8th Annual Oyster Mortality Conference
University of Delaware, Newark, DE
January 23-25, 1966

USBCF Biological Laboratory
Oxford, Maryland
Manuscript Report No. 66-2
January 24, 1966.

A PROPOSED LIFE HISTORY OF (MSX) IN THE AMERICAN OYSTER

by
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ABSTRACT

This paper presents a proposed developmental sequence of (MSX) (Figure 1); based on study of oyster infections occurring over the past five years in Chesapeake Bay. The proposed life history is compared with those of several other species of Minchinia as reported by earlier investigators. Haploid uninucleate stages develop by karyokinesis into multinucleate plasmodia which proliferate in the tissues by plasmotomy. Forms thought to be plasmodia containing gamete nuclei originate by unequal plasmotomy of large plasmodia. These daughter plasmodia fuse to form the prozygote. Fusion of prozygote nuclei occurs within the plasmodium, resulting in the diploid synkaryon stage, which undergoes karyokinetic (possibly meiotic) division. Sporoblasts within the sporont differentiate into spores with the formation of a spore wall and operculum. Encystment of haploid plasmodia appears to occur during times of unfavorable conditions. An atypical sexual process is described, and the occurrence of the various life history stages within the host throughout the year is discussed.

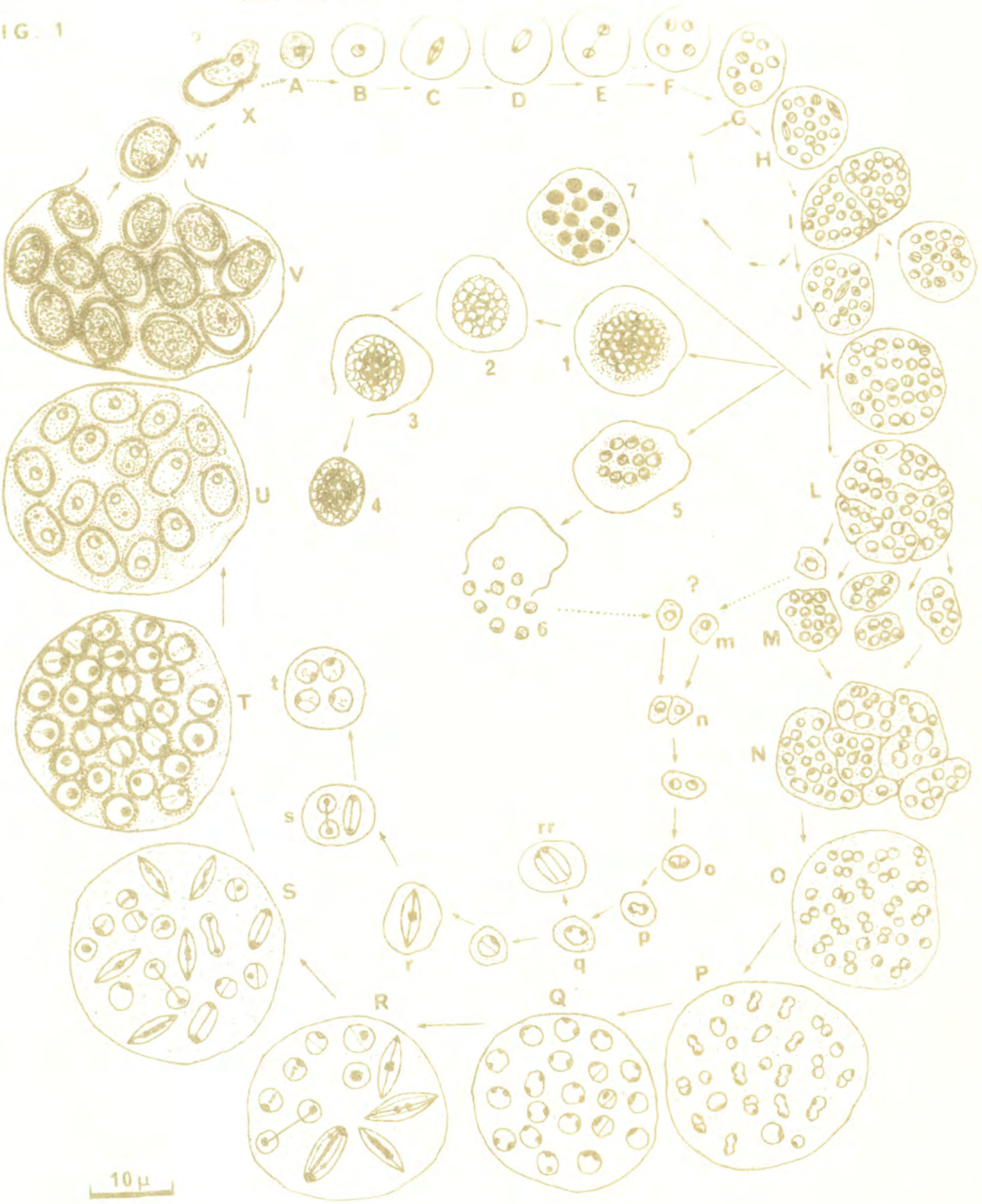
* This is an abstract of a paper titled "A Proposed Life History of (MSX) in the American Oyster", by C. A. Farley, prepared for submission to the Journal of Protozoology. The abstract does not constitute publication, therefore it must not be cited in any way without prior permission from the author.

Legend

Fig.1. Idealized scale drawing of the life history of (MSX) in the oyster, utilizing composite stages representative of the proposed sequence. Dotted lines indicate unclear or unobserved sequences. (A-H) Early karyokinetic development of multinucleate plasmodium from uninucleate amoebula. (I) Plasmotomy producing (J) daughter plasmodia. (K) Enlarged plasmodium. (L) Unequal plasmotomy forming (M) gametic plasmodia. (N) Aggregation and fusion of gametic plasmodia. (O) Prozygote with paired nuclei. (P) Prozygote with fusing nuclei. (Q) Synkaryon stage with enlarged diploid nuclei. (R) First meiotic division, karyokinetic sequence clockwise. (S) Second meiotic division. (T) Early sporont. (U) Immature sporocyst (containing haploid spores). (V) Mature sporocyst and spores. (W) Free spore. (X) Idealized escape of sporoplasm. (1-4) Encystment sequence. (1) Plasmolysis. (2) Formation of dense outer coat. (3) Rupture of plasmodial membrane. (4) Mature cyst. (5,6) Release of nuclei. (7) Moribund plasmodium. (m-t) Atypical sexual sequence and meiosis. (m) Gametes. (n) Fusion of gametes. (o) Prozygote with paired nuclei. (p) Nuclear fusion. (q) Synkaryon with 2 endosomes. (rr) Anaphase figure exhibiting double endosomes and "Kernstabs". (r) Metaphase of 1st. Division. (s) 2nd. Division (may be reduction). (t) Quadrinucleate, post-karyokinetic plasmodium. This form when found usually appears moribund.

PROPOSED LIFE HISTORY OF MSX IN THE OYSTER

FIG. 1



SOME PROPERTIES OF OYSTER HEMAGGLUTININ

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Oyster hemagglutinin is a large molecular weight protein as determined by dialysis and ammonium sulfate precipitation data. When injected into rabbits, precipitins are produced which react to neutralize its hemagglutinin activity. Galactose will selectively inhibit the reaction of oyster blood with rabbit red blood cells, thus suggesting that the reactive site on the red blood cell contains galactose. Partial purification of the hemagglutinin has been accomplished by ammonium sulfate precipitation.

USBCF Biological Laboratory
Oxford, Maryland
Manuscript Report No. 66-4
January 24, 1966.

A HAPLOSPORIDIAN PARASITE OF TAIWAN OYSTERS

by
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ABSTRACT

Pacific oysters (Crassostrea gigas) grown in Taiwan were examined microscopically for the presence of microparasites. Multinucleate plasmodia of a presumed haplosporidian were observed in 9% of the oysters in histological section. The organism closely resembles vegetative stages of the (MSX) organism found in the American oyster (C. virginica) along the Middle Atlantic States of the United States. The possibility that the parasite is identical to (MSX) would end the speculation that (1) MSX is host specific, (2) Pacific oysters are refractive to the disease, (3) that the distribution of MSX is restricted to the Middle Atlantic Coast of the United States.

Fresh squash examinations also showed the presence of: spores of the gregarine Nematopsis (38%); sporocysts and cercariae of the trematode Bucephalus (9%); larval stages of the cestode Tylocephalum (9%); and oocysts containing sporozoites of a presumed coccidian (possibly of the family Aggregatidae) (12%).

USBCF Biological Laboratory
Oxford, Maryland
Manuscript Report No. 66-3
January 24, 1966.

CONCURRENT HAPLOSPORIDIAN INFECTIONS OF THE AMERICAN OYSTER

by
J. A. Couch *

ABSTRACT

The concurrent, natural infection of 4 of 88 oysters by the haplosporidians Minchinia costalis (Wood and Andrews, 1962) and (MSX), is reported from Chincoteague Bay, Virginia. Sporocysts and spores of M. costalis occurred throughout the oyster's tissue, whereas those of (MSX) were restricted to the digestive diverticula; the former species was more abundant than the latter. M. costalis was characterized by fixed sporocysts 13.6 microns in greatest dimensions, and unfixed spores measuring 4.3 by 3.3 microns, whereas fixed (MSX) sporocysts were 37.7 microns in greatest dimension, and unfixed spores measured 8.1 by 5.5 microns. The spore operculum and nucleus of M. costalis are morphologically distinct from those of (MSX). It is suggested, however, that because of otherwise close morphological, host, and ecological affinities, the two may be closely related species.

* This is an abstract of a paper titled " Concurrent haplosporidian infections of the American oyster" by J. A. Couch, prepared for submission to the Journal of Parasitology. This abstract does not constitute publication, therefore it must not be cited in any way without prior permission from the author.

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

Reference No. 66-3

January 18, 1966

AN EPEDEMIC OF DIFFUSED ULCERATIVE GASTRO-ENTERITIS IN OYSTERS FROM TWO LOCATIONS.

(Delivered at the 8th Annual Shellfish Pathology Conference
Newark, Delaware - January 24-25, 1966)

Robert L. Beckett

During the course of an experiment, oysters were collected from various locations and, as a routine, 30 oysters from each location were sacrificed to determine their condition at the time of importation. Thin sections of oysters from Delaware Bay and Pocomoke Sound showed evidence of diffused ulcerative gastro-enteritis of epedemic proportion. Similar ulceration was also found in oysters from other locations but only as an occasional occurrence. All of the oysters were collected at the end of July, 1964, and all were apparently alive at the time of sacrifice.

The ulceration was found in approximately 30 per cent of the Pocomoke Sound oysters and in 20 per cent of the Delaware Bay oysters. The thin sections were made half way between the anterior end of the oyster and the adductor muscle, so it is possible that inflammation and ulceration could have occurred in additional oysters of the samples, but in parts of the alimentary tract which were not sectioned. The percentages mentioned are therefore a minimum estimate.

The sequence of pathological changes in the course of the disease appears to be as follows:

1. Inflammation of part of the alimentary tract epithelium and adjacent connective tissue due to some irritant, probably a bacterium or virus. Spread of this irritant and accompanying inflammation throughout the tract.

2. Sloughing of the epithelium of inflamed areas followed by disintegration of the basement membrane. The lumen of the alimentary tract fills with cellular debris and pus, and in many cases the abscess distends the lumen causing squashing of the adjacent Leydig cells and digestive diverticula.

3. After the basement membrane breaks down, the ulceration spreads radially, eventually causing death of the oyster.

An unusually long bacillus species has been found associated with the inflammation and ulceration, but at this time there is not enough evidence to positively identify this bacillus as the causative agent.

OBSERVATIONS ON EXPERIMENTAL RELATIONSHIPS BETWEEN THE TREMATODE *Himasthla quissetensis* AND SEVERAL MARINE BIVALVES.[1]

Thomas C. Cheng,[2] Carl N. Shuster, Jr.,[3] and Alan H. Anderson[4]

Synopsis

The compatibility of 7 species of marine mollusks -- *Mya arenaria*, *Modiolus modiolus*, *Mytilus edulis*, *Cumingia tellinoides*, *Aequipecten irradians*, *Ensis directus*, and *Crepidula fornicata* -- as second intermediate hosts for cercariae of *H. quissetensis* was demonstrated by Stunkard (1934, 1937, and 1938). He reported encystment in the mantle, gills, and foot of these mollusks. *M. arenaria* is an important second intermediate host in nature (Uzmann, 1951), with 40 to 100% of the clam populations sampled harboring metacercariae of this trematode.

In further exploration of this susceptibility of marine mollusks for *H. quissetensis*, the authors experimentally challenged 8 marine bivalves -- *Crassostrea virginica*, *C. gigas*, *M. edulis*, *Modiolus demissus*, *E. directus*, *M. arenaria*, *Mercenaria mercenaria*, and *Tapes philippinarum* -- with 150 cercariae of this trematode from naturally infected *Nassarius obsoletus*. Cercariae also were exposed, *in vitro*, to sera and tissue extracts from a similar series of bivalves -- *C. virginica*, *C. gigas*, *M. edulis*, *M. demissus*, *M. arenaria*, *M. mercenaria*, and *T. philippinarum*.

Histological examination of bivalves fixed at 32-34 hours post-infection revealed species differences in the responses of the hosts and the parasite. For example, all of the metacercariae found in *E. directus* were encysted in the foot musculature; an empty space surrounding each metacercaria suggested that the parasites were motile during encystment. In *M. arenaria* the majority were found in the matrices of the gill, others in the matrices of palps and gonads (ovaries). A conspicuous non-cellular, parasite-secreted, inner cyst wall was observed. The serum (hemolymph) of each bivalve stimulated cercarial encystment but the rapidity of the encystation varied according to the species. The stimulatory component of the sera was heat labile. All of the tissue extracts (homogenates) caused the death of the cercariae, with time of death related to the species; longevity of the cercariae was longest in the seawater control. The senior author has submitted for publication manuscripts on these studies.

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[1] Presented by Dr. Shuster at the Eighth Annual Shellfish Mortality Conference, held at the University of Delaware on 23-25 January 1966.

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USBCF Biological Laboratory
Oxford, Maryland
Manuscript Report No 66-1
January 24, 1966.

SPORULATION OF (MSX) IN THE AMERICAN OYSTER

by
J.A. Couch, C. A. Farley and A. Rosenfield *

ABSTRACT

During June and July of 1965 plasmodial, pre-spore and spore stages of a haplosporidian parasite possessing characteristics of the genus Minchinia Labbe, 1896 (as revised by Sprague, 1963) were found in 12 oysters from the Manokin River in Maryland, and in four oysters from Chincoteague Bay, Virginia. Plasmodia of the parasite were found in all tissues of the oysters whereas the pre-spore stages and the spores were largely restricted to the epithelia of the digestive diverticula. The plasmodia (4 to 25 microns) possessed variable numbers of nuclei. The nuclei were characteristic of MSX, i.e. they ranged from 1.5 to 2.0 microns and each possessed a distinct endosome against its thin but sharply defined nuclear membrane. A sequence of stages, to be described, originating with typical multinucleate MSX plasmodia in the connective tissue and the epithelium of digestive diverticula gave rise to sporocysts (28 to 54 microns) which contained operculate spores (7.5 by 5.4 microns), each possessing a single nucleus identical in size and morphology to the nuclei described for the MSX plasmodia. In the 12 oysters from the Manokin River there was no evidence of infections by haplosporidian parasites other than MSX. These hosts were from an area where MSX is the only recognized haplosporidian parasite of oysters. The four oysters from Chincoteague Bay possessed plasmodia, sporocysts, and spores identical with those diagnosed as MSX from the Manokin River. In addition these oysters contained readily identifiable sporocysts and spores of Minchinia costalis (Wood and Andrews) which were easily distinguished from the MSX stages.

Based on the information presented here and the considerable precedent of similar life cycles (not identical) as is found in the literature dealing with other species of the genus Minchinia, we propose that the plasmodia diagnosed as MSX gave rise to the spore described here.

* This is an abstract of a paper titled "Sporulation of (MSX) in the American Oyster" by J. A. Couch, C. A. Farley, and A. Rosenfield prepared for publication in "Science". This abstract does not constitute publication, therefore it must not be cited in any way without prior premission of the authors.

A Preliminary Report on the Possible Serological Differentiation of Malpeque Disease Resistant and Susceptible Oysters

By

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An epidemic disease of oysters, commonly called Malpeque disease, has reduced oyster stocks in Eastern Canada to negligible proportions. One of the features of the disease is that the survivors and their progeny are disease resistant. In contrast oysters from the disease free areas contract the disease and die approximately 2 years after being transferred to the infected areas. Apparently survival depends upon inherent resistance.

Mr. Drinnan and his co-workers are conducting a research program which is aimed at increasing the supply of oyster by producing seed oyster from resistant parents and in breeding a resistant oyster stock. One of the major problems lies in distinguishing resistant oysters from susceptible oysters. The only means now available consists of exposing the oyster to an infected area and noting the development of pustules and eventual death. This consumes a period of approximately two years. The study reported here is an attempt to shorten this period by the introduction of serological techniques. If the serological procedures are applicable the extent of disease resistance in the progeny resulting from any 'cross' would be evident immediately and would accelerate markedly a breeding program.

In the experiments 2 sets of pooled sera were used as antigens. One set was drawn from apparently healthy disease susceptible oysters and the other set was drawn from healthy disease resistant oysters. The antigens were injected into healthy rabbits with Freund's Adjuvant in a graded series of doses and the serum was prepared by standard procedures after a satisfactory titre was reached. The Ring or interfacial precipitin test and agar diffusion techniques were employed to gain the following results.

Table 1. Antigen titres of both homologous and heterologous reactions in the disease resistant and the disease susceptible stocks of oyster

Antigen / Antiserum	Antiserum / Antigen Titres (Ring Test)	
	C	M
C	2560	5120
M	5120	1310720

C = Bras d'Or Stock (The susceptible stock)
M = Malpeque Bay Stock (The resistant stock)

The results of the cross typing, shown in the Table 1, illustrate that there is a considerable difference between the antigen titres of the two groups of oysters.

When the homologous reactions were run in double diffusion agar plates the presence of two major precipitation lines was recorded for the disease resistant stock while only one major line was noted for the susceptible stock. The second major precipitation line becomes very faint or non existent when the resistant antigen is run against the susceptible antiserum. One interpretation could be that the resistant stock contains an antigenic component which is missing or present at a very low level in the susceptible stock. This conclusion is consistent with the data obtained from the ring test.

This is a preliminary report and does offer evidence of definite and reproducible serological differences between the two stocks. We have not yet shown whether these differences are related to disease resistance or racial differences. The study is being continued to resolve this point.

Parallel to this is a study of the antigenic components themselves. A variety of methods are being used to fractionate the oyster serum. At the time of writing starch gel electrophoresis appears the most promising, and six and possibly 7 discrete bands have been observed.

1. Presented at 8th Annual Shellfish Mortality Conference, University of Delaware, Newark, Delaware, January 24-25, 1966.

2. The cooperation of Mr. R.E. Drinnan in supplying oysters and Mr. T. Leung and Dr. P.H. Odense in studying oyster serum in Starch Gel Electrophoresis is gratefully acknowledged.