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43. INCIDENCE OF *PERKINSUS MARINUS* IN *CRASSOSTREA MADRASENSIS*

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ABSTRACT

Perkinsus marinus (Dermocystidium marinum) commonly known as "Dermo" is one of the causative pathogens for widespread mortalities in oysters. In the natural population of oysters at Tuticorin, oyster tissues cultured in fluid thioglycollate medium with dextrose fortified with antibiotics were found to be Infected with ***P. marinus.***

This is the first time that this pathogen has been reportsd from Indian waters. The incidence of infection in oysters ranged from 10 to 60%. The weighted incidence ranging from 0.05 to 0.35 indicates the very light level of Infection.

INTRODUCTION

It is not uncommon to come across reports of diseases of contagious or noncontagious nature occuring among oysters in the natural beds. The noncontagious diseases are caused by the physiological malfunction of organ due to unfavourable environmental condition. The infectious diseases are mostly microbial origin, occasionally due to parasites also. Unfavourable environmental conditions lower the resistance of the oyster paving way for secondary infections by facultative pathogens. Three major causative organisms for the widespread mortalities in the east coast of United States oyster populatin are *Peridnsus marinus, Minchinia nelsoni* and *M. costalis.* (Andrews 1979 Sinderman 1970). *Marteiiia refringens* and *Bonamia ostrea* are known to cause mass mortality along the coast of France. The epizootic disease caused by *F. marinus* occurs m the warm season, causing mortalities in the Gulf and South eastern coastlines of United States (Mackin 1962). It caused havoc in the Pearl Harbour, Hawaii, during 1972, in which mortalities to 90 - 99% of the stock occurred in the oyster *Crassostrea* wirfh/mca (Frederic et al 1973). The examination of monthly sample of oysters C. *madrasensis* collected from natural oyster beds around Tuticorin during 1984-85, brought to light the incidence of *P. marinus* In them. The level of Infection being very light, no mass mortality occurred. But this is the first time that *P, marinus* has been detected in India.

MATERIAL AND METHODS

Methods for detecting P. *marinus* by microscopic examination of fresh tissue or stained sections of the tissue are difficult and time consuming. Therefore, the effective method of culturing them in thioglycollate medium, widely "'^^ in determining the incidence as well as the '"^^"s'ty °\ \:^*iBcX\on* was followed. The organisms do not multiply in the medium,

Dehydrated fluid thioglycollat. (29.3 gm) ^.^^ ^.^^,„^j ^^^^^ ^^^.^^ 2*o*l„ of sodium chloride was used as the medium. lOmi of the medium was taken in a culture tube (15 x 125 mm of 10 ml capacity) with screw cap. Before planting the tissue, the medium was fortified with 200 units of mycostatin and 200mg of Chloromycetin per ml of the medium in order tosupressthe bacterial growth (Ray 1952).

Ffom July 1984 to June 1985, twenty oysters were collected each month from the oyster bed at Tuticorin. They were cleaned and their length measured to the nearest mm. Small pieces (5 x 10 mm) of mantle (near palp) and rectal tissue of the oyster were planted in the culture tube. These tubes were incubated for seven days at room temperature. The jncubated tissues after wiping out the medium in a tissue paper, were placed on a slide with 2 or 3 drops of diluted Lugol's iodine solution, The tissues were teased into small bits and examined microscopically for green, blue and blue-black spheres. Depending upon the number of cells present in the tissues, oysters

were grouped as very light, light, light to moderate, moderate, moderate to heavy and heavy infection and assigned arbitarary values of one half, one, two, three, four and five respactively. The sum of these values divided by the number of individuals in the sample indicated the weighted incidence which combines incidence and estimated intensity of infection provides better index of the degree of infection (Ray 1954b).

DESCRIPTION

'Dermo'is a small nearly spherical spore like body, the size ranging from 2 to 25(A. The most distinctive feature is the presence of very large partially eccentric vacuole which usually contains one large, polymorphic retnngent inclusion body or "vacuoplast" occupying greater portion of cell body (Ray and Chandler 1955).

Dermo" was considered to be Rhinosporidian; under Endomycetalsj (Mackin 1962) considering its mycotic type of infection produced. Mackin and Ray (1966) renamed it as *Labyrinthomyxa marina* because of some gliding cells with iabyrinthulid "tracks,' Plasmodia and vegetative division in propagation. Later not finding any Iabyrinthulid characteristics, Perkins (1976) indicated that D./nsr/nym is a protozoan in the sub-phylum Apicomplexa because of the apical complex consisting of conoid, polar ring, rhoptries and micronemes. In addition to the apical complex, zoospores and trophozoites of ^^^ oyster pathogen possess microspores. Subsequently it was redesignated as *Perkinsus marinus* by Levine (1978).

RESULTS

The number of cellsiin^the infected oyster tissues ranged from 1 to 5 per piece of sample (P'O'1 a and b), with size ranging from 15 to 100|A Cells of size 35 to 50|JI were common. In JulV' 1984 rectal tissue of an oyster had thirty five cells with a maximum size of 100(J>. Clusters ofcells having 9 to 17 cells were observed (Fig.1

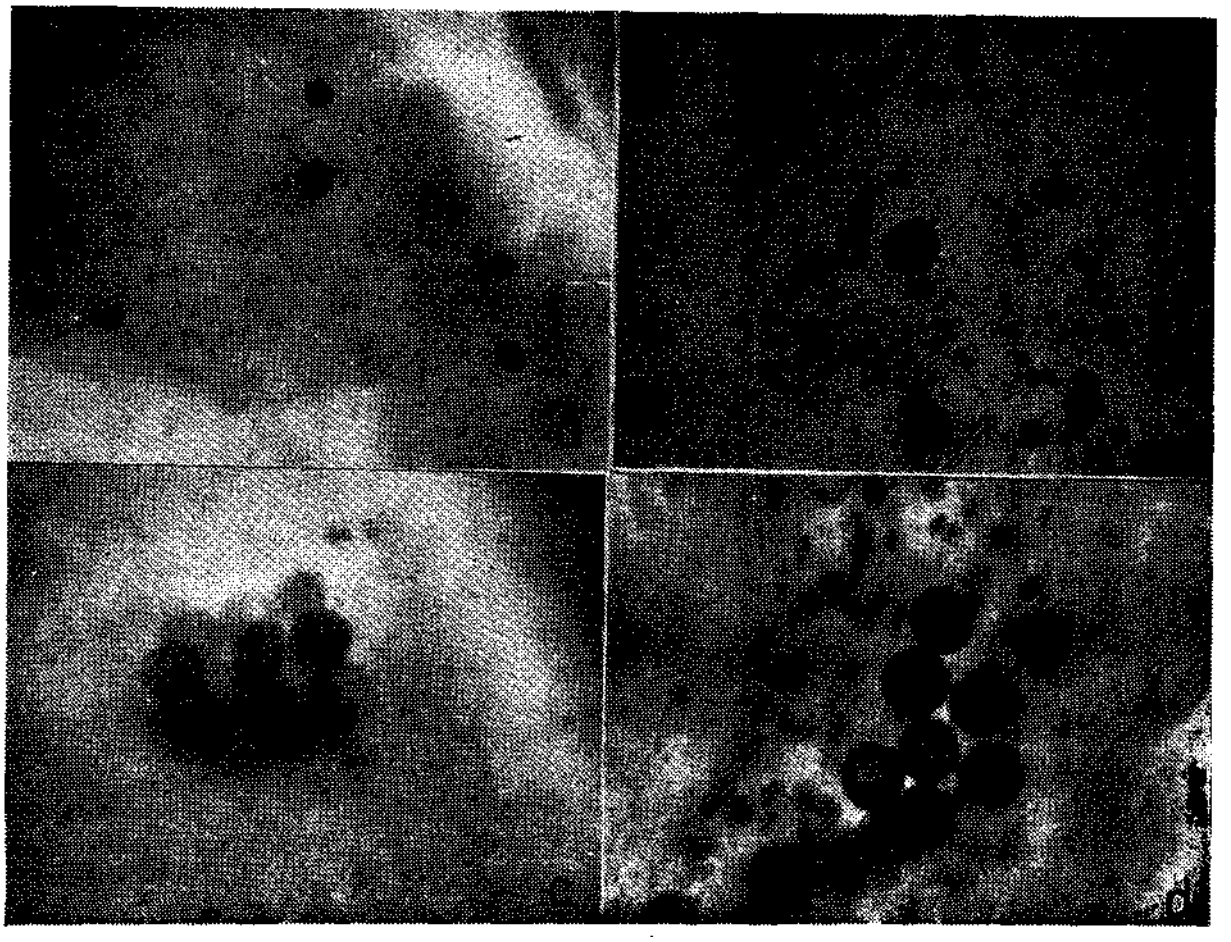

Fig. 1 (a d b). ***Perkinsus marinus*** **in oystsr mantle tissue after seven days of incubation in fluid thioalycollate medium, (C a DJ Cluster of cells in some samples of oyster mantle tissue.**

c & d). The percentage of infection and weighted incidence along with the surface temperature during July 1984 to June 1985 are given in Fig 2. The percentage of infection varied from 10 to 60%, low during February and high in May 1985.

Weighted incidences of 0.05 during February 1985, of 0.10 during November 1984 to January 1985, of 0.15 during March and April 1985 were noted. In the months of August, October 1984 and June 1985 the weighted

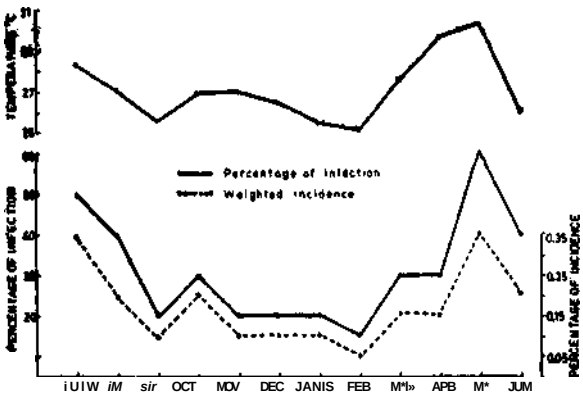

Fig. 2. The monthly percentage Infection and weighted incidence of P. *marinus* Infection In the oyiters, Also monthly temperature shown.

incidence was 0.2. High weighted incidence of 0.35 was observed in July 1984 and May 1985. The range of weighted incidence from 0,05 to 0.35 indicates only very light level of infection.

Temperature and salinity are the important factors in epizootiology of "Dermo disease" (Ray 1954b;. Andrews (1965) stated that *P. marinus* proliferates readily only at temperatures above 25°c. During colder periods, the infection and associated mortalities become reduced because of reduced metabolism of the pathogens rather than elimination of the organism. Low salinity retarded development. Excessively high salinities may also be unfavourable for "Dermo" (Ray 1954b; Ray and Chandler 1955). In the present study, high percentage of infection (60%) and weighted incidence at 0.35 were observed when the temperature was 30.3°c. Percentage of infection was 10-20% and weighted incidence 0.05 to 0.10 during December 1984 to February 1985 when the temperature ranged from 25.1°c-26.5°c ^Fig 2). This indicates broad agreement with the earlier findings of Ray {1954b) on the effect of temperature on *P. marinus*

DISCUSSION

Much attention has been directed towards large-scale oyster mortalities and account of diseases by pathogenic organisms. *P. marinus* is one of the etiological agents for large-scale oyster mortalities. The invasion seems to take place through the gut epithelium and possibly through the mantle. The epithelium is destroyed, the parasite lyses the basement membrane and is distributed by blood to all parts of the body. Mortality results more by acute infections which lyses tissues and clog organ system especially blood sinuses (Mackin 1951).

Ray (1954a) found that the pathogen was transmitted by "proximity method". Hoese (1964) was able to find *P. marinus* in digestive tracts and faeces of fish, oyster drills and crabs that had fed on dying and dead infected oysters, speculating that transmission might be aided by the scavengers. But the mode of transmission is still not firmly established.

Besides being the casuative agent for mass mortalities "Dermo ' inhibits the normal gonad development (Ray et al 1953) and retards the oyster growth (Menzel and Hopkins 1955). Ray et al (1953) found loss of 12-15% meat weight in moderately infected oysters and in heavily infected cases the meat weight reduction was upco 33%.

The occurrence of *P. marinus* was also detected in some of the pearl oysters thus indicating its possible destructive role on the pearl oyster population in the "paars" for the periodical mass mortality of oysters.

Though at present no mortality could be decisively attributed to *P. marinus* in oyster beds, in India, the incidence of the protozoan needs to be regularly monitored considering the epizootological factors.

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