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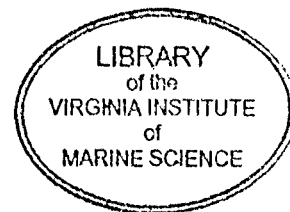
FINAL TECHNICAL LETTER REPORT

FOR

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ENTITLED

Studies of Marine Coccoid Fungi and Protozoa
of the lower Chesapeake Bay, Virginia



BY

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Signed: Frank O. Perkins

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Research supported by grant GA-31014 was initiated to investigate the taxonomy, morphology, and roles of biflagellated coccoid fungi and Protozoa in the lower Chesapeake. As can be seen in the enclosed reprints, considerable information was gathered concerning taxonomy and structure which has shown that the organisms are not fungi, but rather Protozoa. With the exception of Dermocystidium marinum, which is a sporozoan, all species studied (Thraustochytrium spp., Schizochytrium aggregatum, Labyrinthuloides yorkensis, Labyrinthula spp.) are closely related and are unique enough to deserve the new subphylum status of Labyrinthulina as proposed by Olive (1975. The Mycetozoa. Academic Press). They are herein referred to as labyrinthulids.

Labyrinthulids were found in this study to be interrelated in that they all form ectoplasmic nets from specialized organelles, termed sagenogenetosomes. The nets serve to move the cells across the substrate, to penetrate suitable substrates by delivery of soluble lytic agents, and to convey nutrients to the cell body. The nets are distinguished from amoebae ectoplasm by the lack of ribosomes and other organelles in the matrix and from rhizoids by the lack of cytoplasmic organelles and a delimiting wall.

Only one paper has been published on ecology and roles of the organisms (Perkins, 1973. Canad. J. Bot. 51: 485); however, considerable information has been gathered and will be published later. We have found that the labyrinthulids are remarkably ubiquitous in distribution. Biweekly counts of cells in

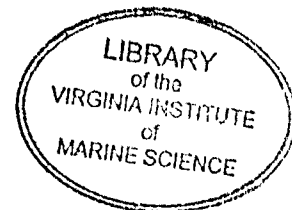
a water column in the York River over a period of 20 months revealed no influence from salinity in the observed range of 14 to 23‰. Thraustochytrium spp. was more prevalent at the higher temperatures (above 21°C) whereas S. aggregatum populations were stable throughout the range of 4 to 29°C and L. yorkensis was most common in the range of 14 to 21°C. Only 10 propagules of Labyrinthula spp. were encountered. Populations of Thraustochytrium spp. and L. yorkensis rose markedly during and following massive pine pollen "fall-out" during April and lasted through August for Thraustochytrium spp. and through May for L. yorkensis. Numbers of labyrinthulids could not be correlated with water turbidity as measured by Secchi disc readings.

In efforts to determine what food sources are utilized by labyrinthulids, eight bait stations were established in the York River, lower Chesapeake Bay, and Atlantic Ocean side of the Eastern Shore of Virginia, covering a salinity range of 0 to 34‰. Twenty-six monthly samples were obtained from baits which consisted of leaves from 14 species of angiosperms, crab chitin, mollusc tissue, polyethylene plastic, and glass slides. Mollusc tissue lasted a few days and chitin one or two months; thus they were renewed at the bait stations only three times to gain repetitive samples. Throughout the study the angiosperms were renewed as they disappeared. Substrate preference analysis showed that the animal materials were highly favored by all species; however, the angiosperm baits were colonized no more frequently than glass and polyethylene by Thraustochytrium spp., S. aggregatum, and L. yorkensis. Labyrinthula spp. were found only one-third as frequently on the non-biological substrates. These results indicated that species of the first three genera were possibly

feeding on microorganisms which colonized the substrates. Laboratory studies in which pure cultures of bacteria, fungi, and Protozoa were mixed with pure cultures of the labyrinthulids yielded inconclusive results. Most isolates could utilize one or more microbes as a food source, but some angiosperm leaves sterilized by propylene oxide were also utilized, whether fresh or skeltonized after exposure in the York River for several months. Artificial seawater was used to eliminate the contribution of dissolved organic compounds. Studies are being conducted to obtain more definitive results; however, at this point it appears that labyrinthulids may feed on microbes as well as feed on angiosperm materials. Their ability to penetrate and internally colonize such materials was established through histological and fine structure studies.

Of the labyrinthulids only Labyrinthula spp. appears to include pathogenic species capable of invading living cells. Isolates were obtained which actively invaded living Zortera marina cells causing host cell death. In these studies blocks of agar containing pure cultures of Labyrinthula sp. were placed around fronds of eel grass without causing mechanical damage to the fronds. Cell invasion was accomplished by only two isolates and the pathogenicity of those isolates was attenuated then eliminated upon repeated subculturing. No isolates, pathogenic to Spartina alterniflora, were obtained.

Comparisons of growth on artificial seawater and 0.22 μ membrane-filtered natural seawater indicate that naturally occurring dissolved organic compounds do not support growth beyond a few cell cycles for all species of labyrinthulids examined.



Bait station studies further indicated the ubiquitous distribution of labyrinthulids in estuaries. It was observed that all species are found at all salinities above 10‰ with only Labyrinthula spp. invading fresh water streams. Fluctuations in salinity to levels below 10‰ were effective in causing either the disappearance or inhibition of the other species since they were, with few exceptions, not encountered at those salinities. Return to higher salinities resulted in rapid reappearance of the organisms within the one month period between samples.

Temperature fluctuations in the range of 5 - 29°C showed that Thraustochytrium spp. are most prevalent at temperatures above 18°C, whereas L. yorkensis occurs in greatest numbers below 18°C. S. aggregatum and Labyrinthula spp. populations were relatively stable over the whole range.

Incidence of the labyrinthulids on baits and in the water column was determined after culturing on agar media. In only three instances were labyrinthulids observed by direct observation of uncultured baits; therefore, the possibility was investigated that cell sizes of the microbes are smaller in the estuaries than on culture media, thus preventing detection on uncultured baits. After growth on propylene oxide-sterilized angiosperm leaf explants in various stages of degeneration, it was determined that cell size ranges were the same as in cultures grown on agar media. Thus the inability to detect labyrinthulids by direct observation of baits was probably due to their colonization of internal moribund and dead cells as opposed to epiphytic colonization. Histological sections of baits indicate that this is correct. Nevertheless,

large numbers of labyrinthulids are never found concentrated in or on any substrate. They are nearly always present but in small numbers.

Fine structure studies of Dermocystidium marinum have shown that the oyster pathogen is a sporozoan as evidenced by the presence of a conoid and micronemes in the zoospores and micropyles in the vegetative stages. There is no evidence that D. marinum is related to the labyrinthulids. One other species of Dermocystidium, as yet undescribed, was found in Macoma balthica. Five other species of Chesapeake Bay bivalve molluscs were found to be uninfected by Dermocystidium spp. The M. balthica pathogen will be described in a future paper. It differs from the oyster pathogen in that it is active throughout the year, not just during the summer and fall, and it routinely forms presporangia in living host tissue.

Numerous attempts to culture D. marinum showed that none of the cell-free media support growth of the pathogen. Only oyster cells maintained in vitro served as a suitable medium. Efforts are now underway to find a suitable cell line which will support growth. Of special interest are the ultrastructural changes which occur during host cell penetration by zoospores, particularly as they relate to the conoid and micronemes. Attempts to observe penetration in oyster cells were unsuccessful. In spite of the fact that massive infections of oysters can be induced using zoospore suspensions, the numbers of penetrations were too small to detect at the fine structure level.

Research initiated during this study will continue in the future using VIMS funds. An atlas of labyrinthulid and Dermocystidium spp. cytology and ultrastructure will be published as well as descriptions of new species of labyrinthulids already established in culture.

Scientific collaborators connected with this grant were Dr. Frederick Y. Kazama, assistant professor, Southeastern Massachusetts University (Formerly at VIMS) and Dr. James P. Amon, assistant professor, Wright State University (Initially a VIMS graduate student at start of grant).

Publications derived from this grant and those in preparation are:

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19. _____. 1975. Ultrastructure of Labyrinthuloides
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20. _____. 1976. An atlas of labyrinthulid cytology
and ultrastructure (in preparation).