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Summaries of twenty-five years of MSX studies in Chesapeake Bay, 1959 to 1983

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MSX in Chesapeake Bay

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Introduction

Thirty years ago the disease of oysters caused by Haplosporidium nelsoni appeared suddenly in Delaware Bay (Haskin et al. 1966) and spread rapidly throughout the bay and into high-salinity areas of Chesapeake Bay (Andrews and Wood 1967). The disease has not abated in virulence and intensity despite severe genetic selection of Delaware Bay oysters over many years (Haskin and Ford 1979). In fact, it spread far up Chesapeake Bay during periods of drought (Farley 1975) and is now causing mortalities in New England waters. From the beginning of epizootic mortalities, biologists have relied on natural or laboratory selection of genetic resistance to control the disease (Ford and Haskin 1982), but during years of intensive activity of the pathogen, resistant oysters both in Delaware and Chesapeake bays were overwhelmed. This failure of genetic resistance after prolonged, severe natural selection is unexplained for the mechanisms of resistance are not known.

Haplosporidium nelsoni, widely known as MSX, has many peculiarities in its life cycle as a pathogen (Andrews 1982a). Its life cycle is not understood in nature although patterns of infection and mortality are well known as to timing and duration. The pathogen has not been cultured in the laboratory and artificial infections have not been achieved. Furthermore, the spore stage is known (Couch et al. 1966) but the pathogen rarely sporulates (Andrews 1979), therefore, the source of infection remains unknown. This has led to much speculation about alternate hosts but none has been found. Alternate hosts have not been demonstrated in any haplosporidan species (Andrews 1984).

The annual cycle of MSX exhibits some peculiar patterns of infection and mortality (Andrews 1966). A protracted infection period of five months, from May through October, results in two patterns of development of disease which contain hidden dangers for oyster culture in Chesapeake Bay. Infections acquired from May through July become clinical in four to six weeks and begin killing oysters by 1 August or slightly earlier in severe epizootic years (Ford and Haskins 1982). Infections initiated during August through October remain sub-clinical for at least three months and most years until April or May of the following year (Andrews 1982b). The cause of this long incubation period is obscure because the change in infection pattern occurs in late summer when continuous high temperatures and high salinities prevail. Some years late-summer infection fails but early-summer ones rarely do. In Delaware Bay infection patterns are more continuous throughout summer and fall and often without the long incubation period; this leads to speculation about low dosage as the cause of delayed clinical infections (Ford and Haskin 1982).

Another character of MSX is its wide occurrence throughout high-salinity areas in both bays (Haskin and Ford 1982), yet it is apparently not contagious from oyster to oyster. Presence or absence of oysters has no apparent effect on occurrence of infection or prevalence levels. In Delaware Bay, rare plasmodia are commonly found localized in gill epithelia suggesting that infective particles are relatively scarce and that low dosage may produce infections (Ford and Haskins 1982). These factors of wide and rather uniform distribution of infections and failure of transmission among contiguous oyster populations probably induced many investigators to consider alternate hosts as necessary (Andrews 1981).

The authors of this paper have each studied MSX disease for about 30 years. Support for monitoring and studying the disease has declined to a low level at a time when severe mortalities are occurring in both bays and the industry is in crisis. An overview of the disease and a description of the unknown factors associated with 30 years of continuous monitoring of epizootic mortalities may stimulate others to undertake needed research. Much data has been accumulated to document epizootiological factors and tentative positions on life cycle (Ford and Haskin 1982; Andrews 1984a). Where interpretations tend to differ for the two bays, both positions are given. For example, annual cyclic patterns of MSX activity have been observed in Delaware Bay but none were discerned in Chesapeake Bay (Ford and Haskins 1982). A large literature exists on MSX disease, but it is not our purpose to review it in detail. An enormous collection of mortality and disease-prevalence data has been accumulated at middle Atlantic coast laboratories and diagnoses are documented by thousands of permanent, stained-tissue slides. Our purpose is to review these data and point out needed research areas for understanding the disease.

HISTORY OF MSX IN CHESAPEAKE BAY

The first mortality from H. nelsoni in Chesapeake Bay occurred in early summer of 1959 in private oyster beds in Mobjack Bay and Egg Island areas of the Bay proper. These deaths resulted from infections initiated in the late-summer of 1958 just two years after MSX first appeared in Delaware Bay. It is certain that this was the first large mortality in Chesapeake Bay because a survey of these same beds was made in February 1959 when mortality was lower than usual. During 1960 the disease spread throughout the lower bay from sting ray point at the mouth of the Rapphannock River to the bay mouth and up the James and York rivers where salinities exceeded 15 °/oo. Bayside creeks on Eastern Shore of Virginia were invaded also. The disease was present on seaside of the Delmarva peninsula, but not serious in the Virginia sector until the late 1960s. Virginia planters using private beds in Indian River and Rehoboth Bay in Delaware experienced serious losses to MSX in 1959.

After 30 years of monitoring MSX in the field, critical salinity levels can be estimated for disease activity. Experience in the James River indicates that 15 °/oo salinity is required to permit infection during early summer and often lower salinities permit subpatent infections which remain hidden until November or December. No mortality occurs from these late infections because high river discharge in winter and spring always depresses salinities below the 10 °/oo for expulsion of MSX. Those few years when MSX mortality occurred were drought years (1964, 1980, 1986-1987) (Andrews 1964, 1983). Salinity at the mouth of the Rappahannock River does not usually exceed 18 to 20 °/oo in late summer and except for drought years there has been little mortality by MSX. Studies of Delaware Bay seed oyster

area, where a summer salinity gradient of 15 to 20 $^{o}/oo$ usually prevails, confirm these key levels in respect to MSX activity; expulsion of the pathogen occurs below 10 $^{o}/oo$, infection occurs at about 15 $^{o}/oo$, but serious mortality does not occur until 20 $^{o}/oo$ is reached in late summer (Haskin and Ford 1982; Andrews 1983).

These salinity levels define MSX activity quite well in large estuaries with high spring runoffs that depress the levels each year. In coastal plains estuaries with little winter-spring runoff, Chesapeake Bay waters tend to dominate salinity levels; consequently, when droughts occur and Bay waters are salty, these small rivers such as the Piankatank, Great Wicomico and Choptank rivers do not get a spring period of low salinities. During normal years, these estuaries have salinities too low in early summer to permit infection by MSX; however, late-summer salinities are often adequate to allow late-summer infections; these infections often remain subclinical until May of the following year. If these estuaries get enough fresh water from runoff or the Bay to expel MSX (<10 $^{o}/oo$), the infections never appear; but if the Bay salinities are high, as occurred in six of eight years during the 1980's, serious mortalities occur in June and July quite suddenly and unexpectedly (Andrews 1982b).

Two three-year periods of drought in Chesapeake Bay have permitted MSX to do great damage to the oyster industry in Chesapeake Bay. Except for the James River, most seed areas in Chesapeake are in small, tortuous coastal plains rivers where retention of larvae is relatively high. The Great Wicomico and Choptank rivers are good examples. The first drought from 1980 to 1982 resulted in severe losses to MSX in the Great Wicomico River in 1982-1983 (Spring of 1983). The disease progressed into Maryland by 1982 with some losses but a wet spring eradicated all infections in April and May

1983. The second three-year period of drought from 1985 to 1987 caused severe losses in all rivers of Virginia and extended up as far as Eastern Bay in Maryland with heavy losses. It was notable that not until the third year in each of these prolonged droughts did MSX become severely active with high mortalities. Three of the drought years (1980, 1985, and 1986) were winter-spring droughts which set conditions for early-summer infections and late-summer mortalities with no freshwater discharge to intervene. Unfortunately, Perkinsus marinus increased greatly in abundance throughout its extensive enzootic area which is far greater than H. nelsoni area. P. marinus is not as easily controlled by freshwater discharges and may be a problem for many years even if salinities return to normal. The James River seed area was deeply invaded by P. marinus in 1987. Fortunately this pathogen has been essentially eliminated from Delaware Bay after its importation in Virginia seed oysters during the early 1950s.

Life Cycle and Pathology

Sporulation

The life cycle of MSX is obscure because all studies in field and laboratory were based on natural infections. Consequently, the origin and dosage of infective particles are unknown and the infective stage has not been demonstrated. Unlike most haplosporidians, which are commonly found in sporulation stage, MSX rarely achieves this stage in oysters. Because spores are presumed to be the usual infective stage in sporozoans, searches for alternate hosts have been pursued by many investigators, and use of antibody techniques provides a method for rapid search of many possible hosts. Failure to infect oysters using scarce spores has increased tentative theories that other hosts must serve as sources of infective particles. However, transmission was not accomplished in other pathogens of marine species where haplosporidian spores were readily available--e.g., chitons (Pixell-Goodrich); H. costale in oysters (Andrews unpublished data); and H. sp. in Panopeus herbstii (Perkins unpublished data). Spores are numerous and readily available in Teredo navalis and are similar in size and shape to those of H. nelsoni (Hillman 1978, 1982). Transmission of H. pickfordae, which parasitizes three freshwater snail species, was reported by Barrow (1965), but his controls were suspect considering the long incubation periods that occur in H. costale and H. nelsoni before infections become clinical.

Haplosporidium nelsoni was not named for ten years after its discovery as a virulent pathogen of oysters because spores were rare and confined to epithelia of digestive tubules. Andrews (1979) reported less than one case of sporulation per 2,000 infected oysters and 44 cases during 16 years of

processing 170,000 stained-tissue slides of live oysters and gapers. These cases of sporulation were scattered throughout the year although cases in June and July were most common. A slight increase in abundance of sporulation stages occurred during the mid-1960's when a period of drought permitted MSX to move far up Chesapeake Bay and attack highly susceptible oysters. The disease was exceptionally severe during that period with high mortalities. By selecting sick and moribund specimens from thousands of young, susceptible oysters, Couch et al. (1966) were able to recognize and describe sporulation of H. nelsoni. Farley (1967) proposed a tentative life cycle. Unlike most haplosporidians where sporulation occurs throughout all tissues, H. nelsoni confines sporulation to epithelia of digestive tubules. Before sporulation occurs, plasmodia must migrate from connective tissues to epithelia of tubules and apparently most parasites in Leidig cell tissues do migrate; but plasmodia are systemic and occur also in mantle and gill tissues where migration is more difficult. Plasmodia are not uncommon in epithelia of the digestive tract when sporulation is not occurring and these are probably sites of infection although gills are presumed to be main infection sites. Restriction of sporulation to tubule epithelia limits the quantity of spores that can be produced compared to other haplosporidian pathogens and this may facilitiate sporadic release of spores from live oysters as epithelia are destroyed by enlarging sporonts. Plasmodia in the multi-nucleate stage enlarge and undergo rapid nuclear division with a punctate appearance of chromatin material which results in up to 50 or more spores formed in sporocysts. This enlargement of sporonts occur between epithelial cells and causes protusions into the tubule lumina. There is evidence that oysters may live several months after sporulation has occurred. Nearly all plasmodia received the signal to migrate and to

sporulate, but it does not begin until the epithelial site is reached. Those few plasmodia that remain in Leidig cell tissues do not enlarge or sporulate.

The process of sporulation by H. costale is similar to that in MSX with all plasmodia enlarging into the punctate stage, but all tissues including mantle and gill become sites. Sporulation of H. costale occurs regularly in late May and early June each year and promptly kills oysters with massive numbers of large sporocysts--often before spores are mature. These sporulation sites for the two haplosporidian species are distinctive as physiological processes of great importance in the life cycles of the pathogens. The spore wrappings and tails used as taxonomic characters to separate haplosporidian genera (Sprague 1963; Perkins and Banning 1981) probably have less physiological importance than sporulation processes (Andrews 1984).

The rarity of sporulation by MSX in oysters justifies detailed descriptions of those few situations where it has occurred. In 1976, a dry year when MSX mortalities were high in Virginia, sporulation occurred in 39% of thousands of small oyster spat less than one-inch long in one tray lot (Andrews 1979). These spat, hatchery-reared from a highly susceptible stock of Rappahannock River oysters, were set in mid-May and held in a disease-free pond for early growth. A tray of these spat was moved to an MSX-enzootic area of the York River for exposure on 8 July 1976. When examined on 20 September only 9 weeks after first exposure, 40% of the spat had died and survivors were 88% infected with MSX (Table 1). Infections were more intensive than usual with 24% already serious (heavy and moderate) and were killing spat rapidly. A second batch of the same brood of spat was transplanted from the pond sanctuary to the York River on 16 August 1976.

Patent MSX infections were delayed until December and little mortality occurred until May-June 1977 when infections had become intensive (Table 1). This is typical seasonal timing for oysters exposed in areas enzootic for MSX after 1 August each year. In this later lot, only one case of sporulation occurred in 93 MSX infections during 1977. Over 60 other trays of oysters of all ages were held in close vicinity to the dying spat but exhibited no increase in the rate of sporulation from earlier years.

It is stimulating to speculate on the prevailing conditions that produced thousands of infections and sporulations in spat in one tray, but oysters in nearby trays developed only plasmodial infections at the usual level. The source and dosage of infection must have been similar for all trays because MSX rarely shows signs of localized infections. What factors induced sporulation in this lot but not in other susceptible oysters nearby? A similar event occurred in Delaware Bay waters when Myhre (in Haskin 1972), following the progress of MSX in susceptible and resistant spat. He reported sporulation in 4 of 34 susceptible spat whereas remission of infections occurred in resistant spat. These current-year spat were infected in the summer and fall of 1967 and exhibited sporulation in spring 1968.

The sources and dosages of MSX required to produce infections are urgently needed to predict the changes of intensity and distribution that occur with rather small changes in salinity and possibly other environmental factors in Chesapeake Bay. It is important to learn how to culture the pathogen in the laboratory and to determine the infective stages and assess dosage required to initiate infection. MSX appears to infect all populations of oysters within enzootic areas regardless of their location or abundance. There is no evidence that direct transmission occurs from oyster to oyster. Localized plasmodia occur frequently in gill epithelia, but do

these few plasmodia penetrate into connective tissues and blood sinuses to initiate systemic infections? Most Acetospora (Marteilia refringens in France, H. costale, H. sp., in P. herstii, H. tumefacientis in Mytilus californianus, and H. sp. in C. gigas in Korea appear to infect primarily the digestive tract initially, and they often concentrate there for development and sporulation. MSX and H. costale appear to initiate infections with few plasmodia and then increase in abundance by multiplication in the host. If this is the method of parasitation, why do some infections regress or remain chronic even in susceptible oysters? Once the pathogen is placed in culture in the laboratory, answers to these questions may be obtained. It appears fairly certain from field studies and many thousands of tissue slides that MSX does not kill oysters until the organism is systemic in blood sinuses and connective tissues; however, it kills with light infections in susceptible oysters and with early deaths in populations upon first exposure.

Pathology of H. nelsoni Disease

Genetic resistance to H. nelsoni disease developed naturally in native oysters in Delaware Bay and in York River, Virginia after intensive selection over many generations (Haskin and Ford 1979; Ford 1985; Andrews 1984). In the York River, prevalence of MSX infection rarely exceeds 10% in resistant oysters (vs 40 to 50% or higher in susceptible oysters) and localized infections are few. The following description of pathology refers to susceptible oysters that are readily killed by the disease.

H. nelsoni disease causes the usual gross signs of growth stoppage and poor meat condition typical of other diseases such as that caused by Perkinsus marinus (Andrews 1963; Farley 1968). During the early years in Chesapeake Bay, MSX-caused abcesses in oyster mantle tissue that interfered

with normal calcification of new shell, thereby leaving brown conchiolin blisters on the inside surface of shells. These were always rare and seldom seen in later years. A quick way to make diagnoses of systemic infections is to make fresh blood smears. An improved method is being assayed for accuracy at the respective laboratories of the authors now. A method of following the progression of diseases in live oysters is to weigh them weekly underwater which measures shell growth. Growth ceases during the third or fourth week after initial infection (exposure in early summer).

Stained-tissue slides are the most accurate method of diagnosing all stages of infection; however, even this method does not diagnose early infections during long incubations periods that may extend up to eight months. The first observation of disease in stained tissues is the abundance of hyaline hemocytes in areas where plasmodia have invaded. This occurs only in systemic infections. Therefore, gill, mantle, and digestive-tract epithelia must be checked for localized plasmodia. Multinucleate plasmodia often aggregate between gill and mantle epithelial cells as if they are unable to cross the basal membrane; it is uncertain whether these localized clusters eventually produce systemic infections, and oysters seem to be unaware of them. It is possible that the digestive tract is a more important site of infection where tubule epithelia and phagocytes are engulfing food particles regularly. Phagocytosis of MSX plasmodia is not an important defense by oysters partly because of their size. Because light infections (1 or 2 plasmodia per 100x field) often kill oysters, toxic effects may be more important than lysis of tissues and blockage of blood sinuses that occur in Perkinsus marinus disease. Field studies are not conducive to understanding the pathology of MSX disease in slides. If infections occur early enough in the season, gonadal development is

interrupted. Distribution of plasmodia is achieved through blood sinuses, and plasmodia increase by multiplication of nuclei and plasmotomy.

Epizootiology of *H. nelsoni* in Susceptible Oysters

H. nelsoni has a long period of infestivity in oysters that extends from mid-May to the end of October. This five and one-half month period of infection was defined in nature by transplanting susceptible, disease-free oysters to enzootic areas at frequent intervals followed by intensive sampling to determine when the disease could be diagnosed clinically by presence of plasmodia (Andrews 1966). Two subperiods were defined based on timing of infection and subsequent patterns of mortality. Before about August 1st, infections became patent in four to six weeks and mortalities began occurring about August 1st typically. In Virginia, prevalence of infection averaged about 30 to 40% in August and this level persisted through fall and winter despite concurrent mortalities of 30% or higher--because new infections became patent sporadically throughout late summer and fall. There was no strong synchrony of incubation or mortality periods such as occurred in *H. costale* (Andrews and Castagna 1978). During years of intensive MSX activity, infections and mortalities appeared a week or two earlier.

Susceptible oysters imported to an enzootic area after 1 August and before 1 November became infested immediately, but a delay of three to nine months occurred before infections were patent (Andrews 1982b). Late-summer infection failed completely during a few years, and in most others infections became clinical in April or May of the following year; during some years of intensive MSX pressure (high prevalences and mortalities), some infections appeared in late November or December but most remained hidden until April or May. Then high rates of mortality occurred in synchrony in June and early July after rapid development to intensive infections. The highest levels of infection and mortality often occurred in

late spring after late-summer infestivity. Groups of oysters transplanted in August, September and October exhibited synchrony in development of infections and mortalities usually. It may be significant that the first massive mortalities of oysters from MSX occurred in late spring or early summer both in Chesapeake Bay (1959) and Delaware Bay (1957) (Haskin et al. 1965, 1966; Andrews and Wood 1967).

Highly susceptible, disease-free oysters from low-salinity areas of the James River seed beds were monitored in trays and sampled regularly both at the Virginia Institute of Marine Science (Andrews and Frierman 1973) and at the Cape May, Laboratory of Rutgers University (Haskin and Ford 1979) in enzootic areas for 30 successive years. Allowing for some differences in site conditions and techniques, we found that MSX caused higher prevalences and greater mortalities at Cape May, NJ than at Gloucester Point, VA. Also, Ford and Haskin (1982) consistently found higher proportions of epithelial and localized infections in Delaware Bay than occurred at Gloucester Point (Andrews and Frierman 1973). Susceptible oysters died more commonly from light-intensity infections in Virginia although exchange of stained slides indicated comparable diagnoses. Planted beds of resistant native oysters in high-salinity waters were available to Rutgers scientist for observation, whereas after the first year or two, no commercial plantings were made in enzootic areas of lower Chesapeake Bay because only highly susceptible seed oysters were available for planting. In Delaware Bay native resistant oysters, too, exhibited more chronic epithelial and localized infections than were found in laboratory-bred resistant oysters in Virginia. Also, Ford and Haskin (1982) found higher prevalences (40 to 80%) and higher annual mortalities (60 to 80%) in susceptible oysters than were found in Virginia. If mortalities in Virginia were calculated for a whole year

levels of 50 to 60% were common. If the calculation was begun August 1 when deaths first occurred in spring transplants of susceptible oysters, total annual mortality approached 80% providing late-summer infections occurred. Because it usually takes two years to grow James River seed oysters to marketable size in Virginia, these levels of losses were not tolerable and oystermen cased planting in enzootic areas.

Three periods of prolonged drought (1963-1967, 1980-1982, and 1985-1987) extended the range of MSX throughout most of Virginia waters and deep into Maryland estuaries. It usually took one or two years of high salinities in these low-salinity areas before MSX caused epizootic losses. The major mortalities during recent droughts occurred in 1982 and 1987. One wet winter-spring season can bring salinites back to normal as occurred in 1983 and stop mortalities in most typical low-salinity areas. There were three winter-spring droughts during the 1980s (1985, 1986) which allowed MSX to spread so widely. Native oysters have not been selected for resistance in areas above the mouth of the Rappahannock River in Chesapeake Bay, therefore, they were extremely vulnerable. During normal years, spring salinities in these areas decline below 10 $^{0}/_{oo}$ which kills the pathogen or allows oysters to expel it. Also, salinities usually stay below the 15 $^{0}/_{oo}$ value during late spring and early summer thereby inhibiting MSX infection and proliferation. By late summer, salinities are usually adequate for infection, but if spring lows reach 10 $^{0}/_{oo}$ for a week or two, the disease is over come and no damage occurs. Prolonged droughts, such as occurred during the 1980s, permit MSX to become effective both in early and late-summer infections which is disastrous for these low-salinity areas where oysters are currently planted and grown for market. However, the late-summer infections are most likely to succeed in Chesapeake Bay where many

estuaries and most seed areas are coastal plains rivers with little freshwater runoff, therefore, their salinities are dominated by Bay waters.

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Late-summer infections are much more erratic in occurrence and intensity than early-summer ones for unknown reasons. They seem to occur most intensively in drought periods during which they develop patent cases in late fall or early winter. Often MSX does not become patent from late-summer exposure until the following April or May. MSX seems to intensify and spread more widely during the second or third years of prolonged droughts, indicating a delayed response to increased salinities. A late-summer infection that occurred in 1966, during the long drought of 1963 to 1967, exhibited the timing patterns of prevalence and mortality when the disease was most intense (Figure 4). A group of disease-free Deep Water Shoal oysters that was transplanted to Gloucester Point on 17 August 1966 showed 28% MSX infections on 20 October--most of which were already advanced in intensity. By 14 December 1966, prevalences of 64% and 80% in duplicate trays occurred. However, the death rate was low through winter and spring and slowly climbed to 5% per month in May; 40 of 47 gapers collected during this period prior to 1 June had MSX infections, mostly moderate and heavy ones. Then during June and July the death rate soared to 35 to 50% monthly. By 1 August new, early-summer 1967 infections began killing Horsehead Rock oysters transplanted to the endemic area in March (Figure 3). The severe selection of the Deep Water Shoals population by late-summer infections (16% in spring and 62% more in June and July) depressed mortality from early-summer exposure in this lot as compared to the spring-imported Horsehead lot. There is not much synergistic effect on mortality between the two infection periods because early-summer infections do not become patent until about mid-July usually.

R.C

EPIZOOTIOLOGICAL METHODS —

The invasion of H. nelsoni into Delaware and Chesapeake Bays during the late 1950s and early 1960s required considerable sampling of planted beds to follow the progression of the disease by seasons and areas. Mortalities were estimated by counts of live oysters and boxes (empty hinged shells) in bushel samples dredged from public and private oyster beds. This requires considerable knowledge and experience with oyster beds and fouling organisms to determine cumulative mortalities. Age and history of oysters on beds were essential for understanding mortality and prevalence values. In Delaware Bay, monitoring of seed oyster and planted beds in enzootic areas was important for advising oyster planters on timing of planting and harvesting. In Chesapeake Bay, oyster planting ceased in the enzootic area and only areas usually free of the disease were planted after 1960.

Both laboratories began monitoring oysters in trays where accurate mortalities could be obtained and source and ages of oysters and previous history of MSX exposure were known. In Delaware Bay monitoring trays were held on the Cape May shore near low tidal level. In Virginia, legged trays were set beside stakes on oyster beds. Both native and susceptible imported oysters were held for monitoring along with resistant strains. Both laboratories used susceptible James River seed oysters from disease-free, low salinity areas as controls. This permitted comparison of annual prevalences of the disease and mortalities caused by it. Gapers were recovered by regular examinations of all oysters in trays. Only by daily examinations can a high proportion of gapers be recovered with meats because crab and fish scavengers soon extract the tissues. If predation and smothering are excluded, the tray method provides reliable data on disease

activity. In Virginia, the tray method was necessary because oysters were not planted in enzootic areas after 1960.

At first, intensive sampling of gapers and live oysters around the year was necessary. But as epizootiological data became available, key periods for sampling became evident. After taking samples of disease-free oysters at the time of import for background, samples were taken in late July or early August to document pre-mortality prevalence of MSX and again in late October or early November to determine levels of infection that ~~prevailed~~ occurred before winter temperatures prevailed.

Winter stress caused mortalities of infected oysters in March or April. During intensive MSX years, a December sample provided evidence of early appearance of late-summer infections becoming clinical, and sampling in May was essential to confirm prevalence before a June and July mortality period. Often additional samples were taken in late summer and early fall because early-summer infections do not have a fixed incubation period, therefore, they may become patent at any time between 1 August and 1 November.

All gapers were processed because they provide evidence of cause of death. Winter gapers need to be separated from those collected during the warm season because they are more abundant and a smaller proportion of deaths are caused by diseases. Any gaper with some intact tissue can be diagnosed for systemic infection of MSX and should be processed. Crowding oysters in trays is not detrimental to disease studies providing smothering is avoided; regular examinations help to avoid such losses.

Because MSX becomes a systemic disease during its development to fatal intensities, any tissue of oysters may be processed into stained slides for diagnoses but most laboratories use a full cross-section just posterior to the palps and the whole cross-section is examined for diseases. When

oysters in trays are sampled or lost, or the number of oysters is increased, mortality must be calculated for each period and added using instantaneous rates ~~for each period and added using instantaneous rates~~ to calculate seasonal or annual mortality.

Effects of H. nelsoni on Oyster Culture in Chesapeake Bay

There has been a progressive decline in the Chesapeake oyster industry since MSX appeared in the Bay. MSX killed over 90% of all oysters on public and private beds in the enzootic area in two or three years after the disease first appeared in 1959. After spring plantings in 1960 were killed that summer, private planters no longer could use susceptible James River seed oysters. Furthermore, setting declined in the large, flushing-type rivers because broodstocks were destroyed. The most critical loss involved private plantings of oysters in Hampton Roads which appear to have been critical broodstocks for the seed area. Setting in the seed area declined to about one-tenth the rates that occurred during the 1950s. No resistant seed stocks were available in Chesapeake Bay.

The long drought of the mid-1960s (1963-1967) increased mortalities from MSX throughout Virginia estuaries and from the Patuxent and the Choptank rivers down bay in Maryland. Reduced oyster populations and higher salinities in the James River seed area permitted the remaining oysters to become larger which encouraged their sale as soup and market oysters. The prices of seed and market oysters remained low ($1.50 to $4 a bushel) until inflation began about 1975. Meanwhile, the 1960's drought had discouraged private planting in rivers above the typical enzootic zone. After 1972 when Hurricane Agnes killed many oysters in low-salinity areas, another problem of predation by cownose rays arose to discourage planting. Soon after MSX appeared, shucking houses in Virginia began importing oysters in the shell from the Gulf of Mexico coast. This increased steadily as locally-grown oysters became inadequate to meet demand. Poor recruitment in Maryland caused a reduction in supply of oysters many of which were traditionally

shucked in Virginia. With an increase in price of market oysters during the inflation of the mid-1970's, the cost of growing and marketing oysters also increased. By 1980, most growers in the Rappahannock River and other low salinity areas had almost ceased planting--except for a few in very low salinity areas such as the potomac River tributaries and the upper Rappahannock River. Then about 1985, packers began importing fresh shucked oysters by air from the west coast of North America. Local production is estimated now to be less than 15% of oysters marketed out of Virginia. The remaining James River seed oysters, after the drastic high salinities produced by droughts during the 1980s, were mostly killed by the two diseases (caused by H. nelsoni and Perkinsus marinus) in the lower half of the seed area and are being fished out in the upper half and sold as small market oysters. The future looks bleak when salinities return to normal because neither seed oysters nor market oysters will be available. Dry periods and salty waters usually favor setting of oysters in the small coastal plains estuaries that provide most seed areas in the Bay for Maryland and for some of Virginia. These small estuaries do not require large populations of broad-stocks for favorable recruitment. They could revive the oyster industry quite rapidly when weather returns to normal to expel the diseases. Some good sets in these low-salinity estuaries during the 1980s have been destroyed by MSX and P. marinus such as occurred in the Great Wicomico River in 1982-1983, and is even more intensive during the 1985 to 1987 drought.

Occurrence of H. nelsoni on Seaside Virginia

H. nelsoni has been present on seaside of Eastern Shore of Virginia since oysters were first sampled in 1959 for diagnoses by stained-tissue slides. After H. costale was discovered and its epizootiology described (Andrews et al. 1962), separation of it from MSX was possible by the occurrence of sporulation and timing of patent infections during the months March through June. Experiments showed that H. costale infected before 1 August each year, but infections remained subclinical until about April of the following year. Diagnoses of plasmodial infections of H. costale that became intensive during May could always be confirmed by occurrence of sporulation between 15 May and 1 July. From 1 July to ~~A~~about 1 April, only MSX plasmodia occurred in oyster tissues and mortalities during this period were ascribed to MSX disease. Although plasmodia of the two pathogens can usually be separated, there are slide preparations and over-lapping of diagnostic traits that cause difficulties in spring.

Native oysters on seaside are a separate genetic race from Chesapeake Bay oysters and they exhibit fast growth, thin shells, and intensive intertidal spatfalls. These are ecological adaptations to high-salinity conditions that also favor predators and diseases. In this high-salinity environment, in most years MSX had low prevalence and no recognizable peak of mortality (Andrews and Castagna 1977). Death rates from July through March remained below 5% per month usually. Annual mortality was often less than 10% in native oysters and quite similar in James River seed oysters which were also monitored in trays. (Table 2) There were a few years during the late 1960s and 1970s when mortalities of 20 to 30% occurred with prevalences to match. However, there seemed to be a gradual increase in MSX

activity on seaside until mortalities of 30 to 40% occurred from 1979 to 1981; although prevalences of infection also increased, they did not always correspond with mortality rates. MSX failed in some years on seaside and remission was observed frequently. Remission occurred only in the plasmodial stage; after sporulation began, all plasmodia sporulated in all tissues and oysters were killed promptly. Often spores are not mature when oysters die with intensive numbers of immature sporonts. Because gapers disintegrated rapidly, it is not clear what proportion of spores matured in sea water.

Sporulation in Haplosporidum nelsoni

The chief restriction to the determination of the life cycle of H. nelsoni is the inability to grow the pathogen in culture for production of artificial infections. Both stage and dosage for infection could be determined when this is achieved. In nature, infection occurs regularly over a period of 5-1/2 months usually, therefore it would seem that artificial infection could be achieved easily. The factors that determine sporulation are unknown. Sporulation has occurred rarely in nature with Crassostrea virginica as the host, therefore, speculation has persisted that there must be another more favorable host. None has been found in searches among a variety of invertebrate species closely associated with oysters such as scavengers and other molluscs.

Most other species of the phylum Ascetospora, order Balanosporida, that are pathogens in molluscs, exhibit regular sporulation, thereby providing the presumptive infective stages for the old sporozoan group called haplosporidans (Sprague 1970, Lauckner 1983, Andrews 1984). Except for questionable transmission in 3 species of freshwater snails of Minchinia pickfordae (now H. pickfordae) (Barrow 1965), attempts to infect using spores have failed (Pixell-Goodrich 1915, Andrews unpublished data, Perkins). Spores from H. nelsoni, H. costale, and Haplosporidium sp. in Panopeus herbstii, have been tried without success. Spores seem to be available throughout the warm season in H. herbstii (Perkins unpublished data) and Teredo navalis (Hillman 1978, 1982). Spores in H. nāvalis are similar in size and shape to H. nelsoni as are spores from Korea in Crassostrea gigas.

The tissue site of sporulation may be significant for the method of release of spores (Lauckner 1983, Andrews 1984). Most Balanosporida species in bivalves sporulate throughout the connective tissues or Leydig cells to the extent that tissues are packed with sporocysts and release of spores in quantities does not occur until dead oysters disintegrate. Haplosporidium costale and H. armoricanum are similar in this respect and have similar sizes of spores but different spore wrappings. The pathogen in Teredo navalis packs sporocysts into connective tissues also. In contrast, H. nelsoni and the species from Korea sporulate in digestive tubule epithelia in such packed numbers that gut lumens tend to be occluded and epithelia to be disrupted. This may allow spores to be released from living oysters because C. virginica appears not to be killed promptly after sporulation occurs; this is in contrast to H. costale, which produces short, sharp mortalities in late spring after sporulation. It appears that the pathogens that utilize systemic sporulation produce far more spores than those using epithelia as the site. In addition there is the necessity for H. nelsoni plasmodia to migrate from the Leydig tissues to tubule epithelia, and plasmodia in gills and mantle may be lost in this activity.

Sporulation is rare in H. nelsoni and unique and puzzling as to timing and the conditions that produce it. The periods when sporulation increased slightly were those of dry years, high salinities and intensive mortalities caused by the disease. Near the end of the drought period of 1963 to 1967, sporulation cases became more frequent than the < 1 per 2000 infected oysters observed in Virginia from 1959 to 1964 (Andrews 1979). Sporulation seemed to occur most commonly in young, highly-susceptible oysters which had never been exposed to the disease before. The slight increase in cases that occurred in Maryland (Couch 1966) and in Virginia (Andrews 1979) followed three years of

drought and intensive infection and mortality levels in an expanded areal distribution that exposed populations of highly-susceptible oysters for the first time. Curiously, Myhre (in Haskin 1972) found several cases of sporulation in susceptible spat of the 1967 year class in early summer of 1968. These spat became infected in the fall of 1967 and exhibited rare spores (4 in 34 spat) in mid-June 1968. Resistant spat monitored concurrently discarded their infections in spring.

In 1976, a dry year with high mortalities in susceptible James River oysters from H. nelsoni (60%), sporulation appeared in current-year spat in the York River in mid-September. The tray was packed with thousands of spat < 1 inch long which were bred in the hatchery from highly-susceptible Rappahannock River broodstock. These spat were held in a salt-water pond free of the disease for two months and moved to an enzootic area of the York River July 8, 1976. By September 20, 40% had died and 88% of live oysters had infections of which 39% were in sporulation. No increase in sporulation was found in 60 other trays of oysters of all ages and susceptiblities and that were within 50 m of the spat tray. A second lot of the same brood of spat was moved from the pond to the York River on 16 August and only one case of sporulation was found during the following year (Table 1).

Why does H. nelsoni fail to sporulate individual routinely? Failure seems to have no relation to the intensity of disease in oysters. What kind of dosage and what circumstances would permit thousands of spat to become infected simultaneously? Where did the infective particles originate? There were no fouling organisms on the tray or oysters only nine weeks after exposure except sea squirts. Why did the lot first exposed August 16 not exhibit sporulation later although infections were delayed until December as expected for importations after the first of August (Andrews 1982). The level

of infection was lower in the lot imported later but the disease was still intensive during the following year. If the pathogen requires higher temperatures or salinities, these were provided in oysters held on seaside of eastern shore of Virginia for 28 years. One is forced to the conclusion that some subtle factor in the interaction of host and parasite triggers or blocks the sporulation process. Perhaps some change in enzyme activity or amino acids such as reported by Feng et al (1970) for infected oysters holds the key. Could dosage be the critical factor?

See Table p 57

MSX, A Most Peculiar Pathogen

Jay D. Andrews

When the haplosporidan called MSX first caused epizootic mortalities in lower Delaware Bay in late spring of 1957, it was already exhibiting unusual traits for this sporozoan group of pathogens. After 30 years of study, we know that the infection period for this spring mortality was probably in late summer or fall after 1 August 1956. MSX has a continuous infection period from mid-May to 1 November, but the late-summer infections are not consistent from year to year and they are followed by 6 to 8 months of localized or hidden infections before patency occurs in April or May of the following year. In Chesapeake Bay, late-summer infections fail completely some years and the period of incubation before clinical diagnosis is possible varies from 3 to 8 months. The co-generic species H. costatum exhibits this trait of long incubation in a regular annual cycle with May-June mortalities.

A second peculiar trait is the failure of MSX to sporulate because most pathogens of the group are known only by their spores which presumably are the infective stage. Sporulation was extremely rare in Delaware bay where natural resistance of native oysters and prolonged chronic infections could be expected to allow the pathogen to complete its life cycle. In Chesapeake Bay, sporulations is also rare, but slight increases in occurrence during the dry mid-1960's in Maryland facilitated the description of spores (Couch et al., 1966) and naming of the pathogen (Haskin et al., 1966). Sporulation was found most often in young, highly susceptible oysters in Maryland and Virginia. During monitoring of over 100 laboratory-bred progeny lots at Gloucester Point, only one lot of three-month-old spat exhibited significant sporulation (39%, Andrews, 1979). Other groups of oysters of all ages and

degrees of susceptibility were being monitored only meters away from these spat. What caused MSX to sporulate in just one tray of oysters?

The site of sporulation of MSX in the epithelia of digestive tubules is also unique because H. costalis and most haplosporidans sporulate throughout the connective tissues of the hosts. The plasmodia appear to migrate from Leidig cell tissues to the epithelia where they enlarge into sporonts packed between host cells and bulge out into the lumen. Stained slides show little evidence of damage to epithelial cells except crowding, but there must eventually be loss of epithelia into the intestinal lumen. The seasonal timing of sporulation is unconfirmed although the most cases occurred in June and July. It appears that sporulation does not cause immediate death as in H. costalis which limits attempts to ~~construct~~ determine seasonality of a life cycle (Andrews, 1979).

The source of infective stages of MSX is still a mystery. The failure of production of spores has led most researchers to speculate that there must be an alternate host. There appears to be no proximity effect on infection, and isolation from other oysters does not prevent infection. Early-summer infection is wide-spread throughout the enzootic areas and in dry years when salinities are high, the disease moves far up Chesapeake Bay in a year or more. If it exists, an alternate host must also be an ubiquitous species, but does it too move up the Bay when salinities are higher than usual? Attempts to infect oysters with spores have failed and experiments with other pathogen-host situations where spores are readily available have also failed (Pixell-Goodrich 1915). No alternate host has been found for other haplosporidans. Spores of the same size and description have been found rarely in Asiatic oysters; the pathogen of shipworms (Teredo sp.) appears to be similar to H. nelsoni (Hillman). The long period of infection is peculiar for MSX also.

Furthermore, the 5 1/2 to 6 month infection period is divided into two periods by the progression of the disease after exposure of oysters. Early-summer infections develop rapidly and in 5 to 6 weeks oysters begin dying. With no apparent change in environmental conditions around 1 August, oysters imported in late summer may not develop clinical infections until May of the following year. Is dosage involved? Oysters transplanted to enzootic areas in September and October have the same timing and level of infection as those first exposed in August. What kind of alternate host provides infective stages for 6 months and perhaps with two different patterns of development? The pathogen needs to be brought under culture in the laboratory; unfortunately, oyster tissues were cultured for several months without growth but MSX did not multiply in them.

Diseases of Oysters during Droughts (Chesapeake Bay)

Jay D. Andrews
August, 1988

Two diseases of oysters, MSX and Dermo, (Minchinia nelsoni and Perkinsus marinus) have severely limited the culture of oysters in lower Chesapeake Bay during the past 30 years. Since 1959, when MSX invaded Chesapeake Bay, few native oysters have been produced on public beds in Virginia and planting of James River seed oysters on private beds has declined drastically. Most of this decline has been blamed on MSX which in endemic areas causes about 50% annual mortality of susceptible oysters such as James River seed. This mortality rate is intolerable for seed stocks that require two or three years to mature to marketable size.

Seasonal weather is an important factor in limiting the distribution of oyster diseases because salinity is a regulatory factor on these pathogens. MSX requires about 15 o/oo salinity to infect oysters and usually does not cause serious mortalities unless summer salinities reach 18 to 20 o/oo. However, these limitations are coupled with seasonal fluctuations in salinities that allow expulsion of the disease in spring at < 10 o/oo for ten days or more. Prior to the mid-1980's, the James River seed area had light MSX mortality in the fall in only two or three of 25 years, and expulsion always occurred in spring. Also, after 1960, seed oysters were

transplanted only to growing areas which usually had spring salinities low enough to expell MSX if infected oysters were transplanted.

During periods of drought, such as occurred in the mid-1960's and the 1980's, increased salinities allowed MSX to spread rapidly up Chesapeake Bay into populations of highly susceptible oysters. For years of normal rainfall in the Chesapeake drainage area, the endemic area on the western shore is confined essentially to waters below the mouth of the Rappahannock River. The disease spreads throughout upper Virginia and many Maryland waters during drought years and recedes as rapidly when salinities return to normal.

Dermo cannot compete with MSX in the endemic area of the latter disease because it is usually slower to spread and requires dense populations of oysters to thrive. It was depressed in the lower bay after MSX killed most oysters and planting of private beds ceased. However, Dermo has a lower salinity tolerance (12 to 15 o/oo) for infection and to cause mortality of oysters. Therefore, it has a far wider endemic area than MSX and persists in nearly all Virginia estuaries (Rappahannock, Piankatank and Great Wicomico rivers) and those estuaries of the lower bay area of Maryland (mouth of Potomac, Choptank and Patuxent rivers). Furthermore, Dermo is suppressed but not easily exterminated by low salinities at any level. It can persist several years at low seasonal salinities without causing appreciable mortalities. Nearly all oyster growing areas reach the 12 to 15 o/oo salinity level during average summers which allows some multiplication of Dermo.

The weather during the 1980's has been catastrophic for oyster populations because of diseases. Seven of the nine years during the decade

have brought drought in the Chesapeake Bay drainage area and four of those years have also been winter-spring droughts which caused salinities to increase far above normal. Only the years 1983 and 1984 brought some relief from drought. These two years eliminated MSX in most areas above its endemic zone but it did not stop Dermo which has been steadily advancing up bay in area and intensity.

The pathogenicity of Dermo is illustrated in the James River where it has found the two requirements needed for its spread and to cause high mortalities. Dermo needs high densities of infective stages to establish infections. the James River seed area provided relatively abundant live and dead oysters for easy transmission. With adequate salinities (highest ever recorded) the pathogen killed whole beds of oysters. The increase in infective stages has apparently allowed the pathogen to spread from bed to bed rather than to be confined to particular beds by infections that spread from oyster to oyster which was the usual sequence.

The fourth consecutive year of drought is now in progress and recent samples indicate that no oysters will survive the 1988 season at Wreck Shoal in the middle of the seed area. Furthermore, the disease is spreading to new beds farther upriver with two or three months of Dermo activity left this year for new generations of infections. No seed oysters were obtained from the James River in 1987 because the small oysters were sold for marketing at a high price (> $20/bushel). However in 1985 and 1986, James River oysters infected with Dermo were transplanted to low-salinity tributaries of the Potomac River where it has become endemic during the drought years. Oyster production in Chesapeake Bay is at an all time low

and it is not clear how long it will take to confine these dieases to their usual limited areas and intensities.

- Notes on MSX Activity in Mobjack Bay

J. D. Andrews

May 1988

During the 1950s, two large Virginia oyster companies (Miles and Ballard) planted large acreages of private oyster beds between New Point Comfort and Old Point Comfort along the western shore of Chesapeake Bay. These companies that planted and shucked their own oysters also had large beds in the deep, open areas at the mouth of Mobjack Bay. Our tray monitoring station was located off the mouth of Davis Creek near Old Point Comfort light house in about 15 feet of water. Many of these bay beds were relatively deep for oyster culture with depths up to 20 or 25 feet.

These planters had stocks of two or three years' plantings on the beds, and each August or September there was a survey to determine which beds were ready to be harvested. We (VIMS biologists) were fortunate for a few years during the 1950s to be invited along on the dredge boats to observe growth and mortality and to collect samples for Dermo tests. P. marinus was the only important disease killing oysters during the 1950s.

In August 1959, the survey trip revealed recent mortalities of 30 to 40% on all beds except those planted in the spring of 1959. This was the first indication that MSX had been introduced into Chesapeake Bay -- presumably from Delaware Bay where it had begun causing mortalities in the spring of 1957. The disease was not found at Gloucester Point in 1959 and it was rare in Hampton Roads; but it spread to its full normal range in 1960. This is from the mouth of the Rappahannock River downbay and up the York River to West Point and up the James River to the James River bridge. Oysters were planted as usual in the spring of 1960 despite our warnings of

the catastrophe occurring in Delaware Bay from MSX. The high death rate during that summer and fall of 1960 destroyed most oysters on Mobjack Bay and Chesapeake Bay beds and they have never been replanted during a period of 30 years.

The Mobjack Bay station was one of many sites monitored in Chesapeake Bay for virulence of the disease in susceptible James River seed oysters. During these first two years of 1959 and 1960, James River seed oysters already planted by the Miles Company in Mobjack Bay were dredged and placed in trays for monitoring. Thereafter, disease-free oysters from Horsehead or Deep Water Shoals beds, areas free of diseases until the invasion of 1986-87 into the seed area, were transplanted in spring each year to Mobjack Bay for monitoring in trays. No oysters on planted beds were available after 1961.

The disease caused by P. marinus has been endemic in Mobjack Bay since 1940 at least, and it appeared in the planted oysters there collected for tray monitoring in 1959 and 1960. Because all the oysters on the Mobjack Bay beds were dead or buried by 1961, Dermo rarely occurred in our trays thereafter; therefore, the high death rates can be attributed mostly to MSX. In trays where predation and smothering were eliminated MSX infections were common in live oysters and gapers diagnosed for the disease in stained slides. Dermo can still be found on pilings and other man-made structures along the shores of Mobjack Bay and in its tributaries. It reappears on planted beds after renewal of culture as occurred in the upper sector of Mobjack Bay during the 1980s. Recent planting was possible because native oysters exhibited considerable genetic resistance to MSX after nearly 30 years of selection; however, these oysters were overcome by the high intensity of MSX activity during the 1985-87 drought period.

In Table 1, successive importations of susceptible James River oysters are shown by years of monitoring along with mortalities and prevalences of the two pathogens in live oysters and gapers. In the gaper column, the occurrence of Dermo is shown because most dead oysters were found to be negative for this disease and nearly all were killed by MSX.

After about 1970, many lots of lab-bred oysters were being monitored at Gloucester Point in the York River for resistance to MSX (up to 65 tray lots each year); therefore, the intensity of monitoring disease in Mobjack Bay had to be curtailed. When visits to trays were a month or more apart, few gapers with residue meats were recovered.

Mobjack Bay, with large open areas free of native and planted oysters after 1961, was one of the few areas in Virginia where MSX could be monitored free of interference by Dermo. This was possible because both shells and live oysters tended to sink into the bottom or be covered by substrate from strong east winds out of Chesapeake Bay. After the first few years, oyster lots were usually held only one year for monitoring and trays were easily lost by loss of stakes in storms and by anchorage of fishing boats. If they had been held for several years, Dermo may have become established. It will be seen, however, that Dermo tended to decline even in tray lots already infected -- probably because high death rates by MSX tended to eliminate Dermo-infected oysters. Lot MJ6 was held six years with only one dubious rare infection by Dermo. After 1960, it was rare to find any Dermo in samples of live oysters from beds or trays.

In comparing mortalities through the 25 years of tray monitoring one must be aware that some trays were lost or not followed through full years. Whereas most of the mortality occurred from August to October inclusive, there was always some mortality from MSX in late winter (March & April).

Furthermore, fall imports do not begin dying appreciably until the following June and July. Therefore, in fall imports both fall and spring infections cause deaths within the 1 April to 31 March annual period and often mortality was substantially higher than when only early-summer infections were involved. In all four years that fall imports were transplanted to Mobjack Bay, MSX infections occurred in fall, and early-summer mortality was high. During some years, MSX fails to infect fall imports during the first fall.

See Table p 42

Table 1. Mortalities caused by diseases (*Minchinia nelsoni* & Dermo) in James River seed oysters in trays in Mobjack Bay. Prevalences in live oysters and gapers indicate that MSX was the primary cause of deaths. All importations were of James River seed oysters free of diseases except as noted. Fall imports are marked with an asterisk.

Tray No.	Year	Annual Mortality % (1 Apr-31 Mar)	MSX in Live Oysters %	Dermo in Gapers[x] H	M	L	N	Remarks
MJ1	1960[1]	44.5	ns	0	0	0	10	JR planting in Mobjack
	1961	61.5	ns	3	2	0	13	
MJ2	1960[2]	51.8	ns	4	3	2	1	JR planting in Mobjack
	1961	43.0	ns	2	3	4	14	
	1962	38.0	ns	5	0	0	1	
	1963	19.4	ns	0	0	0	1	
	1964	19.0	ns	0	0	0	1	
MJ3[3]	1960	16.0	ns	0	0	1	8	JR planting
MJ4[4]	1960	35.2	4 2 Nov.60	12	1	1	0	

*Fall importation of oysters.

[x]Only gapers collected in warm season after 1 July are reported. After 1971 Mobjack Bay trays were examined at wide intervals and few gapers were recovered.

[1]Hurricane muddied tray and smothered half of oysters.

[2]James R. oysters planted in Mobjack in 1957 and exposed to MSX in 1959, therefore selected by MSX one year. Dermo well established by 1960.

[3]Oct. 1959 James River planted seed put in tray 22 Jun. 60 but tray lost after 16 Aug. visit.

[4]James R. oysters planted May 1959 and trayed 22 Jun. 60. Dermo established.

Table 1 (continued)

Tray No.	Year	Annual Mortality % (1 Apr-31 Mar)	MSX in Live Oysters %	Dermo in Gapers[x] H	M	L	N	Remarks
MJ5[5]	1961	55.8	ns	0	0	1?	26	Horseheads
	1962	61.8	ns	0	0	0	5	
MJ6[6]	1962	66.2	48 5 Sep.62	0	0	0	21	Horseheads
	1963	62.8	44 19Jun.63	0	0	0	5	
	1964	52.7	ns	0	0	0	7	
	1965	45.4	ns	0	0	0	6	Combined old oysters
	1966	34.6	ns	0	0	0	5	
	1967	16.0	46 7 Jun.67 4 22Sep.67	--				
MJ7*	1963	58.9	32 11Jul.63	0	0	1	44	21 Sep.62 import
	1964	45.0	88 12Nov.63	0	0	0	10	to 4 Sep.64
MJ8	1963	40.8	4 14Mar.63 4 28May 63 28 6 Aug.63 64 1 Oct.63	0	0	0	16	to 11Dec.63 Aug.62 plants
MJ9	1963	43.6	12 6 Aug.63 64 1 Oct.63	0	0	0	29	Horseheads
	1964	70.0	32 29Apr.64 76 28May 64	0	0	0	20	
MJ10*	1964	71.9	0 11Dec.63 8 29Mar.64 8 28May 64 40 26Jun.64	0	0	0	46	10 Sep.63 import Horseheads
MJ11	1964	65.6	12 26Jun.64 64 4 Sep.64 64 18Nov.64	0	0	0	58	Horseheads

[5]Tray lost after 7 Nov. 62.

[6]Early deaths in spring (17%) not caused by MSX -- perhaps salinity shock; oysters added from younger lots during 1964 and 1965 where numbers got too low.

Table 1 (continued)

Tray No.	Year	Annual Mortality % (1 Apr-31 Mar)	MSX in Live Oysters %	Dermo in Gapers[x] H	M	L	N	Remarks
MJ12*	1965	70.0	12 18Nov.64 60 5 Feb.65 52 16Mar.65 84 7 Jun.65 60 23Jul.65	0	0	0	33	16 Sep.64 import Horseheads
MJ13	1965	64.7	4 7 Jun.65 28 23Jul.65 52 25Oct.65	0	0	0	36	Horseheads
	1966			0	0	0	11	
MJ14*	1966	73.1	20 25Oct.65 44 14Jan.66 68 11May 66 52 7 Oct.66	2	0	0	35	24Aug.65 import Horseheads
MJ15	1966	66.8	0 20Jun.66 44 8 Aug.66 52 7 Oct.66 52 20Feb.67	0	0	0	30	Horseheads
	1967			0	0	0	4	
MJ16	1967	56.4	0 7 Jun.67 44 31Jul.67 40 22Sep.67 21 19Jan.68 41 22May 68	0	0	0	19	Horseheads
MJ17	1967	63.6	0 23Jun.67 52 31Jul.67 56 16Aug.67 36 8 Sep.67 64 19Jan.68	0	0	0	31	Potomac R. oysters
MJ18	1968	66.7	0 13Jun.68 44 4 Sep.68 24 17Apr.69	0	0	0	23	Deepwater Shoal oysters

Table 1 (continued)

Tray No.	Year	Annual Mortality % (1 Apr-31 Mar)	MSX in Live Oysters %	Dermo in Gapers[x] H	M	L	N	Remarks
MJ19[7]	1968	39.9	0 13Jun.68 16 15Aug.68 20 2 Oct.68	0	0	0	11	Potomac R. oysters to 2 Oct.68
MJ20[8]	1969	40.9	0 30Jun.69 28 14Aug.69 40 30Dec.69		--			Deepwater Shoal oysters to 30Dec.69
MJ21	1970	55.7	0 9 Jun.70 0 29Jul.70 36 18Aug.70 12 8 Sep.70 20 25Sep.70 36 19Nov.70	0	0	0	11	Horseheads to 19Nov.70
MJ22	1971	52.6	4 16Jul.71 40 10Aug.71 56 31Aug.71 32 27Sep.71 0 16Nov.71 16 29Mar.72	0	0	0	5	Deepwater Shoal oysters
MJ23	1972	22.2	0 2 May 72 0 20Jul.72 8 30Aug.72 20 13Oct.72 32 16Jan.73					Hurricane Agnes fresh water Horseheads to 16Jan.73
MJ24	1973	53.6	0 14Jun.73 76 27Aug.73 28 26Sep.73 24 23Oct.73 29 8 Jan.74	0	0	0	1	Horseheads to 8 Jan.74

[7]Tray lost after 2 Oct. 68.

[8]Tray closed out 30 Dec. 69.

Table 1 (continued)

Tray No.	Year	Annual Mortality % (1 Apr-31 Mar)	MSX in Live Oysters %	Dermo in Gapers[x] H M L N	Remarks
MJ25	1974	59.9	56 31Jul.74 36 28Aug.74 32 25Sep.74 24 30Dec.74	--	Horseheads to 30Dec.74
MJ26	1975	52.9	28 4 Aug.75 52 3 Sep.75 25 6 Nov.75 40 7 Oct.75	--	Rainbow Rock oysters to 7 Nov.75
MJ27	1976	38.3	0 2 Jul.76 8 12Aug.76 16 31Aug.76 24 6 Oct.76 36 17Nov.76	--	Wreck Shoal oysters to 17Nov.76
MJ28	1977	48.1	24 19Jul.77 16 9 Aug.77 24 31Aug.77 16 20Sep.77 28 18Oct.77 40 2 Dec.77	--	Pt. of Shoals oysters to 19Apr.78
MJ29	1978	6.5	0 15Jun.78 0 18Jul.78 12 11Aug.78	--	Tray lost after 11Aug.78
MJ30	1979	54.3	0 17Jul.79 28 1 Aug.79 12 27Aug.79 36 12Sep.79 28 2 Oct.79 40 17Oct.79 44 23Nov.79		Horseheads
MJ31	1980	?	0 14Jul.80 32 20Aug.80 44 12Sep.80		Horseheads Tray turned over
MJ32	1981	55.8	24 29Jun.81 36 15Jul.81 52 31Jul.81 52 10Sep.81 48 29Sep.81 72 27Oct.81		Horseheads to 27Oct.81

TABLE 2

MSX in Gapers[1] from James River oysters in Mobjack Bay trays

J. D. Andrews

August 1988

Tray No.	Year	No. Gapers	No. MSX infections	% with MSX	MSX intensity H-M-L-R	Comments[2]
MJ5	1964	6	4	67	0-0-4-0	Horseheads
	1965	10	4	40	0-1-3-0	"
MJ6	1964	23	14	61	5-3-5-1	"
	1965	2	1	50	0-0-1-0	"
	1966	14	9	64	3-1-5-0	"
	1967	6	4	67	2-1-0-1	"
MJ7	1963	10	9	90	4-2-2-1	"
	1964	46	37	76	11-5-17-4	"
MJ8	1963	3	2	67	1-0-1-0	Aug 62 plants
	1964	3	1	33	1-0-0-0	(miles)
MJ9	1964	54	36	67	13-4-13-6	Horseheads
	1965	8	7	88	1-0-6-0	
MJ10	1964	39	23	59	4-4-12-3	"
	1965	21	10	48	0-1-7-2	
MJ11	1964	52	46	88	19-5-21-1	to 23 Jul 65
	1965[3]	52	42	81	4-9-24-5	
MJ12	1965[3]	26	19	73	4-6-7-2	fall 1964 import
MJ13	1965	29	23	79	10-2-10-1	
	1966[4]	56	44	79	21-3-20-0	to 29 Aug
MJ14	1966[4]	60	44	73	24-1-17-2	fall import to 7 Apr '67
MJ15	1966	27	21	78	16-2-3-0	
	1967	30	23	77	12-2-8-1	
MJ16	1967	17	12	71	1-9-2-0	
	1968	19	12	63	0-2-8-2	
MJ17	1967	56	42	75	9-15-15-3	Potomac R. to 29 Apr '68
MJ18	1968[6]	11	7	64	0-1-5-1	Deep Water Shoal
MJ19	1968	10	5	50	0-2-2-1	Potomac R.
MJ20	1969	0	-	-	-	Deep Water Shoal
MJ21	1970	18	6	33	1-3-2-0	See live oysters
MJ22	1971	6	3	50	1-0-1-1	Deep Water Shoal
MJ23	1972	0	-	-	-	"
MJ24	1973	1	1	100	1-0-0-0	"
MJ25	1974	1	1	100	1-0-0-0	"
MJ26	1975	6	4	67	2-2-0-0	Rainbow Rock
MJ27	1976	3	2	67	0-2-0-0	Wreck Shoal
MJ28	1977	2	2	100	1-2-1-0	Pt. of Shoals
MJ29	1978	4	4	100	10-4-1-3	Rainbow Rock
MJ30	1979	20	18	90	10-4-1-3	See live oyster
MJ31	1980	9	3	33	0-1-2-0	"
MJ32	1981	19	17	89	7-3-6-1	"
TOTALS		779	560	Av. 72	190-98-230 -42= 560	

[1]All gapers included after deaths be··· (late July) from first exposure to MSX. Winter gapers regularly show lower MSX infection levels than summer ones. Trays MJ1 to MJ4 omitted because planted oysters in Mobjack were trayed.

[2]All lots were imported Horsehead oysters unless otherwise designated.

[3]There was a strong late-summer MSX infection and June-July '65 mortality. (Many gapers missing from records.)

[4]Early late-summer infection appeared very early (25 Oct) and killed intensively in Jun-Jul 1967.

[5]Gapers not saved in 1966--why?

[6]Monitoring large numbers of trays with lab-bred progeny to assay resistance to MSX required fewer visits to Mobjack Bay trays. Samples of live oysters were taken at each visit to monitor MSX activity thereafter.

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Legends

Figure 3. Mortality and MSX prevalence levels in an early-summer importation of Horsehead Rock oysters. These were moved from a low-salinity area of the James River seed area to Gloucester Point, an enzootic zone for the disease in the York River, Virginia. The duplicate trays of susceptible oysters were transplanted in March, 1967. Prevalences (%) of MSX in samples of 25 live oysters are shown above arrows indicating date of sampling. total seasonal mortalities are given below tray designations for the period between hash marks mostly caused by MSX.

Figure 4. Late-summer infections of MSX between 1 August and 1 October, 1966 caused mortality in May and June which is blended into MSX kills beginning about 1 August from early-summer 1967 infections. Compare timing and level of mortality for the two patterns of infection. Note that infection levels were much higher from late-summer exposure to MSX. These were Deep water Shoal oysters from the lowest-salinity bed in the James River seed area.

Fig. 3 Horsehead Rock Oyster (spring import

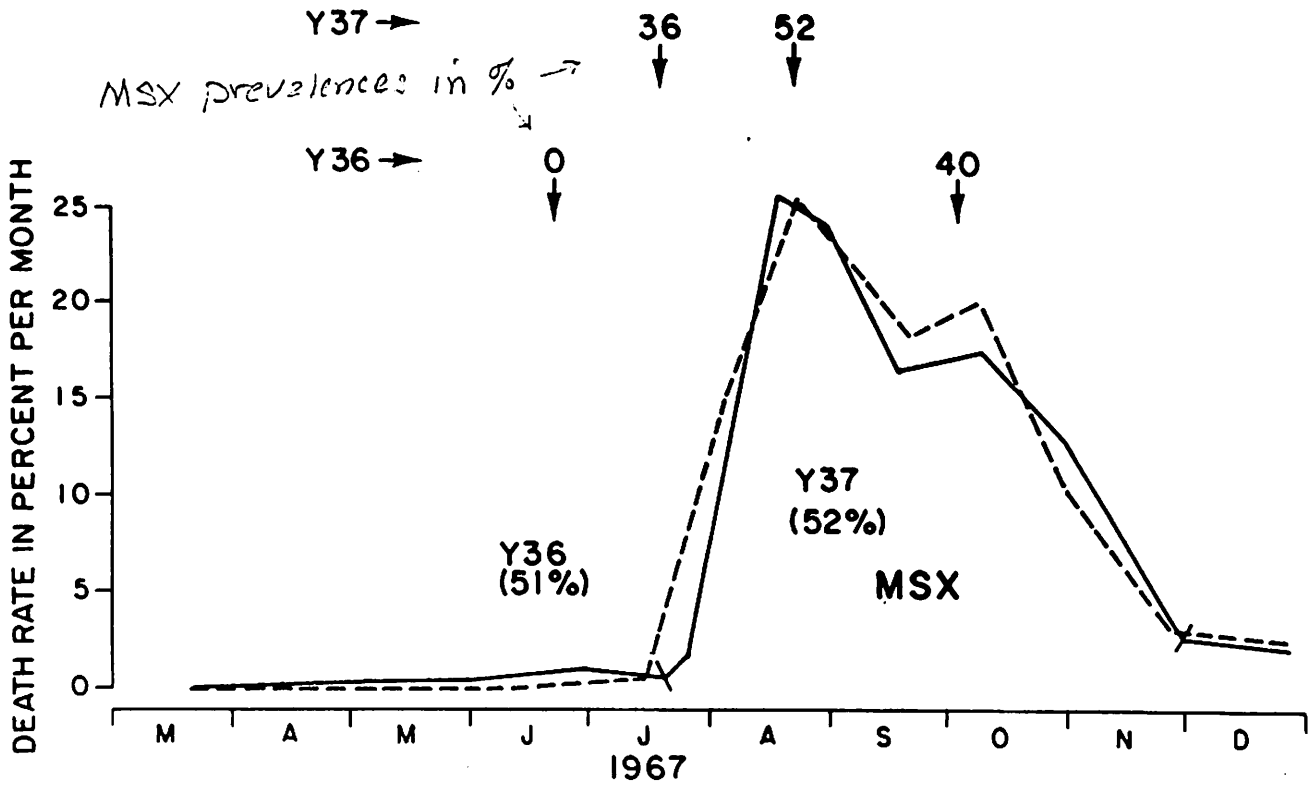

Fig. 4 Deep Water Oyster [illegible] (Fall Impor

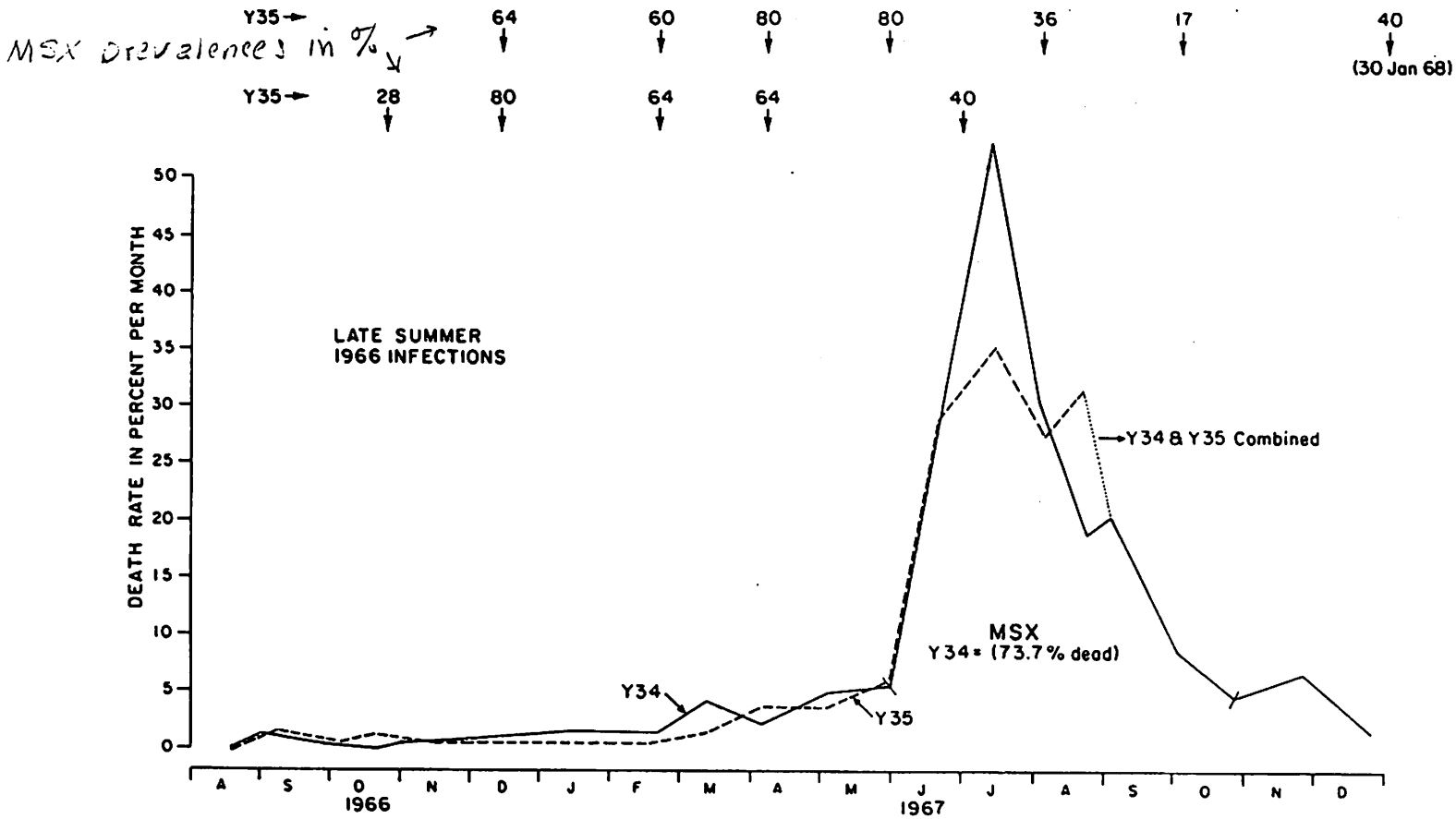

Table 1. Occurrence of sporulation of MSX in highly susceptible Rappahannock River oysters, Tray P171C and 171X. Tray P171C was moved from Ames Pond, a sanctuary free of MSX, to Gloucester Point, Va. 8 July 1976 for exposure to MSX. Tray 171X was moved to the York River 16 August 1976.

	Date of Sample	Slide Numbers	Number Oysters Sampled	MSX Infections		
				No.	Intensity H-M-L-N	No. in Sporulation
P171C	20 Sep 76	141, 391-415	24	22	11-8-3-0	6
(8 Jul 76)	21 Sep 76	149, 453-77	25	21	8-7-6-0	8
	16 Dec 76	151, 123-46	24	18	4-13-1-0	9
			73	61	23-28-10-0	23
P171X	23 Sep 76	149, 593-617	25	0	-	0
(16 Aug 76)	16 Dec 76	151, 148-72	25	6	1-1-4-0	0
	11 Feb 77	151, 219-43	20	7	1-1-5-0	0
	16 May 77	151, 538-87	50	22	17-1-3-1	0
	24 Jun 77	151, 934-83	50	19	15-3-1-0	0
	5 Aug 77	153, 017-41	25	17	11-4-1-1	0
	12 Aug 77	153, 323-47	25	5	2-1-0-2	0
	7 Nov 77	154, 609-33	25	11	7-3-1-0	1
	2 Dec 77	154, 894-09	16	6	2-3-0-1	0
			261	93	56-17-15-5	1

Tray P171C had 83.6% infection and 37.7% of infections exhibited sporulation

Tray P171X had delayed infections; over a year 39.4% of the oysters were infected and only 1 had sporulation

Comparison of Mortalities Between Susceptible and MSX-Resistant Oysters in Trays at Gloucester Point, Va., an Endemic Area for the Disease

J. D. Andrews
18 August 1987

Spring-imported MSX-susceptible oysters from the James River seed area were monitored each year at Gloucester Point, Va. (York River) from 1959 to 1986. The oysters were held in legged trays at stakes on an abandoned public oyster bed in an area endemic for the disease. Only in 1972 when Tropical Storm Agnes reduced salinities to 10 to 12 $^{0}/_{oo}$ was MSX inhibited at this station. Usually two trays of 500 2- to 3-inch oysters each were monitored at biweekly to monthly intervals. Predation was virtually absent although drills sometimes crawled into the trays when new spat attracted them. Frequent handling prevented predation and smothering was rare and mostly when trays were overturned in winter.

The predominant cause of mortality was MSX which has been monitored regularly by live-oyster samples and gapers that were sectioned and stained as permanent slides. The trays were isolated from each other by 50 ft. or more to avoid infection by Dermo, but frequently by late summer of the second or third year the disease became established in trays. Once established, Dermo became the primary cause of deaths in dense tray populations. This occurred in susceptible and MSX-resistant stocks alike and there was no way to eradicate Dermo or reduce its effects. However, lots of 500 resistant oysters permitted monitoring for up to five or six years before the population declined to the minimal count of 100 oysters for accurate mortality rates. Pairs of trays of susceptible oysters were usually combined after a year or two to extend the period of monitoring to

two or three years. This was adequate time for these 2- and 3-year old seed oysters to reach maturity for market.

There was no problem in maintaining resistant-oyster lots for the necessary three to four years to attain marketable size because their death rates were much lower. All gapers were processed but lower death rates resulted in fewer gapers being collected from resistant oysters than from susceptible lots.

New susceptible oysters were transplanted to Gloucester Pt. around 1 April each year -- mostly to allow full adaptation to salinities and improvement in glycogen storage before MSX began infecting in May. In the attached tables, annual mortalities were calculated from 1 April one year to 1 April the following year. Old boxes were deducted from the counts when found; they resulted from low-salinity, anaerobic kills which appeared as closed boxes usually filled with mud. These did not sound hollow, and therefore were hard to detect until spring growth of new shell failed to occur.

Laboratory-bred resistant oysters were set at various times through late spring and summer. They were taken within days after setting to an artificial pond with limited water exchange with the Ware River. Whether on shells or free spat (cultchless), these progeny were not brought back to the York River until after 1 October when natural setting had ceased, and in early years some lots were left in the pond until March of the following year. After rather futile early attempts to count spat on shells, it was found that first-year mortality was quite low; therefore, trays were cleaned and signs of mortality observed without counts until spring when growth of spat soon made accurate counts possible. Sea squirt fouling made monitoring spat during the first summer almost impossible at Gloucester Point. This

system of keeping new batches of spat in the sanctuary during the first summer may have avoided early infections of MSX which occurred so commonly in Delaware Bay studies. We have observed infected spat in natural beds in Virginia but rarely in our tray-grown stocks -- either susceptible or resistant races. Batches of susceptible progeny bred in the laboratory as control groups were treated the same way so that exposure to MSX did not occur appreciably until an age of about one year was attained.

Annual mortalities from diseases in susceptible oysters averaged 50% the first year and d not decline much in the two succeeding years (Table 1). During the first year, deaths were caused almost entirely by MSX because high prevalences of the disease occurred in gapers. All gapers were tested for Dermo by the thioglycollate method of Ray (1952). During many years, the combined mortalities of the first two years reached 75% (about 50% each year), and the third year resulted in total losses approaching 90%. Only 7 of 23 lots had enough oysters left to complete monitoring for the third year, but Dermo became increasingly important and distorted data on MSX during additional years.

Annual mortalities for oysters selected for resistance to MSX showed much lower rates (Table 2) and they tended to decline as successive generations were selected and bred. The last year of laboratory breeding of resistant oysters at VIMS was 1978 in the fifth generation of selection. Considerable inbreeding had occurred by then -- both deliberately and as a result of too few parents being used. The average annual mortality was about 11% for 32 lots monitored over 14 years. Average cumulative mortality was 25% over three years.

Using Haskin's "survival ratio," I find that an average of only 21% of susceptible oysters survived three years whereas 75% of resistant oysters

survived that period of exposure to MSX. This gives a survival ratio of 3.57, that is, over 3 1/2 times more resistant oysters survived than susceptibles. If one omits the two low-mortality years of 1978-79 (Y99 + Y100), the ratio becomes 75/13 = 5.8 times as many resistant oysters survived as did susceptible ones.

It will be noted (Table 2) that only 17% of gapers from resistant lots had MSX infections whereas the prevalence of MSX in susceptible gapers was 85 to 90%. Therefore, a major part of deaths of resistant oysters were caused by background events including some Dermo in some lots by the late second or third year. It is discouraging to note that in 1986 and 1987, MSX is killing native oysters in Mobjack Bay, the only area in Chesapeake Bay where natural resistance to MSX has been found to be effective most years.

The level of MSX infection in lab-bred resistant oysters is shown in Table 3. These are average prevalences in live oysters based on samples of hundreds of oysters over the two or three years of monitoring. Peak prevalences usually occurred in May before mortality from late-summer infections began. Prevalences appeared to decline slightly as additional selection by generations occurred. Even if localized infections were missed in our protocol, the rarity of systemic infections suggests a high level of resistance. Note again the low percentage of MSX infections in gapers (Table 2).

Table 1. Annual mortalities in susceptible James River oysters at Gloucester Point, Va., 1960 to 1979. Samples of live oysters and gapers indicate that most deaths were caused by MSX. Perkinsus marinus was sometimes a minor factor in late summer of the second or third year of monitoring as indicated.

Mortality %

Tray No.	1st Year	2nd Year	3rd Year	Cumulative Totals
Y14	42	55	47	86
Y15	57	54	52	90
Y22	67	48	49	91
Y63	52	48	31	82
Y99	25	23	40	65
Y100	30	20	43	68
Y10	57	53	--	86
Y16	45	55	--	75
Y17	38	54	--	71
Y19	53	57	--	80
Y28	66	38	--	79
Y36	61	57 (to 7 Nov)	--	83
Y48	64	48 (a little Dermo)	--	81
Y61	51	25* (to 28 Jul)	--	63
Y62	55	38* (to 28 Jul)	--	72
Y69	57	32* (Agnes flood)	--	71
Y73	13* (Agnes)	36*	--	44*
Y74	14* (Agnes)	38*	--	47*
Y87	71	30* (to 4 Nov)	--	80
Y88	51	30*	15*	71
Y98	45	39	--	66
Y103	59	45	--	77
Y105	62	64	--	86
Average	53	47	44	79 (3 yr) 75 (2 yr)

* Omit from averages because weather was abnormal or record for the year incomplete.

Table 2. Annual mortalities of lab-bred MSX-resistant live oysters at Gloucester Point, Va., 1964 to 1978 in percentages.

Tray No.	1st Year	2nd Year	3rd Year	4th Year	Cumulative Total	% Gapers With MSX
P7	28	12	12		45	34
P10	12	8	14		30	21
P16	7	16	--		22	45
P31	14	25	18d		47	40
P32	11	18	15d		38	29
P34	4	9	18		29	10
P35	12	12	--		23	38
P37	5	4	6		14	6
P40	11	32	17		50	39
P51	6	11	--		16	11
P52	13	19	6	16	44	20
P55	14	12	--		24	4
P65	0	12	20	4	33	25
P67	0	15	6		21	8
P68	5	5	6	8	22	9
P72	7	10	--		16	1
P73	17	8			24	4
P77	11	13	11		31	18
P84	9	13			21	20
P85	8	17			24	23
P86	7	14			20	4
P88	13	20			30	0
P90	2	11			13	8
P91	4	12	--		16	13
P92	4	11			15	28
P93	3	6			9	--
P96	3	11			14	29
P97	2	10			12	27
P99	1	7	10	12	26	23
P100	12	11	9		29	12
P101	6	9	12		24	5
P102	14	17	--		28	11
P105	8	--	--		--	0
P106	5	--	--		--	0
Average	7.3	12.8	12.1		25.3	17.0

d = Dermo present

Table 3. Prevalences and mortalities of MSX-resistant oysters (lab-bred progeny) at Gloucester Point, Va., from 1964 to 1972 during 2 or 3 years of selection by MSX.

Tray No.	Yearclass	Years Monitored	MSX Av. Infection %	Cumulative Mort. %	Av. Mort./ Year %
P7	1964	1965-67	9.2	44	17
P10	1964	1965-67	3.3	30	11
P16	1965	1966-67*	10.0	22*	12
P31	1966	1967-69	12.0	47	19
P32	1966	1967-69	29.0	38	15
P34	1966	1967-69	0.7	41	10
P35	1966	1967-68*	14.5	23*	12
P37	1967	1967-69	4.2	15	5
P40	1967	1967-69	9.5	50	20
P51	1967	1968-70	2.9	36	13
P52	1967	1968-70	4.4	34	13
P55	1968	1969-70*	0.4	24	13
P65	1969	1969-71	0.7	30	11
P67	1969	1969-71	13.8	20	7
P68	1969	1970-72	0.0[a]	15	5
P72	1970	1971-72*	1.5	16	8
P73	1970	1971-72*	0.0[b]	24	12
P74	1970	1971-73	4.0	49[c]	19
P75	1970	1971-73	4.4	55[d]	23
P77	1970	1971-73	3.7	31	11
P84	1971	1972-73*	4.0	21*	11
P85	1971	1972-73*	4.0	24	12
P86	1971	1972-73*	0.0	20*	10
P88	1971	1972-73*	0.0	30*	16
P90	1971	1972-73*	0.0	13*	7
P91	1971	1972-73*	0.8	16*	8
P92	1971	1972-73*	2.4	15*	8
P93	1971	1972-73*	0.0	9*	5
P96	1971	1972-73*	0.8	14*	7
P97	1971	1972-73*	0.6	12*	6
P99	1971	1972-74	0.0	17	6
P100	1972	1973-75	0.0	29	11
P101	1972	1973-75	0.0	24	9
P102	1972	1973-74*	10.6	28	15
Averages			4.5	26.9	11.4

*Monitored only 2 years before tray closed out or Dermo invaded.
[a]375 live oysters tested and no MSX found.
[b]300 oysters tested.
[c]Had Dermo in 1971 which increased mortality.
[d]Unexplained mortality first two years - inbred siblings.

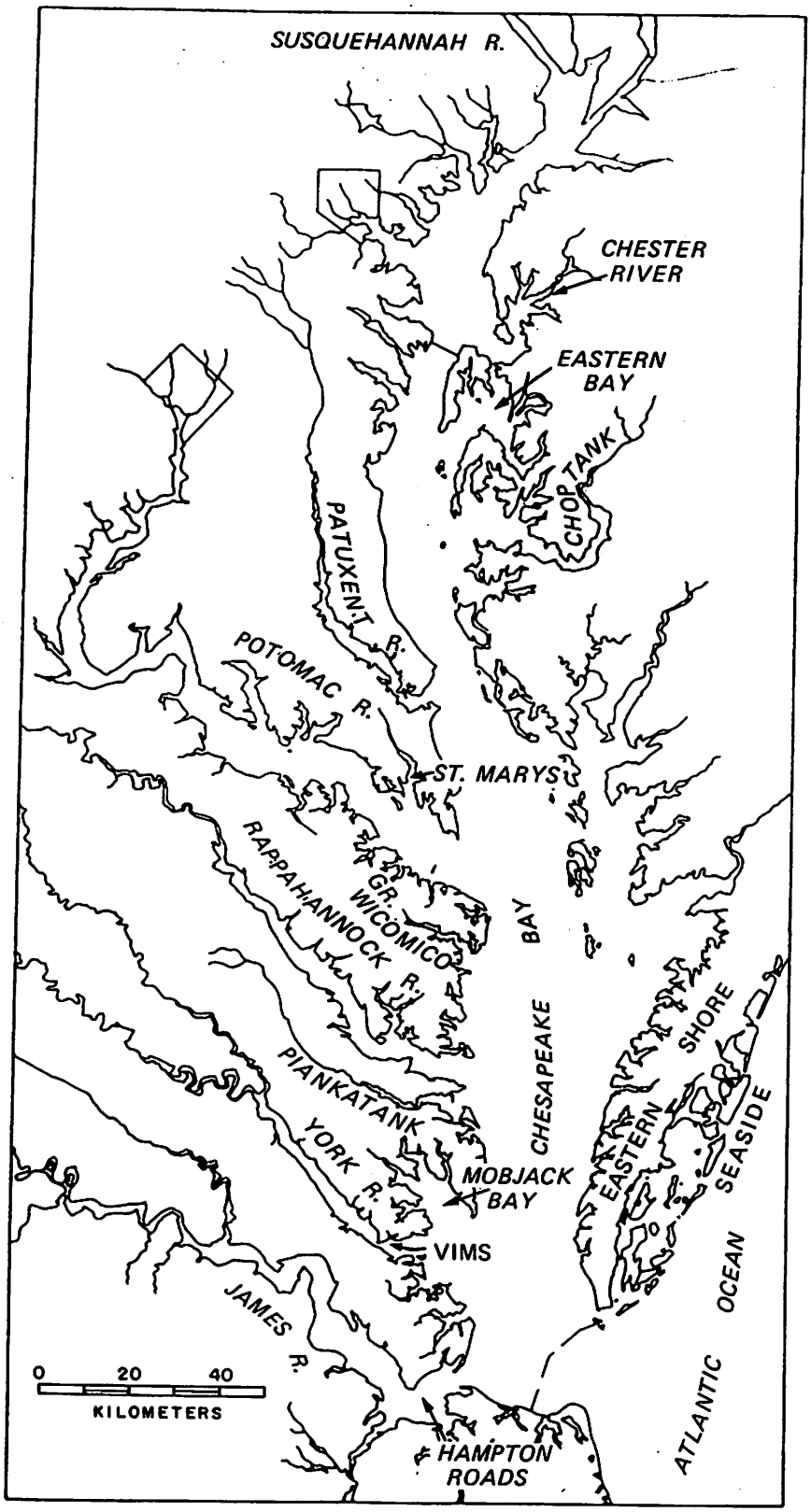

Fig. 1. Chesapeake Bay

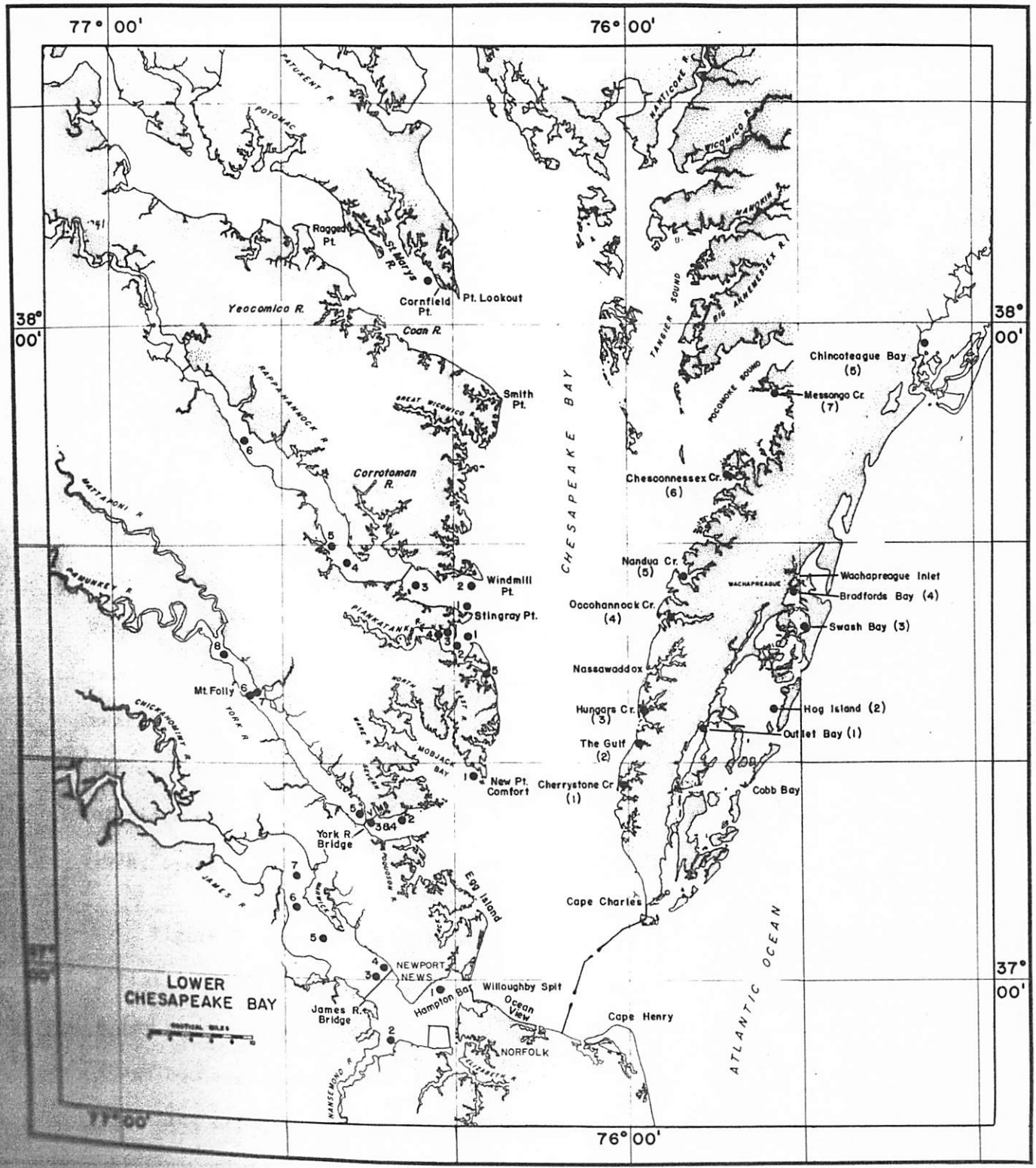

Figure 1. Map of stations monitored for D. marinum and MSX. Sampling stations for oysters taken from public and private beds, and trays of oysters to determine prevalences and mortalities of diseases in Lower Chesapeake Bay.