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EPIZOOTIOLOGY OF *PERKINSUS MARINUS* DISEASE OF OYSTERS IN CHESAPEAKE BAY, WITH EMPHASIS ON DATA SINCE 1985

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ABSTRACT Since 1987 *Perkinsus marinus* has been the most important pathogen of the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay because of its widespread distribution and persistence in low salinity areas. The pathogen became established on all oyster beds in the Chesapeake Bay as a result of natural spread during the consecutive drought years from 1985 to 1988 or by movement of infected oysters during the same period. Elevated salinities resulting from drought conditions and concomitant warm winters allowed *P. marinus* to proliferate in what were historically low salinity areas. Oyster mortality was high on most beds and landings of market oysters declined to record low levels in both Maryland and Virginia during the late 1980s and early 1990s. The seasonal periodicity of *P. marinus* is primarily controlled by temperature. Both prevalence and intensity of infections begin to increase in June as temperature increases above 20°C and overwintering infections begin to proliferate. Maximum values of prevalence and intensity occur in September immediately following maximal summer temperatures. Infection regression occurs during winter and spring as temperature declines resulting in minimum prevalence and intensity values in April and May. Prevalence and intensity of *P. marinus* infections in oysters from the James River, VA, over a five year period were significantly correlated with temperature when temperature data were lagged three months. Temperature explained 39% of the variability in prevalence and 46% of the variability in intensity. The relationship between temperature and annual variability in *P. marinus* abundance is somewhat obscure, in part because of the difficulty separating salinity and temperature effects. Nonetheless, data from 1988 to 1994 from the James River, VA, suggest that abnormally warm winters have a more significant impact on summer *P. marinus* abundance than abnormally cold winters. Salinity is the primary environmental factor that controls local distribution and intensity of *P. marinus* infections. Long-term oyster disease monitoring along a salinity gradient in the James River, VA, revealed a statistically significant relationship between salinity and *P. marinus* prevalence and intensity. *P. marinus* infections remain light in intensity and no oyster mortality results if salinity is consistently less than 9 ppt. However, infections may persist for years in low salinity areas. If summer/fall salinities range from 9 to 15 ppt some infections may progress to moderate and heavy intensity, but oyster mortality is relatively low. If summer/fall salinities are consistently greater than 15 ppt, moderate and heavy infections may be numerous and oyster mortality may be high. Field studies in the York River, VA, suggest that new *P. marinus* infections are acquired from July through early October, but peak infection acquisition occurs during late August and is correlated with oyster mortality. The early infection process in oysters and the role of zoospores in transmission dynamics in nature are poorly understood. No direct link between oyster defense mechanisms and control of *P. marinus* infections has been established. If oyster defense mechanisms do modulate *P. marinus* infections, the components have not been identified. There is little evidence to support the common perception that pollution is responsible for the dramatic increase in *P. marinus* abundance since 1985. Pathogen abundance is clearly correlated with salinity increases resulting from drought conditions in the late 1980s, although there may be subtle effects of toxicants or poor water quality on the host/parasite interaction.

KEY WORDS: *Perkinsus*, oyster disease, annual cycle, transmission, epizootiology, salinity effects, temperature effects

Since 1987, *Perkinsus marinus* (Mackin et al. 1950), the causative agent of Dermo disease, has been the most important pathogen of the eastern oyster *Crassostrea virginica* (Gmelin) along the east coast of the United States south of Delaware Bay. The origin of *P. marinus* is obscure, but it probably always has been an associate of oysters. It was first reported in Chesapeake Bay oysters in 1949 (Andrews and Hewatt 1957). The pathogen was first described as *Dermocystidium marinum* because of apparent affinities with fungal parasites of freshwater fishes (Mackin et al. 1950). It was later reclassified as *Labyrinthomyxa marina* because of observations of gliding cells similar to those present in slime molds (Mackin and Ray 1966). Ultrastructural observations (Perkins 1976) of an apical complex in the motile zoospore stage led Levine (1978) to establish the new genus *Perkinsus* for the pathogen within the phylum Apicomplexa. Taxonomic placement of *P. marinus* in the Apicomplexa has been controversial because of the presence of a number of morphological and life cycle characteristics more typical of the Mastigophora (flagellates) than of the Apicomplexa (Vivier 1982). Molecular sequence data (Fong et al. 1993, Goggin and Barker 1993) and a recent phylogenetic analysis based on sequence data (Siddall et al. 1995) do not support inclu-

sion of *P. marinus* in the phylum Apicomplexa, but suggest a recent common ancestry with the dinoflagellates.

Along the east coast of the United States prior to the late 1980s, *P. marinus* was restricted to high salinity portions of coastal bays and estuaries south of Delaware Bay, although it apparently was absent from the seaside bays of the eastern shore of Virginia and Maryland (Andrews 1988). In the Chesapeake Bay, *P. marinus* was prevalent in the lower Bay, but was restricted to the mouths of the major tributaries in Virginia and southern Maryland (Fig. 1). There were a few localized concentrations of *P. marinus* in Maryland, primarily in Fishing Bay and Eastern Bay. The pathogen was observed locally in Delaware Bay in the mid-1950s, as a result of importing infected oysters from Chesapeake Bay, but it never caused significant mortality in oysters and appeared to die out as importations stopped in the late 1950s (Ford 1992). North of Delaware Bay the parasite was absent or at least undetectable.

In endemic areas *P. marinus* has always been responsible for some oyster mortality, but it did not significantly affect harvest most years because of the large natural sets on public beds and good seed-oyster availability for private planters in Virginia. An excellent review of the history of research on this pathogen and of

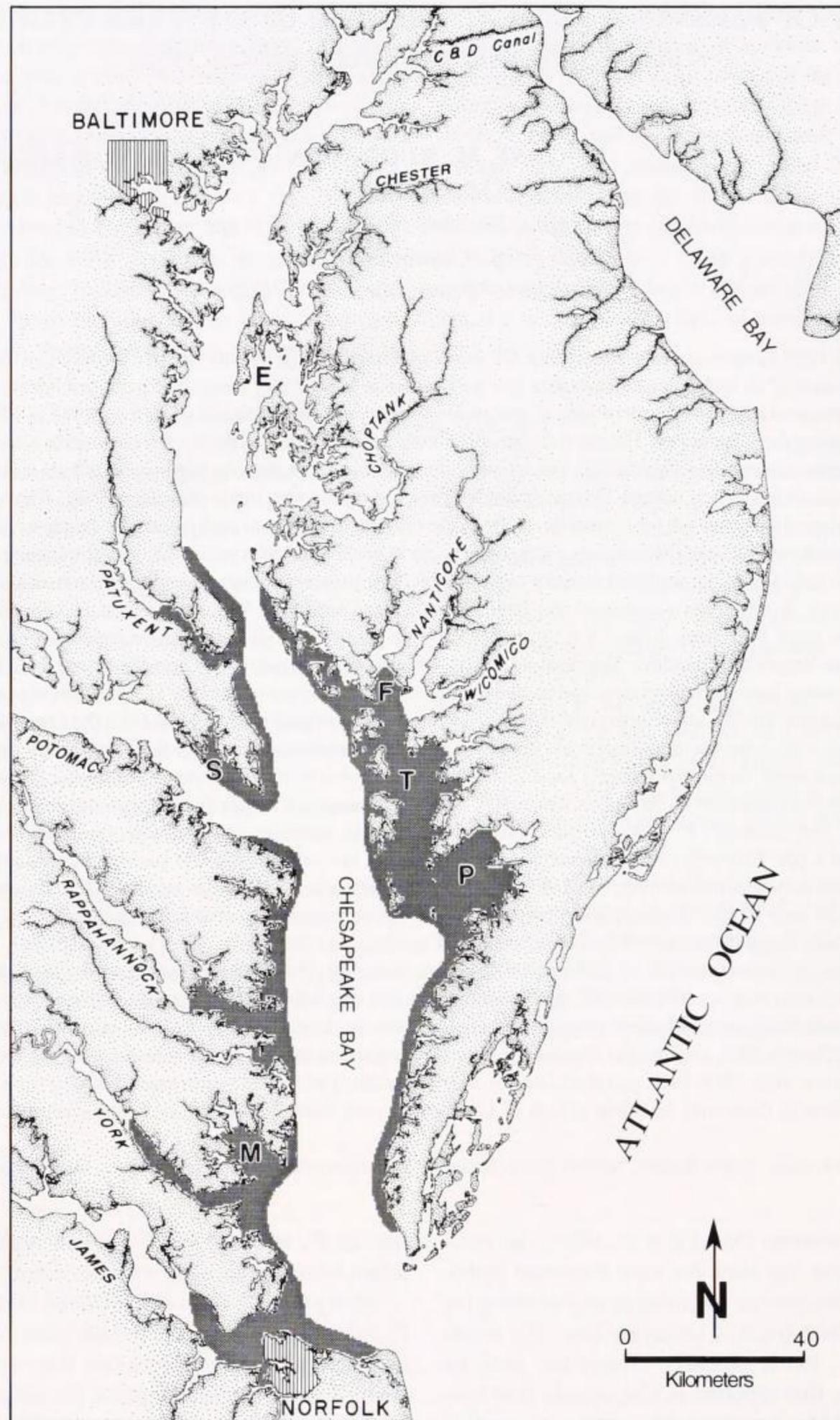


Figure 1. Distribution of *P. marinus* in Chesapeake and Delaware Bays prior to its spread during the 1980s. E = Eastern Bay, F = Fishing Bay, M = Mobjack Bay, P = Pocomoke Sound, S = mouth of St. Mary's River, T = Tangier Sound. Data from Andrews (1981) and Krantz and Otto (1981).

P. marinus epizootiology in Chesapeake Bay prior to the late 1980s was provided by Andrews (1988).

It has long been known that the distribution and local abundance of *P. marinus* are controlled by environmental conditions (Andrews 1988). During the late 1980s and early 1990s the distribution and epizootiology of *P. marinus* in the Chesapeake Bay deviated from historical patterns as the result of four consecutive drought years and concomitant warm winters from 1985 to 1988. During that period, *P. marinus* spread to all productive oyster grounds in Chesapeake Bay either by natural processes or by

movement of infected oysters. Elevated salinities and warm winters allowed the pathogen to survive in areas that historically were disease-free. Although drought conditions have abated and rainfall patterns have returned to more or less typical conditions, with wet winters and springs, especially during 1993 and 1994, *P. marinus* continues to persist tenaciously in most areas of the Chesapeake Bay. The presence of the pathogen throughout the James River seed area in Virginia has been especially troublesome because infections develop to lethal levels when seed oysters are transplanted to high salinity growout areas.

The purpose of this review is to summarize the current distribution of *P. marinus*, to discuss the current understanding of the epizootiology of the pathogen in Chesapeake Bay, with emphasis on changes since 1985, and to discuss the impact of this pathogen on the oyster resource of the Chesapeake Bay. We will focus on environmental controlling factors, as they are particularly important in Chesapeake Bay, and we will attempt to identify areas where data are especially lacking and where research needs to be focused.

PRESENT DISTRIBUTION OF *P. MARINUS*

As of late 1994, *P. marinus* is known from as far north as Wellfleet Harbor, Cape Cod Bay, MA (Ford 1996), south throughout the bays and estuaries along the east coast of the United States, including virtually all oyster beds in Delaware and Chesapeake Bay, and throughout the Gulf of Mexico as far south as Tabasco, Mexico (Burrison et al. 1994a, Soniat 1996). In the mid-1980s, *P. marinus* had not been reported north of Chesapeake Bay; thus, the present distribution represents either a major northward expansion of *P. marinus* or a significant increase in abundance of the parasite in areas where it may have been present but was undetectable.

Although *P. marinus* was reported periodically from native oysters in Delaware Bay in the mid-1950s, probably as a result of importing infected oysters from Chesapeake Bay or other southern areas, it never became established and has never been responsible for significant oyster mortality (Ford 1992). This situation changed in 1990 when *P. marinus* became abundant in Delaware Bay and was also found in Great Bay along the Atlantic Coast. Abundance and distribution within Delaware Bay increased during 1991 and significant oyster mortality occurred then and in subsequent years. Prevalence of *P. marinus* in New Jersey coastal bays during 1991 ranged from 30% in Dry Bay, Manasquan and Tuckerton, 50% in Raritan Bay and 85% in Great Bay. As of 1994, the parasite is abundant on all oyster beds on the north shore of Delaware Bay, including the seed beds (Fig. 2); it seems to be much less abundant along the southern, Delaware shore (Ford 1996).

In Delaware Bay, it appears that *P. marinus* spread from undetected localized foci as a result of unusually warm winters during the period (Ford 1992), although effluent into the Maurice River from shucking houses processing *P. marinus*-infected oysters from the Gulf of Mexico may have also contributed to the spread. Because of the drought conditions and concomitant warm winters, the pathogen was able to become established and it has now replaced *Haplosporidium nelsoni* (MSX), although perhaps temporarily, as the most important oyster pathogen in Delaware Bay (Ford 1996).

The spread of *P. marinus* northward into Long Island Sound was probably also facilitated by warm winters. The pathogen may have spread from undetected localized foci of infection established in the past by importation of infected oysters from southern areas, but possibly also by recent movement of infected oysters, although recent movements have not been documented. Prevalence and intensity of *P. marinus* are high in oyster samples from some areas of Long Island Sound and the south shore of Cape Cod, for example Cotuit, MA, and oyster mortality attributed to this pathogen has been relatively high in some areas (Ford 1996).

In the Chesapeake Bay, *P. marinus* spread into historically low salinity areas during the prolonged drought of the late 1980s and it is now present on all public oyster beds in both Virginia and Maryland (Figs. 1 and 2), although significant oyster mortality is

restricted to those areas where salinity is above about 12 ppt for most of the summer and fall. The parasite is also now present in the bays along the seaside of the eastern shore of Virginia and Maryland, probably as a result of moving infected oysters to those locations from Chesapeake Bay.

Unfortunately, Virginia scientists were not aware of the spread of *P. marinus* during 1985 and 1986. Dr. Jay Andrews had retired in 1984 and the oyster disease monitoring program that had been underway since 1959 was terminated. The first indication of the spread was very high mortality in September 1986 in oysters transplanted from the James River seed area to three tributaries along the south shore of the Potomac River, the Coan and Yeocomico Rivers and Machodoc Creek. Disease analyses revealed high levels of *P. marinus* in all three areas (>90% prevalence, 2.6–3.4 weighted prevalence). The source of the seed was revealed as Miles ground in the lower portion of the James River seed area (Fig. 3) and subsequent analyses of oysters from that site revealed high prevalence (96%) of *P. marinus* although most infections were light (weighted prevalence = 1.36) (Burrison 1987). It became clear that the parasite had spread into the lower seed areas and had been moved to the growout areas in infected seed oysters. The drought conditions allowed *P. marinus* to spread into the seed area and also allowed it to flourish in the growout areas because salinity was favorable (>12 ppt) in those areas as well. During the seven year period from 1985 through 1991, only 1989 was considered a wet year. The growout tributaries off the south shore of the Potomac River had previously been free of significant mortality caused by *P. marinus* although the parasite was observed in these areas during some years (Andrews 1981). Oysters in the lower portion of the James River seed area were known to harbor *P. marinus* periodically (Andrews and Hewatt 1957), but prevalence and intensity were always low.

By 1988, intensity of *P. marinus* infections in endemic areas had increased dramatically and oyster mortality was high, especially during 1987 and 1988. In addition, favorable salinities allowed the pathogen to spread into new areas and by 1991 *P. marinus* had spread to most oyster growing areas of the Chesapeake Bay including Maryland either by natural processes or by movement of infected oysters (Table 1). Oysters in previously non-enzootic areas were highly susceptible to *P. marinus*, infection prevalence and intensity were unusually high, and mortality was high on both planted grounds and public beds in favorable salinity. The parasite was present at Wreck Shoal (WS) (Fig. 3) in the middle of the James River seed area in 1986 and had spread to Deepwater Shoal (DWS), the uppermost oyster bed in the James River by 1988. Prevalence and intensity of *P. marinus* continued to increase in the James River through 1991. Similarly, the pathogen spread throughout the Rappahannock River and was present at Ross Rock, the uppermost oyster bed by 1992, although both prevalence and intensity were very low at that site.

A similar up-bay spread of *P. marinus* occurred in Maryland through the 1980s and early 1990s (Figs. 1 and 2) from foci of infection in Tangier Sound, Holland Strait, Tar Bay and near the mouth of the St. Mary's River. By 1987 the parasite had spread up the main stem of the Bay to Swan Point north of the mouth of the Chester River and throughout Fishing Bay and the mouth of the Choptank River. In the Potomac River the parasite spread to the mouth of Clements Bay during 1987. During 1988 *P. marinus* spread throughout the Choptank and Little Choptank Rivers and further up the Potomac River to the mouth of the Wicomico River. By 1992 the pathogen had spread throughout the Chester River and

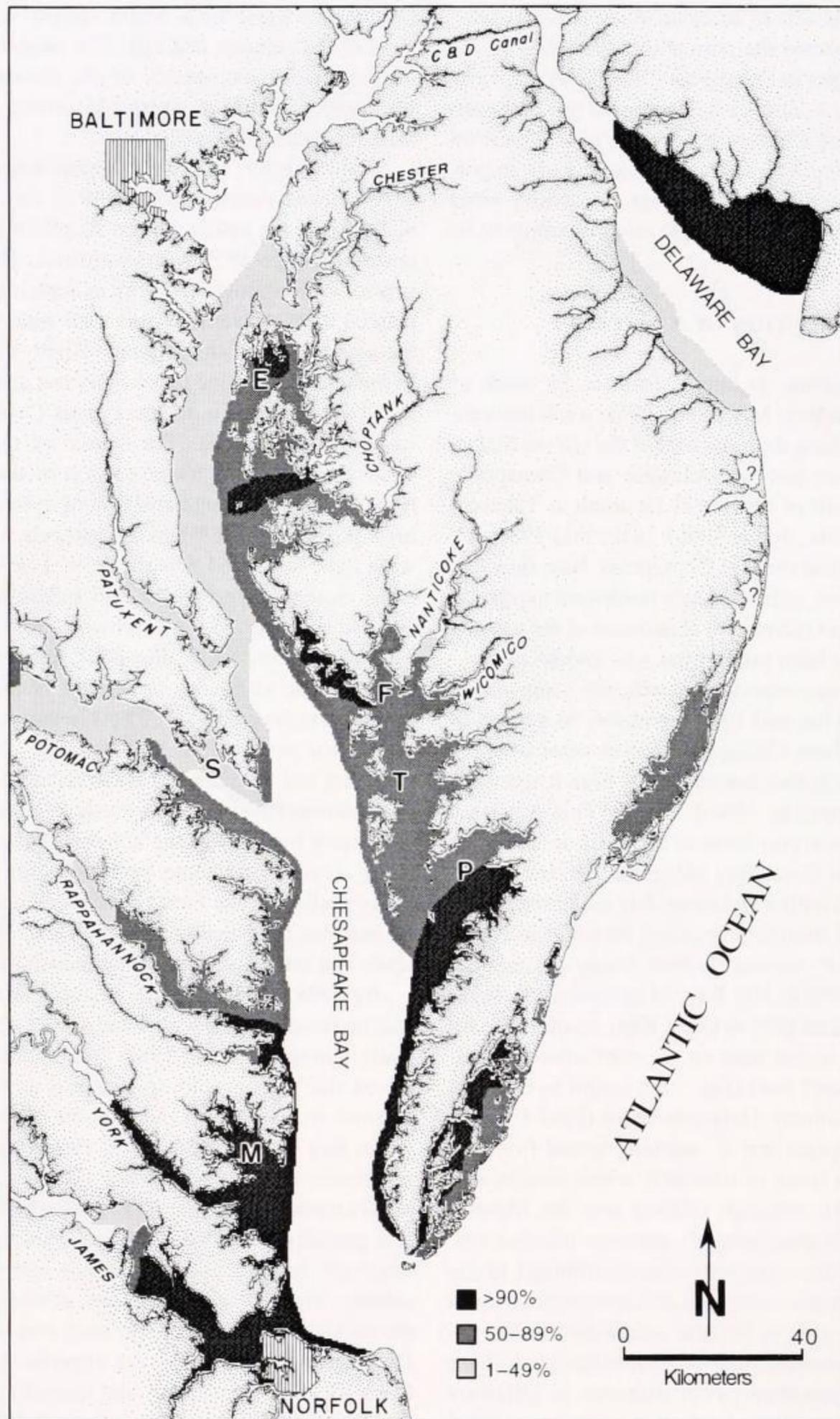


Figure 2. Present distribution of *P. marinus* in Chesapeake and Delaware Bays. Shading indicates maximum annual prevalence. E = Eastern Bay, F = Fishing Bay, M = Mobjack Bay, P = Pocomoke Sound, S = mouth of St. Mary's River, T = Tangier Sound. Data from Ragone Calvo and Burreson (1995), G. E. Krantz (personal communication) and S. E. Ford (personal communication).

was present on every productive oyster bar in Maryland (Krantz 1993). Intensity of infections during summer and fall increased each year in previously invaded areas and oyster mortality was greater than 50% in areas with favorable salinity including most areas south of Kent Point (Krantz 1990, Krantz 1992, Krantz 1993).

The spread of *P. marinus* into areas in the lower Chesapeake Bay where it was historically absent seems to have been a long-term acquisition. Unusually high spring runoff during 1993 and

1994, a very wet July in 1994 and a cold winter in 1993-94 had little effect on the subsequent fall prevalence of *P. marinus* in the James River, VA (Table 1), although intensity of infections declined somewhat from a peak in 1991. Prevalence and intensity of *P. marinus* infections did decline to a greater extent in the upper Bay in Maryland during 1994 (Table 1). The historical absence of *P. marinus* in the upper Bay and upper reaches of the major tributaries suggests that the pathogen will eventually be eliminated from these areas if normal environmental conditions of cold win-

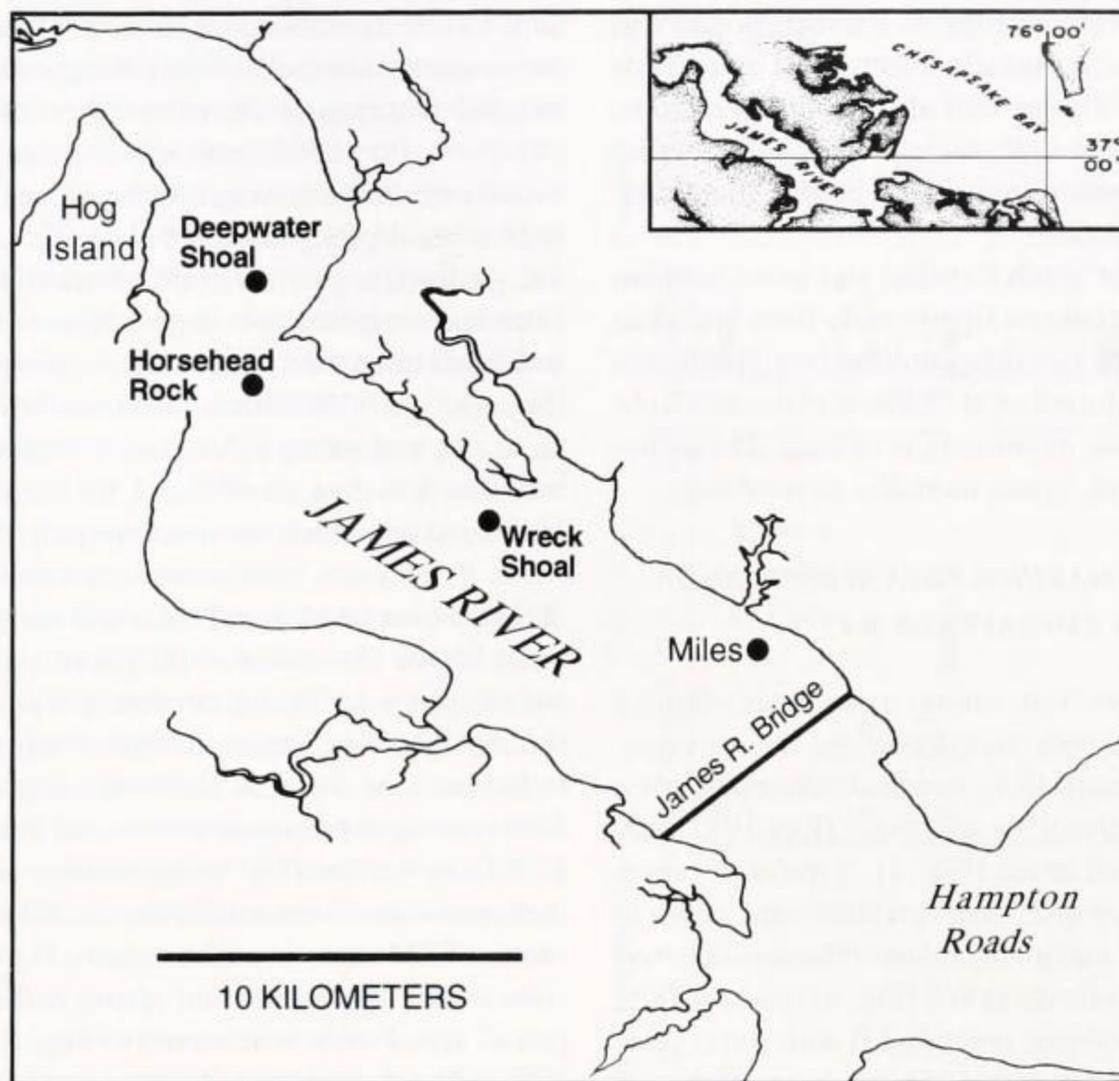


Figure 3. James River, VA, showing locations of various monitoring stations.

ters and wet springs continue, but monthly monitoring in Virginia during the 1990s has demonstrated that the decline will be slow and may take a decade or more. Unfortunately, with the present widespread distribution of *P. marinus*, any drought period will allow the pathogen to increase in abundance and will only prolong the problem.

South of Chesapeake Bay, *P. marinus* has always been present in bays and estuaries including intertidal oyster beds. The drought conditions of the late 1980s also caused a dramatic increase in abundance of *P. marinus* in North Carolina. Oyster mortality attributable to *P. marinus* was first documented in the fall of 1988 in southern North Carolina. From 1988 through 1992 the pathogen

TABLE 1.
Prevalence (% infected) of *P. marinus* at various locations in Chesapeake Bay^a

Location	1980	1986	1989	1991-92 ^b	1994
Virginia					
James River, Wreck Shoal	0	0	100	100	100
James River, Horsehead Rock	0	0	48	100	96
James River, Deepwater Shoal	0	0	8	88	56
Rappahannock River, Broad Creek	04	84	44	100	64
Rappahannock River, Smokey Point	0	04	44	100	46
Rappahannock River, Bowlers Rock	nd	0	40	88	16
Rappahannock River, Ross Rock	0	0	0	24	0
Maryland					
Swan Point	0	0	03	23	03
Chester River, Old Field	0	0	10	37	20
Eastern Bay, Bugby	0	0	100	100	63
Choptank River, Cooks Point	0	0	23	100	90
Choptank River, Sandy Hill	0	0	53	100	83
Patuxent River, Broomes Island	50	50	57	100	40
Potomac River, Cornfield Harbor	75	nd	nd	100	77
Potomac River, Ragged Point	0	nd	93	90	10
Potomac River, Lower Cedar Point	0	0	03	10	83
Holland Straits	80	nd	nd	100	57
Tangier Sound, Old Woman's Leg	80	nd	23	100	73

^a Data from Andrews (1981), Burreson (1987, 1990, 1992, 1993), Krantz (1990, 1992, personal communication), Krantz and Otto (1981) and Ragone Calvo and Burreson (1995). nd = no data.

^b For most locations, either 1991 or 1992 was the year of highest prevalence.

spread northward along the eastern edge of Pamlico Sound and then across to the western side, eventually infecting all oyster beds and causing high mortality. Oyster mortality from *P. marinus* continued during 1993 and 1994 in Pamlico Sound, but mortality seems to have declined in southern areas near Bogue Sound (M. Marshall, personal communication).

The status of *P. marinus* in South Carolina and more southern states does not seem to have changed significantly from historical levels although there have been few data published on distribution and intensity in these areas (Burrell et al. 1984, Crosby and Roberts 1990) and extensive disease monitoring is lacking. The pathogen is present and causes some oyster mortality in most areas.

ANNUAL CYCLE OF *P. MARINUS* PREVALENCE AND INTENSITY IN CHESAPEAKE BAY

Samples of non-spat oysters from natural oyster beds exhibit a pronounced seasonal cycle in both prevalence and intensity (expressed as weighted prevalence) of *P. marinus* infections when diagnosed with the fluid thioglycollate technique (Ray 1952, Ray 1966) of mantle, gill and rectal tissue (Fig. 4). Typically, prevalence and weighted prevalence of *P. marinus* infections begin to increase in June. On average, maximum values of both parameters are reached in September; prevalence at WS (Fig. 4) reaches 100% every year and weighted prevalence reaches 2.0 with some years above 3.0. These values contrast with 1954 when prevalence of 60% and weighted prevalence of 1.5 were considered intense infections (Andrews and Hewatt 1957). The relative contribution of multiplying overwintering infections and acquisition of new infec-

tions to this increase is not clear, but based on timing of *P. marinus* transmission (see below) it appears that the early summer increase is primarily the result of proliferation of overwintering infections. Prevalence may remain high through January, but intensity, measured as weighted prevalence, usually declines sharply in October if peak values are above 2.5. This decline is probably due, at least in part, to death of heavily infected oysters. Prevalence and weighted prevalence values decline through the winter and reach minimum values in late spring, typically April or May (Fig. 4). Since 1988 some infections have been found throughout the winter and spring in Virginia except at locations where salinity becomes less than about 5 ppt for extended periods. Detectable overwintering infections are contrary to the situation prior to 1985 when *P. marinus* infections were either absent or undetectable during winter (Andrews 1988), and are probably the result of the much higher abundance of the parasite since 1985. However, the winter/spring decline in prevalence is in part an artifact of the low sensitivity of the standard fluid thioglycollate medium (FTM) technique (see Fig. 5). Recently, Ragone Calvo and Burreson (1994) using antibody detection and Bushek et al. (1994) using total body burden fluid thioglycollate analyses have also shown that prevalence does not decline as dramatically during winter as routine FTM assay would suggest. However, intensity does decline during late winter and spring and all infections during that period are of very low intensity (Fig. 5) (see also Bushek et al. 1994). The decline in intensity is partly the result of mortality of moderately and heavily infected oysters during winter, but intensity values decline even in areas where infection intensity is relatively low and where no mortality occurs. It is not known if this

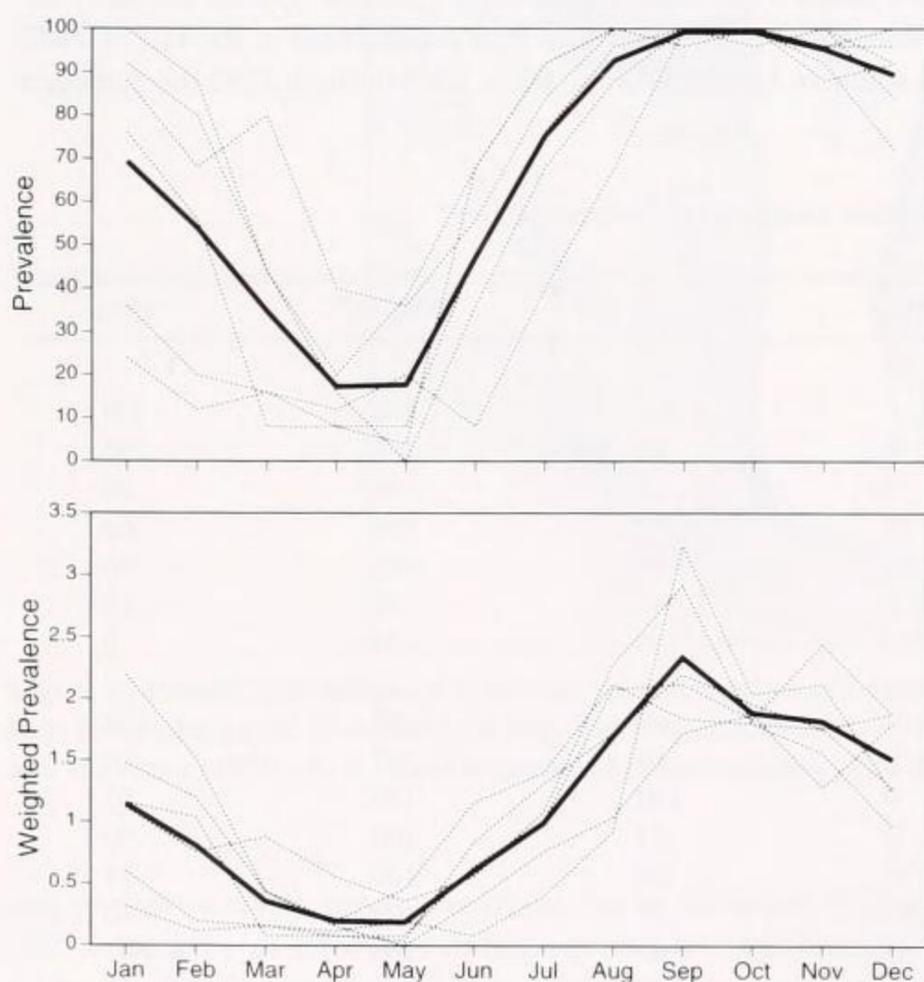


Figure 4. Annual cycle of *P. marinus* prevalence (top) and infection intensity, expressed as weighted prevalence (bottom), in Chesapeake Bay oysters. Dotted lines demonstrate year-to-year variability for years 1988–94. Bold line represents the average of all years, 1988–94. Prevalence and intensity were determined using the FTM method described by Ray (1966). Oysters ($n = 25$) were sampled monthly from Wreck Shoal, James River, VA.

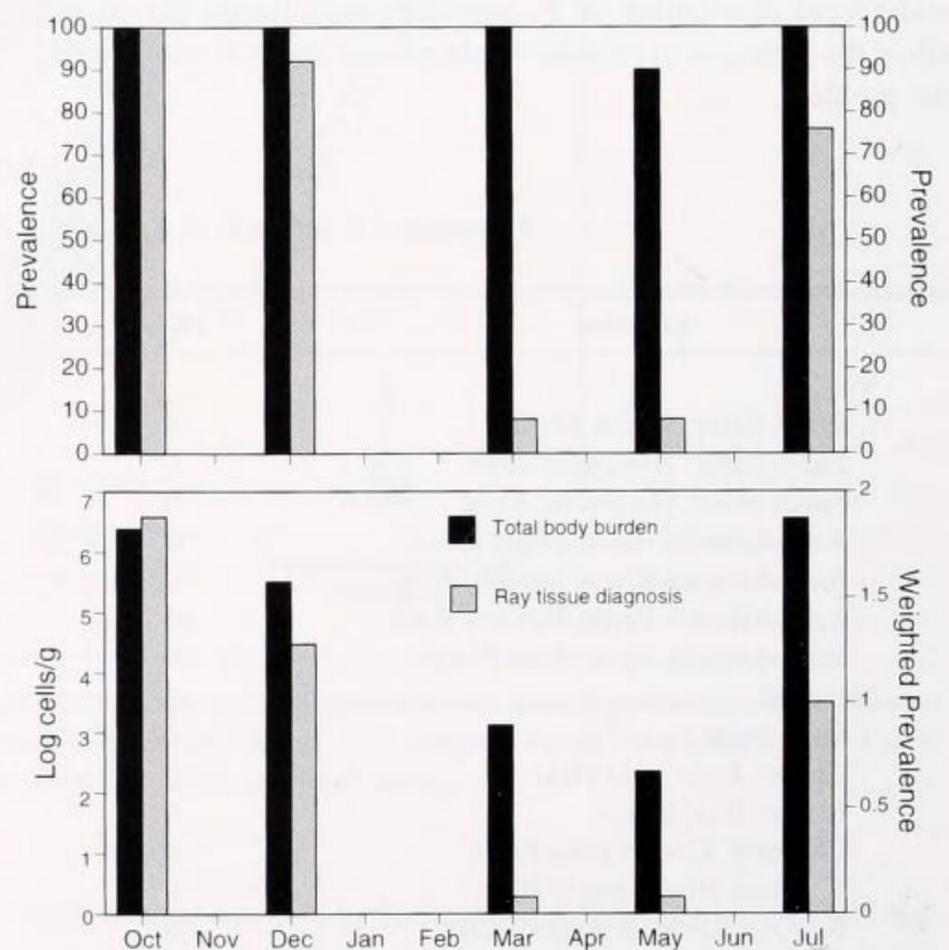


Figure 5. Seasonal prevalence (top) and intensity (bottom) of *P. marinus* as determined by standard Ray tissue FTM assays and by total body burden estimations. Intensity is expressed as weighted prevalence for standard FTM assays (right axis) and as log₁₀-transformed cells per gram wet tissue weight for body burden estimations (left axis). Oysters were sampled from Wreck Shoal, James River, VA. Sample size was 25 for standard FTM assays and 20 for total body burden assays.

decline is the result of active defense processes by the oyster or passive processes related to tolerance of *P. marinus* to temperature and salinity although previous researchers have suggested that parasite cells are actively eliminated (Andrews 1988).

Maximum and minimum prevalence and weighted prevalence values during the annual cycle and the timing of increases and decreases are affected by the local temperature and salinity regimes (see next section) although the general pattern remains consistent in all areas. For example, while prevalence values have reached 100% at Wreck Shoal in the James River every fall since 1988, they reached 100% at Horsehead Rock, an upriver station in lower salinity, only during 1991. In addition, annual prevalence values usually peak one or two months later and begin to decline one to two months earlier in these low salinity areas.

There seems to be an oyster size threshold required for *P. marinus* infection in nature. Spat less than about 30 mm in shell height are rarely found infected using routine FTM assay (Burrenson 1991). This apparent size threshold may be the result of low sensitivity of FTM assay, but more likely is due to reduced filtering capacity of small oysters. It does not appear to be the result of innate resistance to the parasite, as Andrews and Hewatt (1957) have demonstrated that small oysters acquire infections when dose is high. Infections with *P. marinus* are known to be dose dependent and small oysters probably don't filter enough water to acquire sufficient infective stages of the parasite in nature.

ANNUAL CYCLE OF *P. MARINUS*-INDUCED OYSTER MORTALITY

In areas of favorable salinity (>12 ppt) oyster mortality resulting from *P. marinus* infections usually begins about the first of August and continues through early winter although most oysters die in late August and September. The proliferation of *P. marinus* is temperature dependent and abundance within an oyster increases so long as temperature is above about 20°C; thus, an unusually warm spring or fall will prolong the development period of the pathogen and result in greater oyster mortality.

The mortality pattern of oysters placed into salinity regimes conducive to parasite development depends on the prior history of *P. marinus* infection. Uninfected oysters larger than about 30 mm shell height usually acquire *P. marinus* infections during mid to late summer of the first year. Mortality is usually low because declining water temperature during fall prohibits development of most infections to lethal levels, but mortality as high as 40% may occur if oysters are about 50–60 mm shell height. High mortality, often greater than 90%, will occur in these oysters during the second summer if environmental conditions are favorable for *P. marinus* development (Fig. 6). This mortality pattern is drastically different than that prior to 1985 when significant oyster mortality from *P. marinus* did not occur until the third summer after initial infection. Management strategies proposed by Andrews and Ray (1988) to harvest oysters after two summers of growout were successful prior to the 1980s, but have not been as effective since 1986 because high mortality occurs during the second summer of exposure.

Spat that are less than about 30 mm shell height during late summer/early fall will usually not acquire *P. marinus* that summer and they can often be grown to market size before significant mortality from *P. marinus* occurs. Aquaculturists can reduce mortality caused by *P. marinus* by spawning oysters late and delaying

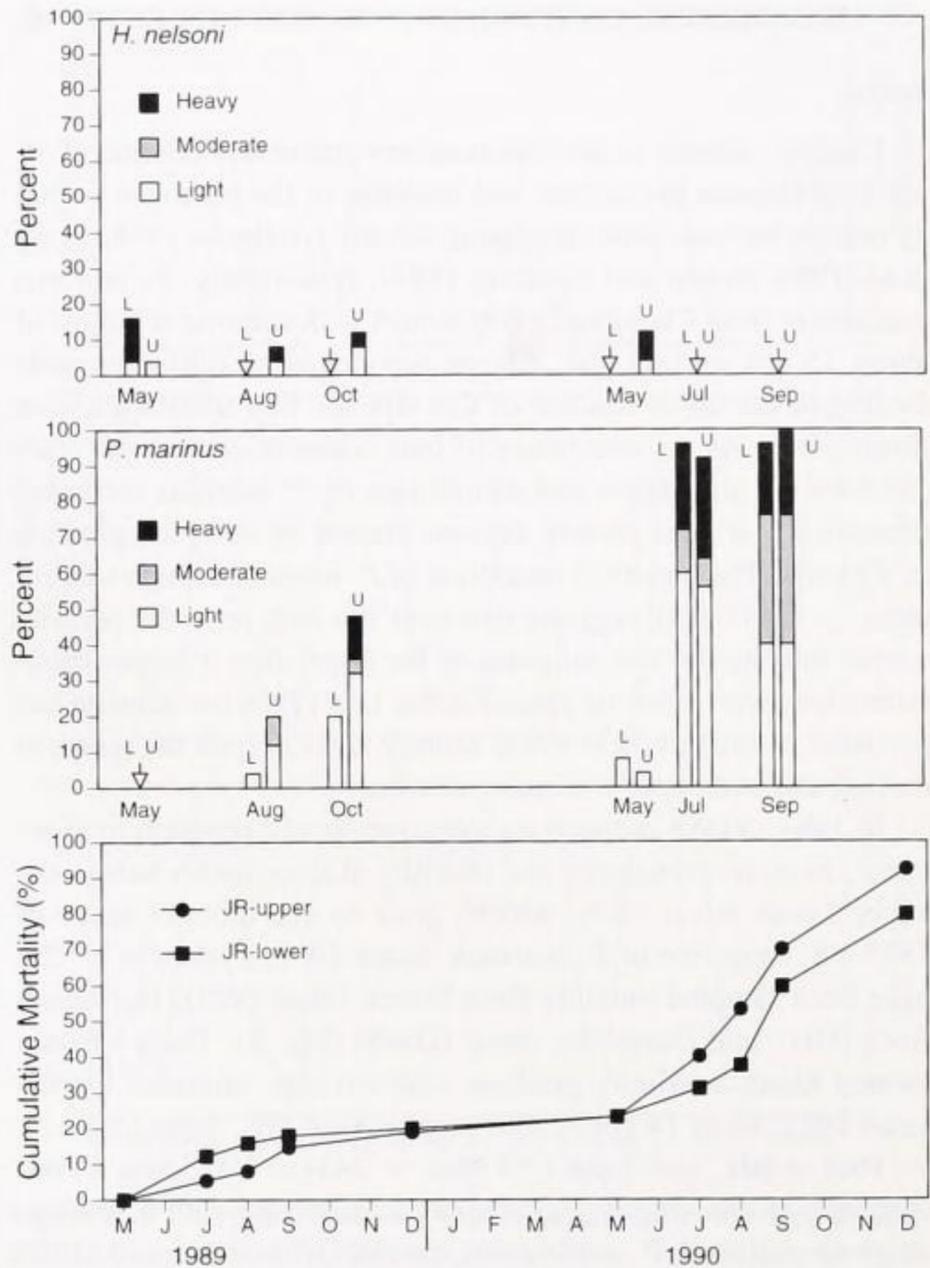


Figure 6. Disease-associated cumulative oyster mortality (bottom) and prevalence and intensity of *H. nelsoni* (MSX) (top) and *P. marinus* (middle) in hatchery-reared juvenile oysters deployed in the lower York River, VA. Prevalence of *H. nelsoni* was very low, especially during 1990, so mortality can be attributed to *P. marinus*. For parasite data, prevalence is indicated by total bar height and percentage of sample in each intensity category by shading. Sample size = 25. Arrows indicate samples examined but no infections found. Two oyster stocks are compared in each graph—upper James River (U) and lower James River (L). Mean shell height in July 1989 was 42 mm for both groups.

placing them in *P. marinus*-enzootic waters until late September. In this situation most spat avoid infection by *P. marinus* but still grow well until winter. They will acquire *P. marinus* infections during the next summer, but mortality will be low. Experience has shown that oysters can reach market size by the following spring and can be harvested before high mortality results the following summer (M. Luckenbach, Virginia Institute of Marine Science [VIMS], personal communication).

Because *P. marinus* is present on all seed-oyster bars in the Chesapeake Bay, oysters should not be moved from seed areas to high salinity growout areas. Light infections will intensify and high mortality will almost certainly occur the first summer after transplantation. Nor should *P. marinus*-infected oysters be moved to low salinity with the expectation that the pathogen will be eradicated. Monthly monitoring in the upper James River, VA (Ragone Calvo and Burrenson 1994), has clearly shown that *P. marinus* can survive long periods (weeks to months) of salinity below 5 ppt and days to weeks in fresh water.

ENVIRONMENTAL CONTROL OF *P. MARINUS* INFECTIONS

Salinity

Clearly, salinity is an important environmental control of *P. marinus* because prevalence and intensity of the pathogen within an estuary increase with increasing salinity (Andrews 1988, Craig et al. 1989, Soniat and Gauthier 1989). Historically, *P. marinus* was absent from Chesapeake Bay waters with summer salinities of about 15 ppt or less and a large proportion of oyster grounds located in the upper reaches of Chesapeake Bay tributaries were disease-free. As a consequence of four consecutive drought years 1985–88 the abundance and distribution of *P. marinus* increased dramatically and the parasite became present on all oyster grounds in Virginia. The historical restriction of *P. marinus* to high salinity areas (>12–15 ppt) suggests that over the long term the parasite cannot tolerate the low salinities of the upper Bay or upper tributaries; however, since its spread in the late 1980s the parasite has persisted in most of these lower salinity areas despite the return to normal and even below normal salinities.

In 1987, VIMS initiated an intensive survey program to monitor *P. marinus* prevalence and intensity at three oyster bars in the upper James River, VA, which, prior to the drought years of 1985–88, were free of *P. marinus*. Since 1987, oysters ($n = 25$) have been sampled monthly from Wreck Shoal (WS), Horsehead Rock (HH), and Deepwater Shoal (DWS) (Fig. 3). These bars are located along a salinity gradient with average salinities for the years 1987–94 of 14 ppt (± 4.3 , $n = 318$) at WS, 9 ppt (± 4.1 , $n = 166$) at HH, and 7 ppt (± 4.0 , $n = 245$) at DWS. As a consequence of abnormally high salinities associated with below average streamflows, *P. marinus* invaded WS in the summer of 1986 and within a year prevalence at the site was 100%. The parasite spread upriver to HH during the summer of 1987 and was first observed at DWS in the summer of 1988. *P. marinus* spread through HH and DWS more slowly than at WS, but since 1990 peak fall prevalences have ranged from 40 to 88% at DWS and from 88 to 100% at HH (Fig. 7).

In addition to affecting the local distribution and abundance of *P. marinus*, salinity also has a significant effect on *P. marinus* infection acquisition and intensity. Paynter and Burreson (1991) found that juvenile cultured oysters deployed at a low salinity site (8–10 ppt) did not acquire infections while those at moderate (12–

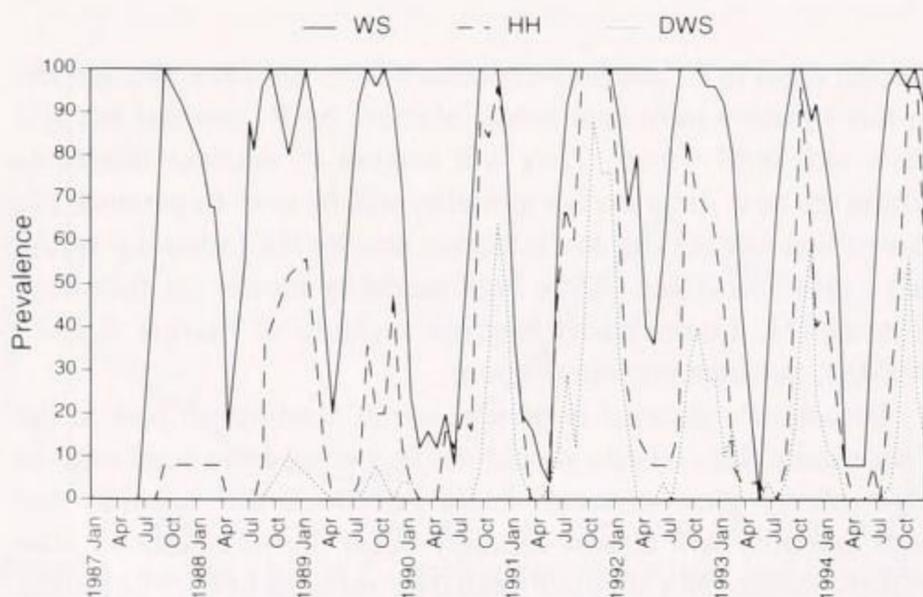


Figure 7. Prevalence of *P. marinus* in oysters sampled along a salinity gradient in the upper James River, VA. Oysters ($n = 25$) were sampled monthly from WS, HH and DWS. Diagnoses were made using standard FTM assays. Average salinities for period 1987–94 at WS, HH and DWS were, respectively, 14, 9 and 7 ppt.

15 ppt) and high (16–20 ppt) salinity sites did acquire infections. Furthermore, infection intensity at the moderate salinity site was lower than that at the high salinity site. Similarly, the limiting effect of low salinity on *P. marinus* prevalence and intensity is observed in native James River oyster populations. At WS, the area having the highest salinity, infections overwinter at a higher prevalence and intensity and increase as the water temperature warms at a much faster rate than at the lower salinity areas, HH and DWS (Fig. 8). During the summer months infections in WS oysters generally progress to moderate and heavy intensity in response to high temperatures and salinities and disease-associated mortality results. For instance, during the late summer and fall months of 1994 salinity at WS ranged from 12 to 20 ppt and moderate to heavy *P. marinus* infections were observed in 12–30% of the oysters sampled each month (Fig. 8). Prevalences and infection intensities decrease in an upriver direction from WS indicative of the limiting effect of low salinity on *P. marinus*. Generally, only a few moderate to heavy infections are observed at HH and infections at DWS rarely progress to moderate and heavy intensity. This was apparent in 1994 (Fig. 8) when summer and fall salinities ranged from 8 to 15 ppt at HH and from 5 to 12 ppt at DWS. While environmental fluctuations may alter the severity of *P. marinus* epizootics from year to year, the general trend of increasing prevalence and intensity in a downriver direction persists.

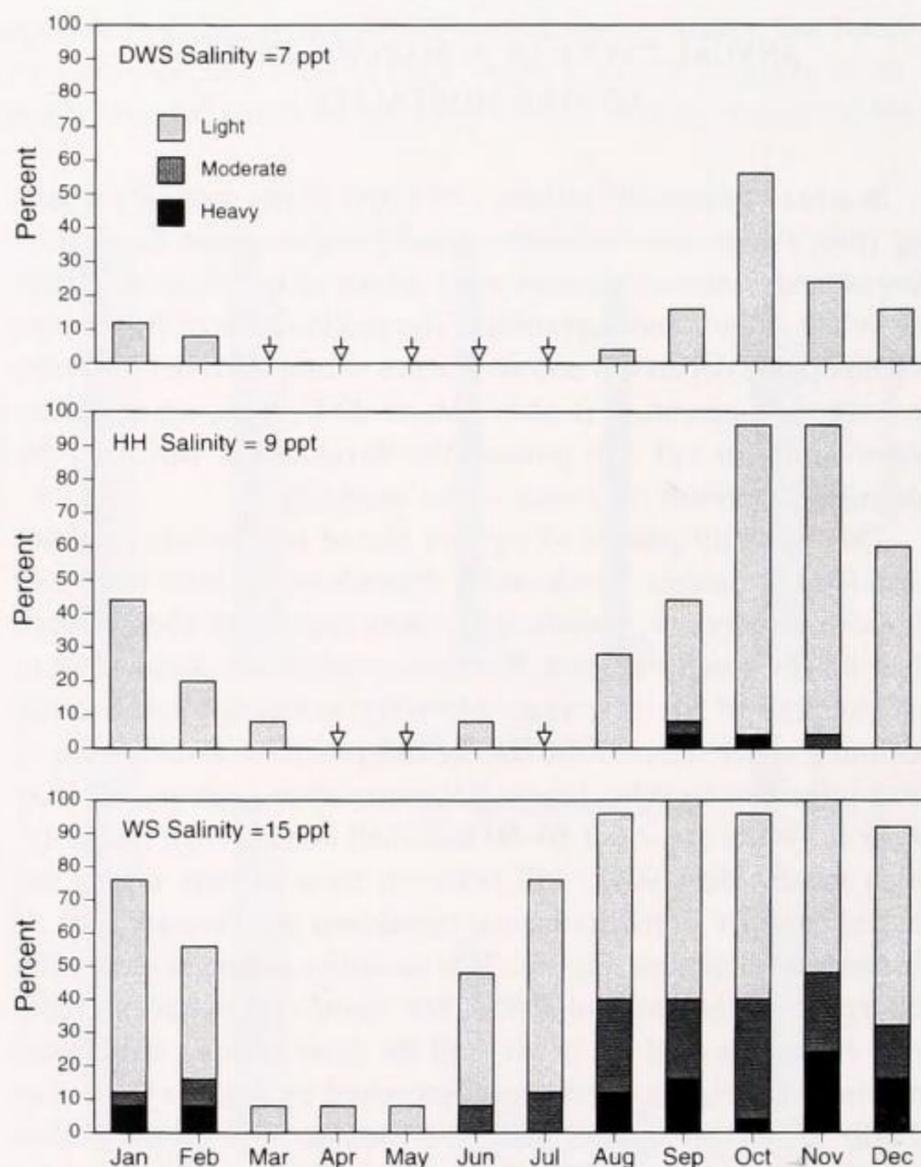


Figure 8. *P. marinus* prevalence (total bar height) and percentage of sample in each intensity category (shading) in oysters sampled along a salinity gradient in the upper James River, VA, in 1994. Oysters ($n = 25$) were sampled monthly from WS, HH and DWS. Diagnoses were made using standard Ray tissue FTM assays and infection intensity was categorized as light, moderate and heavy. Arrows indicate samples examined but no infections found. Salinity is the average for 1994 based on two to three observations per months.

Based on these studies of *P. marinus* infection patterns along the James River salinity gradient and in other Chesapeake Bay tributaries, critical salinity regimes for *P. marinus* activity can be defined. These studies indicate that: 1) if summer and fall salinities are consistently less than 9 ppt, *P. marinus* may persist but infections are limited to light intensity and no oyster mortality results; 2) if summer and fall salinities vary from 9 to 15 ppt, some infections may progress to moderate and heavy intensity, but associated oyster mortality is relatively low; and 3) if summer and fall salinities are consistently greater than 15 ppt, moderate to heavy infections may be numerous and oyster mortality may be relatively high.

Preliminary statistical analysis of the relationship of salinity and *P. marinus* infection intensity and prevalence in James River oysters was conducted using a Spearman rank correlation analysis. The analysis was based on 180 observations which included monthly determinations of prevalence for a five year period, 1990–94, at three oyster beds, WS, HH, and DWS (Fig. 3). Twenty-five oysters were collected from each site each month and examined for *P. marinus* by culturing rectal, gill, and mantle tissue in FTM following the method described by Ray (1966). Infection intensities were ranked as light, moderate, and heavy and assigned numerical values of 1, 3 and 5 according to the scale of Mackin (1962). The numerical intensity values, which included 0 for negative diagnoses, were then averaged for the determination of weighted prevalence. Salinity was recorded at each site 1–3 times each month and monthly means were determined.

The results of the correlation analysis demonstrated a highly significant ($p < 0.0001$) and strong correlation between salinity and *P. marinus* prevalence and intensity in James River oysters (Spearman rank corrected $\rho = 0.729$ and 0.727 , respectively) (Fig. 9). A subsequent linear regression analysis indicated that salinity accounts for 51% of the variability in prevalence. Only a limited number of field studies employing statistical analyses of data have been conducted. Significant positive correlations between salinity and *P. marinus* prevalence and intensity have been observed in Gulf Coast oysters (Soniati 1985, Craig et al. 1989, Soniati and Gauthier 1989) and in South Carolina oysters (Crosby and Roberts 1990). In the Gulf of Mexico, salinity (0–34 ppt) was observed to account for only 20% of the site-to-site variability in *P. marinus* infection (Craig et al. 1989) and in South Carolina salinity (29 to 35 ppt) was only weakly correlated with infection (Kendall rank tau = 0.094) (Crosby and Roberts 1990). The present analysis of the relationship between salinity and *P. marinus* activity in the James River suggests that salinity may play a more significant role in regulating *P. marinus* in the Chesapeake Bay than in more southern waters, although differences in the correlation results may also be attributed to differences in salinity regime and in experimental design, particularly sampling frequency. More rigorous statistical analysis of James River data should help to further our understanding of the role of salinity in regulating *P. marinus* prevalence and intensity.

The association of salinity with *P. marinus* prevalence and intensity has been addressed by several researchers. Mackin (1951) suggested that high flushing rates, typical in the upper reaches of estuaries, dilute infective pathogen cells thereby limiting the ability of water-borne infective stages to infect oysters. Thus, the absence of *P. marinus* from low salinity areas was attributed to the absence or scarcity of infective cells (Ray and Mackin 1954, Mackin 1956, Andrews and Hewatt 1957). Andrews (1988) related the Chesapeake Bay distribution of *P. mari-*

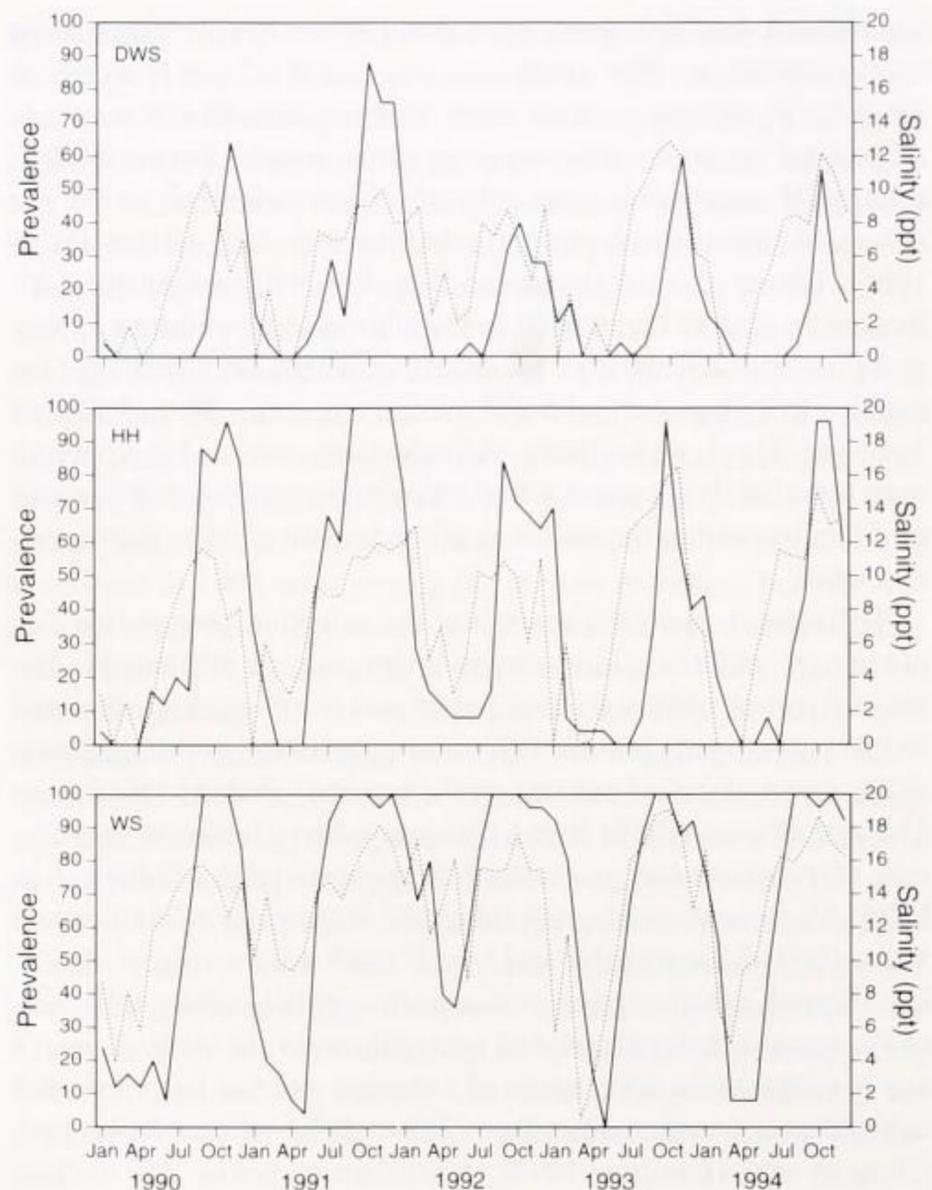


Figure 9. *P. marinus* prevalence (solid line) and mean monthly salinity (dotted line) at DWS, HH and WS, James River, VA. Oysters ($n = 25$) were sampled monthly and prevalence was determined using standard Ray tissue FTM assays. Mean monthly salinity was calculated from measurements recorded one to three times each month.

nus to the physical circulation dynamics of specific Chesapeake Bay tributaries. He asserted that large flushing-type rivers, such as the James River, are not as favorable to the pathogen as small coastal plain tributaries, such as the Choptank River, because large discharges of fresh water during the winter and spring reduce the period of salinities favorable to *P. marinus* and dilute the concentration of infective cells.

While dilution of infective particles by fresh water discharge may be an important factor influencing the distribution of *P. marinus* it is not entirely responsible for the reduced disease levels observed in low salinity areas. In recent years many laboratory investigations have documented an inhibitory effect of low salinity on various aspects of *P. marinus* epizootiology. Scott et al. (1985) found lower mortality in infected oysters held in the laboratory at 8–10 ppt than in oysters held at 21–25 ppt. Their results support the work of Ray (1954) in which *P. marinus* in artificially infected oysters tolerated low salinity (10–13.5 ppt) exposure but development of infections and subsequent mortalities of oysters were delayed relative to the high salinity (26–28 ppt) control group. Ragone and Bureson (1993) exposed naturally infected oysters from the upper James River, VA, to three low salinity treatments, 6, 9 and 12 ppt, and found no reduction in *P. marinus* prevalence at any of the treatments after eight weeks of exposure. However, development of infection was retarded at 12 ppt compared to the high salinity (20 ppt) control and infection intensity did not increase at 6 and 9 ppt. Oyster mortalities after eight weeks were

significantly less at 6 and 9 ppt than at 12 or 20 ppt, which were nearly equivalent. This study suggests that 9–12 ppt is a critical range for *P. marinus* activity supporting recent field observations. Although *P. marinus* infection progression may be limited by low salinity, *P. marinus* is quite tolerant of low salinities, unlike *H. nelsoni* which is intolerant of salinities less than 10 ppt (Ford 1985). Chu et al. (1993) succeeded in artificially establishing infections in oysters maintained in the laboratory at salinities as low as 3 ppt. Prevalences of *P. marinus* five weeks after challenge by mantle cavity injection with 10^6 meronts were 50, 70 and 82% at 3, 10 and 20 ppt, respectively. All infections observed at 3 ppt and most found at 10 ppt were of low intensity, suggesting that parasite proliferation within the host was limited relative to the high salinity control.

Differences in oyster mortality and infection progression between high and low salinity environments may be attributed to the direct effect of salinity on host and/or parasite physiology. Several in vitro investigations have helped us gain a better understanding of the direct effect of salinity on *P. marinus*. Perkins (1966) and Chu and Greene (1989) found that low salinity inhibited sporulation of prezoosporangia isolated from oyster tissue cultured in FTM. The recent, successful culture of *P. marinus* (Gauthier and Vasta 1993, Kleinschuster and Swink 1993, La Peyre et al. 1993) has allowed a more rigorous examination of the salinity tolerance of *P. marinus* in the absence of host influences. In vitro, *P. marinus* is tolerant of a wide range of salinities and has been reported to proliferate at osmolalities from 340 to 1930 mOsm (10–60 ppt) (Dungan and Hamilton 1995). Osmolalities below 340 mOsm were not tested. Maximal proliferation was observed at 790 mOsm (25 ppt) and near-maximal proliferation occurred within the range of 475–960 mOsm (15–30 ppt) (Dungan and Hamilton 1995). While cultured *P. marinus* cells exhibit growth at salinities as low as 10 ppt they are relatively intolerant of acute hypoosmotic shock. Burreson et al. (1994b) exposed *P. marinus* meronts harvested from 22 ppt culture media to 0, 3, 6, 9, 12 and 20 ppt artificial seawater. After a 24 hour exposure period at 28°C, viability was assessed using the vital stain neutral red. Percent mortality was 99% at 0 ppt, 90% at 3 ppt, 70% at 6 ppt, 43% at 9 ppt, 20% at 12 ppt and <5% at the 22 ppt control treatment. The effect of salinity on percent mortality was highly significant. When the osmotic concentration of the various seawater treatments was adjusted with sucrose to the equivalent of 22 ppt, percent mortality was low in all treatments and no different than the optimal control condition demonstrating that low salinity-induced mortality was caused by a decrease in osmotic pressure, not a decrease in sodium or another important ion. The low survival of cultured *P. marinus* cells at 6 ppt is surprising considering the documented ability of the parasite to survive in oysters at 6 ppt and 20°C for a period of eight weeks (Ragone and Burreson 1993) and may not be relevant to natural conditions in which changes in osmotic condition are likely to be more gradual and mediated by host responses.

In summary, within the Chesapeake Bay region *P. marinus* activity is greatly influenced by salinity. Prevalence and intensity of the pathogen intensify during drought years during which low stream flows cause above average salinities in upper tributary waters. In general, prevalence and intensity of *P. marinus* increase in a downriver direction. Infections are restricted to low intensity in areas consistently having salinities of less than 9 ppt, while high intensity infections and associated oyster mortality often occur during the summer and fall in areas with salinities greater than 12–15 ppt. Once established in a low salinity area the parasite

tenaciously persists and has been observed to tolerate salinities <5 ppt for a period of at least three months and to quickly respond to exposure to favorable salinities as evidenced by increases in prevalence and intensity. Laboratory studies have demonstrated that *P. marinus* survival, infection progression and pathogenicity are salinity limited, supporting recent field observations.

Temperature

Temperature appears to be the most important environmental factor affecting the large scale geographic distribution of *P. marinus* (Ray and Mackin 1954, Andrews and Hewatt 1957, Quick and Mackin 1971). The northern limit of *P. marinus* is believed to be controlled by minimum winter temperature (Andrews 1988). Maximum summer temperatures and/or the duration of temperatures above 20–25°C are probably also important, but the role of minimum or maximum temperature on the geographic distribution has not been rigorously investigated.

Within the Chesapeake Bay, seasonal temperature changes are largely responsible for the seasonal periodicity of the annual *P. marinus* cycle. Winter temperatures, which on average (1947–90) are below 5°C for eight weeks, are associated with a regression in tissue infection levels resulting in spring minimums in infection intensity and prevalence. Infections begin to intensify in late spring as water temperature exceeds about 20°C and parasite proliferation occurs (Andrews 1988). In Chesapeake Bay, temperatures favorable to parasite proliferation, >20°C, occur for about 20 weeks and temperature may exceed 25°C for a period of 10 weeks. The highest parasite prevalences and intensities are observed in September immediately following maximal summer temperatures. The occurrence of high prevalences and intensities at high temperature most likely reflects temperature associated increases in parasite multiplication rate but may also relate to temperature associated depressions in host defense capabilities and physiological condition. In high salinity environments, infections intensify to lethal levels and mortality usually occurs from August through October. Infection intensity declines as temperatures decline in winter (Figs. 10 and 11), however, the parasite is known to persist patently at temperatures as low as 0–5°C (Andrews 1988). Winter water temperature in the Bay typically averages 4–5°C, but may be as low as 1°C or less for extended periods during unusually cold winters. Body burden analysis allowed the documentation of a remarkable decline from December to May in number of meronts per gram wet weight of oyster tissue; however, prevalence remained at 90–100% (Fig. 5). These residual infections rapidly proliferate as temperatures rise in late spring.

Numerous field and laboratory experiments have focused on the relationship between temperature and *P. marinus* infection intensity and prevalence. *P. marinus* infection in South Carolina oysters was significantly but weakly correlated with temperature (Kendall rank correlation coefficient = 0.283) (Crosby and Roberts 1990); temperature explained 16.7% of the variability in infection intensity. This result contrasts with those of Burrell et al. (1984) and Craig et al. (1989) in which no statistically significant relationship between temperature and intensity was found in South Carolina and Gulf of Mexico oysters, respectively. Differences in these results have been attributed to differences in frequency and interpolation of temperature measures (Crosby and Roberts 1990).

In the James River tributary of the Chesapeake Bay, *P. marinus* intensity and prevalence clearly follow seasonal fluctuations in water temperature (Fig. 10). Preliminary statistical examination of

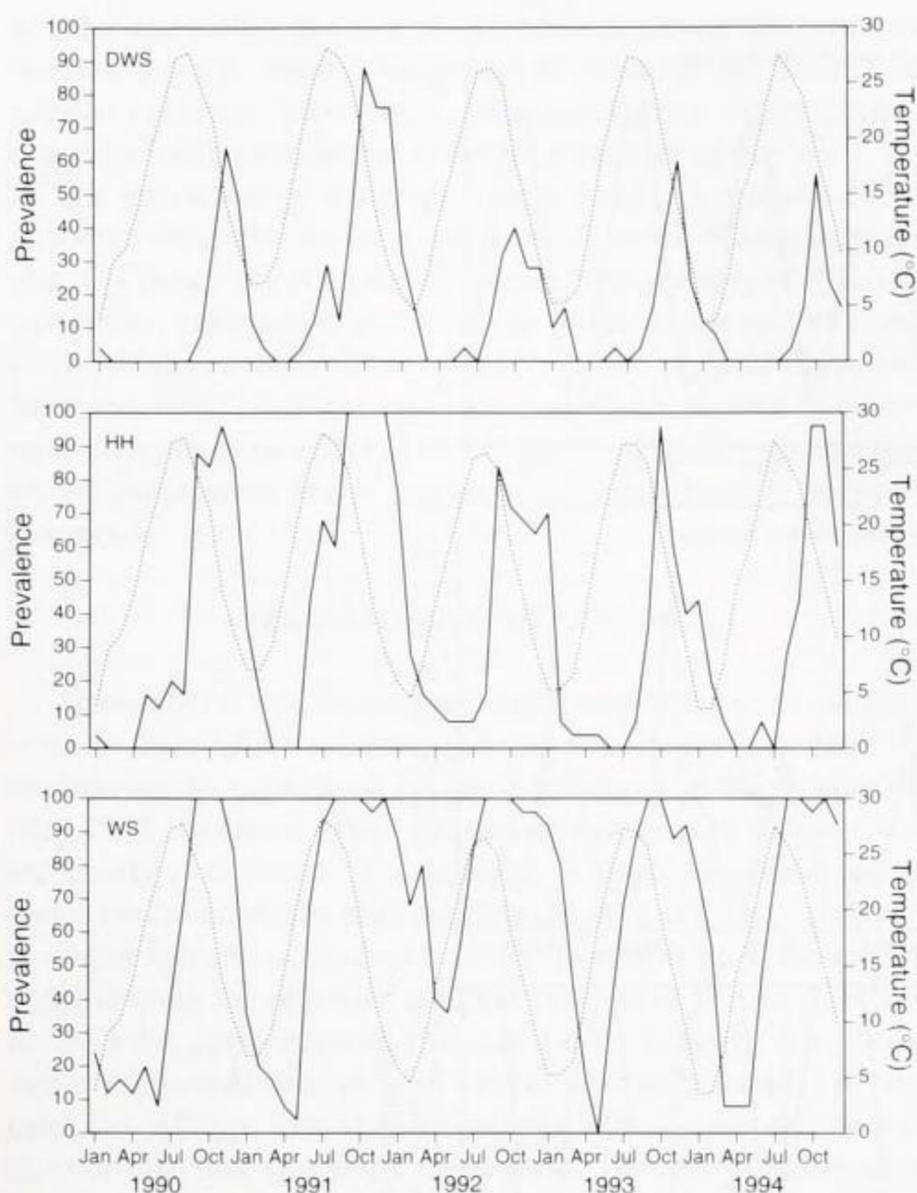


Figure 10. *P. marinus* prevalence (solid line) and mean monthly water temperature (dotted line) at DWS, HH and WS, James River, VA. Oysters ($n = 25$) were sampled monthly and prevalence was determined using standard FTM assays. Mean monthly temperature was calculated from temperature measurements recorded at six minute intervals at the VIMS York River monitoring station.

this relationship was conducted using a Spearman rank correlation analysis. Data for *P. marinus* are the same as described for the statistical analyses of salinity relationships. Mean monthly water temperature was calculated from water temperatures recorded at six minute intervals by a continuous metering system at the VIMS York River monitoring station.

The initial correlation analysis failed to find a statistically significant relationship between mean monthly water temperature and *P. marinus* prevalence or intensity ($p > 0.05$). This result agrees with the findings of Soniat (1985) and Craig et al. (1989) and contrasts with the findings of Crosby and Roberts (1990) which indicated a statistically significant but weak correlation between water temperature and *P. marinus* intensity. However, when the James River data set was reanalyzed with temperature lagged by two to four months, a significant correlation between water temperature and *P. marinus* prevalence and intensity was found. The relationship was strongest when the temperature was lagged three months (eg. April prevalence and January temperature); water temperature was strongly and significantly correlated with both prevalence (Spearman $\rho = 0.704$, $p < 0.001$) and weighted prevalence (Spearman $\rho = 0.706$, $p < 0.001$). Regression analysis indicated that when lagged three months temperature explained 39% of the variability in prevalence and 46% of the variability in weighted prevalence.

The contribution of temperature to the year-to-year variability in *P. marinus* activity is not well understood. Minimum winter

temperature, while thought to control the geographic distribution of the pathogen, is not clearly associated with year-to-year variability of *P. marinus* epizootics in the Chesapeake Bay. During an eight year (1987–94) monthly parasite survey of James River oysters (described above, see salinity discussion) extreme above and below average fluctuations in winter temperature were observed. The relationship of these temperature fluctuations to the subsequent summer epizootics is somewhat obscure, in part because it is difficult to separate the effects of salinity fluctuations from temperature fluctuations. The coldest winters in terms of average winter temperature and duration of weekly average temperatures below 5°C were the winters of 1987–88 and 1993–94 (Fig. 11). Regardless of the cold winter, subsequent summer prevalences and intensities in 1988 were among the highest recorded, but this was also an abnormally dry year. In 1994 winter water temperatures were below 5°C for a period of eight weeks and $1\text{--}2^{\circ}\text{C}$ below the long term average for six of the eight weeks. This unusually cold temperature regime seemed to have little negative impact on *P. marinus* as 1994 had the third highest average summer intensity and prevalence was still greater than 96% at WS for a five month period from August through December (Fig. 11). During the winter of 1989–90 record low temperatures were observed in December but after the first week of January water temperatures were generally above average. The low December temperatures may have contributed to the relatively early decline of overwintering infections and to the relatively slow rise in prevalence during the summer; however, salinity was also relatively low during the period.

Abnormally warm winter temperatures may have a more significant impact on *P. marinus* activity than abnormally cold temperatures. The winter of 1991 was the warmest winter during our survey period (Fig. 11). Mean weekly temperature never went below $5\text{--}6^{\circ}\text{C}$ during the winter and temperatures were $1\text{--}3^{\circ}\text{C}$ above the long term average throughout the year. Overwintering prevalences were low, but prevalence rapidly increased with the onset of warm summer temperatures and remained above 90% for six months at WS (Fig. 11), making 1991 the worst year in terms of average summer prevalence. The cool fall of 1990 and low 1990 prevalences may be responsible for the low 1990–91 overwintering levels, while the abnormally warm winter water temperatures combined with dry conditions probably caused high summer prevalences in 1991. However, 1991 summer temperatures were also above average and probably also contributed to the high summer parasite level (Fig. 11).

The association between temperature and *P. marinus* infection has been the focus of several laboratory investigations. Andrews and Hewatt (1957) reported that at 15°C the development of established infections was retarded and new infections did not appear. Similarly Fisher et al. (1992) found infection progression and oyster mortality were reduced in oysters held at 18°C compared to those held at 27°C . More recently, Chu and La Peyre (1993) exposed oysters held at 10, 15, 20 and 25°C to *P. marinus* through mantle cavity injections of 10^6 meronts obtained from infected oyster tissue. Infections were observed in oysters from all treatment groups; however, prevalence declined with decreasing temperature and moderate and heavy infections were only observed in oysters at 20 and 25°C . Forty-six days after challenge *P. marinus* prevalence was 23% at 10°C , 46% at 15°C , 91% at 20°C and 100% at 25°C .

The influence of temperature on *P. marinus* infection intensity and prevalence may relate to host and/or parasite activity. Both

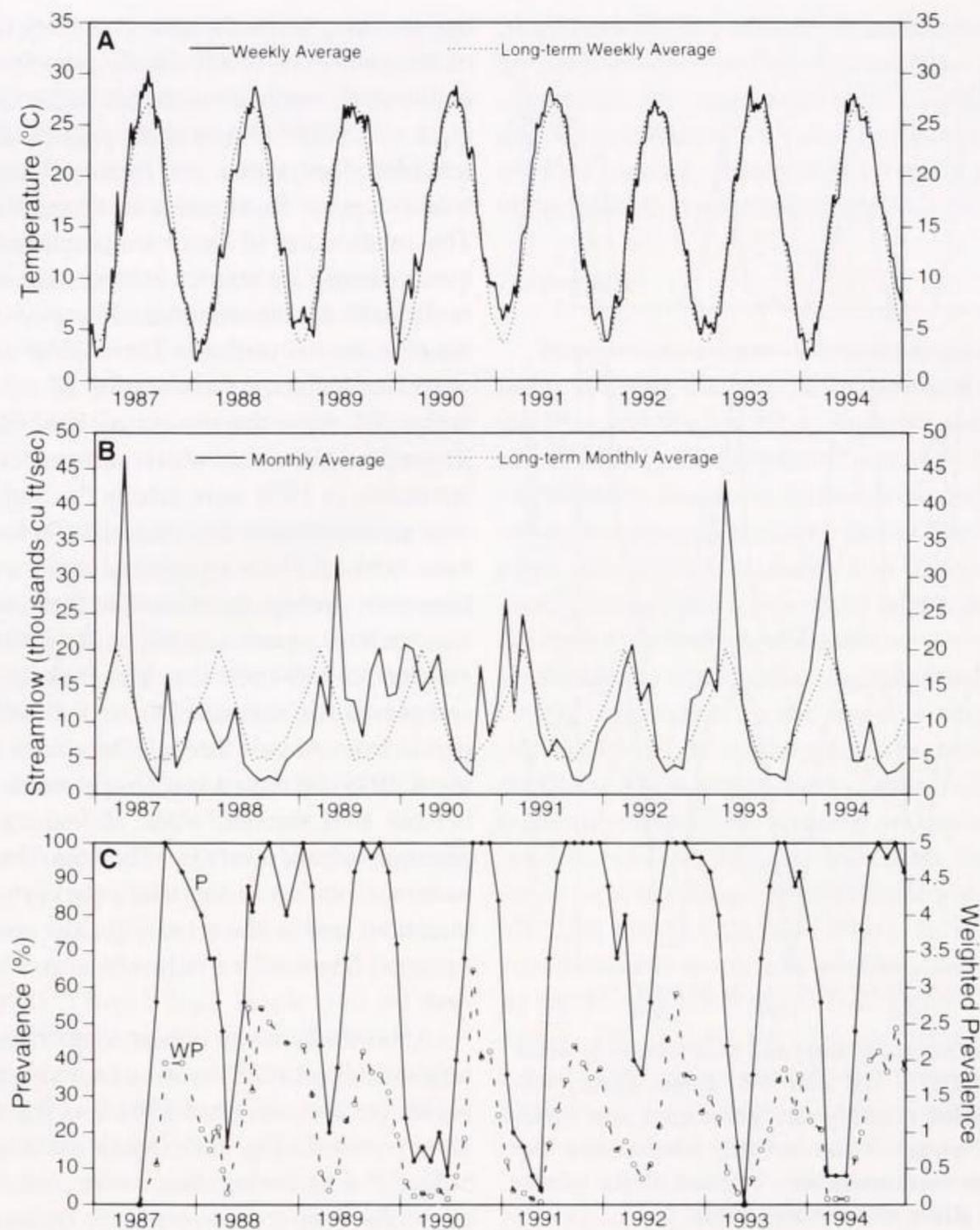


Figure 11. Year-to-year variability of water temperature (A), James River streamflow (B), and prevalence (P) and weighted prevalence (WP) of *P. marinus* (C). In Panel A, weekly temperature averages for each year are contrasted with the long term mean weekly temperature for the period 1947–94. In Panel B, monthly streamflow averages for each year are contrasted with the long term mean monthly streamflow for the period 1951–95. Water temperature is from the continuous record of the VIMS York River Monitoring Program; streamflow data were obtained from the U.S. Geological Survey.

cellular and humoral oyster defense activities have been shown to be affected by environmental temperature (Fisher 1988, Chu and La Peyre 1989, Chu and La Peyre 1993). Unfortunately, the role of these putative oyster defense activities in combating *P. marinus* remains speculative. In vitro culture of *P. marinus* has afforded an opportunity for analysis of temperature effects on the parasite in the absence of host influences. Proliferation of *P. marinus* in culture was near maximal at room temperatures between 15 and 35°C and optimal at 35°C. Minimal proliferation occurred at 10 and 40°C, and no proliferation occurred at 4°C (Dungan and Hamilton 1995). In a study conducted by Burreson et al. (1994b) cultured *P. marinus* cells were quite tolerant of a 24 hour exposure to temperatures as low as 1°C at high salinity (22 ppt). Survival at 1 and 5°C treatments was greater than 90% based on a vital stain assay and did not significantly differ from that at 10, 15 and 28°C treatments (Burreson et al. 1994b). Temperature appeared to have a greater effect when exposed cells were in lower salinity conditions. A general trend of higher mortality at lower temperature was observed at 6, 9 and 12 ppt, however, the temperature effect was only significant at 9 ppt. It was suggested that cold temperatures may inhibit metabolic processes such as free amino acid release

which may enable some cells to survive at lower salinities. Prior to the culture of *P. marinus*, in vitro studies on the effect of temperature were limited to the assessment of sporulation of presporangia isolated from thioglycollate-incubated infected tissue. Viability was determined by the presence of motile spores within sporangia. Sporulation was optimal at 25–35°C (22 ppt) (Perkins 1966). Both the maximum percent sporulation and the rate of sporulation were greatly reduced at 20°C (60% of optimal) and no sporulation occurred at temperatures less than 18°C (Perkins 1966). In a similar experiment Chu and Greene (1989) observed that prezoosporangia survived at 4°C for up to four days but did not survive below 0°C for one day.

In summary, it appears that in the Chesapeake Bay region *P. marinus* activity and annual periodicity are largely controlled by seasonal temperature fluctuations. This conclusion is supported by a strong statistically significant correlation between temperature and *P. marinus* prevalence and intensity. It is difficult to precisely define the effect of temperature on year-to-year variability of *P. marinus* infections based on analyses conducted to date. However some trends are apparent. Abnormally cold winter temperatures may hasten the decline in infection intensity during the winter

months and delay the rise in prevalence during the summer months, but they have little impact in reducing the severity of summer epizootics. Conversely, abnormally warm winter temperatures probably increase the severity of summer epizootics.

The interaction of temperature and salinity is probably more important than either factor acting alone. Recent evidence suggests that, in Chesapeake Bay, the prevalence and intensity of *P. marinus* decline much more rapidly during winter in low salinity areas (<10 ppt) than in high salinity areas (>18 ppt) (Ragone Calvo and Burreson 1994). Laboratory investigations are needed to determine the synergistic effect of temperature and salinity fluctuations on the progression and/or regression of established *P. marinus* infections.

TRANSMISSION DYNAMICS

Although it is well documented that transmission of *P. marinus* is direct from oyster to oyster and that any life cycle stage of *P. marinus* seems capable of initiating infections in the laboratory (Ray 1954, Andrews 1988), the natural dynamics of transmission are poorly understood. Transmission is dose dependent and it seems to take unusually high concentrations of any life cycle stage to initiate infections (Andrews 1988). Transmission is thought to occur through the digestive tract because initial foci of infection occur in the gut epithelium (Mackin 1951), although this observation needs confirmation with careful laboratory studies. In any case, the cell type that actually initiates infection and the mechanism of infection are poorly understood. The role of flagellated, free-swimming zoospores in initiating infections in nature is especially problematic. Zoospores certainly don't seem to be required for initiation of infections, as infections result from exposure to isolated meronts or even minced, infected oyster tissue. However, the transformation that may occur after merozoites or minced tissue are added to an aquarium or injected into the mantle cavity of an oyster are unknown. The occurrence of early infections in the stomach epithelium suggests ingestion of infective stages, not penetration of gill or mantle by zoospores. But perhaps zoosporulation occurs in the gut lumen and released zoospores penetrate in localized areas of the gut epithelium. It appears that a very high dosage of zoospores, on the order of 1×10^5 , is required to initiate an infection (Andrews 1988). This dose seems high for an efficient parasite but may be an artifact of the experimental designs employed. Zoospores must have some function or their production wouldn't have evolved. Maybe they are a dispersal mechanism and are only produced in nature under certain conditions that are not presently understood.

Inoculation of non-zoospore stages into the mantle cavity of oysters has demonstrated that meronts produce higher prevalences and higher intensities of infection than prezoosporangia (Voley and Chu 1994), but the pattern and process of infection were not followed in these studies. A high proportion of *P. marinus* cells in an oyster occur within host hemocytes and it has been proposed that hemocytes that scavenge the epithelial surface of the oyster gut lumen phagocytose ingested *P. marinus* cells and then migrated through the epithelial layer and into the oyster carrying the parasite with them. An innovative study with intubated fluorescent polystyrene beads has demonstrated that such events do occur (Alvarez et al. 1992). Once inside an oyster and under favorable environmental conditions, *P. marinus* multiplies within hemocytes, eventually killing the hemocyte and releasing the *P. marinus* cells. These cells are phagocytosed by other hemocytes and

the cycle repeats; eventually pathogen cells are carried throughout the oyster. The developmental cycle of *P. marinus* within oysters is relatively well understood and recently has been reviewed by Perkins (1991, 1993), but studies that examine the initial infection process in oysters are critically needed.

Even though it is known that transmission is direct, in nature there is a poor understanding of the source of infective stages, the dose required to initiate infections and the duration of the infection window. The prevailing conceptual model is that transmission occurs during periods of high oyster mortality in summer and early fall as infective *P. marinus* cells are disseminated upon death and decomposition of infected oysters (Andrews 1988). However, dead, gaping oysters are consumed rapidly by scavengers (Hoese 1964) and probably don't decompose naturally and release *P. marinus* cells into the water. The parasite can survive passage through the gut of scavengers (Hoese 1964), but the role of scavengers in spreading infections is unclear. In the Gulf of Mexico, transmission of *P. marinus* can occur via the ectoparasitic snail *Boonea impressa* (White et al. 1987), but for the Chesapeake Bay no vectors have been identified. Dissemination of *P. marinus* in fecal matter from live oysters seems likely, given the destruction of gut epithelium observed in live, heavily infected oysters, but is poorly documented. Mackin (1962) proposed that *P. marinus* overwinters as a free hypnozoospore in the sediment and that annual epizootics are initiated by release of infective cells in the spring. Andrews (1988) countered that if this were true, imported uninfected oysters should develop infections in June or early July rather than late July or August as he observed. Nevertheless, the presence of a saprobic stage should not be ruled out. Recently, flow cytometric techniques have been developed that may allow quantification of disseminated *P. marinus* cells in the water column (Roberson et al. 1993). Such data should provide insight into seasonality of infection pressure.

Field experiments to assess the timing of infections are underway at VIMS. Separate groups of uninfected oysters are being exposed in the lower York River for two week periods throughout the year and are then warmed in the laboratory for four weeks to allow infections to develop to detectable levels. Results for 1994 indicate that the highest infection pressure occurs during the last two weeks of August and the first two weeks of September (Fig. 12), a period that corresponds closely with maximum oyster mortality; however, some infections were acquired as early as late June.

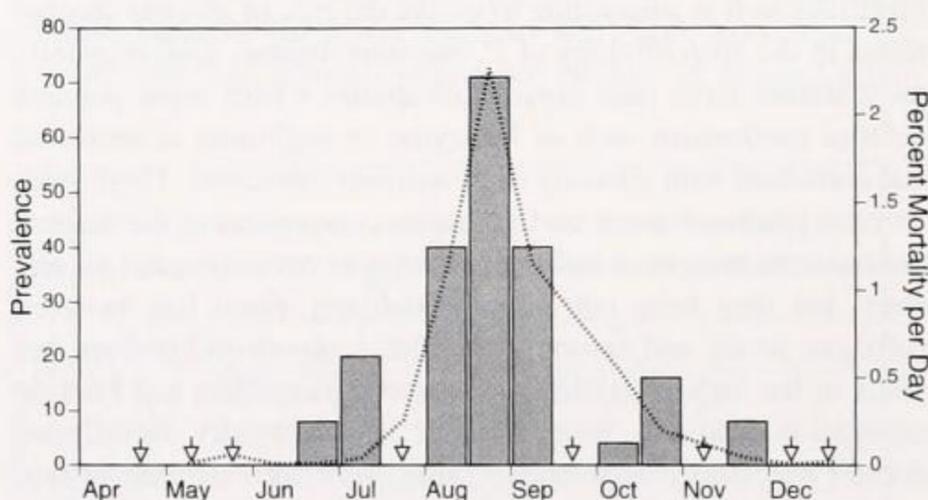


Figure 12. *P. marinus* infection acquisition (bars) in uninfected sentinel oysters deployed for two week periods in the lower York River, VA, and percent mortality per day of local infected oysters (line). Bars represent the prevalence of infection in sentinel oysters as determined by total body burden assays. Arrows indicate no new infections during the period.

There has not been much laboratory research conducted on the effect of environmental variables on transmission dynamics, but meaningful experiments are difficult to perform in the laboratory because of the difficulty of simulating natural conditions and the artificial nature of the challenge used in most experiments. In experiments where *P. marinus* meronts were injected into the mantle cavity of oysters held at various temperatures and salinities, transmission did occur at temperatures as low as 10°C (salinity = 17.5 ppt) and salinities as low as 3 ppt (temperature = 21.0°C) (Chu and La Peyre 1993, Chu et al. 1993). These results clearly show that infection by *P. marinus* is possible at low temperature and low salinity conditions. However, there is little evidence that infections occur under these conditions in nature, probably because of an absence of infective cells in the water or low oyster filtration rates. Andrews and Hewatt (1957) found that new infections were not acquired in the field at salinities ranging from 1 to 13 ppt and Paynter and Bureson (1991) found no infection acquisition in the field at salinities ranging from 8 to 12 ppt. These results suggest that although it is possible for *P. marinus* to infect oysters at relatively low temperature and salinity conditions, such transmission probably does not occur in nature. However, it must be remembered that diagnosis during these studies was by routine FTM assay; more sensitive diagnostic techniques may yield different conclusions.

Movement of *P. marinus* into historically low salinity areas occurred during drought periods when salinities were elevated. Now that the pathogen is present and persisting on all oyster beds it is important to determine if transmission is occurring in areas where salinity has returned to more normal conditions. Field studies using uninfected sentinel oysters in low salinity areas are probably the best method to determine whether transmission is occurring in low salinity areas. Other critical research areas for transmission dynamics include elucidation of the early infection process including cell type and infection site, timing of infections in nature and the role of environmental variables.

THE ROLE OF OYSTER DEFENSE MECHANISMS

Considering all of the research that has been conducted on the role of oyster defense mechanisms in controlling *P. marinus* infections it is perhaps surprising how little is known about the topic. If oyster defense capabilities do control levels of *P. marinus* in oysters, the components and mechanisms involved have not been identified so it is impossible to assess the role of defense mechanisms in the epizootiology of *P. marinus* disease. Unfortunately, most studies have been correlative studies where some putative defense mechanism such as lysozyme or agglutinin is measured and correlated with intensity of *P. marinus* infections. These studies have produced much useful data on components of the defense mechanisms in oysters and their relation to environmental parameters, but they have not demonstrated any direct link between pathogen levels and serum or cellular components, perhaps because of the high variability of measured parameters and because correlation analysis, even when it is statistically significant, doesn't necessarily demonstrate cause and effect. Because of typical highly variable results, investigators have been reluctant to rule out any component, but recently Chintala et al. (1994) have demonstrated, and clearly stated, that the particular serum agglutinins studied play no role in defense against *P. marinus*.

More innovative, directed studies are needed to determine the role of the various hemolymph components that have been postu-

lated as important in defense against *P. marinus*. Experimental manipulation of the defense components and subsequent monitoring of infection progression, compared to untreated control oysters, should shed light on the role of specific hemolymph components. For example, employing monoclonal antibodies as blocking agents of putative defense components, passive transfer of purified components into oysters or incubation of *P. marinus* with serum components prior to injection into oysters may enable determination of the roles of these components. Unfortunately, much preliminary research may have to be done before meaningful studies can be conducted.

One critical need is to determine how *P. marinus* avoids intracellular killing by hemocytes. Although *P. marinus* is not an obligate intracellular parasite, most cells in oysters are found within hemocytes. Obviously, hemocytes recognize the parasite as foreign and phagocytose individual cells. However, there seems to be no intracellular killing of the parasite or at least the multiplication ability of the parasite during summer far outweighs any killing. Rather, the parasite multiplies within hemocytes and eventually bursts the cell membrane releasing more individual cells that get phagocytosed by other hemocytes and carried throughout the oyster in hemolymph. Oyster hemocytes are known to produce the typical free oxygen radicals (ROIs) involved in intracellular killing by vertebrate phagocytes (Anderson et al. 1992, Anderson 1994) but they seem to be ineffective against *P. marinus*, at least during periods of active parasite multiplication, possibly because *P. marinus* suppresses ROI production (Volety and Chu 1995).

THE ROLE OF POLLUTION AND WATER QUALITY IN *P. MARINUS* ABUNDANCE

One of the questions most often asked of oyster disease researchers concerns the role of declining water quality in the increase of oyster diseases during the recent past. Most oystermen and many of the lay public consider pollution to be a critical factor in disease processes and blame it solely for the increase in oyster diseases. However, there is little evidence to support their claim. In the Chesapeake Bay there is no correlation between water quality or the level of pollution and disease abundance. Abundance of *P. marinus* is just as high in relatively unpolluted areas as in polluted areas of equivalent salinity. For example, Tangier Sound is one of the areas in Maryland hardest hit by oyster diseases and it was characterized by the EPA Chesapeake Bay Study as having the best water quality in Maryland. Similarly in Virginia the abundance of oyster pathogens is high wherever salinity is favorable and many of the areas where oysters were decimated by disease, such as Pocomoke Sound, are relatively pristine.

Pathogen abundance clearly correlates with salinity levels and the dramatic increase in abundance in the late 1980s can be explained by drought conditions and resulting increased salinity with concomitant warm winters as a secondary factor. As discussed in earlier portions of this review, a variety of laboratory studies and field observations support the primary role of salinity and temperature in modulating *P. marinus* abundance.

Nonetheless, even relatively unpolluted areas today are not as pristine as they were even 20 years ago so some subtle effects of pollution or water quality cannot be completely ruled out. Pollution effects are known to modulate host defense mechanisms in aquatic vertebrates (Anderson 1990), but since the role of oyster defense mechanisms, if any, in controlling *P. marinus* infections is not understood, it cannot be concluded that pollution suppresses the oyster's ability to inhibit the pathogen.

Although pollution clearly is not one of the primary factors responsible for recent increases of oyster diseases, there has been very little research on the potential subtle effects of toxicants on *P. marinus* disease progression. Only recently have studies been completed that suggest some effect of toxicants on *P. marinus* disease development. Winstead and Couch (1988) reported rapid proliferation of *P. marinus* in oysters exposed to high concentrations (600 mg l^{-1}) of the carcinogen *n*-nitrosodiethylamine when compared with unexposed control oysters. Chu and Hale (1994) found elevated *P. marinus* prevalence in oysters exposed to water soluble fractions derived from estuarine sediments grossly contaminated with polycyclic aromatic hydrocarbons and then challenged with *P. marinus*. These results suggest some effect of these chemicals on either pathogen multiplication or host condition or defense mechanisms, but it is difficult to determine the environmental relevance of these studies because it is unclear how the concentrations utilized in the experiments compare to actual levels of these compounds found in the water column in nature.

Tributyltin (TBT) has also been shown to enhance *P. marinus* disease progression and increase cumulative oyster mortality during experiments using environmentally relevant levels of TBT. Maximum prevalence and intensity levels of *P. marinus* occurred sooner in oysters exposed to 100 ppb TBT for five months and exposed to *P. marinus* after one month than in oysters not exposed to TBT and infected with *P. marinus* similarly (Anderson et al. 1995). In addition, mortality was higher in oysters that were both exposed to TBT and also infected with *P. marinus* than in oysters either infected but unexposed or exposed but uninfected. Similar results were obtained in experiments using 30 and 80 ppb TBT (Fisher et al. 1995) although the experimental design was somewhat different from that of Anderson et al. (1995).

These studies suggest that environmental toxicants may have some effect on disease development in highly polluted areas, but as the authors emphasize, there is no evidence that the dramatic increase in abundance of *P. marinus* since 1985 is the result of increased environmental pollution. Undoubtedly, further research will better clarify the subtle interactions among oysters, disease agents and environmental contaminants.

OTHER FACTORS INFLUENCING THE EPIZOOTIOLOGY OF *P. MARINUS*

There are other factors that potentially may influence the epizootiology of *P. marinus* in the Chesapeake Bay, but little research has been done that is specific to the Bay. For example, recent modelling studies in the Gulf of Mexico (Powell et al. 1996) suggest that timing of food availability is important to enable oysters to outgrow the parasite (Soniati 1996). Because of the longer growing season in the Gulf of Mexico than in the Chesapeake Bay, nutrition may be more critical in the Gulf of Mexico than in the Bay. Another factor that potentially may influence the epizootiology of *P. marinus* disease in Chesapeake Bay is the well-documented summer hypoxia, but no research has been done on this interaction.

It is well known that many other species of molluscs in Chesapeake Bay harbor cells of *Perkinsus* sp. (Andrews 1954). The taxonomic status of *Perkinsus* in these other hosts has not been clarified, but if they are *P. marinus* then these other molluscs could serve as reservoir hosts for the pathogen. The significance of putative reservoir hosts in the epizootiology of *P. marinus* disease is unknown. Studies are needed to determine if lethal *P. marinus*

infections can be induced in oysters using *Perkinsus* cells isolated from other mollusc species.

EFFECT OF *P. MARINUS* ON THE OYSTER RESOURCE OF CHESAPEAKE BAY

Prior to 1985 *P. marinus* had little significant impact on the Maryland oyster industry because the pathogen was uncommon in Maryland. There were localized foci of infected oysters in the St. Mary's River in the 1960s and high mortality had decimated the local population by the late 1970s. The parasite was reported in Fishing Bay in the 1970s and in Eastern Bay in 1981. In Virginia, where *P. marinus* historically was restricted to the lower Bay and mouths of major tributaries, significant, but tolerable, oyster mortality occurred in these areas, especially in high salinity areas or during dry years. Nonetheless, harvest in Maryland and Virginia varied between 2 and 3.5 million bushels annually during the 1930s, 1940s and 1950s (Fig. 13). In Maryland the harvest was primarily from public oyster beds, but in Virginia over 80% of the harvest came from private planters who planted disease-free seed oysters from the Upper James River to growout grounds in the lower Bay areas of Mobjack Bay and Hampton Roads. Productive public beds occurred in the York River and Rappahannock River, Pocomoc Sound and various small tributaries along the western shore of the Bay (Andrews 1988). Typically, private planters in Virginia held seed oysters for three years on growout grounds, but as knowledge of *P. marinus* epizootiology increased and it was learned that most mortality occurred during the third year, planters began harvesting after only two years of growout (Andrews 1988). This early-harvest disease-avoidance strategy worked well during the late 1950s and annual harvest in Virginia varied from 3 to 4 million bushels during that period.

The sudden epizootic of *H. nelsoni* (MSX) in Mobjack Bay beginning in 1959 (Haskin and Andrews 1988) and the resulting high oyster mortality caused private planters to eventually abandon the traditional growout areas in the lower Bay by the mid-1960s and move operations to lower salinity areas in the Rappahannock River and small tributaries along the south shore of the Potomac River such as the Coan and Yeocomico Rivers. The 1970s were generally wet and levels of both *H. nelsoni* and *P. marinus* were reduced; from about 1967 through 1981 oyster harvest in Virginia was more or less stable at about 1 million bushels annually (Fig. 13)—greatly reduced from pre-1960 levels because of reduced growout acreage resulting from abandonment of the traditional growout areas in the lower Bay. In Maryland, good spat sets coupled with low pathogen abundance because of reduced salini-

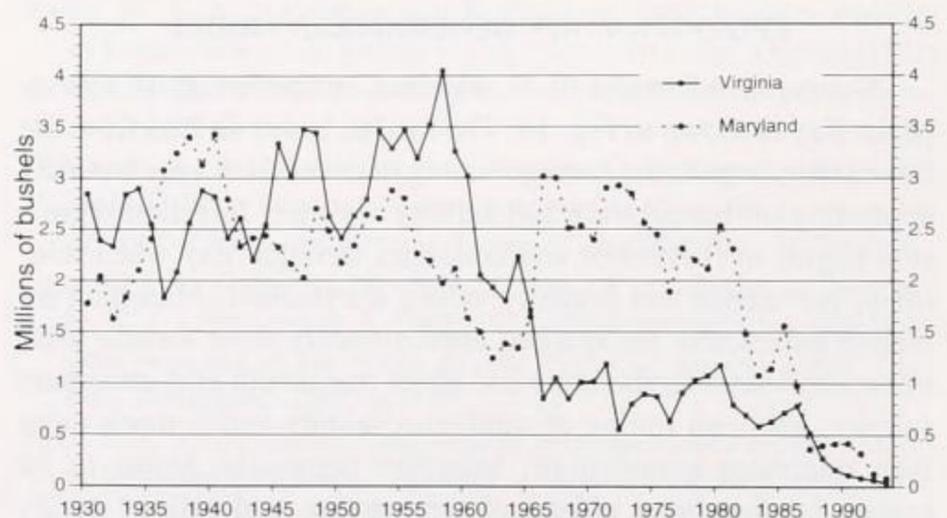


Figure 13. Annual market oyster landings in Chesapeake Bay from 1930 to 1994.

ties during the 1970s produced harvests between 2 and 3 million bushels annually during the 1970s (Fig. 13).

Both 1980 and 1981 were dry years; *P. marinus* intensified and *H. nelsoni* spread into Maryland for only the second time since it first appeared in the Bay in 1959. Mortality was high in areas where salinity was favorable for the pathogens and oyster landings declined in both states from 1982 through 1984.

The four consecutive drought years from 1985 through 1988 were catastrophic for oyster resources in both Maryland and Virginia. As outlined above, *P. marinus* spread to most oyster growing areas of the Chesapeake Bay including Maryland either by natural processes or by movement of infected oysters. Oysters were highly susceptible to *P. marinus* and mortality was high on planted grounds in Virginia and on public beds in both Virginia and Maryland. *H. nelsoni* also intensified during 1987 and 1988 and contributed substantially to the mortality in both states. The end result in Virginia was the virtual elimination of oysters from public beds in the lower Bay and from all but the uppermost reaches of the major tributaries. Current estimates are that less than 5% of traditional public oyster beds in Virginia are productive and these are all in the upper James River. Public beds were depleted by disease, and private planters, fearing losses from planting infected seed oysters, were (and still are) reluctant to transplant oysters for growout. Because of the absence of oysters in other areas of the lower Bay, harvesting pressure increased significantly in the upper James River, VA, beginning in 1986. Annual harvest increased in Virginia during 1986 and 1987 (Fig. 13) because of the large numbers of oysters in the upper James River, but the stocks were rapidly fished out and harvest plummeted in subsequent years. Since 1988, over 95% of the public market-oyster harvest in Virginia has come from the James River. Although there have been some restrictions placed on harvesting in Virginia, no quotas have been adhered to and remaining critical broodstocks in Virginia are being fished heavily. In Maryland, approximately 79% of the harvest during 1993–94 came from areas north of the Bay Bridge and 66% of the harvest came from the Chester River. As a comparison, during the 1973–74 season in Maryland, only 2.1% of the harvest came from the Chester River. These harvest figures from low salinity areas in both Virginia and Maryland demonstrate the impact of disease on the oyster resource in high salinity areas of the Chesapeake Bay.

The oyster resource rebounded in Maryland during 1994–95 and harvest increased for the first time in three years. Wet springs during 1993–94 resulted in lower salinity in Maryland, reduced levels of *P. marinus* and increased oyster survival in high salinity. In Virginia, no improvement was observed during 1994–95.

EPIZOOTIOLOGY GENERALIZED MODEL

A generalized model of *P. marinus* epizootiology in Chesapeake Bay is shown in Fig. 14. The model, based on data from the last decade, represents average timing of events that vary annually depending on temperature and salinity regimes. Infection regression begins in November and continues through May when minimum prevalence and intensity values are reached. Minimum infection parameters are reached approximately three months after minimum winter temperature and about one month after minimum salinity, although timing of minimum salinity varies much more than minimum temperature. Infection regression seems to be caused by the direct effects of temperature and salinity on *P. marinus* survival. The role of oyster defense mechanisms in infection regression is unknown but cannot be ruled out.

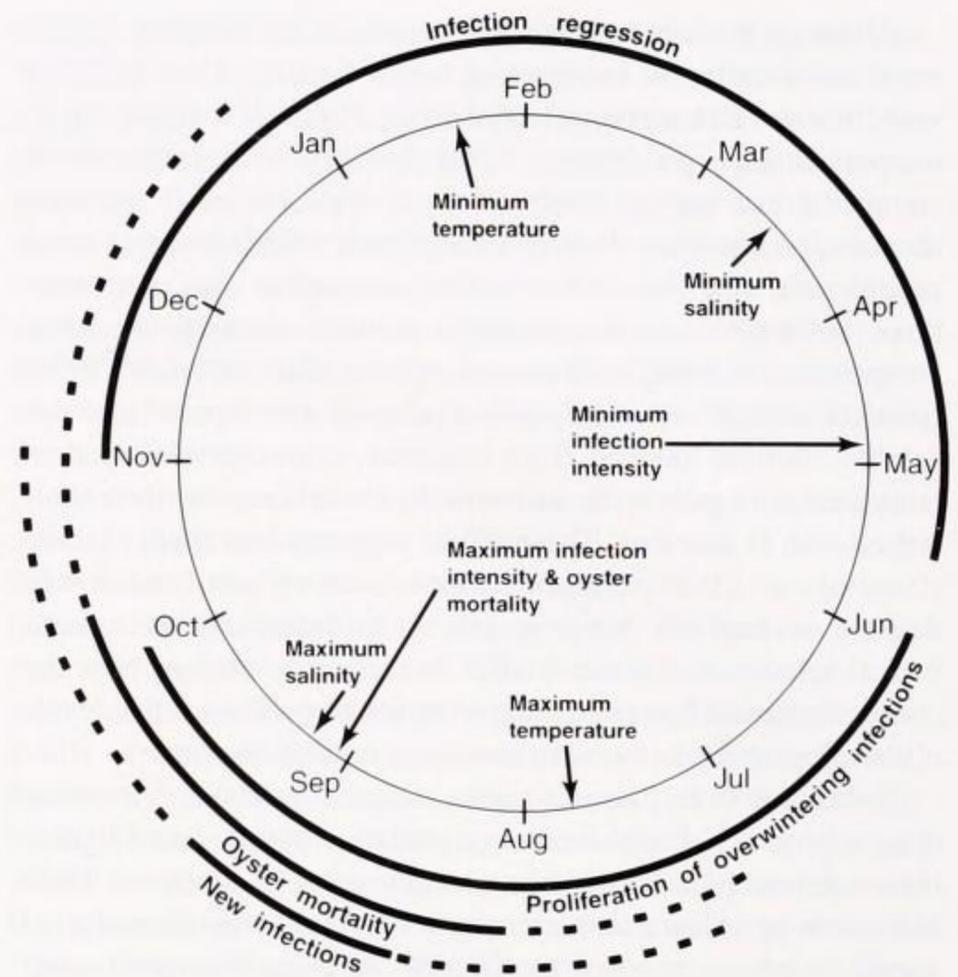


Figure 14. Generalized summary of *P. marinus* epizootiology in Chesapeake Bay. Dashed lines represent months of reduced activity compared to months represented by solid lines.

Infection prevalence and intensity begin to increase in June as water temperature increases above 20°C and overwintering infections begin to proliferate. Increase in prevalence and intensity from June through most of August seems to be due almost entirely to the proliferation of overwintering infections. Infection proliferation probably continues until October in oysters that don't die from the disease. Maximum prevalence and intensity occur in September, approximately six weeks after maximum water temperature, if salinity is greater than about 15 ppt, but values may peak one or two months later in lower salinity. Maximum values reached depend on the salinity regime.

A warm spring allows early proliferation of overwintering infections and oyster mortality may begin by early July, but under typical conditions most oysters die in August and September. Some mortality may continue at a reduced level until January or even later depending on fall temperatures. Total oyster mortality depends on the temperature and salinity regime and on the infection history.

New *P. marinus* infections are acquired by oysters shortly after oyster mortality commences and the correlation between new infections and oyster mortality suggests that the dying oysters are the source of infective stages. Most new infections are acquired during the last two weeks of August and the first two weeks of September, corresponding with the period of greatest oyster mortality. Because temperatures are greater than 25°C during this period and infections develop rapidly, there may be one or more cycles of oyster mortality/new infections/proliferation between late July and early October.

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