolor kit).

Tissues for histopathology were fixed either in Carson's fixative (100 ml of formol 36.5%, 23.4 g of NaH_2PO_4, $2H_20$, 5 g of NaOH, 900 ml of water, pH = 7.2) for light microscopy or, for transmission electron microscopy (TEM), in 1.25% glutaraldehyde/2% paraformaldehyde solution in 0.1 M sodium cacodylate buffer (pH 7.4), followed by 1% osmium tetroxide in the same buffer Osmolarity was raised to 1100 mOsm with sucrose (primary fixative) or with sodium chloride (secondary fixative). For light microscopy, samples of heart, hepatopancreas, gills and gonads were processed and embedded in paraffin; 3 µm sections were stained with hematoxylin and eosin. Tissues and pelleted hemolymph samples were prepared for TEM using an automatic processor and embedded in LX 112. Thin sections (60 to 90 nm) were contrasted with lead citrate and aqueous uranyl acetate by means of an LKB Ultrostainer. Grids were examined with a Jeol 1200 CX electron microscope.

Parasite detection was performed for epidemiological surveys by light microscopy of stained heart smears. Smears were as reliable and sensitive as tissue sections, but much more rapid and easy to do, particularly for field preparation of samples.

The seasonal dynamics of this dinoflagellate disease were studied between June 1986 and 1987 using monthly sampling with trawls. Prevalence of infection was considered with individual estimations of infection intensity. No dinoflagellate counts were made. Three degrees of infection — light, moderate and high — were recognized. Light infection levels meant only a few dinoflagellates were observable among crab hemocytes. In highly infected samples, dinoflagellates were predominantly or exclusively present in high numbers. In intermediate cases, the infection was classified as moderate.

RESULTS

Clinical signs

Dying crabs showed no obvious external signs of disease with the exception of a pale pink colour of the abdomen. Creamy and yellowish hemolymph and deliquescent tissues were frequently observed internally. Such animals are unmarketable because the cooked meat has a bitter flavour, similar to the condition described for 'bitter crab disease' (Meyers et al. 1987). The characteristics of the condition are the same in France as for Tanner crabs *Chionoecetes bairdi* in Alaska, USA.

Histopathology and parasite morphology

Light and electron microscopy of organs from dying crabs revealed parasites invading the hepatopancreas, gonads and muscles, as well as other tissues. Severe infections caused total disorganisation of the tissues. Occasionally, haplosporidian infections were detected in heart smears.

Light microscopic examination of fresh hemolymph permitted separation of the crab hemocytes, which generally attached to the glass slide. The dinoflagellate parasites remained in suspension and appeared as refringent uninuclear or polynuclear cells. In highly infected samples, hemocytes were totally replaced by dinoflagellates.

On stained smears of hemolymph or tissues, dinoflagellate cells were 12 to 25 µm in diameter with a single nucleus containing clumped or granular chromatin (Fig. 2). In multinuclear forms, nuclei frequently contained 5 V-shaped chromosomes. Fig. 3 shows parasite cells which appear to be in the act of nuclear and cytoplasmic division. As in the uninuclear cell stages, the cytoplasm of the plasmodia was granular and vacuolated. Although 578 specimens examined were infected with parasite vegetative stages, no motile dinoflagellate spore was ever observed. *In vitro* attempts to induce sporulation were unsuccessful. For that purpose, 1 ml blood from an infected crab was injected into a healthy crab.

Electron microscopy of the vegetative stages of the parasite further showed the clumped chromatin of nuclei and the nuclear membrane (Fig. 4). The vacuolated cytoplasm contained a few mitochondria but no trichocysts as have been observed in several other dinoflagellates (Bursa 1959, Bouck & Sweeney 1966). In each month during the year, about 20% of all crabs were lightly infected by a haplosporidian species. The Haplosporidia do not seem to be pathogenic.

Epizootiology

Table 1 shows sample dates and locations (see also Fig. 1), as well as the fishing technique used, the number of individuals collected and infection prevalence. These data show that infection varied with locations as outlined below.

One sample of 42 crabs was collected from Galicia (Spain) in April 1987. Fishing of *Necora puber* is an important activity in this area (González-Gurriarán 1985) and no dinoflagellates were detected.

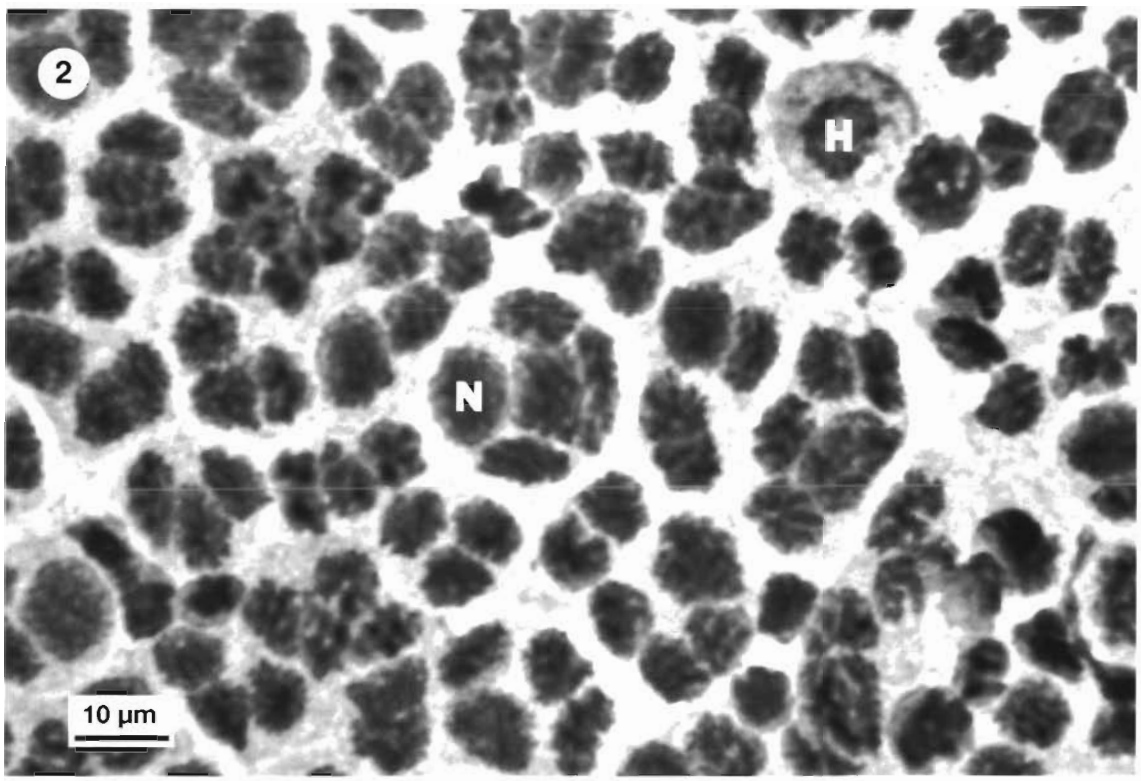
In Seine Bay (north Brittany), only 1 crab with a dinoflagellate infection was observed in samples collected during February 1987 and 1988. However, based on the epizootiological data from Mor-Braz shown below, this low rate may not necessarily be representative of dinoflagellate infection levels, since it is suspected that infected crabs die between October and January.

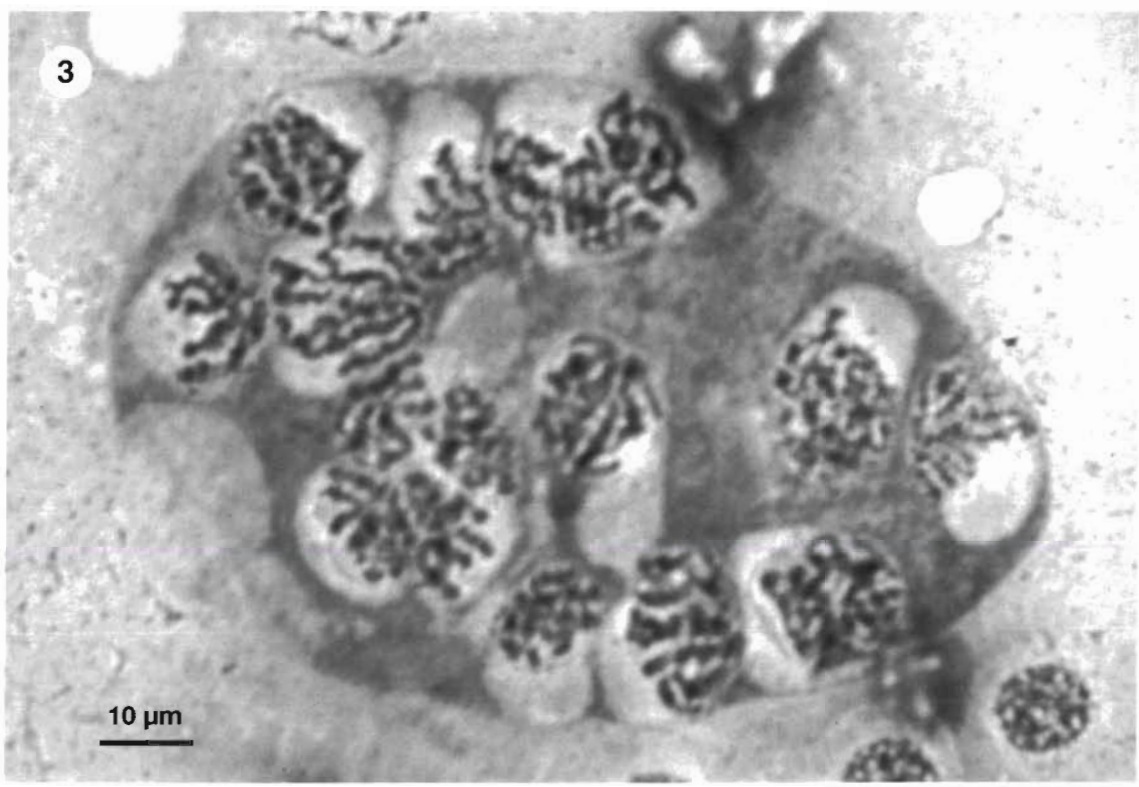
Dinoflagellates were observed in 7% of one sample of 44 crabs collected by hand in the Brest area (west Brittany) in February 1988.

The epizootiological survey of dinoflagellate disease was the most extensive in Mor-Braz (south Brittany) because of the local importance of crab fishing activity. Eleven sites and 46 samples (n = 9 to 90, depending on fishing technique) were examined.

The relationship between prevalence of infection and crab collection technique was investigated. Sample collections using trawls and crabpots were performed simultaneously in 2 neighbouring areas: one at the South of Rhuys peninsula (location 5; Fig. 1, Table 1) and one at the South of La Trinité-sur-Mer (location 3; Fig. 1, Table 1). By using Cochran or Bartlett tests (Statgraphics 1991), variances were proved to be homogeneous ($p = 0.658$) and variance analyses showed significant differences ($F = 13.9 > F\ 1.4 = 7.71$ at 5% and $F = 13.9 < F\ 1.4 = 21.2$ at 1%) of infection prevalences according to fishing technique. Consequently, trawls were considered more efficient for determining infection rates since they permitted non-selective catching of active and listless crabs.

Prevalences of dinoflagellates in crab samples varied significantly by month of collection [homogeneity of variances established by Cochran and Bartlett tests (Statgraphics 1991), $p = 0.605$ and $p = 0.658$ respectively; variance analysis: $F = 14.19 > F\ 1.4$ at 5% and $F = 14.19 < F\ 1.4$ at 1%]. Thus, fluctuations in prevalences from 10 to 80% during a 6 mo period suggested an epizootic phenomenon (Fig. 5). This hypothesis was strengthened by data related to infection intensities (Fig. 6). Indeed, the highest intensities were noted in samples taken in November and December 1986, just before a major decrease in prevalence. Consequently, it may be assumed that the death of highly infected





Figs. 2 & 3. *Necora puber* infected by *Hematodinium* sp. Smear from the heart of a heavily infected crab. Fig. 2. Multiple dinoflagellate nuclei (N) in plasmodia are compact and host hemocytes (H) are very rare. Fig. 3. Dividing nuclei showing chromosomes surrounded by a white, circular zone

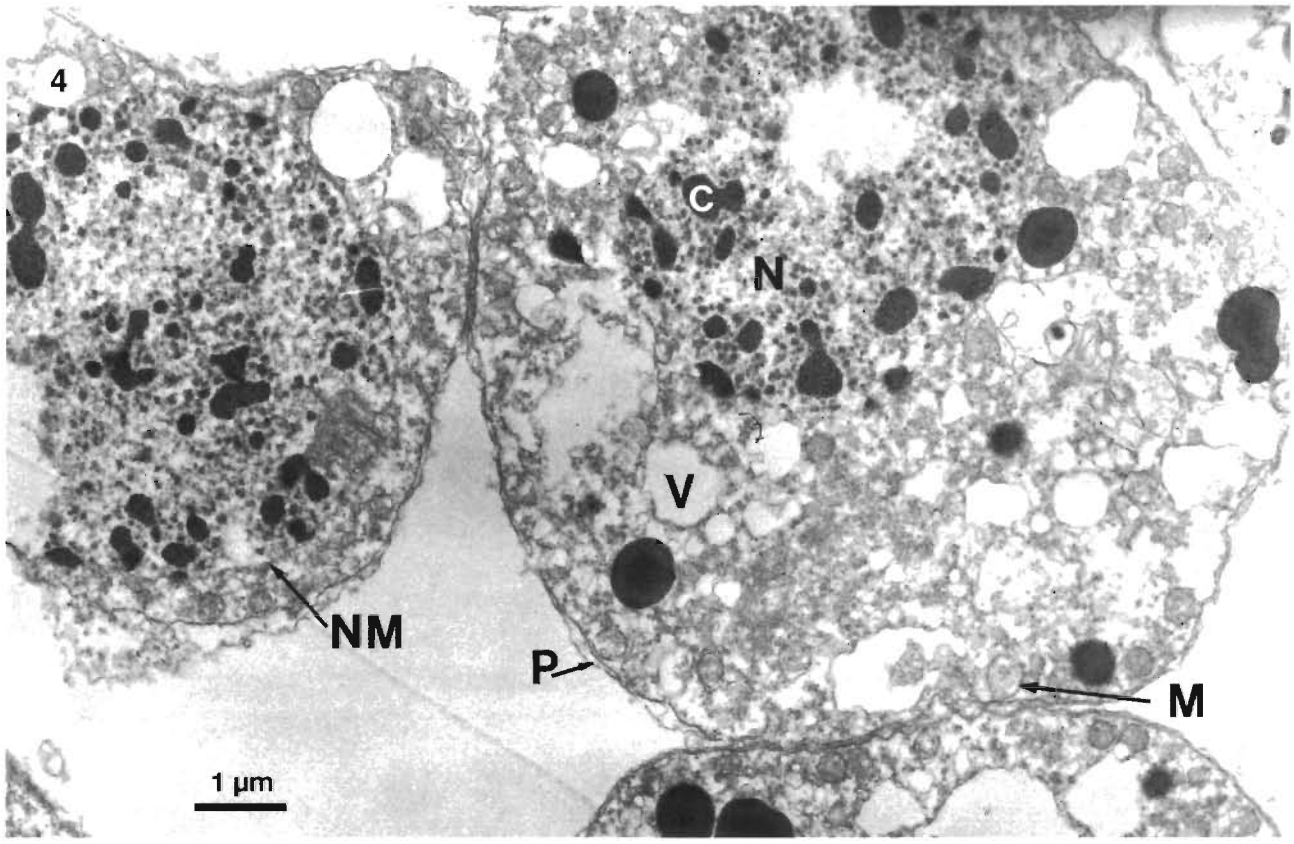


Fig. 4. *Necora puber* infected by *Hematodinium* sp. Typical dinoflagellate vegetative stage from the hemolymph of an infected crab. Transmission electron micrograph. The nucleus (N) is large, with dense chromatin surrounded by a membrane (NM). The cytoplasm contains polymorphic vacuoles (V) of different sizes. There are few mitochondria (M). A pellicle (P) borders the dinoflagellate cell

crabs led to the subsequent decrease in prevalence. Such a hypothesis concurs with observations of fishers who have found many dead crabs during winter.

In samples of adult crabs collected in June and July 1986, 34% had a soft shell indicating that they were newly molted. About 43% of these soft-shell crabs were infected compared to only 14% in hard-shell crabs. This difference in prevalence was statistically significant (χ^2 test = 7.4 > 3.84 at 5%).

No relationship was found between infection prevalence and sex or size of crabs. However, 47% of the smallest crabs examined (15 to 20 mm carapace width) manually collected in February 1987 from La Trinité shores were found to be infected. This shows that at any age, crabs can be infected.

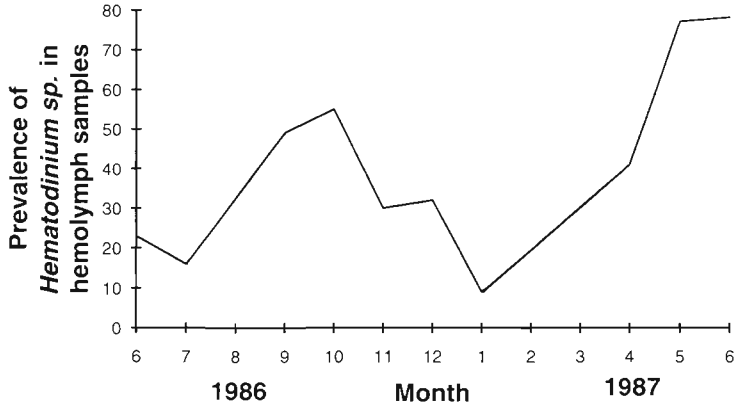


Fig. 5. Monthly prevalence of *Hematodinium* sp. infection in *Necora puber* collected by trawl during 1986 and 1987 from Mor-Braz, France

DISCUSSION

In Brittany, fishing for molluscs and crabs is a traditional and economically important activity which has been severely depressed by epizootics over the last 20 yr.

The parasite studied here is related to the genus *Hematodinium* (Dinoflagellata: Syndinidae) on the basis of morphological and ultrastructural characteristics of uninuclear

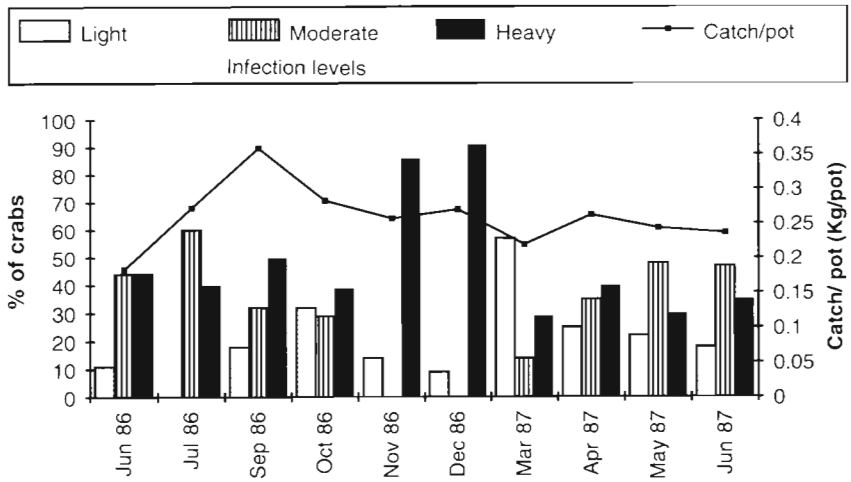


Fig. 6. Monthly intensity of *Hematodinium* sp. infection in *Necora puber* collected by trawl during 1986 and 1987 from Mor-Braz, compared to catch per crab pot

cells and plasmodia (Chatton & Poisson 1931, Newman & Johnson 1975, MacLean & Ruddell 1978). This dinoflagellate differed from a similar *Hematodinium* parasite observed in a sympatric crab, *Cancer pagurus* (Latrouite 1988), in that it lacked trichocyst organelles. This difference may be species-related, or it might have been due to the season of the investigation. The disease caused by a *Hematodinium* dinoflagellate in *Necora puber* appears similar to dinoflagellate-caused diseases described from blue crab *Callinectes sapidus*

Table 1. Percentage of *Hematodinium* dinoflagellate infection of *Necora puber* in relation to date, fishing method and the areas of collection (numbers in parentheses refer to loctions in Fig. 1). Fishing methods: T, trawler; H, handpicked; P, pot; SP, shrimp pot

Month	Location	Method	No. of crabs	No. infected	%
1986					
Jun	South Rhuys P. (5)	T	22	14	64
Jun	South Rhuys P. (5)	T	90	21	23
Jun	South Rhuys P. (5)	H	15	0	0
Jul	South Rhuys P. (5)	T	31	5	16
Jul	La Trinité (3)	P	20	1	5
Aug	St Jacques (6)	P	55	0	0
Sep	St Jacques (6)	P	24	1	4
Sep	La Trinité (3)	P	29	8	28
Sep	South Rhuys P. (5)	T	59	29	49
Oct	South Rhuys P. (5)	T	76	42	55
Nov	South Rhuys P. (5)	T	46	14	30
Dec	South Rhuys P. (5)	T	34	11	32
Dec	St Jacques (6)	P	25	7	28
1987					
Jan	Le Croisic (10)	T	22	2	9
Jan	La Trinité (3)	P	18	0	0
Jan	La Trinité (1)	H	29	0	0
Feb	Seine Bay (13)	T	40	1	2
Feb	La Trinité (3)	P	30	26	87
Feb	La Trinité (1)	H	30	14	47
Mar	Le Croisic (10)	T	46	14	30
Apr	South Rhuys P. (5)	T	46	19	41
Apr	Galicia (Spain)	P	42	0	0
May	La Trinité (1)	H	16	3	19
May	La Trinité (3)	P	29	16	55
May	South Rhuys P. (5)	T	30	23	77
Jun	South Rhuys P. (5)	T	36	28	78
Aug	La Trinité (1)	H	22	5	23
Aug	La Trinité (3)	P	34	4	12
Sep	La Trinité (1)	H	30	5	17
1988					
Feb	Seine Bay (13)	T	45	0	0
Feb	Brest (12)	H	44	3	7
Feb	St Jacques (6)	P	53	32	60
Mar	G. of Morbihan (2)	P	37	21	57
Mar	La Trinité (3)	P	38	21	55
Mar	Le Croisic (11)	T	46	28	61
Mar	Le Croisic (9)	P	38	11	29
Mar	Grand Mont (4)	P	42	12	29
Apr	Le Croisic (10)	T	41	25	61
May	St Jacques (6)	P	15	2	13
Jun	South Rhuys P. (5)	T	33	9	27
Jun	La Trinité (3)	P	32	8	25
Jul	Le Croisic (10)	T	32	6	19
Nov	La Trinité (3)	SP	36	2	6
Nov	St Jacques (6)	SP	9	7	78
1989					
Jan	Quiberon Bay (8)	T	25	14	56
Mar	Quiberon Bay (8)	T	32	26	81
1991					
Oct	Quiberon Bay (8)	T	30	18	60
Nov	Quiberon Bay (8)	T	28	19	68
1992					
Jan	St Jacques (6)	P	23	0	0
Feb	Quiberon Bay (8)	T	38	0	0
		Total:	1743	578	

and the Alaskan Tanner crab *Chionoecetes bairdi* (Meyers et al. 1987, Eaton et al. 1991). To better understand the taxonomical relationships between these parasites, morphological data will have to be complemented by biochemical and immunological characterizations.

Crab collection by trawl proved better than pots for reliably determining infection prevalences. Crabs were caught independently of their activity, which is strongly decreased in the final stages of the disease.

It is noteworthy that minimal infection prevalences followed the disappearance of the crabs with the greatest infection intensities; this disappearance coincided with mass crab mortalities. The histopathological and epizootiological data together reinforce the probability that this dinoflagellate infection caused the drop in crab production in Mor-Braz. The periodicity of the disease and a possible seasonal correlation have yet to be clearly defined, as has recently been done for a similar disease in the Alaskan Tanner crab (Meyers et al. 1987, Eaton et al. 1991). The infection process will also have to be determined, considering that prevalences were higher in newly molted crabs and that only vegetative stages were observed. It is probable that the soft crab stage is more susceptible to infection by the infective stage of the dinoflagellate.

These data reinforce previous information related to the role of dinoflagellates as regulators of crab populations. Thus, it appears crucial to develop epizootiological surveys to anticipate the risks of epizootics and to appropriately manage the fishery around such occurrences.

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