ATTENDANCE

FOURTH ANNUAL SHELLFISH MORTALITY CONFERENCE
Chesapeake Biological Laboratory
Natural Resources Institute of the University of Maryland
January 22 - 23, 1962

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SUMMARY OF OYSTER MORTALITY STUDIES - 1961

Oyster Research Laboratory
N. J. Agricultural Experiment Station
and
Dept. of Zoology
Rutgers - State University of New Jersey

(Prepared for 4th Annual Mortality Conference - Jan. 22-23, 1962)

Field, tray and laboratory studies previously reported for the Delaware Bay area have all been continued, although emphasis on various aspects of the work has changed.

I. Delaware Bay Seed Beds and Leased Grounds

D. Haskin, W. A. Richards, J. H. Myhre

By far the most important development in this area is the spring planting of approximately 166,000 bushels of seed oysters on the planted grounds. Five grounds of these were sampled routinely once a month following planting. Unexplained (non-predation) mortality was low--usually under 2% monthly--from June through November. The plants have now been marketed after an unusually fine growing and fattening season. Yields of high quality oysters were excellent, averaging slightly better than bushel for bushel turnout. Drills were active and destroyed as high as 16% of the oysters monthly at peak of season. At the monthly sampling, collections of oysters from each ground were fixed for histological study. These have been worked up through November for only one ground and, to date, only one oyster positive for "MSX" has been found in the spring of '61 plants.

Grounds with old surviving oysters of the 1958 planting, and of the experimental plantings of 1959 are still under study. In spite of the low incidence of "MSX" in the newer stocks there is evidence of a substantial amount of "MSX" in the older stocks. Detailed description of the levels of infection during the apparent decline of the Delaware Bay epidemic will await further work-up of the bay samples collected.

II. Experimental Tray Studies

H. Haskin, W. Canzonier, S. Y. Feng

In 1960 although mortality levels had declined sharply in experimental plantings of oysters on the leased grounds, kills continued undiminished at epizootic levels in our tray stocks at the Cape Shore. In 1961 this situation changed. Beginning with a James River stock imported in October of 1960, all introductions have had negligible "MSX" mortalities to date. James River stocks imported in August and September of 1960 became infected. Appreciable death of the August imports occurred in fall and winter 1960-61 and heavy kill over the 1961 summer. The September import had

its first heavy kill in June, 1961. Monthly imports of susceptible James River stocks were repeated from March to September in 1961 (courtesy of Dr. Andrews, Virginia Institute of Marine Sciences); and various other known susceptible stocks from upper Delaware Bay, the Navesink River and Long Island Sound were also brought in. None of these have yet shown epizootic levels of kill in sharp contrast to the results obtained with similar importations in 1959 and 1960.

Older tray stocks continued to die at a low level in spring and early summer and at an appreciably higher level in late summer and fall. Apparently much of this latter kill may be correlated with Dermocystidium infection.

III. Laboratory Studies

A. Tray Stocks - W. Canzonier

"MSX" incidence was monitored in tray stocks from fall 1960 through 1961 by fresh smear technique as well as examination of preserved tissue.

Gapers from stocks introduced in August and September, '60 exhibited infections as high as 100% through fall and winter and summer of '61. These stocks also experienced considerable mortality over the period.

Gapers of old survivors in '61 ran from 25 - 50% infected from February-July in contrast to as high as 83% in '60 for the same period.

Gapers of spring '61 imports, however, range between 0 - 25% infected for the period of July-October. This is in sharp contrast with previous years of '59 and '60 where infection in gapers for this period were 85 - 100%.

Examination of living samples of spring '61 imports revealed infection levels between 0 and 20% for July-October whereas incidences as high as 50 - 70% were shown by similar stocks in '59 and '60.

Fresh examination and culture of gapers indicate Dermocystidium to be of major importance in late summer-fall mortalities of tray stocks.-- Old stocks running as high as 100% infected in gapers examined and new imports as high as 37%.

Examination of samples of living oysters showed "mycelial disease" (Mackin) in considerable abundance in new imports in November, '60 with an associated weakening of oysters. Living samples of spring imports also show a high incidence (80 - 100%) in July and August with almost complete absence by September-October.

B. Bay Stocks and Trays - John Myhre

The development of "MSX" infection in trays in an experimental stock introduced into the lower Bay in August, 1960, has been under intensive study. The population was sampled at daily, then weekly, and finally monthly intervals for a year and three months. First infections were recognized 3 weeks after introduction of the stock. "MSX" apparently invaded through the gill, causing extensive epithelial damage. Infection apparently continued to build up through the entire fall. A decline in infection levels occurred at time of a significant mortality in spring. This appeared to be followed by a period of increased infection. This study is continuing.

C. Dermocystidium on the Planted Grounds - D. Kunkle

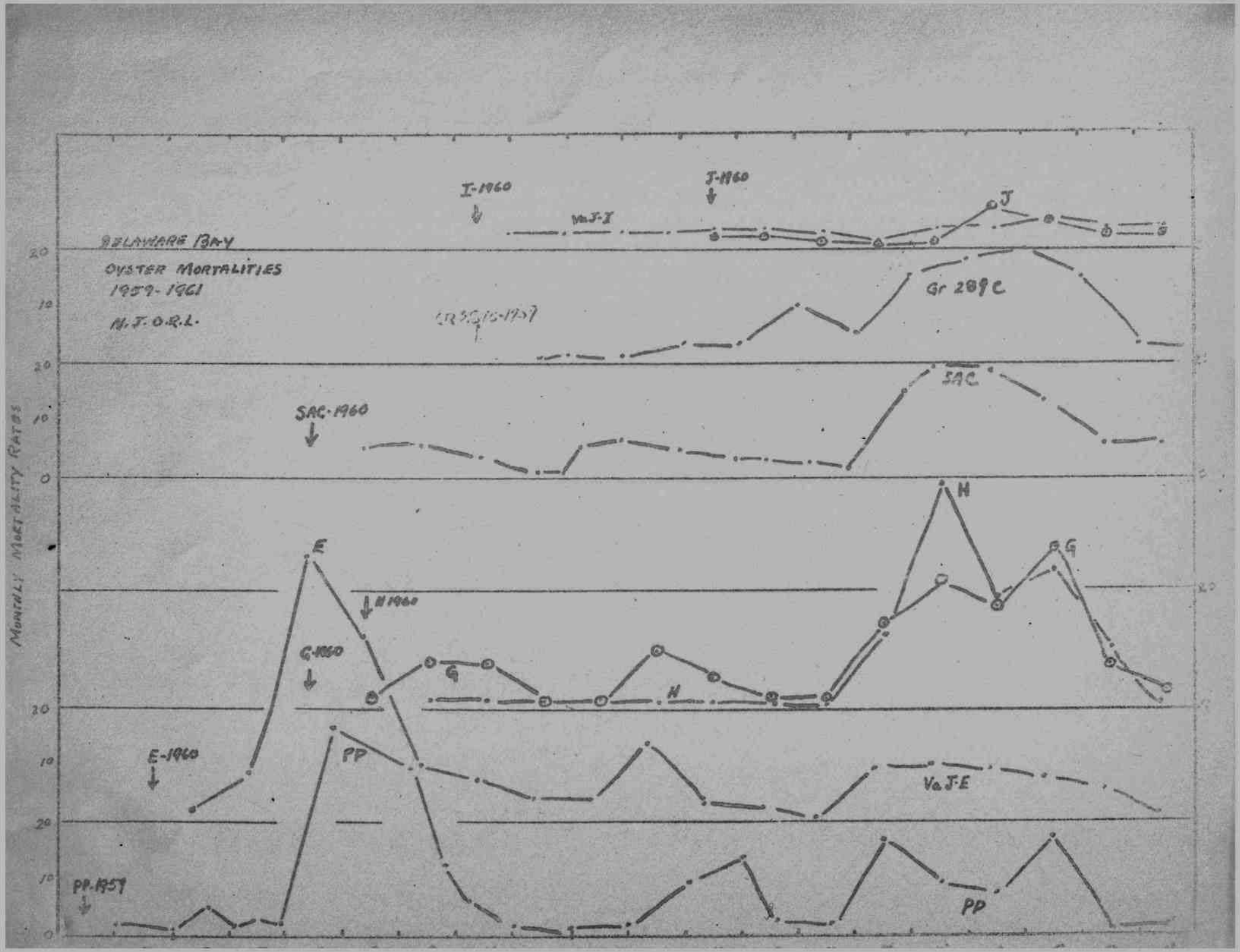
Dermocystidium levels in the bay have continued to decline since the first surveys conducted in 1955, as shown by an annual late summer-early fall sampling program. In 1960 approximately 2% of the oysters sampled were infected and average weighted incidence was less than 0.02. We are inclined to believe that the higher levels reported earlier were maintained by the importation of heavily infected stocks from the lower Chesapeake.

D. The Oyster Gut as an Environment - S. Y. Feng

pH of oyster stomach fluid, heart blood and shell liquor were studied. Observations were made on intact and partially denuded oysters held under dry cold and wet cold conditions. Through the 50-day period of study Hexamita were present in stomach fluid samples (pH 6.5 - 6.9) though in low numbers (ca. 500 per oyster) and at no time were Hexamita found in heart blood samples (pH 6.9 - 7.1)

E. New (?) Oyster Associates

Three organisms not previously seen in oysters by us will be demonstrated.


DELAWARE BAY
OYSTER MORTALITIES
1959-1961
N.J. O.R.L.
I-1960
J-1960
Vs J-I
J
Gr 209 C
SAC-1960
SAC
H
E
H 1960
G-1960
G
H
E-1960
PP
Vs J-E
PP-1959
PP
MONTHLY MORTALITY RATES
20
10
0

NATURAL RESOURCES INSTITUTE, UNIVERSITY OF MARYLAND
Chesapeake Biological Laboratory, Solomons, Md.
L. Eugene Cronin, Director

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SEARCH FOR ANOTHER HOST OF MSX

Victor Sprague

Chesapeake Biological Laboratory

INTRODUCTION

There are several conspicuous gaps in our knowledge of MSX which seriously impede progress in work with this important parasite of oysters. Some of them are as follows:

1. An infective stage, or stage which seems suited for the function of initiating the infection in a new individual (oyster or possible alternate host), has not been recognized.

2. Although MSX looks like a haplosporidian, the spore, a stage of decisive importance in identification, has not been demonstrated.

3. Attempts to transmit the infection from one oyster to antther have been conspicuously unsuccessful and there is no clue as to the mode of transmission.

4. The method of dispersal is completely mysterious.

5. Distribution seems to have some relation to salinity, but we do not know whether salinity is a primary factor or only incidental to some more basic factor.

Undoubtedly, all persons working with MSX have given much thought to developing theories adequate to explain the mysteries. Keeping in mind the principle that the best theory is the simplest one which will explain the facts, and claiming no monopoly on any theory, we at Chesapeake Biological Laboratory favor the idea that MSX is a typical haplosporidian normal to another host, occurring sometimes in oysters but being usually incapable of developing to the spore stage in this unnatural host. On the basis of this theory we would expect the problems listed above to be solved approximately as follows:

1. In the normal host a spore would be commonly present and it would be known a priori to be an infective stage.

2. The morphology of the spore would provide the basis for classification.

3. With the spore available it should be possible to infect oysters in the laboratory.

4. The method of dispersal would be found to relate to the habits and methods of dispersal of the normal host.

5. The geographical distribution of the normal host would be found to be a basic limiting factor in distribution of the parasite, although not necessarily the only one.

If the theory supported here is the simplest one that will account for the facts or, conversely, is not contradicted by known facts, finding the supposed other host seems to be a matter of critical importance to the progress of some of the major phases of the MSX research. This theory is, therefore, being presented in some detail at this time for the primary purpose of provoking further thought and discussion as to its merits. If this theory cannot be eliminated on a priori grounds and no better one is in view, a vigorous effort to find MSX in another host seems fully warranted.

METHOD OF APPROACH

Since there are hundreds of species of organisms associated with oyster beds, it is highly desirable to limit the problem by having a knowledge of which organisms are most likely to be hosts of Haplosporidia and concentrating efforts on them. To provide such information the appended list of Haplospordia, with host and habitat, has been compiled. Appended, also, is a list of appropriate references. The list is believed to include most of the known Haplosporidiidae, the family which MSX seems most closely to resemble.

Of the more than thirty species of Haplosporidiidea listed here, most are in molluscs and annelids. Few are in Crustacea, Nemertea, Tunicata and Insecta. These groups of organisms, in approximately this order, may be the ones on which efforts might most profitably be concentrated. There is no implication, however, that others should be neglected.

After collecting such organisms, especially from areas where oysters are commonly infected with MSX, there are at least two general procedures which can be followed. These are examination of the suspected host organisms and infection experiments. Both can, and probably better should, be carried out at the same time.

Examining the suspected organisms could be made into an impossibly large task, especially if one should undertake indescriminately to section great numbers of them without employing first a screening process. Screening can be done by means of gross inspection and fresh smears. Fortunately, it has been noted that many of the Haplosporidiidae show gross signs of their presence. They may cause grossly visible hypertrophy of the affected organs and, most likely, the millions of spores, typically brown in color, will cause striking discoloration of the same organs.

Spores can be easily detected by microscopic examination of fresh smears without any kind of treatment. Although there are many sporozoan spores which can not readily be assigned to their proper taxonomic group, the chances are that spores of Haplosporidiidae will be easily recognized as such by anyone reasonably familiar with the Sporozoa. The lantern slides of spores of Haplosporidium sp. shown at this time illustrate typical spores of this genus. Spores of other species in this and related genera are variations of the same basic structure. The essential features are a single, more or less spherical sporoplasm, enclosed by a thick shell. There may be an opening at one end for escape of the sporoplasm and this may be covered by a lid or operculum. There may be long appendages. There are never polar capsules or polar filaments as in the Cnidosporidia, this being the characteristic for which the group was named Haplosporidia. "Haplo" is from the Greek word for "simple."

Plasmodial stages should be recognizable in fresh smears with addition of methylene blue to show up structural detail, a method which Rutgers University workers have shown to be quite reliable for demonstrating MSX. After this screening process with gross inspections and fresh smears, then it would seem to be the proper time to concentrate on preparing and studying sectioned materials.

At the same time, crude infection experiments can be carried on by holding oysters together in the same aquaria with any or all of the various organisms being investigated as possible other hosts of MSX. When and if infections arise in the oysters and/or possible stages of MSX are found in the other hosts by microscopic examination, the experiments can be appropriately refined and possibly some conclusive evidence can be obtained.

PRELIMINARY STUDIES AT CHESAPEAKE BIOLOGICAL LABORATORY

We have examined about 1 to 50 of each of the following organisms, listed approximately in decreasing order of number examined: Serpulids, Eurypanopeus, Branchiodontes, Nassarius, Mytilus edulus, Crepidula, Anomia, Laevicardium, amphipods, Lyonsia, Panopeus, Rhithropanopeus, Eupleura, Neanthes, Urosalpinx, Mya, Arca, Molgula, Stylochus, nudibranchs, anemones, Ensis, Mulinia, Polydora, Cliona, Microciona, Leptosynapta, nematodes. Nothing resembling a haplosporidian was found, although various other parasites (gregarines, Microsporidia, trematodes, ciliates) were seen.

In August, 1961, a crude infection experiment was set up at Public Landing and another at Solomons. Healthy oysters were placed in aquaria with various other organisms, especially serpulids, from areas where MSX is known to occur. Thus far there are no positive results to report.

Recently, we have directed special attention to Mytilus edulus, host of Haplosporidium mytilovum Field, 1924. The parasite, living in the egg of its host, has a striking resemblance to MSX, as judged by Field's rather sketchy illustrations. We think this is the best lead we have had and we plan to pursue it vigorously, although chances of finding the parasite this late in the season (early November) are not good because most of the mussels have completed spawning. We are finding a few mussels with some eggs and have added this mollusc to the experimental aquaria.

GENERAL DISCUSSION

The list of parasites appended includes organisms which the authors assigned to genera in the Haplosporidiidae. There is no implication, however, that the present writer considers them all to be properly placed as to genus. On the contrary, there are certain reservations. For example, H. sp. Debrone, 1935, which looks much like MSX, did not have spores in the material studied. It can, therefore, be only provisionally considered as a species of Haplosporidium. MSX could, with as much reason, be assigned to the same genus. Likewise, in H. mytilovum Field no spore seems to have been observed. Field figured certain structures which he called spores but the figures and descriptions of these structures are completely unconvincing.

He either used the term "spore" in a very loose and non-technical sense or figured the spores very poorly, for the objects he regarded as spores were almost certainly nothing but nuclei of the plasmodium, similar to certain nuclear stages in MSX which one by wish thinking might be tempted to designate as spores. Therefore, it is not certain that H. mytilovum is in the right genus. It is possible there is an unrecognized group of parasites related to Haplosporidium but without spores. It is more plausible, however, to suppose that spores in some species simply have not been demonstrated.

In reviewing the literature, it also seemed evident that there is so much structural difference in spores of species assigned to genus Haplosporidium that some species should be removed to other genera. This, however, is a matter for more detailed treatment at another time. For our present purpose it is sufficient to note that MSX and species previously assigned to Haplosporididae all appear to be closely related.

If our searches should reveal that MSX is identical with a typical haplosporidian, complete with spores, and normally occuring in another host, a number of our problems might be solved in about the manner already indicated. On the other hand, if it develops that certain haplosporidian-like parasites, including MSX and possibly Haplosporidium sp. Dehorne and H. mytilovum Field, really have no spores then we are dealing with a new group of parasites and must deal also with some new problems relative to such matters as infective stage, mode of transmission and means of dispersal. It would, in any event, be a great step forward if we only knew whether we are dealing with new parasites and new problems or merely with peculiar manifestations of better known cases of parasitism.

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Provisional List of Species of Haplosporidiidae Caullery et Mesnil, 1905

Species	Name	Host Classification	Habitat
Haplosporidium C. et M., 1899			
1. H. heterocirri C. et M., 1905	Heterocirrus viridis	Annelida	Marine
2. H. scolopli C. et M., 1905	Scoloplos mulleri	" , Aricidae	"
3. H. marchouxi C. et M., 1905	Salmacina dysteri	" , Serpulidae	"
4. H. potamillae C. et M., 1905	Potamilla torelli	" , Sabellidae	"
5. H. sp. C. et M., 1905	Polymnia nesidensis	" , Terebellidae	"
6. H. caulleryi Mercier et Poisson, 1922	Nereilepas fucata	" ,	"
7. H. sp. Dehorne, 1935	Nereis diversicolor	" , Nereidae	"
8. H. vejdovskyi C. et M., 1905	Mesenchytraeus flavus	" , Enchytraeidae	Fresh water
9. H. limnodrila Granata, 1913	Limnodrilus udekemianus	" , Tubificidae	" "
10. H. cernosvitovi Jirovec, 1935	Opistocysta flagellum	" ,	" "
11. H. aulodrili Jirovec, 1940	Aulodrilus pleuriseta	" ,	
12. H. chitonis (Lankester, 1885)	Chiton fascicularis and	Mollusca	Marine
	Craspedochilus cinereus	"	"
13. H. dentali Arvy, 1950	Dentalium	"	"
14. H. tapetis Vilela, 1950	Tapes decussatus	"	"
15. H. mytilovum Field, 1924	Mytilus edulus	"	"
16. H. pickfordi Barrow, 1961	Heliosoma, Physa, Lymnea	"	Fresh water
17. (SSO) H. sp. Wood and Andrews (undescribed)	Crassostrea virginica	"	Marine
18. H. sp. " " " "	" "	"	"
19. H. bayeri Weiser, 1947	Cleon rufulum (larva)	Insecta, Ephemeroptera	Fresh water
20. H. ecdyonuris Weiser, 1947	Ecdyonurus venosus (larva)	" "	
21. H. typographi Weiser, 1954	Ips typographus	" , Coleoptera	
22. H. periplanetae Georgevitch, 1953	Blatta orientalis	" , Orthoptera	Land
23. H. aselli Pfugfelder, 1948	Asellus aquaticus	Crustacea, Isopoda	Fresh water
24. H. gammari Van Ryckeghem, 1929	Gammarus pulex	" , Amphipoda	
25. H. sp. Sprague, 1954	Panopeus herbsti	" , Decapoda	Marine
26. H. nemertis Debaisieux, 1919	Lineus bilineatus	Nemertea	"
27. H. ascidiarum Duboscq et Harrant, 1923	Parascidia elegans and	Chordata, Tunicata	"
	Amaroucium proliferum		
Urosporidium C. et M., 1905			
28. U. fuliginosum C. et M., 1905	Syllis gracilis	Annelida, Syllidae	"
Anurosporidium Caullery et Chappellier, 1906			
29. A. pelseneeri C. et C., 1906	Cercaria lutea (in Donax trunculus)	Trematoda	"
Haplosporidiidae of undetermined genus			
30. A haplosporidian hyperparasite, Mackin and Loesch, 1955	Bucephalus (in oysters)	"	"
31. A haplosoridian hyperparasite, M. and L. 1955	Sporocysts(in Donax Variabilis	"	"
32. A haplosporidian hyperparasite., Guyénot, 1943	Sporocysts(in Barnea candida)	"	"