1988

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American Fisheries Society Special Publication 18:5–22, 1988

Uncertainties and Speculations about the Life Cycle of the Eastern Oyster Pathogen *Haplosporidium nelsoni* (MSX)

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Abstract.—For 30 years, the pathogen *Haplosporidium nelsoni* (MSX) has been causing serious mortalities of eastern oysters *Crassostrea virginica* in the Delaware and Chesapeake bays of the eastern USA. Its life cycle is largely unknown, and methods for control are wanting. Breeding of resistant eastern oyster strains, at this time, offers the best hope for some degree of control of the disease. Although haplosporidians are known by their spores, controlled transmission, with one possible exception, has not been achieved in any of the 30 recognized species. *Haplosporidium nelsoni* rarely sporulates in eastern oysters, and this and other observations led to early speculation that another host probably exists. Based on apparent effects of environment on *H. nelsoni* abundance, particularly in Delaware Bay, we are attempting to profile a hypothetical alternate host. Recent increases in abundance and activity of MSX in Chesapeake and Delaware bays and in Virginia rivers are associated with droughts, but this is not true elsewhere. Distribution of *H. nelsoni* along the Atlantic coast appears to have spread slowly southward from Chesapeake and Delaware bays, but is not identified with eastern oyster mortalities in southern localities. To the north of the original epizootics, *H. nelsoni* has been scattered along the Long Island, Connecticut, and Massachusetts coasts for 30 years or more, usually without occurrence of serious mortalities of eastern oysters. Foci of increasing *H. nelsoni* activity with mortality, over the past 5 years, are of great concern in these areas. Careful examination of changes in these northern areas may contribute significantly to our understanding of the relationships between *H. nelsoni* and its environment.

In spring 1957, the disease of the eastern oyster *Crassostrea virginica* caused by *Haplosporidium nelsoni* (commonly called MSX) appeared without warning in Delaware Bay, USA (Haskin et al. 1966; Ford and Haskin 1982). It spread rapidly throughout the bay, killing 90–95% of all eastern oysters on the planting grounds and 60% on the seed beds within 3 years (Figures 1, 2). Two years later, in spring 1959, *H. nelsoni* was associated with serious eastern oyster mortalities in the high-salinity areas of Chesapeake Bay (Andrews and Wood 1967); 30–50% of the 122 million kg of eastern oysters in Mobjack Bay–Egg Island areas were destroyed. Within the next 2–3 years, total mortalities reached 90–95% in the lower bay; however, losses on the James River seedbeds were negligible (Andrews 1964). The disease has not abated in virulence or intensity since that time. It spread far up the Chesapeake Bay during periods of drought and is now causing mortalities in Long Island and New England waters. Resistance to mortality has developed in Delaware Bay native eastern oysters (Haskin and Ford 1979) and in wild stocks in the lower Chesapeake Bay (Andrews 1968; Farley 1975), but, during years of intensive activity of the pathogen, native resistant eastern oysters in both estuaries have been overwhelmed. Current understanding of survival mechanisms is reviewed by Ford (1988, this volume).

Haplosporidium nelsoni has many peculiarities (Andrews 1982, 1984b). Its life cycle is not known, although patterns of infection and mortality are well known as to timing and duration (Andrews 1966; Ford and Haskin 1982). The pathogen has not been cultured, and controlled transmission of infection has not been achieved. Furthermore, the spore stage is known (Couch et al. 1966; Perkins 1968) but is rarely found in eastern oysters (Andrews 1979; Ford and Haskin 1982), and the source of infective stages remains unknown. This lack of information has led to much speculation about other hosts (Ford and Haskin 1982; Andrews 1984a, 1984b; Burreson 1988, this volume), but none has been found. The wide dispersion of infective materials throughout the high-salinity areas of both bays, whether large

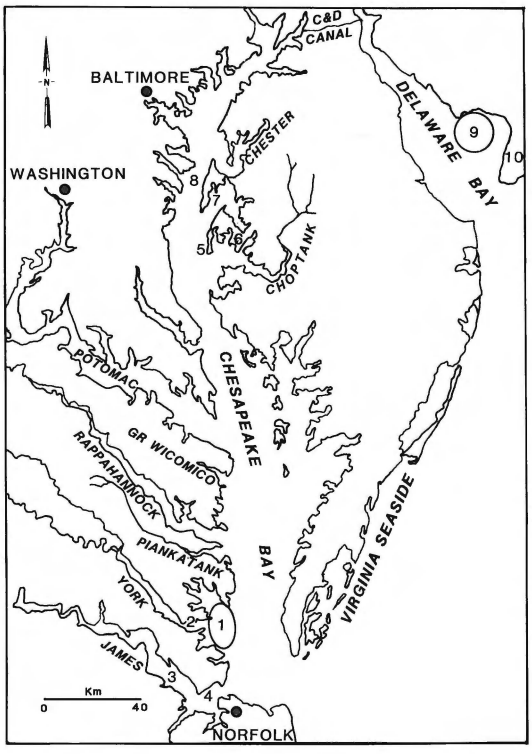


FIGURE 1.—Map of Chesapeake and Delaware bays. 1 = Mobjack Bay–Egg Island area of 1959 MSX outbreak; 2 = Virginia Institute of Marine Science Laboratory; 3 = James River seed beds; 4 = Hampton Bar; 5 = Tilghman, Maryland; 6 = Oxford Laboratory; 7 = Eastern Bay; 8 = Bay Bridge; 9 = MSX outbreak area in 1957; 10 = Rutgers Cape Shore Laboratory.

eastern oyster populations are present or absent, and the apparent lack of contagious transfer among eastern oysters strengthen the speculations about the existence of an alternate or reservoir host for *H. nelsoni*. Alternate hosts have not been demonstrated for any haplosporidian species (Andrews 1984a, 1984b), nor has any controlled transmission of a haplosporidian parasite disease been reported, with one possible exception (Barrow 1965).

The annual cycle of infection and mortality is basically the same in both Chesapeake and Delaware bays, although there are some differences in timing and detail of interest to the eastern oyster industry. Some of these differences are also of interest because they lead to questions of *H. nelsoni* dosage and of influences of temperature and salinity on host–parasite reactions. In any event, they lead to speculations that can be explored.

Data accumulated over the past 30 years have documented epizootiological factors and opinions on life cycle. Our purpose is not to review the enormous literature on *H. nelsoni* disease, but to review some of the data in general, emphasize several important gaps in our knowledge, and speculate on explanations of some of the unknowns and uncertainties. When interpretations and data analyses differ for Delaware and Chesapeake bays, alternative positions will be given.

Recent History of *H. nelsoni*

The pressure of *H. nelsoni* on the eastern oyster resources of the U.S. east coast has not only continued but has intensified since the early outbreaks. In the early outbreaks and rapid spread, the pathogen did not kill eastern oysters on the uppermost beds in both Delaware and Chesapeake bays and in tributary rivers and creeks. Low salinities were probably setting the limits for *H. nelsoni* penetration. Since then, extensive studies of infection and mortality patterns along the salinity gradients in Delaware Bay, Chesapeake Bay, and the James River have established that: (a) above a salinity of 20‰, *H. nelsoni* is not inhibited in its activities; (b) below 15‰, infections are generally rare, and development is inhibited; and (c) below 10‰, *H. nelsoni* cannot survive in eastern oysters (Andrews 1964, 1983; Haskin and Ford 1982; Ford 1985; Ford and Haskin 1988). Therefore, in examining areas of changing *H. nelsoni* activity, we should ask first if there has been a corresponding change in salinity regimes. In the drought of the mid 1960s, *H. nelsoni* invaded Maryland eastern oyster beds and moved up Chesapeake Bay as far as Tilghman, Maryland (Farley 1975). For the first time, it killed oysters on the James River seedbeds and was active in Delaware Bay on the uppermost productive seedbed (Figures 1, 2). All three of these examples of expanding range were predictable because increased salinities occurred in areas where low salinities previously had restricted the parasite. At the end of the drought, *H. nelsoni* retreated to its earlier boundaries coincident with the return of normal river flows. Occurrences of intensified *H. nelsoni* activity that cannot be explained by increased salinity are puzzling.

Increased *H. nelsoni* activity, culminating in increased eastern oyster mortality, may be influenced by factors such as temperatures favorable for the development of critical stages in the *H. nelsoni* life cycle, for host response (Ford 1988), or for the release of infective stages. The influence may be on the magnitude and the timing of infective dosages available to eastern oysters. Many

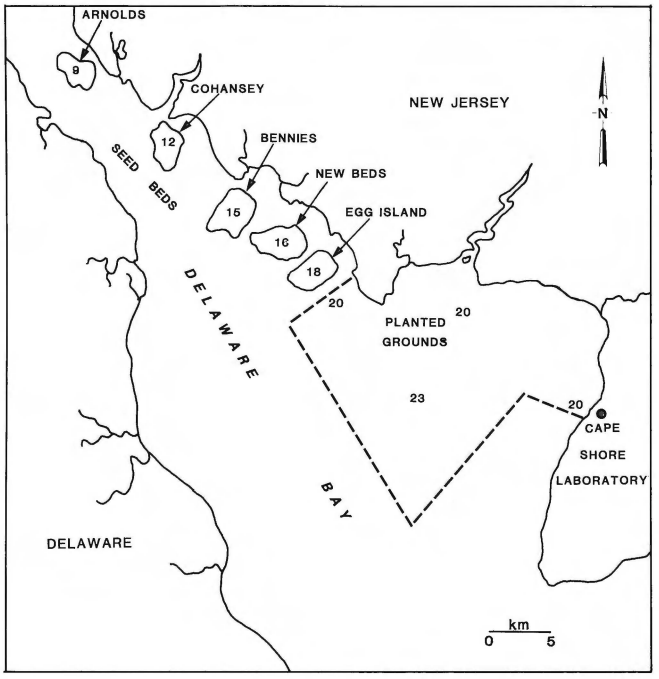


FIGURE 2.—Delaware Bay showing major eastern oyster seedbeds, planting grounds, and location of Rutgers University Cape Shore Laboratory. Numbers shown indicate bottom salinities (‰) at mean river flows.

factors that stress eastern oysters directly may also result in *H. nelsoni*-related mortality when *H. nelsoni* pressure alone would not be fatal. These stresses may include shell repair after damage by enemies such as boring sponges, scavenging crabs, and *Polydora* spp. Noxious algal blooms and failures in phytoplankton production that can reduce eastern oyster condition may make the animals susceptible to disease. Diversion of energy reserves into gametes may also leave less reserves available to meet the immediate energetic demands imposed by *H. nelsoni* disease. These factors underlie proposals to produce triploid east coast eastern oysters to reduce losses due to disease.

Two enzootic areas of recently increased *H. nelsoni* activity not attributable to salinity changes have been closely linked in long-term studies that provide excellent data for assessing *H. nelsoni* activity. One area is on tidal flats in lower Delaware Bay off the Rutgers Cape Shore Laboratory and the other is in the York River near the Virginia Institute of Marine Science Laboratory (VIMS) at Gloucester Point, Virginia (Figure 1). Since spring 1959, we have placed seed of susceptible James River eastern oysters in trays at these two locations in advance of the early summer infection period for *H. nelsoni* (late May–June). Salinities in the two locations are quite similar: Cape Shore, 18–22‰ and up to 26‰ in drought; VIMS, 17–24‰ and up to 25‰ in drought. Live and dead eastern oysters have been carefully monitored for *H. nelsoni* prevalence and intensity. The James River eastern oysters were not previously exposed to *H. nelsoni* and were transplanted as uninfected individuals to both test locations. At Cape Shore and at VIMS, James River imports also served as the highly *H. nelsoni*-susceptible constant controls against which the performance of *H. nelsoni*-resistant strains being developed at these locations could be compared. Details of tray handling and calculation of cumulative mortalities for Cape Shore and VIMS groups were presented elsewhere (Andrews 1968; Haskin and Ford 1979).

The mortality patterns at VIMS for susceptible eastern oysters exposed to *H. nelsoni* in early summer (May and June) are illustrated in Figure 3. Infections usually appeared in July as shown by increased prevalences. Mortality usually began about the first of August, peaked in late August and September, and declined sharply in October and November. Typically, there was a small end-of-winter kill in March or April of eastern oysters

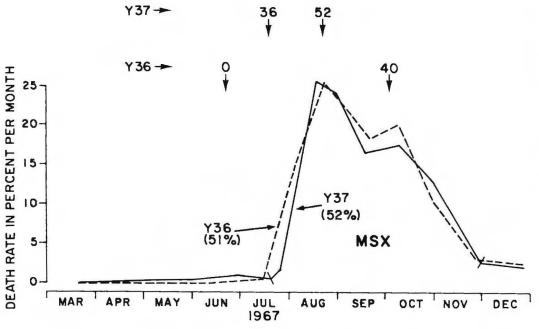


FIGURE 3.—Mortality of, and MSX prevalence in, duplicate lots (trays Y36 and Y37) of MSX-susceptible eastern oysters transplanted from the James River to Gloucester Point in March 1967, before the early-summer infection period for MSX. Percent prevalences of MSX in samples of 25 live eastern oysters are shown above the vertical arrows, which indicate sampling dates. Total MSX-related mortalities of eastern oysters in each lot during mid-July to end November (hash marks) are in parentheses.

with advanced infections. The June to December first-season mortality averaged 52% for the two tray lots of eastern oysters.

The mortality pattern for late-summer lots, that is, lots imported after the first of August, is shown in Figure 4 for VIMS. Late-summer infections usually remained subpatent until spring, but in 1966 they appeared in October. Slight mortality occurred in March and April followed by a June–July peak. Prevalence of the disease was high throughout the winter and spring of 1966 without causing appreciable mortality. The August 1967 mortality shoulder was caused by June 1967 infections; total mortality by the end of the year was nearly 74%.

The Cape Shore mortality patterns for early summer infections were essentially the same as those at VIMS, but the total mortalities were usually greater (Figure 5). Infections in late August or September imports developed more rapidly at Cape Shore than at VIMS and often killed eastern oysters that same fall. At Cape Shore, fall infections (September–October) killed some eastern oysters as early as March, but losses were usually much heavier in June.

First season (June–December) cumulative mortalities at both locations showed both similarities and differences (Figure 5). In the York River, the 3-year peak in 1965–1967 was related to the drought. The decreased mortality in 1972 was attributed to the freshwater input by Hurricane Agnes, and the 1973 low may have indicated a residual salinity effect of that big freshwater discharge.

Cape Shore mortalities were definitely more variable from year to year than those in the York

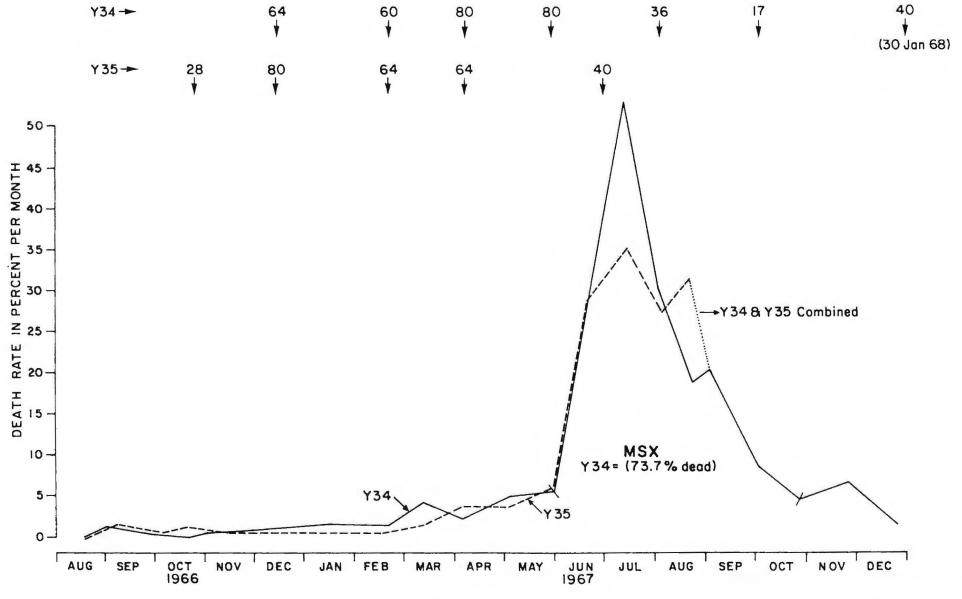


FIGURE 4.—Mortality of, and MSX prevalence in, duplicate lots (trays Y34 and Y35) of eastern oysters transplanted from Deep Water Shoal to Gloucester Point before the late-summer infection period for MSX. Percent prevalences of MSX in samples of 25 live eastern oysters are shown above the vertical arrows, which indicate sampling dates.

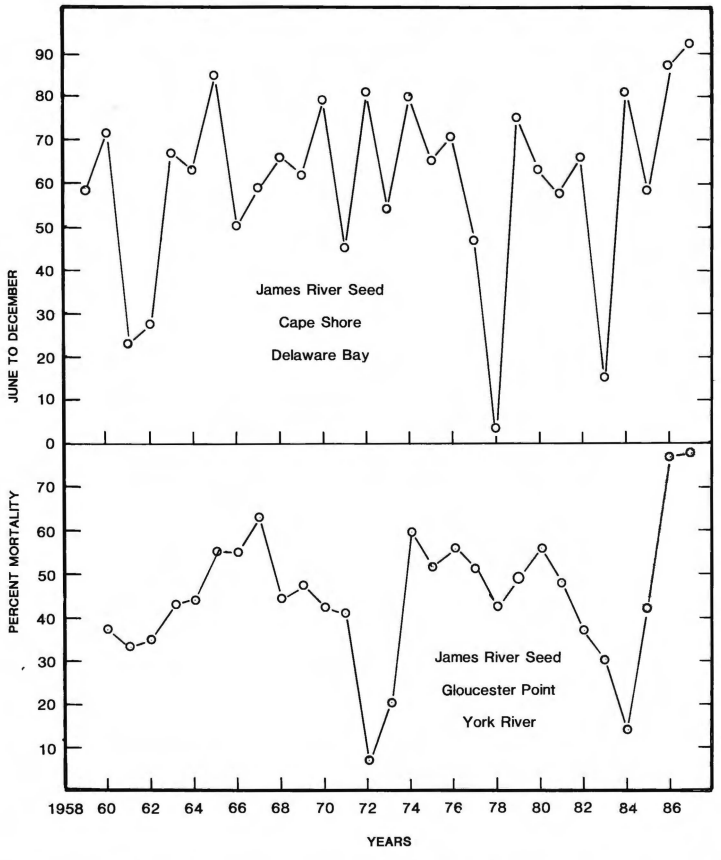


FIGURE 5.—Cumulative mortalities of eastern oysters during June–December at Cape Shore, Delaware Bay, and at Gloucester Point, York River. James River eastern oysters were placed in trays shortly before the MSX infection period.

River. They indicated little or no influence of Delaware River flows, reflecting neither the drought of 1963–1967 nor the eight consecutive years of higher-than-average runoff in 1972–1979. The extremely low mortalities in 1961, 1962, 1971, 1978, and 1983 occurred after unusually cold winters (Ford and Haskin 1982). We speculated that the extremely cold winters influenced *H. nelsoni* activity by killing reservoir hosts from which the infective stages were released (Ford and Haskin 1982).

Low-mortality periods following unusually cold winters have not been apparent in the York River. For the 28 years that records have been kept, the average June–December mortality at VIMS was 42% compared with 58% at Cape Shore. Except for the four cold winters, the Cape Shore mortalities ranged generally from 60 to 80%. Few VIMS mortalities exceeded 60%, and it is clear that selection pressure for survival against *H. nelsoni* was greater at the Cape Shore. Mortalities caused by *H. nelsoni* have persisted in both locations and have intensified dramatically, especially in the last 2 years.

Except for a few years in the early 1960s, Delaware Bay oystermen have continued to plant eastern oysters brought downbay from the seedbeds in May and June. Every year, the Rutgers Shellfish Laboratory has followed a number of these plantings for determination of *H. nelsoni* prevalences and mortalities from time of planting until harvest (Ford and Haskin 1982). June–December mortalities of these planted native eastern oysters for each year were averaged for comparison with the mortalities of James River imports described previously (Figure 6).

As at the Cape Shore tray station, mortalities on the planting grounds obviously did not reflect the periods of drought or high runoff described above. This conclusion leads to speculation that

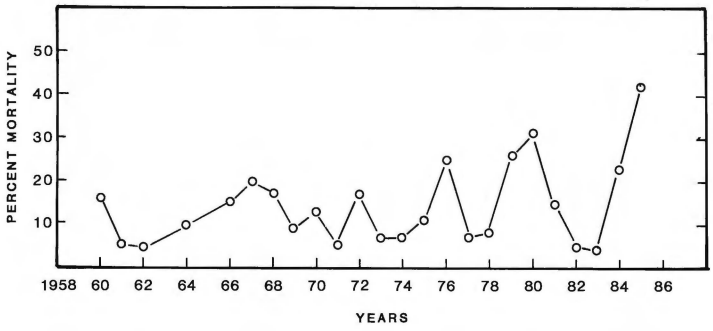


FIGURE 6.—First-season nonpredatory eastern oyster mortality on Delaware Bay planting grounds from 1958 to 1986. Eastern oysters were transplanted from the seedbeds in May and June each year and were monitored on selected grounds until harvested.

there is a variable discharge of infective particles by reservoir hosts that leads to variable mortalities in an ever-favorable salinity regime. Four of the five lowest points on the graph of mortality on planted grounds (Figure 6) coincide with the four lowest points of mortality in the Cape Shore trays (Figure 5), and these points correlate strongly with preceding cold winters. Since 1972, mortality peaks have become more severe after each low-mortality period. The upward trend in Delaware Bay mortalities is remarkably similar to that found for James River seed exposed to *H. nelsoni* on the Virginia seaside (Figure 7).

The highest first-season mortality ever recorded on Delaware Bay planting grounds occurred in 1985 and was related to drought (Figure 6). It was a direct result of intense 1984 *H. nelsoni* infections extending upbay to all seedbeds in the dry fall followed by the lowest winter–spring flows on record in 1984–1985 (Table 1). Normal spring flows probably purge *H. nelsoni* from seedbeds of eastern oysters when eastern oysters begin to feed in mid to late March. With seedbed salinities 3–4‰ higher than usual, this purging did not occur on the lower seedbeds in the spring of 1985, and, for the first time, New Jersey oystermen planted native seed already heavily infected with *H. nelsoni*. In late May 1985, 65–80% of the eastern oysters on the lower seedbeds were systemically infected (Table 2). They died in large numbers over the next 2 months, before the onset of new summer infections. Reduced river flows, with their corresponding salinity increases, are not sufficient of themselves to permit *H. nelsoni* to move farther upbay. An abundance of *H. nelsoni* infective stages must also be present. For example, in June 1980, a severe drought led to low river

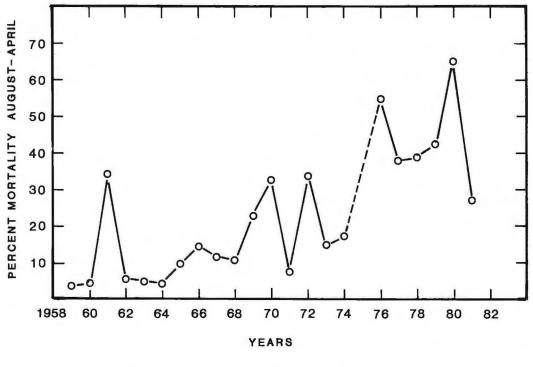
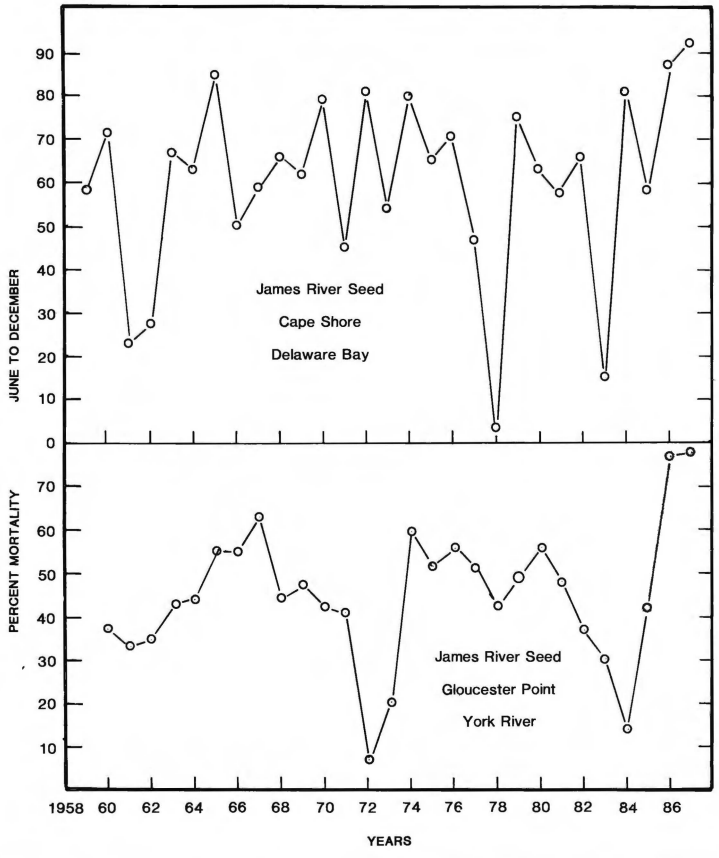


FIGURE 7.—Mortality of eastern oysters due to MSX at Virginia seaside, 1958–1981. James River seed oysters were placed at seaside each year in trays. Mortalities are cumulative for the period August to April.

TABLE 1.—Mean monthly Delaware River flows at Trenton, New Jersey, during the drought of 1984–1985 compared with the long-term means of 1913–1983. (USGS District Engineer 1913–1985.)

Month	Mean flow (m^3/s)	
	1984–1985	1913–1983
Aug	168	169
Sep	104	150
Oct	103	189
Nov	100	291
Dec	197	343
Jan	171	343
Feb	191	365
Mar	273	595
Apr	187	638
May	215	395
Jun	171	252
Jul	132	198
Aug	100	
Sep	262	
Oct	273	
Nov	501	



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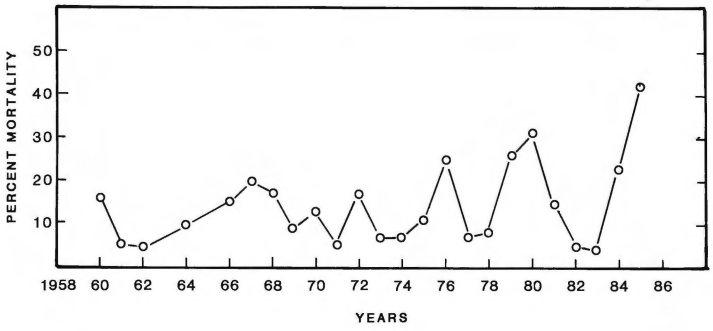

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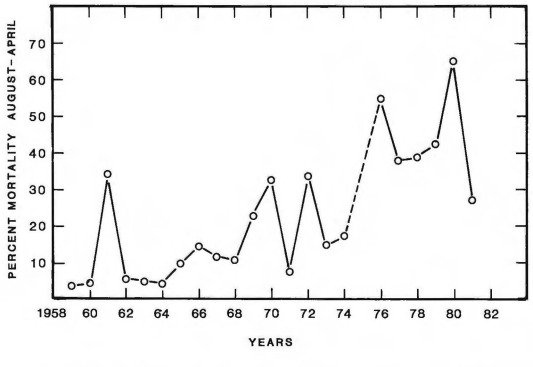

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Sep	262	
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TABLE 2.—*Haplosporidium nelsoni* winter prevalence (%)[a] and cumulative annual nonpredatory mortality (%)[b] of eastern oysters on four Delaware Bay seedbeds, 1980–1986.

	Arnolds bed		Cohansey bed		Bennies bed		Egg Island bed	
Year	Prevalence	Mortality	Prevalence	Mortality	Prevalence	Mortality	Prevalence	Mortality
1980	0	6	10	21	55	26	50	13
1981	10	19	21	14	65	33	45	19
1982	0	7	15	2	25	4	55	5
1983					10[c]	14[c]	25	10
1984	10	11	20	11	65	12	85	26
1985	60	45	50	41	65	46	80	62
1986		13		26	86	37		21

[a] Sample usually taken in December.
[b] July to June.
[c] Sample taken from adjoining bed.

flows that continued below long-term averages through April 1981. River flows through the fall and early winter were substantially lower than in corresponding periods of the 1984–1985 drought when higher *H. nelsoni* prevalences and mortalities occurred on the seedbeds (Table 2). These relationships support the conclusion that an increase in infection pressure is not directly correlated with an increase in salinity.

The Virginia seaside is another monitoring area receiving James River seed imported by VIMS over a period of at least 25 years. The seaside bays have high salinities, and there is no question of restraint of *H. nelsoni* development by low salinity. Over the first 5 or 6 years of import, although a few infections occurred, mortalities associated with *H. nelsoni* were so low (about 5% annually) that a tentative conclusion was reached that salinities of about 30‰ were inhibiting development of *H. nelsoni* (Andrews and Castagna 1978). From about 1969, however, *H. nelsoni*-related mortalities trended upward, though with wide swings, to the 40–60% range (Figure 7). Because a second haplosporidian parasite of eastern oysters, *Haplosporidium costale* (SSO), complicated the seaside picture in spring, the *H. nelsoni*-related mortalities were sorted out for the period from August to April. The upward trend in *H. nelsoni*-related mortality in this area cannot be related to salinity changes. Perhaps *H. nelsoni* was new to the region in late 1958, and, after several years, became abundant enough to cause substantial eastern oyster mortalities. Another possibility is that a reservoir host population was producing and releasing ever-more infective particles.

Expanded Range Along the East Coast of North America

As river runoffs returned to normal levels after the mid-1960s drought, *H. nelsoni* receded from its upbay and uptributary excursions in the Chesapeake and Delaware bays. More recently, two 3-year periods of drought in the mid-Atlantic region (1980–1982 and 1985–1987) have permitted *H. nelsoni* to extend its range even further than in the 1960s and to do great damage to the eastern oyster industries.

Because of the presence of *H. nelsoni*, nearly all eastern oysters in Chesapeake Bay are grown in areas where late-summer salinities do not exceed 18–20‰. Most areas above the mouth of the Rappahannock River (Figure 1) have late spring and early summer salinities less than 15‰, which inhibit or delay *H. nelsoni* infections. As a result, infections are more likely to occur in late summer, after salinities increase. These infections usually do not become patent or serious until spring of the following year. In average or wet years, however, many of these areas have early spring salinities below 10‰, which purge eastern oysters of infections. Successive dry years during 1980–1982 and 1985–1987 have permitted both early- and late-summer infections to proliferate and cause serious mortalities. Late-summer infections are most insidious during dry periods because salinities in the coastal plains estuaries such as the Great Wicomico and Piankatank in Virginia and the Choptank in Maryland (Figure 1) are controlled by the Chesapeake Bay regime and not by local freshwater discharges.

In 1982, an intensive spatfall occurred in Great Wicomico River, but in late summer, native eastern oysters (including the spat) became infected with *H. nelsoni* and died in May–June 1983. This mortality would not have occurred with average salinities, but, in spring 1983, the salinities never fell below 10‰. The winter and spring of 1983 was wetter than average, but three preceding drought years had allowed Chesapeake Bay salinities to

become unusually high, 20‰ at the Bay Bridge in Maryland in the summer of 1982. Higher bay salinities were still controlling salinities in the coastal plain estuaries in spring 1983. In the James and Rappahannock rivers, fall infections also occurred but were mostly purged by low salinities in April and May.

The pattern of mortalities caused by *H. nelsoni* epizootics in Virginia and Maryland has been essentially repeated in the drought years of 1985–1987. The losses in all rivers of Virginia have been severe. In Maryland, heavy losses extended into the lower Choptank River and Eastern Bay, and *H. nelsoni* was also reported in the lower reaches of the Chester River (Figure 1).

Shortly after *H. nelsoni* appeared in Chesapeake Bay, it was reported in North Carolina waters (Albemarle Sound). This occurrence was not surprising because North Carolina planters had traditionally imported eastern oyster seed from Virginia. More recently, *H. nelsoni* has been identified in eastern oysters from South Carolina and Georgia. Some mortalities have been reported in these areas, although they have not been extensive, and prevalences remain low. Just a few years ago, samples from South Carolina and Georgia were negative. The pattern of positive *H. nelsoni* samples now indicates that it is gradually spreading southward but has probably not made big jumps. In both states, *Perkinsus marinus* is also present, and attributing mortalities due to *H. nelsoni* is difficult (C. A. Farley and F. Kern, National Marine Fisheries Service, Oxford, Maryland, personal communication).

In 1985, a sample of 30 eastern oysters from the St. Johns River system near Jacksonville, Florida, had three eastern oysters with *H. nelsoni*, one of which had a heavy infection. In spring 1986, four of 30 eastern oysters from Biscayne Bay, Florida, had *H. nelsoni* gill infections. A 1987 sample had none (R. Hillman, Battelle Laboratory, Duxbury, Massachusetts, personal communication). These Florida reports mark the known southern limit of *H. nelsoni* at this time.

To the north of Delaware Bay along the New Jersey Coast, the two small eastern oyster producing areas, Great Egg Harbor and Great Bay, have been plagued with *H. nelsoni* since the late 1950s (Figure 8). Test samples, wherever taken in the back bays, have been positive. A surviving eastern oyster population in the Navesink River, relict from an industry that ended shortly after World War I, was free of *H. nelsoni* until 1980 when a substantial kill occurred. A small eastern

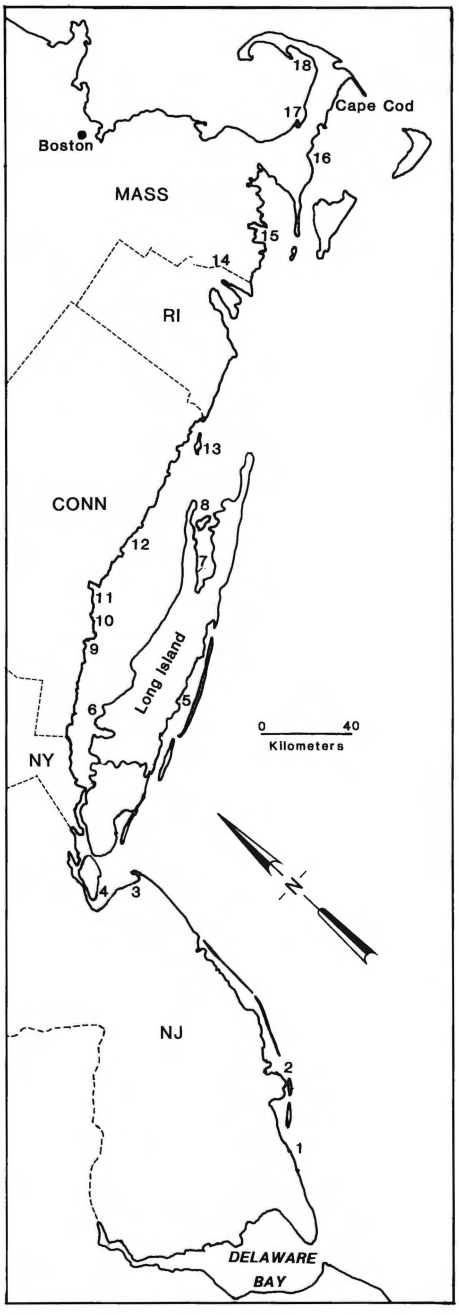

FIGURE 8.—Map of the east coast of the USA from Delaware Bay to Cape Cod. With the exception of Fishers Island, MSX has been found in each identified location. 1 = Great Egg Harbor; 2 = Great Bay; 3 = Navesink River; 4 = Raritan Bay; 5 = Great South Bay; 6 = Oyster Bay; 7 = Peconic Bay; 8 = Gardiners Bay; 9 = Bridgeport; 10 = Milford; 11 = New Haven; 12 = Hammonasset River; 13 = Fishers Island; 14 = Swansea; 15 = West River; 16 = Cotuit Harbor; 17 = Barnstable; 18 = Wellfleet Harbor.

oyster population in Raritan Bay, sampled irregularly between 1979 and 1988, averaged about 10–30% *H. nelsoni* prevalence (Farley, personal communication; Rutgers Shellfish Laboratory, unpublished data).

In the Long Island area, Great South Bay eastern oyster grounds (Figure 8) were sampled in 1965, and three grounds had *H. nelsoni* with prevalences ranging from 25 to 54%. A principal grower in that area at that time estimated annual mortalities in hatchery-reared stock at 15% (Rutgers Shellfish Laboratory, unpublished data).

Along the Connecticut shore of Long Island Sound, *H. nelsoni* prevalence of 40% was found in eastern oysters in Milford harbor in 1960 and 12% prevalence in 1974. New Haven harbor eastern oysters were positive for *H. nelsoni* when examined in 1966 and 1967. In 1985, prevalences of 18 and 32% were reported in Bridgeport harbor and the Hammonasset River, respectively (C. A. Farley and F. Kern, personal communication).

In Massachusetts, *H. nelsoni* was first found in eastern oysters from Wellfleet harbor in 1967 and then in follow-up samples in 1969 and 1970 (Krantz et al. 1972). Krantz et al. (1972) suggested that *H. nelsoni* was introduced by importation of eastern oysters from an epizootic area. They also noted that its presence in native eastern oysters in 1969 and 1970 indicated that it had become enzootic in Wellfleet harbor. *Haplosporidium nelsoni* persisted at Wellfleet with prevalences of 12 and 28% in 1975 and 1978, respectively (C. A. Farley and F. Kern, personal communication). Major eastern oyster mortalities were reported there in 1982, 1985, and 1987 (B. Chapman, Shellfish Constable of Wellfleet, Massachusetts, personal communication). Presently, substantial mortalities are reported at Barnstable and Swansea in addition to Wellfleet, although they have not yet been linked to *H. nelsoni* by histological examination (F. Germano, Division of Marine Fisheries, Sandwich, Massachusetts, personal communication). In 1985, prevalences of 48 and 93% were reported in the West River near New Bedford harbor and in Cotuit harbor, respectively (C. A. Farley and F. Kern, personal communication).

A nagging question over the past 20 years has been, "What is preventing massive epizootics, like those in Chesapeake and Delaware bays, from occurring in the eastern oyster areas extending from Great South Bay, Long Island, to the tip of Cape Cod?" Since 1983, there seem to be some shifts in this situation, as illustrated by two well-documented examples of serious eastern oyster mortalities associated with *H. nelsoni,* one in Long Island and a second on Cape Cod. A third occurrence of mortality in eastern bays of Long Island is not so clearly due to *H. nelsoni.*

In the Flower Hatchery at Bayville, Long Island, spat are set on fragments of shell and then rafted in trays for 6 weeks or longer. The clusters of spat are then planted directly on hard bottom grounds in Oyster Bay that have been previously suction-dredged to remove drills, starfish, and other predators. Within 2.5 years, they usually reach marketable size. Over the years, returns by number have averaged about 30% but range from 15% in bad years to 50% in good years. In any one season, there are usually three year classes on the grounds, and in summer 1983, when *H. nelsoni* appeared, these were year classes 1981, 1982, and 1983. Unusually heavy mortalities continued through 1984 and into 1985. On final tally of harvests, approximately 90% of all three year classes had been lost (J. Zahtila, Franklin B. Flowers & Sons, Bayville, New York, personal communication). Histological examination of samples of eastern oysters by one of us (H. H. H.) and by the Oxford Laboratory (C. A. Farley and F. Kern) established involvement of *H. nelsoni* in these losses.

The Cape Cod eastern oyster operation in Cotuit harbor traditionally depended totally on Connecticut eastern oyster growers for wild seed. In recent years, part of the seed has been produced in a small eastern oyster hatchery of the Ocean Pond Corporation, Fishers Island, New York. In 1984, wild eastern oyster seed of several year classes from the Hammonasset River in Connecticut were planted in Cotuit harbor over the summer and early fall. Some of these eastern oysters were probably infected with *H. nelsoni.* A sample of Hammonasset seed taken from Cotuit harbor in November, had 6% prevalence of *H. nelsoni* (F. Perkins, Virginia Institute of Marine Science, Gloucester Point, personal communication). Hatchery-reared seed that had been rafted on Fishers Island was also imported at the same time. Over the winter and spring the Hammonasset eastern oysters were harvested. A sample of 50 eastern oysters taken in the spring, presumably the Fishers Island seed, had 15 *H. nelsoni* infections. Losses in the summer were heavy, about 40%, and a sample examined after the mortality had a 92% prevalence.

Before the 1985 mortality, eastern oysters from Ocean Pond on Fishers Island had been examined

in 1968, 1972, 1976, and 1978, and all were negative for *H. nelsoni*. Two additional samples in January and September 1985 were also negative. Eastern oysters from Hammonasset River were negative in 1978 and 1983, but in December 1985, 32% of a sample had *H. nelsoni* (C. A. Farley and F. Kern, personal communication).

In 1986, experimental trays of Fishers Island eastern oysters were placed in Cotuit harbor monthly and sampled monthly from April 1 to November 1. A preliminary report on the timing and early development of *H. nelsoni* infections in these eastern oysters indicated close conformance with the earlier findings of investigators in New Jersey, Maryland and Virginia (L. Leibovitz, Marine Biological Laboratory, Woods Hole, Massachusetts, personal communication).

All evidence indicated that seed brought from Ocean Pond in 1986 was not infected with *H. nelsoni*. Therefore, the tray studies demonstrated that *H. nelsoni* was established in Cotuit harbor and able to infect imported susceptible eastern oysters. Probably, *H. nelsoni* was introduced into Cotuit harbor with the Hammonasset eastern oysters or possibly, infective stages entered the harbor in the coastal flow in the summers of 1984, 1985, and 1986. This speculation is based on the unpredictable occurrences from the 1960s to 1985 of *H. nelsoni* in so many scattered locations spread along the coast of Connecticut and the entire southern coast of Massachusetts.

Mortality of eastern oysters in Peconic and Gardiners bays in late summer and fall of 1985 was massive—a loss of 80% of a planting of market-size eastern oysters valued at $1–1.5 million—but the role of *H. nelsoni* in that mortality is not clear. Most of the seed eastern oysters came from wild stock in New Haven harbor and Norwalk, Connecticut, with smaller additions of hatchery-reared seed from Maine and the Shinnecock Indian hatchery on Long Island. Brown tide, a bloom of a recently identified small phytoplankter *Aureococcus anorexefferens,* appeared in 1985 in eastern Long Island bays. Brown tide is considered responsible for widespread mortality of the bay scallop *Argopecten irradians* and the destruction of eel grass by shading (Cosper et al. 1987). During the period of bay scallop mortality, the eastern oyster plantings were checked weekly without evidence of deaths. The owner thought his eastern oysters had escaped damage from the algal bloom, but as the bloom was dissipating, the eastern oysters "died within a period of 1–2 weeks." After the mortality, in mid-October 1985, two samples of survivors were sent to the Rutgers Shellfish Research Laboratory. Samples containing 20 eastern oysters each from a Cedar Beach ground and a Long Beach ground had 3 and 2 lightly infected eastern oysters, respectively. Because survivors of an *H. nelsoni*-caused epizootic are usually highly infected (Ford and Haskin 1982), we do not believe that *H. nelsoni* was the primary cause of the massive kill. However, light *H. nelsoni* stress, added to the burden of the noxious alga, might have been enough to trigger the high mortality.

Resistance with Increasing Disease Pressure

After the early epizootic mortalities in the lower Chesapeake Bay in 1959 and 1960, Virginia planters retreated from grounds in Mobjack Bay and Hampton Roads to areas of lower salinity in tributary rivers. Because no major planting areas of low salinity were available in Delaware Bay, the industry largely confined its planting from the seedbeds to the upper edge of the traditional planting grounds. By the mid 1960s, the natural seed—offspring of survivors of the heavy *H. nelsoni* pressure of 1957–1959—had about three times more resistance to mortality caused by *H. nelsoni* than the preepizootic population. With this level of resistance, the use of larger seed of eastern oysters from the lower seedbeds, and a shorter planting time (harvesting spring plants in the following fall and winter), the Delaware Bay industry managed to survive, although it was greatly stressed and suffered reduced production (Haskin and Ford 1983). The generally upward trend in mortalities from about 1972 (Figure 6), capped by the peak in 1986, has discouraged further planting of natural seed and has raised questions as to why the resistant native seed was overwhelmed by *H. nelsoni*. Parallel experiences with native eastern oysters from the surviving populations in Mobjack Bay and Hampton Roads led to the same questions for the Chesapeake Bay.

We use the term "resistance" to mean resistance to death from disease caused by *H. nelsoni*. It does not imply resistance to infection but rather the capacity to restrict parasite numbers to tolerated levels (Ford 1988). If two stocks of eastern oysters are exposed for a time to infection with *H. nelsoni,* and one has 10% survival and the other 30%, we conclude that the second group is three times more resistant to mortality than the first. Resistance in eastern oysters is not absolute; rather, it may differ among stocks or individuals.

TABLE 3.—Mortality of seven Cape Shore (Delaware Bay) tray stocks of eastern oysters, June–December 1986.

Stock	Mortality (%)
Control—1986 imports of James River seed	88
Offspring of James River seed[a]	93
Control—1986 imports from Maryland	91
Offspring of Maryland import seed[a]	98
Cape Shore wild seed[a]	41
Resistant yearlings, 6th generation[a]	5
Resistant yearlings, 6th generation[a]	26

[a] 1985 year class.

TABLE 4.—MSX-related mortality of six 1978 year-class groups of eastern oysters at Cape Shore (Delaware Bay) over a 3-year test, October 1978–July 1981.

Stock	Mortality (%)
Virginia control, susceptible	97
Navesink control, susceptible	98
F_6 Virginia, resistant[a]	60
F_4 Long Island, resistant[b]	35
F_4 and F_5 Long Island, Delaware Bay, resistant[b]	39
Wild Cape Shore set, resistant	54

[a] Produced at Virginia Institute of Marine Science Laboratory.
[b] Produced at Rutgers Cape Shore Laboratory.

The level of resistance to *H. nelsoni* mortality in an eastern oyster population or in an inbred line may be expected to reflect the rigor with which it has been selected through generations of exposure to *H. nelsoni* infections. Surviving older wild eastern oysters in areas of high salinity in both Chesapeake and Delaware bays are being rigorously selected against *H. nelsoni* disease. However, newly set spat in these locations may derive partly from eastern oysters outside the immediate area. In all low-salinity creek and river tributaries of Delaware Bay and on the seedbeds, eastern oysters are under little or no pressure due to *H. nelsoni*. As the larvae of these eastern oysters swim, they mix with all other eastern oyster larvae in the estuary, including those produced by survivors of heavy selective pressure in the lower bay. The natural set, wherever it occurs, comes from this mix (Haskin and Ford 1982). In contrast, laboratory-reared resistant eastern oysters have been held in the lower bay (Cape Shore) where selection against *H. nelsoni* is very intense. Each generation in a resistant line was exposed for at least 2.5 years before the survivors of that generation became parents for the next. Broodstock was spawned, and larvae were raised and set in the laboratory, without mixing with wild larvae from the native bay eastern oysters. The hardiest of these lines after six generations are about three times as resistant as the present Delaware Bay wild seed, and they are about ten times more resistant to *H. nelsoni*-caused mortality than the original Delaware Bay wild stock. Mortalities of several stocks in trays on the Cape Shore flats over the 1986 June–December test period illustrate this point (Table 3).

In 1986, *H. nelsoni* pressure in lower Delaware Bay, judged by mortality in susceptible control stocks, was higher than in any earlier year (Figure 5). In an average year, the first year June–November mortalities in the James River seed imports would have been about 60%; in the Cape Shore wild set, about 15–25%. Losses of susceptible controls over that same 5-month period in 1986 approached the long-term average of 93% for a 33-month period (Table 3). In the presence of this intense disease pressure, mortality for the sixth generation of one of the best Delaware Bay lines was 5%, for another line (mixed Delaware Bay and Long Island stock) it was 26%. Thus, in contrast to four susceptible controls, the rigorously selected resistant lines were not overwhelmed.

In earlier years, the authors developed *H. nelsoni*–resistant lines and exchanged groups of eastern oysters with each other for tests at Cape Shore and at VIMS. In May 1978, a fifth generation resistant line, with a record of about 10% annual mortality over 2 years at VIMS, was sent to Cape Shore for breeding and testing. In that summer, *H. nelsoni* pressure at Cape Shore was very slight (Figure 5), and testing of the yearling spat of the Virginia resistant line extended through the 1981 season. Results of these tests were compared with results from tests with offspring of two imported susceptible controls, with two resistant lines under development at the Cape Shore, and with Cape Shore wild set of the 1978 year class (Table 4). The offspring of the line selected under the test conditions at VIMS had the highest mortality of the four resistant groups including the wild Cape Shore set.

The severe eastern oyster losses in both Chesapeake and Delaware bays in recent years are not an indictment of disease-resistant oysters. They simply indicate that the disease pressure is now higher than we have seen before and certainly higher than the infection pressure against which those eastern oysters have been selected. We are encouraged that some of the Rutgers selectively bred resistant lines were able to withstand the increased pressure.

Even the best stocks, held under continuing *H. nelsoni* attack for several years, will begin to have substantial mortalities caused by *H. nelsoni,* and, after 5–6 years, most stocks will have died as a result of advanced infections (Ford and Haskin 1987). Growers of eastern oysters can be encouraged that the most resistant eastern oysters can effectively localize and tolerate infections until they reach market size. With *H. nelsoni* under control, they can feed, grow, reproduce, and develop as a high quality eastern oyster.

Environmental Effects on *H. nelsoni* Disease

Variation in *H. nelsoni* activity along the east coast is difficult to understand but no more so than in the bays in which *H. nelsoni* first appeared 30 years ago. Eastern oyster mortalities caused by *H. nelsoni* in James River seed are substantially higher in Delaware Bay at the Cape Shore than in the York River at Gloucester Point (VIMS), even though both are enzootic waters with very similar salinity and temperature regimes. This observation indicates that other factors controlling *H. nelsoni* activity may be different in the two areas. The Cape Shore intertidal area consistently receives the heaviest setting of eastern oysters in the bay. Eastern oyster growth rates there are high and correlate well with phytoplankton populations (measured by total chlorophyll) that are the highest in the bay (W. Canzonier, Rutgers Shellfish Laboratory, Port Norris, New Jersey, personal communication). The hydrographic system that concentrates larvae and phytoplankton at the Cape Shore may also concentrate the infective stage of *H. nelsoni,* and this system may not have a counterpart in the York River at Gloucester Point.

Although salinity and temperature data indicate somewhat similar habitats in these two locations, there are faunal indicators that are probably more sensitive than our physical measurements. For example, the eastern oyster pathogen *Perkinsus marinus* was not found north of the lower Chesapeake Bay until imported to the Delaware Bay with seed eastern oysters from Virginia and Maryland in the early 1950s. With an embargo on all eastern oysters for commercial imports and exports after the 1957–1959 *H. nelsoni* epizootic, *P. marinus* died out in Delaware Bay, and the northern boundary of this pest is currently reestablished in the Chesapeake Bay. This boundary probably indicates a sensitivity to temperature regimes that are milder in the Chesapeake Bay area. Andrews (1988, this volume) discusses temperature relative to *P. marinus*. Ford and Haskin (1982) also suggested that the winter temperatures of the lower Chesapeake Bay never become low enough to reduce *H. nelsoni* activity in a cyclic pattern such as that in Delaware Bay.

The exceptionally high simultaneous mortalities of 1986 and 1987 in susceptible stocks at Cape Shore and VIMS (Figure 5) suggest changes in a common controlling factor that is probably climatological. The prime factor could be the drought commencing in the fall of 1984 and extending through 1987. Because salinities in both locations are well within the range for optimal parasite activity under normal conditions, the infective dosage may have peaked during 1986 and 1987. Furthermore, drought may have permitted an increase in supply of infective particles at both locations.

An earlier deduction based on *H. nelsoni* prevalence patterns in lower Delaware Bay (Ford and Haskin 1982) led to the conclusion that the source of infective stages was downbay and that the stages were diluted with increasing distance upbay from this source. An extension of this hypothesis is that a reservoir host was held downbay by unfavorable salinities. A direct result of persistent drought and increasing salinities would be to permit upbay migration of such a host. This migration would increase the concentration (dosage) of infective stages, released by this host, throughout the bay. This scenario, assuming that migration of the reservoir host requires one or more reproductive seasons, would explain observations that the increased *H. nelsoni* activity upbay does not occur until the second or third year of drought in Chesapeake Bay.

The upbay movement of the host would not only extend the range for infective stages but in all probability would increase their abundance within the area of release. The quick increase in *H. nelsoni* activity in Delaware Bay, compared to the Chesapeake Bay, in times of drought may reflect its smaller size and its consequent reduced reserve of freshened water.

The complex role of temperature in eastern oyster–*H. nelsoni* interactions is also not clearly established. Earlier experimental work by Myhre (1973) and Douglass (1977) in the Rutgers Laboratory has been discussed (Ford 1988). Douglass (1977) demonstrated in mortality-resistant stocks of eastern oysters that *H. nelsoni,* typically restricted to gill epithelia, becomes systemic as fall temperatures drop below 18–20°C. As temperatures rise in spring, infections in resistant oysters

may be suppressed or eliminated. Shifts in temperature regimes, such as an early fall or a late winter with delayed spring warming, could influence *H. nelsoni* infections, causing mortality rates to rise or fall.

Two further cold winter relationships to *H. nelsoni* levels are possible. (1) In comparing mortalities at Cape Shore and VIMS (Figure 5), the 1972 low mortality at VIMS was attributed to the influence of Hurricane Agnes. A similar low in 1984 has not been explained. Both the Gloucester Point lows of 1972 and 1984 follow cold winter lows, at the Cape Shore. The other two Cape Shore cold winter lows, 1961–1962 and 1978, coincide with smaller but distinct drops in mortality at Gloucester Point. These are probably coincidental but perhaps a look at Virginia coastal climatological data would be justified. (2) In considering coastal relationships of *H. nelsoni,* Ford and Haskin (1982) pointed out that the parasite had been present in several locations in Long Island and southern New England since at least the 1960s without substantial eastern oyster mortalities. Arguing from the cold winter syndrome of *H. nelsoni* cycles in Delaware Bay, they suggested that the colder winters to the north might prevent full-fledged disease development. The outbreaks of *H. nelsoni* in Long Island and Cape Cod described above overlap in the years 1983–1986 and, therefore, invite a study of temperature trends in that area.

Life Cycle Clues to Recent Range Expansions of *H. nelsoni*

We are certain that the *H. nelsoni* stage that infects oysters is waterborne and can be spread through the waterways. The longer the infective stage remains viable, the greater the distances it can travel by this route. Spreading would be expected to continue until boundaries set by temperature, salinity, or some other factor are reached. If this stage is released by infected eastern oysters, the spread within these boundaries would be most quickly accomplished by the movement of infected eastern oysters to the new areas.

On the other hand, if there is an obligate reservoir or intermediate host species that supplies the *H. nelsoni* life cycle stage infective for eastern oysters, that species must be established within the range of the eastern oysters. If that reservoir host species is not resident in the area, transport of infected eastern oysters alone to virgin territory would then not permit transmission and establishment of *H. nelsoni*. Presumably *H. nelsoni*-infected eastern oysters that are moved to virgin territory might also carry with them the reservoir or alternate host which would then have an opportunity to become established in that territory. Moreover, *H. nelsoni* may not be species specific for its presumed alternate or reservoir host, that is, more than one species might host the infective stages of *H. nelsoni*.

The *H. nelsoni*-caused mortality of 1985 and 1986 in Cotuit harbor followed an importation of infected eastern oysters from Connecticut in 1984. In 1985, the mortality included both the Connecticut imported seed and the disease-free seed brought in from Ocean Pond in 1984. In 1986, Ocean Pond eastern oysters that were brought in experimentally at monthly intervals also became infected and had heavy mortality. The *H. nelsoni*-caused mortality of the Ocean Pond eastern oysters established that transmission of *H. nelsoni* occurred within Cotuit harbor. Based on *H. nelsoni* activity in other areas, we think it very unlikely that it was directly transmitted from one eastern oyster to another. The presence of *H. nelsoni* in Ocean Pond eastern oysters in spring 1985 indicates infections existed in fall of 1984. This would mean very quick cycling from eastern oyster to alternate host to eastern oyster if the alternate host required exposure to parasitized eastern oysters before it could become infected. Probably other species dredged from Hammonasset River accompanied the eastern oysters to Cotuit. Among these may have been the host species already carrying *H. nelsoni* infective stages that were released upon arrival to infect previously unexposed Ocean Pond eastern oysters. The Cotuit oyster planter indicated that he had brought eastern oyster seed from the Hammonasset River for several years before the 1984 import. Two earlier samples from the Hammonasset River (1979 and 1983) were negative for *H. nelsoni*. One sample in October 1985 had a prevalence of 32%. Another possibility is that alternate or reservoir hosts from the Hammonasset River had been established in Cotuit harbor with the earlier imports. Other possibilities are considered at the end of this article.

The most important advance yet to be made in our understanding of *H. nelsoni* biology is to work out its complete life cycle. That knowledge may point the way to control of *H. nelsoni*. Many of the gaps in our information were indicated in the introduction. Our frequent reference to a hypothetical reservoir host also emphasizes that we

really do not know the source of *H. nelsoni* infectivity for eastern oysters.

Although most haplosporidians commonly undergo sporulation in their hosts, *H. nelsoni* rarely achieves this stage in eastern oysters. Because of the scarcity of spores and the consequent uncertainty of its affinities, *H. nelsoni* was not named until 8 years after its discovery. Andrews (1979) reported 44 cases of sporulation in 170,000 slide preparations of stained tissues from living and recently dead eastern oysters over a period of 16 years. When only infected oysters were considered, the sporulation rate was less than one case per 2,000. These cases of sporulation were scattered throughout the year, although those in June and July were most common. In the Delaware Bay area, "fewer than a dozen cases. . .among the many thousands of tissue slides and fresh smears" were reported (Ford and Haskin 1982). Most of these cases were in yearling eastern oysters and invariably in the epithelia of the digestive tubules. Sprague (1965) hypothesized that *H. nelsoni* "may normally occur within some associated organisms living in the vicinity of oyster populations and may sporulate regularly to provide forms infective to both oysters and the other host."

During the drought of the mid-1960s, *H. nelsoni* moved far up Chesapeake Bay and attacked highly susceptible eastern oysters with a small increase in the abundance of sporulation stages. Whether the ratio of sporulation to numbers of eastern oysters examined increased is not clear. By selecting sick and moribund specimens from thousands of young susceptible eastern oysters, Couch et al. (1966) were able to recognize and describe sporulation of *H. nelsoni* after which Farley (1967) proposed a tentative life cycle.

Unlike most haplosporidians where sporulation occurs in all tissues, *H. nelsoni* confines sporulation to the epithelia of the digestive tubules. Therefore, all prespore stages either migrate to the digestive diverticula or develop in them (Farley 1967; Andrews 1979). Multinucleate plasmodia enlarge and undergo nuclear division, and the chromatin material acquires a punctate appearance before 50 or more spores are formed in the sporocysts. This enlargement of sporonts occurs between epithelial cells and forms protrusions into the tubule lumina. Such restricted sporulation limits the quantity of spores that can be produced and may facilitate release of spores from live eastern oysters sporadically as the epithelia are destroyed by the bulging sporonts. Eastern oysters may live several months after sporulation.

Sporulation by *H. costale* (SSO), the seaside organism, is more typical of haplosporidians in general. All plasmodia enlarge when the punctate stage is reached, and sporulation occurs in all tissues, including mantle and gill. Sporulation in *H. costale* occurs regularly in late May and early June each year. Eastern oysters with massive numbers of sporocysts die rapidly, often before spores become mature. Distinctive sporulation sites for the two species indicate that different biochemical or physiological processes may be occurring in spore formation.

The rarity of sporulation by *H. nelsoni* has led to some speculation that *H. nelsoni* is not really adapted to parasitism of the eastern oyster and that the eastern oyster is an accidental host. Such opinions are refuted by an interesting account of a massive sporulation in eastern oysters in Virginia in 1976 (Andrews 1979). Of thousands of highly susceptible, hatchery-raised young (25 mm) spat in a single tray, 39% had *H. nelsoni* sporulation. They were set in mid-May, held in a disease-free pond for early growth, and then transferred to the York River enzootic area on 8 July 1976. Nine weeks later, on 21 September, 40% had died, 88% of the survivors were infected with *H. nelsoni,* and 39% were in sporulation. A second group of the same brood was transplanted to the York River on 16 August 1976. In this group, patent infections did not appear until December, and little mortality occurred until May and June 1977 when infections were intensive. In this second lot there was only one case of sporulation in 93 infections diagnosed during 1977.

At the time the first tray was placed in the York River, there were 75 other lots of oysters in the vicinity, some within 50 feet. None of these exhibited sporulation, although *H. nelsoni* activity was intense in several lots of susceptible eastern oysters.

The question raised by these observations is what induced sporulation in this one lot of eastern oysters while others in nearby trays developed only plasmodial infections. Most likely, the differences in the two lots were genetic. Among the 75 lots in the York River, no others were newly set Rappahannock River stock receiving first exposures to *H. nelsoni* infectivity. Even within the Rappahannock gene pool, individual variation in response to *H. nelsoni* challenge would be great.

Such a spectacular sporulation event leads to a certain uneasiness. Could we all have been miss-

ing such events occurring in young spat in their first exposure to *H. nelsoni,* events that could have produced infective stages to account for the massive mortalities in our bays? We do not believe so. Surely such events would have been detected by the large-scale monitoring programs with accompanying histological studies in the three states of Virginia, Maryland, and New Jersey. The highly susceptible control stocks, spawned and examined year after year in the disease-resistance breeding programs at VIMS and at the Rutgers Laboratory, would also have been prime candidates for such sporulations, if they were occurring.

Although at least 30 species of *Haplosporidium* are known (La Haye et al. 1984), none of these have been transmitted to their respective hosts under controlled conditions, with the possible exception of *H. pickfordae* in freshwater snails (Barrow 1965). The complete life cycles are not known for any of these species.

We hope that the immunological technique being adapted by our colleagues will enable us to search for the hypothetical reservoir or alternate host(s) of *H. nelsoni* (Burreson 1988). To date, we have avoided a grueling systematic histological search through the hundreds of possible candidates in and around our coastal estuaries. The enzyme-linked antibodies, DNA probes, or both promise to reduce the drudgery and to speed the search for *H. nelsoni.*

It is certainly time to intensify and concert the efforts of investigators along the coast to resolve the many questions remaining about the life cycle of *H. nelsoni.* We need to lift our eyes and perhaps open our imaginations to some of the exciting discoveries in related fields. Sweeney et al. (1985) working with a microsporidian parasite (*Amblyospora* sp.) infecting the Australian encephalitis mosquito vector *Culex annulirostris,* demonstrated stages in an intermediate copepod host necessary to complete the life cycle of the microsporidian. This discovery is cited as the first evidence of alternate host involvement in the life cycles of microsporidia. Another spore is formed in the copepod, and this spore is then infectious to larval mosquitoes.

Andreadis (1985), working in Connecticut, demonstrated that haploid spores from another species of *Amblyospora* parasitic in another mosquito species, are also transmitted to an alternate copepod host. He reported that members of the genus *Amblyospora* have at least three distinct developmental cycles, each producing a different spore.

A discovery of particular relevance, for those who have been frustrated for nearly 30 years in pursuit of the *H. nelsoni* life cycle, concerns the whirling disease of salmonid fish which has been known and studied for 80 years (Wolf and Markiw 1984). The causative agent was recognized as a myxosporean named *Myxosoma cerebralis* which produces an abundance of spores in trout. However, the spores were not infectious to other fish. This myxosporean disease of fish is initiated by an organism known since 1899 as an actinosporean, parasitic in a tubificid oligochaete. Wolf and Markiw (1984) showed conclusively that "instead of being considered as representatives of separate classes in the phylum *Myxozoa,* the myxosporean and actinosporean are alternating life forms of a single organism." They suggest that, if the host worms could be eradicated by selectively lethal chemicals, the whirling disease of trout may be prevented.

We stated earlier that *H. nelsoni* is a poorly adapted parasite in the eastern oyster. Wolf and Markiw (1984) noted that *Myxosoma cerebralis* infections were well tolerated in the parasite's original host fish, the brown trout *Salmo trutta.* In a new host, the rainbow trout *S. gairdneri,* the parasite produces the virulent whirling disease. They note that the rainbow trout was introduced into Europe in the late 1800s and that *Myxosoma cerebralis* was accidently brought to the USA in the 1950s. *Haplosporidium nelsoni* may prove to be nonvirulent in another host or perhaps even in a parallel host, such as the Pacific oyster *Crassostrea gigas.*

Profile of an Alternate or Reservoir Host for *H. nelsoni*

Recent findings in parasite life cycles encourage us to attempt a profile of an alternate or reservoir host of *H. nelsoni.* Based on observations of *H. nelsoni* activity, what deductions can we reasonably make about its source, that is, the hypothetical host that releases the stage infective for eastern oysters?

Observation 1.—Infection intensity (dosage) of *H. nelsoni* in eastern oysters is independent of location or size of eastern oyster populations. Infection pressures actually appear to be increasing in recent years as the eastern oyster populations diminish in our bays.

Deduction 1.—This observation may indicate that the oyster has no obligate role in the life cycle of *H. nelsoni.* That is, it is an accidental host and irrelevant to the cycling of *H. nelsoni* in the bays. This deduction would be in line with Sprague's

(1965) earlier suggestion that an organism in proximity to the eastern oysters is supplying the infective stage both for the eastern oysters and for that other organism.

Observation 2.—In Delaware Bay, there is a pattern of timing of *H. nelsoni* infections in oysters in summer and early fall with first infections in the lower regions of the planting grounds and later infections progressing slowly upbay in a wave. This pattern has not been observed in the lower Chesapeake Bay.

Deduction 2.—The source (host) of the infective stages is in the lower bay, either on or below the lowest planting grounds. This may indicate that the host is restricted to higher salinity areas.

Observation 3.—In times of drought, higher prevalences and mortalities of eastern oysters are delayed in upper Virginia and Maryland sectors of Chesapeake Bay until the second or third year of the drought. In Delaware Bay, the delays are shorter.

Deduction 3.—In drought periods, the salinity-limited host may move upbay as the salinity increases, probably with a jump in population size each reproductive period, especially if there is a pelagic larval stage. A substantial upbay movement of the host would then require 1 or 2 years to establish larger populations. The upbay migration of the host then would increase the concentration of infective material in upbay and tributary areas.

Observation 4.—In Delaware Bay, the years of lowest *H. nelsoni* prevalence and mortality follow unusually cold winters, indicating that the host may be damaged, killed, or inhibited by low temperatures.

Deduction 4.—The host in the lower bay is vulnerable to damage by cold because it inhabits shallow water on shoals, rock jetties, or bases of light houses, or in salt marshes. Prolongation of the cold period may also increase winter casualties, even in deepwater populations.

Observation 5.—The reduction in *H. nelsoni* activity in Delaware Bay does not occur until a full year after the cold winter, for example, the 1983 low was preceded by an unusually cold January in 1982.

Deduction 5.—(a) Perhaps the simplest deduction would be that the infective stages were released in average numbers before, during, or even after the winter damage to the host and persisted until the usual eastern oyster infection period begins in late May or June. Lack of infections during the following summer could be related to the time required to rebuild the host population. (b) An alternate deduction is one that would require a two-host alternating cycle in addition to the eastern oyster. In this scenario, host A would release infective materials supplying both the eastern oyster and host B. Host A is not cold sensitive and would release its infective materials in the season immediately following the cold winter. The eastern oyster would receive its dosage of *H. nelsoni* particles. But the damaged or decimated host B population would not be able to receive and process its usual dosage. The host B survivors would be producing a reduced amount of infective materials to cycle to host A. The reduced infection of host A would then be reflected in its reduced output of infective materials for the eastern oyster population.

If the eastern oyster is indeed an accidental host for *H. nelsoni,* there is a consequence of practical importance for management. *Haplosporidium nelsoni*-infected oysters by themselves would not be effective in spreading the disease into a new area. Rather, the true host(s) would be the effective carrier(s) of the infective stages. Maintenance of the disease in the new area would require infection of the true host species in that area, if already resident. If not resident, the true host carrier to the new area would have to become established to maintain the *H. nelsoni* population.

However, there is no direct evidence that the eastern oyster is an accidental host for *H. nelsoni.* The speculation is based on the premise that if the eastern oyster is an obligate host, the supply of infectious stages should diminish with the reduction of the high-salinity eastern oyster population. There is no certainty, however, that a small residual eastern oyster population could not be remarkably productive of *H. nelsoni* infectious stages.

Acknowledgments

We are indebted to staff members C. Austin Farley and Fred Kern, U.S. National Marine Fisheries Service Laboratory, Oxford, Maryland, and to Frank Perkins, Director of Virginia Institute of Marine Science (VIMS) for unpublished information on the distribution of *H. nelsoni* along the east coast from New England to Georgia. Robert Hillman, Battelle Laboratories, Duxbury, Massachusetts, extended that range to Florida. Frank Germano, Division of Marine Fisheries, Sandwich, Massachusetts, provided information on eastern oyster mortalities along the southern Massachusetts coast, and Billy Chapman, Shellfish Constable of Wellfleet, Massachusetts, updated the eastern oyster mortalities there. Details

of the Cotuit harbor outbreak were provided by George C. Matthiessen, Ocean Pond Corporation, and J. R. Nelson, Cotuit Oyster Company. *Haplosporidium nelsoni* experiences of the Bayville Hatchery of Frank Flower & Sons were detailed by David Relyea and Joseph Zahtila. Jack Mulhall, Long Island Oyster Company, discussed with us the details of his eastern oyster losses in eastern Long Island bays. Recent data on diseases in Virginia were provided by E. M. Burreson, VIMS. George Krantz, Director of the Oxford Laboratory, has released to us the most recent information on *H. nelsoni* distribution in Maryland waters. Searching criticisms by several anonymous reviewers were constructive and much appreciated by the authors.

This is publication F-32504-3-88 supported by state funds of the New Jersey Agricultural Experiment Station and publication 1490 of VIMS.

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