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Foreword

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Over 45 years have elapsed since John G. Mackin, H. Malcolm Owen, and Albert Collier of the Texas A & M Research Foundation and the Louisiana Department of Wildlife and Fisheries first noted that a protistan parasite was associated with mortalities of *Crassostrea virginica* found in the area of the Mississippi River delta. Due to the presence of cells of a parasite with a large eccentric vacuole containing a prominent inclusion, they concluded that the protist was a species of the genus *Dermocystidium* and named it *Dermocystidium marinum*. In the next two decades, it was well documented that the parasite was the causative agent of the oyster mortalities first observed in the Gulf of Mexico coastal waters and, in fact, could be found in oysters from Texas to New Jersey as well as other bivalve molluscs in that range. Although there were many researchers who contributed to our knowledge of the parasite during the 1950s and 1960s, Jay D. Andrews, John G. Mackin, and Sammy M. Ray provided the majority of information. Ray facilitated investigations of the parasite by providing the fluid thioglycollate medium (FTM) technique by which rapid and inexpensive detection of cells of the organism could be accomplished in large numbers of oyster tissue samples due to marked enlargement of the pathogen in the culture medium. Although not yet rigorously evaluated, there is good evidence that enlargement occurs without cellular multiplication. Almost 40 years later the Ray technique was redesigned to permit a quantitative estimation of the numbers of cells in selected tissues and in whole oysters by incubation in FTM followed by digestion in an NaOH solution and counting the number of *Perkinsus marinus* cell walls in the digest.

Detailed epizootiological and experimental ecological studies by Andrews, Mackin, and Ray yielded information of value to oyster growers and managers of oyster populations. It was soon determined that transmission of infections occur from oyster to oyster and the pathogen is most virulent at higher temperatures and salinities approximating 20 to 30°C and 20 to 30 ppt, respectively.

The structure and life cycle of the parasite was examined in greater detail in the 1960s and 1970s using electron microscopy. The demonstration of zoosporulation in sea water of cells (hypnospores) which had enlarged in FTM yielded the observation that infective, biflagellated zoospores were released. These cells were found to have an apical complex and other apicomplexan structures. Thus, evidence was presented that the organism is closely related to the Apicomplexa. Norman D. Levine in 1978 renamed the parasite *P. marinus* and placed it in a new class Perkinsea in the phylum Apicomplexa. A curiosity which remains to be explained is the fact that for about 20 years after its discovery, zoosporulation could be readily induced to occur in *P. marinus* under laboratory conditions by most cells of a hypnospore population. Since the late 1980s, this was found to occur in less than 1% of a hypnospore population and zoospore release failed to occur. On the other hand, isolates of *Perkinsus* spp. hypnospores derived from other bivalve hosts readily zoosporulate in sea water.

During the 1980s until present, research activity involving *P. marinus* and other species in the genus increased markedly on several fronts and excellent progress has been made. Epizootiological studies and investigations in experimental ecology both in the field and laboratory have centered around the effects of salinity and temperature in controlling expression of the disease, thus expanding on the

extensive studies reported earlier by Mackin and co-workers. The workers who followed them have confirmed that increased salinity and temperature enhance expression of the disease, and they have greatly expanded upon our knowledge of the details of that paradigm as well as the exceptions. Salinity has emerged as a more dominant factor than temperature in some studies and conditions. In other studies, temperature has been found dominant. The laboratory component of temperature and salinity studies has centered mainly around observations of hemocyte function and composition of hemocyte populations. Large-scale, field observations have revealed that epizootics of *P. marinus* in oysters are not induced simply by fluctuations in temperature and salinity but rather some other stimulatory factor or factors such as limited food supply or recruitment that occurs just before or at the same time as elevated temperatures and salinities. Thus, progress is being made toward constructing climatic models to predict the activity of the pathogen. Of significance may be the recent observation that *P. marinus* proliferation is enhanced by excess iron accumulation in the host. It is known that there is an increase in iron levels in oysters in the summer when *P. marinus* causes elevated mortalities. Thus, researchers will undoubtedly have to consider many more factors than just salinity and temperature in their modelling efforts.

Oyster hemocyte and *P. marinus* interactions have been evaluated *in vitro* by measuring reactive oxygen intermediates (ROI) primarily as expressed by the luminol-enhanced chemiluminescence (CL) response and by assaying for hemocyte lysosomal enzymes. Although there are conflicting results, it appears that *P. marinus* can either prevent ROI production or neutralize ROI in hemocytes with one hypothesis being that acid phosphatase produced by *P. marinus* inhibits superoxide radicals released by hemocytes. In the future, there will undoubtedly be increased research activity directed toward understanding how *P. marinus* is able to survive and multiply in hemocytes, recognizing that the hemocytes are the primary line of host defense against microbial agents. Related to this are ongoing investigations to identify and quantify substances that are produced by the pathogen and result in destruction of oyster cells.

The question as to whether anthropogenic chemicals in growing waters predispose oysters to mortalities caused by *P. marinus* continues to be one of major importance to managers and users of the estuarine environment. For over a century, oyster farmers and harvesters have cited pollution as the primary reason for the decline in oyster production with enhancement of microbially induced disease by pollution being a focus of their complaints. However, the evidence to support or refute their claims is not yet sufficient. In recent years, some insight has been obtained in working with compounds such as tributyltin and sediments contaminated by polynuclear aromatic hydrocarbons. It appears that some anthropogenic compounds can enhance the proliferation of *P. marinus* in oysters and can suppress the CL response. Furthermore, the matter of soluble iron (mentioned above) needs to be considered. It has been suggested that increased iron levels in industrially contaminated waters and/or sediments may enhance the expression of the disease. Therefore, the long-standing complaints of the oyster harvesters may prove to be correct. However, much is left to be determined before proof is forthcoming and informed management decisions can be made relevant to this issue.

Also of importance to managers and users of oyster populations is the question of whether *Perkinsus* sp. or spp., which are found in most (all?) other bivalve mollusc species co-existing with *C. virginica*, are *P. marinus* or another species of *Perkinsus*. There is probably at least one other species of *Perkinsus* in bivalve molluscs associated with *C. virginica*. It is found in *Macoma balthica* and *Macoma mitchelli* and is probably *Perkinsus atlanticus*. It can be induced to infect *C. virginica* most easily when its zoospores are fed to oysters. The observation of *Perkinsus* cells in other bivalve molluscs may in large part involve a carrier relationship with the pathogen, but multiplication of *P. marinus* is known to occur in many of those presumptive carriers. Whether they cause mortalities in those bivalves remains to be seen. This information is of interest to governmental regulators of bivalve mollusc transportations between estuaries and must be more completely investigated.

Recently, evidence has been obtained that there are probably strains or races of *P. marinus* with the Gulf of Mexico strains being less virulent than those along the mid-Atlantic Ocean coast of the U.S. Such preliminary information requires further clarification so that more informed decisions can be made concerning transportation of oysters.

Although some excellent biochemical and physiological studies were conducted using *P. marinus* cells isolated from infected oyster tissue, the lack of axenic cultures inhibited pursuit of such research. The problem was solved in late 1992 and early in 1993 followed by publication in 1993 of semi-defined culture media formulations by three different laboratories within months of each other. The contributions were significant and, as expected, have resulted in improved ability to investigate the biological characteristics of the pathogen. A further refinement has been made with the formulation of a defined culture medium which will permit even greater biochemical and physiological characterizations. The only word of caution has been that the few studies of transmission of infections using cultured cells have resulted in the observation that such cells do not appear to be as infective as *P. marinus* isolated from oysters and used directly in challenge experiments without being cultured. Identification of a culture medium that yields cells of the same infectivity as uncultured ones must be accomplished to lessen uncertainty as to whether naturally occurring characteristics are being observed when cultured cells are used. It is known that the cytological characteristics of many of the cells in culture differ in terms of size and cytokinesis from those observed in oyster tissues.

It has been established that infections of *P. marinus* occur from oyster to oyster, the developmental cycle in the oyster appears to have been well characterized, and it is known that zoosporulation can occur outside of the host to yield zoospores that are infective for other oysters. Nevertheless, the question has remained as to whether saprobic development can occur free of the host. The fact that the pathogen can be cultured in a variety of media leads one to suggest that *P. marinus*, as well as other species of *Perkinsus*, is a faculative pathogen. With the provision of fluorescein-labeled specific antibodies to *P. marinus*, cell DNA labeling with propidium iodide, and the use of flow cytometric analyses, it is now possible to detect cells of *Perkinsus* (not just *P. marinus*) in water and sediment samples. This will undoubtedly lead to greater insights into the life cycle with answers to the question of whether there is multiplication of the pathogen free of its host. Evidence that this may occur comes from the observation that enlarged cells (hypnospores) in sea water may not zoosporulate but rather may form hyphal-like outgrowths into which the cytoplasm flows and subdivides into daughter cells that are released into the sea water. These daughter cells are morphologically dissimilar to those found in the host. Whether these cells are saprobic forms in the life cycle or must enter a host to continue development remains to be determined.

Whereas most investigators accept that *Perkinsus* spp. are related to the Apicomplexa, the taxonomy and phylogeny of the pathogens remain a subject for scrutiny and reevaluation. In light of new phylogenetic alignments of the Protista and recent findings by molecular biologists studying nucleic acid base sequences of *P. marinus*, as well as the reinterpretation of the morphology of the pathogen by others, it is now realized that pathogens in the genus belong either with the Dinoflagellata, the Apicomplexa, or some intermediate taxon yet to be described. It has already been suggested that the Apicomplexa arose from the dinoflagellates with *Perkinsus* spp. being an early diverging group in the evolution of the Apicomplexa. This hypothesis may prove to be accurate when an adequate number of species in the two higher taxa are thoroughly evaluated.

The reader of this special issue of the *Journal of Shellfish Research* will find most of these research accomplishments described in greater detail in the papers that follow as well as other aspects not covered in this introduction. The accomplishments are considerable and much valuable information will undoubtedly continue to be provided in the years ahead. As measured by publications, the rate at which new information was being provided reached its highest level in the early 1990s and continues today. This is due in large part to funding from NOAA and in particular from NOAA's National Oyster Disease Research Program which has provided over $6 million in funding before being terminated this year. The U.S. Congress funded the Program with a special appropriation following an initiative by former Congressman Roy Dyson with particularly strong support from the Virginia and Maryland delegations; therefore, many of us who have worked on *P. marinus* are indebted to them. Once commercially viable answers to oyster mortalities caused by *P. marinus* have been found, oyster harvesters and farmers will also have these Congressional representatives to thank for much of the progress made in attaining that goal.

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