

BLACKBOARD AGENDA - MORTALITY CONFERENCE

March 28-29, 1983

1. The current MSX situation in Maryland and Virginia
2. Other hosts for MSX:  
    who has looked at what? when? where? how?
3. Life cycle considerations
4. Environmental parameters -  
    Requirements and limitations for MSX
5. Taxonomic considerations
6. Techniques, variations, standards
7. Other parasites, hosts, diseases of all sorts
8. Resistance - Evidence for? does it exist?  
    Define Tolerance: Mutations

COMMENTS ON AND ASSESSMENT OF  
THE CURRENT OYSTER MORTALITIES IN MARYLAND

April 1983

The following assessment is based on several considerations: 1. Our understanding and interpretation of reports by Maryland workers attending a two-day conference on shellfish mortality held at the Rutgers Shellfish Laboratory, Bivalve N.J., March 28-29, 1983; 2. The results of testing oysters from Eastern Bay, MD for resistance to MSX in Delaware Bay during the summer of 1982; 3. The results of histological examination of Eastern Bay oysters collected in May 1982 and in January 1983; and 4. Our experience with MSX in Delaware Bay since its discovery here in 1957.

We fully acknowledge that we do not know Chesapeake Bay as well as we know Delaware Bay; that there are important differences between the two estuaries; and that we may be unaware of some of these differences. With this caveat, however, we hope that the following comments will be helpful to those in Maryland who have the responsibility for dealing with the current oyster mortalities.

1. Correlation of MSX prevalence with oyster mortalities.

Histological data for Chesapeake oysters, reported by Sally Otto (Maryland Department of Natural Resources) showed fall 1982 MSX prevalences between 0 and 56%, with most being less than 30%. Half or more of the infections were advanced or terminal, and would most likely result in death. These samples were collected between August and November 1982. We understand that the mortalities were occurring--at least in some areas--over this period and that they continued on through the winter. Thus, the prevalence data should be a reasonably good reflection of how many oysters had MSX around the time they were dying. Our own examination of samples collected from Eastern Bay (Parson's Island) in May 1982 and in January 1983 showed prevalences of 20 to 30%. At both times, 10 to 12% of the sample consisted of heavily (and probably lethally) infected individuals. Thus, it is clear that MSX is potentially responsible for some of the current oyster kill.

However, the presence of MSX does not always correlate with the areas of heaviest mortality (mortality data courtesy of George Krantz, University of Maryland). For instance, MSX has not been found in an area of heavy kill in the Chester River, and while it is present throughout the Tangier Sound region in prevalences ranging up to 15%,

mortalities greater than 20% have occurred only in the upper portion of the Sound.

A major problem with interpreting the mortalities is a lack of gapers for histological study. We believe that diagnosis of the parasite in dead oysters is vital to confirming an association of MSX with mortality. Our laboratory did examine 5 gapers received in a shipment of oysters collected in early January 1983 from Eastern Bay. Three had MSX, but only one had an infection heavy enough to have killed it. The other two had light MSX and we judge that some other factor, most probably combined with MSX, killed these. The two MSX-negative gapers probably died of something other than this pathogen.

(In Delaware Bay oysters, MSX infections become lethal when they reach the moderate to heavy, systemic stage. We do find light MSX infections in gapers, but these are most frequent in late winter and early spring--a time of "winter kill" even before MSX--and we have interpreted these to reflect a combination of stresses acting to kill relatively lightly infected individuals that might otherwise have survived.)

Given these relatively low prevalences (particularly of advanced infections), we find it difficult to believe that MSX is responsible for oyster mortalities reported to exceed 50% in some areas. This, plus the lack of consistent correlation of MSX presence with high mortality leads us to agree with some Maryland investigators that agents other than MSX are responsible for a good deal of the kill.

## 2. Other possible causes for the mortalities

The information made available to us at the Bivalve meeting makes it clear that there are several possible causes for the mortalities besides MSX.

A. Prevalence of the oyster pathogen Perkinsus marinus or "Dermo" equals or exceeds that of MSX in many areas of the Chesapeake from the Choptank River south on the eastern shore, as well as in the lower Patuxent and Potomac Rivers on the western shore (Sally Otto's data). "Dermo" has previously been correlated with high mortalities in the upper Chesapeake. However, "Dermo" appears less frequently than MSX in the Miles River and in Eastern Bay where there have been high kills.

B. "Physiological Stress Syndrome", an unusual proliferation of collagenous tissue and of blood cells, accompanied by a general deterioration of all tissues without any recognized pathogen, is found in oysters throughout the bay (Sally Otto). This condition is believed to be associated with mortality. It is prevalent, for instance, in the Chester River where there has been heavy mortality with no (recognizable) MSX or "Dermo".

C. Areas of low dissolved oxygen have been found recently in the upper Chesapeake and are reported to coincide closely with areas of

heaviest recent kill (George Krantz). Although we are not entirely clear about when and where the low dissolved oxygens have occurred, they are undoubtedly a complicating factor in understanding current mortalities.

D. We understand that the new technique of harvesting oysters by SCUBA is extremely efficient -- far more so than tonging. According to George Krantz, it is possible for the divers to cull while on the bottom -- that is, to take only oysters and no boxes. Given the reported efficiency of removal of live oysters, the proportion of boxes to oysters would rapidly increase and any mortality estimates would be biased accordingly.

E. We were surprised to learn from George Krantz that James River oysters have been planted in many of Maryland's sub-estuaries recently, including Eastern Bay. The transplants may be responsible for some of the MSX presence in these estuaries, since we do know that James River oysters, even from the upper bars, carry low levels of the parasite.

### 3. Salinity and MSX in the Chesapeake

In Delaware Bay, we have a very clear and regular pattern of decreasing MSX prevalence with decreasing salinity in an upbay direction. The same has been shown in the James River by J.D. Andrews, and in the upper Chesapeake during the mid-1960s drought, by C.A. Farley. We are therefore puzzled by the present pattern of "patchy" MSX distribution in the upper Chesapeake. The finding that prevalences are higher in the upper portions of the bay than in the lower sections is contrary to all previous experience.

In Delaware Bay, we now find relatively little mortality caused by MSX infections that occur in water below 20 ppt salinity and on this basis, we would not expect to see heavy MSX-caused mortality at salinities present in areas of heavy oyster kill in the Chesapeake. However, in 1958-59, when MSX first moved upbay in the Delaware, mortalities of 50-60% occurred in salinities as low as 14-15 ppt (Cohansey Bed, fall salinities), possibly because the then-unselected oysters were so susceptible to the disease. Eastern Bay oysters exposed to MSX at our Cape Shore Laboratory over the summer of 1982 proved to be the most susceptible imports we have yet tested. All the oysters became infected and more than 90% died from MSX between June and November.

The question then arises as to whether the extreme susceptibility of the Maryland oysters is resulting in heavy MSX kill at salinities low enough to inhibit parasite activity in moreresistant stocks. Following from this is the question of whether susceptibility and/or low salinity allows the parasite to kill at much lower intensities than it would otherwise.

Unfortunately, we lack reliable histological data from the 1958-59 period when MSX moved into low salinity water in Delaware Bay, so that we are not sure what disease prevalences were that

corresponded to the observed mortalities. However, two other pieces of information may help to answer the above questions. 1. Regardless of location along the salinity gradient (10 to 25 ppt), lethal MSX infections for Delaware Bay natives are systemic, and moderate to heavy in intensity. 2. Of the 23 gapers collected from Eastern Bay oysters exposed to MSX in Delaware Bay last summer, 20 died with moderate to heavy systemic infections. The remaining 3 had light systemic lesions. Thus, salinity does not seem to affect the level at which MSX becomes lethal, at least in moderately resistant oysters; and the lethal infection intensity is the same for very susceptible oysters as it is for more resistant ones.

#### 4. Resistance to MSX

We are troubled by suggestions that the lower prevalences in down-bay oysters are the result of resistance to MSX that developed at the time MSX was last a problem in Maryland -- in the mid-1960s. Austin Farley found high MSX levels (up to 70%) and heavy mortalities on Marumsco Bar at that time, and the survivors of that kill were undoubtedly more resistant to MSX kill than had been the original population. However, it is difficult to believe that in the ensuing 15 or more years without MSX pressure, this resistance would not have been diluted by larvae from unselected upbay parents.

Results of testing native seed from various parts of Delaware Bay, an admittedly much smaller and simpler estuary than the Chesapeake, indicates a very thorough mixing of larvae so that oysters setting anywhere in the bay have about the same level of resistance to MSX, even though high salinity populations in the lower bay regularly experience far greater selection pressure than do upbay stocks.

In any event, it is premature to conclude that differences in MSX prevalence between upper and lower regions of the Chesapeake are the result of differences in resistance to MSX between the resident oyster stocks. This presumes exposure of all stocks to equal MSX infective pressure. If MSX infective agents are that well-distributed around the entire Bay, it is difficult to conceive of totally discrete and isolated oyster stocks in the various sub-estuaries. The transport of infective particles could also provide for the transport of early stage oyster larvae which move throughout the water column.

While we do not doubt that larval mixing in the Chesapeake is less thorough than in the Delaware, we do believe that significant mixing of larvae from selected and unselected parents has most probably occurred in the 15+ years since the last major incursion of MSX into the upper Chesapeake.

#### 5. Recommendations

It is obvious that the causes for the current oyster mortalities in Maryland have not been sorted out, and we think that the most important job for Maryland investigators is to do this as quickly as possible. This should involve a somewhat different approach to field sampling than now exists, with emphasis on frequent and thorough

sampling of certain key stations. It is probably not necessary to sample as many different locations as are now being checked. Collections of accurate and consistent mortality data should be continued. We stress the need to determine "recent" mortality (as is presently done by George Krantz, and by the Oxford Laboratory) which can be cumulated over time to provide estimates of total mortality. (It is then extremely important to be able to define the recent mortality interval with up-to-date information about scavenging and fouling prior to the time of collection.) Total kill should be expressed as cumulated recent mortality, since this defines the period during which the mortality has occurred. Total box counts are not a good measure of mortality because of the uncertainty about the age of "old boxes". We suggest that trays of oysters be used in addition to samples from the bottom. Trays could help eliminate confusion caused by harvesting, and they also provide very accurate mortality information.

In addition to mortality data, frequent samples for histological examination, and the collection of gapers for detection of disease agents in dead oysters should receive high priority. Also very important is the collection of reliable hydrographic data at each station. It is clear that information on salinity and dissolved oxygen are going to be crucial to interpreting the other data.

The hypothesis that lower bay oysters are resistant to MSX should be thoroughly tested before large scale transplants are made of these oysters to repopulate depleted upbay areas. This should involve exposing these stocks to MSX side by side with known susceptible oysters and determining their relative survival.

We also believe that a strong effort should be made to determine how much of the reported mortalities are caused by harvesting pressure or practices.

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April 12, 1983

DISCUSSION NOTES  
MORTALITY CONFERENCE  
RUTGERS SHELLFISH RESEARCH LABORATORY  
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2. Other Hosts

In the earlier years of MSX, all lab groups were looking at a variety of common oyster associates for any evidence of Haplosporidan involvement. All together an impressive list of animals has been examined, but, with few exceptions, these were occasional or "one shot" examinations. At present, all known hosts of Haplosporidans are invertebrates and Frank Perkins felt that a search for an alternate host for MSX should begin by concentrating on filter feeding invertebrates.

Austin Farley and Fred Kern have systematically looked at a variety of bivalves, e.g. Anomia simplex, Mytilus edulis, Geukensia demissa, Mercenaria mercenaria and Macoma sp. in MSX territory with such frequency and over a long enough time that they can probably eliminate these as candidate hosts. Sally Otto has examined many slides of Mercenaria mercenaria and Mya arenaria. Frank Perkins reported that a VIMS researcher, Mary Beth Saffo (sp.?) has studied the parasites of Molgula in Virginia rather thoroughly without finding any Haplosporidans.

Gene Burreson pointed out that spores are the key to the life cycle of Haplosporidans, and he proposes to work first with SSO where spores are found regularly and in abundance in the oyster host.

5. Taxonomic Considerations

Frank Perkins emphasized the value of spore ornamentation in the taxonomy of Haplosporidans and pointed out that it supports Vic Sprague's splitting of MSX from Minchinia. Austin Farley felt that more emphasis should be placed on the physiological characteristics of the parasite (and its relationship to its host) such as whether sporulation is in the digestive diverticula only, or occurs elsewhere in the host. Austin pointed out that MSX sporulates in the epithelium of the digestive diverticula (of the oyster) and the same is true of Minchinia tumefaciens in Mytilus californianus.

6. Techniques, Variations, Standards

In the workshop session, Austin Farley demonstrated the technique he has developed for the rapid preparation of permanent stained slides of molluscan blood cells.

Questions were raised about the uniformity between laboratories in diagnosing MSX prevalences, intensities and mortalities. Sample size necessary for reliable histological diagnosis was discussed and the general consensus seemed to be that samples should include between 20 and 50 individuals, but that the exact number would depend on the

abundance of the parasite in the oysters sampled. In other words, the rarer the parasite, the larger the number of oysters needed to accurately estimate MSX levels.

Difficulties in ageing oyster boxes and the necessity for frequent and consistent sampling of beds for accurate cumulation of mortality figures were emphasized. The MSX prevalence figures for Delaware Bay may be higher than for some other areas because of the detailed study of the entire oyster section (including gill tips). Rare and very light infections may be missed by some of the other study techniques used.

A lively discussion revolved around the value of continued monitoring of oysters for MSX. Most conferees felt that is essential to be able to define what kills oysters or other species when large mortalities occur, and that monitoring remains essential to this ability. However, other types of research should accompany the monitoring effort because it is also essential that we find out more than just when and where MSX is present.

#### 7. Other Parasites and Diseases of all Sorts

Austin Farley showed slides of various pathological conditions of Mytilus edulis collected from several northeast estuaries.

Austin and Fred Kern reported that SSO is common in oysters along the northeast coast.

4. & 8. Covered in lengthy discussion of Item 1 (the Maryland situation).