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Sporulation of haplosporidan parasites : notes and tables

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Sporulation of Haplosporidan Parasites

(Notes and Tables)

J. D. Andrews

VIMS

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revised slightly

Notes on Sporulation of MSX in Tray Oysters

J.D. Andrews

14 April 1983

Susceptible James River oysters at Gloucester Point averaged 42% prevalences of MSX over the first year after importation during the years 1960 to 1980. Average mortality the first year (1 Apr to 31 March) was 56% excluding 1972 when MSX infections failed to occur at normal prevalences because of low salinities following Hurricane Agnes. If this annual death rate is added to prevalence levels about 1 June in the following year from late-summer infections which could be high as 50%, a total infection level of 75 to 80% was initiated during one summer. Those oysters which acquired late-summer infections usually died in June and July of the second year; therefore, 80% of susceptible oysters could become infected and die in one year beginning 1 August when the first deaths from MSX occur. Prevalences from late-summer infections are somewhat lower after 50% of the oysters have been killed by MSX from early-summer infections, but the decline is not as great as might be expected. One of the mysteries of MSX is why so many oysters get MSX during their 2nd year of exposure; why did not all susceptible oysters get the disease the first year?

The rarity of sporulation of MSX in Crassostrea virginica is another mystery which has greatly hampered studies of the life cycle of the pathogen. All the cases of sporulation stages found over 23 years of routine sampling and sectioning of 168,000 oysters is given in Table 1. In the early years of epizootic MSX mortalities, we were

processing over 10,000 live and dead oysters each year but finding only one or two sporulation-stage infections. Beginning in 1966, a few more infections in sporulation were found; this slight increase peaked in 1967 then reverted to the super-rare status. In 1976, one tray of 3-month-old spat exhibited sporulation levels of 40%, but all other oysters including many lots of spat remained in the rare category.

Over 23 years, 104 cases of MSX sporulation were found; this is about 1 case per 2000 infected oysters except for the one tray of spat. The sporulation stage occurred more commonly in gapers than in live oysters, because we probably processed > 10 times as many live oysters as gapers. The occurrence of sporulation cases in all months of the year suggests that oysters were not dying promptly when it occurred. There is a tendency for one case to be followed by another one in the same tray of oysters a few months later. For example, gapers from Tray P95A in January and June probably were derived from simultaneous sporulation but one oyster survived longer than the other. Restriction of sporulation to the epithelia of liver tubules makes longer survival possible in contrast to systemic sporulation of SSO in all connective tissues and prompt deaths over a period of about 30 days only.

It appears that sporulation is most likely to occur in young, susceptible oysters. Susceptible oysters with sporulation-stage infections numbered 85 cases compared to 15 cases in resistant oysters. Occurrence of sporulation appears to decline with age of

oysters. The age of oysters was known for progeny bred at the VIMS hatchery, but James River oysters were typically 2 or 3 years of age when transplanted to high-salinity areas for monitoring MSX. We handled far more young oysters than old ones, however. Sporulation occurred at all sites of tray stations except in low-salinity areas. It appears that sporulation was more rare on Eastern shore of Virginia, but over the first 15 years MSX was not very active on Seaside and after the first year or two of MSX kills, oysters were no longer planted in Bayside creeks.

The distribution of occurrence of sporulation cases by months and seasons suggests that June and July are the probable normal period for sporulation of MSX. Far more deaths and greater sampling of live oysters occurred in late-summer and fall when MSX killed oysters most intensively. The lot of Rappahannock River spat demonstrated that MSX can sporulate in August and September in C. virginica, but that may be an abnormal time for oyster species that are normal hosts.

Table 1. Chronological Occurrence of Sporulation Stage Infections of *Minchinia nelsoni* (MSX) in Live and Dead Oysters in Virginia, 1960 to 1982.

Date Sampled	Tray No.	Source & Location of Oysters	Resistant Susceptible	Live or Gaper	Age years
3 Nov 60	60J	WS at VIMS	S	G	2
20 Mar 61	J6	WS at HB	S	G	3
22 Mar	J2	BS at BS	S	G	3
26 Jun	S16 & 17	Machipongo at HIB	S	G	3
13 Nov 63	Y17	HH at Tillages	S	G	2
16 Nov	B23	HH at Gulf	S	G	3
30 Nov	B23	HH at Gulf	S	G	3
22 Jun 64	S37	LI at Bradfords	S	G	3
4 Sep	MJ9	HH at Mobjack	S	G	3
18 Sep	MJ11	HH at Mobjack	S	L	2
5 Apr 65	MJ11	HH at Mobjack	S	G	2
21 May	Burton Bay	Piankatank (Yrlgs)	S	L	1
20 Oct	Y23	HH at VIMS	S	L	2
10 Jun 66	P2A	Egg Is. at VIMS	R	G	2
16 Jun	P4A	Potomac at VIMS	S	G	2
20 Jun	P5A	LI at VIMS	S	G	2
20 Jun	P6	HH at Gl. Pt.	S	L	2
1 Jul	P5A	LI at VIMS	S	3L	2
1 Jul	Y25	HH at Tillages	S	G	3
15 Dec	P18	HB at Gl. Pt.	R	L	3
4 Jan 67	Y31	Potomac at Gl. Pt.	S	G	3
17 Jan	P18	HB at Gl. Pt.	R	G	2
16 Feb	P22	P7 at VIMS (2 yr)	R	G	2
23 Feb	Y32	Potomac at Gl. Pt.	S	G	3
7 Jun	MJ6	HH at Mobjack	S	L	7
13 Jun	P25	Mobjack at VIMS (2 yr)	R	L	4
21 Jun	P10	Mobjack at VIMS (3 yr)	R	L	3
27 Jun	P6	HH at Gl. Pt.	S	2L	3
29 Jun	P28	HH at Gl. Pt.	S	L	2
12 Jul	P10	Mobjack at VIMS	R	L	3
12 Jul	Y34	DWS at Tillages	S	G	3
26 Jul	weighed lot	HH at VIMS	S	L	3
30 Aug	P27	Deep Rock at Gl. Pt.	S	G	3
22 Sep	MJ16	HH at Mobjack	S	L	2
9 Oct	Y37	HH at Tillages	S	G	2
30 Oct	S54	Machipongo at HIB	?	L	3
18 Jan 68	P32	Mobjack at Tillages (2 yr)	R	L	2
6 Mar	MJ16	HH at Mobjack	S	G	3
31 Mar	P30	Mobjack at Tillages	R	L	2
18 Jun	P40	P10 at Tillages	R	L	1
20 Jun	P30	Mobjack at Tillages	R	G	2
7 Jun 69	Y44	Potomac at Gl. Pt.	S	G	4
8 Oct	P53	LI at Tillages	S	L	2
8 Oct	P57	Potomac at Tillages (1 yr)	S	L	3
31 Jul 70	S72	Native at Swash	S	L	2
17 Feb 71	P66	West R. Md at Tillages	S	G	2
30 Jun	P64	West R. Md at Gl. Pt.	S	L	3
31 Aug	MJ22	DWS at Mobjack	S	L	2

Date Sampled	Tray No.	Source & Location of Oysters	Resistant Susceptible	Live or Gaper	Age years
28 Sep	P80	P40 at Tillages (1 yr)	R	L	1
28 Sep	P81	West R. Md at Tillages (1 yr)	S	L	1
17 Nov	P76	West R. Md at Tillages	S	G	1
1 Jun 72	S86	Natives at Swash	?	L	1
17 Jul 72	S86	Natives at Swash	?	L	1
24 May 73	B45	Seaside Natives	S	L	2
8 Jun 73	S93	Natives at Chinco	?	L	3
13 Sep 73	S95	W.S. at Chinco	S	G	2
29 Jan 74	Y77	HH at Tillages	S	G	3
29 Jan 74	P95A	Y69 at Tillages	S	G	3
22 May 74	Y79	WS at Tillages	S	L	3
30 May 74	Y78	WS at Tillages	S	G	3
13 Jun 74	P95A	Y69 at Tillages	S	G	3
20 Jun 74	P31	EI at Tillages	R	G	8
29 Oct 75	J37	Rainbow at HB	S	L	2
6 Nov 75	MJ26	Rainbow at Mobjack	S	L	2
3 Jun 76	S105	W.S. at Bradfords	S	G	2
12 Aug 76	P104	P80 at Tillages	R	L	4
7 Sep 76	P104	P80 at Tillages	R	L	4
20 Sep 76	P171C	~~P80 at Tillages~~	S	6L	0
21 Sep 76	P171C	Rapp. at Tillages	S	8L	0
7 Oct 76	P171X	Rapp. at Tillages	S	3G	0
7 Nov 76	P171X	Rapp. at Tillages	S	L	0
29 Nov 76	J42	Rainbow at HB	S	L	2
16 Dec 76	P171C	Rapp. at Tillages	S	2G	0
16 Dec 76	P171C	Rapp. at Tillages	S	8L	0
11 Feb 77	Y88	W.S. at Tillages	S	2G	3
27 Oct 81	MJ32	Horseheads at Mobjack	S	L	2
3 Jun 82	Y113	Horseheads at Tillages	S	L	3
28 Jun 82	Y113	Horseheads at Tillages	S	G	3

Symbols: HH=Horsehead; WS=Wreck Shoal; BS=Brown Shoal; LI=Long Island; HB=Hampton Bar; DWS=Deep Water Shoal; NJ=New Jersey; EI=Egg Island; Chinco=Chincoteague Bay; R=Rappahannock River; MJ=Mobjack Bay

Susceptibility to MSX: Y (York River) lots from James River were all susceptible oysters; P lots were mostly resistant oysters but some lots were progeny of susceptible James River stocks for controls; MJ (Mobjack Bay), and J (James River) were always susceptible oysters; S=Seaside and B=Bayside of Eastern Shore, Va., native oysters are usually undetermined as to susceptibility or resistance.

Age of oysters: James River oysters imported to monitor MSX activity averaged about 2-3 years (2 inch oysters); these were used in Y, J, R, MJ tray series. All P lots were from oysters bred in the VIMS hatchery.

Total No. of sporulation cases 104

Total in live oysters 63, Gapers 41

Total in susceptible 85 and resistant 15 unknown 4 oysters

Total on Western Shore of Bay 92 Seaside 9 Bayside 3

Total cases by age of oysters <1 yr. 28, 1 yr. 7, 2 yr. 31, 3 yr. 32, >3 yr. 6

28 cases in P-171C, Rappahannock River 3-month-old spat; this does not include many smear diagnoses - only slide cases.

*Distribution of Sporulation Stage Infections by Months and Seasons:

January	5	Winter	14
February	5		
March	4		
April	1	Spring	28
May	4		
June	23		
July	9	Summer	19
August	3		
September	7		
October	7	Fall	14
November	7		
December	1		
Total	76	Total	76

*Does not include 28 cases in susceptible Rappahannock River spat (Tray P171) which first appeared in September and were found in samples each month through December 1976.

SPORULATION OF MINCHINIA NELSONI IN OYSTERS

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1976

Sporulation of the oyster pathogen Minchinia nelsoni has been rare in occurrence and rather mysterious for a haplosporidan. Most species of haplosporidans are known primarily by their spores with limited knowledge of other stages. The group is less notorious than microsporidans as agents of disease in invertebrates. However, even allowing for the "wastebasket" status of the order Haplosporida, recognized by Caullery (1953), Sprague (1967), and Perkins (1976), the group parasitizes a wide range of hosts. These include mollusks, annelids, nemertines, trematodes, nematodes, tunicates, and crustaceans among aquatic taxa. Other species of the genus Minchinia have exhibited typical spores and regular sporulation. Minchinia costalis, called SSO, was found in oysters on Seaside of Eastern Shore, Virginia, while searching for M. nelsoni (Andrews, Wood and Hoese, 1962). It sporulated regularly in May-June each year, and it provided an example of what was expected in the epizootiology and histology of haplosporidans.

The first case of Minchinia nelsoni sporulation in oysters was found in a gaper from lower Chesapeake Bay on 3 November 1960. The spores were very similar in morphology to SSO but twice the size (8 $m\mu$ long). Only about one case of sporulation per thousand plasmodial infections of MSX was found prior to the mid-1960's in Virginia (Table

1). In January 1963 at the Fifth Annual Mortality Conference, Walt Canzonier of Rutgers University described "Spores associated with MSX" and distributed a mimeographed summary. Three oysters from Chesapeake Bay and Delaware Bay with MSX plasmodia in connective tissues had spores in epithelia of digestive diverticula. At that time it was not appreciated that some haplosporidans attack primarily the digestive tract and sporulate in the epithelia of that organ system. An example is Minchinia pickfordae in five species of Great Lakes snails (Barrow, 1965). At the Seventh Annual Mortality Conference in January 1965, (Mimeographed reports), Andrews referred to six cases of haplosporidan spores associated with MSX in oysters. The pathogen, known for seven years, had not been given a scientific name, and investigators were reluctant to draw conclusions from such rare occurrences of spores. Thousands of plasmodial cases had been diagnosed at several laboratories.

In July 1965, Couch, Farley and Rosenfield found spores in 12 oysters from the Eastern Shore of Maryland. This led to the naming of Minchinia nelsoni by Haskin, Stauber and Mackin (1966), and a description of sporulation by Couch et al (1966). It is now apparent why sporulation was so rare in Delaware Bay and lower Chesapeake Bay and yet increased in occurrence in fringe areas of the range of MSX in Maryland. The pathogen achieves sporulation in young susceptible oysters more frequently than in older resistant oysters. The timing of sporulation is not fixed to an annual cycle as in SSO. These circumstances were contrary to expectations for a virulent parasite believed to be a poorly-adapted pathogen and newly introduced.

In 1965, MSX invaded the upbay western-shore tributaries of Chesapeake Bay in Virginia, and spread into Maryland (Farley 1975) where only susceptible oysters were present. The drought years of 1963 to 1967 brought higher salinities which permitted the pathogen to invade and devastate beds of susceptible oysters not previously exposed to epizootics of the disease. Heavy losses occurred throughout Virginia and in southern Maryland waters. The drought also affected salinities in Delaware Bay waters but unexposed susceptible oyster stocks were not available in quantities for infection.

After the drought years, MSX dropped out of Maryland and the upper-tributaries of Virginia, and became confined again to the lower Chesapeake Bay. Sporulation and the rare scurfy shells associated with mantle lesions caused by MSX became scarce again (Table 1). After four years (1971-1974) of low-salinity regimes in Virginia, which depressed MSX activity, the disease returned to intensive epizootic conditions in 1975-1977 and what may be called normal distribution or range of activity. Infections were reduced at Gloucester Point during the wettest years, but MSX remained enzootic from York River and Mobjack Bay down the Chesapeake Bay system. Fringe areas of the range (Rappahannock and Great Wicomico rivers) exhibited a few infections but no substantial mortalities. Spring purging of infections by oysters in April-May occurred in fringe areas where salinities were low <10 °/oo (Andrews 1983).

Sporulation in Susceptible Spat

On 20 September 1976, Tray P171 holding Rappahannock River spat, reared at VIMS and being monitored at Gloucester Point, were found to

be dying excessively. Thousands of uncounted free spat in the tray yielded 9 quarts of boxes when sorted and mortality was estimated at 40%. These spat were brought to Gloucester Point from Ames Pond an MSX-free sanctuary on 8 July 1971 for monitoring in offshore trays. No apparent deaths were observed in a routine check on 23 August 1976. Fresh smears of digestive tubule tissues revealed sporocysts of MSX in 16 of 74 unselected live spat (21.6%). Four of seven gapers in the same lot had spores. These spat were only four months old and 20 to 40 mm long. MSX had infected them and gone in to sporulation in 10 weeks (8 July to 20 September 1976). A later importation from the pond of the same lot of spat on 16 August 1976 exhibited no mortality and no patent cases of MSX in the fall of 1976. This later lot had spat twice the size of the P171 group by 1 November 1976. Disease had drastically stunted the MSX-infected spat. By 1 November 1976, only a few hundred spat were left in tray P171 and a 90% mortality had occurred within four months after first exposure to MSX.

In 18 years of monitoring MSX in Virginia, this explosive epizootic was unprecedented. The high mortality of young susceptible spat and the occurrence of sporulation in them was contrary to my exceptations and to my tentative theories of resistance and susceptibility of oysters. The timing of sporulation was also unexpected. Previous occurrences of spores were erratic in timing but a trend toward June-July cases was noted in scattered cases summed over many years. None of the previous episodes of sporulation and mortality had occurred so soon after first exposure to MSX. Unlike SSO, the pathogen MSX did not require an annual cycle to achieve

sporulation. However, spores produced in oysters still appeared too infrequently to be important in instigating new infections. Early deaths within a month or two after first exposure in early summer and prolonged mortality seasons may help explain why MSX has a long period of infectivity in contrast to SSO.

In trying to understand why this incident of abundant sporulation had not occurred until the 13th year of handling laboratory-reared spat, several explanations are possible but none satisfactory. Most spat lots monitored have been genetically resistant groups from heavily MSX-selected parent lots. Control lots from susceptible parents were bred each year, but these also exhibited greater survival after early field exposure than importations of older seed oysters introduced directly from low-salinity areas. James River seed stocks were used also as controls but it is apparent that Rappahannock River and particularly Potomac River stocks were much more susceptible to MSX. Most of the progeny lots bred in the laboratory at VIMS were held through the summer in Ames Pond for protection against wild spatfall and smothering by fouling organisms. Most lots were not brought back to the York River until fall (after 1 October). No MSX was ever found in Ames Pond. A few lots were brought back in June or July and thereby exposed to MSX in early summer but most of these lots were genetically resistant to the disease. It seems to require an intensive year of MSX, such as 1976 was, to induce infections in spat. Tray P171 oysters may been a very susceptible lot by the chance genetics of parentage. High susceptibility was noted previously in a lot derived from Long Island Sound parents in 1968. However, if

susceptible control lots were not exposed early enough in their spat year, why did they not experience MSX sporulation as yearlings? Sampling of live oysters for diseases was particularly intensive on susceptible control groups through the years.

My concept of possible life cycles in Delaware Bay disease was based upon a knowledge of Seaside Disease caused by SSO which exhibited a regular, carefully-timed sequence of events with sporulation and oyster deaths occurring in June one year after infection. I presumed that MSX was a poorly adapted pathogen which killed its host before sporulation was achieved. If this were true, one would expect sporulation in oysters that were resistant to the disease and able to live for nearly a year after initial infection. A small number of oysters did survive June infections that persisted into the following June or July before they died. Infections were always very intensive by this time but sporulation was rare as usual.

Some hints that resistant oysters were not the best source of spores came from other areas. In Maryland, Farley (personal comunication 1976) smeared hundreds of live oysters after selecting sick and poor ones and obtained some 80 cases of sporulation in late October and November 1966. He believed that sporulation occurred in late June and again from late October through December (Farley, 1975) and new infections were expected to follow in ensuing months. These were native oysters in Maryland most of which are highly susceptible to MSX. Virginia studies do not provide any evidence for new infections after 1 November although early-summer infections may first

become patent in late fall because of low salinities that reduce development of the disease.

In 1966, Myhre (Haskin 1972) exposed resistant and susceptible lots of laboratory-reared 1966 spat to MSX in early September in nature in Delaware Bay. The following June, 4 cases of spores among 34 infections of MSX were found in 10-month old susceptible spat. The resistant spat had discarded earlier MSX infections. This coincides in timing with Virginia data for 1966-1967 where 15 of 19 spore cases occurred in June or July and the other 4 were mid-winter cases in gapers (Table 1). Had these last four sick oysters survived the winter as most did, they would have appeared as spore cases in live oysters in June with the other cases. There is little evidence that sporulation of MSX brings oysters to a pathogenic crisis involving immediate deaths such as occurs in SSO. Sporulation is localized by site for MSX and is not promptly disabling or lethal in most cases.

Significance of Sporulation Site

What are the circumstances that cause MSX plasmodia to migrate to epithelia of digestive tubules for sporulation? Most early localized infections occur in the gill epithelial from which they gain access to blood sinuses for systemic distribution. Large plasmodia are frequently found in intestinal and tubule epithelia when oysters have severe infections. Occasionally, localized infections are found in tubule epithelia before infections become systemic. Massive infections of epithelia in all tubules have never been found except when sporulation is in progress. Only a small portion of plasmodia

are phagocytized or surrounded by hemocytes. Most are too large for phagocytes to engulf and transport to tubule epithelia for explusion. Epithelial mucosa do not appear to be regular sites for occurrence of plasmodia in most early systemic infections of MSX; SSO plasmodia occur rarely in epithelia even in advanced cases. SSO appears to establish initial infections through the gut epithelia rather than through gill epithelia as MSX does.

Sporulation of MSX is confined to mucosa of tubule epithelia. Plasmodia in connective tissues and blood sinuses show no evidence of attempts to sporulate, but they do collect around digestive tubules. Some plasmodia always remain in connective tissues. In tubule epithelia, MSX plasmodia enlarge to 3 to 4 times their size in connective tissues to become sporonts (30 μ to 50μ). The sporonts become much distorted in shape in the crowded spaces between epithelial cells, and they usually cause epithelial tissue to bulge into tubule lumen or underlying connective tissues (fixed specimens). In fresh smears, sporocysts released from pressure are globular and mulberry-like and the cyst wall is quite tough. Meanwhile, plasmodia in connective and other tissues do not enlarge or change their appearance.

All plasmodia located in tubule epithelia generally progress synchronously to sporulation but all stages of sporonts may be found simultaneously. At the initiation of sporulation, chromatin material becomes distributed in a punctate pattern abundantly throughout the cell with nuclear membranes faint or absent. Presumably this is the

paired-nuclei state reported by EM studies (Perkins, 1976) and may preceed a reduction division. The occurrence of sporulation appears to have little relation to intensity of infections in other tissues. Most tubules show one or more sporonts in each 6-8μ thick cross-section but none in ciliated ducts usually. This rather uniform and wide distribution of sporonts in digestive tubules suggests a determinate transfer process by plasmodia rather than chance passage or occurrence in epithelia.

Despite no obvious mechanism for getting plasmodia into tubule epithelia except by their own efforts, it seems unlikely that initial infection through digestive tubules and retention of plasmodia there could be the source of sporonts. The low occurrence of localized plasmodial infections in these organs is incompatible with the density of sporonts in sporulation. However, mere presence of plasmodia in epithelia does not induce sporulation; the stimuli or causes that instigate it are unknown.

The invasive particles for MSX and SSO infections are not determined, yet uninucleate haplosporidan stages are found sparingly in June-July in oysters on Seaside where SSO is infecting. These stages are located in the epithelia of digestive tubules and sometimes can be seen breaking into underlying connective tissues and becoming multi-nucleate. When massive infections of MSX occur it is likely that some invasive stages do involve digestive epithelia (most early infections are in the gills) and these may remain and develop to sporulation there. This may be what happened to the spat in tray P171

in the summer-fall of 1976. At least 90% were infected; a sample of live-oysters on 21 September 1976 had an incidence of 92% after nearly half the spat had died. With thousands of small spat jammed into one tray, there must have been an abundance of infective particles to produce so many infections in so short a time at one fixed location. Presumably the infective stages are water-borne and are not from a localized source, therefore a chance swarm of infective particles is unlikely. July is the month during which infection patterns change from quickly clinical ones (one month from first exposure) to incipient and hidden infections in exposures after 1 August. It is intriguing that spat of the same lot as P171, imported to the York River 16 August, did not exhibit patent infections (0%) by mid-winter 1976-77. They did show plasmodial infections in the following spring typical of late-summer infections of MSX.

Table 1. Chronological Occurrence of Sporulation-Stage Infections of *Minchinia nelsoni* (MSX) in Live and Dead Oysters in Virginia, 1960 to 1982.

Date Sampled	Tray No.	Source & Location of Oysters	Resistant Susceptible	Live or Gaper	Age years
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17 Jul 72	S86	Natives at Swash	?	L	1
24 May 73	B45	Seaside Natives	S	L	2
8 Jun 73	S93	Natives at Chinco	?	L	3
13 Sep 73	S95	W.S. at Chinco	S	G	2
29 Jan 74	Y77	HH at Tillages	S	G	3
29 Jan 74	P95A	Y69 at Tillages	S	G	3
22 May 74	Y79	WS at Tillages	S	L	3
30 May 74	Y78	WS at Tillages	S	G	3
13 Jun 74	P95A	Y69 at Tillages	S	G	3
20 Jun 74	P31	EI at Tillages	R	G	8
29 Oct 75	J37	Rainbow at HB	S	L	2
6 Nov 75	MJ26	Rainbow at Mobjack	S	L	2
3 Jun 76	S105	W.S. at Bradfords	S	G	2
12 Aug 76	P104	P80 at Tillages	R	L	4
7 Sep 76	P104	P80 at Tillages	R	L	4
20 Sep 76	P171C	P80 at Tillages	S	6L	0
21 Sep 76	P171C	Rapp. at Tillages	S	8L	0
7 Oct 76	P171X	Rapp. at Tillages	S	3G	0
7 Nov 76	P171X	Rapp. at Tillages	S	L	0
29 Nov 76	J42	Rainbow at HB	S	L	2
16 Dec	P171C	Rapp. at Tillages	S	2G	0
16 Dec	P171C	Rapp. at Tillages	S	8L	0
11 Feb 77	Y88	W.S. at Tillages	S	2G	3
27 Oct 81	MJ32	Horseheads at Mobjack	S	L	2
3 Jun 82	Y113	Horseheads at Tillages	S	L	3
28 Jun 82	Y113	Horseheads at Tillages	S	G	3

*Distribution of Sporulation Stages of Infection by Months and Seasons:

January	5	Winter	14
February	5		
March	4		
April	1	Spring	28
May	4		
June	23		
July	9	Summer	19
August	3		
September	7		
October	7	Fall	14
November	7		
December	1		
Total	76	Total	76

*Does not include 28 cases in susceptible Rappahannock River spat (Tray P171) which first appeared in September and were found in samples each month through December 1976.

Symbols: HH=Horsehead; WS=Wreck Shoal; BS=Brown Shoal; LI=Long Island; HB=Hampton Bar; DWS=Deep Water Shoal; NJ=New Jersey; EI=Egg Island; Chinco=Chincoteague Bay; R=Rappahannock River; MJ=Mobjack Bay

Susceptibility to MSX: Y (York River) lots from James River were all susceptible oysters; P lots were mostly resistant oysters but some lots were progeny of susceptible James River stocks for controls; MJ (Mobjack Bay), and J (James River) were always susceptible oysters; S=Seaside and B=Bayside of Eastern Shore, Va., native oysters are usually undetermined as to susceptibility or resistance.

Age of oysters: James River oysters imported to monitor MSX activity averaged about 2-3 years (2 inch oysters); these were used in Y, J, R, MJ tray series. All P lots were from oysters bred in the VIMS hatchery.

Total No. of sporulation cases 104

Total in live oysters 63, Gapers 41

Total in susceptible 85 and resistant 15 unknown 4 oysters

Total on Western Shore of Bay 92 Seaside 9 Bayside 3

Total cases by age of oysters <1 yr. 28, 1 yr. 7, 2 yr. 31, 3 yr. 32, >3 yr. 6

28 cases in P-171C, Rappahannock River 3-month-old spat; this does not include many smear diagnoses - only slide cases.

SIGNIFICANCE OF SPORULATION SITE

J. D. Andrews

VIMS

Since MSX sporulates only in epithelia of digestive tubules, it could be reasoned that failure of plasmodia to reach this site results in rarity of sporulation. yet when sporulation does occur nearly every tubule x-section shows one to several sporocysts suggesting that MSX has collected there actively. It is unlikely that plasmodia in transit through epithelia during initial infections become situated in tubule epithelia for sporulation. More probable is that hemocytes try to discharge pathogen cells across epithelia layers although most plasmodia are too large for one hemocyte to engulf. Phagocytosis of plasmodia is not conspicuous and appears to be an inadequate defense mechanism against MSX. How then do plasmodia collect in tubule epithelia? Why do many remain in connective tissues adjacent to the sporulation site with no attempt to sporulate? If MSX plasmodia were attempting to reach epithelia for subsequent discharge of spores into the gut lumen, sporulation would be expected in gill, digestive tract, and mantle epithelia. The enlarged sporocysts do pouch out conspicuously into the lumen of digestive tubules although the cyst walls seem quite tough when smearing for spores. Most remain intact when oyster tissues are teased out. MSX plasmodia enlarge by 3 to 4 times to become sporonts of 30 μ to 50 μ. The sporonts become much distorted in shape to fit crowded spaces between epithelial cells (fixed tissues). Released from the tissues in fresh smears, sporocysts are globular. Meanwhile, plasmodia in connective tissues do not enlarge or change their appearance.

In contrast, SSO shows no attraction for epithelia and usually leaves mucosa uninvaded and intact even when connective tissues are severely disrupted by large numbers of sporocysts. All SSO plasmodia sporulate in synchrony and the high intensity of infections results in rapid death. Sporulation of MSX disrupts feeding mechanisms but does not result in prompt death.

One could speculate that MSX is a parasite of the digestive tract normally, but that its pathogenicity for the American oyster is high and poor defense mechanisms permit it to invade all tissues. The necessary conditions of stimuli for sporulation are usually missing and it kills the host without completing its life cycle. This does not preclude infection of additional hosts by means other than spores.

Notes on Life-cycles of Haplosporidans

J. D. Andrews

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January 1978

The failure of artificial infections is not proof of need for alternate hosts because conditions required for development of sporoplasms may not be met. On the other hand, sea water provides a favorable medium for survival and dispersal of all stages of a pathogen. Since most widespread aquatic diseases of bivalve mollusks are probably acquired through water-pumping activities, there is likely to be exposure of many species to infective particles. The possibility of other bivalve hosts seems more likely ~~than~~ than of a true alternate host with specialized parasite stages. Thus, ten species of bivalves in Virginia were found infected with Dermocystidium - like parasites when this disease of oysters was abundant and widespread. (Andrews, 1954) There may be several pathogen species in this group but mud crabs and polychaete worms probably acquired presporangia of D. marinum by eating dead oysters. Oysters, which filter large quantities of water, must be exposed to a variety of pathogens, some of which could be mildly pathogenic or at least slowly phagocytized. A great variety of protein substances have been shown to be quickly removed from lymph fluids by oyster phagocytes.

Pathological Evidence

Most localized infections of MSX, presumed to be new infections, are found on gills. They may be restricted to epithelia or simply localized in one or more gill lamellae. Often the plasmodia are congregated around the junction of two lamellae at the base of the gills where fine food particles are collected and passed forward to the mouth. This implies that infective particles are collected out of the water by filtration of food items.

Sometimes plasmodia of MSX will occur along the whole length of a gill lamella, but confined to the epithelia and situated just outside the basal membrane. These may occur by the hundreds in one cross-section. They are typically rather large multi-nucleated plasmodia not in obvious process of multiplication. Does each plasmodium represent an infective particle or has multiplication occurred in the epithelia? If the latter process has been active, how do the plasmodia migrate the length of a gill lamella (in epithelia) without blood sinuses? Furthermore, if infective particles are numerous, how can the other lamellae of the same oyster escape infections? There is no evidence of hemocyte transport of plasmodia in the epithelia although localized reaction indicates the oyster is aware of the infection. Localized early infections (epithelial only) could be expected more frequently in the digestive tract than they occur. Early invasion of the gills by the pathogen or resistance by the host must limit intestinal infections.

Methods of Transmission

Most marine diseases for which direct transmission is known depend on close proximity to of the hosts for infections. Dermocystidium marinum is a good example of this among oyster parasites. Because of dispersal by tidal waters and necessity for hundreds or thousands of infective particles to establish infections, isolation of 50 feet provides an effective quarantine for several years usually.

Haplosporidan diseases have not responded to attempts at artificial infections by investigators. This has led to the supposition that intermediate or other hosts are missing in the attempts. A review of known and presumed facts for each pathogen will facilitate discussion.

Minchinia nelsoni (MSX) is the best known of the oyster pathogens in this group. It has been studied over a wide geographical range on the mid-Atlantic Coast of North America for over 20 years now. Thousands of infections have been examined each year in stained slides and fresh smears by many biologists. Yet the stage of transmission and infection has not been observed. No empty spore case has been found lingering on a gill surface. Some facts and presumptions bearing on infection are listed for perusal:

1. Infections are acquired through the food-filtering process. Early infections occur through the gills usually but sometimes in the digestive tract.
2. Plasmodia with two to many nuclei are the only stage seen commonly in oysters.

3. Sporulation is rare and lacks regular seasonal timing. It is localized in digestive tubule epithelia and does not cause immediate death.
4. Infections occur during five warm months and subsequent deaths may occur from 6 weeks to 12 months later. Some deaths occur from light infections.
5. Mixing of infected and disease-free oysters in close proximity does not alter regular timing of infections or deaths.
6. Oysters in isolated trays with no other oysters around attain infections at the same levels as those on beds of the host species. This tends to eliminate sedentary species as potential alternate hosts.
7. Timing and levels of infections and deaths of oysters in trays miles apart are quite similar every year.
8. Epizootics from MSX move up and down estuaries tens-of-miles in particular years although low levels of infections tend to persist within known ranges of the disease. These changes are clearly salinity regulated. Oysters discharge (eliminate) MSX in low (<10 o/oo) and high (> 30 o/oo) salinities regularly.
9. Disease-free, susceptible, and resistant lots of oysters are available for monitoring timing and intensity of infections.
10. No new exotic estuarine species is known for Chesapeake Bay, particularly conspicuous large mobile predators or scavengers such as blue crabs that could transport diseases to isolated oysters.

11. Early-summer infections (May-June) never failed in 18 years, but late-summer ones (after 1 August) have failed some years in endemic areas. Presumably low-level of infections causes the 3-9 month delay in appearance of clinical infections.
12. Intensive selection results in resistant strains of oysters. Spat or first-summer oysters do not acquire infections routinely. Young susceptible oysters are most likely to exhibit sporulation of MSX. The pathogen kills oysters with relatively light infections and sporulation is not strongly linked to mortality.

The scarcity of spores, the failure of direct transmission with close proximity of oysters, and the long-distance dispersion of infections could readily be attributed to dispersal by a mobile or a tide-carried intermediate host. The failure to find either such a host, or a new exotic species linked to periods or places of infection, is frustrating too. Blue crabs have been examined quite extensively, but the haplosporidan in trematodes causing pepper crabs belongs to the genus Urosporidium. Furthermore, oysters in trays on barren sandy bottoms without the sedentary organisms usually associated with them get infections readily in relative isolation. There is no evidence of spotty distribution of infections, or delays in their occurrence that would be expected by chance if a mobile carrier such as blue crabs were involved. Infective particles seem to be pervasive throughout the endemic area for five continuous months. Infections occur immediately after

introduction of susceptible oysters. Hundreds of trays of oysters in a great variety of habitats over 18 years have provided this overview of infections of MSX.

The failure to find spore cases associated with infections and scarcity of spores in oysters suggests that naked "sporoplasms" may be involved in MSX infections. Their survival in salty estuarine waters is no great problem although their origin in such numbers as to blanket-cover many square miles of habitat to cause infections is more difficult to understand. Could plasmodia released by disintegration of dead oysters release multiple infective agents? These are the only MSX stage abundant in parasitized oysters.

The possibility that MSX is a highly infective pathogen requiring few infective particles to establish disease seems to be strong. This may be the explanation for the long incubation period, up to 9 months, which characterizes late-summer and fall infections. Localized infections involving only a few plasmodia in a short area of one gill are fairly common during periods of early infections. Why they are so commonly large multi-nucleated plasmodia even in epithelial infections is not understood.

MSX is a virulent pathogen with great capacity to kill oysters in Chesapeake Bay despite its inability to complete its life cycle in oysters. Probably the timing of its life cycle is shortened and disrupted by the high pathogenicity to oysters newly exposed to a new disease in the Bay. Whether newly introduced from another continent (Asia) or a new strain

resulting from mutation, it has overcome barriers of distance and low populations of oysters to cause a catastrophic disease.

Minchinia costalis (SSO) is much less enigmatic in its life cycle. Patterns of infection and mortality are quite fixed in timing and duration. Infections occur during or shortly after mortalities in June-July. Incubation requires about 9 months before clinical cases are observed in March of the following year. Mortalities occur in May-June and all clinical cases disappear thereafter. The essential facts about SSO include:

1. A short season of infection-June-July.
2. A long incubation period of localized or sub-clinical infections. No infections occur after 1 August.
3. Rapid development of infections in spring (Mar-May) to intensive cases.
4. Sporulation in most oysters, especially those that die.
5. Maturation of spores is variable but often is not achieved even in gapers.
6. Young oysters (first year) are not attacked usually, just as in MSX.
7. Most years, several isolated bays exhibit similar infection and mortality levels, and the same timing.
8. The disease occurs only in high-salinity waters (mostly $>$ 30 o/oo).
9. Rapid proliferation produces numerous tiny plasmodia ($<$ 5 um) with one or two nuclei in April-May.

10. Sporulation occurs in all plasmodia throughout connective tissues of oysters synchronously unlike MSX. Epithelia are usually avoided by plasmodia in systemic infections and they tend to remain intact without any sporonts as cases reach climax.
11. Sporulation is fatal to oysters whereas plasmodial infections may be discarded.

Spores could well be the source of infections for SSO and the timing fits the period of disintegrating gapers. Still SSO like MSX exhibits a long period of hidden or localized infections which are difficult to diagnose or find. Again the evidence suggests that rather few infective particles are required to establish infections which then proliferate rapidly at a subsequent favorable period. A fixed annual cycle with all stages exhibited regularly suggests that SSO is a more adapted parasite than MSX.

Minchinia amoricana is the newest haplosporidan pathogen to be found in oysters. It appears to be much like SSO in stages and tissues of infection. Only 3 (4 now) cases have been reported by Van Banning of which two had sporulation throughout connective tissues. The spores are slightly larger than those of SSO and sporonts are reported as larger with more spores. The timing of infection and mortality cannot be surmised from so few cases which seem to have arisen in French waters. It seems likely that French imports of C. gigas have contributed substantially to eventual cosmopolitan distribution of oyster diseases, hence it behooves us to seek methods of immunizing our brood stocks and possibly wild stocks eventually. There is no other alternative short of replacing native stocks by exotics and that will raise

a host of other cultural problems. The situation in Southern France with the replacement of C. angulata by C. gigas, and the associated problems, is a dramatic example of the dangers inherent in using exotic imports for replacements.

Marteilia refringens in Brittany exhibits a puzzling set of characteristics which may reflect doubt on its classification as a haplosporidan. Perkins found haplosporosomes but others in the U. S. doubt its relationship. The differences from MSX and SSO are:

1. It causes a disease mostly localized in the epithelia of the digestive tract. This is not unusual for a haplosporidan.
2. It is slow to kill oysters by comparison and lacks the intensive infections found in MSX and SSO cases.
3. It seems to multiply by internal budding with one to three stages of cell-within-cell of the pathogen.
4. Prof. Balouet's data for 1977 suggest a short infective period of 2-4 weeks about 1 September. This probably corresponds in timing with the peak water temperatures in an oceanic climate.
5. Mortality also occurs in Aug-Sept. of the 2nd year at peak temperatures. This suggests that starvation from damage to the digestive tract is the cause of death rather than toxicity or intensity of general tissue damage as in MSX and SSO. Poor oysters are reported before death in the 2nd year (Guy Maheo).
6. Sporulation occurs in the 2nd year but it is not clear whether it is related to deaths and what the timing is.

The salinity relationships of Marteilia disease are of concern considering their restrictions on MSX and SSO diseases. From Guy Maheo's description, it seems to kill more in shallow enclosed areas where there is some dilution from freshwater runoff (river mouths) in Brittany. The areas most open to the ocean (Binic the seed area, Cancale, Carantec, Roscanvel, and even Aber Benoit and Aber Wrach where Martelia disease was first noticed have few or no infections and little mortality. Although these mortality areas are subject to salinity fluctuations down to 25o/oo when runoff is high (fall and winter mostly?), this may be the cold period when temperature limits Marteilia anyway. On the other hand, the open sea beds may benefit from dispersal and dilution of infective particles whatever the source. It is not surprising therefore that infected oysters imported from France to the Oostershelde do not result in deaths and tend to show regression of the disease. Presumably absence of undetected alternate hosts in the open-water regions of France is not an explanation for rarity of disease. Guy Maheo reports severe losses in 1976 when a 3-1/2 month drought in Western Europe increased temperatures as much as 5°C and probably also increased salinities. Higher temperatures in the Oostershelde or ~~over~~ the Brittany Coast should favor the disease if the necessary "critical mass" of disease is present.

It is significant that C. gigas planted in the high-mortality area of Landevennec failed to obtain any infections when O. edulis transplanted at the same time did. This appeared to be the situation in Delaware (USA) where C. gigas were held

without losses in an area being decimated of C. virginica by MSX in the 1960's. The data are obscure and unpublished by the investigator, who was severely criticized for having C. gigas in W. Atlantic waters.

Spores of MSX in Rappahannock River Spat

21 September 1976

J. D. Andrews

Yesterday, the field men spent much time sorting Tray 171, containing Rappahannock River set of this year, of its boxes. Some 9 qts. of spat died since our last check on 23 August 1976. This morning, I had Curtis bring in 100 spat for Mike to make smears of the digestive tubules. At this hour (2 pm), he has found spores in 7 of 20 sick oysters-spores usually scarce but good clumps of about 6μ spores in one I looked at.

The background history of this lot is significant!

1) These are susceptible oysters by source.
2) They are young oysters.
3) They were moved from pond to Tillages 8 July 1976.
4) A second lot of the same batch (some set later) were brought to Tillages 16 August 1976 and they have none dead and are vigorous oysters twice as big as the earlier lot.

Obviously, these spat have acquired MSX and are dying at a high rate 8 to 10 weeks later with spores mature in some. The spores are 6 to 8μ in length, and usually in sporocysts (sporangia). In most spat the spores are scarce. The walls of the sporocysts seem quite tough.

We are thinking about trying infection experiments in closed aquaria by feeding or injecting spores. Dr. Perkins will try to clean them up by centrifuging. We should use Horsehead oysters and fix a control sample. They should be held for at least 4-6 weeks if possible. How poor are

they? (Was not possible to clean by centrifuge because small spat and immature spores - also Perkins is busy.)

Sporulation of MSX

J. D. Andrews

September 1976

Although sporulation is rarely achieved in oysters that survive MSX infections for a full year, it can occur in as little as eight weeks from infection. It appears that environmental conditions for sporulation are not favorable rather than that resistance of oysters is preventing it. What circumstances permit sporulation in the epithelia of digestive tubules but not in other tissue? This is a major deviation from the patterns of SSO where plasmodia in all tissues progress to sporulation. This restriction to digestive gland epithelia could be interpreted as an adaptation of the parasite to an organ where ready access for discharge of spores is accomplished. Such an adapted host would not need to kill its host.

Minchinia nelsoni kills most infected oysters unless environmental conditions, particularly salinity, are modified to favor the host. However, sporulation of MSX in limited areas of one tissue does not seem to accelerate deaths of oysters. Oysters with spores in mid-winter live until June of that year and die from heavy plasmodial infections. In contrast, SSO begins sporulation in mid-May and all oysters subjected to sporulation die before mid-June. Infections and sporulation are massive and systemic in oysters with SSO. It is significant that oysters with either MSX or SSO infections usually die before spores of the pathogens are mature. This has implications for the continuation of life cycles by the parasites. Meats of dying oysters are ingested or dispersed within hours after the oyster shell gapes.

Diagnosis of Sporocysts

It is impossible to dissect out the thin epithelial layer of digestive diverticula. A pippete with a long finely drawn tip was found most effective to rupture the tubules and suck up a bit of tissue and fluid for fresh smear examinations. The tiny white sporocysts under low power were spherical with tough elastic walls not easily broken. The diameter of sporocysts in squashes was 25μ to 40μ and each sporocyst was estimated to have 30 to 50 spores. The granular aspect of sporocysts filled with spores was distinctive in proper lighting. Mature spores were not abundant enough to give a distinctive color to digestive tubules although the organs were usually whitened in comparison to tubules in feeding oysters.

History of MSX Spores

23 September 1976

We found spores this week in live oysters of tray P171 which contains Rappahannock River spat set early (June) this year. About 40% (Curtis Kough estimate) of the oysters have died mostly in September apparently. Mike found sporangia in 7 of 21 "sick" oysters Tuesday and I found 3 in 29 oysters unselected. Only 2 cases had any abundance of sporangia and mature spores.

This is the shortest known period from time of exposure (8 July 1976) to observed sporulation and spores (20 September 1976). It shows for the first time that spores can develop without going through an annual cycle.

We are considering infection experiments but can't find enough mature spores for Perkins to centrifuge and try to clean up and concentrate them. Everyone has tried infection experiments with gapers and infected oysters, but I'm not sure anyone has with spores. Haskin has not had access to live spores. Oxford has had live spores in 1965 and October - November 1966. We have never had live spores before although Perkins may have had from the gapers I provided.

I have been reading the mortality conference records for clues to infection experiments. Only those in Nature are described. However, I encountered references to MSX spores in addition to Walt Canzonier's 1963 account which proves that the rare early cases of spores (2 in Delaware Bay and 1 at Ocean View) were displayed at the conferences. In January 1962, at Solomons, Barrow was trying to make enlarged nuclei of plasmodia into spores. Carriker asked "How do you think MSX should be classified?" Barrow: "Not as Haplosporidium if there is no spore."

Myhre: "Would you care to comment on the spore that you were shown last night?" Barrow: "It is definitely a haplosporidian and the size is comparable to what we should expect for MSX."

Then Barrow goes back to his nuclear-spore. Mackin says: "The spores John Wood (M. costalis) and Hal (M. nelsoni) have mentioned are not the same at all. One has a rounded lid and the other has a flat one. There seems to be 3 spores not 2." Hal says: "I'm not clear on the timing of these cycles." Barrow: "Shizogony lasts longer and occurs in summer. Sporogony occurs suddenly in the fall." (He is giving the cycle seen in Minchinia pickfordae in freshwater snails.) Sprague: "...I can't accept these bodies as spores..." (This refers to Barrow's nuclei-spores.)

Then John Wood mentions the finding of spores much larger than those of SSO and talks about the haplosporidan in crabs that causes pepper crabs. Victor Sprague asks if there is any chance either of the spores you mentioned is the MSX spore? John answers "I wouldn't be surprised if the larger one might be." Hal says: "We have a slide of spores, probably the same ones you mentioned from a Delaware native oyster that was heavily infected with MSX." John: "The one I was speaking of may have had MSX in it also." It did! JDA! Then after John describes SSO, Stauber raises his seaside theory that resistant seaside oysters plus different environment may have caused MSX to sporulate there and that we were looking at the same pathogen. History has proved him wrong, but the Rutgers people gave me a hard time for years about this. I suppose the argument continues for Haskin doesn't accept my high-salinity inhibition of MSX on Seaside and invokes a resistance mechanism. How about James River oysters then? They exhibit SSO spores.

In January 1965, I made mention in a mimeographed hand-out of the life cycle of MSX of 6 cases of Minchinia spores and Walt Canzonier's report on 3 of them with MSX plasmodia. The following June-July, Couch and et al made smears of sick oysters from Manokin River, etc. and found spores in some 20 oysters. They set about immediately to name MSX secretly by publication in Science. They never have acknowledged the rare cases discovered by Haskin and us. The year 1965 was an intensive one for MSX which pushed into Maryland and attacked susceptible oysters which appear to be necessary to get many spores. Also, it is apparent to me now that young oysters are more likely to have spores -perhaps because they resist MSX more than do older oysters.

MSX Sporulation

J. D. Andrews

September 1976

1.) Rarely achieved even in oysters that live for a year.

2.) May occur at any time within 2-3 months after infection.

3.) Does not kill oysters as a result of sporulation - winter cases in live oysters would have persisted until June deaths probably.

4.) Requires a special set of circumstances not present in most oyster tissues - never sporulates except in epithelia of digestive tubules.

5.) Most spores do not mature in live oysters??

6.) The "punctate stage" of multiplication seems not to be adequately recognized as the beginning of sporulation by other investigators.

7.) Since many (usually ≈ 50 spores) are formed per sporangium or sporocyst-not like species in chitons and worms which have 4 and 8 spores that may result from sexual reproduction.

8.) Look for orange-white discoloration - sporulation usually too early or too light for discoloration by spores.

9.) Spores seem far too scarce to cause infections in widely scattered oysters! Yet other stages have never been shown to produce infections either.

10.) Barrow did mention sporulation in gut of snails in 1964 at Solomons and I failed to pick it up in his discussion - info was kept secret for Oxford publication in later years.

11.) It is interesting to speculate how and what infective particles were acquired by thousands of spat almost simultaneously in Tray 171, whereas most trays of oysters only experienced a limited infection the first year and more the second year.

NOTES ON MYHRE'S SPAT EXPERIMENTS

18 August 1976

J. D. Andrews

I had almost forgotten about Haskin's 1972 report to the National Marine Fisheries Service. I either forgot most of the contents or never read most of it. Myhre's studies are the only ones that stimulate me much.

In 1966, John exposed 3 resistant and 2 susceptible lots of lab-reared 1966 spat in early September and the following early August found the resistants with only 4 infections in 50 oysters whereas the susceptibles had 35 in 50. I would have concluded that these were June 1967 infections, just appearing, since 25 of them were localized.

John set up 3 lots of each category from 1967 spat groups and began exposing them in early September of that year. He found 2 infections 24 days after 1st(?) exposure in one lot. Unfortunately, he did not sample in November of December that year, but found high infection levels in the period 15 January to 15 February 1968. These increased in number by late April but most infections remained localized (he cut at least 5 sections about 750 μ apart). By June 26th, the resistant oysters had gotten rid of most infections, whereas the susceptibles had systemic infections. By 31 July, the susceptibles had about equal numbers of general and localized infections hence new ones had occurred and lots of deaths too. He concludes that probably 100% of both categories of spat got localized infections "in the winter" but only in susceptibles did these progress to general infections.

He examined only 10 spat per sample but the prevalences were so high that this number if adequate to show the trends. He found a high proportion of localized infections and suggests that those in small spat were subpatent until late April.

The work is impressive altho I have to allow for John's tendency to speculate beyond reason. Here are some of the items that impress or disturb me:

1) I am amazed at this intensity of infection in spat. We have not seen its equivalent in Chesapeake Bay. Neither do we have mortalities of spat or yearlings in the range of 50 to 80% from MSX or any cause!
2) If we are getting subpatent infections in the fall (and I strongly believe we do in susceptible oysters), why do we not encounter more localized infections in the gills. We find an occasional one but it is a rarity.
3) The decline of sub-patent infections in resistant oysters after 29 April parallels my experience in low salinities where infections disappear about 1 May. But this is not known to occur in high salinities, except Seaside where regression is common.
4) We have never found the high proportion of localized infections they find. By selecting the time of late July or early August we can find some, just when new infections are appearing.
5) How could I forget that John found 4 cases of sporulation 15 June of MSX in spat out of 34 infections! One was a concurrent SSO & MSX spore case.

The regression of MSX infections in Delaware Bay is very similar to what we have seen on Seaside of Eastern Shore, particularly Hog Island Bay. Salinities at the Cape May Lab are high, particularly in drought years, and this may be the cause of regressions as I think it is on Seaside. The occurrence of a few cases of SSO in lower Delaware Bay is also an indication of salinity conditions being high. I wonder if salinity could be a factor in early development of MSX cases from late summer infections, for our early ones came in dry years. Also could it be a factor in slow development from localized to systemic cases. We have attributed these events to infection pressure. In passing, it is noteworthy that spat set in the summer exhibited an SSO case which together with the recent case we found in a Horsehead oyster imported to Eastern Shore on 22 November that was in plasmodial stage on 1 June of following year.

I am quite puzzled by the relatively heavy death rates of resistant oysters to MSX in the first year or two in Delaware Bay usually 40 to 50% at least. Over 50% of these spat died altho I question whether MSX was the cause in resistant oysters and I have always believed that Cape Shore trays are bound to have smothering and predation losses, not to mention the winter storage conditions in creeks. Ice must be avoided. Our system usually avoids these problems.

Notes on *Minchinia americana* from paper by P. VanBanning

J. Invert. Pathol. 30:199-206

J. D. Andrews

Two spore cases (19 August 1974 and 21 July 1975) and one plasmodial case (26 January 1976) in oysters of *Ostrea edulis* transplanted from France to Holland in spring(?). He made light and electron preparations of spores.

Spores with haplosporosomes and spherules and long tails! Seen in fresh squashes. Size of spores 5.5 x 5.0u fresh and 4.5 x 4.0 fixed. (Short, almost round-mature?)

Brown, discolored oysters with sporocysts in connective tissue, especially of visceral mass (systemic?); sporocysts 35-50 u in size (same as MSX but larger than SSO). Spores 100 to 150 per sporocyst. V dense in the photo with lots of mature spores! High counts of spores.

Plasmodial *v large* (17-25u) scattered in connective tissues of digestive tract.

The projections (tails) on *Minchinia* spores I have not seen. He puts too much emphasis on brown color, especially for separating species. MSX has some color but only in tubules where spores are and often there are not enough mature spores even in SSO to cause discoloration.

The new species has sporonts and sporocysts 2-3 times as large as SSO and in the same range as MSX. The striated spore wall is like in SSO but wall is not striated in MSX. He places the filaments or *tails* as being larger and with bases like in *M. chitonis* whereas SSO and MSX have only filaments, no tails.

Table 1. Comparison of haplosporidan parasites

	M. armoricana	SSO *M. costalis*	MSX *M. nelsoni*	*M. louisiana* in mud crabs (*Panopeus*)	*M. ehitonis*	*M. nemertis*
Size of Sporocyst	35-50u	9-23u	-	-	40-75	-
No. of Spores	100-150	8-51	30-50	50-100	>100	± 50
Size of Sporont	30-45		25-40		60	30
Localization in host	connective tissue	all tissues	digestive diverticula	gut wall blood incises	-	-
Spore wrappings	2 tails	filaments	filaments	-	2 tails	
Color	brown	-	-	brown	brown	-
Spore wall	spore wall straited	not	straited			
Type Sporulation	mass sporulation systemic	mass systemic	sporulation rare	mass systemic dig. tract	mass dig. tract	-

Notes on *Minchinia americana* from Paper by P. Van Banning J. Invert Pathol. 30: 199-206

Two spore cases (19 Aug 74 & 21 Jul 75) and one plasmodial case (26 Jan 76) in oysters of *Ostrea edulis* transplanted from France to Holland in spring(?) J. P Andrew

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Table 1. Comparison of haplosporidan parasites

	M. americana	SSO *M. costalis*	MSX *M. nelsoni*	*M. louisiana* in mud crabs (*Panopeus*)	*M. chitonis*	*M. inemertis*
Size of Sporocyst	35-50 μ	9-23 μ	–	–	40-75	–
No. of Spores	100-150	8-51	30-50	50-400	>100	± 50
Size of spore	30-45		25-40		60	30
Localization in host	connective tissue	all tissues	digestive diverticula	gut wall blood sinuses	–	–
Spore wrappings	2 tails	filaments	filaments	–	0 tails	
Color →	brown	–	–	brown	brown	–
Spore → wall	spore wall striated	not striated	striated			
Type → sporulation	Mass sporulation systemic	Mass, Systemic (tails)	sporulation rare	mass, systemic dig. tract	mass dig. tract	–

The projections on *Minchinia* spores I have not seen. He puts too much emphasis on brown color, especially for separating species. MSX has some color but only in tubules where spores are & often there are not enough mature spores even in SSO to cause discoloration.

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South Atlantic
(Region 03)

The states of the South Atlantic Region are largely rural in character and do not face the intense urban pressures of the northeastern states. Florida, however, is an exception. It must deal with the conflicting needs for urban, recreational, and industrial growth. Florida's fragile marine and nearshore environment has been imperiled by man's activities. Other South Atlantic states must also respond to increasing demands for recreational, residential and industrial shoreline development. The problem of shoreline destruction and increasing water pollution are faced by all South Atlantic states. Interstate compacts in the region include the Atlantic States Marine Fisheries Compact and the Coastal Plain Regional Commission. Innovative legislation in the region is typified by Florida's Environmental Land and Water Management Act.

North Carolina

Administrative Organizations
Department of Administration
Department of Natural and Economic Resources
Office of Marine Affairs
Marine Science Council
Division of Health Services

Major Statutes (General Statutes of North Carolina)
Environmental Policy Act (§ 143B-282 (1974))
Coastal Area Management Act of 1974 (§ 113A-100 (1974))
Sedimentation and Pollution Control Act (§ 113A-50 (1974))
Wetlands Protection Act (§ 113-230 (1974))

Coastal Zone Management Act Responsibility
Department of Natural and Economic Resources
Office of Marine Affairs
Department of Administration
Coastal Resources Commission

Problem Areas
Balancing need for economic and transportation development with wildlife and fisheries needs.
Maintaining water quality.
Providing shoreline recreation opportunities.

South Carolina

Administrative Organizations
Wildlife and Marine Resources Department
Department of Health and Environmental Control

Summary of *Minchinia armoricana*

J. D. Andrews

25 Jan. 1977

Paul van Banning of the Netherlands Institute for fishery Investigations, Haringkadel, I J Muiden - 1620, the Netherlands has reported a new pathogen from 3 live oysters imported from Brittany to Holland, 1974-1976. This is the first haplosporidan from oysters in Europe and the first in *Ostrea edulis*. Two cases were advanced sporulation with 4.0-5.5 μ spores, large sporocysts (35-50 μ) and 100 to 150 spores per sporocyst. The spore walls are striated as in SSO and large "tails" are present on the spore cover in fresh smears. The pathogen is systemic with sporulation in all connective tissues but not epithelia.

It appears that oysters imported in the spring had the infections and July-August occurrence of spores would about match June-July temperatures and seasonal timing here for SSO. Well-developed spores would suggest that it is an acclimated species perhaps with an annual infection and mortality period. The disease is too rare to cause noticeable mortality. The emaciated brownish meats of the infected oysters were conspicuous to compensate for rareness.

A table comparing 6 *Minchinia* species by sizes of various stages and e.m. structure and tissue of sporulation is interesting.

Minchinia sp. in Shipworms (*Teredo*)

J. D. Andrews

5 August 1983

Hillman has published three papers giving prevalences of a haplosproidan in shipworms. Three species of *Teredo* were found to be infected but not *Bankia*. The parasite is systemic in all connective tissues. It seems to have a short life cycle with infections most abundant from October to December each year. Spores are large, similar in size and shape to those of MSX (8-10μ), and nearly always present. Because Hillman's artificial wooden blocks were usually left only six months, there appears to be a life cycle much shorter than one year - perhaps adapted to a short life-span of shipworms.

Two of three species of shipworms parasitized by the pathogen are subtropical species introduced to New Jersey by boats and dependent on warm water effluents of power plants for necessary survival temperatures. Distribution of shipworms including *Teredo navalis*, the most abundant species, was quite irregular in Barnegat Bay although some 30-40% of all *Teredo* were infected with *Minchinia* sp. The parasite was also found in *T. navalis* near the effluent area of a power station in Long Island Sound. Earlier studies of *Teredo* in New Jersey did not reveal the parasites. Hillman speculates that *Minchinia* sp. may have been introduced with the exotic subtropical shipworms.

EM studies of the spores are being pursued by Haskin and associates at Rutgers to determine if it could be *M. nelsoni*. It is unlikely that shipworms constitute a reservoir of infective particles for MSX in Chesapeake Bay where *Teredo* is irregular in occurrence and not abundant. *Bankia* is the common shipworm here.

Woolever, Patricia. 1966. Life history of a Haplosporidian Nephridiophaga blattelae (Crawley) n.comb., in the Malpighian tubules of the German cockroach, Blattella germanica. J. Protozool. 13(4):622-642.

1) Infected disease-free cockroaches with spores from tubules of infected specimens. Peroral infections. Infections first appeared in 15 days and were well-established by 30 days. Only third reported attempt at infections (details).
2) Interspecific infections-failed thus indicating specificity of hosts and pathogens. (4 species of cockroaches)
3) Early infections were intracellular but soon move to lumen of tubules and are attached to brush edge of epithelia. Increase mostly by schizogony and not plasmotomy.
4) Doubts sexual process and feulgen test indicates only one nucleus (not paired).
5) Woolever considers it a haplosporidan without polar capsule and filament.
6) Sprague reports this species is a microsporidan.

Table A. Early data Oxford Md. MSX in Live Oysters

	Area	Date	Sal.	Temp.	No. in Base	No. Selected Oysters	No. Infected	No. With Spores	No. Uninfected	Prevalence of Spores in Selects	Prevale of Spor Random in Bush
Res	Marumsco	10/13/66	19	18.0	(300) ½bu	37 F	17	0	20	0	0
	Marumsco	10/13/66	20.36	17.8	687 (½bu)	53 F	51	3	2	5.7%	0.43
	Marumsco	10/17/66	19.31	17.0	1270 (2bu)	130 FC	113	10	17	7.7%	0.79
Sus	Marumsco	10/23/66	20	15	(700) 1bu	154 F	147	12	7	7.8%	(1.70
	Marumsco	11/2/66	(20)	(12)	(700) 1bu	90 FR	88	6	2	6.7%	(0.86
	Marumsco	11/8/66	20.52	10.0	685 1bu	144 FRC	118	10	26	7.0%	1.47
	Marumsco	11/17/66	19.22	10.9	843 (1bu)	26 F	23	4	3		Not Com
Pub	Manokin	10/18/66	17.57	15.5	663	5 C	1	0	4	0	0
	Manokin	10/26/66	18.49	15.0	584 bu	37 C	9	5	28	13.5%	0.86
Res	Manokin	11/4/66	18.60	11.0	491	20 R	18	3	2	15 %	0.61
	Manokin	11/9/66	18.7	10.18	886	41 C	28	1	18	2.4%	0.26
	Tangier Sound										
	Kedges	10/18/66	20	15.5	88	2 C	0	0	2	0	0
	Lambstone	10/19/66	(19)	16	182 bu	13 R	6	0	7	0	0
	Kedges	10/27/66	18.93	(15)	577 bu	53 F	45	4	8	7.5%	0.7%
	Lambstone	10/27/66	18.75	15	148 bu	6 R	5	0	1	0	0
	Holland Strait	10/18/66	19	(15)	728 bu	11 C	2	0	9	0	0
	Holland Strait	10/27/66	18.72	14.8	(700)	54 R	43	1	11	1.8%	(0.14%
Sus	Honga River	10/26/66	18.98	(15)	319 bu	37 R	34	6	3	16 %	1.9%
	Honga River	11/4/66	19.22	11.4	550 bu	55 RC	41	10	14	18 %	1.8%
	Honga River	11/9/66	18.7	11.79	825 bu	59 RC	51	6	8	10 %	0.98%
	Honga River	11/16/66	18.84	10.7	575 bu	56 F	55	10	1	18 %	1.75%
	Totals - 21 samples				(9731	1083	890	91	193	10 %	0.78%

table A. Early data Oxford Md, MSX in live oysters