

LIFE CYCLE STUDIES OF *HAPLOSPORIDIUM NELSONI* (MSX):

SPORES AND NON-OYSTER HOSTS

(Grant # NA26FLO382-01)

January 1, 1993 to March 31, 1995

Final Report

to

NOAA
National Marine Fisheries Service
Northeastern Regional Office
One Blackburn Drive
Gloucester
MA

by

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June 30, 1995

INTRODUCTION

Since its identification as the cause of devastating mortalities of eastern oysters, *Crassostrea virginica*, in the mid-Atlantic beginning in the late 1950s, the causative agent, *Haplosporidium nelsoni*, has been under intensive investigation. During the early years in particular, a significant fraction of this effort was devoted to transmission experiments and attempts at describing the parasite's life cycle. Despite the concerted efforts of a number of laboratories in at least 3 states, experimental transmission was not achieved and the life cycle remained a mystery. After the first intensive studies provided no positive results, investigations were curtailed for many years. A number of fairly recent developments have stimulated a new round of studies, including the project reported here. These developments are

- 1) the finding of regular sporulation by *H. nelsoni* in spat of the eastern oyster (Barber et al., 1991; Burreson, 1994) (previous surveys, which concentrated on older oysters, had reported extremely low spore prevalences);
- 2) results of a workshop on the "Life Cycle and Transmission of *H. nelsoni* " held at the Haskin Shellfish Research Laboratory in March, 1992 (funded by NOAA/ODRP) in which a group of outside experts urged that a renewed effort be made to search for potential non-oyster hosts and made some suggestions as to how to go about this (see below and Final Report to NOAA "Studies On Life Cycle Stages Of The Oyster Parasite *Haplosporidium nelsoni* [MSX]" Grant # NA90AA-D-FM460, March 1993); and
- 3) the finding of ingested haplosporidan spores, resembling those of *H. nelsoni* in size and shape, in the guts of oysters inhabiting waters enzootic for the pathogen and suggesting a widespread distribution of these spores in the water column (Barber and Ford, 1992).

OBJECTIVES

The project included three objectives:

- 1) to initiate a methodical, but restricted, search for potential alternate or intermediate hosts of *H. nelsoni* by collecting and screening at selected periods and locations a) a number of small bivalve species (alternate host similar to oyster spat in which *H. nelsoni* spores are

produced) and b) zooplankters (intermediate host dissimilar to oysters, which could act as a dispersal mechanism for the parasite);

- 2) to continue our survey of oyster spat to document annual variation in *H. nelsoni* infection prevalence and spore production, and to collect spores for analysis; and
- 3) to characterize and identify haplosporidan spores, identical to *H. nelsoni* at the light microscope level, that we have found ingested by oysters throughout Delaware Bay in numbers suggesting very high abundance in water.

BACKGROUND

1) The Alternate or Intermediate Host Hypotheses

Early studies on the life cycle and transmission of *H. nelsoni* (Sprague, 1962; Canzonier, 1968) concluded that 1) transmission of the parasite in nature is not dependent on a nearby source of infected oysters; 2) the spore stage, presumed necessary part of the transmission process, was extremely rare (<1% of infected [adult] oysters); and 3) experimental transmission could not be achieved. These results led to speculation that an intermediate host might be involved, or that oysters might be an incidental host and that a "normal" or alternate (reservoir) host might be present, or both (Sprague, 1962; Farley, 1965; Farley, 1967; Andrews, 1968; Ford and Haskin, 1982; Haskin and Andrews, 1988).

Our recent findings have added an important element to what we know of the life cycle: *H. nelsoni* infections acquired by oyster spat (oysters < 1 year old) are highly likely to produce spores once they become infected (Barber et al., 1991). Infection rates of small oysters are relatively low, however. In our surveys since 1988, the maximum prevalence in spat has been 30% (in 1988) and overall, spores have been found in only about 5% of all spat sampled. Whereas this is still a large number of spat, with the potential of producing a large number of spores, we have found no correlation between the historical abundance of spat in any given year and subsequent *H. nelsoni* infection prevalence in the general oyster population of Delaware Bay. Further, neither we nor others (Andrews, 1979) have been successful in infecting oysters with known *H. nelsoni* spores. Thus, the need to consider a life cycle involving other hosts remains compelling.

During the "Workshop on the Life Cycle and Transmission of *H. nelsoni* " in 1992, several deductions and recommendations were made by life cycle experts with experience in other host-parasite systems. Among these were the following:

- 1) It is likely that an alternate or intermediate host, or both, exists for *H. nelsoni*, but we should not give up on looking at the possibility of direct transmission via spores produced in oyster spat (Fig. 1A).
- 2) Because spores are so infrequent in adult oysters, the oyster may be an abnormal/adventitious host. In this case, an alternate (normal) host would exist that would most likely be very similar to the oyster (i.e., a sessile bivalve) and the seasonal infection cycle would probably also be similar, except that *H. nelsoni* would produce spores in this host (Fig. 1B).
- 3) If an intermediate host exists, it is likely to be quite different from the oyster and possibly one that is itself highly mobile or is dispersed by water currents (i.e., zooplankton, including larval forms - Fig. 1C). The parasite must have some mechanism to maintain itself near potential hosts (oyster or other similar estuarine species) in the estuary. The potential host is not likely to be a commercially valuable fish species because these have been examined extensively for parasites. Small non-commercial species are candidates, but haplosporidans have never been found in a vertebrate host.
- 4) An *H. nelsoni* spore produced in another host might not exactly resemble that produced in oysters, but would be similar enough to be classified in same phylum. Spore size should not be considered an immutable criterion for differentiating between species.
- 5) It is also possible that both an intermediate and an alternate host are involved (Haskin and Andrews, 1988) (Fig. 1D).

At the outset of the project, we recognized that we could not possibly screen all potential host species. Our approach was to select certain groups that met the above criteria and to sample them at specific locations and times chosen to maximize the likelihood of finding a host if it exists.

2) Spores in Oyster Spat and the Direct Life Cycle Hypothesis

We outlined above the arguments for a life cycle involving at least one other host; however, we do not want to dismiss the importance of *H. nelsoni* spore production in oyster spat. Although total infection prevalence in spat is typically low, and consequently so is that of spat producing spores, the number of spores formed in individual spat may be up to 1.5 million (we found a mean of 1.6×10^5 in spat examined from 1991 and 1992 collections). It is difficult to estimate the total number of spat present in Delaware Bay during any given year, but based on extensive dredge sampling on Delaware Bay seed beds over the past 35 years, we estimated that a figure of 100 spat per m^2 to be a reasonable average. From this, we calculate that there are 10^{10} - 10^{12} spat in Delaware Bay on an "average" year. If 5% each produce 1.6×10^5 spores, the total number of spores would give a (cumulative) concentration of several hundred spores L^{-1} of Delaware Bay water in an "average" year. These very rough calculations suggest that *H. nelsoni* spores from infected spat could be produced in sufficient quantity to be significant elements of the life cycle. Spores are produced (and released) from spat during the period when oysters are becoming infected, and in some years, at least, the potential number of spores produced in this manner may be very high. They may directly infect other oysters or may mirror spore production in an alternate ("normal") host. Thus, we believe it important to continue some level of monitoring for *H. nelsoni* spores in oyster spat.

3) Ingested Haplosporidan Spores and their Significance

In 1989, while examining stained sections of eastern oysters collected in Delaware Bay, we noted an operculated spore, apparently ingested and resembling in size and shape that of *H. nelsoni*, within the digestive tract lumen of an oyster. With funding from the NOAA Oyster Disease Research Program (Studies On Life Cycle Stages Of The Oyster Parasite *Haplosporidium nelsoni* [MSX] - Grant #NA90AA-D-FM460), we undertook a systematic search for ingested spores in archived tissue slides of oysters collected over the past 34 years in Delaware Bay. We found capped haplosporidan spores in the digestive tract lumina (stomach, mid-gut, and intestine) of 818 of 3292 (25%) oyster tissue sections examined from all locations sampled in Delaware Bay, including sites as far up the estuary as oysters grow (Barber and Ford, 1992). They were present in oysters of all ages from April through December, but predominated during months when water temperature was above $10^{\circ}C$. They were present during the first outbreak of MSX disease (1958) and in locations to the north and south of Delaware Bay where *H. nelsoni* is enzootic. The mean (SE) length and width of ingested spores (N=193) was 5.5 (0.1sd) x 7.5 (0.1sd) μm . In comparison, the mean size of *H. nelsoni* spores produced in infected Delaware Bay oysters (N=76) was 5.3 (0.1sd) x 7.5 (0.1sd) μm . The widespread distribution of these spores, their resemblance to those of *H. nelsoni*, and epizootiological links with known *H.*

nelsoni infection patterns, made it desirable to determine whether they are, in fact, a stage in the life cycle of that parasite.

METHODS

1) Non Oyster Hosts

The search for non oyster hosts followed two paths. The first was directed at a potential intermediate host; the second, at a potential alternate (reservoir) host.

Intermediate Host Search. We hypothesized that an intermediate host would be a mobile species and chose to examine zooplankton, as was suggested at the workshop. We further hypothesized that an intermediate host would produce spores at about the time that oysters are becoming infected (early summer). Therefore, zooplankton samples were collected by boat during 1993 and 1994 at 4 locations in lower Delaware Bay (Fig. 2; Table 1). At each station, a 500 μm plankton net with a 0.5-m opening, was towed about 1 m below the surface for 15 (in 1993) to 20 (in 1994) minutes. Our original plan to pump water through a net was scrapped as being too destructive of the relatively large zooplankters. On two cruises in 1993, we used a General Oceanics digital-mechanical flowmeter (model 2030 series) to estimate the volume of water sampled during a 15-minute tow ($=85.7 \text{ m}^3 \pm 9.1 \text{ sd}$, $N=6$). Extrapolating to 20 minutes indicates that each 1994 tow collected organisms from about 115 m^3 . Most of the samples were collected during the day, but on two occasions, collections were also made at night to determine whether this would increase the numbers of mysid shrimp (which rise to the surface at night) taken. Samples were immediately fixed in Davidson's fixative.

In the laboratory, the samples were fractionated as well as possible to remove fish eggs, debris, and ctenophores. The remaining sample was scanned under a binocular scope for evidence of discoloration in zooplankton that could be caused by a large number of spores. The samples were then embedded in paraffin, sectioned, stained, and examined microscopically. Any recognizable parasites or pathology were recorded.

Alternate (Reservoir) Host Search. In this search, we hypothesized that *H. nelsoni* infections in an alternate host would resemble, epizootiologically, those in oysters. Because we have found that *H. nelsoni* appears to sporulate regularly in oyster spat, but not in adult oysters, we further hypothesized that some morphological characteristic of small size, or the enhanced metabolic rate of small organisms, may be the stimulus for spore production. Thus we

concentrated our sampling efforts on bivalves in the <30 mm or less size range. We also hypothesized that spore production should be at least as frequent as it is in oyster spat, if not considerably more prevalent.

Samples were collected from the intertidal flats at the Haskin Shellfish Research Laboratory (HSRL) Cape Shore Station in lower Delaware Bay, a site where *H. nelsoni* infection prevalence is typically very high. Collections were made at low tide every two weeks from May to September. Sediment was dug to a depth of about 10 cm and passed through a 1 mm sieve. All live organisms were removed and fixed in Davidson's fixative. The sampling effort was not quantified, but at each date, two individuals spent the entire low tide period (1 to 3 h) collecting organisms. Additional organisms that were a by-catch of dredging activities in the lower Bay were examined on occasion. All organisms (non-bivalves included) were embedded in paraffin, sectioned, stained, and examined microscopically for parasites and pathology.

2) Spores in Oyster Spat

Spat were collected every 7 to 10 days from May through August, and every 2 weeks in April and September, from the intertidal flats off the Cape Shore Laboratory. From each collection, all spat were shucked. Fresh smears of those showing evidence of disease (e.g., emaciation, shell stunting, or pale digestive gland) were examined microscopically for the presence of *H. nelsoni* plasmodia and spores. Twenty-five to forty of the remaining spat were fixed in Davidson's, embedded, sectioned, and stained for more precise determination of infection and spore prevalence.

Pieces of digestive gland containing sporulating stages were then fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) plus 4% (W/V) sucrose, overnight at 4 °C. Tissues were washed 4 times (30-40 min. each) in the cacodylate buffer with sucrose, cut to 1-2 mm, post-fixed in 1% osmium tetroxide in cacodylate buffer with sucrose, and dehydrated through a graded series of alcohols. Fixed tissues were embedded in either Epon 812 or Quetol 651. Sections for light microscopy were cut at 0.5 μm , and stained with 1% toluidine blue adjusted to pH 11.0, and checked to verify the presence of spores. Sections for transmission electron microscopy (TEM) were cut at 900 Å, and stained with 2% aqueous uranyl acetate for 60 minutes, followed by lead citrate for 10 minutes. Examinations and photomicrographs were made using a Jeol 100CX II. Tissue preparation for scanning electron microscopy (SEM) was identical, except that pieces of digestive gland were mounted on stubs using colloidal graphite, critical point dried, and coated with gold/palladium by vacuum evaporation. Examination and photomicrographs were made using a Hitachi S-450.

3) Ingested Spore Identification

At numerous times during the project, oysters were collected from various areas of Delaware Bay, including some suspended from the HSRL dock on the Maurice River (Fig. 2). In the laboratory, they were shucked and a portion of the stomach contents removed and examined microscopically for the presence of haplosporidan spores. Samples with high numbers of spores were processed as above for electron microscopy. Sections of bivalves and benthic organisms examined in connection with the search for a non oyster host were also examined for the presence and abundance of ingested spores.

Originally, we had planned to test ingested spores in histological section with an antibody to *Minchinia teredinis* (a haplosporidan present in Delaware Bay that resembles *H. nelsoni*) available from Dr. Gene Burreson at the Virginia Institute of Marine Science (VIMS). Since that time, gene probes for *H. nelsoni* have been developed by us, and by Dr. Burreson and his associate Nancy Stokes (Fong et al., 1993; Stokes and Burreson, in press). Stokes and Burreson subsequently perfected a method for *in situ* staining of paraffin sections using the probe and agreed to examine some of our sections containing ingested spores. In preparation for this, we destained known ingested spores-containing slides with potassium permanganate and oxalic acid. As controls for the destaining procedure, we processed slides with known *H. nelsoni* spores in the same manner. Additional controls were freshly made slides from embedded tissues of oysters diagnosed as containing *H. nelsoni* and *H. costalis* spores and plasmodia as well as unidentified ingested spores.

RESULTS

1. Non Oyster Hosts

Zooplankton samples were collected on 18 occasions in 1993 and on 10 dates in 1994 (Table 1). Sampling during the middle summer was decreased in 1994 because most of the samples were fish eggs. On each date, 4 stations, on a rough upbay-downbay transect, were visited. Copepods of several species and larval shrimp predominated in the spring samples; larval crabs were most numerous in summer (Tables 2 and 3). Samples collected in July were nearly all fish eggs and were not counted. Although we did not count all the individuals processed histologically, we fixed, embedded, sectioned, and examined microscopically samples containing hundreds of zooplankters (Table 3). Despite the large number of individual zooplankters examined, only two recognizable parasites were found.

A microsporidian parasite was found infecting copepods at three different stations on two dates in June and one in September 1994. Infections were confined to the musculature where they could be heavy; only spore stages were present; and the prevalence was low (2 to 6%). It was impossible to identify the species of copepod in section. However, the only species present in the September was *Pseudodiaptomus pelagicus*, and this species was predominant in the June samples as well. This is a common marine and estuarine copepod that tolerates a wide range of salinities, but peaks in abundance between 15 - 20 ppt.

Another microsporidian was seen in a single nereid worm (probably *Nereis virens*) collected from the plankton in June, 1993. In this case only meront and sporonts, rather than spores, were present, and the host tissue was very disrupted. Polychaetes were extremely rare in plankton samples leading us to believe that this individual may have been moribund because of the heavy infection.

Over 1200 individual bivalves and other benthic organisms were collect and examined histologically during the study (Table 4). Although the species representation was far from equal (e.g., 586 *Tellina* sp. vs 2 *Lyonsia* sp.), it did represent the relative frequency as well as the total abundance of these species at the collection site. Trematodes and cestodes were common in the bivalves and were also found in *Diopatra* sp. and *Balanus* sp. No recognizable protozoans were found.

2) Spores in Oyster Spat

During 1993 and 1994, nearly 1500 oyster spat or yearlings from 32 collections, were examined by fresh smear for the presence of *H. nelsoni* spores (Tables 5 and 6). Of these, 1259 were also examined by histological section. No spores were found in any samples and, for the most part, total infection prevalence was very low.

As part of other projects, total prevalence of *H. nelsoni* was determined in oysters at the Cape Shore site and in locations farther up in the Delaware Bay.

1) Screens of fresh hemolymph from large, susceptible (Martha's Vineyard and Connecticut) oysters deployed each spring at Cape Shore.

1993 (summer dates): 25-80%

1994 (summer dates): 25-30%

2) Cape Shore experimental oysters (1992 year class)

1993 (November): 0 (resistant) - 8-15%(susceptibles)
1994 (August): 4-5% (resistant and susceptible)

3) Fall Seed Bed samples

1993 (October): 0 to 15%
1994 (October): 0 to 5% (4 plasmodia found in 1 oyster - among 160 examined)

Comparison of *H. nelsoni* infection prevalences in Delaware Bay native stocks during the study period (1993 and 1994) with previous years shows that they were among the lowest on record (Fig. 3). Four earlier periods of reduced parasite activity were all preceded by cold winters, whereas the winters preceding the study period were unusually warm, although decreasing from their 1990 high (Fig. 3A). Although there is no statistical correlation between spat set in Delaware Bay and *H. nelsoni* prevalence the next year, we plotted spat abundance on the lower seed beds (where we have a record going back to 1959) and *H. nelsoni* prevalence to aid in visualizing any longer-term relationship or trend (Fig. 3B). None appeared. The 1993-94 low occurred during a period when there had been almost no oysters in the lower Bay for a couple of years, as did the previous low in 1991-92 (Fig. 3C).

3) Ingested Spore Identification

Transmission and scanning electron microscopy of known *H. nelsoni* spores were examined for comparison with the unknown ingested spores (4A-E). Although some electron microscopical studies of *H. nelsoni* sporulation had been performed in the 1960s (Perkins, 1968; Rosenfield et al., 1969), the rarity of spores had precluded further ultrastructural studies. Our recent findings of regular spore formation in small oysters provided the opportunity to examine the fine structure of one of the most intensively studied haplosporidans in greater detail. Of particular interest were the filaments because of their taxonomic significance and their possible function as a floatation device.

Spore wall formation in *H. nelsoni* is initiated by the deposition of a thin layer of electron dense material around each nucleus with associated cytoplasmic elements (Fig. 4B). Subsequent deposits of electron-dense granules form nodes that are non-contiguous. As this process continues, the wall becomes contiguous around the sporoplasm, additional deposits are laid down, and the wall assumes a periodic banding (Fig. 4C). As the spore nears maturity, material of lesser electron-density is deposited on the periphery of the outer wall. The final deposits of material on the outer spore wall form tubular structures, indicative of the onset of formation of episporic ornaments. These tubules often appear to run perpendicular, rather than parallel to the outer wall surface, and are positioned in the same manner as the episporic ornaments as seen on

the fully mature spore (Fig. 4A, D and E). The material in the filaments coalesces during lysis of the extraspore cytoplasm, exposing the spore filaments, and they develop their characteristic internal structure as described by Perkins (1968). Scanning electron micrographs of immature spores, before the onset of lysis of the extraspore cytoplasm show that the episporic ornamentation is visible at this stage of development (Fig. 4D). After lysis of the episporic cytoplasm the spore is considered to be fully mature and the ornamentation is fully exposed (Fig. 4A and E).

We continued to find ingested haplosporidan spores in both fresh and fixed material, but not in the abundance of earlier years and nearly all of those found were the large spores, not the smaller variety that we believe may be those of *H. nelsoni*. In all the fresh gut smears we examined we found only 30 of the small variety, compared with 2500 of the large. We examined, by scanning electron microscopy, the stomach contents from oysters identified as having ingested spores by fresh smears. However, we were not able to locate spores in these samples, probably because their overall abundance was relatively low. Thus we were not able to use electron microscopy as an identification aid.

Several slides sent to Burrenson and Stokes at VIMS were examined using the *H. nelsoni* ssRNA gene probe. No cross reactions were evident against either large or small ingested spores. At this point, failure of the probe to react with ingested spores is not considered definitive evidence because there has been difficulty obtaining reactions with any spores, probably because the thick spore wall prevents entry of the probe. In fact, positive control, known-*H. nelsoni* spores did not react, although plasmodial stages in the same samples reacted very strongly (N. Stokes, VIMS, personal communication, October, 1994). Stokes and Burrenson did not believe that further analyses would be useful at the time because of the apparent inability of probes to penetrate the thick spore walls. There remains a chance of using *in situ* PCR on tissue sections containing ingested spores to amplify DNA and increase the chances of detection (N. Stokes, personal communication, January 1995)

DISCUSSION

Despite histological examination of several thousand individual potential hosts, we did not find any parasites that we could link to *H. nelsoni*. The most abundant parasite that we did see was a microsporidian in a copepod. Copepods are secondary hosts for several microsporidian species that also infect mosquitoes (Andreadis, 1985; Sweeney et al., 1985).

Although the spores in the copepod hosts are somewhat different than those in the primary hosts, they are still recognized as members of the phylum Microspora. Similarly, the causative agent of Whirling Disease in salmon also infects tubificid worms, an intermediate host, in which it forms a morphologically different spore. Spores in the two hosts were originally classed in separate orders, but still in the phylum Myxozoa (Wolf and Markiw, 1984). Thus, we do not consider the microsporidians in the copepods and the nereid worm as potential stages in the life cycle of *H. nelsoni*.

"Disappearance" of *Haplosporidium nelsoni* and its effect on the project

At the outset, we recognized that this project had an above average element of risk that we would not reach the goal of identifying another host. To mitigate this possibility, we designed our search to thoroughly examine certain groups of organisms, which we felt had good potential for being second hosts, at certain times of the year and in certain locations that would maximize our chance of finding such a host. If we didn't find anything, we expected that we could at least eliminate, with a reasonable degree of confidence, certain species from the "candidate species list". Unfortunately, because *H. nelsoni* prevalence in oysters was almost non-existent during the study period, we are unable to do this. The lack of *H. nelsoni* in oysters, in fact, makes it nearly impossible to interpret our results, as a number of scenarios are possible:

- 1) A non-oyster host was among the species examined, but the low *H. nelsoni* prevalence during the study (as measured in oysters) precluded finding the parasite in this host.
- 2) A non-oyster host was among the groups (i.e., small bivalves or zooplankters) examined, but was scarce or absent during the study.
- 3) A non-oyster host exists, but is not among the organisms that we examined.
- 4) The life cycle is direct, with oyster spat providing the infective stages (spores).

It could be argued that the low prevalence of *H. nelsoni* in Delaware Bay native oysters during and before the study period was not due to lack of infection pressure. In 1984-86, and upbay incursion of the parasite caused heavy mortalities - the first substantial mortalities on the seed beds since 1958-59. Perhaps this selective pressure resulted in an incremental jump in resistance and lower infection levels. Certainly, an increase in resistance may have occurred, but since 1989, the prevalence of *H. nelsoni* in unselected oyster stocks exposed annually at the HSRL Cape Shore Station as an index to infection activity, has also been unusually low and was

particularly so during 1993-94. Thus, we must conclude that infection pressure (e.g., the abundance of infective particles) was truly very low.

The prevalence of a parasite in a secondary host may be only a few percent, so that a large number of organisms must be examined to find the parasite. In this situation, however, the abundance of the secondary host is typically very high (e.g., small worms that may number in the thousands per m² of sediment (Bellerud et al., 1995)) or copepods that are similarly abundant in the water column. Thus, the total number of infected individuals in the environment is large enough to perpetuate the parasite. The presumed low number of *H. nelsoni* infective particles present in 1993-94 suggests that the total number of infected second hosts would be similarly low - either because the host numbers were low or because their infection rate was low. In either case, the chances of our locating such a host would have been much reduced compared to a year in which infection pressure was more typical.

A change in the long-term infection pattern of H. nelsoni

An aspect of the study that deserves consideration is the departure from a 30-year pattern of *H. nelsoni* infections in Delaware Bay oysters. From the start of the monitoring program in 1958 to the early 1990s, there were 5 cycles of low and high prevalence that were roughly correlated with winter temperature. A cold winter was followed, a year or two later, by unusually low prevalence in the oyster population. This pattern was sufficiently pronounced that it was linked, speculatively, to a hypothetical second host population, which was damaged because of the cold (Ford and Haskin, 1982; Haskin and Andrews, 1988). After each low, there was a rapid and extreme increase in prevalence - to a level higher than in the previous cycle. According to this pattern, then, *H. nelsoni* prevalence should have rebounded from its 1988-89 low after the temperature increased (see Fig. 3A). Instead, prevalence remained essentially flat, and relatively low (30%) for 5 years, and then fell to almost 0% in 1994. This was the lowest recorded level since 1962.

The single most obvious correlate with this anomaly was the onset, in 1990, of an epizootic caused by *Perkinsus marinus* (Dermo) in Delaware Bay. Infection levels built in the middle and lower Bay that year resulting in localized mortalities, which increased the following year and continued through 1993 as the epizootic spread upbay. In addition to the loss of oysters through mortality, the Dermo disease epizootic caused a cessation of seed oyster transplants from the upper Bay to the lower Bay leased grounds, which lasted from 1992 through 1994. This followed a three-year (1987-1989) ban on seed planting after the last major MSX epizootic in the mid 1980s, which also caused major mortalities and depleted the seed supply.

During six of the eight years between 1987 and 1994, then, no seed transplants were made to the lower Bay. Consequently, the adult oyster population in the lower Bay (where high salinity favors *H. nelsoni*) was smaller than at any time since 1959-60, after the first *H. nelsoni*-caused epizootic had killed most of the oysters and halted seed planting. In 1993 and 1994, in fact, there were almost no oysters at all on the leased grounds. One planter made a survey of his grounds in 1994 and reported catching only half a bushel of oysters after a half-day's dredging.

It may be only coincidental that the two periods of lowest measured *H. nelsoni* prevalence in Delaware Bay co-occurred with the two periods of lowest adult oyster population size in the lower Bay. It may also be coincidental that prevalences rose as recruitment to the seed beds improved beginning in the late 1960s and allowed the industry to plant large numbers of seed oysters during the 1970s and early 1980s. A link between these two events would imply one of the following:

1) The number of oysters present in the lower Bay, where salinities are favorable for *H. nelsoni* survival and development is directly important to the maintenance of *H. nelsoni* densities in the Bay. This conclusion is contrary to much previous evidence that oyster density is not a factor in the transmission of infections - or, presumably, in the ability of the parasite to perpetuate itself (Haskin and Andrews, 1988). Because most planted oysters are adults, this argument implies that adult oyster infections, which are almost exclusively plasmodial infections, are significant in the life cycle. Given the number and variety of unsuccessful attempts to transfer plasmodia from infected to uninfected oysters and the fragility of these stages that we have observed in laboratory manipulations, it seems inconceivable that plasmodia could be the infective stage transported between hosts in the water, or in or on a vector. Could plasmodia survive ingestion by a secondary host and then infect that host? Parasites with two-host life cycles in which one host becomes infected by eating the other typically produce spores in both hosts and it is the spore that is consumed, excysts, and infects the new host (Wolf and Markiw, 1984; Sweeney et al., 1985). Spore-forming protozoans with direct life cycles also involve ingestion of spores (reviewed in Sindermann, (1990).

2) The number of oysters present in the lower Bay, where salinities are favorable for *H. nelsoni* survival and development is indirectly important to the maintenance of *H. nelsoni* densities in the Bay. This could occur if aggregations of living oysters attract or provide habitat for a second host. A species that inhabits the oyster bed would not be very mobile and would not fulfill the hypothesized requirement of an intermediate host - that it help maintain infective stages in the estuary by carrying them upbay. A swimming species (e.g., fish) or a benthic species that travels long distances (e.g., some crabs), but congregates around living oyster beds

to feed could meet this requisite. Thus, we may not have focused on the right types of organisms in our search.

Spat and spores again

The fact that *H. nelsoni* sporulates in small oysters is now known, but the relevance to its life cycle remains unknown. We must not discount the possibility that these oysters life stages are an important key to the life cycle puzzle. The arguments against spat involvement are that *H. nelsoni* prevalence in these small oysters is often low (by virtue of their small size, they encounter few infective stages relative to larger oysters) and their abundance (in Delaware Bay) does not correspond to *H. nelsoni* infection cycles. However, we can draw an analogy from the secondary host data in other systems - that large numbers of hosts compensate for low infection prevalence. Thus, large numbers of spat with relatively low infection rates could produce large numbers of spores. In the Chesapeake Bay, two reports of sporulation in spat give prevalences of 35-40%, which is similar to our maximum of 30% (Andrews, 1979; Barber et al., 1991; Burreson, 1994). The first year we began systematically examining spat (1988) was actually the beginning of the current "low" in *H. nelsoni* infection pressure. Our studies show that once a spat becomes infected there is a high probability that the infection will proceed to sporulation. Thus, in years before 1988 when infection pressure was higher, there is a high likelihood that spat produced a large quantity of spores.

Under the direct life cycle scenario with spat the source of spores, two pieces of the puzzle remain unanswered. 1) Why is there no correlation between spat set and subsequent *H. nelsoni* prevalence in the general oyster population? 2) Because most spores would be disseminated in the lower Bay where the highest spat prevalences would occur, would they not be in danger of being transported out of the Bay? If spat are important sources of spores, it seems that these spores are not immediately or directly infective to oysters. Perhaps spores are capable of surviving for long periods (years?) in the environment so that the production in any one year would not necessarily correspond to infections that year.

Scanning electron microscopy of *H. nelsoni* spores shows numerous filaments attached to the wall of mature spores. That spore ornamentations such as these may aid in flotation is suggested by our finding of ingested haplosporidan spores in oysters suspended high in the water column (Barber and Ford, 1992). There is a net upbay movement of water on the inshore New Jersey side of Delaware Bay, but the bulk of the water has a fairly rapid, net downbay movement. Under these circumstances, could the passively floating spores find their way upbay without some form of conveyance? It should be recalled that we found ingested spores that

resemble those of *H. nelsoni* in size and morphology well up estuary in Delaware Bay, and as a matter of fact, equally distributed between upper and lower Bay (Barber and Ford, 1992). Another host or vector as the missing piece of the puzzle would answer both of the above questions.

Finally, the dissemination and transmission of a parasite depends not only on the availability of appropriate hosts, but on the external environment. Temperature seems to play a role in long-term *H. nelsoni* infection cycles, at least in Delaware Bay, and salinity is a clear influence on infection development, but there must be many other prevailing or infrequent events that we have not yet considered that affect the *H. nelsoni* life cycle.

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Table 1. Dates on which zooplankton samples were collected.

1993	1994
13-Apr	11-Apr
28-Apr	20-Apr
6-May	3-May
10-May	23-May
17-May	1-Jun
25-May	7-Jun
2-Jun	22-Jun
7-Jun	24-Aug
14-Jun	13-Sep
21-Jun	20-Sep
22-Jun *	
30-Jun	
30-Jun *	
6-Jul	
13-Jul	
20-Jul	
26-Jul	
26-Aug	

* Night samples

Table 2. Relative abundance of zooplankton in water samples collected in lower Delaware Bay during 1993.

	5/10	5/17	5/25	6/2	6/7	6/14	6/21	6/30	8/26
SHRIMP									
<i>Crangon septemspinosa.</i>		+++	+++	+++					
<i>Mysid sp.</i>			+	++	+	+++	+++	+++	
CLADOCERA (spp.)				+					
OSTRACA (spp.)				+	++	++			
COPEPODS									
<i>Acartia tonsa</i>	+++	+++	+	+	+		++	+	
<i>Pseudodiaptomus pelagicus</i>		+			+				
<i>Temora longicornis</i>		+		+					
<i>Labidocera aestiva</i>	+		++			++		+	
<i>Centropages typicus</i>	+		+						
<i>Oncaea venusta</i>						+			
<i>Metrida longa</i>	+								
AMPHIPODS									
<i>Gammarus sp.</i>							+		
<i>Corophium sp.</i>									+
CRAB LARVAE									
unident.						++	++++	+++	++
<i>Porcelana sp.</i>							+		
EGGS (fish)	++	++	++++	++++	++++	++++	++++	++++	++

+ rare/uncommon
 ++ few
 +++ common
 ++++ abundant

Table 3. Zooplankton abundance (L^{-1}) in plankton samples collected for histological analysis in 1994. Sites are shown in Figure 1.

Date coll.	Site	Larval				Ostracod	Larval	Fish
		Shrimp	Copepods	Amphipods	Cladocera		Crabs	Eggs
11-Apr	EIP	53	1518	0	0	26	0	40
	BAR	700	1090	0	80	40	0	0
	DPW	1739	419	0	106	102	0	0
	UBS	147	320	0	25	0	0	0
15-Apr	EIP	59	5630	0	0	0	0	0
	BAR	535	3693	0	79	36	0	327
	DPW	733	3406	0	182	50	0	56
	UBS	847	2500	0	0	8	0	0
3-May	EIP	1076	2640	0	0	0	0	56
	BAR	610	490	0	0	30	0	150
	DPW	1353	548	0	0	2	0	310
	UBS	440	2190	0	0	0	0	45
23-May	EIP	0	1065	0	0	0	0	1760
	BAR	72	1208	0	0	0	0	424
	DPW	1264	1432	0	0	0	0	208
	UBS	130	429	0	0	0	0	13
1-Jun	EIP	1720	1242	0	0	0	0	0
	BAR	552	112	0	0	0	0	2128
	DPW	178	1297	0	0	0	0	9900
	UBS	336	8	0	0	0	0	860
8-Jun	EIP	180	500	0	0	0	370	250
	BAR	7	92	0	0	0	7	9900
	DPW	264	1531	0	0	0	343	9900
	UBS	440	90	0	0	0	210	3890
22-Jun	EIP	114	7	0	0	0	211	62
	BAR	64	2	0	0	0	6000	36
	DPW	203	30	0	0	0	1000	3080
	UBS	6	4	0	0	0	1500	1016
24-Aug	EIP	0	8	128	0	0	264	0
	BAR	0	0	76	0	0	851	0
	DPW	0	5	160	0	0	3220	16
	UBS	13	13	0	0	0	2508	990
13-Sep	EIP	0	20	50	0	0	40	0
	BAR	4	0	0	0	0	40	0
	DPW	0	10	0	0	0	306	0
	UBS	297	33	7	0	0	139	7
20-Sep	EIP	0	7	13	0	0	67	0
	BAR	0	4	0	0	0	264	0
	DPW	11	114	0	0	0	604	11
	UBS	66	92	0	0	0	26	686

Table 4.1 Small bivalves and other benthic species collected and examined histologically during 1993 and 1994. Location codes on Figure 2.

Date	Loc.	# Exam.	PARASITES				INGESTED HAPLOSPORIDAN SPORES			
			Bucepha- lus	Other Trem & Cestodes	% Buceph.	%Other Trem & Cestodes	Ind w/ spores	Total lg spores	Total sm spores	% Ind w/ spores
BIVALVES										
<i>Tellina agius & versicolor</i>										
5/4/93	CS	14	1	4	7%	29%	1	1	0	7%
5/28/93	CS	105	1	5	1%	5%	19	18	2	18%
6/11/93	CS	57	0	14	0%	25%	16	21	0	28%
6/29/93	CS	125	1	35	1%	28%	9	8	1	7%
7/14/93	CS	47	0	22	0%	47%	0	0	0	0%
7/27/93	CS	58	2	23	3%	40%	1	0	1	2%
8/11/93	CS	13	0	6	0%	46%	0	0	0	0%
8/25/93	CS	29	0	7	0%	24%	0	0	0	0%
5/18/94	CS	27	0	0	0%	0%	4	3	1	15%
5/23/94	UBS	2	0	0	0%	0%	0	0	0	0%
6/8/94	CS	10	0	1	0%	10%	1	1	0	10%
6/21/94	CS	23	0	8	0%	35%	3	3	0	13%
7/5/94	CS	22	0	17	0%	77%	2	2	0	9%
7/19/94	CS	23	0	17	0%	74%	0	0	0	0%
8/1/94	CS	4	1	4	25%	100%	0	0	0	0%
8/15/94	CS	8	0	2	0%	25%	1	1	0	13%
8/30/94	CS	12	0	3	0%	25%	4	3	1	33%
9/15/94	CS	7	0	2	0%	29%	1	1	0	14%
TOTALS		586	6	170	1%	29%	62	62	6	11%
<i>Gemma gemma</i>										
6/11/93	CS	13	0	0	0%	0%	0	0	0	0%
6/29/93	CS	88	1	3	1%	3%	0	0	0	0%
7/14/93	CS	7	0	1	0%	14%	0	0	0	0%
7/27/93	CS	1	0	0	0%	0%	0	0	0	0%
8/11/93	CS	1	0	0	0%	0%	0	0	0	0%
8/1/94	CS	25	0	1	0%	4%	0	0	0	0%
8/30/94	CS	6	0	0	0%	0%	0	0	0	0%
TOTALS		141	1	5	1%	4%	0	0	0	0%
<i>Haminea solitaria</i>										
8/11/93	CS	19	0	5	0%	26%	0	0	0	0%
8/25/93	CS	12	0	4	0%	33%	0	0	0	0%
TOTALS		31	0	9	0%	29%	0	0	0	0%

Table 4.2 Small bivalves and other benthic species collected and examined histologically during 1993 and 1994. Location codes on Figure 2.

cc## INDDate	Loc.	# Exam.	PARASITES				INGESTED HAPLOSPORIDAN SPORES				
			Bucepha- lus	Other Trem & Cestodes	% Buceph	%Other Trem & Cestodes	Ind w/ spores	Total Ing sp	Total Sm spores	% Ind w/ w/ spores	
BIVALVES											
<i>Ensis directus</i>											
6/11/93	CS	3	0	2	0%	67%	0	0	0	0%	
6/29/93	CS	21	0	6	0%	29%	0	0	0	0%	
7/14/93	CS	38	0	3	0%	8%	0	0	0	0%	
7/27/93	CS	54	0	10	0%	19%	0	0	0	0%	
8/11/93	CS	4	0	4	0%	100%	0	0	0	0%	
8/25/93	CS	5	0	1	0%	20%	0	0	0	0%	
4/20/94	CS	20	0	20	0%	100%	0	0	0	0%	
6/21/94	CS	5	0	5	0%	100%	0	0	0	0%	
7/5/94	CS	2	0	2	0%	100%	0	0	0	0%	
8/15/94	CS	4	0	3	0%	75%	0	0	0	0%	
9/15/94	CS	1	0	0	0%	0%	0	0	0	0%	
TOTALS		157	0	56	0%	36%	0	0	0	0%	
<i>Mullinea lateralis</i>											
5/28/93	CS	2	0	2	0%	100%	0	0	0	0%	
6/11/93	CS	1	0	0	0%	0%	0	0	0	0%	
6/29/93	CS	2	0	2	0%	100%	0	0	0	0%	
7/14/93	CS	1	0	1	0%	100%	0	0	0	0%	
7/27/93	CS	11	0	2	0%	18%	0	0	0	0%	
8/3/93	CS	9	0	0	0%	0%	2	3	3	22%	
8/11/93	CS	1	0	1	0%	100%	0	0	0	0%	
8/25/93	CS	1	0	1	0%	100%	0	0	0	0%	
7/5/94	CS	6	0	3	0%	50%	0	0	0	0%	
8/1/94	CS	3	0	0	0%	0%	0	0	0	0%	
8/30/94	CS	13	1	4	8%	31%	1	1	0	8%	
9/15/94	CS	6	0	3	0%	50%	0	0	0	0%	
TOTALS		56	1	19	2%	34%	3	4	3	5%	
<i>Anadara ovalis</i>											
8/1/94	CS	4	0	0	0%	0%	0	0	0	0%	
<i>Tagelus plebeius</i>											
4/20/94	CS	4	0	4	0%	100%	0	0	0	0%	
8/30/94	CS	2	0	0	0%	0%	1	1	0	50%	
TOTALS		6	0	4	0%	67%	1	1	0	17%	
<i>Nucula proxima</i>											
5/23/94	UBS	13	0	0	0%	0%	0	0	0	0%	
<i>Lyonsia hyalina</i>											
5/23/94	UBS	2	0	0	0%	0%	0	0	0	0%	

Table 4.3 Small bivalves and other benthic species collected and examined histologically during 1993 and 1994. Location codes on Figure 2.

				PARASITES				INGESTED HAPLOSPORIDAN SPORES			
				Bucepha	Other Trem & Cestodes	%	%Other Trem & Cestodes	Ind w/ spores	Total Ing Sp	Total Sm Sp	% Ind w/ sp
				lus		Buceph	& Cestodes				
POLYCHAETES											
<i>Diopatra cuprea</i>											
8/1/94	CS	2	0	0	0%	0%	0	0	0	0%	
8/15/94	CS	14	0	3	0%	0%	0	0	0	0%	
TOTALS		16	0	3	0%	19%	0	0	0	0%	
<i>Glycera dibranchiata</i>											
4/20/94	CS	4	0	0	0%	0%	0	0	0	0%	
6/18/94	CS	2	0	0	0%	0%	0	0	0	0%	
6/28/94	CS	5	0	0	0%	0%	0	0	0	0%	
7/5/94	CS	2	0	0	0%	0%	0	0	0	0%	
8/1/94	CS	1	0	0	0%	0%	0	0	0	0%	
8/15/94	CS	1	0	0	0%	0%	0	0	0	0%	
TOTALS		15	0	0	0%	0%	0	0	0	0%	
<i>Hydroides diathus & Filograna implexa</i>											
5/26/94	UBS	132	0	0	0%	0%	0	0	0	0%	
<i>Platynereis dumerilii</i>											
5/18/94	CS	3	0	0	0%	0%	0	0	0	0%	
6/28/94	CS	3	0	0	0%	0%	0	0	0	0%	
7/5/94	CS	2	0	0	0%	0%	0	0	0	0%	
TOTALS		8	0	0	0%	0%	0	0	0	0%	
NEMERTEANS											
<i>Cerebratulus lacteus</i>											
6/8/94	CS	1	0	0	0%	0%	0	0	0	0%	
6/21/94	CS	1	0	0	0%	0%	0	0	0	0%	
7/5/94	CS	1	0	0	0%	0%	0	0	0	0%	
8/1/94	CS	1	0	0	0%	0%	0	0	0	0%	
8/15/94	CS	5	0	0	0%	0%	0	0	0	0%	
8/30/94	CS	1	0	0	0%	0%	0	0	0	0%	
9/15/94	CS	2	0	0	0%	0%	0	0	0	0%	
TOTALS		12	0	0	0%	0%	0	0	0	0%	
ANEMONES											
<i>Actinothoe modesta & Edwardsia elegans</i>											
4/20/94	CS	6	0	0	0%	0%	0	0	0	0%	
6/8/94	CS	1	0	0	0%	0%	0	0	0	0%	
6/21/94	CS	3	0	0	0%	0%	0	0	0	0%	
7/5/94	CS	2	0	0	0%	0%	0	0	0	0%	
8/1/94	CS	2	0	0	0%	0%	0	0	0	0%	
8/15/94	CS	7	0	0	0%	0%	0	0	0	0%	
TOTALS		21	0	0	0%	0%	0	0	0	0%	
BARNACLES											
<i>Balanus sp.</i>											
9/13/94	EI	15	0	5	0%	33%	0	0	0	0%	

Table 5A. Comparison of prevalence data in preliminary (fresh) and final (combined fresh/tissue section) examinations of 1992 set collected in 1993

COLL. DATE	N		PREVALENCE*					ALL SPORULATING INFECTIONS
			ALL INFECTIONS	LIGHT INF.	ADV INF	SPOR. INF	ADV W/ SPORES	
5/12	30	PRELIMINARY	0%	0%	0%	0%	0%	0%
5/12		FINAL	13%	75%	25%	0%	0%	0%
5/25	32	PRELIMINARY	0%	0%	0%	0%	0%	0%
5/25		FINAL	0%	0%	0%	0%	0%	0%
6/4	30	PRELIMINARY	0%	0%	0%	0%	0%	0%
6/4		FINAL	0%	0%	0%	0%	0%	0%
6/18	31	PRELIMINARY	0%	0%	0%	0%	0%	0%
6/18		FINAL	3%	100%	0%	0%	0%	0%
6/23	28	PRELIMINARY	4%	0%	100%	0%	0%	0%
6/23		FINAL	7%	50%	50%	0%	0%	0%
7/1	29	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/1		FINAL	3%	100%	0%	0%	0%	0%
7/7	25	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/7		FINAL	0%	0%	0%	0%	0%	0%
7/14	26	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/14		FINAL	0%	0%	0%	0%	0%	0%
7/21	27	PRELIMINARY	4%	0%	0%	0%	0%	0%
7/21		FINAL	7%	0%	0%	0%	0%	0%
7/28	29	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/28		FINAL	3%	0%	0%	0%	0%	0%
8/6	30	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/6		FINAL	7%	0%	0%	0%	0%	0%
8/18	32	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/18		FINAL	6%	0%	0%	0%	0%	0%
9/2	24	PRELIMINARY	0%	0%	0%	0%	0%	0%
9/2		FINAL	0%	0%	0%	0%	0%	0%
9/14	24	PRELIMINARY	0%	0%	0%	0%	0%	0%
9/14		FINAL	29%	86%	14%	0%	0%	0%

*Categories "ALL INFECTIONS" and "ALL SPORULATING INFECTIONS" are based on all oysters examined; categories "LIGHT", "ADVANCED", and SPORULATING" are based on infected oysters only; category "ADVANCED W/ SPORES" is based on advanced infections only.

Table 5B. Comparison of prevalence data in preliminary (fresh) and final (combined fresh/tissue section) examinations of 1993 AND 1994 set collected in 1994.

COLL. DATE	N	1993 SET	PREVALENCE					ALL SPORULATING INFECTIONS
			ALL INFECTIONS	LIGHT INF.	ADV INF	SPOR. INF	ADV W/ SPORES	
4/21	96	PRELIMINARY	0%	0%	0%	0%	0%	0%
4/21		FINAL	0%	0%	0%	0%	0%	0%
5/11	17	PRELIMINARY	0%	0%	0%	0%	0%	0%
5/11		FINAL	0%	0%	0%	0%	0%	0%
5/13	22	PRELIMINARY	0%	0%	0%	0%	0%	0%
5/13		FINAL	0%	0%	0%	0%	0%	0%
5/18	82	PRELIMINARY	0%	0%	0%	0%	0%	0%
5/18		FINAL	0%	0%	0%	0%	0%	0%
6/7	122	PRELIMINARY	0%	0%	0%	0%	0%	0%
6/7		FINAL	1%	0%	0%	0%	0%	0%
6/23	134	PRELIMINARY	0%	0%	0%	0%	0%	0%
6/23		FINAL	0%	0%	0%	0%	0%	0%
7/5	55	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/5		FINAL	2%	0%	0%	0%	0%	0%
7/19	46	PRELIMINARY	0%	0%	0%	0%	0%	0%
7/19		FINAL	4%	0%	0%	0%	0%	0%
8/1	75	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/1		FINAL	1%	0%	0%	0%	0%	0%
8/15	66	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/15		FINAL	2%	0%	0%	0%	0%	0%
8/30	25	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/30		FINAL	4%	0%	0%	0%	0%	0%
1994 SET								
8/30	81	PRELIMINARY	0%	0%	0%	0%	0%	0%
8/30		FINAL	0%	0%	0%	0%	0%	0%
9/13	125	PRELIMINARY	0%	0%	0%	0%	0%	0%
9/13		FINAL	0%	0%	0%	0%	0%	0%
9/15	113	PRELIMINARY	0%	0%	0%	0%	0%	0%
9/15		FINAL	0%	0%	0%	0%	0%	0%
9/25	60	PRELIMINARY	0%	0%	0%	0%	0%	0%
9/25		FINAL	0%	0%	0%	0%	0%	0%
10/7	76	PRELIMINARY	0%	0%	0%	0%	0%	0%
10/7		FINAL	0%	0%	0%	0%	0%	0%
10/11	38	PRELIMINARY	0%	0%	0%	0%	0%	0%
10/11		FINAL	0%	0%	0%	0%	0%	0%
10/25	34	PRELIMINARY	0%	0%	0%	0%	0%	0%
10/25		FINAL	0%	0%	0%	0%	0%	0%

*Categories "ALL INFECTIONS" and "ALL SPORULATING INFECTIONS" are based on all oysters examined; categories "LIGHT", "ADVANCED", and SPORULATING" are based on infected oysters only; category "ADVANCED W/ SPORES" is based on advanced infections only.

Table 6. Summary, by year and year class, of the maximum total infection prevalence, maximum prevalence of advanced infections, and maximum prevalence of spores of *Haplosporidium nelsoni*, along with the dates at which the maxima occurred.

Sample year	Year class	Total examined	Number of dates	Max prevalence	Date	Max adv prevalence	Date	Max spore prevalence	Date
1988	1987	195	8	35%	15-Jul	30%	31-May	25%	20-Jun
	1988	181	8	no data	no data	no data	no data	28%	7-Dec
1989	1988	392	15	15%	30-Aug	5%	30-Aug	5%	30-Aug
1990	1989	1086	11	15%	17-Jun	13%	21-Jun	6%	27-Jun
	1990	701	4	2%	9-Oct	1%	17-Sep	0%	
1991	1990	3249	18	21%	8-Jul	4%	9-Jun	4%	9-Jun
	1991	1333	7	5%	13-Dec	1%	25-Nov	0%	
1992	1991	3102	19	8%	21-May	7%	21-May	3%	21-May
	1992	188	2	0%		0%		0%	
1993	1992	397	14	29%*	14-Sep	14%	14-Sep	0%	
1994	1993	740	11	4%	19-Jul	0%		0%	
	1994	527	7	0%		0%		0%	

* Prevalence during yearling stage; maximum as spat was 13% in May 1993 (see Table 5A).

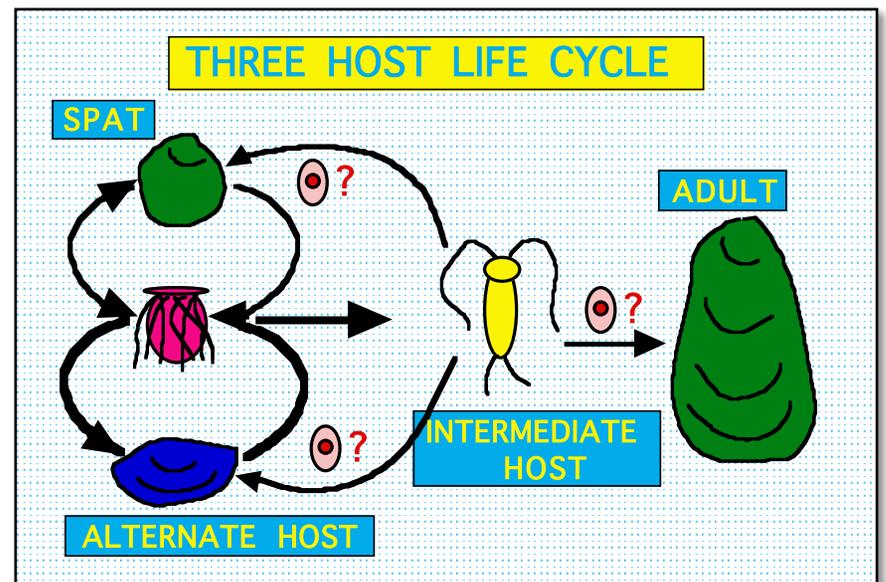
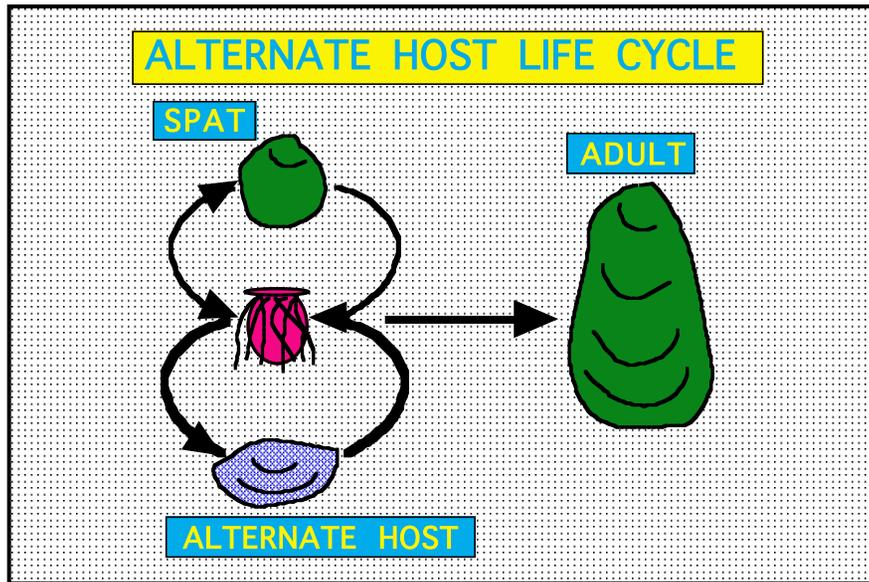
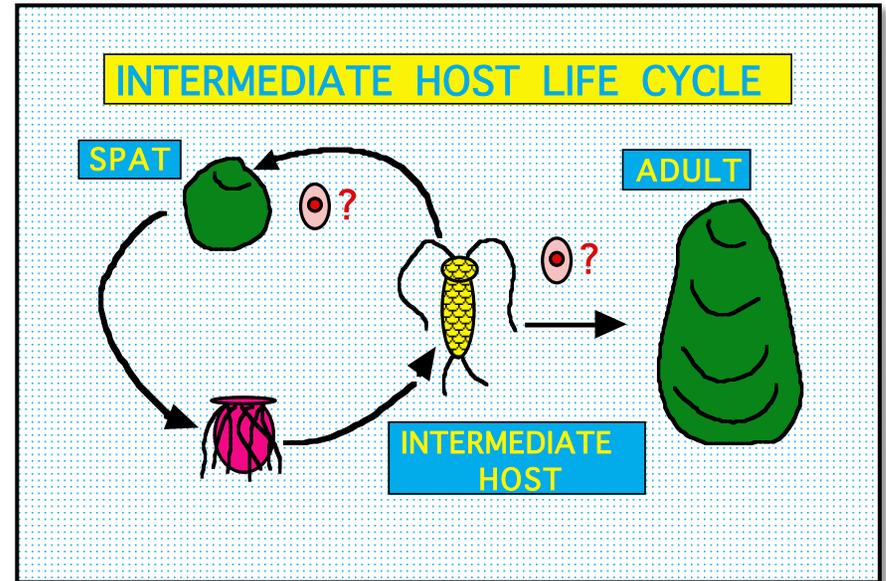
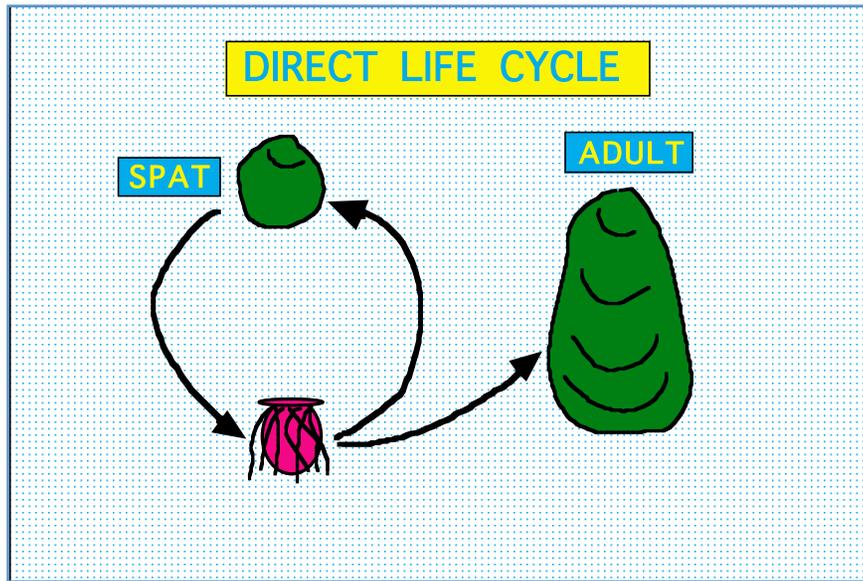


Figure 1. Schematic diagrams of various life cycle hypotheses for the oyster parasite *Haplosporidium nelsoni*.

Figure 2. Zooplankton ● and small bivalve ○ sampling stations in lower Delaware Bay.

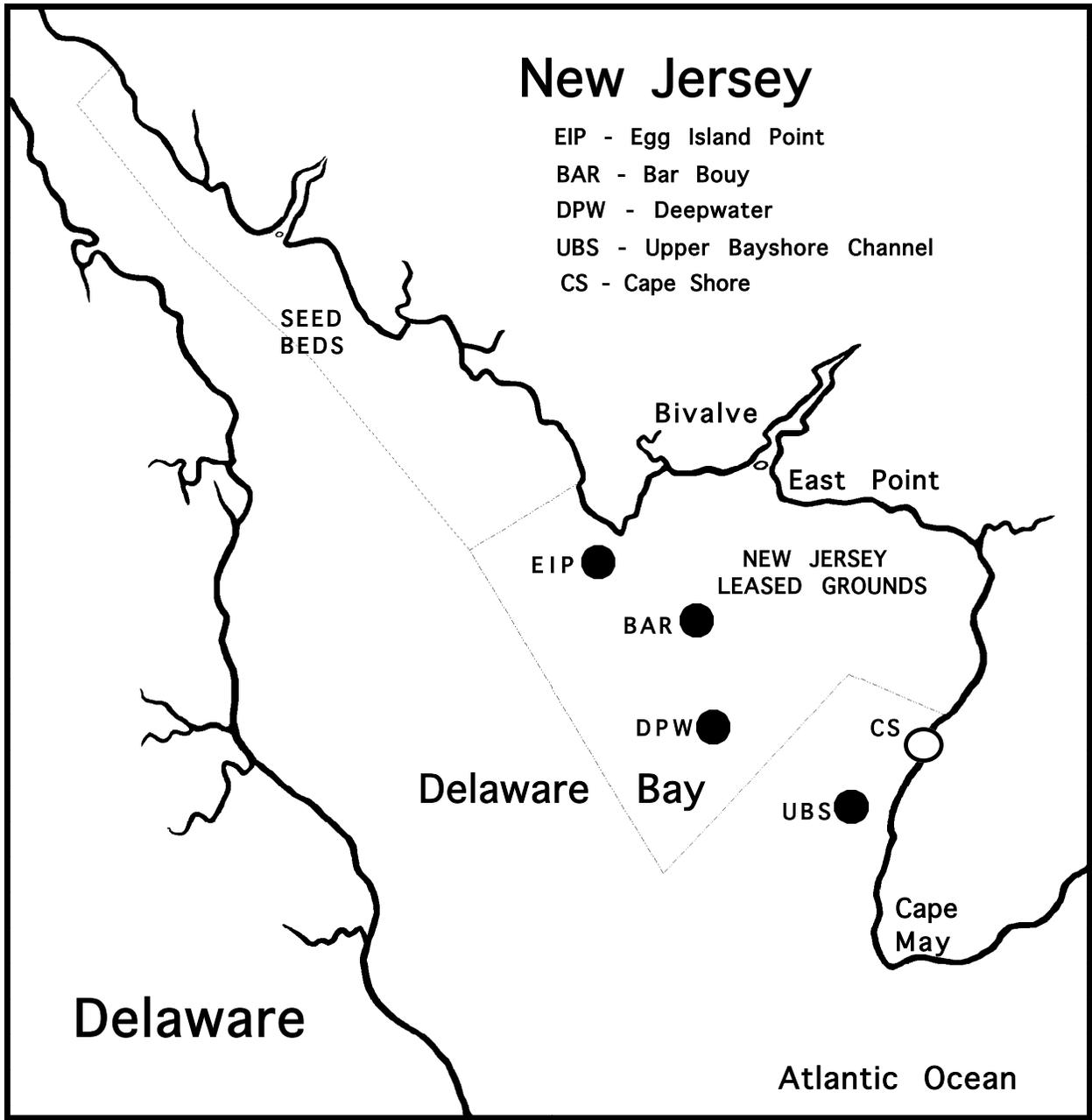


Figure 3A. Mean annual prevalence of *Haplosporidium nelsoni* on Delaware Bay planted ground/lower seed beds and mean winter (December - March) air temperature at Millville, NJ, about 12 miles north. Mean annual prevalence was obtained by averaging late fall and late spring prevalences on planted oysters (mostly first year) except in years in which no plantings were made, when post-first year plants were used - or in 1992, 1993, and 1994, when lower seed bed oysters were used.

Figure 3B. Mean annual prevalence of *Haplosporidium nelsoni* on Delaware Bay planted ground/lower seed beds, and mean annual abundance of oyster spat on lower seed beds.

Figure 3C. Mean annual prevalence of *Haplosporidium nelsoni* on Delaware Bay planted ground/lower seed beds, and reported landings of oysters from New Jersey planted grounds.

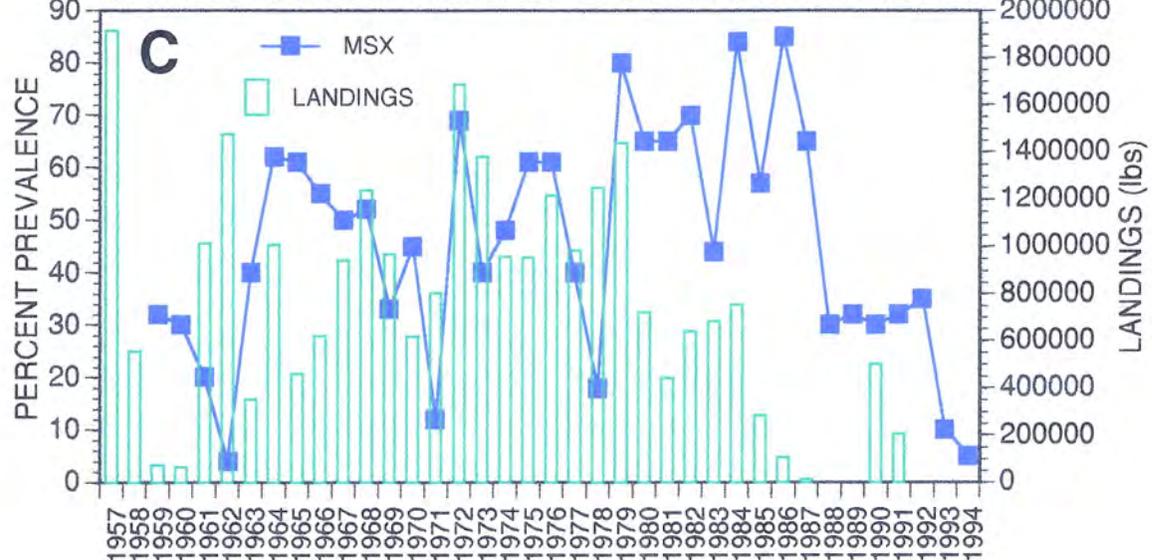
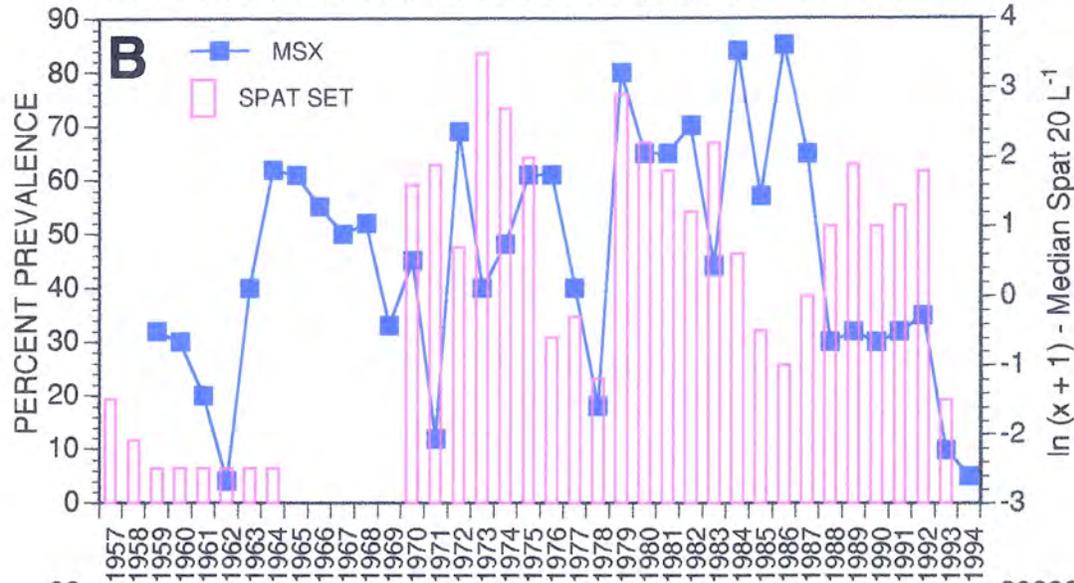
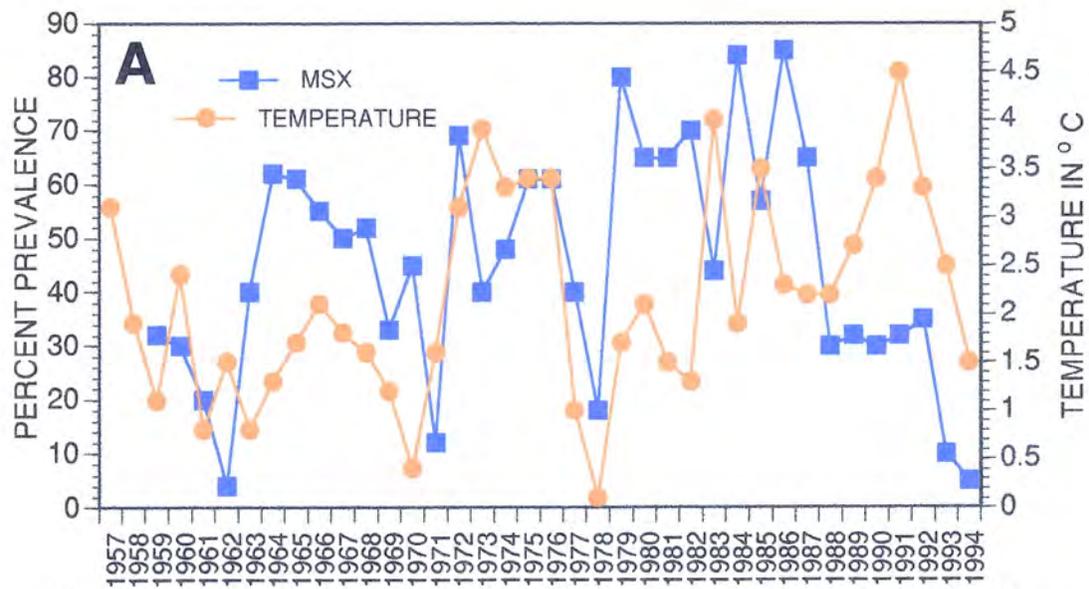


Figure 4A. TEM photomicrograph of a mature *Haplosporidium nelsoni* spore. Maturation is complete when the spore wall becomes thick and continuous around the sporoplasm, and the extraspore cytoplasm lyses showing the filaments (arrows) arising from the wall surface and lid.

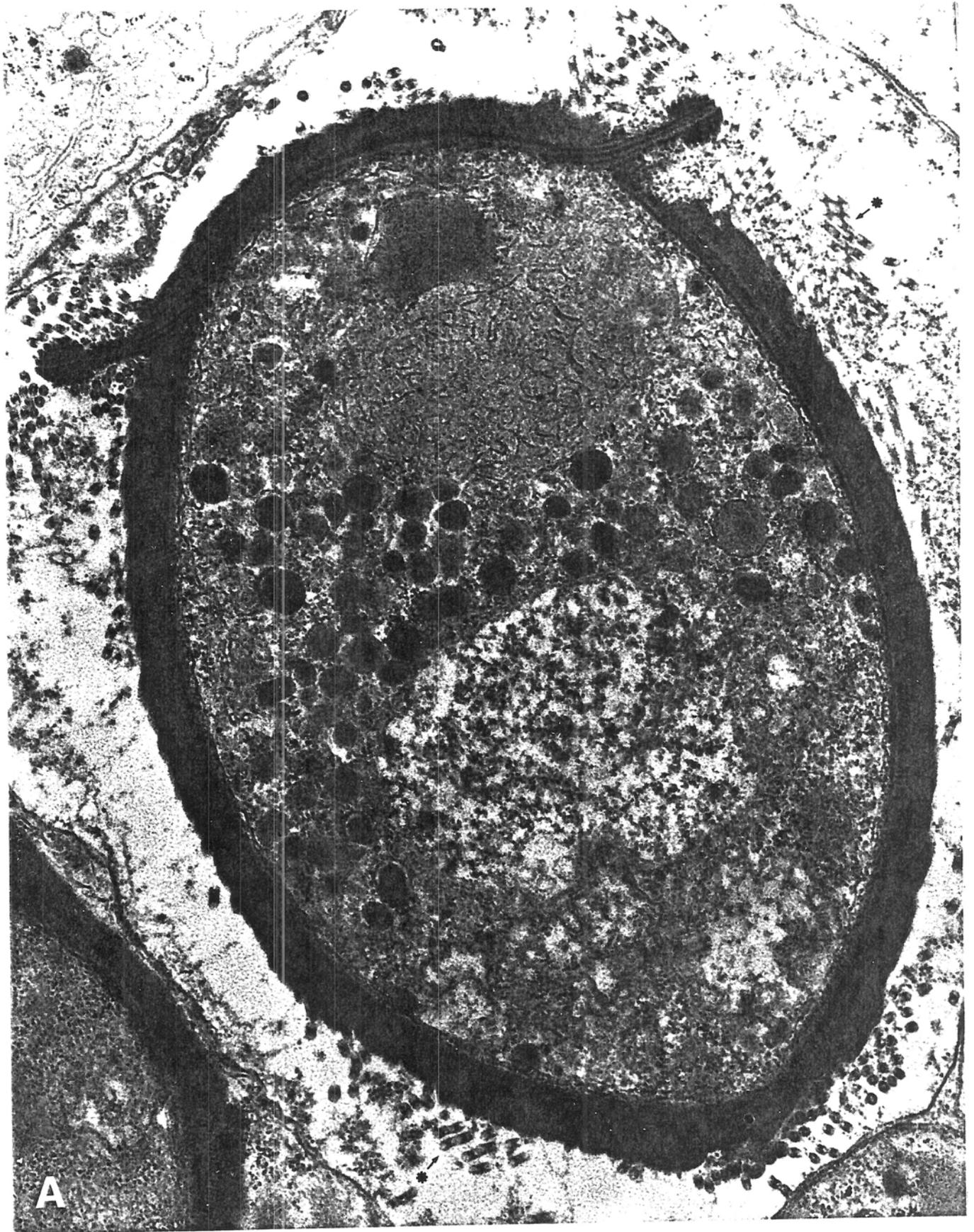


Figure 4B. Low magnification TEM photomicrograph of a *Haplosporidium nelsoni* sporocyst showing the onset of spore wall formation around each nucleus in a multinucleated plasmodia.

Figure 4C. High magnification TEM photomicrograph of an early stage spore, showing incomplete spore wall and lid formation.

Figure 4D. SEM photomicrograph of an fully formed, but immature spore, before the onset of lysis of the extraspore cytoplasm. Filaments are visible within the cytoplasm.

Figure 4E. SEM photomicrograph of a section through a sporocyst. Fully exposed filaments are visible on the mature spore (arrow) after complete lysis of the extraspore cytoplasm.

