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OYSTER MORTALITY STUDIES IN VIRGINIA. VII. REVIEW OF EPIZOOTIOLOGY AND ORIGIN OF *MINCHINIA NELSONI* [1, 2]

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ABSTRACT

Intensive epizootics in Crassostrea virginica *caused by* Minchinia nelsoni *(MSX) show no signs of abating in lower Chesapeake Bay. Prevalences of the pathogen have commonly exceeded 50% in susceptible stocks during the first year of exposure; mortalities of 50 to 70% occurred during the first year and slightly lower losses in succeeding years. Disease activity increased in isolated lots of oysters from 1963 to 1966 during a drought period with high salinities. Native and planted oysters were extremely scarce in the lower Chesapeake Bay hence density of populations appears not to be important for disease activity. Seasonal patterns of death rates have remained stable through eight years of observations but morbidity has occurred earlier in drought years. These patterns are influenced by time of import of unexposed susceptible oysters to areas of disease prevalence.*

Laboratory-bred progeny from susceptible (unexposed) and selected (by MSX) parents were held to marketable size in areas where MSX is intensively active without serious losses (10 to 20% annually). Early exposure appears to be important for subsequent survival of large oysters; hence, acquired resistance is postulated.

M. nelsoni *has not produced epizootics of oysters in the high-salinity environment of the Seaside of the Eastern Shore of Virginia;* M. costalis *(SSO) regularly causes a sharp mortality there in May-June each year. Since these congeneric sympatric pathogens are very similar morphologically, it is postulated that their annual life cycles are similar, with June the period of sporulation. MSX appears to be highly virulent since death of the host usually occurs before sporulation which is rare.*

A hypothesis on the origin of a virulent strain which arose in Delaware Bay in 1957 and appeared to spread to Chesapeake Bay in 1959 is based on seed oyster transplantations between the areas.

INTRODUCTION

Nearly ten years of activity of the oyster pathogen, *Minchinia nelsoni,* in the estuaries of the middle Atlantic coast have been observed. Mortalities first appeared in Delaware Bay in 1957 and the pathogen was first observed by Stauber in 1958 (Haskin, Canzonier and Myhre, 1965). Early patterns of prevalence and mortality were reported by Haskin in 1959 (personal communication). The disease was found in Chesapeake Bay in 1959 (Andrews and Wood, 1967) where it quickly became epizootic. In 1966, the organism was named and described as a haplosporidian by Haskin, Stauber and Mackin (1966), and further linked with this group of parasites by Couch, Farley and Rosenfield (1966) on the basis of rare spores. The morphology and life cycle are incompletely known and sporulation is rare. Most information is based on field studies and collections. Laboratory cultures and infections have not been attained. The organism seems to be highly virulent and extensive damage has been inflicted on the oyster in-

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dustry of the middle Atlantic states, particularly in Chesapeake and Delaware Bays.

The epizootiology was described by Andrews (1966) from data collected through 1963. Subsequently, three dry years have brought some changes in the intensity and the seasonality of "MSX," as the organism is commonly called. Additional information on the effects of age and history of oysters is presented. The program of research has been broadened from epizootiology of susceptible oysters to include a search for resistant strains of oysters. A discussion of possible origins and life cycles is offered from the meagre clues provided by field studies. Finally, some findings that may permit oystermen to limit losses in afflicted areas are presented.

This paper presents evidence and discussion on three primary subjects: 1) confirmation, changes and refinements of epizootiological concepts given earlier; 2) evidence for innate and acquired immunities in oyster populations; 3) hypotheses on the origin and life cycle of the pathogen. The dramatic appearance and persistent depredations by *M. nelsoni* demand some attempt to rationalize its origin and history.

METHODS

Disease activities have been monitored with cohorts of oysters in plastic and wire mesh trays. Wild oysters transplanted from disease-free areas are referred to as "imports." The designation "progeny" is restricted to laboratory-bred groups spawned from parents chosen for their history of exposure to MSX. Groups of 300 to 1000 oysters have been held on oyster grounds in legged trays. Regular examinations and counts have provided accurate death rates and eliminated predation and physical causes of deaths. Little attempt has been made to control density of oysters. Each cohort was held as a separate population until numbers declined to about 100 oysters. Samples of live oysters were taken sparingly and all gapers (dead oysters with meats) were examined. Methods of handling trays and processing oysters have been described previously (Andrews, 1966; Andrews and Wood, 1967).

Several stations have been established in Virginia waters and often duplicate trays have been maintained at one or more stations (Andrews and Wood, 1967). "Off VIMS" refers to an oyster bed one-quarter mile offshore of the laboratory. Data from VIMS pier trays have been avoided because of *Dermocystidium* occurrence. Seasons of importation and histories listed in graphs are important factors for various cohorts. Seasonal patterns of morbidity and mortality were the chief parameters sought. Conclusions are drawn only when differences were large in magnitude. "Background" losses are acknowledged (usually less than 10 per cent annually) but are not explainable. Deaths from the fungus organism, *Dermocystidium*, were excluded for the most part by isolation of trays (Andrews, 1965). Many samples of live oysters were examined each year from private and public beds to follow disease activities in various commercial operations. These tests have aided greatly in confirming that tray data were representative of oyster beds.

Mortalities are expressed as death rates per month (30 days) regardless of period between examinations. In line graphs, the death rate is plotted at the end of the period of observation, hence refers to the period preceding the point. Death rates were obtained by dividing number of deaths during the period by number alive at the beginning of the period. Plotted death rates cannot be added to obtain cumulative mortalities. Total cumulative mortalities for peaks are often given below the curves with end points chosen as illustrated by vertical bars in Figure 1. Prevalences in all graphs are given as number of cases of MSX per 25 live oysters. No data are given for *Dermocystidium* because it was not involved in these studies.

In referring to the history of oysters, the terms early-and late-summer exposure (or infections) are used because a sharp break occurred in patterns of morbidity and mortality about 1 August (Andrews, 1966). Early-summer exposure applies to all oysters imported to an endemic area from November through July whereas late-summer infections were initiated in August, September and October.

The term "selection" is used in the phenotypic sense of choice of breeding stock by the activity of MSX and has no implications of phylogenetic changes because genotypes are unknown. The terms infectiveness or infectiousness refer to the ability of the pathogen to produce disease.

RESULTS

MSX in Susceptible Imports

The patterns of prevalences and mortalities for the years 1960-63 were derived from exposure of susceptible James River oysters. Each year new lots of the same oyster stocks were imported and observed until depleted. Intensity of MSX activity increased in the drought years of 1964 to 1966. Timing of events was earlier and death rates were higher but the basic patterns prevailed (Andrews, 1966). The following data confirm earlier observations under conditions of higher infective pressure by MSX.

Mortality data from spring and late-summer imports of susceptible oysters are compared for two-year periods in Figure 1. Spring imports are

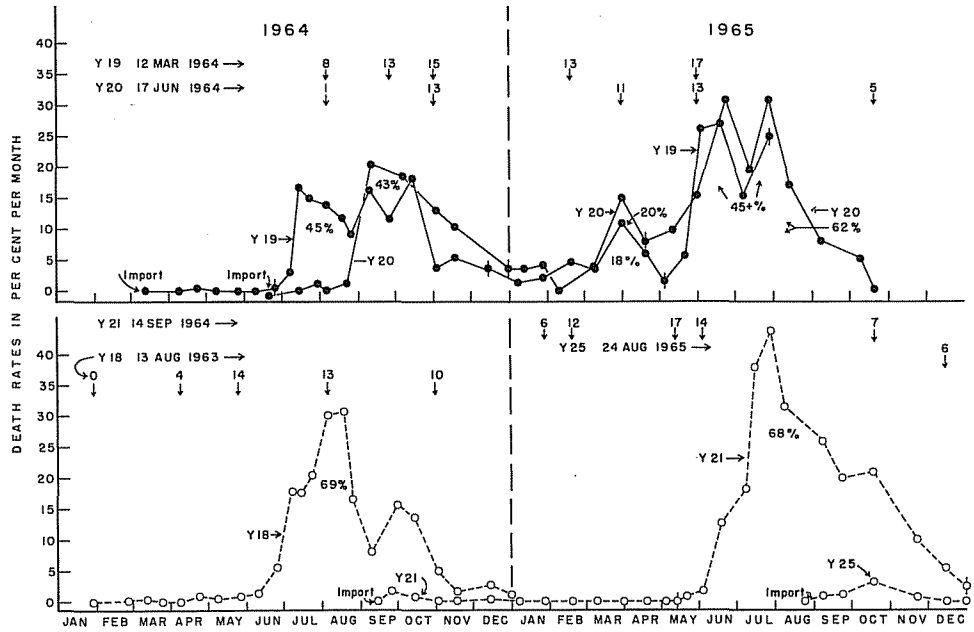

FIG. 1. *Death rates of susceptible oysters, imported 1) winter and spring (upper graph), 2) late-summer and fall (lower), for first two years. Cumulative mortalities for major peaks are given as percentages for periods delineated by vertical bars through points. Prevalences of MSX in cases per 25 live oysters are given above arrows designating dates of sampling. Station off VIMS.*

depicted in the upper half of the graph and fall imports in the lower half. The March imports (Y19) exhibited the earliest first-summer deaths in seven years of monitoring. Deaths began before mid-July whereas early August is typical. Oysters imported in mid-June (Y20) began dying about six weeks later, approximating the minimum period from infection to first deaths. Death rates were high in late summer and fall but declined as temperatures dropped. In the second year, a late-winter peak in March was followed by severe mortalities the second summer beginning in late May. Typically, death rates declined earlier in the second fall than in the first.

In contrast, late-summer imports of 1963 (Y18) began dying only slightly earlier than spring imports of 1964 but death rates were higher. Without the high prevalence in Y18 in May, one might suspect that infections occurred in both groups in May and June. It is possible that intensity of infection was boosted by infective particles acquired in early summer. A double mortality peak with the later one smaller is typical (Y18). The late-summer import of 1964 (Y21) repeats the pattern. Note that no mortality occurred for nine or ten months after import in either year. Infections were not clinical until May in the years 1960 to 1963 except for an occasional localized one. After 1963 deaths began as usual in June although infections began appearing earlier each year. In 1964, the first clinical infections were found in early April, whereas in 1965 infections appeared in January 1965 or earlier. The trend of late-summer infections becoming clinical earlier in drought years continued in Y25 which had 6 of 25 live oysters infected in December 1965 (Fig. 1). Many more cases had appeared by May 1966 (Fig. 2). Late-summer imports did not show mortality prior to June until 1967 (Y35, not shown).

A comparison of mortality peaks in 1965 (Fig. 1) suggests that most June deaths in Y19 and Y20 were from infections initiated in the early summer of 1964. These were apparently the last oysters to die of those first exposed and infected in May-June 1964. Perhaps late-summer infection pressure aided in their eventual demise. Y21 oysters imported in September 1964 died mostly in July 1965, matching the second peak in spring imports.

Prevalences of MSX are shown above arrows indicating dates of sampling (Fig. 1). Levels of infection in spring imports reached about 50 per cent during the first summer mortality peak and did not subside appreciably until after the second summer of losses. By this time some 75 per cent of

the imported stocks were dead. In late-summer imports, infections became clinical well ahead of deaths although this did not occur in earlier years (Andrews, 1966).

The same patterns occurred in 1966 with higher mortalities — particularly in spring imports (Fig. 2). Y28 and Y29 are duplicate trays except that the latter was located at AMOCO station three miles below Gloucester Point in the York River. It will be noted that the late-summer import (Y25), although showing patent infections in early winter, did not exhibit deaths until June of the year after import. Y35 had high prevalences in fall and winter and deaths began in winter and accelerated slowly through the spring of 1967 (not shown). By 1 June 1967, Y35 and a duplicate tray (Y34) had about 15 per cent mortality from MSX. Death rates increased steadily through cold winter and spring months without following the typical end-of-winter pattern. Both mortalities and prevalences in 1966 appeared to be higher than in 1964 and 1965.

MSX activity in 1966 is compared for several additional lots of oysters in lower Chesapeake Bay in Figure 3. Both time of import and source are variables in these groups although all were free of MSX initially. Horsehead oysters, imported in March 1966, were monitored in Mobjack Bay (MJ15) and Hampton Bar in James River (J14). Prevalences and mortalities were slightly lower at these stations than at VIMS but the timing and patterns were similar. A striking contrast was obtained with MSX-free Potomac River oysters imported 29 June 1966 during the period of early-summer infectivity (Fig. 3). Susceptibility is possibly higher than in James River imports because these Potomac River oysters were collected 50 miles above the occurrence of MSX. However, it is believed that the timing of the import is the major cause of the excessive activity by MSX. At the peak of this kill on 23 August 1966, 40 of 42 gapers had MSX and the other two were too rotten for diagnosis. After 70 to 80 per cent of the Potomac oysters had died (two trays with 700 oysters each initially), prevalences of MSX in the survivors

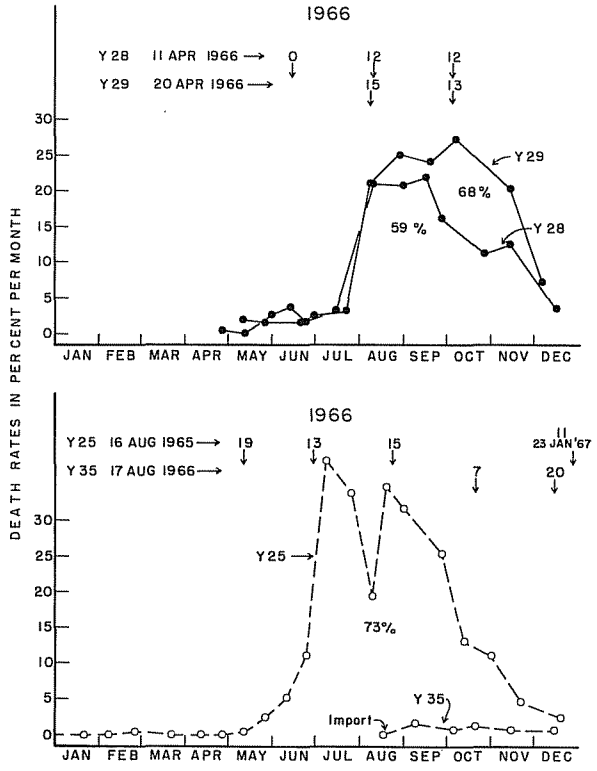

FIG. 2. *Death rates and prevalences of spring and late-summer imports in 1966. All were susceptible oysters imported from Horsehead Bar in James River to VIMS and AMOCO (Y29 only) stations.*

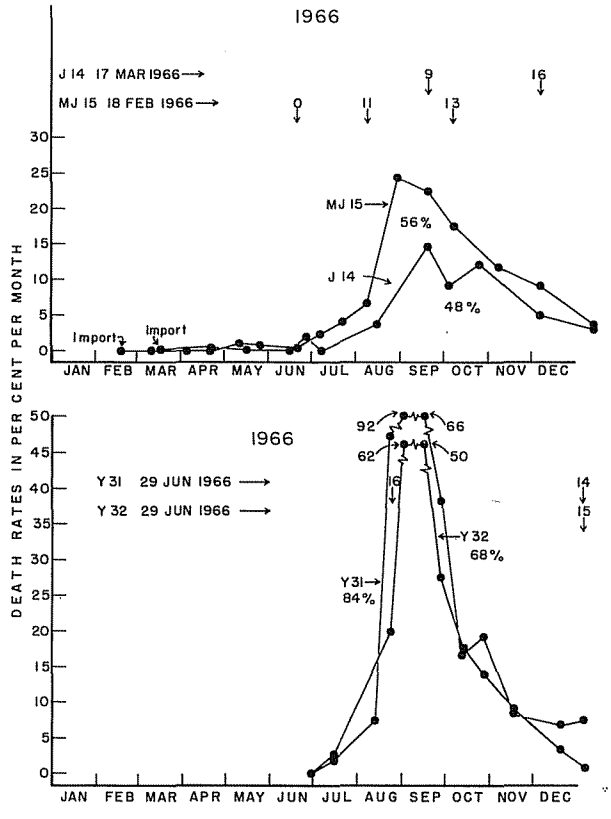

FIG. 3. *MSX activity in spring imports at Hampton Bar (J14) and Mobjack Bay (MJ15), and June imports of Potomac River susceptibles off VIMS. 1966.*

were over 50 per cent in January 1967. Note that prevalences were not much higher in Potomac oysters than in other groups but death rates were very high and more peaked in August and September. These groups of oysters illustrate the importance of timing and history of imports if severe losses are to be avoided, as the next lots further demonstrate.

MSX in Native Oysters and Progeny in Endemic Areas

One important factor in the epizootiology of a disease is the age of host organisms. It was noted early that young oysters had low death rates but it was assumed this was related to size and that MSX gradually selected populations more vigorously as oysters became larger. This was the experience with the disease caused by *Dermocystidium*. MSX infections in spat were observed in the 1958 epizootic in Delaware Bay (Haskin, personal communication, 1960).

For most studies of MSX patterns, James River oysters 2 to 3 inches in length were imported. James River is the primary source of seed oysters in Virginia although other seed areas have been developed since 1963. In lower Chesapeake Bay, small seed oysters are subject to predation; hence the use of large susceptible James River seed oysters is natural and practical.

In March 1965, Virginia opened, for the first time, public beds of the Piankatank and the Great Wicomico rivers for seed oystering. Beginning in 1963, these beds had been planted with reef shells obtained by a suction dredge. Samples of seed oysters in January and April 1965 showed the 1963 yearclass to be 60 per cent infected with MSX and small 1964 yearclass spat 20 per cent infected on the same bed. Due to crowding, few oysters were over an inch in length. A group containing both yearclasses was moved to VIMS in November 1964. Incidence of MSX declined in spring and summer of 1965 without much mortality and two years of monitoring revealed that losses were relatively low. These observations increased interest in the use of small, young oysters for rehabilitation of oyster beds although attempts to breed disease-resistant oysters were begun in the summer of 1962.

Other observations and reports indicated that native oysters in areas epizootic for MSX were producing some marketable oysters. The Nansemond Ridge area of Hampton Roads produced a sizable crop of oysters between 1960 and 1965. Deep Rock bed off the mouth of the Piankatank River was observed to have good survival and marketable oysters in the epizootic years of 1964 to 1966. Additionally, a native spatfall in September 1964 on pilings at Gloucester Point has grown nearly to market size without appreciable losses — in the same waters where imported susceptibles concurrently exhibited mortalities of 60 to 70 per cent per year. Fortunately, some data have been accumulated to explain these contrasting experiences with MSX.

Two groups of native trayed oysters exposed to the severe epizootics of 1965 and 1966 are shown in Figure 4. Y27 contained Deep Rock oysters almost marketable in size. It is clear that the 50 per cent prevalence of MSX when imported to VIMS in March was greatly reduced in April and May, and a light mortality of short duration indicates recovery of these oysters from infections. Tray Y33 containing VIMS piling oysters (1964 yearclass) showed no evidence of a typical summer mortality and prevalence of MSX was low. It is important to note that little selection of the piling population occurred unless it happened at a very small size when "boxes" (attached valves of dead oysters) were easily detached.

A comparison of susceptible imports and laboratory-bred progeny is given in Figure 5. Susceptible oysters are represented by trays of new imports in 1965 and 1966. The progeny group P10 was bred in the summer of 1964 and averaged three inches in the fall of 1966, hence represents MSX-exposed progeny as yearlings in 1965, two-year olds in 1966, etc. Death rates and MSX prevalences reveal dramatic differences in susceptible and exposed lots. P10 is perhaps the most resistant of 13 progeny groups of the 1964 yearclass being monitored. It was bred from rare survivors of millions of bushels of oysters decimated by MSX in Mobjack Bay. Tray P10 has not shown any of the mortality peaks typical of epizootics caused by MSX and prevalence has been consistently low (June 1967).

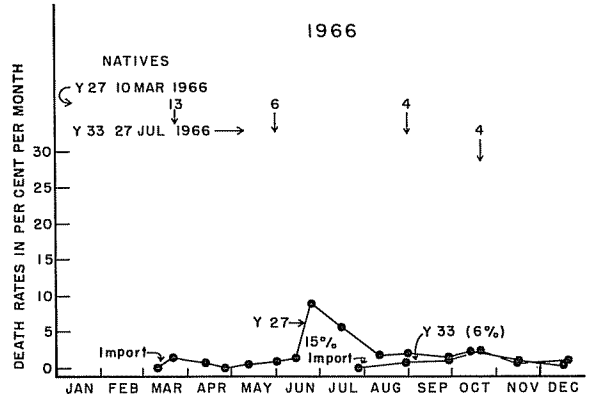

FIG. 4. *MSX activity in native oysters from Deep Rock (Y27) and VIMS pier (Y33) in 1966. Y33 oysters were placed in trays in July but had been exposed at VIMS from setting.*

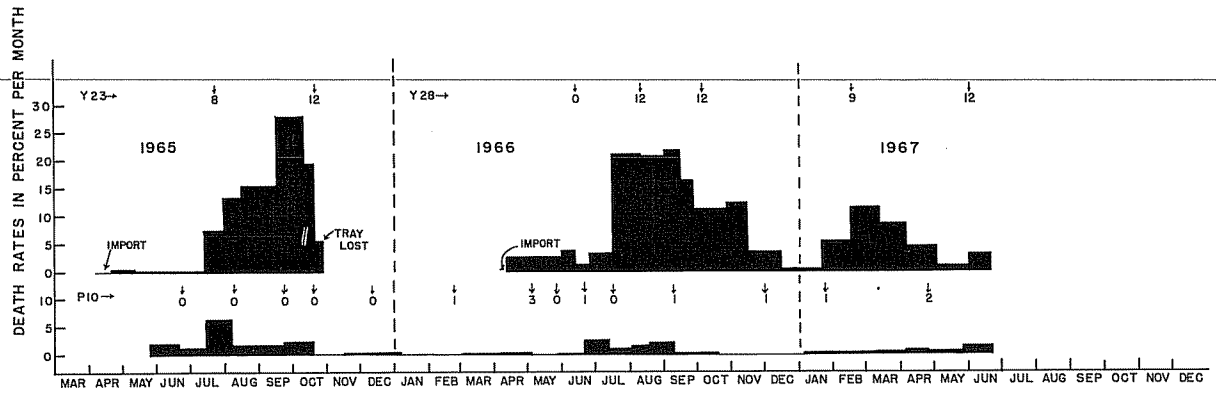

FIG. 5. *Susceptible oysters (Y23, 1965 imports and Y28, 1966 imports) versus laboratory-bred progeny (P10) off VIMS. Horseheads were older and slightly larger in both years but all selection by MSX shown on this graph. VIMS station.*

More typical of the level of MSX in progeny are groups P6 and P7 whose parents were the survivors of several years of MSX selection at VIMS in trays (Fig. 6). These groups show small mortalities at expected times of MSX-caused deaths, although the peak was very late in 1965 as yearlings, and peaks are barely detectable in 1966 as two-year olds. The winter loss of P7 is not typical of progeny, and factors other than MSX were involved. Prevalences, which usually were only one-third or one-fourth as high as in susceptible oysters, peaked in May (typical) but losses were not commensurate. Total losses in trays at two and one-half years of age and market size were about 35 per cent in each tray.

Low prevalences and mortalities were not confined to oysters reared from MSX-selected parents (survivors of epizootics). It is probable on the basis of population sizes alone that the native oysters which survived epizootics (in three separate river systems) were derived mostly from upriver breeding populations which had little or no selection by MSX. An example of a progeny group reared from unselected (susceptible) parents is P14 shown in Figure 7. These oysters bred in February 1965 from Horsehead parents are essentially comparable in size to 1964 progeny. Low prevalences and late moderate mortality were exhibited in 1966. The two groups of progeny depicted in the upper graph of Figure 7 are from MSX-selected parents. Both were held in Ames' Pond until March 1965, then moved to Horsehead Rock in the upper James River seed area where MSX is not prevalent. In April 1966 at a size of nearly three inches, P13A was moved to VIMS for exposure to MSX. Mortality and prevalences were surprisingly low although typical in timing. Background levels of mortality in disease-free low-salinity areas are exhibited by P13, which was a control group held at Horsehead Rock.

P14, raised from unselected parents, appears to have as good a record of resistance to MSX as progeny bred from selected parents. It was one of the few groups held at VIMS from time of setting,

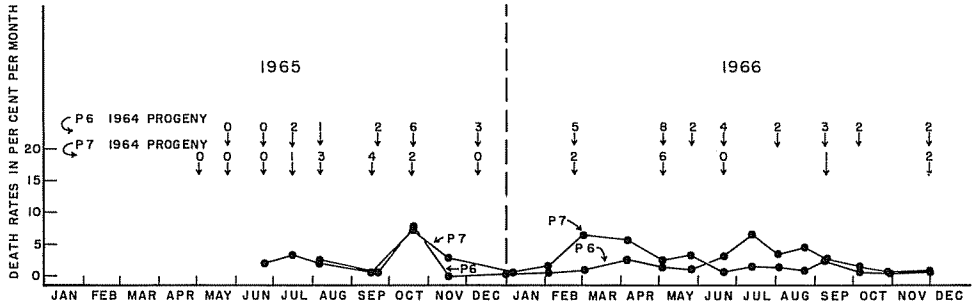

FIG. 6. *MSX activity in laboratory-bred progeny at VIMS (P6 and P7). Average size in 1966 was 65 mm in June and 75 mm in November.*

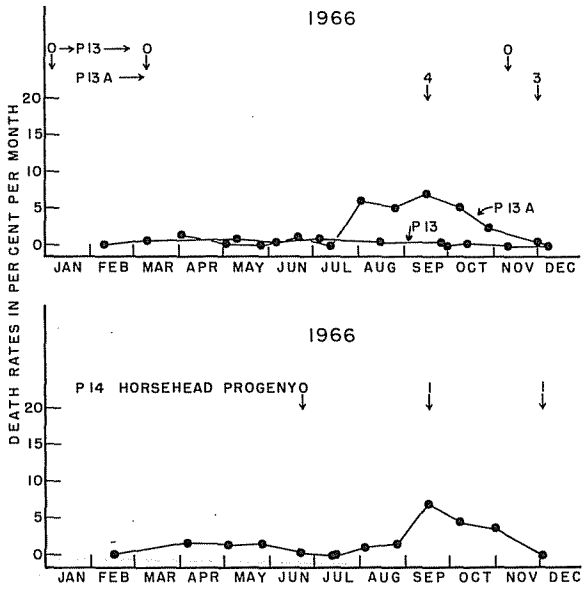

FIG. 7. *MSX activity in progeny from selected (P13 and P13A) and unselected (P14) parents. VIMS station except the control group P13 at Horsehead, James River, an area free of MSX.*

hence exposed intensively to MSX.

MSX Activity on the Seaside of Eastern Shore

Mortality patterns and prevalence data indicate very little MSX activity in the bays of Seaside of Virginia. Monitoring for MSX on the Seaside in 1959 and 1960 resulted in the discovery of Seaside Disease of oysters caused by *Minchinia costalis* (Andrews, Wood and Hoese, 1962). It was reported in 1962 that oysters on the Seaside did not exhibit the epizootic patterns of MSX and subsequent data have sustained this conclusion through 1966. In 1962, prevalence data for gapers and live oysters were presented indicating *M. costalis* (SSO) was much more active than *M. nelsoni* (MSX) in native oysters. Since there is a possibility that native oysters have developed some resistance to MSX, data from susceptible James River imports were chosen to demonstrate the inactivity of MSX on the Seaside. No attempt has been made to compare the level of activity of SSO in the six years illustrated.

James River oysters, imported to the Seaside in 1959, survived unexpectedly well for 12 to 14 months (Figs. 8 and 9). A sharp mortality, caused by SSO, occurred in May-June in 1960 but there was no evidence of epizootics from MSX in either year. Prevalences of MSX were low in live oysters. Fall samples are particularly significant because SSO is not clinically evident then. Four relatively isolated bays on the Seaside gave similar patterns of little mortality and low MSX morbidity during a two-year period. High prevalences of SSO were found in gapers during the May-June epizootics in 1960 as demonstrated previously for native oysters (Andrews *et al.*, 1962).

Two imports in later years are depicted in Figure 10. The mortality patterns and low morbidity from MSX again demonstrate the inactivity of *M. nelsoni* on the Seaside. The graphs also show that oysters go through their first SSO epizootic without losses and that this exposure apparently initiates infectons which result in an epizootic the next May-June period. The Long Island oysters were imported as spat hence were rather small and young for a full-scale SSO epizootic in 1963.

Mortality and prevalence data are given for two trays of native oysters in Hog Island Bay (Fig. 11). Mortalities were low and erratic except for the deaths caused by *M. costalis* each June. Deaths

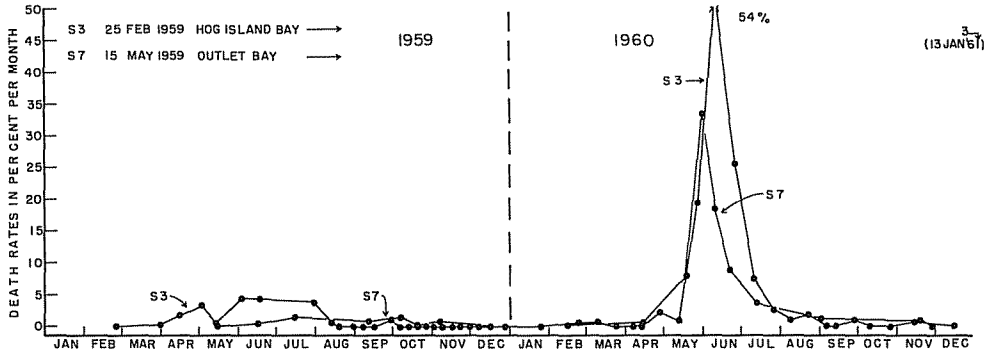

FIG. 8. *Susceptible Horsehead oysters on the Seaside of Eastern Shore Virginia (S3 and S7). The mortality peak in 1960 was caused by SSO. MSX was not active as indicated by absence of typical mortality peaks and scarcity of infected oysters.*

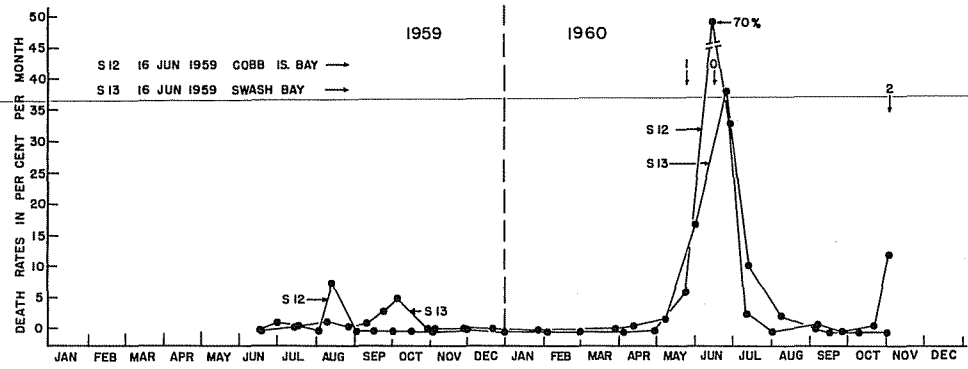

FIG. 9. *Horsehead oysters in two additional Seaside bays reveal little MSX but a strong SSO kill in 1960 (S12 and S13).*

in tray S49 for the period ending 30 September 1966 are of unknown accidental cause and should be disregarded. Prevalences in these groups, although low, suggest that MSX played a minor role in the 1966 deaths in S49. The summer death curve of this tray is spread much wider than in typical SSO epizootics.

MSX has not caused enough deaths in eight years of monitoring tray oysters on the Seaside to produce a recognizable peak in mortality curves. Exposure of susceptible oysters indicates that the environment is unsuitable in some way. The regular occurrence of MSX on the Seaside suggests that its failure to become epizootic may not be due to scarcity of infective materials although nothing is known of dosage. In contrast, SSO is an important cause of deaths but can be avoided for nearly two years by proper timing of imports.

DISCUSSION

The epizootics of oysters caused by *Minchinia nelsoni* show no signs of abating after eight years of devastation. My first description of the epizootiology of MSX was based on five years of field studies (1959-1963). The oyster industry in lower Chesapeake Bay was destroyed in these first years of MSX activity which were considered catastrophic. Yet, the past three years (1964-66) have brought increasingly intensive activity during a period of drought in the Bay. The range of MSX, or at least the area of damage, has been extended up the Bay and its tributaries in irregular patterns not associated with salinity alone. In 1964, the James River seed area was invaded seriously but not in later years. In 1965, the disease caused extensive damage in Pocomoke Sound, the lower Maryland tributaries of the Bay, and in the upper Rappahannock River — all of which had been marginal areas for MSX in earlier years. These extensions of MSX activity were not maintained in 1966 and 1967.

Epizootiology

The studies of recent years have essentially confirmed the patterns of morbidity and mortality re-

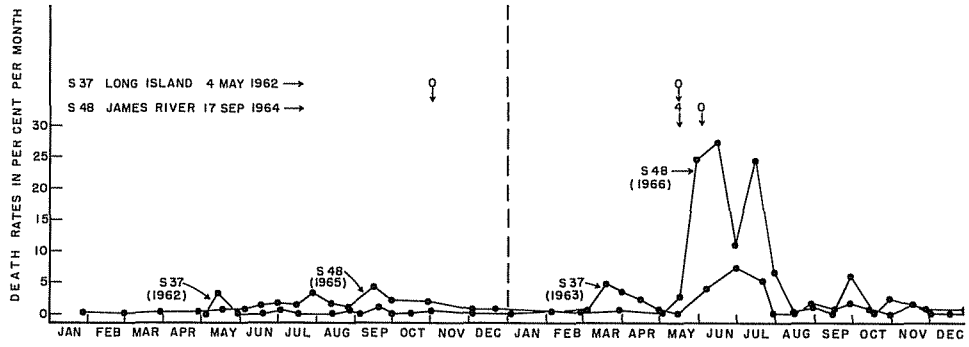

FIG. 10. *Imports monitored from 1962 through 1966 revealed typical SSO peaks but very little MSX activity (S37 and S48).*

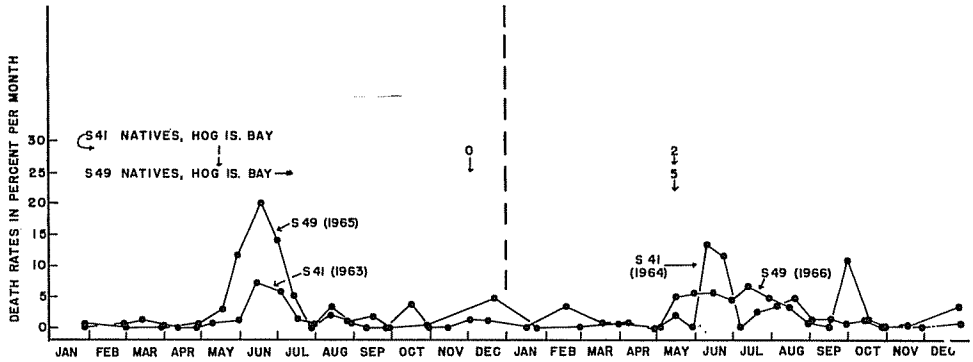

FIG. 11. *MSX activity in native oysters (S41 and S49) is similar to that in susceptibles—without clear mortality peaks from MSX and with low prevalences.*

ported for MSX earlier. A clearer understanding of the epizootiology has resulted from exposure of oysters of a greater variety as to age and history. Increasing intensity of the disease challenged susceptibles, resistant progeny and old survivors and provided new insights of timing of infections and sporogony. Sporulation, although still rare, has occurred more frequently in resistant progeny than in susceptible oysters. Mostly the susceptible oysters were monitored in earlier years to obtain patterns of mortality and morbidity. The life cycle is slowly being pieced together without the benefits of cultures and infections in the laboratory. MSX has been active in Delaware Bay for ten years. Delaware and Chesapeake bays are the two major areas damaged by MSX and massive collections of data are available for both. It would be very useful to have careful comparisons of data and of interpretations of epizootiology.

Patterns of Mortality

Patterns of mortality have remained essentially stable in timing but death rates have increased in recent years. Season of import is critical in subsequent time and level of deaths. Winter and spring imports begin dying from mid-July to late August. Oysters imported in winter and spring exhibited lower first-year deaths than those brought in during the early-summer period of infectivity (June and July). This was observed in earlier years but not appreciated fully until the 1966 experience with Potomac River oysters (Fig. 3).

For many years the absence of deaths (and patent infections) in late-summer (August through October) imports was an enigma. These groups usually lived for eight to ten months with deaths beginning in June of the second year. In recent years of increased MSX activity, this pattern has been broken. The change is more evident in prevalence data than in death rates but August 1966 imports began dying from MSX in November and December 1966. The death rates accelerated slowly from about 1 per cent per month in the late fall to 5 per cent per month in May without a distinctive pattern. These rates are very low for oysters 60 to 80 per cent infected with MSX, and most diseased oysters followed the usual pattern of high mortalities in June and July of the second year. The apparent occurrence of two separate types of infections seems to resolve now into a matter of intensity of infectivity or dosage followed by temperature reduction. The end-of-winter mortality peak is absent in late-summer imports despite high prevalences.

Patterns of Morbidity

Prevalence of MSX in live oysters has increased during the drought years of 1964-66. Susceptible Horsehead Bar oysters have been used for monitoring prevalence each year. In earlier years, 50 per cent prevalence was about maximum but in recent years samples exceeding this level are common in all seasons.

Winter and spring imports acquired typical June infections, evident in July, except that clinical infections have occurred slightly earlier in drought years — particularly in 1964 when deaths began before mid-July. The minimum observed period from import to deaths for populations has been six weeks; hence MSX infectivity must have been intense by 1 June in 1964. Imports in mid-June that year resulted in late infections and deaths beginning 1 August.

Late-summer imports have shown progressive changes in timing with increases in infective pressure as shown by susceptible controls. Previously, infections were apparently acquired promptly after import but were not clinically detectable until May of the following year. Beginning with the first of four drought years in 1963, a gradual change in the time of appearance of clinical infections was observed. In 1963, oysters imported 14

September had no infections in late January 1964 when first sampled. By early April 1964, infections were evident and prevalence reached 56 per cent in late May before deaths began. In 1964, mid-August imports already had 25 per cent clinical infections in late January 1965 and nearly 50 per cent in February. It is probable that some infections were clinical cases in the late fall of 1964 but no sampling was done because patent infections were not expected. In 1965, August imports showed 25 per cent infections in December and prevalence reached over 60 per cent in May 1966. Finally, in 1966 mid-August imports had begun to die in late October with 30 per cent infections and prevalence climbed to 80 per cent in December.

In recent years intensive MSX activity has demonstrated that throughout the warm season some susceptible oysters developed clinical infections in about two months after import. This implies that dosage of infective materials is variable by seasons and years. Early summer (late May to early July) appears to be consistently the period of most intensive infectivity and the shortest incubation periods follow. Incidence of MSX (percentage of oysters infected in a given period) is higher from early summer exposure than prevalence data indicate because deaths remove morbid oysters continuously. In late-summer infections, deaths are usually delayed for 8 to 10 months, hence the highest prevalences are observed in May. Cool temperatures intervene and delay the development of late-summer infections hence no major change in mortality patterns occurred even in the years of high MSX activity.

The conclusions from these many years of observations are that interaction of dosage, susceptibility of oysters (including effects of timing of importation) and temperature produced the patterns of infectivity and mortality described. These factors tend to obscure a pattern of continuous exposure at variable levels of infective particles. Apparently infections are established only during the warm season from May through October when oysters are dying most rapidly. This does not preclude exposure during the cold months of November to May at a low level of infective particles.

Susceptibility of oysters and dosage of infective particles are only very crudely measured in field studies by monitoring stocks of common history and origin at one station. I must assume steady virulence of MSX to conclude that dosage has increased during the drought years. Susceptibility has probably not increased, although it appears to be modified by time of import and age (size) of oysters at first exposure. Evidence on the importance of early exposure is presented below.

Contagiousness

Perhaps the most intriguing and dismaying features of MSX in lower Chesapeake Bay are the persistent patterns of widespread infections without regard for number or proximity of other oysters. Susceptible oysters held in trays with infected lots do not obtain infections earlier or in greater numbers than others grown in relative isolation. Most epidemiologists would probably regard this as evidence of another host whether alternate or one independent of oyster populations. The long period of infectiousness of MSX (about 5 months) and continuous infection pressure are factors of interest in this regard. The obvious dispersion of micro-particles in tidal estuarine waters makes it difficult to imagine direct transmission over widely spaced areas where oysters are relatively scarce. Yet the period of apparent infective pressure matches the period of oyster deaths from MSX rather closely. If another host is involved, it must release infective elements for a long period also. Large highly motile and widely distributed organisms, such as blue crabs and scianid fishes which are attracted to oyster communities, seem most likely as possible alternate hosts. The many varied situations where oysters have become infected tend to exclude fouling organisms and bottom infauna if proximity is required. Oysters essentially free of oyster associates have been imported and infected before fouling organisms could accumulate. Sandy, shelly and muddy bottoms show no apparent differential effect on infection activities.

Innate vs Acquired Resistance

Progeny of two types of parental populations were monitored in our program to select oysters resistant to *M. nelsoni.* Unexposed susceptible stocks and survivors of MSX-ravaged populations were chosen for breeding. The survivors from epizootic areas were estimated to comprise less than 10 per cent of the originial populations after five years of MSX activity. Evaluation of resistance in progeny tests was based on the phenotypic characters of mortality and prevalence rates. As controls and as indicators of MSX activity, native Horsehead oysters grown for two to four years in an area free of the pathogen were imported. In the years 1964 to 1967, MSX generated intensive infective pressure in these susceptible controls.

Progeny of both susceptible and selected parents exhibited low levels of MSX activity and of mortality. At first this was attributed to their small size and young age, but all progeny continued to exhibit resistance to MSX as they grew to market size at ages of two and three years. Progeny were reared in trays off VIMS in the same environment in which controls succumbed to MSX (Fig. 12). It is important to note that early selection of progeny did not occur. Spat were tested when moved from the pond to VIMS and MSX was

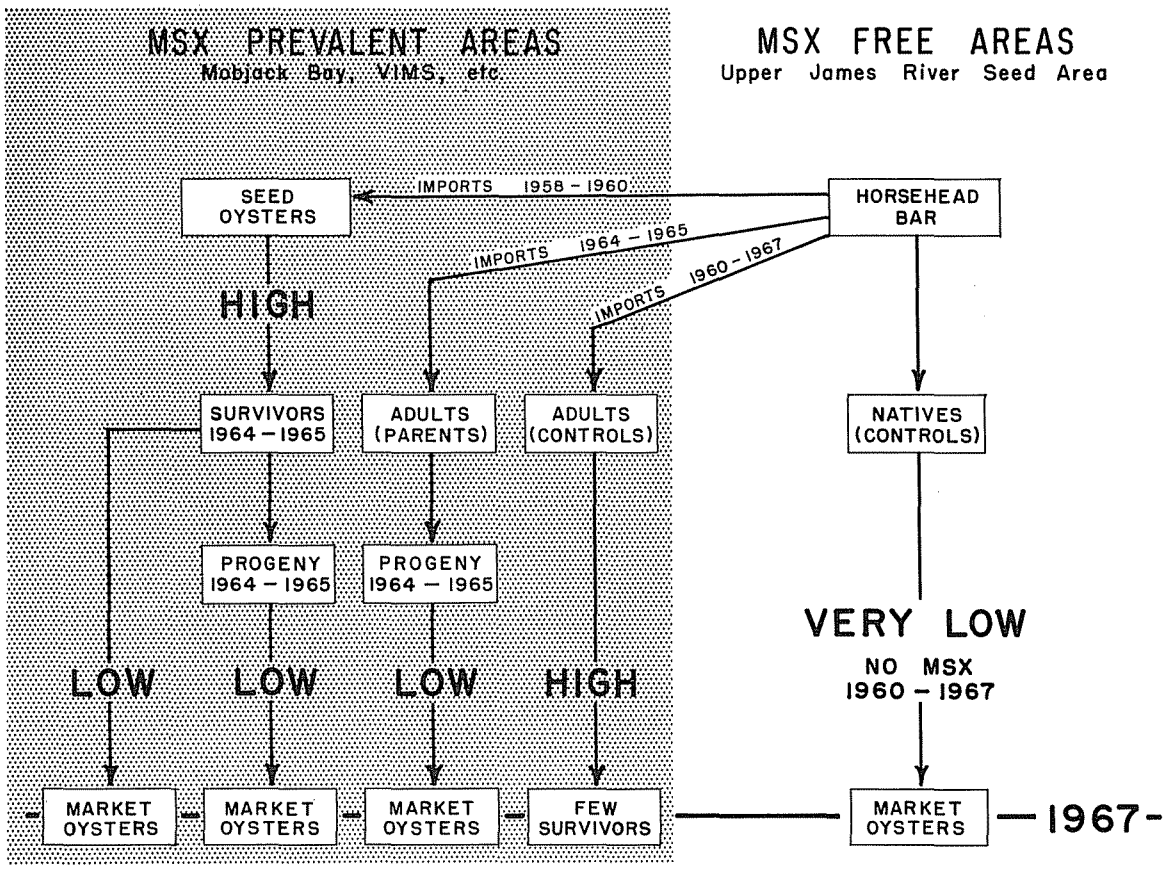

FIG. 12. *Flow diagram showing results of exposure of populations (rectangles) of oysters to MSX. The terms "low" and "high" indicate large differences in both rates of death and prevalences of MSX. All imports were of mature 2 to 3 inch oysters which were 2 or more years of age. Note that progeny, both of survivors and unselected parents, resisted MSX successfully whereas adult imports (controls) succumbed in the epizootic area at VIMS.*

absent. History and source of parental oysters was less important for survival than early exposure to an environment where the disease was active.

Native oysters of unknown parentage in MSX-prevalent areas also exhibited excellent survival. Native oysters from three rivers have been monitored in trays at VIMS with similar results. A wild set on VIMS pilings in 1964 was particularly impressive in the survival of oysters to market size. A trayed lot confirmed our observations that death rates were low and infections scarce in 1965 and 1966.

Survival of progeny and native oysters to market size in areas of intensive MSX activity where imported susceptibles had high death rates suggests that acquired resistance is involved. Horsehead susceptibles and their progeny — from the same gene pool but reared in different environments — exhibited differences in MSX prevalences and mortalities, hence I presume that they had different immunological experiences. Small young oysters, regardless of history or source, seem to withstand attacks of MSX easier than large oysters. Size influences the responses of oysters to environment more profoundly than age. In an environment of known high infective pressure, it is presumed that high metabolic rates in small young oysters enabled them to withstand prevalent dosages of MSX infective particles. Without knowing the mechanisms involved, it seems probable that exposure of progeny to infective particles or occurrence of subclinical infections produced immunities. Lack of knowledge of mechanisms in invertebrates does not invalidate the hypothesis of acquired resistance. Stauber (1959), reviewing immunity in invertebrates, concluded that acquired resistance must yet be demonstrated in oysters.

These studies suggest that survival of early gen-

erations in MSX-prevalent areas may be enhanced more by acquired than innate resistance. The immediate practical applications of early exposure to reduce losses from the disease caused by *M. nelsoni* make it imperative that this aspect of resistance be explored and exploited fully. If effective in practice, it reduces the immediate need for producing seed from genetically resistant strains of oysters under controlled conditions of hatcheries and ponds. The effects of predation on spat and young oysters must be considered by oystermen in Chesapeake Bay where most seed oysters are large when planted. Most seed oysters originate in low-salinity sanctuaries where disease is absent or scarce, hence sources of stocks exposed to MSX must be considered.

Comparison of Life Cycles of MSX and SSO

SSO and MSX appear to be poorly adapted parasites of oysters. Both tend to kill their hosts before sporulation is completed. A carefully attuned parasite, such as *Bucephalus,* exhibits low pathogenicity and does not upset the feeding ability of its host — at least until late in the parasitic relationship when the parasite has had ample time to complete its reproductive cycle. Oysters show little reaction to *Bucephalus* although the parasites castrate their hosts and rob them of nutritive materials.

A comparison of the two *Minchinia* parasites of oysters reveals several important differences in adaptation of life cycles. These factors of seasonality, exposure, incubation period, intensity of infections and their implications about life cycles will be discussed. SSO is much more regular in its seasonal patterns of mortality and prevalence. This sharp seasonality was a primary reason for describing *M. costalis* as a separate organism in 1962. However, without sporulation stages and spores, which were common and regular in occurrence, the description of SSO as a new species would have been risky in 1962. Plasmodial stages were not easily separated from those of *M. nelsoni;* the characters used were recently described by Couch (1967). SSO disappears from oysters (clinically) in July and does not reappear usually until March and April, although occasional infections have been seen as early as December. Sporulation occurs rapidly in May and June and oysters die or recover in close synchrony. In contrast, MSX occurs in oysters throughout the year and kills them continuously in patterns related to time and intensity of exposure, susceptibility and temperature.

SSO appears to require exposure of oysters to an epizootic to initiate infections. MSX produces infections in newly-imported oysters for at least the five continuous warm months and probably oysters are exposed to infective particles throughout the year although this has not been demonstrated. Since oysters are dying most rapidly during these five months of demonstrated infectivity, this is indirect evidence that oysters are the source of infections. The problems of dosage and source of infective particles are not easily solved in field studies.

SSO exhibits a long incubation period (6 to 9 months) during which neither clinical evidence of the organism nor physiological evidence of reaction by the host can be observed. MSX studies have yielded a confused picture on period of incubation to clinical cases. Individual oysters exposed in early summer may show MSX in 5 weeks or 5 months, whereas oysters first exposed in late summer may show physiological effects in two weeks (under water weighing) but delayed appearance of clinical cases may extend to nine months. Again dosage and susceptibility are probably involved in ways now obscure and produce puzzling patterns of prevalences and mortalities.

Once patent, SSO infections develop rapidly, become intensive and kill oysters promptly. Tissues of nearly all organs are filled with sporocysts and are quite disrupted, but usually epithelia are left intact and not invaded. MSX can kill with equal rapidity but no clear relation between intensity of infections and deaths is seen. Susceptible oysters which die in the first summer often have light infections with little disruption of tissues. Those oysters which survive with persistent infections until late winter or early summer of the following year (one year after infection) usually have intensive infections and reactions to match. Yet tissue disruption is not as serious as in SSO cases.

In conclusion, it is postulated that the normal life cycle of *Minchinia* species in oysters is an annual affair with mortalities and new infections occurring in early summer (June mostly in Virginia). SSO is moderately adapted to its host in terms of seasonality, level of mortality and limitation of morbidity in oyster populations. This pathogen is limited to high-salinity areas in its environmental range. Its pathogenicity is high and a large proportion of infected host individuals die before sporulation is completed. Since oyster meats are rapidly destroyed after death, only a small proportion of gapers produces mature spores. The potential number of spores is very high.

MSX appears to be a closely related species parasitizing oysters in more brackish waters. Apparently, some recent event of mutation or hybridization has increased its virulence greatly. This virulent strain is so pathogenic that the annual cycle is interrupted very early in most oysters by death of the host. Sporulation is rare but it has been observed in nearly all months of the year. June appears to be the month when sporulation normally occurs. Timing of infections, deaths and

sporulation have all been disrupted by high pathogenicity. The usual fate of such a destructive parasite as MSX would be to decimate its host population, hence reducing its own occurrence. But MSX continues to thrive with greatly reduced oyster populations, and the source of infective particles and the mode of transmission remain a mystery.

Hypothesis on Origin of MSX

The origin of marine epizootics is always difficult to explain. However, it seems worthwhile to record the fragments of information available and to attempt an explanation of events. In Virginia we have positive evidence that MSX was not important as a disease agent in the decade of the 1950's prior to 1959. The preponderance of data consists of mortality records of many groups of oysters over many years. The patterns of oyster deaths were clearly linked to *Dermocystidium marinum*. Losses were confined to the late warm season each year. A few hundred permanent slides of gapers confirm the absence of MSX and high prevalence of *D. marinum*.

A tenuous but important link in the evidence on origin is the finding of two pre-epizootic cases of MSX — one in 1953 among 49 fungus-killed oysters six years before MSX became active. One must conclude that *M. nelsoni* was endemic but quite inactive in the 1950's. If this assumption is accepted, upon slender evidence, the question arises as to how MSX became epizootic. A theory of cyclic activity with fluctuations in susceptible host populations seems improbable with more than a decade of known inactivity when oysters were abundant followed by eight years of continuous epizootics.

In 1959, while monitoring for MSX, the haplosporidian parasite, *Minchinia costalis*, was discovered and later named by Wood and Andrews (1962). Seaside organism (SSO), as this pathogen was called, has never been found on the western shore of Chesapeake Bay although it appears to be endemic on Eastern Shore, particularly on Seaside. The pattern of a sharp peak of mortality in late May and June was a dominant reason for separating SSO from MSX. In addition, sporulation stages were observed progressing to typical haplosporidian spores. Considerable effort was expended in convincing colleagues that two separate organisms were involved, for the early plasmodia are remarkably similar.

In 1966, Couch *et al.* associated some rare *Minchinia* spores with MSX. These spores, found only in epithelia of liver tubules whereas smaller SSO spores occur in all tissues within epithelial walls, were observed first at VIMS in 1960. Occurrence of MSX spores has been extremely low in gapers and live oysters, but always typical MSX plasmodia have been present with spores (about 20 cases now on slides). Despite the rarity of spores (fewer than 1 per 1,000 cases of MSX prior to 1966), I believe the sequence of sporulation stages and invariable occurrence of MSX plasmodia are strong evidence of correct identification as a haplosporidian. The similarity of morphology and the placing of both pathogens in the genus *Minchinia* reinforce the concept of closely related species. I agree with Couch (1967), who described concurrent infections, that affinity of the two pathogens is close.

It is worth noting some other similarities and differences between MSX and SSO. Both exhibit a frequent failure to complete their cycle to a mature resting spore. However, SSO usually produces sporulation stages whereas MSX rarely does. In our paper on the epizootiology of SSO (Andrews *et al.*, 1962), we reported over half of the gapers with spores but our criterion was to report the most advanced stage seen. Many gapers had only immature spores and often few of these. Therefore, most SSO materials were being released to open waters as sporocysts without mature spores. In some recent years, spores have been comparatively difficult to find in smears and sectioned gapers during SSO epizootics although cysts of sporoblasts are common. A further similarity is the occurrence in both pathogens of a stage without distinct nuclei but with numerous punctate chromatin granules (probably schizogony) preceding formation of sporoblasts. This stage is distinctive in morphology but it is never observed unless sporogony occurs. Enlargement of plasmodia to sporocysts begins with this stage.

Field experiments by carefully timed imports indicate that periods of infectivity match those of oyster mortalities for both MSX and SSO. The infective period for SSO appears to be less than two months, with June the most important month. Although oysters are infected with SSO during a June mortality period, the pathogen is rarely found before March of the following year. In contrast, MSX kills oysters around the year but chiefly in the warm months, June through November, inclusively. Evidence has been presented that high levels of infection of MSX can be attained by exposure in any one of these six months except Novmber. MSX, too, tends to exhibit "hidden" infections for periods up to nine months.

The purpose of these discussions of similarities of MSX and SSO drawn from epizootiological studies is to demonstrate the close relationship of the pathogens. It is ironic that in the early 1960's my efforts were directed at proving differences in the pathogens whereas now I am emphasizing congeneric relationships. On Seaside, the pathogens are living sympatrically but only SSO produces typical infection and mortality patterns there.

The next episode of our reconstruction of the

origin of MSX is based upon well known events and their timing but presumes interbreeding of MSX and SSO for which there is no evidence. The history of seed oyster planting from the Seaside of Eastern Shore to Delaware Bay is well known. In the first half of the decade of 1950-60, over half the seed planted in Delaware Bay came from Seaside where SSO is prevalent. Although generally limited to environments nearly oceanic in salinities, SSO does live in Bayside creeks and lower Delaware Bay (Haskin, personal communication). In view of the sudden appearance of a virulent strain of MSX in Delaware and Chesapeake bays only two years apart, it seems quite logical to me that a new strain of *Minchinia* could have arisen in Delaware Bay from the proximity of native and Virginia oysters. No evidence that MSX was present in Delaware Bay prior to 1957 is available, but if endemic in Chesapeake, transplanting would almost surely have scattered it along the coast within its tolerance range.

If we assume that a new virulent strain arose, it could be expected to follow epizootiologically the patterns of a newly introduced disease. The patterns of kill are very similar in both bays (Haskin *et al.*, 1965). Mortalities began in late spring and early summer of 1957 and 1959 in Delaware Bay and Chesapeake Bay, respectively. The following summer severe mortalities occurred in all beds within the salinity tolerance of MSX. In both bays the initial large-scale infection occurred in late summer (well documented for Chesapeake Bay) and typical patterns of infection and mortality were established in both bays the second summer. In each bay a localized center of infection was observed from which the disease spread. In Delaware Bay this center consisted of imported Seaside oysters but in Chesapeake Bay it occurred in native transplants no different from beds in surrounding areas. It is hard to believe that this close parallel of events two years apart could be accidental or caused by natural changes or cycles.

Perhaps an important part of this hypothesis of a new virulent race of MSX lies in the environmental tolerances of the two pathogens — for lack of accurate knowledge of causes, often referred to as "salinity tolerance." It has been noted that SSO requires high salinity environments whereas MSX flourishes best in moderate salinities and does not produce typical epizootics in the optimum areas for SSO. The fluctuations in MSX activity at Cape May (Haskin *et al.*, 1965) are notable in comparison with persistent activity in lower Chesapeake Bay. I suspect lower Delaware Bay is marginal in some years for both MSX and SSO although the reasons are vague. One can only speculate that this environment provided suitable conditions for mutation or hybridization which gave rise to a virulent race.

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