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Epizootiology of the Disease Caused by the Oyster Pathogen *Perkinsus marinus* and Its Effects on the Oyster Industry

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Abstract.—Perkinsus marinus is a protozoan parasite that causes a major disease of eastern oysters *Crassostrea virginica* from Chesapeake Bay south along the Atlantic coast of the USA and throughout the Gulf of Mexico. It is a warm-season disease that kills eastern oysters at temperatures above 20°C. The pathogen requires salinities of at least 12–15‰ to be active, but it persists tenaciously when low temperatures and salinities occur during winter and spring. Prolonged droughts that increase salinities cause extensions of the range of disease. In the Chesapeake Bay, mortalities begin in June and end in October, and up to 50% of native susceptible eastern oysters are killed each year. Most infections are acquired by eastern oysters in proximity to disintegrating dead eastern oysters. Massive populations of prezoosporangia are released into marine waters and eventually produce thousands of zoospores, which are infective when ingested by eastern oysters. The disease is controlled by isolating new beds from infected eastern oysters and by early harvest before the pathogen becomes established. Seed areas in low-salinity waters usually provide disease-free eastern oysters, but beds must be monitored regularly to avoid transplanting infected eastern oysters. In the Gulf of Mexico where warm temperatures persist through much of the year, control is much more difficult. Eradication is difficult unless introductions of infected eastern oysters are avoided and summer warm seasons are short and relatively cool to prevent the pathogen from multiplying in eastern oysters. The presence of *P. marinus* in Chesapeake Bay has been monitored for 37 years; the disease is established in most eastern oyster-growing areas of Virginia and in many tributaries of Maryland. The disease thrives on densely planted private beds of eastern oysters but persists through wet periods of weather on sparsely populated public beds and artificial structures along shores where there is recruitment of eastern oysters. Unfavorable temperatures essentially eradicated it in Delaware Bay after importations of infected seed eastern oysters from Virginia were discontinued.

Extensive losses of eastern oysters *Crassostrea virginica* due to disease occur in the Gulf of Mexico and Chesapeake Bay, USA (Andrews and Hewatt 1957; Mackin 1962). Extensive mortality and prevalence data from eastern oyster beds and intensively monitored trays of eastern oysters substantiate these losses. Management methods for control and avoidance of disease caused by the protozoan *Perkinsus marinus* were developed long ago (Andrews 1966, 1979), but oystermen and regulatory agencies are not applying these practices. Harvesting and transplanting policies are restated in this paper together with recommendations for laborious, but necessary, seasonal testing of seed and market eastern oysters of individual beds. Accurate histories of beds are required for proper analysis of management techniques. A summary of the epizootiology of the disease was given by Andrews (1976a).

Perkinsus marinus was the dominant pathogen of eastern oysters in Chesapeake Bay during the 1950s. In 1959, a highly pathogenic parasite, *Haplosporidium nelsoni* (MSX) (Haskin et al. 1965), was apparently introduced into Chesapeake Bay (Andrews and Wood 1967). It spread rapidly in 1960 and caused high mortalities that stopped all eastern oyster culture where salinities exceeded 20‰ in late summer (Andrews and Frïerman 1974). The rapid decline of eastern oyster populations throughout lower Chesapeake Bay caused *P. marinus* to decline in activity in high-salinity areas where eastern oysters were no longer planted. To show the interactions between the two diseases, new prevalence and mortality data are presented for periods before and after the new disease became enzootic. The contrasts in parasitic activities and methods of control of these two eastern oyster pathogens provided many interesting epizootiological studies (Mackin 1962; Andrews 1965, 1967). The consequences for the eastern oyster industries are discussed; 27 years after the invasion of MSX in Chesapeake Bay, beds in high-salinity areas have not been replanted, and private culture continues to decline, partly from fear of these two diseases. In years when MSX abates, *P. marinus* increases in importance.

The stages of *P. marinus* are well known (Mackin and Boswell 1956; Perkins 1976, 1988, this volume), but their functions in the life cycle are unclear. Zoospores develop in sporangia outside of eastern oysters, yet they have not been seen in stained tissues of the hosts. They may be the major stage for infection in nature particularly from distant sources. Confusion persists in the names of stages seen in eastern oysters and during thioglycollate tests of tissues for diagnostic purposes. Now that the taxonomic affinity of *P. marinus* is firmly established in the phylum Apicomplexa (Perkins 1988), clarification of the names and functions of the life cycle stages may be expected soon.

History of Disease Research

The disease caused by *P. marinus* was discovered in the Gulf of Mexico in 1948 (Mackin et al. 1950). For 30 years it was called *Dermocystidium marinum* in the belief that it had fungal affinities. The signet-ring stage seen most commonly in eastern oysters, called trophozoites or prezoosporangia (aplanospores of Mackin 1962 and Lauckner 1983), resemble funguslike protistans parasitic on freshwater vertebrates. These vegetative cells have a large vacuole with a dense inclusion and a peripheral nucleus, and they multiply in eastern oysters by successive division into multinucleated clusters of trophozoites. The discovery by electron microscopy of the production of biflagellated zoospores with organelles called apical complexes (Perkins and Menzel 1966; Perkins 1976) led to the recognition of the true affinity of *P. marinus* to Apicomplexa (Levine 1978). A recent review of the epizootiology of this warm-season pathogen and its new classification was given by Lauckner (1983).

The search for the causes of eastern oyster mortalities in the Gulf of Mexico began in 1948 after oystermen filed damage suits for millions of dollars against oil companies (Mackin et al. 1950; Mackin and Sparks 1962). Four biologists, J. G. Mackin, S. H. Hopkins, R. W. Menzel, and H. M. Owen, who worked at the Virginia Fisheries Laboratory, Gloucester Point, during World War II, organized extensive literature and field researches in Louisiana. The Texas A&M Research Foundation, College Station, which was supported financially by some oil companies, published many reports on purported disease agents and on literature searches (see Mackin 1962 for list). Mackin and his associates came to Virginia during the summer of 1949 and found *P. marinus* in eastern oysters growing in environments away from oil fields. Consequently, another associate, W. G. Hewatt of Texas Christian University, Fort Worth, came to Virginia during the summer of 1950 to initiate eastern oyster mortality studies by the tray method (Hewatt and Andrews 1954). The first trays were established in June 1950 at the Virginia Institute of Marine Science (VIMS) pier, and *P. marinus* has been monitored continuously since 1950 (Andrews 1980). Many thousands of eastern oyster tissues were sectioned or tested for diseases both in the Gulf of Mexico (Mackin and Sparks 1962) and in Virginia, and these regions became centers for disease studies. Stained tissue sections of gapers (dying eastern oysters) and live animals amounted to 170,000 at VIMS by 1983 when I retired.

The researchers in the Gulf of Mexico encountered two problems in their studies of *P. marinus* (Mackin 1953). First, the pathogen was so widespread geographically in the Gulf of Mexico that disease-free eastern oysters had to be obtained from New England for their experiments. In Virginia, large, annual freshwater discharges in rivers with large drainage areas, such as James River, provided disease-free oysters from low-salinity areas around the year. Also, the diagnosis of disease was difficult, and few eastern oysters had been sectioned anywhere in the U.S. because the process was expensive and tedious. Furthermore, the usefulness of stained tissues was limited to advanced infections because only they could be diagnosed accurately for incidence and intensity.

In 1952, S. M. Ray attempted to culture *P. marinus* at Rice Institute, Houston, Texas, and discovered the thioglycollate test, which is a sensitive method for diagnosis and for rating the intensity of infection (Ray 1952, 1954a, 1966). Use of this growth medium causes prezoosporangia to enlarge and develop walls that stain blue-black when Lugol's iodine solution is added. Mackin (1962) devised a rating system that combines prevalence and intensity of infections into a single expression, weighted incidence (WI). Values of one, three, and five are assigned to light, moderate, and heavy infections, respectively (Ray 1954a), and the total divided by the number of eastern oysters gives an average. A value of 0.5 signifies mostly light infections with little or no mortality; 1.0 means generally light infections, but some cases will be severe, and some mortality may occur; a value of 1.5 or higher for live hosts indicates that most eastern oysters are infected, and those with severe cases die. Deaths tend to keep WI from rising much higher than 1.5 in live

eastern oysters, but gapers typically exhibit values around 4.0 when 80–90% of eastern oyster deaths are caused by *P. marinus*. These values are useful for predicting the extent of mortalities, when they are combined with the date of sampling and the prevailing water temperatures. The larger the number sampled, the more accurate WI becomes; samples of 25 live eastern oysters were routinely taken at VIMS. Gapers are difficult to collect on eastern oyster beds in summer because crabs, worms, and small fishes regularly remove meats in about 1 d after eastern oysters gape. A much higher proportion of gapers can be obtained during winter, when scavengers are dormant, or by searching trays daily as I did during warm seasons of the 1950s at VIMS (Andrews 1980).

Infection experiments began in the Gulf of Mexico and in Virginia as soon as Ray's (1952) thioglycollate test was available. The maceration of infected gapers in a blender provided infective inoculant that was injected into the mantle cavity through holes bored in the shells of eastern oysters or that was fed to eastern oysters in closed aquaria (Ray 1954a; Andrews and Hewatt 1957). Numerous infection experiments conducted during the 1950s were summarized by Ray (1954a), Ray and Chandler (1955), and Mackin (1962).

Distribution of *Perkinsus marinus*

P. marinus ranges from Tampico Bay, Mexico, along the southeastern coast of the U.S. to Delaware Bay (Mackin 1962; Andrews 1976b, 1979). Delaware Bay is now believed to be free of the disease it causes owing to an embargo placed on imports of eastern oysters from more southern areas. In the Gulf of Mexico, the distribution of the disease includes all coastal areas (Quick and Mackin 1971; Andrews and Ray 1988, this volume). The gulf coast is subject to wide annual and seasonal variations in rainfall with corresponding fluctuations in salinities, but few areas retain salinities low enough to preclude *P. marinus* from persisting during wet periods and thriving during dry ones (Mackin and Hopkins 1962; Andrews and Ray 1988).

Northward along the Atlantic coast, most estuaries or bays where eastern oysters are grown have high salinities until Chesapeake Bay is reached. *Perkinsus marinus* is prevalent in most areas including intertidal beds. South Carolina eastern oysters exhibited considerable resistance to the disease in Chesapeake Bay (Andrews and McHugh 1956). However, the seaside bays of the eastern shore of Virginia are free of *P. marinus* for unexplained reasons. This absence of *P. marinus* is usually true of Chincoteague Bay, which is mostly in Maryland (Andrews 1980).

In Chesapeake Bay (Figure 1), the pathogen is firmly established in the lower bay, all rivers in Virginia, Pocomoke Sound, and northward into Maryland as far as the St. Marys River, Choptank River, Patuxent River, and Eastern Bay (Andrews and Hewatt 1957; Andrews 1980; S. Otto, Maryland Department of Fisheries, unpublished data). The upper parts of major rivers, including the James, Rappahannock, and Potomac rivers, are free of the disease because large discharges of fresh water during winter and spring reduce the summer period of favorable salinities for *P. marinus*. In contrast, the smaller rivers with only coastal plain drainage areas are dominated by bay salinities which are often favorable to the pathogen. These small estuaries include the Great Wicomico, St. Marys River on the Potomac River, Choptank River, and all small tributaries in lower Maryland draining from eastern shore land. *Perkinsus marinus* activity fluctuates up and down with dry and wet years in these waters that have marginal salinities for the maintenance of the disease (Andrews 1980). There was a major extension of the disease throughout Chesapeake Bay during the prolonged drought of 1985–1987, including all seed areas and much of the James River seed area. Flushing and dilution of infective particles tend to limit infections to local beds where recruitment of eastern oysters is regular; therefore, large flushing-type rivers are not as favorable to the pathogen as small, shallow coastal plains tributaries. However, this normal slow pattern of spread changed during the recent droughts to one of rapid spread from bed to bed and area to area as the disease invaded more densely populated seed areas, probably because of an increase in abundance of infective stages. In Delaware Bay, the disease disappeared from commercial beds a few years after importations of infected eastern oysters from Virginia ceased.

During the 1980s, three winter–spring droughts (1981, 1985, and 1986) in the Chesapeake Bay drainage area were followed by dry, warm summers that caused exceptionally high mortalities attributed mostly to *P. marinus*. The successive dry years of 1985–1987 caused severe losses mostly attributable to *P. marinus* throughout Virginia, and few surviving eastern oysters were left on public or private beds for harvest or broodstock. Since the advent in 1959 of the disease caused by MSX in Chesapeake Bay (Andrews and

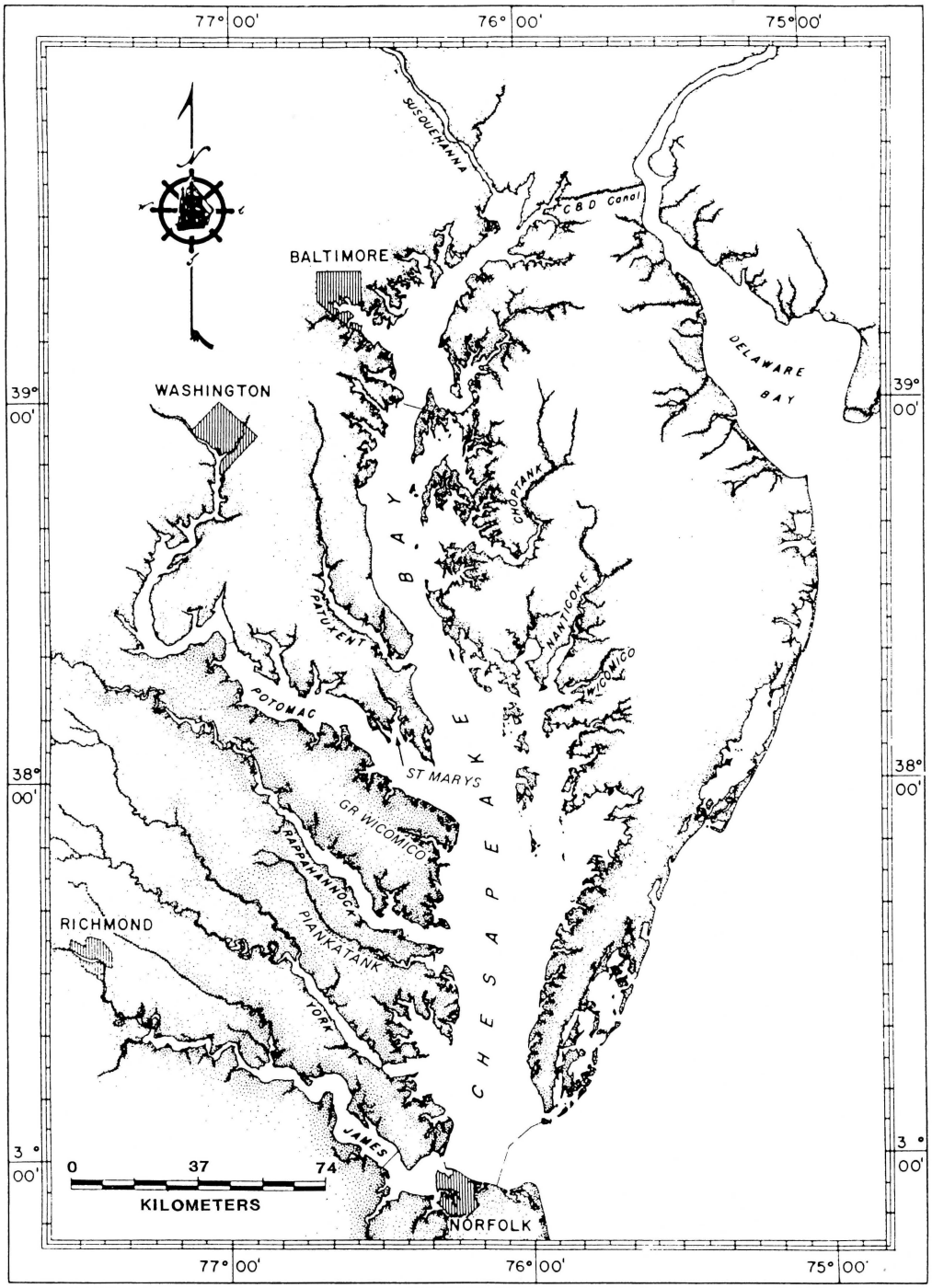

FIGURE 1.—Map showing major tributaries of the Chesapeake Bay region, Maryland–Virginia, USA, where *Perkinsus marinus* is enzootic. The upper ends of the large rivers of the bay are still free of the disease despite a great increase in abundance and a wider distribution of the parasite during the two drought periods of the 1980s.

Wood 1967), eastern oysters are grown only in areas where summer salinities do not exceed 20‰. However, even these low-salinity areas (<10‰ in spring, which allows eastern oysters to expel MSX) are not safe in Chesapeake Bay from either disease during drought periods. Mortalities have been so severe in Virginia that during the 1986–1987 and 1987–1988 eastern oyster harvests, Virginia tongers worked in the James River seed area catching small eastern oysters (sold as seed in 1985–1986 for $3/bushel) for market shucking at $12–$22/bushel (1 bushel = 35.2 L). Unfortunately, *Perkinsus*-infected eastern oysters from the lower section of the seed area were transplanted to private beds in the Machodoc and the Yeocomico rivers (Potomac tributaries usually of low salinity). This transplantation resulted in heavy mortalities from *Perkinsus* disease during summer and fall of 1986 and 1987 (E. M. Burreson, VIMS, personal communication). Several wet or normal years of fallowing these beds may eradicate the pathogen. Transplanting infected seed eastern oysters is the most rapid and frequent method by which *Perkinsus* disease is spread.

Host Species of *Perkinsus marinus*

Besides *Crassostrea virginica, P. marinus* infects *Dendostrea frons* and *Ostreola equestris,* which live offshore in the Gulf of Mexico (Ray and Chandler 1955). Uzmann (U.S. Fish and Wildlife Service) at Milford, Connecticut, transmitted it to *Ostreola conchaphila* (Ray 1954a). Probably, *Perkinsus* will infect almost any species of oyster because dosage is so intensive, but the degree of pathogenicity remains to be determined. The persistent effort to import *Crassostrea gigas* to New England and Canada suggests that testing of susceptibility of that species and of *Ostrea edulis,* grown in Maine, should be conducted.

Perkinsus marinus, as known in southeastern USA, provides no threat to eastern oyster culture on temperate coasts with oceanic-type climates, such as western Europe and western North America. It requires temperatures of 20°C or higher to multiply in eastern oysters; below this temperature eastern oysters expel the pathogen. Eastern oysters infected with *P. marinus* were introduced into Hawaiian waters and caused mortalities (Kern et al. 1973). *Perkinsus marinus* was also reported from Adriatic waters in the Mediterranean area (Da Ros and Canzonier 1985).

The occurrence of *Perkinsus*-like cells in a large variety of other bivalve molluscs, some scavengers such as mud crabs (xanthids), and nereid worms has caused much speculation about new species or alternate hosts (Ray 1954b). Andrews (1955) found these cells in 12 of 16 bivalves tested from the York River. Often, all specimens in samples of 25 bivalves had a few iodine-stained cells, and these cells persisted in some species for long periods without evidence of pathogenicity or seasonality. Most other bivalves were tested in 1954, a year of intensive culture of eastern oysters in the York River and of severe *Perkinsus* mortalities. The large quantity of prezoosporangia released by eastern oyster gapers plus the estimated 1,000–2,000 zoospores produced per large sporangium (Perkins 1976) make it likely that scavengers and filter-feeding organisms would encounter *P. marinus* stages. Yet these organisms do not appear to be suitable hosts or reservoirs (Ray 1954a). Because the *Perkinsus*-like parasite in *Macoma balthica* was abundant and multiplying (Valiulis and Mackin 1969), it was described as a new species (Mackin and Ray 1966).

The series of labyrinthulid species described by Mackin and Ray (1966) were probably contaminants and not *P. marinus*. There may be races of *P. marinus* as well as races of eastern oysters along the Atlantic coast, but they have not been satisfactorily defined. Growth patterns and resistance to diseases are measurable genetic traits of eastern oysters that have been observed to vary from one region to another (Andrews 1968; Haskin and Ford 1979). Other interactions between hosts and pathogens can be expected to vary accordingly.

Epizootiology of the Disease Caused by *Perkinsus marinus*

Life Cycle and Regulatory Factors

The two most important environmental factors regulating the life cycle of *P. marinus* are temperature and salinity (Mackin and Boswell 1956). These factors have been extensively studied in the Gulf of Mexico and in Virginia (Hewatt and Andrews 1956; Mackin 1956; Andrews and Hewatt 1957; Mackin and Hopkins 1962). This pathogen causes a warm-season disease, although obscure infections (few cases or of low intensity) persist through winter at water temperatures of 0–5°C. It also survives winter salinities less than 5‰ although 12–15‰ are required for multiplication in eastern oysters (Andrews and Hewatt 1957). Seasonal and annual variations of temperature and salinity between the Gulf of Mexico and Chesapeake Bay cause important differences in the

respective seasonal cycles of the disease (Mackin 1956; Andrews 1980).

In Virginia, *P. marinus* begins its annual cycle when overwintering infections, not easily disclosed by thioglycollate tests, begin multiplying actively in June at temperatures above 20°C. These overwintering infections are difficult to monitor, and much more study of them is needed. From January through May, thioglycollate tests show only rare cases of infection in eastern oysters which had up to 100% infection the previous October. An exception occurred in 1987 when 80–90% of eastern oysters carried infections through the winter and began development in June (Burreson, personal communication). If such eastern oysters are placed in heated aquaria (30°C) in spring, a few infections develop, and these eastern oysters begin dying in about 1 month. Because live eastern oysters may discharge infective stages and gapers may deteriorate before their removal, satisfactory testing for overwintering cases requires isolation of each eastern oyster in warm water. Most eastern oysters with severe infections do not survive winter conditions, and most other infections are rare or nonclinical by late December when winter temperatures occur. There is no reason to expect undetected stages, but how the pathogen overwinters and how abundant it is are unknown. Advanced infections typically persist during early winter when most light cases have disappeared. Eastern oysters with advanced cases fail to recover from the disease in late fall, and most appear to die during the winter stress period. The rare survivors of advanced cases may be carriers of overwintering infections. Overwintering of the disease requires more study. The few eastern oysters with overwintering cases develop severe infections by late July or early August, and their deaths initiate a second generation of disease which is fatal in late August or early September. Often, all acclimated eastern oysters (having spent 1 or 2 years in an endemic area) in crowded trays have *Perkinsus* infections by 1 November of the second or third year. When the critical water temperature decreases to 20°C, deaths cease. The physiological balance between host and pathogen is altered in favor of the host, and most light infections are eliminated by mid-December. The persistence of infections through winter and the extent of survival are not clear.

The weather in September and October is a critical factor that determines the mortality level during late summer and fall. If water temperatures remain high, more infections develop into lethal ones. The pathogen multiplies fastest at temperatures between 25 and 30°C which persist for 3 months or longer during Virginia summers and much longer in the Gulf of Mexico.

In Virginia, eastern oyster planters typically obtain disease-free oysters from low-salinity seed areas which are transplanted in fall and winter. If growing beds are cleaned of old eastern oysters or fallowed 1–2 years to allow them to die, local infection sources are eliminated, and few if any infections are acquired from distant sources the first summer of culture. Under these conditions, mortality is low. If infections are acquired from local foci during the first summer in endemic areas, they cause deaths in late August or September; therefore, second-generation infections are too late to become serious before temperatures decline. If overwintering infections occur from the first summer of exposure, they become clinical the following June, and first deaths occur in July or early August during the second year. Prevalences and intensities increase rapidly with each additional generation; therefore, mortalities are highest in September and October.

In the Gulf of Mexico, winter temperatures often remain high enough for most infections to persist as clinical cases throughout the winter, although intensities usually decline (Mackin 1953). Infection cycles begin earlier and continue longer with higher mortalities there than in Chesapeake Bay. Planters there have learned to obtain large seed eastern oysters which are planted after summer temperatures decline and harvested before the next summer when pathogen activity exacts a heavy toll.

Salinity is almost as critical a factor as temperature in Chesapeake Bay; *P. marinus* tolerates quite low salinities during the cold season, although in normal years, most eastern oysters overcome patent infections in late fall. A large sector of the lower bay and adjacent tributaries provide adequate salinities (above 12–15‰) throughout warm seasons during all years. Nearly all areas where eastern oysters are grown in Virginia and many tributaries in lower Maryland provide adequate salinities during late summer; if they remain below 15‰ during early summer, few deaths occur, and disintegrating gapers do not materialize to provide high infective dosages. The cycles of infection and mortality are delayed, and late infections do not achieve lethal levels because fall temperatures stop multiplication of the pathogen. In the Gulf of Mexico, even wider fluctuations of salinity that occur seasonally and

annually cause critical conditions for control and management of the disease.

Transmission of Perkinsus marinus

In the Gulf of Mexico, many infection experiments were conducted in aquaria and in trays in open waters, as were reviewed by Mackin (1962). For the most part, experiments in Virginia confirmed results obtained in the Gulf of Mexico (Andrews and Hewatt 1957). The proximity studies of Ray (1954a) and Andrews (1965, 1967) were attempts to assess dosage and timing of infections. Mackin (1962) found that a dose of 500 prezoosporangia was required to produce substantial mortality from *P. marinus* infections. Increasing the dosage to 5×10^5 decreased the lag time before infected eastern oysters died. Pathogen cells were cultured in thioglycollate medium for 24 h or more before injection to enlarge them for staining and easy counting and to stop reproduction. M. H. Roberts (VIMS, personal communication) found that 1×10^5 zoospores were required to induce infections. These dosages may be comparable if each sporangium produces 1,000–2,000 zoospores (Perkins 1976). This conclusion does not imply that continuous smaller dosages may not induce infections over a period of time. With high dosages, serious infections are produced in 3 or 4 weeks (Roberts, personal communication). In vitro production of zoospores from prezoosporangia enlarged in thioglycollate culture occurs regularly in seawater outside of eastern oysters. After 1 or 2 d in thioglycollate culture, zoosporangia take 4–5 d to produce zoospores at warm temperatures; the process is temperature dependent (F.-L. E. Chu, VIMS, personal communication). What happens inside live eastern oysters when they are injected with macerated tissues from infected gapers is unclear. The 3- to 4-μm zoospores have not been seen in stained tissues; however, they may be easily overlooked because of their small size.

Infection occurs typically through the digestive tract as indicated by the location of foci of infection in sectioned live eastern oysters (Mackin 1951). Epithelial cells of the digestive tubules and hemocytes phagocytize the pathogens and probably facilitate transport to connective tissues and to blood sinuses through which the disease is spread to all tissues of the body. Multiplication in tissues is rapid by successive fissions of trophozooites (aplanospores of Mackin 1962 and Lauckner 1983) in clusters or clumps which separate to become typical pathogen cells engulfed by hemocytes. At temperatures of 25–30°C, the parasite develops rapidly; occlusion of blood sinuses and lysis of tissues cause death in about 1 month. The density of pathogen cells is great upon death of eastern oysters. Deaths are hastened among eastern oysters that are near disintegrating infected gapers; this phenomenon was observed in tray studies where positions of eastern oysters were fixed. Infections from remote foci may occur but probably develop more slowly in normal years (Mackin 1962). Studies to determine the relationship between infective dosage, distance from source, and rate of expulsion by eastern oysters at various temperatures are urgently needed to determine effective isolation distances for commercial plantings. The massive spread of *P. marinus* throughout Chesapeake Bay during the 1980–1982 and 1985–1987 droughts provided excellent opportunities to study the rapid, long-range transmission of the disease in nature. Experiments with trays and aquaria, which are necessarily point sources of infective particles, and fixed times of exposure probably do not simulate all conditions in natural waters.

In nature, dosage is difficult to estimate. Long experience with tray eastern oysters that are separated various distances to avoid *P. marinus* infection, provides some conclusions on the effectiveness of isolation. For 20 years, about 60 trays of eastern oysters were monitored annually for diseases at Gloucester Point, Virginia, on an old public eastern oyster bed in the York River. The legged trays were set by stakes about 15 m apart in three rows. New lots of disease-free eastern oysters were imported each year from low-salinity areas of the James River. All ages of eastern oysters from spat to 10-year-old survivors of diseases were monitored. After the second or third year at this enzootic site, most tray lots acquired *P. marinus* infections, and deaths rapidly depleted the stocks.

Eastern oysters were routinely grown to market size during 1 or 2 years after importation without serious losses due to the pathogen. Thousands of gapers and frequent live eastern oyster samples were tested by thioglycollate culture to confirm causes of deaths. Some native eastern oysters on the bottom harbored *P. marinus*, and older infected tray lots were interspersed with new importations. Yet, distances of 15 m between trays prevented or delayed infection for 2 years while data on MSX were being collected.

During the summer of 1957, a proximity (or isolation) experiment was conducted in open waters near Gloucester Point on bottom that was free of eastern oysters. One control and one experi-

TABLE 1.—Proximity experiment in nature near Gloucester Point, Virginia, USA, with disease-free eastern oysters held on sandy bottom in trays 15 m apart to observe transmission of *Perkinsus marinus*. Eastern oysters infected in the laboratory were added to the experimental trays only. Monthly observations of mortality were made 8 July–4 November 1957; then samples of live eastern oysters were taken to determine prevalence and intensity of the disease. Intensity ratings: H = heavy, M = moderate, L = light, and N = no infection.

Group	Number tested in group	Frequency (%) of infections by intensity				Weighted incidence[a]	Mortality 8 Jul–4 Nov (%)
		H	M	L	N		
Control							
1	25	0	4	16	80	0.28	5.6
2	25	0	4	20	76	0.32	
Experimental							
1	25	4	12	52	32	1.08	26.9
2	25	8	12	64	16	1.40	
Laboratory-infected[b]							
1	14	0	64	36	0	2.29	32.7
2	19	0	37	63	0	1.74	

[a]Weighted incidence combines intensity and incidence ratings by assigning values of 5, 3, and 1 to heavy, moderate, and light infections, respectively, and dividing the total by the number of eastern oysters rated.

[b]Laboratory-infected eastern oysters were marked with paint to distinguish them from originally disease-free experimental animals in the same trays.

mental tray of disease-free eastern oysters were spaced 15 m apart. Each tray had a divider in the middle to segregate two groups of animals. Forty-nine eastern oysters exposed to *P. marinus* infection in aquaria were added to the experimental tray, some to each group, to induce infections by proximity. The trays were examined monthly to record mortalities (Table 1). The control tray had low prevalences and low mortality when live eastern oysters were sampled in November. The experimental groups exhibited moderate values for prevalences, mortalities, and weighted incidences. The survivors among the laboratory-infected eastern oysters had higher values for all three measures of disease activity. The timing of the experiment was a little late for the pathogen to cause first-generation mortalities in nature, but the effects of the pathogen's proximity to, and isolation (by 15 m) from, potential hosts were demonstrated clearly.

An infection experiment in open waters was conducted at Gloucester Point in 1965 at the peak of a 3-year drought that favored *P. marinus* even in low-salinity areas where most eastern oysters were being grown (Andrews 1967). Four trays were spaced 5 m apart on sandy bottom with 400 disease-free eastern oysters in each tray and separated into two lots by a divider board. Eastern oysters marked with paint were fed tissue from infected gapers that was macerated in a blender; then 2, 10, or 61 of these eastern oysters were added on 18 June to one end of three trays, respectively. The central tray was kept as a control. Eastern oysters infected with both MSX and *P. marinus* were found in all compartments, and mortality was high. Incidence and intensity of *P. marinus* infection increased in proportion to the number of paint-marked eastern oysters introduced, as did total mortality. Most early deaths were caused by MSX, but by late August, *P. marinus* began killing about 0.5 of the dying eastern oysters, as gapers collected by scuba divers showed. Two additional trays of disease-free eastern oysters, from the same James River collection, placed 100 m offshore on a barren, sandy bottom, had only 1 of 50 eastern oysters infected with *P. marinus*. Remote outside sources of infection probably did not contribute to this locally induced epizootic. This experiment provided evidence that transmission of *P. marinus* tends to be localized and that isolation of beds is a useful strategy for controlling the disease during normal years.

The distances required between beds of eastern oysters to prevent epizootic mortalities by *P. marinus* are not known. Extensive sampling for 37 years has revealed that most public beds, where some recruitment occurs, are major reservoirs of infected eastern oysters. Overharvesting and reduction of setting rates have greatly depleted and thinned stocks on public beds in Virginia in recent decades; yet the disease persists. Piers and pilings also serve as foci of infection if any eastern oysters are attached. In small rivers where eastern oyster beds are crowded near the shore, these structures can be a continuous source of infection. *Perkinsus marinus* will never be eradicated from estuaries with marginal salinities because the disease can persist with few or no deaths for

several years until the next dry period. Low temperatures do not extirpate the disease either.

In Virginia, young eastern oysters (2 years old) usually do not acquire *P. marinus* infections until their second summer in an endemic area (Ray 1954b; Andrews and McHugh 1956). If dosage is high, eastern oysters of any size or age can become infected (Andrews and Hewatt 1957). It is not clear whether small eastern oysters do not collect enough infective stages or their rapid metabolism permits expulsion of the pathogen more rapidly. Most eastern oysters can overcome patent infections when temperatures are too low for the pathogen to multiply. Why overwintering infections are not expelled too is a mystery. Mackin (1962) believed that sporangia overwintered on the bottom and released infective stages in early summer. If this were true, eastern oysters imported from low-salinity areas in spring should develop infections in June and July rather than in late July or early August when eastern oysters with overwintering infections die. Because transmission is direct from one eastern oyster to another, proximity usually is required to ensure the large dosage required to establish infection.

A host of scavengers live on eastern oyster beds to feed on oysters killed by predators or disease. Blue crabs and mud crabs (xanthids) kill small eastern oysters, and nereid worms, spider crabs, and several small fishes, such as blennies, gobies, and clingfishes, are also quick to snatch bits of flesh out of gaping oysters. Recently, *Boonea impressa* has been added to the list of organisms found to have *Perkinsus*-type cells in their tissues (White et al. 1987). This finding is not unexpected, because these ectoparasitic snails puncture the edge of eastern oyster mantles to suck juices. The role that any of these scavengers has as a source of infection or as a reservoir for *Perkinsus* disease remains to be shown. Probably, scavengers cannot make major contributions to the high dosage necessary to produce infections.

Prevalence and Mortality Data

The clearest data on prevalences and deaths caused by *P. marinus* in Virginia were collected during the 1950s before MSX caused epizootic mortalities in Chesapeake Bay. Many private and public beds were sampled during the 1950s for thioglycollate tests, but box counts provided unsatisfactory mortality data because it was difficult to determine the period during which eastern oysters died. In contrast, tray studies provided detailed and accurate data on prevalences and deaths. Furthermore, it was possible to select disease-free stocks and to transplant them to enzootic areas at optimal times for determining infection periods and duration of morbidity.

TABLE 2.—Average annual mortalities in 3–10 trays of disease-free eastern oysters imported from nonenzootic areas to Virginia Institute of Marine Science pier, Gloucester Point, each spring to monitor mortality and prevalence of *Perkinsus marinus* infections in gapers during the 1950s.

Year	Mortality (%)	Number dead	Number of gapers tested	Infection in gapers (%)
1952	25	56	33	94
1953	35	275	251	87
1954	56	675	625	92
1955	37	455	383	87
1956	24	191	143	71
1957	43	103	49	88
1958	29	155	96	78
1959	59	241	129	84
Average	38.5			85.1

The pier at VIMS was selected as the site for *P. marinus* disease studies for convenience in examining trays and collecting dying eastern oysters or gapers. Sea-Rac trays (Chesapeake Corporation, West Point, Virginia) were suspended from three catwalks to hold about 100 market-size eastern oysters. Water depth was about 1.5 m at low tide. Many trays were examined daily throughout the warm seasons for 8 years during the 1950s to recover gapers. Trays of eastern oysters exposed in an enzootic area for one or more summer seasons and newly transplanted disease-free eastern oysters were suspended adjacent to each other. This arrangement provided an optimal density and proximity of eastern oyster groups for *P. marinus* to flourish. This situation was believed to approximate regularly planted eastern oyster beds with some old survivors and plenty of new susceptible eastern oysters. During these years, *P. marinus* caused 80–90% of all deaths in the trays (Andrews and Hewatt 1957). The ratio was always lower on commercially planted beds where smothering and dredge injuries caused some deaths.

Total annual mortality varied from 24 to 59% during 8 years of tray monitoring at the VIMS pier. Average annual mortalities in groups of 3–10 trays of eastern oysters are given in Table 2; this summary excludes lots in their first year of exposure in an enzootic area. Remote sources of infection were not necessary to keep *Perkinsus* epizootics active at the VIMS pier because 85% of the gapers had serious infections (mostly heavy, but some moderate). The two worst mor-

talities (1954 and 1959) were caused by early warm water temperatures in late spring and high temperatures that continued into September and October (Andrews 1980).

Seasonal prevalence and intensity data for live eastern oysters in 1954 are shown in Figure 2. Eastern oysters from three sources in two rivers were sampled monthly for thioglycollate tests. All lots were exposed to *P. marinus* infection in enzootic areas for at least 1 year before the 1954 sampling, and winter samples in 1953–54 showed that the parasite was established. Mortality in the VIMS pier lot (James River seed eastern oysters in trays) for the warm season was 51%, and 94% of the gapers were infected and had a WI of 4.85, an indication of nearly all heavy infections. At peak prevalence on 5 October, 96% of live eastern oysters had infections with a WI of 2.40 or an average intensity of nearly moderate infection. If warm waters had prevailed for another month, nearly all eastern oysters would have died. The apparent absence or low prevalence of the pathogen in late winter and early spring is typical for thioglycollate tests, because these tests do not detect overwintering infections; yet, the method is highly sensitive. Samples were taken near the first of every month shown, yet three winter–spring months showed no infections (Figure 2). Patent infections appeared early in 1954 with 16% infection on 1 June; this event allowed three generations of infections and deaths to occur before declining water temperatures stopped multiplication about 1 November. Heavy infections always remained in low numbers because deaths removed infected individuals continuously during late summer. Remission of infections was slower in late fall and winter than development which occurred during early summer, and often a few severely infected individuals persisted into winter before dying.

Native eastern oysters were sampled from Ferry pier pilings at Gloucester Point, Virginia, for 11 months through 1954 (Figure 2). These were 2-year-olds at a site where older eastern oysters were scarce, yet it is clear that they acquired infections during the summer of 1953 despite their small size as yearlings. Density of eastern oysters was much lower on the Ferry Pier where no trays of older, infected eastern oysters were located, yet overwintering of patent infections was more frequent than usually occurs in enzootic areas. Hoghouse Bar in the Rappahannock River is a public eastern oyster bed that usually has a sparse population of large, old eastern oysters and limited recruitment. Spring salinities at this site are

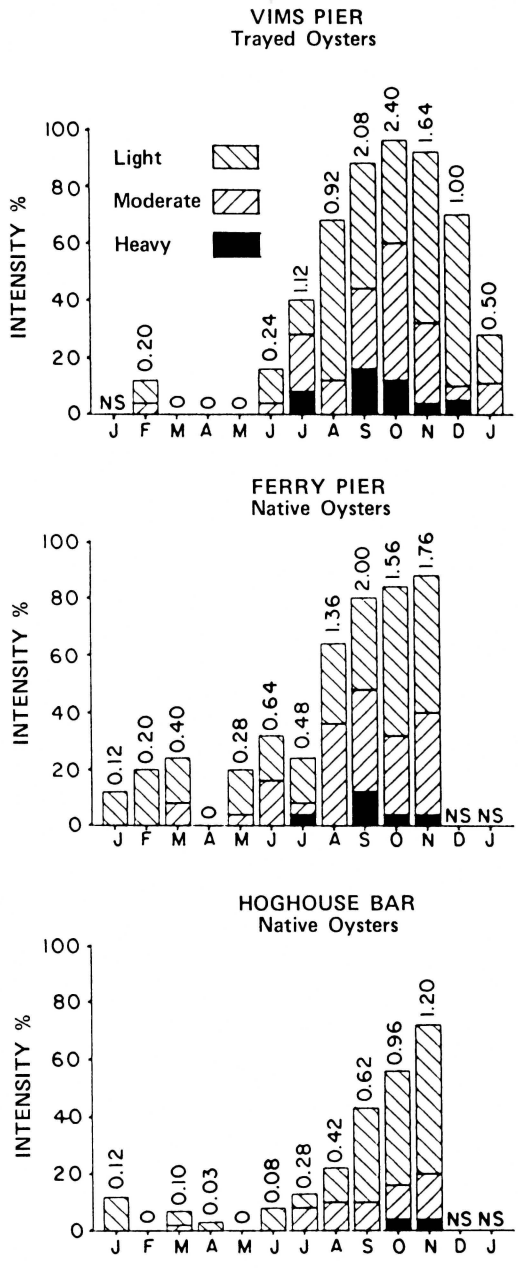

FIGURE 2.—Monthly intensities and prevalences of *Perkinsus marinus* infections in live Virginia eastern oysters during 1954 at three sites where the disease is enzootic. Note the frequent absence of the disease in samples of 25 eastern oysters during late winter and early spring. VIMS = Virginia Institute of Marine Science, York River, Gloucester Point. Ferry pier, York River, is at Gloucester Point. Hoghouse Bar, Rappahannock River, is near Towles Point. Categories of heavy, moderate, and light intensities as defined by Ray (1954a). NS = no samples.

TABLE 3.—Average monthly mortality in tray eastern oysters at Virginia Institute of Marine Science, Gloucester Point, and average monthly prevalence of serious infections (moderate and heavy) of *Perkinsus marinus* in gapers over eight pre-MSX years (1952–1959). Three to 10 trays were monitored each year. For histories of tray lots, see Hewatt and Andrews (1954) and Andrews and Hewatt (1957).

Month	Mortality (%)	Number dead	Number of gapers tested	Prevalence of serious infections (%)
Jan	0.4	22	19	26
Feb	0.4	20	15	13
Mar	0.6	35	24	4
Apr	0.4	27	11	0
May	1.4	78	35	14
Jun	2.1	86	75	39
Jul	6.8	351	262	87
Aug	13.6	723	631	94
Sep	13.1	539	443	97
Oct	6.4	311	171	96
Nov	1.1	29	22	96
Dec	0.9	17	9	89
Total	47.2	2,238	1,717	

often near the lower limit (12–15‰) for *P. marinus,* and development was delayed in 1954, which became a favorable year (high salinities and an extensive warm season) for the disease. The disease did not reach lethal intensities (average WI > 0.50 in a sample of 25 eastern oysters) until about 1 September; therefore, mortality was low for the summer (few boxes). *Perkinsus marinus* persisted for 37 years in the Rappahannock River despite the marginal spring salinities and several hurricane floods that reduced salinities to less than 10‰ for weeks or months (e.g., 1955 and 1972, Andrews 1973).

Disease in the Rappahannock River is typical of many small estuaries with coastal plain drainage basins which exhibited *P. marinus* mortalities mostly when dry years occurred over the Chesapeake Bay watershed. These estuaries include such systems as the Great Wicomico River, the lower Potomac River and its tributaries including St. Marys River (a former eastern oyster seed area now enzootic for *Perkinsus*), the Patuxent and Choptank rivers in Maryland, and Pocomoke Sound. Nether low temperatures nor low salinities will ever eradicate *Perkinsus* from these estuaries if experience during the past 37 years is indicative.

The monthly progression of *P. marinus* was monitored for eight years in 3–10 trays of eastern oysters at VIMS pier (Table 3). The average mortality was low in 8 months of the year. Over eight successive years of daily monitoring, 77% of all dead animals were recovered as gapers. The annual cycle of activity for the disease is shown in Table 3. From July through December, a high proportion of gapers had advanced infections, which indicates that *P. marinus* was the overwhelming cause of deaths in eastern oysters protected in trays from other adverse agents and events. During winter and early spring months when death rates were low (Table 3), the percentage of *Perkinsus*-infected eastern oysters declined (Table 4), but by late May and June, when temperatures were favorable for the pathogen, the level of intensity of the disease increased to begin a new warm season of epizootic mortality. During

TABLE 4.—Prevalence of *Perkinsus marinus* in winter gapers at Virginia Institute of Marine Science pier, Gloucester Point, for 3 years, 1952–1954. Infections were diagnosed by the thioglycollate test as heavy, moderate, light, and negative. Gapers were collected from 18 trays containing about 5,000 live eastern oysters.

Month	Number of gapers collected	Gapers infected (%)	Number of infected gapers by intensity				Infected gapers with heavy or moderate infection (%)
			Heavy	Moderate	Light	None	
Jan	15	53	1	4	3	7	62
Feb	13	31	1	1	2	9	50
Mar	9	44	0	1	3	5	25
Apr	9	33	0	1	2	6	33
May	23	44	3	1	6	13	40
Jun	52	63	17	8	8	19	76

TABLE 5.—First-year prevalence of *Perkinsus marinus* in gapers from trays of disease-free James River (Virginia, USA) eastern oysters transplanted to old eastern oyster beds to monitor MSX. Most dead eastern oysters were killed by MSX. All sites were enzootic for *Perkinsus* disease, but few eastern oysters were left on the beds.

Year	Location	Number of gapers tested	Perkinsus marinus infection (%)	Weighted incidence[a]
1966	James River[b]	54	24	0.80
	York River	381	3	0.13
	Mobjack Bay	156	1	0.06
	Rappahannock River	11	0	0.00
1967	James River[c]	85	3	0.08
	York River	529	4	0.08
	Mobjack Bay	114	0	0.00
	Rappahannock River	3	0	0.00
1968	James River[c]	70	0	0.00
	York River	477	7	0.19
	Mobjack Bay	92	0	0.00
	Rappahannock River	5	0	0.00

[a]Weighted incidence combines intensity and incidence ratings by assigning values of 5, 3, and 1 to heavy, moderate, and light infections, respectively, and dividing the total by the number of eastern oysters rated.
[b]Brown shoal public bed where recruitment continued and *Perkinsus*-infected eastern oysters persisted.
[c]Some trays were at Wreck Shoal, a nonenzootic area where *Perkinsus* is rare.

the pre-MSX period of the 1950s, gapers seldom exhibited light infections (Table 3), but this condition changed after the introduction of MSX disease in 1959–1960 when concurrent *Perkinsus* and MSX infections occurred. These eastern oysters were all acclimated to the enzootic area at least 1 year before the records were collected. About 5,000 eastern oysters were monitored each year to obtain these records.

When MSX disease invaded Chesapeake Bay in 1959, tray operations were moved away from VIMS pier to avoid *Perkinsus* interference with studies of its epizootiology. Legged trays were constructed and were set beside stakes on eastern oyster beds all over Virginia. MSX infects eastern oysters everywhere in lower Chesapeake Bay whereas *P. marinus* tends to be localized and can be avoided by isolation for 1–3 years. Furthermore, *Perkinsus* requires dense populations to cause epizootic mortalities, but MSX is not affected by eastern oyster population size or density. It was not feasible to inspect trays daily at these scattered sites. After MSX had reduced eastern oyster populations in areas enzootic for *P. marinus,* trays of disease-free eastern oysters on outlying public eastern oyster beds showed little activity by *Perkinsus* during the first 2 years of exposure to the two diseases (Table 5). The low number of gapers collected in the Rappahannock River reflected the low mortality caused by both diseases during most years of normal rainfall and the once-per-month examinations for gapers. Mobjack Bay trays were examined monthly too, but they had high mortality from MSX, and many gapers were collected. More gapers are recovered usually during winter when decay is slow and scavengers are inactive than in warmer season when the opposite is true. The disparity is especially great when offshore trays are visited only at 2- or 3-week intervals during the year. In winter, MSX occurs in about 50% of gapers in trays. After MSX killed more than 90% of all eastern oysters on beds in about 3 years, *P. marinus* declined in abundance in the lower bay. Table 6 shows the proportion of eastern oysters killed by the two diseases in 1979 after 20 years of MSX activity. MSX killed most eastern oysters during the first 2 years of exposure in enzootic areas, but, once

TABLE 6.—Causes of death in gapers collected from trays of susceptible James River eastern oysters at Tillages Bed in open waters of York River, Virginia, USA, 1979.[a]

Date imported	Number of gapers collected	Number of gapers dead, by cause	
		MSX	*Perkinsus marinus*
4 Mar 1976	3	0	3
1 Sep 1976	5	0	2
17 Aug 1977	25	7	10
11 Oct 1977	10	5	0
24 Mar 1978	11	9	0
13 Mar 1978	16	12	1
13 Mar 1978	17	7	0
13 Mar 1978	7	6	0
28 Aug 1978	28	12	0
Total	122	58	16

[a]These tray eastern oysters had been exposed in an enzootic area for both diseases 1 or 2 years before these 1979 data were collected.

TABLE 7.—Number and intensity of infection by *Perkinsus marinus* and *Haplosporidium nelsoni* (MSX) in mostly native eastern oysters from public eastern oyster beds in Virginia, 1986. Samples of live eastern oysters contained 25 specimens. Data were provided by G. Burreson, Virginia Institute of Marine Science.

			Prevalence[a]							
			MSX				*Perkinsus marinus*			
			Number				Number			
Location	Site	Month	H	M	L	%	H	M	L	%
James River	Wreck Shoal	Aug	0	0	2	8	0	0	0	0
	Horsehead	Aug	0	0	1	4	0	0	0	0
	Miles beds	Sep	3	0	4	28	1	3	20	96
York River	Poropotank River	Aug	0	0	0	0				67[b]
	Poropotank River	Aug	0	0	0	0				72
	Tucker Ground	Sep	0	1	0	4	16	3	6	100
	Skimino Creek	Sep	1	0	3	16	2	1	5	32
Mobjack Bay[c]										
1985		Dec	2	2	7	44	0	1	8	36
1984		Dec	4	6	7	68	0	1	1	8
Chesapeake Bay	Deep Rock	Aug	8	3	4	60	0	1	0	4
Rappahannock River	Drummond Creek	Oct	0	1	3	16	2	4	15	84
	Morattico Bar	Oct	0	0	1	4	0	0	1	4
	Sharps Wharf	Oct	0	1	0	4	0	0	0	0
Potomac River area	Machodoc Creek	Sep	0	0	0	0	11	8	6	100[c]
	Yeocomico River	Sep	0	0	0	0	13	5	6	96[c]
	Coan River	Sep	0	0	0	0	7	8	8	92[d]
Great Wicomico River	Fleeton Point	Sep	0	1	1	8	6	5	9	80
	Haynie Bar	Sep	1	0	3	16	2	2	16	80
	Cranes Creek	Sep	0	0	4	16	0	1	7	32

[a]Prevalence of disease is the level of infection at a given time: H = heavy, M = moderate, L = light. Prevalences are based on samples of 25 live eastern oysters.
[b]Sample of 21 eastern oysters.
[c]James River transplants in year noted.
[d]*Perkinsus marinus*-infected seed eastern oysters were imported from lower James River in 1984 and 1985.

Perkinsus established residence in older lots, it became the primary cause of deaths (Table 7).

Severe drought in the Chesapeake Bay area from 1985 to 1987 caused serious eastern oyster mortalities by *P. marinus* in low-salinity fringe areas for MSX (Burreson, personal communication). Samples of live eastern oysters were taken from the James River seed area and several low-salinity estuaries where *Perkinsus* disease was prevalent. Because MSX did not extend its range upbay during the first 2 years of drought, *P. marinus* was able to cause enzootic mortalities (Table 7). Prevalences are expressed as cases per 25 live eastern oysters. Only the lower part of the James River seed area had *Perkinsus* infections in 1986, but the disease spread widely in 1987. Private beds in the lower sector were the source of seed eastern oysters transplanted to Potomac River tributaries, where serious mortalities occurred as indicated by the prevalences. The pathogen was severely active in the Great Wicomico River, too. Prevalences in the York River were no surprise because salinities throughout this river are usually favorable for both diseases, but MSX was not as active in 1986 as in typical years. MSX was a serious problem in 1986 only in Mobjack Bay, which is usually highly saline. The data for 1986 prove that *P. marinus* is more persistent in low-salinity estuaries than MSX and more regular in producing mortalities in such areas (Burreson, personal communication). During 1987, the 3rd year of drought, MSX and *P. marinus* both became active in all areas listed in Table 7 and in most of Maryland.

Effect of *Perkinsus* on Eastern Oyster Culture in Chesapeake Bay

During the 1950s, Virginia produced annually 3–4 million bushels of market eastern oysters. A large proportion of these eastern oysters were grown on private beds with seed eastern oysters from the James River. The three largest producers planted eastern oysters in high-salinity waters in Hampton Roads, Chesapeake Bay (deep beds off Egg Island, New Point Comfort, and Wolf Trap), and Mobjack Bay. Other important producing areas were York

River, Rappahannock River, and smaller estuaries along the western shore of Chesapeake Bay from Old Point Comfort up bay and along the Potomac River. Eastern oysters are no longer planted in many of these areas, particularly below the mouth of the Rappahannock River. Maryland produced almost as many eastern oysters but mostly on public grounds; however, losses to the two major diseases were substantial during dry years.

During the period from 1946 to 1960, *P. marinus* was the major cause of mortality among eastern oysters 2 years or more of age. During the early part of this period, oystermen typically held 2- and 3-year-old seed eastern oysters on growing beds for three additional years. Yields were strongly reduced during the 3rd year on beds in high-salinity areas, which comprised about two-thirds of the total acreage of private eastern oyster beds. Yields in areas enzootic for *Perkinsus* were often 0.5 bushel for each bushel of seed eastern oysters planted. In bushel counts, seed with 1,500–2,000 small eastern oysters yielded 300–400 market eastern oysters, which represented an 80% mortality from all causes over 3 years. Most spat and many yearlings were smothered or killed by predators (flatworms, crabs, and oyster drills). During the 1950s, oystermen began to harvest eastern oysters after 2 years on growing beds, which provided better yields. Early harvesting is an important method of disease control.

During eastern oyster company surveys in the 1950s to determine which private beds were ready to be harvested, samples were taken for thioglycollate tests for *P. marinus,* and box counts were made of dead eastern oysters with valves still attached at the hinge. Box counts were typically lower on beds planted only 1 year than those with 2 years of growth. Typical box counts on acclimated eastern oysters (i.e., grown 1 year in an area enzootic for *Perkinsus*) reached as high as 50% in 1954 and 1959.

Yields were often much higher (1 or 2 bushels harvested per bushel planted) from eastern oysters planted in low-salinity areas of the Rappahannock and Potomac rivers. Usually, these areas were free of disease-produced mortalities, except in periods of drought. Oystermen have never considered it practical first to transplant eastern oysters into low-salinity areas to avoid diseases and predators and then to transplant into high salinities for final growth and fattening. Once seed eastern oysters are planted, they are nearly always left on the same bed until ready for harvest. The cost of transplanting is substantial, and often nearly one-third of the eastern oysters planted are not recovered by the dredges used.

When MSX disease, caused by *H. nelsoni,* invaded and spread in Chesapeake Bay in 1959–1960, it quickly became the dominant disease killing eastern oysters (Andrews 1967, 1976b, 1984). In the lower bay, private eastern oyster beds were last planted in the spring of 1960. In 2 or 3 years, MSX had killed over 90% of 2 million bushels of eastern oysters in Mobjack Bay, in the Chesapeake Bay down to Old Point Comfort, and in Hampton Roads. During 26 years of waiting for the disease to subside, oystermen made only small trial plantings in these areas. Eastern oysters resistant to MSX have not developed naturally except in the lower York River and Mobjack Bay. Most beds are too barren of shells and eastern oysters to catch spat; therefore, *Perkinsus* is absent too, but it persists in nearby creeks and on man-made structures along the shores where setting occurs.

Production has declined drastically on both public and private beds (Andrews, in press). With brood stocks in low abundance, setting has declined too. Yet, tongers working on public beds have been allowed to overfish and further deplete the brood stocks. The climax in Virginia was reached in 1986–1987 when mismanagement (i.e., no cull or size limit) and disease induced by drought combined to deplete all public beds, including the seed area in James River. In 1987–1988, this seed area was opened again to remaining tongers. Consequently few seed eastern oysters were available for planters during the 1986–1987 and 1987–1988 seasons. The extension of *Perkinsus* upriver into the lower James River seed area in 1985–1987 produced disastrous results for those planters in low-salinity areas who transplanted infected eastern oysters.

Meanwhile, few private beds are being planted because economics, politics, and high risks of eastern oyster culture have changed management objectives (Andrews, in press). Disease is only one of the reasons why so few private beds are planted in Virginia, even in low-salinity areas where risks are lower in most years. The state should never allow *Perkinsus*-infected eastern oysters in seed areas to be transplanted in such a way as to spread disease and discourage private planting. The seed area should be sampled for prevalence of MSX and *Perkinsus* to determine where and when eastern oysters can be safely transplanted. Monitoring private growing beds for *Perkinsus* is a more difficult program, but must be

pursued. Because *Perkinsus* cannot be adequately assessed from December through June or July, tests must be made during the peak-incidence period in September and October each year. Unfortunately, unlike MSX, *Perkinsus* activity must be tested on each planted bed because it is localized and variable with age, exposure, weather, and location of site.

Private investment on a planted bed of eastern oysters may be as high as $3,000/acre (1 acre = 0.4 hectare), which justifies more attention to diseases and other biological problems than is usually given. The market price of eastern oysters is favorable, but more attention must be given to seed supply and methods of culture and management to revive the industry. Large areas of public and private eastern oyster beds suitable for culture are unproductive in Chesapeake Bay. The states control most seed areas and must insure availability of seed supply. Off-bottom culture, practiced in other regions of the world, would be more expensive in labor and supplies without avoiding problems of disease and fouling. Harvesting of poor-condition seed eastern oysters from James River for marketing has caused a decline in quality of marketed meats. A large proportion of shucked eastern oysters distributed by Chesapeake Bay packers is imported from the Gulf of Mexico and the west coast of the U.S. Public eastern oyster beds are severely overfished, and their recovery from natural setting appears questionable. The states continue to subsidize public oystering by transplanting seed eastern oysters and planting shells for substrate. The huge acreage of barren public grounds cannot be made productive by limited state financing. Eastern oyster planters with leased private grounds seem to be discouraged by high costs and risks of eastern oyster culture. Extreme fluctuations of weather seem to compound these problems by encouraging diseases. Now, seed eastern oysters are scarce, and no strategy for survival of private culture has been found. Rapid human population growth along the shore of Chesapeake Bay has multiple effects on the waters in addition to pollution. The eastern oyster industry is at a crossroad in terms of survival, and diseases are an important facet of efforts to revive it.

Strategies to control the diseases caused by MSX and *Perkinsus* are quite different. Control of MSX depends on movement to low-salinity areas, and, ultimately, on developing and breeding resistant eastern oysters (Ford and Haskin 1988, this volume). Resistance has occurred naturally in Delaware Bay where nearly all stocks of eastern oysters were exposed to the disease and intensive selection has occurred over many years. In Chesapeake Bay, seed eastern oyster stocks are located in relatively low-salinity areas, where little selection occurred; therefore, selection and breeding for resistance must be done at high cost in hatcheries.

Control of *Perkinsus* disease depends on isolation and manipulation of seed stocks before and after they are transplanted to growing areas. The following management procedures are detailed in Andrews and Ray (1988): (1) Avoid use of infected seed eastern oysters. This precaution can set back infection by a full year. (2) Isolate newly planted beds from those with infected eastern oysters. (3) Harvest and fallow beds to allow all infected eastern oysters to die before replanting. (4) Harvest early if beds become infected. (5) Diagnose for disease on public and privately planted beds in September and October each year in enzootic areas, or earlier if summer mortality occurs. If seed eastern oysters are infected, the area should be closed to all transplanting until the beds recover from the disease.

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