Oyster Diseases in Chesapeake Bay

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Introduction

Oyster disease studies have been pursued at the Virginia Institute of Marine Science (VIMS) from 1950 to 1977. The first decade was devoted to *Dermocystidium marinum* which causes a warm-season wasting disease. Zoospores of the pathogen exhibit organelles collectively called the apical complex found only in protozoa of the subphylum Apicomplexa (Perkins, 1976). After 10 years of monitoring mortalities and parasites in Chesapeake Bay, a new disease caused by the haplosporidan *Minchinia nelsoni* appeared in 1959 (Andrews and Wood, 1967). It was discovered 2 years earlier in Delaware Bay (Haskin et al., 1966). A recent overview and bibliography is given by Kern (1976).

Field methods developed in the 1950's were applied to the new disease. These consisted of monitoring imported lots of disease-free oysters in legged trays on natural oyster beds. Disease-free oysters were obtained from low-salinity waters (<15‰) in the James River seed area of Virginia. Legged trays lined with 1-inch mesh hardware netting prevented smothering and predation which were the major interfering problems on natural bottoms.

In 1959, a disease caused by *Minchinia costalis,* a pathogen closely related to *M. nelsoni,* was discovered on Seaside of Eastern Shore, Virginia, in high-salinity waters (Andrews et al., 1962). This disease has a well-defined seasonal pattern of activity, and the pathogen achieves sporulation regularly in May-June each year (Andrews and Castagna, 1978). The life cycles of these two haplosporidans are obscure, and artificial infections have not been achieved. A comparison of epizootiological traits is made for clues to sources of infection which are a persistent mystery. Hypotheses on timing of activities and infective sources are derived from these comparisons. Studies of the two diseases in Chincoteague Bay were made by Couch and Rosenfield (1968).

The chronological history of oyster diseases in Chesapeake Bay, the methods evolved for studying mortalities and prevalences, and clues to life cycles derived from epizootiological studies are offered for comparison with those of the protozoan parasites described by colleagues from Europe and Australia at this symposium.

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ABSTRACT–Three major diseases of oysters have been monitored in Virginia estuaries for 2-3 decades. Dermocystidium marinum, *causing a warm-season wasting disease, was discovered in Virginia in 1950 and continues actively to kill oysters where beds or populations are found in high-salinity waters (>15‰). This disease spreads by close proximity of dying oysters to other oysters, hence each isolated bed must be sampled in early fall annually to document activity of the pathogen. Control involves avoiding infected seed oysters, cleaning beds of all oysters after harvest, and isolation of new beds.*

A new pathogen, Minchinia nelsoni *(MSX), caused catastrophic oyster mortalities in 1959-60, and oyster planting ceased thereafter in a large area of high-salinity (>15‰) waters in lower Chesapeake Bay. A third pathogen,* Minchinia costalis *(Sea Side Organism or SSO), was found almost simultaneously on Seaside of Virginia in high-salinity waters (>30‰). Both these haplosporidan parasites kill native susceptible oysters at rates of 20-50 percent annually. Strains resistant to MSX were selected from survivors by laboratory breeding.*

SSO appears to be an endemic pathogen that causes confined periods of infection and mortality. Sporulation and infection occur regularly each May-June associated with oyster deaths. A long incubation period of 8 months with hidden or subclinical infections characterizes the disease. SSO is confined to high-salinity waters along the seacoast from Cape Henry to Long Island Sound. MSX is a highly infectious pathogen that appears to be new by importation or advent of a virulent strain. Infections occur during 5 warm months (June-October) and deaths occur throughout the year. Direct transmission has not been achieved in the laboratory for either haplosporidan.

Transmission of the diseases and life cycles are still important objectives after 18 years of studies.

Methods of Monitoring Diseases

Most diseases and parasites in estuarine environments are dependent upon water movements in one way or another for dispersion. Examples in Virginia include *Nematopsis, Bucephalus, Pinnotheres* (pea crabs) in oysters, and sacculinids in mud crabs (Xanthidae). Most of these exhibit more intensive infestations or infections near sources of infective stages. *Dermocystidium marinum* may be found in very localized centers of infection such as piers, public oyster beds, and bridges. Dispersal in tidal waters becomes an exceedingly difficult dilution problem for diseases that require dosages of many infective particles to establish infections. *Dermocystidium marinum* is in this category.

The spotty distribution of *D. marinum* disease has always required annual surveys to establish the activity of the disease in particular beds. Thioglycollate tests (Ray, 1952) of all gapers, and samples of live oysters from August through October from public and private beds were necessary. Tray lots of known age, source, and history were monitored to establish patterns of infection, spread, over-wintering, and salinity tolerance (Andrews and Hewatt, 1957). The advantages of monitoring

trays over sampling beds of oysters also include choice of strains (susceptibles, resistants, etc.) and elimination of predation and smothering. The timing of transplanting between disease-free and disease-prevalent areas can be fixed advantageously by use of trays with discrete populations.

The tray method of monitoring became important in studying *Minchinia nelsoni* because natural and planted beds were lacking after 1960 in the disease-infested areas. Those few oysters that survived or set thereafter were selected by the disease in unknown degree thereby distorting disease patterns. The haplosporidans exhibited much more uniform distributions without the patchiness and proximity effects of other oyster parasites. Hence, a pair of trays of oysters gave prevalence and mortality data that applied to rather large areas. The tendency of MSX to fluctuate up and down Chesapeake Bay by large distances from one year to another must still be reckoned with by judicious locations of trays. Susceptible control oysters were always used for these stations monitoring disease activity levels. Typically, live samples were taken every month from these trays in major oyster-growing estuaries.

The legged trays used in Virginia hold about 1 bushel of oysters and lots of 500 are initiated usually. Fouling of the 1-inch mesh liners is intensive and requires monthly examinations in cold seasons and more frequent ones in warm periods. Oysters are double-counted at each visit and gapers and boxes (empty shells) removed. The trays are located on oyster beds (often barren) beside a marker stake. Samples of 25 live oysters are processed into permanent stained sections on slides. Except for *D. marinum,* infections are all diagnosed from permanent slides. Blue crabs or spider crabs are kept in some trays of older oysters to keep down fouling by sea squirts and sponges. Small fishes (blennies, gobies, clingfish) scavenge gaper meats in the trays (scuba observations).

Each group of tray oysters is treated as a distinct population of known history and origin without mixing until numbers of oysters fall below 100 and no longer give reliable mortality estimates. Monthly mortalities are calculated for each 15- to 30-day period of observation based on the number alive at the beginning of the interval. Annual mortalities are calculated using instantaneous rates. Prevalences are given as number of cases per 25 live oysters, or in percentages. Incidence of infections per unit of time is difficult to obtain as is morbidity (cases to deaths). At the VIMS Pier, diseases are monitored seasonally by weighing individually marked oysters underwater weekly for shell growth (Andrews, 1963).

History of Oyster Disease Studies in Lower Chesapeake Bay

Dermocystidium marinum Era—1950's

Disease studies began in Virginia with the discovery of *D. marinum* in oysters in 1950. The thioglycollate assay technique (Ray, 1952) permitted epizootiological studies of this disease in the 1950's when microtechnique facilities were lacking or inadequate. It is not known when *D. marinum* became endemic in Chesapeake Bay. Increased mortality rates about 1940 may have reflected its introduction. Chesapeake Bay oysters are more susceptible to the disease than South Carolina native oysters (Andrews and McHugh, 1956).

The 1930 winter mortality of oysters in Mobjack Bay (Prytherch[1]), discovered in March, was not caused by *D. marinum* for it does not kill oysters in late winter. The timing of this mortality was confirmed by Dumont[2] (and report dated April 1930) who participated in the investigation. Neither was it caused by *Nematopsis* as proposed by Prytherch (see footnote 1) for these parasites have not been demonstrated to kill oysters in Virginia (Feng, 1958). Prytherch's proposal to limit *Nematopsis* in oysters by reducing mud crab populations was largely effected in the mid-1960's by importation of the sacculinid parasite *Loxothylacus panopaei* into Chesapeake Bay from the Gulf of Mexico in oyster shipments (Van Engel et al., 1966).

In the 1950's, *D. marinum* was the primary cause of oyster deaths in Virginia waters during the warm season. Losses to smothering and predation were large on planted private oyster beds with marginal bottom textures. Yields seldom exceeded 1 bushel for each bushel of seed oysters planted, and often in mesohaline areas (10‰ to 25‰) yields were only one-half bushel. With initial seed-oyster counts of 1,000 to 2,000 per bushel, total losses in 2 or 3 years of culture were 65 to 85 percent of the number planted. *Dermocystidium marinum* caused a major part of these losses in most high-salinity areas. Seed-oyster plantings were usually held 3 years before marketing, which accentuated losses.

Continuous culture on public and private grounds that were intermixed in oyster-growing areas assured continuity of *D. marinum* infections. The only known reservoir of infective particles is in live oysters. Recruitment on public beds and repeated plantings on private beds insured that some old infected oysters were present to spread the disease when they died. Most infections occur from dying oysters in near proximity (<15 m) (Andrews, 1965). *Dermocystidium marinum* spreads very slowly into new areas or new beds without residual infected oysters. Thorough cleaning of beds and fallowing or isolation are the chief control methods. All stages found in oysters are infective (trophozoites and sporangia) as well as zoospores released in seawater.

A scarcity of oysters (hosts) has caused a decline in abundance of *D. marinum* in lower Chesapeake Bay since 1960 when private plantings ceased. It persists as a constant threat, in areas where oyster populations are built up, by living in oysters on pilings and in fringe areas of salinity tolerance of the disease (12-15‰) where natural recruitment of oysters occurs. Overwintering occurs as cryptic stages in

[1]Prytherch, H. F. 1931. Report of the investigation on the mortality of oysters and decline of oyster production in Virginia waters. Mimeogr. rep., 12 p. U.S. Dep. Commer., Bur. Fish.
[2]Dumont, W. H., Bureau of Commercial Fisheries, U.S. Fish and Wildlife Service.

live oysters. The parasite requires high temperatures (>20°C) for multiplication and oysters tend to discharge it in fall at cool temperatures. Low salinities alone inhibit activity but do not eradicate the parasite from oysters. Persistent low salinities or absence of oysters, as in Mobjack Bay, will eliminate the disease in a few years.

The earliest studies were made with Sea-Rac[3] (Chesapeake Corporation, West Point, Va.) off-bottom trays suspended from catwalks at VIMS piers. These trays were examined daily to procure gapers (dead oysters) for diagnoses (Hewatt and Andrews, 1954). The close proximity of trays at the piers insured maximum rates of spread of *D. marinum*. Trays were comparable to infested beds of planted oysters in disease activity. Many beds of oysters were sampled in the 1950's to confirm that *D. marinum* was killing oysters on public and private grounds (Andrews and Hewatt, 1957). Since VIMS pier with its pilings was a continuous reservoir of *D. marinum* infections due to recruitment of oysters, it was necessary to place oysters on abandoned oyster grounds to decrease the interference of this pathogen in *Minchinia* studies.

MSX—A New Pathogen Out of Control, 1959 to 1977

The appearance of *Minchinia nelsoni* in 1959-60 changed the whole industry of oyster culture in Chesapeake Bay (Andrews, 1966, 1968). No longer were planters able to tolerate losses as they had with *D. marinum* kills. Only trial plantings were made after 1960 in high-salinity waters (>15‰). MSX, as *M. nelsoni* was called, replaced and displaced *D. marinum* as the major cause of oyster mortalities. The scarcity of oysters prevented *D. marinum* from spreading actively except in localized pockets. MSX killed oysters at annual mortality rates of 50-60 percent and with peak death rates of 20-25 percent monthly.

Delaware Bay disease, caused by *M. nelsoni*, spread throughout lower Chesapeake Bay in 1 year (1960). It infected over long distances from a Mobjack Bay focus in 1959. The distribution of MSX infections may shift tens of miles upstream in one warm season depending on salinities and unknown factors of infection. These changes reflect level of disease activity more than absence of infections in borderline areas. MSX tends to exhibit rather uniform levels of infections and kills over wide areas with appropriate salinity levels (about 15 to 25‰). Presence or absence of infected oysters and local abundance of oysters have no discernible effect on MSX activity. Proximity of oysters to each other is not a factor as in *D. marinum*. These patterns of uniform infection and mortality make monitoring of the disease relatively easy (Andrews and Frierman, 1974). The lack of planted beds forced use of tray monitoring after the first 2 or 3 years. Source of oyster stocks, amount of selection by MSX, and local strains of oysters became much more important than location of experiments. Fortunately, this pathogen also requires mesohaline waters, hence disease-free stocks were obtainable from low-salinity areas of the James River. The importance of this limiting salinity parameter becomes evident from the problems of French scientists studying *Marteilia refringens* on Brittany coasts characterized by high salinities in all oyster-growing estuaries (Grizel et al., 1974). If controls are not adequate, the timing of mortalities may appear erratic, and periods of infectivity are difficult to determine. Many years of sampling have confirmed the disease-free status of James River control oysters. Long periods of incubation (latent or subclinical cases) for MSX present difficult problems of insuring absence of disease in controls unless long-term monitoring is pursued.

Intensive mortalities of oysters caused by MSX made possible selection of resistant strains by laboratory breeding of survivors. These resistant oysters did not usually show clinical infections in Chesapeake Bay, and mortality in trays was reduced to about 10 percent annually. To utilize these strains in Virginia requires hatchery production of seed oysters which is not yet economically feasible. It is important to recognize the potential of genetic resistance to haplosporidan diseases which may be used in other circumstances and areas where hatcheries are utilized.

Discovery of SSO (*Minchinia costalis*)—an Endemic Pathogen on Seaside—Epizootiology

The discovery of SSO (Wood and Andrews, 1962) provided an opportunity to study an endemic haplosporidan with stabilized patterns of activity. Its restriction to near-oceanic coastal waters with high salinities provided insights into the salinity tolerances of haplosporidans. Concurrent infections of the two *Minchinia* diseases in Seaside bays provided opportunities to study seasonal progressions and regressions.

The climax of SSO enzootics occurs in May-June each year with 20-50 percent mortalities compressed into about a 1-month period (Andrews and Castagna, 1978). There is an annual life cycle with new infections occurring during May-June deaths or shortly thereafter. Infections are hidden until the following March, usually a period of 8 months. Prevalences of 30-40 percent infections are typical in mid-May before deaths begin. Remission of light plasmodial cases occurs in May-June and sporulation occurs in nearly all oysters that die. Old infections are rare by 1 July and new ones remain hidden. Oysters imported for exposure after 1 August do not acquire infections. Feeding and injecting spores did not achieve infections. Successive enzootic kills up to 5 years have been observed in individual lots of oysters, but declining in intensity. Sporulation is often incomplete in gapers.

Numerous examples of SSO activity on Seaside of Eastern Shore are given by Andrews and Castagna (1978). An example of persistent SSO mortality in a particular tray of oysters over a 3-year period is given in Figure 1. Tray S46 contained susceptible James River seed oysters imported 15 May 1964. Three successive enzootics of SSO occurred with no interference by MSX.

[3]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

The May-June mortalities are typical in timing and intensities of death rates. Infections tended to decline as selection occurred, hence death rates were lower in successive years. This suggests that resistant strains can be obtained against SSO as already has been achieved for MSX-caused disease.

Patterns of MSX Activity

Patterns of infectivity and mortality caused by MSX are well-known (Andrews and Frierman, 1974). Disease distribution tends to be erratic in area due to fluctuating salinities and infection pressures. The prime infection period is early summer (late May to mid-July). Incubation takes 4-5 weeks. Deaths begin in 6-8 weeks, usually by 1 August each year. Death rates of 20-25 percent monthly in late summer and fall are followed by declines to <5 percent monthly as cold weather occurs. A late winter kill (March typically) of 10-20 percent occurs of which about two-thirds is attributed to MSX as shown by gapers. The last of the early-summer infection cases die in June-July of the second year with very intensive plasmodial infections.

Oysters imported after 1 August and before 1 November acquire subclinical infections that are hidden or localized. Typically these infections remain subclinical until April or May of the following year, after which they develop rapidly and cause deaths in June-July. These deaths are mixed with some lagging cases from earlier infections in spring imports.

Prevalences are high (often >50 percent) in May before deaths begin. These "late summer" infections fail to occur in some years for unexplained reasons. In years of intensive infection

Figure 1.—Three consecutive SSO enzootics on Seaside in a lot of susceptible James River seed oysters. MSX activity was minimal in this lot with slight mortalities in early fall.

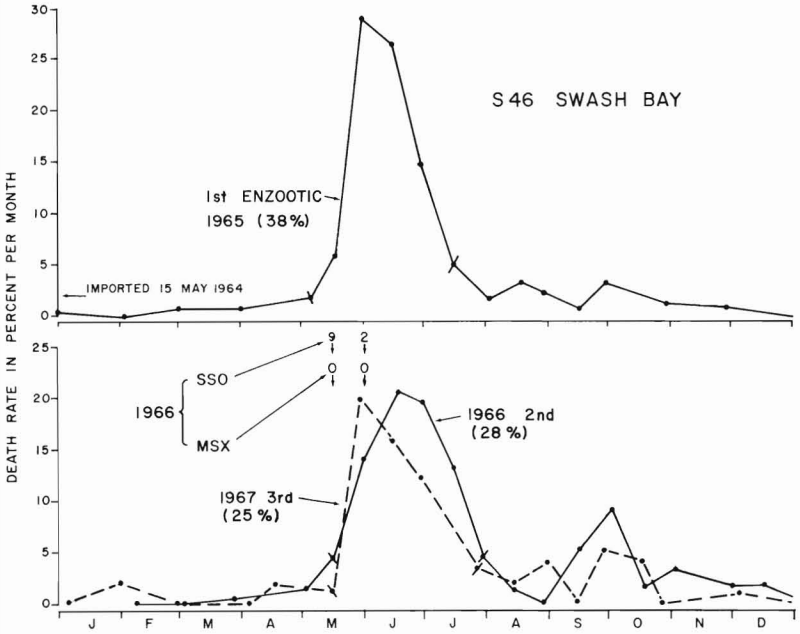
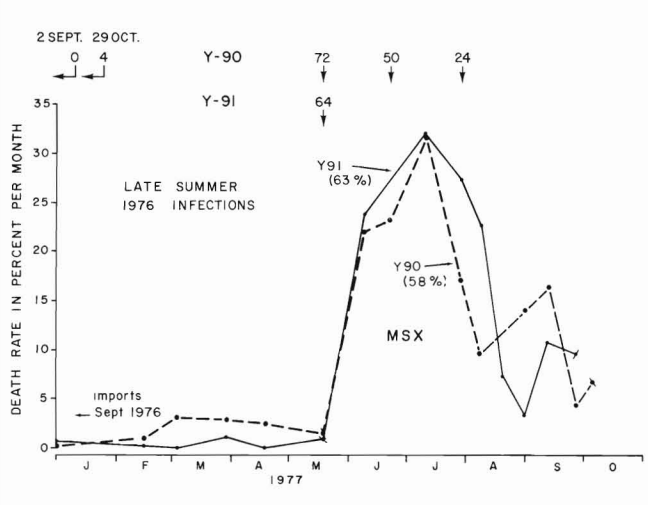
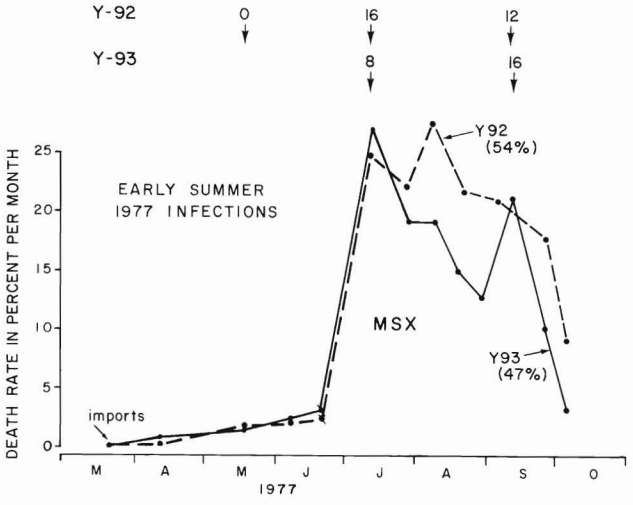


Figure 2 (left) and Figure 3 (right).—MSX kills of late-summer and spring imports of susceptible James River seed oysters. Note that earlier import lot shows mortality beginning a month earlier than spring-import group. Prevalences of MSX in percentages are given above arrows for samples of 25 oysters.





pressure, these late infections may appear clinically in November and December, but most mortality is still delayed until June-July.

Early summer infections in marginal areas of low salinities (<15‰) are delayed in becoming clinical until November or later (Andrews[4]). These infections persist through the winter and are actively discharged by oysters about 1 May when salinities are <10‰. Unless sampling is done, these inhibited infections may never be known, for oysters are not killed. However, transplanting oysters with subclinical infections to high-salinity waters results in patent infections in a week or two in the warm season and delayed patency in the cold season.

In the high salinities (>30‰) of Seaside, MSX infected oysters erratically by year and locality, and then often regressed with few or no deaths. The timing of infections was typical but regression occurred in late summer and spring (Andrews and Castagna, 1978).

Examples of recent MSX mortalities are given in Figures 2-5. Each graph portrays monthly death rates and prevalences in a pair of duplicate tray-oyster lots. Total warm-season mortalities are given under each tray number designation. No *D. marinum* was involved in these lots and deaths were caused almost entirely by MSX. Typical prevalences and annual mortalities are given in Table 1.

A comparison of timing of deaths is given in Figures 2 and 3 for late-summer and spring imports of susceptible oysters. Oysters imported in September 1976 began dying in late May with a peak death rate in early July and a vague second peak in September. About 60

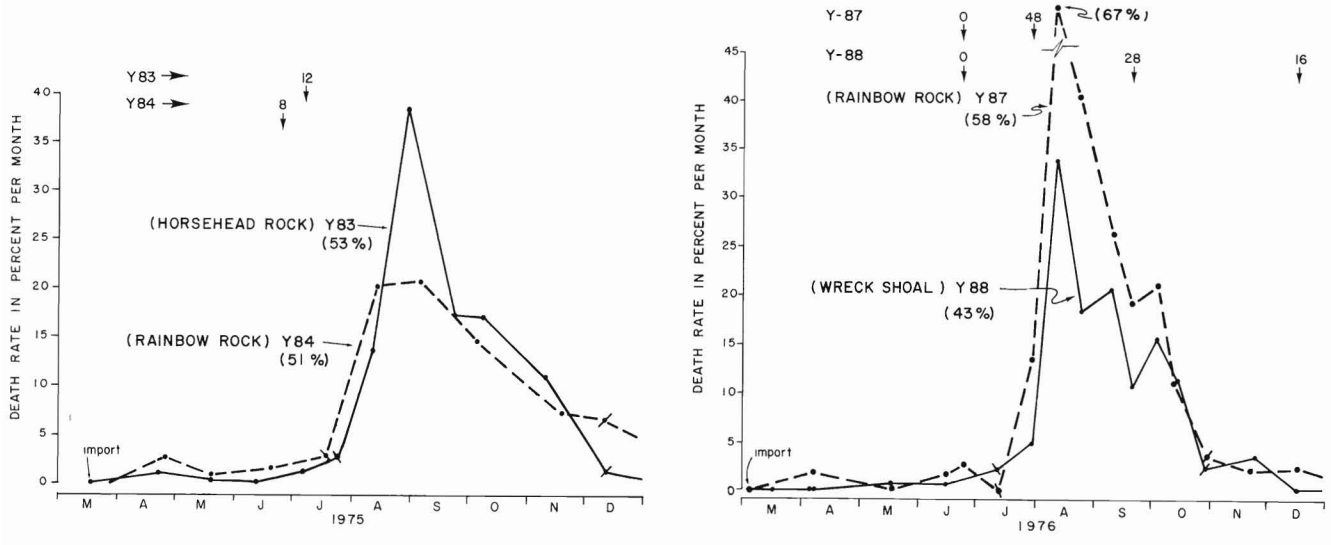
[4]Andrews, J. D. 1965. Fluctuations of MSX (*Minchinia nelsoni*) in the James River and seasonal effects of salinity and temperature. Unpubl. manuscr.

Table 1.—Prevalences of MSX in susceptible oysters in trays at Gloucester Point, Va., York River, 1975-77.

Tray no.	Date of import	Date sampled	Prevalences (%)	Mortality[1] 1st year (%)
Y83	6 Mar. 1975	8 July 1975	12	55
		28 June 1976	0	
Y84	6 Mar. 1975	24 June 1976	8	53
Y85	26 Aug. 1975	6 Apr. 1976	20	[2]7
		13 May 1976	32	
Y86	26 Aug. 1975	13 Jan. 1976	4	34
		10 Aug. 1976	20	
Y87	4 Mar. 1976	24 June 1976	0	65
		29 July 1976	48	
Y88	4 Mar. 1976	26 June 1976	0	46
		20 Sept. 1976	28	
		16 Dec. 1976	16	
Y90	1 Sept. 1976	2 Sept. 1976	0	64
		29 Oct. 1976	4	
		18 May 1977	72	
		22 June 1977	50	
		29 July 1977	24	
Y91	24 Sept. 1976	18 May 1977	64	66
Y92	8 Mar. 1977	19 May 1977	0	44
		13 July 1977	16	
		12 Sept. 1977	12	
Y93	8 Mar. 1977	13 July 1977	8	54
		12 Sept. 1977	16	
		4 Nov. 1977		
Y94	13 June 1977	29 July 1977	48	53
		12 Sept. 1977	16	

[1]Year of import for spring lots (March to March) and year after import for fall lots.
[2]Closed tray 13 May 1976.

Figure 4 (left) and Figure 5 (right).—Trays of susceptible oysters in pairs reveal about 50 percent mortalities from MSX in 1975 and 1976. Typical timings of initiation and of peaks of mortalities are shown by these lots of oysters.



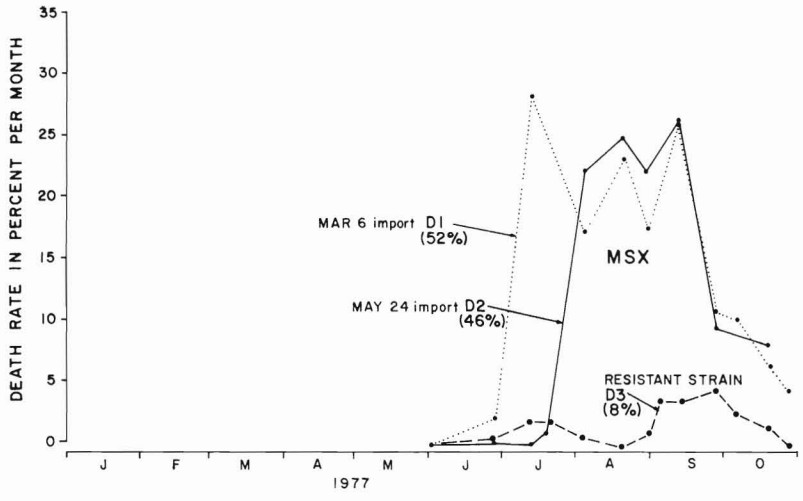
percent of the oysters died. March 1977 imports began dying in early July, a month earlier than the typical 1 August initiation of deaths. The year 1977 was one of very intensive MSX activity. The September 1976 imports were subjected to an intensive additional infection period in June 1977 which elevated death rates to 30 percent per month and accelerated deaths in comparison with those in spring imports in Figure 3. Usually there is a 2-month period between initiation of deaths (1 June) caused by infections acquired in late summer and those from early-summer exposure (1 August). Note that high death rates persisted into August and September in the latter group.

Additional pairs of trays of oysters imported in March 1975 and March 1976 are shown in Figures 4 and 5. These are typical years with deaths beginning 1 August and about 50 percent dead during the first warm season of exposure to MSX. There appear to be slightly higher death rates with origin of susceptible seed oysters further up the James River. Wreck Shoal oysters had a few deaths from MSX on the seed bed in 1976 which is unusual. Rainbow Rock and Horsehead Rock are seed beds progressively further up the river from Wreck Shoal.

The feasibility of breeding strains of oysters resistant to MSX, by selection of survivors as brood stock, is shown in Figure 6. This experiment was intended as a study of interactions of *D. marinum* and MSX in susceptible and resistant populations of oysters. Oysters from tray lots infected with *D. marinum* in the fall of 1976 were added to lots D1 and D3 with D2 as a control. Low temperatures in September to November 1976 permitted most oysters to discard *D. marinum* infections before winter temperatures prevailed. Consequently, the disease was very late infecting experimental oysters in 1977 and only a few deaths in late October in D1 can be attributed to *D. marinum*.

The disparate curves and seasonal mortalities between D1 and D2 (native susceptibles) and D3 (lab-bred selected resistant strain) fully demonstrate the value of genetic resistance to MSX in enzootic waters (Figure 6). These three trays were placed 50 feet apart in a row off VIMS at Gloucester Point, Va. They were exposed to exactly the same conditions, except D1 was imported from James River in March and D2 in late May. The 3-week earlier initiation of MSX-caused deaths in D1 presumably means that infections occurred in early May. This is an earlier infection period than had been demonstrated previously. The resistant-oyster lot (D3) contained oysters of the same size range as the susceptibles, but they had been exposed to MSX since the previous fall of 1976 without deaths. That is, there had been no culling of oysters from the group by MSX or any other agent.

Figure 6.—One resistant and two susceptible lots of oysters responded to intensive MSX activity in 1977. Time of import affected time of first deaths in susceptible oysters.



Comparison of Diseases Caused by *Minchinia costalis* and *M. nelsoni*

Seaside disease (SSO) and Delaware Bay disease (MSX) are active from Chesapeake Bay to Long Island Sound although MSX has been reported from North Carolina and Massachusetts. SSO is confined to high-salinity coastal waters whereas MSX penetrates far up the Delaware and Chesapeake Bays in some years and has been continuously active in the lower bays for 21 and 19 years respectively.

A comparison of epizootiology and developmental stages of the two diseases is presented. *Minchinia costalis* is considered the more adapted pathogen and presented as the norm for life cycles of haplosporidans. Seaside disease differs from Delaware Bay disease as follows.

1) SSO is restricted to high-salinity waters along the seacoast, that is >30‰ usually with a lower limit of about 25‰. The name "costalis," meaning rib or coast, reflects this limited distribution. Oysters imported to VIMS from Seaside in April have exhibited regression of SSO (salinities <20‰). A few cases of SSO have developed in disease-free, imported, susceptible oysters in Bayside of Eastern Shore creeks with salinities of about 25‰. Seaside seed oysters with SSO infections were regularly imported commercially to Bayside creeks prior to the advent of MSX.

In contrast, MSX does not thrive in this environment. It requires salinities of 15 to 25‰ for full epizootic activity. Oysters frequently acquire early summer infections on Seaside but few die and regression occurs. MSX infections and activity are highly variable by years and bays on Seaside. This is not a result of resistance to SSO or MSX, inherited or acquired, for it occurs in susceptible James River stocks.

2) Mortality from SSO is limited to about 1 month (late May to late June typically) with very high monthly death rates. Monthly death rates of 50 percent for the peak 15 days in the first half of June are not uncommon and 90 percent rates have been recorded. The short-term, sharp mortalities occur regularly

in May-June in all Seaside bays from Cape Charles to Cape Henlopen, including Chincoteague Bay. Nearly all deaths are associated with sporulations and very intensive infections.

MSX kills around the year with peaks in late summer (August-September) and early summer (June-July) of the following year depending on time of oyster import and infection. Peak death rates do not usually exceed 25 to 30 percent monthly. Deaths are caused by plasmodial infections of great variability in timing and intensity. There is little synchrony in development of cases and of deaths.

3) Infections of SSO are initiated only during enzootics or shortly thereafter (June-July). Oysters exposed after 1 August do not exhibit SSO kills until 22 months later, that is, after exposure to a May-June enzootic. James River seed oysters can be grown to market size in this time without losses to SSO.

Infections of MSX occur for 5 months (late May through October), although late-summer and fall infections fail to occur in some years. MSX infections also occur during the period of mortalities in the warm season but erratically.

4) SSO sporulates regularly in May-June enzootics. Sporulation results in immediate deaths of oysters. Most gapers and some live oysters exhibit sporulation. Maturation of spores is variable by years, but it often occurs in gapers and sometimes in live oysters. If sporulation is not achieved, regression of plasmodia often occurs, but not after sporulation begins.

MSX sporulates rarely and in young susceptible oysters most commonly. The effect is damaging but not necessarily lethal immediately. Consequently, cases of sporulation have been found in nearly every month of the year, and more often in live oysters than in gapers in 18 years of routine slide reading. In this long series of slides, spore cases have predominated in June as they do for SSO. The occurrence of sporulation has been one case per 2,000 plasmodial cases diagnosed.

5) All plasmodia sporulate synchronously in SSO throughout the connective tisuses of all organs. Infections are intensive at this time and tissues are riddled with sporonts and sporocysts. A typical disease syndrome of clustered cells and large sporulation stages gives a curdled appearance to tissues which is recognizable at low magnification (100×). Epithelia are not involved usually, and the oyster becomes a sack of whitish sporocysts.

MSX sporulation is confined to epithelia of digestive tubules. One to several sporonts and sporocysts are visible in each 7 μm section of all tubules. This implies migration of plasmodia to the tubule epithelia. Numbers of plasmodia left in connective tissues seem low and these do not sporulate there. Sporocysts commonly bulge into the lumen of the tubules. All stages of sporulation may be seen at one time but mature spores are often scarce in live oysters.

6) The incubation period is long (8 months) and relatively fixed in duration in SSO. Between June-July infections and appearance of early plasmodia in March, only occasional uninucleate haplosporidan cells in epithelia of digestive tubules are seen. Infected oysters do not grow new shell in spring.

Incubation period for MSX varies from about 4 weeks to 10 months. In part, this is related to time of exposure of oysters. Salinities <15‰ retard infections. Presumably, infection pressure is involved in rapidity of disease development, for in years of intensive losses, deaths often begin early (July). Late-summer infections are always delayed, but may appear from November to May, again related to intensity of infective pressure as implied by prevalences and death rates.

7) All stages of SSO average smaller in size than those of MSX with spores averaging about 4 μm and 8 μm long, respectively, and sporocysts ranging from 10 to 20 μm and 30 to 50 μm in diameter, respectively. Plasmodia are extremely variable in size and difficult to distinguish between the two pathogens. Rapid multiplication of SSO tends to produce many <5 μm plasmodia with few nuclei whereas MSX cells are often 10 μm or larger with many nuclei. Overlapping sizes occur.

Notes on Life Cycles

The prime questions about life cycles are: 1) What is the source and the cellular form of infective particles? 2) Is there an alternate host? 3) What is the explanation of the hidden infections? and 4) How may infections be initiated artificially? Farley (1967) proposed a life cycle for *M. nelsoni,* but those questions have not been resolved satisfactorily.

The most important evidence bearing on life cycles from epizootiological studies is determination of infective periods from field studies. SSO infects in June-July during or immediately after enzootics in May-June. It has a relatively short infection period compared with the 5 months for MSX. The long incubation periods tend to confuse and obscure the timing of infections. Both pathogens produce infections during periods of oyster mortality. Any stage of the pathogen being discharged by live or dying oysters could be infective. There is little evidence of discharge of spores of SSO by live oysters. Gapers killed by SSO have an abundance of sporonts or spores. General disintegration of connective tissues is required to release them, and this does not happen until oysters become gapers as evidenced by stained-slide preparations.

MSX does not produce enough spores in oysters to sustain widespread infections. Hence, there is a widespread belief in an alternate host, although none have been demonstrated for haplosporidans. Failure of infection experiments led Pixell-Goodrich (1915) to conclude that *M. chitonis* in chitons required another host. Barrow (1965), working with *M. pickfordi* in freshwater snails, claimed direct transmission, but his experimental snails were survivors of an earlier enzootic and may have been infected earlier in nature. Again, a long incubation period may have been involved.

The scarcity of spores has been a handicap in attempts to produce MSX infections. Feeding spores in aquaria and inoculations into the mantle cavity have been tried sporadically whenever spores were available without success. This was tried at VIMS with both MSX and SSO in the mid- and late-1960's with fresh spores directly from gapers. But MSX spores became very rare again until 1976.

Sporulation in Susceptible Spat

An unexpected and isolated event in 1976 provided a new view of sporulation of MSX in oysters. On 20 September 1976, a tray containing thousands of about 1-inch spat was found to have about 40 percent mortality. These 4-month-old spat had been reared in the laboratory at VIMS in May and held in a pond (a sanctuary from MSX) until transferred to the York River on 8 July 1976. There were no deaths on 23 August 1976. Fresh smears of digestive tubule tissues on 21 September 1976 revealed 16 cases of advanced sporulation in 74 unselected live spat (21.6 percent). A live sample taken at the same time for slide material had 88 percent MSX infections (43 in 49 oysters) and 39 percent were in sporulation. This was unprecedented in 18 years of monitoring oysters for MSX, both native and lab-bred stocks. None of 75 other lots of oysters in trays in the same vicinity (some only 50 feet away) exhibited sporulation or anything unusual, although MSX activity was intensive in susceptible oysters.

MSX had infected the spat and developed to sporulation in 10 weeks. A second batch of the same lot of susceptible spat was imported from the pond to the York River on 16 August 1976. These did not die or show MSX infections through the fall and winter until January 1977, but died of MSX in June-July of 1977 without sporulations. This was typical timing for late-summer infections, and it shows that the 8 July lot acquired infections in the York River after importation.

This experience suggests that sporulation of MSX is more likely to occur in young, susceptible oysters. The parents of the spat lot were susceptible oysters from the Rappahannock River being used as controls for MSX activity. This is evidently what happened in Maryland in the drought years of 1965 to 1966 (Couch et al., 1966) when far more sporulation cases occurred there than had ever been encountered in Delaware Bay and lower Chesapeake Bay before 1976. That is, the oysters were young susceptibles although not spat. Also Myhre's (1972) experiments with susceptible and resistant spat in Delaware Bay are pertinent. He found four cases of sporulation in susceptible spat in June 1967 following exposure to MSX in September 1966.

Occurrence of Sporulation of MSX

The occurrence and rarity of MSX sporulation in Virginia deserves some documentation (Table 2). The first case was in a gaper found 3 November 1960. Except for 1966 and 1967, only 1 or 2 cases were encountered each year in about 10,000 oysters processed (<1 case of sporulation per 2,000 cases of MSX). Of 44 cases of sporulation found in 16 years before 1976, approximately one-third were in resistant oysters and two-thirds in susceptibles. Many more resistant oysters were processed into slides. No selection or special effort to obtain sporulation cases was made. Oysters were preserved primarily for prevalence data. In 1966-67, 15 of 19 spore cases occurred in live oysters in June-July and the other 4 in gapers in mid-winter. Sporulation is localized in the digestive tubule epithelia and is not necessarily disabling or lethal. Hence, deaths depend more upon progression of systemic infections and spore cases are erratic in timing. It appears that the live oysters in June had been carrying spores since January-February of 1967 at least.

Infection experiments with MSX were repeated in the fall of 1976. Sporulation was common in the Rappahannock River spat but mature spores were scarce. No success was achieved with inoculations or feedings of tissue minces, and extra spores remained unchanged (none open or shriveled) in the bottoms of bowls for over a month. Two-month incubation periods were allowed before experimental oysters were sacrificed.

A comparison of life cycles of MSX and SSO reveals long incubation periods in both pathogens. The failure of haplosporidan infection experiments may be linked to these incubation periods. Seasonal imports of disease-

Table 2.—Chronological occurrence of spores of *Minchinia nelsoni*.

Date sampled	Tray no.	Source of oysters	Resistant or susceptible	Live or gaper
3 Nov. 1960	60J	James R.	S	G
20 Mar. 1961	J6	James R.	S	G
13 Nov. 1963	Y17	James R.	S	G
4 Sept. 1964	MJ9	James R.	S	G
18 Nov. 1964	MJ11	James R.	S	L
20 Oct. 1965	Y23	James R.	S	L
10 June 1966	P2A	Egg Is.	R	G
16 June 1966	P4A	Potomac R.	S	G
20 June 1966	P5A	Long Is. Sound	S	G
20 June 1966	P6	Mobjack Bay	R	L
1 July 1966	P5A	Long Is. Sound	S	3L
1 July 1966	Y25	James R.	S	G
15 Dec. 1966	P18	Hampton Bar	R	L
4 Jan. 1967	Y31	James R.	S	G
17 Jan. 1967	P18	Hampton Bar	R	G
16 Feb. 1967	P22	1965 set	R	G
23 Feb. 1967	Y32	James R.	S	G
13 June 1967	P25	1965 set	R	L
21 June 1967	P10	1964 set	R	L
27 June 1967	P6	1964 set	R	2L
29 June 1967	P28	1965 set	R	L
12 July 1967	P10	1964 set	R	L
26 July 1967	Y67	James R.	S	L
30 Aug. 1967	P27	Piankatank	S	G
22 Sept. 1967	MJ16	James R.	S	L
18 Jan. 1968	P32	1966 set	R	L
18 June 1968	P40	1967 set	R	L
8 Oct. 1969	P53	Long Is.	S	L
8 Oct. 1969	P57	1968 set	S	L
31 July 1970	S72	Seaside native	S	L
17 Feb. 1971	P66	Md. 1969 set	S	G
30 June 1971	P64	1969 set	R	L
31 Aug. 1971	MJ22	James R.	S	L
28 Sept. 1971	P80	1970 set	R	L
28 Sept. 1971	P81	Md. 1970 set	S	L
1 June 1972	S86	Seaside natives	S	L
17 July 1972	S86	Seaside natives	S	L
24 May 1973	B45	Seaside natives	S	L
22 May 1974	Y79	James R.	S	L
29 Oct. 1975	J37	James R.	S	L
6 Nov. 1975	MJ26	James R.	S	L
Totals 44 cases in 16 years			15R 29S	14G 30L

free oysters to enzootic areas has permitted definition of infection periods and incubation periods, but the causes and criteria for activating these phenomena are still obscure. The patterns in SSO suggest an annual cycle with a long incubation period being normal. Perhaps MSX in acclimated hosts would also have an annual cycle with sporulation at the time of enzootic kills. It seems to be epizootic and out of control on the mid-Atlantic coast with high virulence and consequently disrupted life-cycle patterns.

What kind of infection source could infect thousands of tiny spat confined and crowded in one tray in the open waters of York River almost simultaneously? Obviously the infective particles had to be abundant and ubiquitous in the area, even if only a few are required to establish an infection. Annual infections of MSX have been persistent in Chesapeake Bay although late-summer ones have failed some years. There was no time for fouling organisms to build up on the tray. Since each spat must filter the infective particles out of the water, it seems hardly likely that an alternate host, such as a blue crab defecating on the tray, was a potential source. If there is an alternate host, the infective particles are waterborne for long distances and the dilution factor must be staggering.

Direct Infections VS Alternate Hosts

The rapid spread of MSX from a localized center in Mobjack Bay in 1959 to all high-salinity areas of lower Chesapeake Bay in 1960 suggests direct transmission from these dying, infected oysters. The alternative explanation for such a rapid expansion of distribution almost requires equally rapid spread of an alternate host. This implies a host new to Chesapeake Bay or an endemic species newly parasitized by MSX without serious reduction of numbers. There has been no evidence of a newly imported exotic species to fill this role. Is it reasonable to assume that mutation of MSX in an endemic host, not requiring oysters as alternate hosts, provided the impetus for epizootics in Delaware Bay and Chesapeake Bay oysters? A strain of *Minchinia nelsoni* was present in Virginia as early as 1953 but with low virulence (Andrews, 1968). From present evidence, I conclude that MSX was introduced with exotic oysters, first into Delaware Bay, and that it spread 2 years later into Chesapeake Bay. If MSX was introduced in oysters, it is unlikely that an alternate or other host is involved. Failure to achieve artificial infections seems to be the major impetus for speculations about other hosts. Scarcity of oysters in high-salinity areas that could provide infective particles is another problem to be explained. The ready achievement of resistant strains of oysters to MSX in laboratory and field populations suggests that oysters can adapt to haplosporidans in a few decades if mutations and exotic strains are excepted and excluded. Direct studies of virulence and life cycle are precluded until artificial infections can be attained. Availability of spores makes SSO the preferred species to use in infection experiments. Manipulation of environmental factors should reveal what criteria favor sporulation of SSO.

[Note added in proof. The name *Dermocystidium marinum* has been changed to *Perkinsus marinus* by Levine (1978) since preparation of this manuscript.]

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