PATHOLOGICAL INVESTIGATIONS IN PENAEID PRAWNS

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DECLARATION

I hereby declare that this thesis entitled "PATHOLOGICAL INVESTIGATIONS IN PENAEID PRAWNS" has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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April, 1986.

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CERTIFICATE

This is to certify that the thesis entitled "BIOLOGICAL INVESTIGATIONS ON PENAEID PRAWNS" is the bonafied record of the work carried out by Shri SUBHASH CHANDRA SONI under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

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P R E F A C E

The widening imbalance between human population and food supplies, changing life-styles, attitudes, tastes and preferences, demographic factors and enhanced industrial requisite have brought greater demand for fish and fishery products throughout the world. This is particularly so in the case of gourmets' favourites such as prawns, lobsters and crabs. To meet this demand, there have been increasing fishing pressures on the wild fish stocks which are in great demand in commerce. This high level of fishing effort, over the years, has led to a decline in the abundance of certain fish stocks and reduction in net returns from their fishery. This situation is presently experienced in the marine prawn fishery of India. In the sixties (1960-69), the annual average marine prawn landings of the country was of the order of 85,100 tonnes. With the increasing fishing effort, the prawn catch enhanced to an annual average of 1,74,100 tonnes during 1970-79, recording the maximum of 2,22,750 tonnes in 1975. However, from the latter half of that decade, there was a downward trend showing wide fluctuations below 20,00,000 tonnes. These fluctuations in the exploited traditional wild stock of prawns coupled with the rising cost of fishing operations and the ever increasing demand, created an interest in

aquaculture of prawns as a means to augment the production and as a source of increased supply of prawns.

Farming of prawns and fishes in the brackish water fields has been a very old and sustaining practice in Kerala, West Bengal, Karnataka and Goa. The general practice followed in this traditional system which covers an estimated area of about 30,000 ha in the country, is to stock the impounded fields with the seed brought in by the incoming tides, to allow them to grow for a short period by feeding on the natural food available in the field, and to harvest them periodically around the new moon and full moon phases. The prawn and fish production in this system is found to vary from about 100 kg to 1000 kg/ha for a season extending to about 6 months. The prawn catch in this culture operation is composed of mainly the species belonging to the genera Metapenaeus and Penaeus, the former predominating the yield. As this system involves indiscriminate and uncontrolled stocking of seeds, relatively shorter time allowed to grow the seed before harvesting and no eradication or control of predatory and competitive species in the field, the quality and quantity of the production have been found to be relatively low. In recent years, an improved system involving eradication of undesirable organisms from the culture base and its preparation appropriately before stocking, and stocking with species that grow fast and command good price and

demand, is being introduced. This semi-intensive system of prawn culture is now rapidly spreading and gaining importance in the country. Further, the realisation of the great growth potential of aquaculture of prawns in the country and its significant role in the rural development has prompted both the central and state governments to assign high priority for its development. everal schemes and projects are being formulated and implemented to bring in appreciable areas under prawn culture and to establish hatcheries and other infrstructural facilities by different maritime states during the eventh five Year lan period.

One of the major factors that limit the successful culture operation and supress its full growth potential has been identified to be the diseases affecting the principal species selected for culture. Even in the natural population, the wide fluctuation of the catch is often assigned to the natural mortality and one of the responsible causes of this natural mortality is found to be the occurrence of diseases. Mortalities, abnormalities and slow growth due to diseases from significant deterrants, and often lead to considerable loss to the production. Prawns, as in the case of other organisms, become susceptible to diseases whenever certain abnormal biological, physiological or environmental changes harming their normal life occur. Good health and growth of the prawns are maintained when the relationship between the prawn,

pathogen and the environment is balanced. When this relationship is disturbed, disease problems arise and reduced growth manifests, and when it deteriorates further, overt disease, poor growth and mortalities occur. Besides biotic and abiotic diseases, prawns are also susceptible to nutritional and genetic diseases. Being generally density dependent, the disease hazards are encountered more in the culture fisheries than in the capture fisheries.

During the past two decades there has been considerable advance in the knowledge of diseases affecting the principal aquaculture species of prawns, their diagnosis and control. However, information on diseases affecting penaeid prawns of India is limited to a few descriptions and lists of parasites and their biological considerations. As India is poised for large scale development of aquaculture of prawns with a stress on high density semi-intensive or intensive culture system, it is natural to expect increasing hazards of diseases in these systems. Since the knowledge of such diseases, their causative agents and etiology is basic to control, the present investigation on the diseases of penaeid prawns has been taken up for this Doctoral thesis.

The thesis is presented in five chapters. Chapter 1 deals with the review of the literature on the penaeid prawn diseases from India and abroad. This is followed by

a chapter on the material and general methods employed during the investigation. The study was initiated by undertaking a base-line survey at certain centres in the southewst and southeast coasts of India, to collect information and to understand the common diseases and abnormalities encountered in the commercially important penaeid prawns. Ten cases of diseases and abnormalities encountered during the survey are presented and discussed in Chapter 3. One of the diseases caused by the microsporidian parasites is found to bring forth considerable economic loss to the penaeid prawns population particularly that contributed by Penaeus semisulcatus, exploited on the southeast coast of India. This disease was taken up for detailed studies and the results of the investigation on the structure, life history, histopathology and other aspects of microsporidiosis in P. semisulcatus and Metapenaeus affinis are provided and discussed in Chapter 4. Finally, in Chapter 5, an attempt is made to discuss the control measures for various diseases of penaeid prawns in the light of the available published information.

The tumour-like outgrowth recorded in P. indicus in the present study forms the first record of such an occurrence from the penaeid prawns of India. The studies on microsporidian parasites and the disease caused by them, presented in Chapter 4, form a comprehensive work. Three species of

microsporidians collected and described in the present investigation are new to science. These aspects, and observations made in the symptoms of microsporidiosis caused by one of the microsporidian parasites, studies on the histopathology of various tissues of the infected prawns, transmission experiments and biochemical analysis of the infected and normal prawn tissue constitute original contribution and considerably add to the present knowledge on the pathology of penaeid prawns of India. Besides, this being the first detailed study on the microsporidian infection in Indian penaeid prawns, it not only provides information on the parasites per se but also their relationship with the hosts.

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PATHOLOGICAL INVESTIGATIONS IN PENAEID PRAWNS

C O N T E N T S

CHAPTER 1

GENERAL INTRODUCTION

It is axiomatic that the information base on diseases in marine fishes and shellfishes has closely followed the development of exploitation of these resources. Most of the scientific studies on the subject have, however, come from only during the past four decades. In 1970, Sindermann, compiling the information available up to that time on the diseases of commercially important species, provided an excellent review and a bibliography. A perusal of this literature reveals that the significant contributions published prior to 1970 on the diseases of crustaceans relate to the works by Reinhard (1956) on parasitic castration and to the accounts by Gordon (1966), Sindermann and Rosenfield (1967) and Johnson (1968). Anderson and Conroy (1968) discussed the role of diseases in the aquaculture of crustaceans.

With the growing importance of crustacean culture during the past one and half decades, a series of studies on the pathology of cultivable species ware carried out, and this paved the way for accumulation of valuable information and considerable expansion of our knowledge about their diseases and the technology of disease control. The most

important works brought out since 1970 were by Bang (1970, 1983), Johnson (1970), Rosen (1970), Sprague (1970, 1978), Sindermann (1971a, 1971b, 1977, 1979, 1981), Alderman (1973), Unestam (1973), Pauley (1974, 1975), Stewart (1974, 1983), AQUACOP (1977), Overstreet (1978, 1979, 1983), Lewis and Leong (1979), Lightner (1981), Couch (1981, 1983) and Johnson (1983a, 1983b, 1984).

Among the different groups of crustaceans, much emphasis of disease investigations has been on prawns and shrimps, obviously due to their economic value and demand. Several excellent reviews by Hutton et al. (1959a), Kruse (1959), Johnson (1977, 1978), Lightner (1975, 1977, 1983), Couch (1978) are now available. Besides these, the valuable studies by Villella et al. (1970), Fontaine (1971), Barkate (1972), Feigenbaum (1975), Fontaine and Dyjak (1973), Fontaine and Lightner (1973, 1974, 1975), Lightner (1973, 1978a), Barkate et al. (1974), Johnson (1974a), Fontaine et al. (1975), Lightner et al. (1975), Delves-Broughton and Poupard (1976), Cacutan et al. (1977), Liao et al. (1977), Lightner and Redman (1977), Nurdjna et al. (1977) and Perez Alvidrez (1977) have greatly contributed to the fund of data on the diseases of these animals. It is also significant to note that most of these works pertain to diseases of either captured or cultured penaeid prawns of America, Hawaii, Polynesia and Japan.

While the knowledge on crustacean diseases in general and on penaeid prawn diseases in particular is fairly developed and progressive in the advanced countries as revealed from the above cited several investigations and reviews, the information on the subject from India is limited. Among the earlier works, the most significant contribution to the knowledge of crustacean parasites was by Chopra (1923). Paradoxically, further interest and endeavours to study the subject came forth only since the last decade.

In the following section, an attempt is made to briefly review the most valuable studies carried out on penaeid prawn diseases abroad and in India.

An overview of the studies carried out abroad

Organisms belonging to different groups such as Viruses, Bacteria, Fungi, Protozoa, trematodes, cestodes, nematodes, and parasitic crustaceans cause diseases in penaeid prawns. Apart from these, dietary deficiencies, environmental stress as well as pollution and toxic algal blooms in the water also bring forth diseases.

Viral diseases

Among the infectious diseases of cultivated penaeid prawns, those resulting from viruses are important. After

the discovery of virus particles in the hepatopancreas of Penaeus duorarum from the northern Gulf of Mexico by Couch (1974a), five diseases of viral etiology have so far been described. These are the three baculoviruses, namely, Baculovirus penaei (BP)(Couch, 1974b; Summers, 1977), monodon baculovirus (MBV) (Lightner and Redman, 1981; Lightner et al., 1983a) and baculoviral midgut gland necrosis virus (BMNV) (Sano et al., 1981); a probable picornavirus, known as infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Lightner et al.,1983b, 1983c; Bell and Lightner, 1983, 1984) and the fifth one, suspected to be a parvovirus and named as Hepatopancreatic Parvo-like Virus (HPV) (Lightner and Redman, 1985). Unnamed virus-like particles have also been observed in an apparently healthy P. aztecus from Mississippi, U.S.A. (Foster et al., 1981).

The BP is reported to occur in several penaeids such as P. duorarum, P. aztecus; P. setiferus, P. vannamei and P. stylirostris cultured on the northern Gulf of Mexico and the Pacific coast of Central America (Lightner, 1993). MBV is principally encountered in P. monodon in Philippines, Taiwan, Tahiti, Hawaii and Mexico (Lightner and Redman, 1981; Lightner et al., 1983a) while the BMNV is found in P. japonicus cultured in southern Japan (Sano et al., 1981). These baculoviruses commonly infect the hepatopancreatic

epithelial cells and less commonly, the anterior midgut epithelium of the host. The infection results in high mortality in postlarvae, juveniles as well as adults. There is also some evidence that the BP causes epizootic mortalities in wild shrimp populations in the Gulf of Mexico (Couch et al., 1975). The viral attack in the epithelial cells causes nuclear hypertrophy, proliferation of nuclear membrane, chromatin diminution and nuclear degeneration. The greatly hypertrophied nuclei of the affected cells contain free virions in the case of BMN disease whereas in BP and MB diseases, occluded polyhedral inclusion bodies are produced. Couch (1970) was unable to increase Baculovirus prevalence in naturally infected P. duorarum by experimentally exposing them to low levels of polychlorinated biphenyl (PCB) insecticide and cadmium. However, Couch and Courtney (1977) were able to induce a 50 per cent increase in Baculovirus prevalence in captive shrimp populations exposed to sublethal levels of PCB. Transmission of Baculovirus penaei in nature probably takes place via cannibalism of the infected prawn by the non-infected ones (Couch, 1978). Oral inoculation was found to be successful in the infectivity trials carried out for Japanese virus, BMNV (Sano et al., 1981).

The IHHNV is reported in P. stylirostris and P. vannamei from Hawaii and in P. monodon from Guam (Lightner

et al., 1983b, 1983c; Bell and Lightner, 1983, 1984). This viral disease is diagnosed by the presence of eosinophilic inclusion bodies within the nuclei of cuticular hypodermis, haematopoietic or connective tissue cells which are completely destroyed in acute cases. The recently discovered HPV is found to affect cultured populations of P. merguiensis in Singapore, P. monodon in Philippines, P. orientalis in China and P. semisulcatus in Kuwait (Lightner and Redman, 1985). It is observed that in P. merguiensis and P. semisulcatus, accumulative mortality rates due to HPV disease in epizootics reach as high as 50 to 100 per cent. Prawns with HPV disease are characterised by poor growth rate, anorexia, reduced preening activity, increased surface fouling and occasional opacity of tail musculature. This disease is diagnosed by necrosis and atrophy of the hepatopancreas, accompanied by presence of large prominent basophilic, PAS-negative, Feulgen-positive intranuclear bodies in affected hepatopancreatic tubule epithelial cells.

Bacterial diseases

Most of the information on the bacterial diseases of penaeids concern with captive and cultured populations. In majority of the cases of bacterial infections, the isolated bacteria such as Vibrio alginolyticus, V. parahaemolyticus and V. anguillarum, Pseudomonas spp., Aeromonas spp., Beneckea sp., Flavobacterium sp., Pasteurella sp., and Moraxella sp. are reported to be chitinoclastic

in nature and characterised by motile, Gram-negative short rods.

The bacteria affect all the life stages of penaeid prawns (Lightner, 1977). Their infection causes localised pits or melanised erosions of cuticle on the general body surface, gills or appendages (Anderson and Conroy, 1968; Rosen, 1970; Cook and Lofton, 1973; Cipriani et al., 1980) or abscesses in the gut, muscle and gills (Lightner, 1978 b) or they produce generalised septicaemia (Lightner and Lewis, 1975; Nelves-Broughton and Poupard, 1976). While some of the bacterial diseases caused by Vibrio are considered to be of primary etiology (Nickelson and Vanderzant, 1971; Cook and Lofton, 1973; Lewis, 1973; Lightner and Lewis, 1975), most bacterial diseases are of secondary etiology with different disease syndromes (Ogusa et al., 1974; Hood and Meyers, 1977; Lightner, 1977, 1978, 1983; Couch, 1978) and play significant role as opportunistic pathogens that typically cause disease in severely stressed prawns or as secondary invaders in prawns with weakened defence mechanisms due to wounds or other diseases (Lightner, 1977).

Of the various species of bacteria causing infection, Vibrio are important in the ecology and survival of cultured penaeid prawn populations (Sindermann, 1971) and have been implicated as a major cause of mortality in juvenile penaeids in culture systems (Sindermann, 1971a).

V. parahaemolyticus, which causes an infectious food poisoning syndrome and gastroenteritis in Japan and in the United States (Krantz *et al.*, 1969, Nickelson and Vanderzant, 1971), has been found to be lethal to experimental populations of brown shrimp, *P. aztecus* by Vanderzant *et al.* (1970). Subsequent studies have shown that haemocoelic infections by other vibrios such as *V. anguillarum* and *V. alginolyticus* would also cause epizootics and mortalities in brown, pink and white shrimp (Lewis, 1973; Lightner and Lewis, 1975). The latter species has been found to be responsible for the mass mortality in a commercial hatchery in the United states in 1972 and 1973 (Lightner, 1975). Leong and Fontaine (1979) have assessed the virulence of four species of *Vibrio* on the white shrimp, *P. setiferus* by dosage-mortality and time-mortality relationships through intramuscular injections. *Vibrio* spp. as well as members of the genera *Beneckea* and *Pseudomonas* with chitinolytic capacities are also responsible for another significant shell disease in cultured penaeids (Cook and Lofton, 1973). Cipriani *et al.* (1980) have transmitted shell disease to the shrimp, *P. aztecus* and *P. setiferus* and concluded that only shrimp with abraded cutile are susceptible to experimental infection. Besides these, chitinoclastic bacteria, *Leucothrix mucor* and *Leucothrix*-like filamentous ectocommensal bacteria are found to infest the gills and appendages of larval,

postlarval, juvenile and adult penaeid prawns in culture systems particularly when stocking density is high, the water is rich with organic substrate and optimum temperature prevails (Ishikawa, 1966, 1967; Barkate et al., 1974; Johnson, 1974a; Lightner, 1975, 1977, 1978a, 1983; Lightner et al., 1975; Steenberger and Schapiro, 1976). In acute cases, filamentous bacterial infestation impairs respiration, feeding, locomotion and moulting, and occasional heavy mortality may occur from hypoxia (Ishikawa, 1966, 1967; Barkate et al., 1974; Lightner, 1977, 1978a, 1983).

Fungal Diseases

Like viruses and bacteria, several species belonging to phycomycetous fungi and a single genus of the imperfect fungi form an equally important group causing disease in all the life stages of the penaeid prawns. Chitriodinium parasiticum is found to be parasitic on the eggs, believed to belong to penaeid shrimps in the Mediterranean region (Cachon, 1960). The infectious phycomycetous fungi, Lagenidium callinectes and related species including Sirolpidium-like fungus have been responsible for epizootics in cultured eggs and larvae of penaeid prawns throughout the world (Couch, 1942; Cook, 1971; Lightner and Fontaine, 1973; Barkate et al., 1974; Bland, 1974, 1975; Lightner, 1975, 1977, 1981, 1983; Baticados et al., 1977; Pascual and Baticados, 1979). Other phycomycetous fungi such as

Atkinsiela dubia in P. aztecus (Lightner, 1981), Halipthoros milfordensis in P. duorarum and P. setiferus (Lightner, 1977; Tharp and Bland, 1977), H. philippinensis in P. monodon (Natai et al., 1980) and an unidentified phycomycete in P. aztecus (Overstreet, 1973) have also been encountered. The infection by Lagenidium and Sirolpidium to the larval shrimp occurs through the parent brood stock or through the carrier hosts in the sea water supply, when a fungal zoospore attaches to and encyst in the egg or the larva (Lightner, 1983). The pathogenesis of this infectious disease, which is also known as "larval mycosis" (Lightner, 1977), involves the sequential production and release of zoospores into the larval rearing medium (Lio-Po et al., 1982). The pathogenesis of the disease has been described in detail by Lightner and Fontaine (1973) and Lightner (1981).

The only recorded imperfect fungus causing serious disease in penaeid prawns is Fusarium solani which has been reported from P. japonicus (Egusa and Ueda, 1972; Fukuyo, 1974; Fukuyo and Egusa, 1974; Fuary et al., 1974; AQUACOP, 1977; Natai et al., 1978), P. aztecus (Johnson, 1974b), P. duorarum (Nimmo et al., 1977), P. Setiferus, P. occidentalis (Lightner, 1977), P. californiensis, P. stylirostris and P. vannamei (Lightner, 1975; Larsmore et al., 1977; Lightner et al., 1979a). This is an opportunistic pathogen, ubiquitous in distribution (Lightner, 1981), and has been responsible for mortalities

in captive populations of several species of penaeids (Johnson, 1983b). It infects dead or damaged tissue, wounds resulting from crowding, gills damaged from chemical treatments or lesions from other disease process such as "shell disease" (Lightner et al, 1979a). Lesions due to Fusarium infection typically begin as inconspicuous focal lesions in the gills or on the appendages or on the exoskeleton proper, and as the lesions expand, they become increasingly inflammed and grossly appear as darkened melanized lesions (Lightner, 1981). These melanized lesions have given rise to the names "black gill disease" (Egusa and Ueda, 1972) or "burn spot disease" (Hose et al., 1984). Once established, the infection is chronic, usually progressive, and eventually leads to death of the infected host due to tissue destruction (Lightner et al., 1979a) by toxins produced by the fungus (Hose et al., 1984). The chistopathology of "black gill disease" caused by F. solani in P. japonicus has been worked out by Bian and Egusa (1981) while Solangi and Lightner (1976) have studied the cellular inflammatory response of P. aztecus and P. setiferus to injected suspension of conidia of F. solani. The pathogenesis of F. solani has been studied in naturally and artificially infected P. stylirostris and P. californiensis by Lightner et al. (1981) and in artificially infected P. californiensis by Hose et al. (1994).

Protozoan diseases

Among the parasites or pathogens causing diseases in penaeid prawns, protozoans are the most common and widespread.

They are found associated with prawns as commensals, symbionts, parasites and pathogens. Sprague (1970), and recently, Couch (1983) have reviewed the protozoan parasites and hyperparasites of decapod crustaceans while Sprague and Couch (1971) have given an annotated list of protozoan parasites, hyperparasites and commensals of decapod crustaceans. The flagellate protozoan Leptomonas sp., an opportunistic invader of weakened shrimp larvae, is reported to cause mass mortality infecting the haemocoel, abdomen and the appendages of protozoeal and mysis stages of P. aztecus (Couch, 1978).

Among other protozoan parasites, Gregarines mainly belonging to the two genera of Nematopsis and Cephalobolus, are found in the wild and the pond reared P. aztecus, P. duorarum, P. setiferus, P. vannamei and P. brasiliensis (Hutton et al., 1959a; Kruse, 1959; Sprague and Couch, 1971; Overstreet, 1973, 1978; Feigenbaum, 1975; Johnson, 1978; Couch, 1978). Although common inhabitants of the digestive tract, they are not thought to cause any significant disease (Johnson, 1978). Haplosporidians are found to be rare in penaeids and the only one report by Delves-Broughton and Poupard (1976) indicates the presence of the spores of Urosporidium in the gut and muscle tissue of a single specimen of P. orientalis. On the other hand, microsporidians are frequently encountered in penaeids causing a serious disease known as "cotton" or "milk shrimp disease" both in the wild as well as pond cultured prawns incurring considerable loss

to the fishermen and fish farmers (Kruse, 1959; Overstreet, 1973, 1978; Johnson, 1978; Lightner, 1977). Prawns with microsporidian infection have distinctly opaque musculature and ovaries and often have dark blue or blackish discolouration due to expansion of the cuticular chromatophores (Lightner, 1983). Lightner (1975) reported about 15 to 16 percent of "cotton shrimp disease" incidences in the commercially reared P. setiferus in Florida and Texas. Sometimes microsporidian infection may be present at epizootic level (Couch, 1983). Four species of pathogenic microsporidians are known to occur in the penaeid prawns: Perezia (=Nosema) nelsoni is found in the muscle of P. aztecus, P. duorarum and P. setiferus (Sprague, 1950; Hutton et al., 1959a; Overstreet, 1973; Lightner, 1975; Couch, 1978); Agmasoma (=Thelohania) penaei infects the blood vessels, foregut, hindgut, gonads and occasionally the muscles of P. setiferus (Sprague, 1950; Hutton et al., 1959a; Overstreet, 1973; Rigdon et al., 1975); a similar but unnamed species infecting ovaries of P. merguiensis has been described by Baticados (1980); a third microsporidian, Thelohania duorara, has been reported to infect muscle, gonads and other organ tissues of P. aztecus, P. duorarum and P. brasiliensis (Iversen and Manning, 1959; Kruse, 1959; Iversen and Van Meter, 1964; Overstreet, 1973) and the fourth microsporidian, Pleistophora sp. and Pleistophora penaei have been found infecting the different tissues of P. aztecus, P. setiferus

and P. duorarum (Baxter et al., 1970; Censtransitch, 1970; Sprague, 1970; Overstreet, 1973).

Besides the above protozoans, the ciliates are found to be very common protozoan associates often encountered in or attached to penaeid prawns. Stalked peritrichs such as Vorticella sp., Zoothamnium sp., Epistylis sp. and Lagenophrys lunatus are generally found attached on the gills, appendages and body surface of the larval, postlarval, juvenile and adult penaeids in culture systems and, when abundant on the surface of the gills, can cause hypoxia and death (Overstreet, 1973; 1978; Johnson, 1974a; Lightner, 1975, 1977; Lightner et al., 1975; Couch, 1978). An encysted form (phoront) of an unidentified apostome ciliate, associated with black gill disease in P. duorarum, has been described by Couch(1978). Heavy infestation of this ciliate occurs on gills of prawns during periods of warm to moderately cool weather when prawns are held under crowded conditions (Couch, 1978). Another ciliate, Parauronema sp., has been observed by Couch (1978) in the haemocoel of protozoea, mysis and juvenile stages of living, moribund and dead P. aztecus. Suctorians such as Acineta sp. (Johnson, 1978), Ephilota sp. (Couch, 1978) and E. geminipara (Cacutan et al., 1979a) have also occasionally been encountered on the body and gills of penaeid prawns. Couch (1983) has pointed out that certain species of the genus Ephilota may act as stressor in infested prawns, while E. gemmicola infestation on

P. monodon larvae in rearing tanks has been implicated for weakening and mortality of the population (Cacutan et al., 1979a).

Metazoan parasites

The metazoan parasites of penaeid prawns comprise of helminth parasites such as digenetic trematodes, cestodes and nematodes and bopyrid isopods. However, these organisms appear to cause insignificant effect on the prawns (Couch, 1978). Cysts and larval stages of the helminth parasites are generally found in the musculature, hepato-pancreas, intestine or haemocoel. The digenetic trematodes recorded in penaeid prawns are Microcephallus sp. (Hutton et al., 1959a, 1959b; Overstreet, 1973) Parorchis sp. (Johnson, 1978) and Opecoiloides fimbriatus (Overstreet, 1973), while the cestode parasites include Prochristianella hispida (=P. penaei)(Kruse, 1959; Aldrich, 1965; Overstreet, 1973, 1978; Sparks and Fontaine, 1973; Young and Kruse, 1974; Feigenbaum and Carmuccio, 1976; Couch, 1978), Parachristianella spp. (Kruse, 1959; Corkern, 1970; Feigenbaum, 1975), Renibulbus penaeus (Feigenbaum, 1975), Cyclophyllidean and Lecanicephalid larvae (Johnson, 1978) and an unidentified cestode larval stage (Hutton et al., 1959a; Kruse, 1959; Overstreet, 1973; Feigenbaum, 1975; Couch, 1978). The important nematode parasites observed in penaeid prawns are Thynnascaris sp. (=Contracaecum sp.) (Hutton et al., 1959a;

Kruse, 1959; Corkern, 1970; Overstreet, 1973; Norris and Overstreet, 1976), Spirocamallanus pereirai, Leptolaimus sp., Ascarophis sp. and Cõroconema sp. (Overstreet, 1973, 1978; Johnson, 1978). The bopyrid isopods have been reported to parasitise the branchial chamber of penaeid prawns in nature (Dawson, 1958; Tuma, 1967; Ahmed, 1978; Cheng and Tseng, 1982; Palisoc, 1982; Abu-Hakima, 1984). Although the bopyrid infestations do not generally inhibit the growth of the hosts, they considerably affect the gonadial development, often causing parasitic castration in the hosts (Tuma, 1967; Abu-Hakima, 1984).

Nutritional diseases

Besides the diseases caused by biological agents described briefly above, several abiotic agents including the environmental imbalances affect the penaeid prawns. With the increasing stress on semi-intensive and intensive culture systems in the recent years, compounded diets to enhance the growth and survival of the farmed population are being frequently used either to supplement the natural food available in the culture base or as a complete diet in controlled culture operation. The deficiency of certain vital ingredients in the artificial diet or the aflatoxins due to their un-scientific preparation and preservation have led to cause certain nutritional deficiency diseases in the farmed stocks. One such disease

reported commonly is the ascorbic acid deficiency disease, popularly known as "black death disease", observed in P. californiensis, P. stylirostris, P. aztecus and P. japonicus (Deshimaru and Kuroki, 1976; Lightner, 1977, 1983; Lightner et al., 1977; Lightner et al., 1979b; Magarelli et al., 1979). Black death disease produces characteristic blackened (melanised) haemocytic necrotic lesions in the epithelial and sub-epithelial connective tissues of the stomach and gills, subcuticular tissues at the junction of the body segments and appendages and the loose connective tissues of hepatopancreas, nerve cord and eye stalk(Hunter et al., 1979; Lightner et al.,1979b). Once signs of the disease become apparent, the affected prawns do not feed and death usually follows within 24 to 36 hours (Lightner, 1977). Deshimaru and Kuroki (1976), Lightner et al. (1979b) and Magarelli et al. (1979) have reported that a dietary requirement of 2000 to 3000 mg of the ascorbic acid per kilogram of feed is necessary to control the disease.

Diseases caused by environmental stress

Stress conditions such as supersaturation of atmospheric gases, low dissolved oxygen levels, sudden temperature or salinity changes, over crowding and rough handling lead to unhealthy state in prawns, and in severe cases, lead to large scale mortalities. "Gas bubble"

disease occurs when the prawns are subjected to waters supersaturated with the atmospheric gases, particularly when the dissolved oxygen level reaches or exceeds 250 per cent of the normal saturation of the medium (Lightner et al., 1974; Supplee and Lightner, 1976; Lightner, 1983). Gas bubbles are formed in the haemolymph and death results if large amount of bubbling occurs (Johnson, 1978). The corrective measures involve vigrous aeration and lowering of the dissolved oxygen level of the water (Supplee and Lightner, 1976). Several other diseases such as muscle nerosis or spontaneous muscle necrosis(Rigdon and Baxter, 1970; Venkataramiah, 1971a, 1971b; Lakshmi et al., 1978), cramped tail condition (Johnson, 1975, 1978; Lighter, 1977; Miao et al., 1977) and broken back syndrome (Couch, 1978) occur due to changes in environmental factors. The spontaneous muscle necrosis follows periods of severe stress such as over crowding, low dissolved oxygen levels, sudden temperature or salinity changes and rough handling (Lakshmi et al., 1978). Shrimps recover in many cases, however, if stress ceases (Couch, 1978). The cramped tail condition appears to be related to sudden increase in the temperature of water and air (Lightner, 1983). The tail is drawn under the body and becomes rigid to the point that it cannot be straightened (Johnson, 1978). Broken back syndrome appears to be related to severe salinity, cold temperature and handling stresses which, in combination, display a characteristic dorsal separation of

the pleural plates covering the third and fourth abdominal segments (Couch, 1978).

Toxic diseases

The toxic diseases manifest mainly from two sources, toxigenic algae and pollution of water by pesticides or industrial chemicals, chlorinated hydrocarbon, petroleum or oil products and certain heavy metals. Blooms of the diatom, Chaetoceros gracilis, certain dinoflagellates and filamentous blue green algae such as Lyngbya calcicola, Spirulina subsala and Microcoleus lyngbyaceus are reported to be toxic to the cultured populations of P. stylirostris, P. vannamei and P. californiensis (Lightner, 1978b, 1983; Lightner et al., 1978; Simon 1978; Lightner et al., 1980). The dinoflagellates may affect prawns during moulting (Sievers, 1969). A dinoflagellate, Amphora sp. may infect the haemocoel of prawn and cause melanization in the gills (Overstreet and Safford, 1980). The blooms of blue-green algae are shown to cause haemocytic enteritis(HE), particularly in juveniles, when necrosis and haemocytic inflammation of the mucosal epithelium of those portions of gastrointestinal tract that lack a chitinous lining occur (Lightner, 1978b, 1983; Lightner et al., 1978). This leads not only to osmotic imbalances and poor absorption of nutrients, but also to secondary bacterial infections (Lightner, 1978b, 1983; Lightner et al., 1978; Lightner et al., 1980).

Toxic responses of penaeid prawns to pollution have been reviewed in depth by Couch (1978, 1979). Several years of experimentation have revealed that penaeids are, in fact, more sensitive to toxic effects of most insecticides than the fishes or molluscs (Couch, 1978). Organochlorines such as DDT, Dieldrin, Mirex and PCBs; organophosphates such as Baytex, Dibrom, Malathion and Parathion and carbamate such as Sevin, have adverse effects on penaeids, usually affecting the physiological processes of hepatopancreas and resulting in death of the animal (Butler and Springer, 1963; Butler, 1966; Duke et al., 1970; Nimmo et al.,1970; Lowe et al., 1971; Nimmo et al., 1971a; Nimmo et al., 1971b; Nimmo and Blackman, 1972; Parrish et al., 1973; Coppage and Matthews, 1974; Couch and Nimmo, 1974a, 1974b; Hansen et al., 1974a; Hansen et al., 1974b; Conte and Parker, 1975; Couch, 1978; Schoor and Brausch, 1980). Although little information is available on the effects of petroleum on penaeid prawns, fuel oils, particularly the napthalenes and sonified crude oil, are known to be very toxic to penaeid prawns as they accumulate in the animal tissues and/or produce necrotic lesions on the body, gills, lining of the gastric mill and eyes (Mills and Culley, 1971; Anderson et al.,1974; Cox et al., 1975; Yarborough and Minchew, 1975; Neff et al., 1976; Minchew et al., 1979).

Penaeid prawns are also sensitive to certain heavy metal pollutants. Exposure of prawns to cadmium causes black gill syndrome by killing the gill cells and consequently lead to the death of the animal (Bahner, 1975; Couch, 1977; Nimmo et al., 1977). Mercury is accumulated by penaeids and may interfere with their osmoregulatory abilities (Couch, 1978). Petrocelli et al. (1974) have experimentally shown that exposure of brown shrimp, P. aztecus to mercuric chloride results in interference of prawn's ability to adjust its internal ion levels to the external changes which may be detrimental to prawns.

Nitrogen, which enters culture systems primarily as organic compounds that are metabolized to ammonia, nitrite and nitrate by resident culture species and/or bacteria, is also toxic to cultured crustaceans including penaeid prawns when present in excess (Armstrong, 1979). Nitrite is the most toxic of the three compounds with effective concentrations of about 0.5mM NO_2/l; ammonia adversely affects the prawns at about 1.1mM ammonia/l and nitrate, the least toxic, at 12.5mM NO_3/l (Wickins, 1976; Armstrong, 1979).

Toxic effects of chemotherapeutic chemicals

Some of the chemotherapeutic chemicals used routinely in the treatment of fish diseases are found to be toxic to penaeid prawns at certain concentrations (Johnson, 1974c,

1976a; Hanks 1976). Gacutan et al. (1979b) have determined the optimum exposure time of P. monodon to furanace baths. Schnick et al. (1979) have listed a number of chemotherapeutants and anesthetics with their relative toxicity to crustaceans including penaeid prawns, while Hatai et al. (1974) dealt with the toxicity of a number of fungicides on the Fusarium causing black gill disease of P. japonicus. According to Lightner (1977), use of potassium permangnate as antibiotic at 5 to 10 ppm for one hour to treat filamentous bacterial gill disease may cause severe gill damage. For the similar disease, Lightner and Supplee (1976) have found that use of Cutrine plus at 0.1 and 0.5 mg/l concentrations was toxic to P. californiensis.

Miscellaneous diseases

Besides the aforementioned diseases, there are reports on several other diseases and abnormalities which are either of uncertain etiology or not believed to be serious diseases of penaeid prawns. These include tumour-like growth (Sparks and Lightner, 1973), hamartoma (Overstreet and Van Devender, 1978), blisters (Lightner, 1977; Johnson, 1978), "golden" shrimp (Johnson, 1978; Lightner, 1983) blue disease (Lightner, 1983; Lightner et al., 1983b), blue or white eye disease (Lightner, 1983), amoebasis of larvae (unclassified amoeba) (Laramore and Barkate, 1979), larval encrustation, multifocal opacities

(Lightner, 1983), gut and nerve syndrome or GNS (Lightner et al., 1984), white pleura disease (AQUACOP, 1977; Lightner, 1983), red disease (Liao et al., 1977; Lightner and Redman, 1985), nerve disease syndrome (Katzen et al., 1984), aflatoxicosis (Lightner et al., 1982; Wiseman et al.,1982) and fatty infiltration of hepatopancreas (Kaiser et al., 1978; Lightner, 1983).

Studies carried out in India

About 62 species of prawns and shrimps belonging to the family Penaeidae occur in Indianwaters. The important of those supporting the commercial marine fishery of the country at present are Penaeus (Fenneropenaeus) indicus* H. Milne Edwards, P. (Penaeus) monodon* Fabricius, P. (Penaeus) semisulcatus* de Haan, P. (Fenneropenaeus) merguiensis* de Man, Metapenaeus dobsoni (Miers), M. monoceros (Fabricius), M. affinis (H. Milne Edwards), Parapenaeopsis stylifera (H. Milne Edwards), P. sculptilis (Heller), P. hardwickii (Miers), Solenocera crassicornis (H. Milne Edwards) and non-penaeid prawns such as Exhippolysmata ensirostris (Kemp),

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* Species name given here follows Holthius, L.B.(1980) FAO species catalogue, Vol. 1 - Shrimps and prawns of the world. FAO Fish. Synop., 125: 1-261 pp. However, in other parts of the thesis, the species are referred to as P. indicus, P. monodon, P. semisulcatus and P. merguiensis without the sub-generic name.

[illegible] stylifocus (H. Milne [illegible], [illegible] tenuipes (Henderson) and Metapenaeus indicus (H. Milne [illegible]). Various aspects of life history, distribution patterns, and [illegible], reproduction, larval development, and [illegible] population, exploitation and fisheries of [illegible] peneid prawns have been extensively dealt with and well documented in a series of species synopsis and other publications by George (1970a, 1970b, 1970c, 1970d, 1972, 1978); Menon (1970), Mohamed (1970a, 1970b, 1973) Rao (1970), Rao (1972) and Kurian and Sebastian (1976). Recently Silas et al. (1984) reviewed the scientific basis for the management of the prawn fisheries in the country. Several accounts are also now available on different aspects of culture of penaeid prawns in the brackishwater regions of the country (Srivastava and Ratnala, 1984; Srivastava, 1985). The technological advances made on the hatchery production of penaeid prawn seed along with a package of practices involved, are given by Silas et al. (1985).

Although a wealth of information on the biology, and on the capture and culture fisheries of penaeid prawns of the country are now available from the above mentioned works, investigations on the diseases of prawns are limited. One of the outstanding contributions on the parasites of decapod Crustacea was by Thomas who, in 1973, described

several bopyrid parasites along with their geographical distribution and keys for identification. Following this, there have been only occasional and isolated studies on diseases of prawns. The noteworthy among these are the bacterial diseases such as myxobacteriosis, haemorrhagic septicaemia, vibriosis and enteric bacterial infection. The myxobacterial infection caused by Chondrococcus sp. is reported in P. indicus, P. monodon, M. affinis and M.dobsoni cultured in earthern ponds in the brackishwater areas while Pseudomonas fluorescence, causing haemorrhagic septicaemia, is encountered mainly in P. indicus and M. monoceros (Mahadevan et al., 1978). Among the bacterial diseases, vibriosis caused by V. anguillarum is the most frequent disease found in P. indicus cultivated in the brackishwater fields (Mahadevan et al., 1978). Recently, brown spot disease caused by Vibrio and Aeromonas sp. are also reported in P. indicus (Lakshmanperumalsamy et al., 1982). The bacterium, Escherichia coli is found to infect the larvae of P. indicus (Mahadevan et al., 1978).

Among the diseases caused by fungi, large scale mortality in the larvae and juveniles of P. monodon raised in the hatchery has been reported due to heavy infection by fungus, Lagenidium sp. (CMFRI report, unpublished). Similarly, the fungi Saprolegnia parasitica and Leptolegnia marina are recorded from the juveniles caught from the

backwaters of Cochin (Gopalan et al., 1980). In the giant freshwater prawn, Macrobrachium rosenbergii, Shah et al. (1977) reported five different fungi, namely, Saprolegnia sp., Achlya sp., Aphanomyces sp., Pythium sp. and Leptomitus sp..

The protozoan parasites reported from the Indian prawns are Zoothamnium rigidium and Stenctor coerulens in M. monoceros (Santhakumari and Gopalan, 1980). Besides these, Epistylis sp. together with Zoothamnium sp. is also encountered in P. monodon causing hypoxia (Issac Rajendran et al., 1982). Occasionally, these protozoans are found to affect the juvenile prawns in the culture ponds where dissolved oxygen level in pond water decreases to 1.0 ppm due to non-flushing of pond water with tidal water (Issac Rajendran et al., 1982).

The "cotton" or "milk shrimp" disease caused by microsporidian parasites in the natural populations of P. indicus, P. semisulcatus, M. monoceros and M. brevicornis caught off Madras, Mandapam, Tuticorin and Cochin has been reported on several occasions (Subrahmanyam, 1974; Thomas, 1976; Santhakumari and Gopalan, 1980; Gopalan et al.,1982; Palaniappan et al., 1982; CMFRI report, unpublished).

Large number of metacercarial cysts infecting M. monoceros inhabiting the Cochin backwaters have been reported by Gopalan et al. (1982) and Syed Ismail Koya

and Mohandas (1982). Instances of isopod bopyrid parasites infesting the branchial chamber or attaching to the appendages have been reported in M. monoceros, P. stylifera, P. semisulcatus and Palaemon tenuipes from natural populations and they were found to affect the primary and secondary sexual organs of the host which remain imperfectly developed or rudimentary or in degenerated condition (Menon, 1953; Sawant and Kewalramani, 1964; Thomas, 1977).

Among the farmed P. indicus in the brackishwater fields, an important disease syndrome reported and being investigated at the Central Marine Fisheries Research Institute is what is referred to commonly as "soft prawns" (Mahadevan et al., 1978; Rajamani, 1982; Rao 1983). This disease syndrome in cultured prawns is generally encountered during adverse ecological conditions such as low salinities and combination of higher temperature and salinities. It is more frequently encountered in culture operations involving relatively higher stocking densities and often results in considerable loss of prawn population in the field and economic loss to the farmers. In a recent study, Natrajan et al.(1982) observed black spot disease on exoskeleton, large number of protozoan cysts on the body, a bopyrid isopod, Probopyrus sp. in the branchial chamber, blisters on the different body parts and a tumour on the carapace of freshwater prawn, Macrobrachium equidens from Nethravathi river of Dakshin Kannada.

Recognising the importance of disease diagnosis and control in the context of rapid development of aquaculture in the country, to facilitate exchange of information and to gather the scattered information available on the diseases of finfishes and shellfishes in India, the University of Agricultural Sciences, College of Fisheries, Mangalore, organised a national symposium on the subject in 1982. At this symposium 6 papers relating to the deseases of prawns were presented. Later, the Central Marine Fisheries Research Institute, Cochin, held a workshop on 'Approaches to finfish and shellfish pathology investigation' in January, 1983 where the guidelines in the identification of disease problems and the rational approaches to be undertaken to tackle the same were discussed.

The various projections attempted on the aquaculture of prawns have indicated great growth potential in India and in this development, an understanding of the diseases affecting the farmed stocks and their control has a significant role to play. In this perspective, the present investigation is taken up to facilitate improvement and accelerated development of culture fisheries for prawns through better management and disease control so as to obtain higher survival and production in the culture operations.

Although diseases, parasites and abnormalities have been observed in several penaeids such as P. indicus, P. monodon, P. semisulcatus, M. dobsoni and M. affinis during the present investigation, frequently encountered cases of diseases have been mainly in P. indicus and P. semisulcatus. Brief notes on these two species are given below.

Penaeus indicus H. Milne Edwards, 1837: (Pl.I, Fig.1). This species is popularly known as Indian white prawn and forms an important commercial species occuring along both the east and west coasts of India. Its general distribution ranges from the east and southeast coasts of Africa to South China, New Guinea and Northern Australia. In the marine region it occurs up to 90m depth and generally inhabits the muddy or sandy bottom. In the adult phase it supports a fishery in the inshore waters, and juveniles are caught from estuaries and backwaters. It also forms an important species in the paddy-cum-prawn farming of Kerala and to a lesser extent in the brackishwater fish farming of west Bengal, Karnataka and Goa. It grows to a maximum total length of 184 mm in male and 228 mm in the female.

Penaeus semisulcatus de Haan, 1844: (Pl.I, Fig.2). Popularly known as green tiger prawn. It is more common on the east coast of India than on the west coast. It supports an important fishery on the southeast coast. Its

PLATE I

Fig. 1. The Indian white prawn, Penaeus indicus H. Milne Edwards, 1837.

Fig. 2. The green tiger prawn, Penaeus semisulcatus de Haan, 1844.

The white streak in the photographs is due to reflection of light.

PLATE 1

1

2

general distribution ranges from Red sea, East and Southeast Africa to Japan, Korea, the Malay Archepelago and northern Australia. In the eastern Atlantic, the species occurs from the eastern Mediterranean through the Suez Canal and is also found all along the coasts of Egypt, Israel, Lebanon, Syria and southern Turkey. In the marine region, it is recorded upto 130 m depth. As in the case of P. indicis, it inhabits muddy or sandy bottom. Adults are found in the marine region and juveniles in the estuaries. It attains the maximum total length of 180 mm in male and 228 mm in female. It plays a role in the rice field prawn farming in the Ganges delta.

CHAPTER 2

MATERIALS AND METHODS

The present work was carried out from August 1982 to December 1984, and it involved a survey of prawn diseases at certain centres along the southwest and southeast coasts of India, the clinical examination to diagnose the disease by means of macro-and microscopical observations and detailed studies on the microsporidian parasites infecting the penaeid prawns. The methods of collection of samples for environmental parameters and of data pertaining to infected/ abnormal prawns as well as the techniques involved for microscopic examination of the specimens, common to all studies, are presented in this chapter. Materials and specific methods employed for experiments on transmission of one of the microsporidian species and for the determination of proximate composition of normal and microsporidian infected prawns have been given in the relevant chapters.

Survey

The survey on prawn diseases was carried out from two regions: (1) from Cochin on the southwest coast of India, and (2) from Mandapam on the southeast coast (Pl. II). At Cochin, the samples of abnormal or diseased prawns were

PLATE II

Fig. 1. Map showing the location of collection sites around Cochin on the southwest coast of India.

Fig. 2. Map showing location of collection sites around Mandapam on the southeast coast of India.

PLATE II

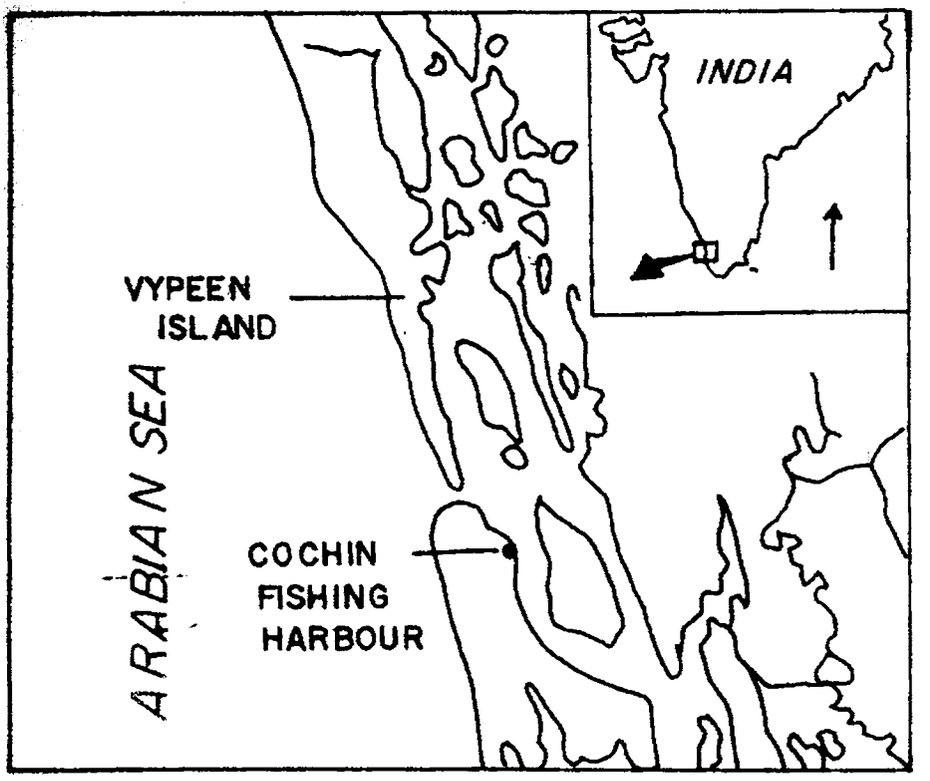


Fig.1

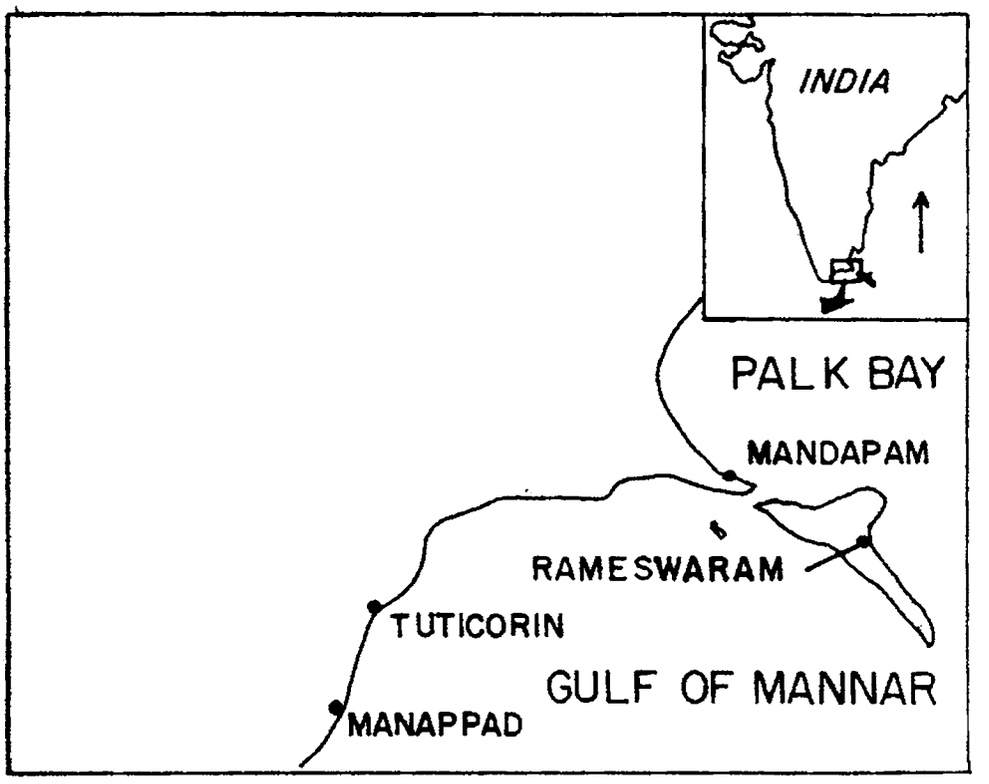


Fig.2

collected from regular fortnightly visits to the prawn culture fields at the Vypeen island and the Cochin fishing harbour. The Vypeen island lies parallel to the mainland of Cochin and is surrounded on three sides by brackishwater while on the western side it faces the Arabian sea. It is about 25 km long, with a total area of 69.63 km^2. The island has extensive marshy low lands, paddy fields, and a network of tidal canals having typically brackishwater. About 1,737 ha of the brackishwater area is used at present for culture of fishes and prawns following the traditional practice. The annual average production of prawns and fishes from these culture fields is estimated to be 1300 tonnes. Out of this, prawns comprising mainly of Penaeus indicus, P. monodon, Metapenaeus dobsoni and M. monoceros contribute to about 86.5 percent and the fishes account for the rest. Majority of the cases of diseases and abnormalities in the penaeid prawns reported in this thesis were collected from the prawn and fish culture fields of Vypeen island during the survey.

The Cochin fishing harbour forms an important fish landing centre for mechanised fishing vessels. From this centre, about 100 to 150 mechanised boats (9.6 meter long and above) operate bottom shrimp trawls off Cochin within 40 meters depth zones and land their catches. The prawn catch landed by these mechanised vessels is principally

constituted by P. indicus, P. monodon, M. dobsoni, M. monoceros, M. affinis and Parapenaeopsis stylifera. A few cases of brown spot disease and ciliate infestation reported in the present study were collectid from this centre.

The survey of diseases in penaeid prawns were also carried out at different fish landing centres along the southeast coast of India. These included Rameswaram, Pamban, Mandapam, Vedalai, Kilakkarai, Arvadi, Tuticorin and Kanyakumari. The prawn catches landed at these centres came either from the Palk Bay or the Gulf of Mannar. The prawns were mainly caught by mechanised fishing vessels (9.14 meter long and above) operating otter trawls. Among the prawn catches, P. semisulcatus was the major species contributing to the prawn fisheries at these centres followed by M. affinis.

In addition to the above, collections were also made in the Palk Bay and the Gulf of Mannar by using "Cadalmin" (13.25 meter long) fishing vessel belonging to the Regional Centre of the Central Marine Fisheries Research Institute (R.C.M.F.R.I.), Mandapam Camp. The postlarvae and juveniles of P. indicus and P. semisulcatus infested with metacercarial cysts were collected from the tidal mudflat near Pamban by using hand operated scoop net. The prawns in the brood stock tanks and the rearing ponds at the Narakkal Prawn Culture

Laboratory (NPCL) of CMFRI in Vypeen island, Cochin, were also examined regularly for diseases, parasites and abnormalities.

Information collected during the Survey

During the survey, the prawn catches were carefully examined either with naked eyes or with the aid of a folding type 10 magnifying lens. The body surface, rostrum, eyes, antennal scale, branchial chamber, appendages, uropod and telson were scrutinized for external signs of any disease, parasitic infection/infestation or abnormality. While collecting diseased or abnormal prawns from the fish landing centres/fishing harbour, information such as the type of gear operated, the locality from where the catch was made, approximate depth, incidence of occurrence of such abnormal prawns in the catch, and seasons of occurrence was collected by enquiry. To facilitate collection of abnormal/diseased prawn samples from the mechanised fishing vessels, each of the vessel was given an ice box with sufficient ice for preserving the samples as soon as they were caught. In case of the material collected from the prawn culture ponds, the information on the type of culture practice followed (monoculture/mixed culture/polyculture, seasonal or perennial culture); nature of the stocked population; water supply, its source, and quality; stocking strategy (wild stocking/stocking of seeds of selected species/ stocking of hatchery raised seed/stocking density); food resources (natural/artifical); and occurrence of any previous

mortality was collected either by actual observation or by enquiry. Besides, the general behaviour of the prawns was also noted. The diseased/abnormal prawns, whenever encountered, were immediately collected from the prawns catches and preserved in large ice boxes, until they were transported to the nearest laboratory, or in suitable fixatives at the collection site itself for further treatment and examination. Besides, in certain cases live prawns were also transported to the laboratory in polythene transportation bags.

At the time of collection of diseased/abnormal prawn samples from the prawn culture fields during the survey, samples for environmental parameters relating to temperature (T), hydrogen ion concentration (pH), dissolved oxygen (DO) and salinity (S) of the surface water of the pond were also collected. The temperature, and hydrogen ion concentration were determined at the site itself. For temperature measurement, an ordinary immersible mercury thermometer graded upto 50°C (accuracy 0.01°C) was used. A "Biochem" make portable pH meter was used for determination of hydrogen ion concentration. Water samples for the analyses of oxygen and salinity were collected in 125 ml clean glass BOD bottles. To determine the oxygen, the water was collected without agitation following usual procedure and precautions, and the water samples were fixed immediately with Winkler's solutions. Later, in the laboratory, the salinity of water

samples was estimated by argentometric method (Strickland and Parson, 1968) and the dissolved oxygen by Winkler method (Strickland and Parson, 1968).

In the laboratory, the prawn samples were analysed for size, sex and maturity stage. The size of the prawn was recorded as total length (TL) measured from the tip of the rostrum to the tip of telson; the sex, on the basis of secondary sexual characters, and the different maturity stages of female on the criteria described by Rao (1968). After recording the data, the samples were subjected to detailed microscopical studies. Laboratory studies were mainly carried out at the Central Marine Fisheries Research Institute at Cochin and Mandapam Camp. Occasional short duration laboratory work was also performed at the Research centre of CMFRI, Tuticorin. Electron microscopic study of microsporidian parasites was carried out at the Indian Institute of Horticultural Research (IIHR), Bangalore.

Histopathological studies

The histopathological studies of the normal and diseased prawns, and their various tissues were made both by light and electron microscopy. The material for the microscopical examination included the animals and tissues fixed in suitable fixatives, smears and micro-sections of different tissues. The techniques followed in the preparation of this material are briefly described below.

Smears: The haemolymph for smears was collected either from the heart using a 1 ml glass syringe fitted with No. 24 hypoderemic needle which was pretreated with an anticoagulant, sodium citrate solution or by cutting the tip of one of the pleopods of the prawn. In either cases, the collected haemolymph was smeared over a clean glass slide using another similar slide. The smear was air dried and fixed in 70% methanol for 5 minutes. It was stained with dilute Giemsa stain (McManus and Mowry, 1960) for 35 to 40 minutes and washed in phosphate buffer (pH 6.8) and air dried.

The tissues such as hepatopancreas, muscle and gonad of microsporidian infected prawns were removed by dissecting the animal, smeared on clean glass slide and fixed in 70% methanol or Bouin's fixative or 30% H_2O_2 or 4% glutaraldehyde for 5 minutes, rinsed with distilled water and air dried. These smears were stained with dilute Giemsa stain or with Heidenhain's haematoxylin as modified by Sprague (Clark, 1981) or treated with the periodic Acid Schiff (PAS) reaction or Feulgen reaction (McManus and Mowry 1960) to study the structure and various cytological properties of microsporidian spores and developing stages.

Fixation of material and fixatives used: Fixation of the prawns and their different tissues were carried out either at the collection site or in the laboratory depending on the situation. When fixation was not possible at the site of

collection, the live prawns were transported to the laboratory in 10 capacity polythene transportation bags filled with the water collected from the same area from where the material was obtained, and oxygen. In the case of moribund or freshly killed specimens, they were brought to the laboratory preserved in ice in an ice box. Fixatives used frequently during the study were 10% neutral buffer formalin, Bouin's fixative and Davidson's fixative.

The whole prawns for histopathological studies were fixed in 10% neutral buffer formalin or Davidson's fixative by one of the following two methods. The fixative, either 10% neutral buffer formalin or Davidson's fixative, was injected directly below the carapace and abdominal regions with a hypodermic syringe prior to immersing the whole prawn in the fixative. In the second method, the exoskeleton of the dorsal aspect of the prawn was cut longitudinally to ensure penetration of the fixative and then the whole prawn was immersed in the fixative at room temperature. When 10% neutral buffer formalin was used, the fixative was changed after 24 hours and then stored in the fresh fixative. In cases where Davidson's fixative was used, the fixation time was 48 hours after which the fixed prawns were transferred to 70% alcohol and stored.

The organs/tissues such as eye, hepatopancreas, gonad, heart, gill, gut or portions of body muscle, for histopathological examinations, were collected from live or moribund or freshly killed prawns and fixed in either Bouin's or Davidson's fixative at least 20 times the volume of the tissue. When an entire organ, such as hepatopancreas, eye, heart or abdominal segment of large prawn had to be fixed, chilled Bouin's fixative was used. All the fixed tissues were directly transferred to 70% alcohol after 36 to 48 hours, and stored in glass tubes at room temperature for further processing.

Examination of fresh and fixed material in the laboratory: For clinical diagnosis of diseases, the whole body as well as the different organs of fresh and fixed animals were first critically examined with the naked eyes for external symptoms, and later, under the dissection or compound microscope. In order to proceed systematically, wet mounts of compressed or squashed tissues were examined from side to side following the methods used for fishes (Lucky, 1977; Roberts, 1978) and lobsters (Fisher et al., 1975). For microsporidian parasites, applying the method given by Vavra and Maddox (1976), spore monolayers were obtained using a combination of liquid paraffin and water.

Decalcification: Materials with hard cuticle such as the abdominal segment of the prawns, eyestalk and entire

postlarvae and juveniles, which were fixed in Bouin's fixative or 10% neutral buffer formalin, were decalcified following acid decalcification method as used by anderson (unpublished laboratory technique's manual, Galveston Laboratory, U. ...). The chitinous tissues fixed in Davidson's fixative does not require decalcification as the fixative itself acts as a decalcifying agent.

Processing of tissues and staining: For cutting sections of the different tissues in paraffin, the dehydration and clearing of the tissues was carried out at room temperature. The tissues were first washed in two changes of 70% alcohol for 1 hour each, dehydrated for 1 hour in 80% alcohol, graded twice in 95% alcohol and in absolute alcohol, cleaned through a mixture of absolute alcohol and chloroform (1:1 V/V) and then passed twice in pure chloroform for 1 hour each. Chloroform was preferred over xylene because it did not leave the tissue hard and brittle. The tissues, after clearing, were left in a mixture of chloroform and paraffin wax (approximately 1:1) at room temperature overnight. Before embedding, 1 hour impregnation in paraffin wax of 56 to 58°C melting point was given twice. The sections were cut at 5 to 7 µm thickness using a manual rotary microtome (Fuji Optics, Japan). After deparaffinising in xylene, the sections were dehydrated through graded series of alcohol and finally in distilled water, and stained with Harris alum haematoxylene (Preece, 1972) or Heidenhain's haematoxylin (Clark, 1981) and counterstained

with 1% alcoholic eosin (Preece, 1972). Some of the sections were also stained with Mallory's triple stain (Mallory, 1944) or dilute Giemsa stain. Occasionally, sections were treated with PAS or Feulgen reaction. Applying the routine procedure, stained sections were dehydrated through the graded series of alcohol and mounted with glass cover slip in DPX through xylene. However, when sections were stained with dilute Giemsa stain, they were air dried and directly mounted in DPX.

Following the normal staining procedures with the Mallory's triple stain, the infected gonadial tissue was found to stain light violet whereas the uninfected muscle and connective tissue, blue. As these overlapping colours often made it difficult to differentiate the tissues clearly, a slightly modified staining procedure with the Mallory's triple stain was employed for some of the serial sections. In the modified procedure, the tissues were overstained in the Mallory's triple stain for about 18 minutes instead of the normal 9 minutes duration. These overstained sections were then treated with saturated aqueous solution of periodic acid for 15 to 20 seconds; rinsed twice in quick succession in distilled water and were then passed on to 90% alcohol for 6 seconds and twice in absolute alcohol for 10 seconds each. The sections were cleared in xylene was usual and mounted in DPX. By this procedure, the infected gonadial tissue was found to stain bright yellow to orange while the uninfected

muscle, connective tissue and the collagen, intense blue, thus facilitating clear differentiation between the uninfected and infected tissues (Pl. XIV, Fig. 1). Further, it was also observed that the nucleus of the early developmental stages of the microsporidian stained deep red and the cytoplasm almost colourless in this staining procedure as against the dark and light shades respectively with the normal Mallory's triple stain.

Light microscopy and photomicrography

The histological sections and smears were studied either with an Olympus monocular compound microscope or with a Carl Zeiss JENA ERGOVAL binocular compound microscope. Cellular measurements were taken with Carl Zeiss microscope fitted with a caliberated ocular micrometer scale with a accuracy up to 1 /um. Photomicrographs were taken with mf camera attachment unit having optical tube factor of 1 on Carl Zeiss Ergoval compound microscope with projection eye pieces 3.2, 4, 5, 6.3, 8 and 10 X and objectives 3.2, 10, 40 and 100 using 24 x 36 mm negative film of 100 ASA. The magnification of the enlarged prints was calculated following the instruction and formula given in the manufacturer's manual (Carl Zeiss-No. 30 - 0 605 g-2) supplied along with the microscope.

Transmission Electron Microscopy

Live or fresh specimens of P. semisulcatus and M. affinis, infected with microsporidian parasite and caught from off the coast of Mandapam in the Gulf of Mannar and Palk Bay, were injected with cold (8°C) 4% glutaraldehyde solution prepared in Millionig's phosphate buffer (pH 7.2) (oberts, 1978) using a 1 ml hypodermic glass syringe fitted with No. 22 needle through the carapace and abdominal regions. ubsequently, the cuticle over the carapace and abdominal region was removed and small pieces of tissues (1 to 2 mm^3) such as abdominal muscle and ovary were fixed immediately in 4% buffered glutaraldehyde solution (pH 7.2). The fixed tissues were kept in a refrigerator at 4°C for 12 hours. Thereafter, the used fixative was replaced with the fresh, cold fixative and stored at 4°C as suggested by reece (1972).

The transmission electron microscopy work was carried out at the Indian Institute of Horticultural Research, Bangalore. In the laboratory, the fixed tissues were further cut to small pieces (< 1 mm^3) and washed several times in quick succession in cold (~5°C) Millionig's phosphate buffer (pH 7.2). Following the washing, tissues were post-fixed in cold (5°C) 1% Osmium tetroxide solution prepared in Millionig's phosphate buffer containing sucrose (pH 7.2) at 4°C for 2 hours. The tissues were then rinsed twice with double distilled water; stained with 2% aqueous uranyl

acetate for 2 hours at 4°C; rinsed twice with double distilled water and dehydrated in 20%, 50%, 70% and 95% acetone, each for 15 minutes; and in pure acetone twice, each of 30 minutes, duration. Tissues were soaked for 2 hours in a 2:1 mixture of acetone and Spurr's resin embedding medium (Spurr, 1969) and then left overnight in a 1:2 acetone and Spurr's resin mixture. On the following day, each tissue was carefully transferred to a plastic capsule arranged vertically in a special holder and Spurr's embedding resin was poured in. These capsules were then left at room temperature for 1 hour and incubated at 50°C for 4 hours, and at 60°C for 48 hours in an incubator. Polymerised resin blocks were removed from the capsule and semi-thin sections of 1 µm thickness were cut on an LKB Ultratome III using the glass knife. These sections were stained either with methylene blue-azure II and basic fuchsin combination (Humphrey and Pittman, 1974) or with 1% toluidine blue (Roberts, 1978) and were examined under the light microscope. Based on content, adequacy of fixation and absence of artifacts, blocks were then selected and cut for ultra-thin sections at 600-600A° in LKB Ultratome III with glass knife. Selected ultra-thin sections were taken on 3-200 copper grids coated with 2% collodion solution in amyl acetate (Hayat, 1970); stained as per the procedure given by Hayat (1970) with 2% uranyl acetate in 50% ethyl

alcohol for 10 to 15 minutes; washed thrice with double distilled water, and stained with 0.4% lead citrate in 0.1 N NaOH for 5 to 10 minutes; followed by quick and thorough wash consecutively in 0.02N NaOH solution and double distilled water. The ultra-thin sections thus prepared and mounted on grids were allowed to dry, and later, examined and photographed with a JEM-100S or a ZEISS 109 Transmission Electron Microscope. Electron micrographs taken on Kodak E. 4489 or Agfaortho 25 Professional film were developed immediately in a high contrast developer and printed on a contrast glossy paper with magnification accurately controlled.

CHAPTER 3

SURVEY OF PENAEID PRAWN DISEASES AT CULTURE CENTRES

The review of literature presented in the "General Introduction" reveals that documented information on the incidence of diseases or on their studies on the Indian penaeid prawns, both in the natural and farmed populations, is scarce. This is perhaps due to the non-occurence of large scale diseases in prawns in nature or whenever mortalities in the natural populations and those encountered in the prevailing extensive farming systems following traditional practice occur, they are compromised either for natural mortality or those caused by abiotic environmental factors. Besides, the difficulties associated in the establishment of disease as the cause of mortalities in the population have also contributed to the relatively little information available in this field in India. This situation prompted the candidate to undertake a base-line survey in the beginning of the investigation with a dual purpose recording the common diseases/anomalies encountered in penaeid prawns of the country in nature as well as in culture operations and to select a disease for detailed study. Initially, the survey was carried out by

regularly visiting the landing centre at the Cochin fishing harbour and prawn culture fields in the Vyeen island, Cochin. Subsequently, the survey was extended to the landing centres in and around Mandapam on the southeast coast of India.

DISEASES RECORDED IN THE PRESENT INVESTIGATION

During the survey, the following ten cases of anomalies and diseases in penaeid prawns were encountered. A brief description and salient features of each of them are presented and discussed.

3.1. Tumour-like growth
3.2. " " Soft prawn Syndrome
3.3. Tail necrosis
3.4. Brown spot disease
3.5. Red rostrum
3.6. Ciliate infestation
3.7. Microsporidiosis
3.8. Helminth parasitisation
3.9. Metacercarial infestation
3.10. Bopyrid infestation

3.1. TUMOUR-LIKE GROWTH
(Plate III, Figs. 1 to 6)

Host: Penaeus indicus, female, measuring 152 mm total length (TL).

External symptoms: A large, bulbous, tumour-like swelling on the left dorsolateral side of the carapace (Pl. III, Figs. 1 and 2).

Material studied: One specimen collected from one of the grow-out ponds of the Narakkal Prawn Culture Laboratory (NPCL) of the Central Marine Fisheries Research Institute (CMFRI), Wypeen island, Cochin.

Date of collection: 12th December, 1994.

Indidence: Rare.

Season: No particular season observed.

Environmental information: Salinity (S) = 29.61 ppt; Dissolved Oxygen (DO) = 3.96 ppm; T = 29.3°C; pH = data not available.

Observations: The tumour-like growth was observed on the dorsolateral side of the carapace between the proximal dorsal rostral spines and the hepatic spine. It appeared as a bilobed tumour, the lobe near the hepatic spine being slightly larger than the other lobe found close to the proximal dorsal rostral spines. Due to the size and

PLATE III

Fig. 1. Cephalothoracic region of Penaeus indicus showing the tumour - like outgrowth (arrow) on the dorsolateral side of the carapace. The pigments in the affected area are comparatively fewer. CP=Carapace; RM=Rostrum; WO=Wounds.

Fig. 2. Same in close-up view.

Fig. 3. Penaeus indicus: Transverse section through one of the lobes of tumour - like outgrowth. AT=Areolar connective tissue; FL=Fibroblast-like cells; FT=Fibrous connective tissue. Bouin-Heidenhain's haematoxylin and eosin*.

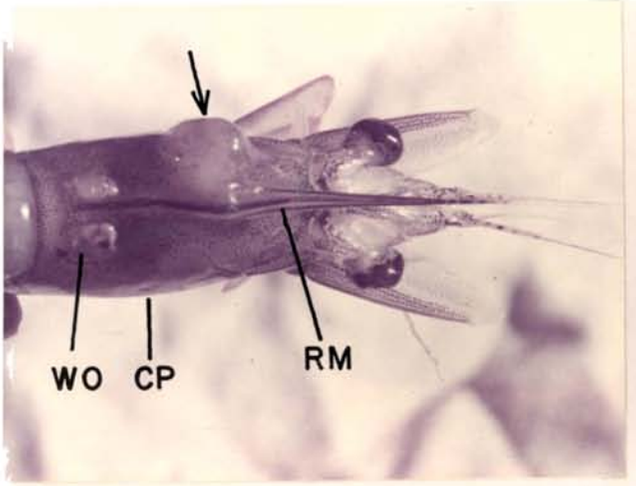
Fig. 4. Penaeus indicus: Amoeboid granular cells (AG) in the tumour - like out-growth. BD=Dark beaded bodies; CS=Collagenous stroma of fibrous connective tissue. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 5. Penaeus indicus: Transverse section of the peripheral part of tumour-like outgrowth showing intense infiltration of haemocytes (HC) at the periphery. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 6. Penaeus indicus: Transverse section of the peripheral part of hepatopancreas showing embedded muscle tissue (MM) at one focus. The connective tissue membrane of hepatopancreas (CT) is seen intending muscle tissue. Arrow shows the infiltrating part of membrane between the hepatopancreatic tubules. Bouin - Mallory's triple stain.

* Indicates fixative and the stain used. This pattern is followed for all the plates.

PLATE III



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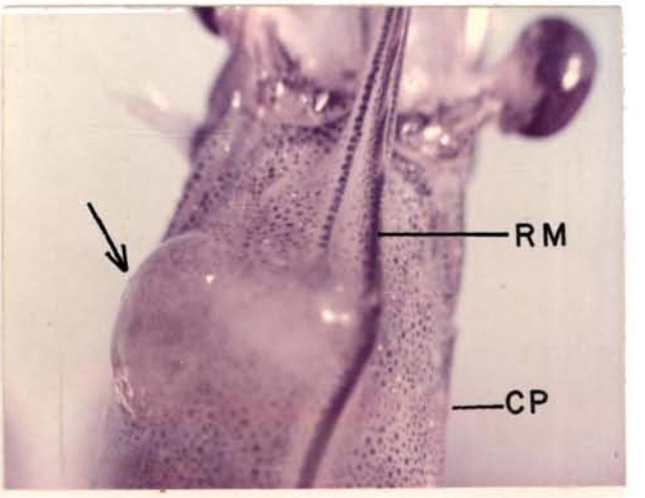

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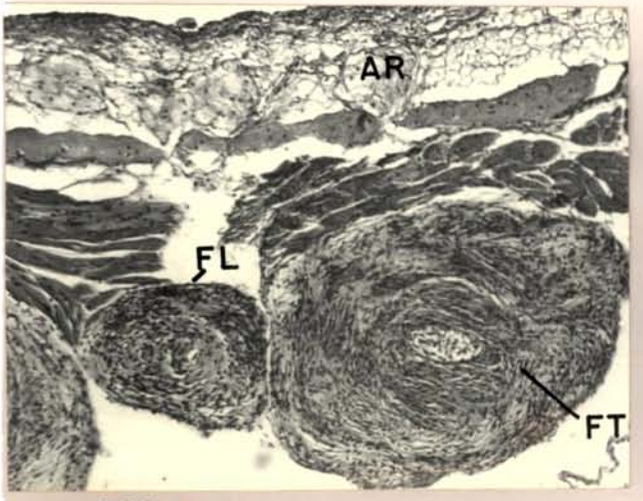

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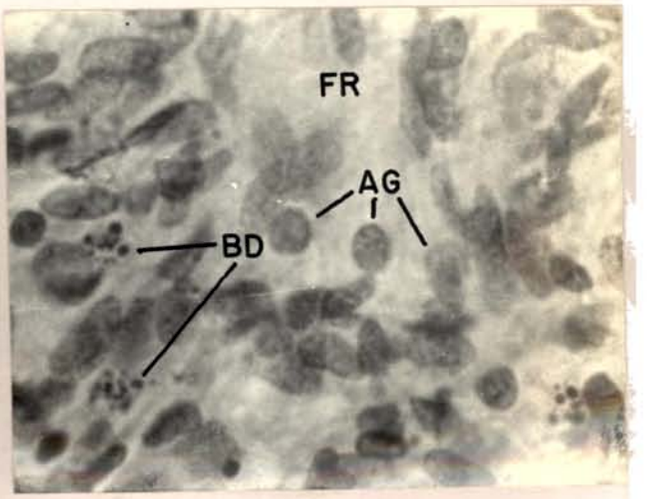

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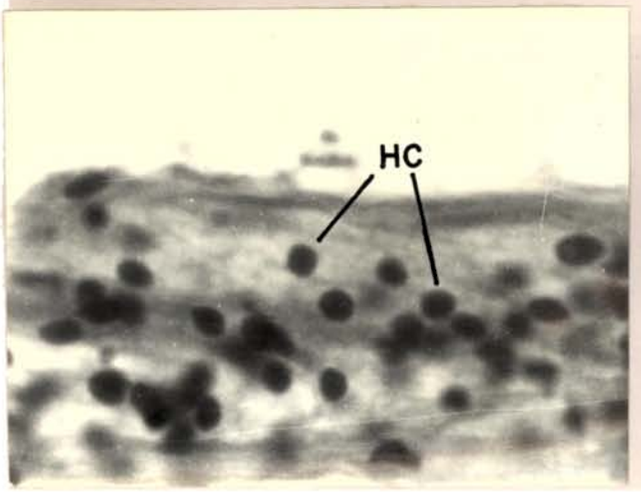

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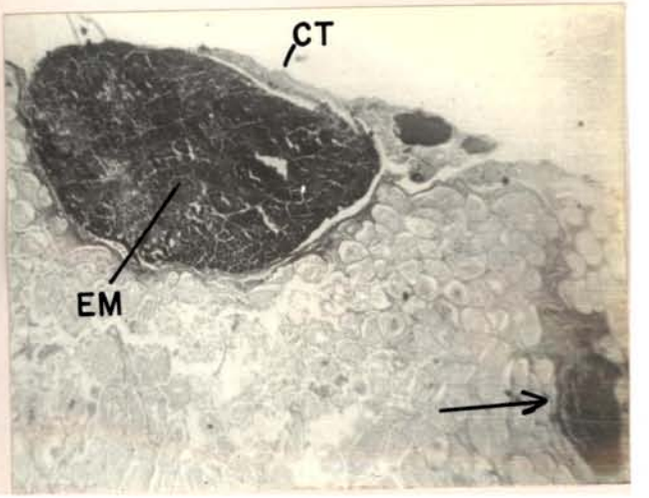

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location of the tumour, the proximal spines of the rostrum were slightly deviated towards the right side of the carapace (Pl. III, Fig. 2). The external surface of tumorous outgrowth was hard as it was covered by the cuticle.

Difference, except for relatively a few pigments, was observed in the colour of the cuticle covering the growth from the adjacent exoskeleton of the carapace (Pl. III, Figs. 1 and 2). Two small wounds were also found located on either side of the posterior region of the post-rostral carinae (Pl. III, Fig. 1).

The larger tumorous outgrowth measured approximately 14 X 7 X 7 mm and the smaller, 12 X 6 6 mm in size. Upon removing the cuticle covering the outgrowth, it was found to be covered by the epidermal and sub-epidermal layers continuous with the normal tissues of the adjacent region of the carapace. The swellings were sac-like structure, relatively firm in consistency. Both the tumorous outgrowths were close to each other and firmly anchored in the underlying muscles of the carapace.

Histological study revealed that the cuticle covering the tumorous growth was relatively thick. The epidermis appeared to be slightly hypertrophied. Below the epidermis at certain foci, areolar (loosely arranged) connective tissue was seen (Pl. III, Fig. 3). Below this, there was

extensive fibrous connective tissue (collagenous stroma) consisted of elongated fibroblast-like cells and interlaced collagen fibres, arranged often in swirling array. The collagen fibres which formed dense stroma of the tumour, were intensely stained pink with haematoxylin and eosin, and blue when stained with Mallory's triple stain. The elongated fibroblast-like cells were found to be highly basophilic when stained with Delafield's haematoxylin. There were also found a large number of amoeboid granular cells (Pl. III, Fig. 4) which were, however, comparatively less basophilic in nature. These cells appeared to be different from the haemocytes present in the tumour as well as in the other adjacent tissues due to their relatively larger size. Such cells were also not noticed in the normal muscle tissues examined from the adjacent muscle or abdominal region of the same prawn. In the collagenous stroma, at certain points, very small dark beaded bodies, about 2 to 5 in numbers, were seen originating from the amoeboid cells (Pl. III, Fig. 4). There was rather intense infiltration of haemocytes in the peripheral region of the tumour (Pl. III, Fig. 5). The loose connective tissue was again present below the fibrous, proliferative connective tissue, containing many scattered muscle fibres and tegumental glands. It was well vascularised and, at certain points, greatly expanded in volume.

Despite careful examination of several sections of the tumour, no parasite or foreign body was found in the tumour. Other organs of the prawn such as ovary, hepatopancreas, heart and abdominal muscle were also examined histologically in order to find out any structural abnormality. However, except the hepatopancreas, all other organs were found to be normal. In the hepatopancreas of the abnormal prawn, a mass of muscle tissue embedded at two foci on the either side of its periphery was seen. On a cursory examination, this muscle tissue was believed to be the surrounding muscle of the hepatopancreas normally present in the cephalothoracic region and would have come along with the organ while it was removed from the prawn and fixed in the fixative. However, after detailed and careful examination of several serial sections of the hepatopancreas of the abnormal prawn and their comparison with the sections of normal hepatopancreas from normal prawns, it was found that the presence of this muscle tissue partly embedded on the periphery of hepatopancreas was an unusual and abnormal feature (Pl. III, Fig. 6). This muscle tissue was constituted of striated muscle fibres. The connective tissue membrane bounding the hepatopancreas was found to indent at one point for a short distance between the hepatopancreatic tubules with an expanded distal portion (Pl. III, Fig. 6). It was at this region that a few fibroblasts and amoeboid granular cells, which were structurally similar to those observed in the tumour, were

noticed. The nucleus of these cells was darkly stained and contained peripheral inclusion bodies in the karyoplasm. Some cells were observed giving rise to small beaded dark bodies. Some of the tubules inside the hepatopancreas were empty and devoid of epithelial cells, probably due to the post-mortem autolytic changes.

Remarks: According to Couch (1978), there have been no invasive neoplasms reported for decapod crustaceans, but tumour-like growths have been reported in lobsters, crabs and a palaemonid shrimp. Recently, Natrajan et al. (1982) have reported a tumour in a specimen of fresh water prawn, Macrobrachium equidens. However, histological study of this abnormality was not given by these authors.

Thus far, the only published report on tumour-like growth in the penaeid prawns was that by Parks and Lightner (1973). These authors found a papilliform tumour-like growth on the right ventrolateral aspect of the sixth abdominal segment in P. aztecus collected from an experimental rearing pond at Palacios, Texas, U.S.A.. The growth was tentatively diagnosed as a benign neoplasm, consisting of grossly hypertrophied and normal tissue elements. In the present case, however, the epidermis was slightly hypertrophied and the extensive areas of fibrous connective tissue, found below the epidermis, did not resemble with that of normal connective tissue underneath the epidermis. The presence of the large

volume of loose connective tissue in the tumour was also unusual. The amoeboid cells found in the tumour along with the fibrous connective tissue appeared to be either abnormal cells or neoplastic cells, since their presence in the tumour was unique and they were not found in the normal muscle or connective tissues nor did they closely resemble the haemocytes.

Large volume of both fibrous and loosely arranged connective tissue present in the tumour indicates the probable origin of the tumour from the connective tissue. The tumour, therefore, described here is tentatively classified as "fibroma" and is tentatively identified as a "benign neoplasm" consisting of fibrous and areolar connective tissue, abnormal amoeboid cells and normal striated muscle fibres.

It may be noted that the present case is the second report on the occurrence of "tumour" or "tumour-like growth" in penaeid prawns, the first being that reported by Parks and Lightner (1973) and incidently, it forms the first record of tumour in penaeid prawns from India.

3.2 "SOFT" PRAWN SYNDROME

(Plate IV, Figs. 1 to 3)

Host: P. indicus ranging in size from 41 to 141 mm TL and P. monodon, 102 and 110 mm TL; juveniles and adults of both the sexes.

External symptoms: The cuticle of the affected prawns, except in the rostrum, becomes thin and fragile; body muscle looses its firmness and the prawns feel soft to touch. The gut in the abdominal portion of the prawns appears wavy, particularly in the first three abdominal segments. Affected prawns are sluggish, show leghargic movements and belated response to external stimuli.

Materials studied: Several specimens of P. indicus ranging in size from 41 to 141 mm TL and two specimens of P. monodon, 102 and 110 mm TL collected from the prawn culture ponds in Aymeen island, Cochin and grow-out ponds at Muthukad near Madras, belonging to the CMFRI, respectively.

Date of collection: 4th February, 1993, 17th May, 1993 and 8th and 10th June, 1993.

Incidence: Moderate to high.

Season: Pre-monsoon and monsoon (February to September).

Environmental information: S = 32.76 ppt; DO = 4.43 ppm; T = 32.5°C; pH = 8.2 - data collected on 10th June, 1993.

Enquiry has revealed that this disease syndrome is generally reported during adverse ecological conditions when low salinities (below 15 ppt) or combination of high salinities (32 ppt and above) and temperatures prevail in the pond water where the prawns are cultured.

Observations: During the period of present investigation, two incidences of "soft" prawn syndrome were encountered; one case was from one of the grow-out ponds of the M/S of [illegible] at Narakkal and the other, from the prawn culture field at Valappu, a fishing village in the Vypeen island. The observations reported here were based on these two cases of "soft" prawn phenomenon.

In the grow-out pond of the [illegible], the prawns were cultured in an earthern pond of 0.1 ha area, which was supplied with the tidal water from the adjoining canal. After eradication of undesirable organisms from the field, the pond was stocked with 5000 seeds (at a stocking density rate of 50,000 seeds/ha) of *P. indicus* produced in the hatchery on 21st March, 1983. The growth of the prawns stocked in the pond was regularly monitored. After 20 days of stocking, the prawns grew to an average size of 52 mm TL. The symptoms of "soft" condition in prawns were first noticed in the field on 7th June, 1983 when the environmental parameters of the pond were: S = 33.27 ppt; O_2 = 3.82 ppm; T = 33.1°C; pH = 7.9. In the subsequent

days of monitoring, the percentage of prawns in "soft" condition was seen progressively increasing. The samples of prawns were collected regularly from the field for detailed study.

At Valappu, the culture was carried-out in an earthern pond of 0.34 ha area which was supplied with the tidal water from the outer canal running adjacent to the field. The pond was stocked with _. indicus seeds produced at the MFCL at Narakkal at the rate of 50,000/ha on 20th April, 1983. Regular monitoring of the growth of parwns in the pond was undertaken and the incidence of "soft" prawns was reported in the pond on 7th June, 1983. As the percentage of the "soft" prawns was found to be relatively high in the samples, harvest of the prawns was carried out on the next day when only 2.5 kg. of healthy prawns as against the estimated production of about 150 kg, were caught from the field.

In the initial stage, it is hard to distinguish the "soft" prawns from the normal ones as they show similarities to the post-moult stage in having fragile exoskeleton and behavioural pattern. As the syndrome advances, they can be easily distinguished from the normal prawns by the characteristic thin and fragile nature of the exoskeleton of the body except the rostrum which alone remains rigid as in the intermoult stage prawns. Further, the body muscle looses the firmness and the prawn feels soft

to touch. In the more advanced stage, the "soft" prawns develop a wavy intestine, particularly in the anterior region of the abdomen, which is discernible in the affected live prawns through the semi-transparent exoskeleton. The gut in this region also appears slightly enlarged and dark greenish as compared to the unaffected prawns. Such prawns show sluggish movement and do not swim properly as in the case of normal prawns.

The samples of the "soft" P. indicus were analysed for biological and histological characteristics. The biological observations included the size, weight and gut content analysis. The size range of the "soft" P. indicus collected from the grow-out pond at NIOT, Narakkal was from 92 to 141 mm TL in females and 75 to 138 mm TL in males. The weight of the "soft" prawn measuring 109 mm TL was 4.92 g and that measuring 138 mm TL was 6.96 g. The gut content analysis of the "soft" prawns showed that the gut was always filled with ingested food material and no case of empty or half-filled gut was found. The gut content was principally composed of detritus and blue-green algae. The hepatopancreas of "soft" prawns appeared to be comparatively smaller in size than that of the normal prawns of similar size group.

The histological observations were made on the hepatopancreas and midgut. Haemolymph smears prepared from

the "soft" prawns were stained with dilute Giemsa stain and examined microscopically for the possible presence of any haemolymph parasite. None of the 20 smears examined revealed the presence of any parasite in the haemolymph. Histological examination of five samples of hepatopancreas from affected prawns revealed that the tubules of the central part of the hepatopancreas were devoid of epithelial cell lining and appeared almost empty. Only a thin basophilic membrane binding the individual tubules was present (Pl. IV, Fig. 1). This membrane was straight and plain in certain cases, whereas in others, the membrane was wavy and striated with transverse bands of connective tissue throughout the length of the tubules. There was light to moderate infiltration of haemocytes in the tubule. The empty tubules were filled with fine particles which were intensely stained with eosin. Occasionally, a circular eosinophilic nodular body was also present in the lumen of such tubules (Pl. IV, Fig. 2). Although, the epithelial cells were found in the tubules of the peripheral region of hepatopancreas, the secretory and absorptive vacuoles in these cells were either very small and less in number or totally absent. This observation indicates the possibility that proper storage of nutritional reserves may not be taking place in the hepatopancreas of "soft" prawns. In the region of the hepatopancreas beneath the gut, there was a dense accumulation of crumbled connective tissue debris (Pl. IV, Fig. 3).

The histological structure of the wall of the midgut of "soft" prawns was similar to that of the normal prawns. Algae and detritus were present in the lumen of the gut as the consumed food items. No parasite or foreign body was observed in any of the histological sections of the hepatopancreas and midgut.

Light infestation on the gills of "soft" prawns by filamentous bacteria, Leucothrix sp. and a peritrich ciliate, Zoothamnium sp. was frequently observed in many of the specimens but their infestation did not seem to be apparently correlated with the severity of the "soft" prawn disease syndrome.

The moisture content of the body of "soft" prawns was found to be 82 to 83 percent of the body weight (7 observations) whereas in the normal prawns, the moisture content ranged between 71.4 and 74.6 percent of the total body weight. "Soft" prawns were thus found to contain about 10 percent higher moisture content than that of the normal prawns.

Remarks: The occurrence of "soft" prawns in the culture fields is a seasonal phenomenon found generally during the pre-monsoon and monsoon seasons in the Vypeen islan . It occurs mostly in juvenile prawns measuring above 40 mm and during adverse ecological conditions of low salinities or combination of higher salinity and

temperature. Further, the "soft" prawn phenomenon does not occur simultaneously in all the ponds of a particular locality. It may be that the prawns stocked in one pond of a locality may show symptoms of softening and subsequently leading to mortality of the stocked population within 5 to 6 days, whereas prawns stocked in other adjoining ponds of the same locality may not develop any symptom of the syndrome and are found to grow in the normal pattern. Although frequently reported in the prawn culture operations in the Vypeen island during the summer and monsoon seasons, reliable estimation of the production loss due to this phenomenon is not available at present.

The etiology of "soft" prawn syndrome is not known at present. Similarly, the pathogenesis of this phenomenon is also not clearly understood. Biochemical studies on "soft" prawns was carried out by Rajamani (1992), who observed that non-protein nitrogen (NPN) was relatively higher in "soft" prawns as compared to that of healthy prawns. This indicated that the increase in content might be due to endogenous protein metabolism caused by changes in the ecosystem during the period of adverse ecological conditions.

PLATE IV

Fig. 1. Penaeus indicus: Transverse section of the hepatopancreas of a "soft" prawn. Tubules in the central part of hepatopancreas(left) lack the epithelial cell lining and appear empty, however, the thin membrane(TM), binding the individual tubule persists. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 2. Penaeus indicus: Transverse section of the central part of hepatopancreas of "soft" prawn to show the rounded, eosinophilic nodular bodies(arrows) in the lumen of the tubules(LT). Bouin-Heidenhain's haematoxylin and eosin.

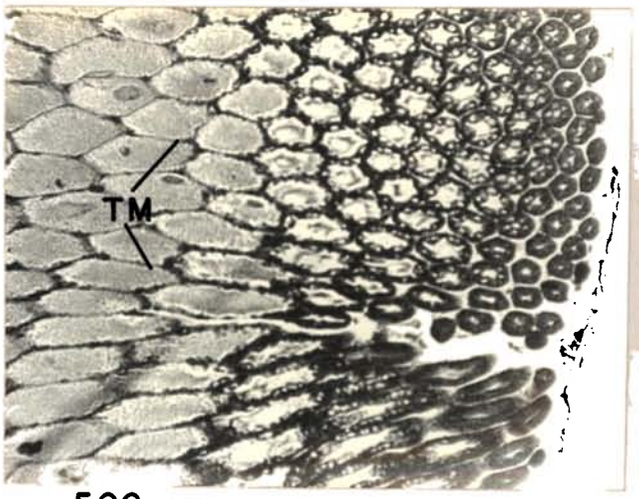
Fig. 3. Penaeus indicus: Transverse section of the hepatopancreas of "soft" prawn showing crumbled connective tissue debris(arrows) below the passage of the midgut. MW-Wall of midgut. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 4. Penaeus indicus with tail necrosis. Note the muscular degeneration associated with necrotic condition in the region of sixth abdominal segment, telson and uropods (arrow).

Fig. 5. Penaeus indicus: Brown spot disease on the exoskeleton of abdomen(arrows) in a pond reared female(above) and a male(below).

Fig. 6. Penaeus indicus showing the red rostrum syndrome.

PLATE IV



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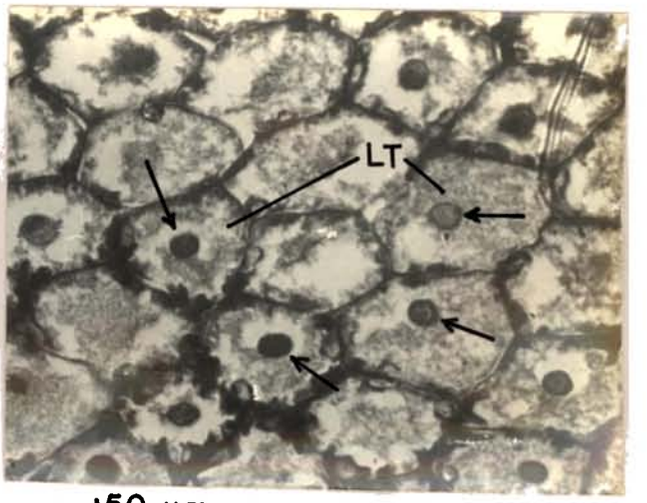

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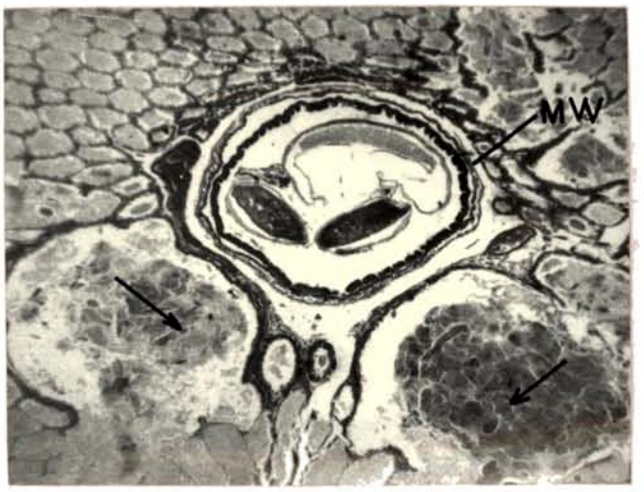

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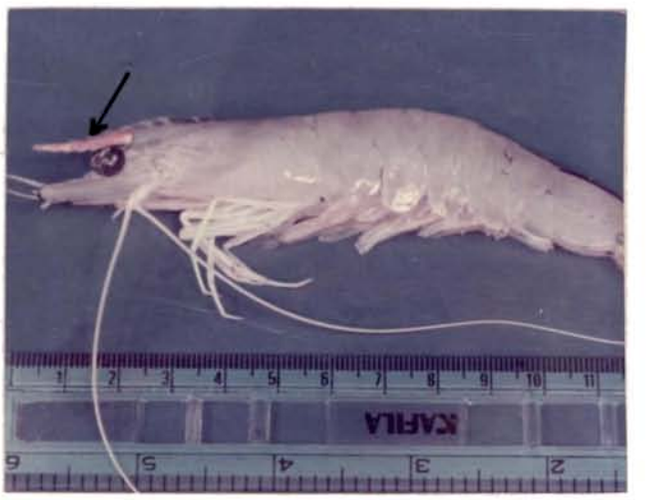

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3.3 TAIL NECROSIS

(Plate IV, Fig.4)

Host: P. indicus of both the sexes ranging in size from 123 to 147 mm TL.

External symptoms: Colour of the sixth abdominal segment, telson and uropods changes from translucent to opaque white and finally pinkish-brown. The affected parts become necrotic and gradually degenerate (Pl. IV, Fig. 4).

Material studied: 3 specimens.

Date of collection: 9th May, 1983.

Incidence: Rare.

Season: Summer (March to May).

Environmental information: Water parameters of the pond - S = 29.14 ppt; DO = 3.46 ppm; T = 29.5°C; pH = 7.4; Water parameters of the tank (initially) - S = 15.35 ppt; DO = 2.11; T = 32.4°C; pH = 8.2; Water parameters of the tank (after changing the water) - S = 25.21 ppt; DO = 4.19 ppm; T = 30.0°C; pH = 8.1.

Observations: The tail necrosis condition was observed when the prawns appearing normal were subjected to different rearing medium. 13 specimens of normal live, adult P. indicus were collected from a stocking pond in Vypeen

island for some physiological studies and were held in a plastic bin containing about 25 l of brackishwater (S = 29.14 ppt; DO = 3.46 ppm; T = 29.5°C;pH = 7.4) collected from the same pond from where the prawns were caught. These live prawns were transported in a van to the laboratory where they were transferred to a circular plastic pool of 300 l capacity filled with a mixture of sea water collected off Cochin and fresh tap water (S = 15.35 ppt; DO = 2.11 ppm; T = 32.4°C; pH = 8.2). The condition of tail necrosis was noticed on the next day. Out of the 13 prawns, only 3 prawns developed white necrotic lesions in the last abdominal segment, telson and uropods. After about 9 hours, these lesions turned pinkish-brown. These prawns were then transferred to another circular plastic pool of 300 l capacity containing fresh, aerated brackishwater (S = 25.21 ppt; DO = 4.19 ppm; T = 30.0°C; pH = 8.1). After 6 hours of transferring the prawns to the tank, the affected parts became totally brown. One of the prawns was removed from the tank and sacrificed for detailed microscopic examination. The other 2 prawns survived in the tank for four days.

The opaque and white appearance of the last abdominal segment, when the disease was in progress, was first confused with the microsporidiosis which produces similar symptoms. However, microscopic examination of the wet mounts and impression smears from the affected parts revealed extensive degeneration of the muscle fibres and complete absence of microsporidian or any other parasite.

The necrotic condition in these prawns, during their four days of survival, was found to confine to the last abdominal segment and the tail fan (telson and uropods). The other abdominal segments as well as the carapace of the prawns were found to be normal. However, the prawns were unable to swim in the normal manner probably due to necrosis in the tail fan.

Remarks: Necrosis is a term given to a condition in penaeid prawns, which is characterised by whitish opaque areas in the striated musculature, especially in the distal abdominal segments. With the advancement of the necrotic condition, the muscles are found to turn brown and begin to degenerate.

The condition, similar to that observed in the present case, had also been reported from P. aztecus and P. californiensis in U.S.A. (Lakshmi et al., 1978; Lightner, 1977), and Palaemon serratus in U.K. (Delves-Broughton and Poupard, 1976). The condition follows periods of severe stress resulting from overcrowding, low dissolved oxygen levels, sudden salinity or temperature changes or rough handling (Lightner, 1983). It usually begins from the tip of the tail (Overstreet, 1978). Such prawns are reported to recover in the initial stage of the tail necrosis if stress factors are reduced, but it may be lethal if large areas are affected (Lightner, 1983). Overstreet (1978) pointed out that once the tip of the tail acquires a

totally chalky appearance, the shrimp usually dies.

In the present case, affected prawns did not recover from the tail necrosis condition despite improving the water quality by increasing the salinity, dissolved oxygen level through aeration and lowering of temperature of the water. While the exact factor of the stress which brought about this condition could not be ascertained, it seemed that sudden change in the water temperature, salinity or dissolved oxygen level were responsible for the tail necrosis since initially there was a large variation in the water quality of the tank (S = 15.35 ppt; DO = 2.11 ppm; T = 32.4°C; pH = 8.2) as compared to that of the pond from where the prawns were collected (S = 29.14 ppt; DO = 3.46 ppm; T = 29.5°C; pH = 7.4). Couch (1970) opined that this syndrome might be related to oxygen starvation of muscle tissue when the shrimp is pressed to its physiological tolerance limits for high or low temperatures or hyperkinetic muscular activity. Lakshmi et al. (1973), in their experimental study on the effect of salinity and temperature changes on spontaneous muscle necrosis in P. setiferus, found that no pathogen was involved for this condition and indicated that both salinity and temperature change had an impact on necrosis; the incidence of necrosis and subsequent mortality of necrotic shrimp appeared to be directly related to the magnitude of the changes.

The tail necrosis condition may serve as a good indicator to assess the general fitness of prawns prior to stocking in nursery or grow-out ponds.

3.4 BROWN SPOT DISEASE

(Plate IV, Fig. 5)

Host: P. indicus, juveniles and adults of both the sexes ranging in size from 47 to 163 mm TL.

External symptoms: Dark reddish brown, eroded lesions of varying shapes and sizes usually on the exoskeleton of the abdomen (Pl. IV, Fig. 5), pleopods, pereopods and uropods; occasionally, lesions are also found on the carapace.

Material studied: Several specimens of prawns caught off Cochin and landed at the Cochin Fishing Harbour; prawn culture ponds in Vypeen island, Cochin; prawns held in rearing tanks in the laboratory of CMFRI, Cochin.

Dates of collection: Many occasions during October-November, 1982 and January to September, 1983.

Incidence: Low.

Season: Throughout the year.

Environmental information: The disease is found to occur in water having a wide range of salinity (5 ppt to 32 ppt),

dissolved oxygen (1.98 ppm to 4.32 ppm), temperature (28.6°C to 31.3°C) and pH (7.6 to 8.3).

Observations: The melanised cuticular lesions were seen mostly on the pleural plates of abdomen and sometimes on the surface of the appendages. These lesions did not have a definite shape or size. Generally, only one or two lesions were observed, occasionally up to five lesions were noted, however, their distribution was multifocal.

When examined microscopically, the cuticle in the affected parts was damaged and eroded, yet the underlying soft tissue was unaffected by necrotic cuticular brown spots. Necrosis and melanisation were quite conspicuous in the cuticle. Often, very minute spherical and coma-shaped free bacteria, probably chitinoclastic Vibrio spp. (Delves-Broughton and Poupard, 1976), were noted in the damaged cuticle. Fungal hyphae were not found in any of the lesions.

The disease appeared to be non-infectious as the prawns having brown spots, when kept along with apparently normal prawns for 24 days, the latter did not develop similar lesions on their body. The brown spots were usually lost after the affected prawns moulted.

Remarks: Brown spot disease, also known as "shell disease", "burned spot disease" or 'rust disease' is of geographical

occurrence in marine waters of the world and found in both natural as well as cultured populations of shrimps and many other crustaceans (Lightner, 1977). Rosen (1970) has extensively reviewed the shell diseases of decopod crustaceans.

A variety of causes such as bacterial species which produce extracellular lipases, proteases and chitinases; fungi; nitrogenous waste products; nutritional and developmental abnormalities, which result in damage to the epicuticular layer, have been suggested for brown spot disease (Brock, 1983).

The brown spot disease observed in the present case appears to have resulted from mechanical injury rather than primary bacterial disease. Damage to the exoskeleton may result from cannibalism, injuries during ecdysis or imperfect ecdysis, aggressiveness and excessive handling. High stocking density in culture system and rearing tanks often enhance the chances of mechanical injury. Bacterial accumulation in the melanised lesions would have occurred after the epicuticle disrupted due to damage, thus the bacteria observed in the lesions are not primary causative agents of brown spot disease. Cipriani et al. (1980) were unable to experimentally produce brown spot disease in penaeids with bacterial species isolated from clinical cases of the disease until isolation was preceded by

mechanical disruption of the epicuticle layer. The epicuticle, which is considered to be the primary line of defense in crustacean exoskeleton (Johnson, 1980), is always involved in the brown spot disease. Epicuticle contains polyphenolic sustances and is mostly inert to microbial attack. When this is subjected to a mechanical erosion, the underlying chitin is exposed to degradation by chitinoclastic bacteria (Welves-Broughton and Poupard, 1976). Lakshmanaperumalsamy et al. (1982) isolated species of Gram-negative bacteria, Vibrio and Aeromonas, from the blackened lesions of brown spot disease in P. indicus caught from Cochin backwaters. Chitinoclastic bacteria are ubiquitous in the natural environment and form a normal part of the microbial flora of both living and dead crustaceans (Lightner, 1977).

The dark brown colouration observed in the affected parts of the exoskeleton is due to melanin formation which, as Brock (1983) discussed, is the result of a host response to injury and does not by itself suggest a particular etiology.

Although brown spot disease was not found in large number of prawns from a particular locality and the disease itself did not seem to be fatal, on the individual level it may sometimes be harmful in indirect ways. The progressive destruction of exoskeleton may permit loss of haemolymph (Couch, 1978) and eventual death of the prawn due to

secondary pathogens such as the fungus Fusarium sp. (Lightner et al., 1979a), osmotic imbalances or difficulty in moulting due to adhesion between old and new cuticles (Lightner, 1977).

3.5 RED ROSTRUM

(Plate IV, Fig. 6)

Host: P. indicus of both the sexes ranging in size from 117 to 153 mm TL.

External symptoms: Reddish discolouration of the rostrum (Pl. IV, Fig. 6).

Material studied: Seven specimens collected from culture ponds in Vypeen island, Cochin.

Date of collection: 24th August, 1983.

Incidence: Rare.

Season: Post-monsoon (August-September).

Environmental information: S = 23.36 ppt; DO = 3.79 ppm; T = 29.2°C; pH = 7.8.

Observations and remarks: Prawns with red rostrum were encountered only rarely in the culture ponds during the post-monsoon period. During the collection of such specimens, it was interesting to note that the diatom,

Peridinium sp. was found abundant in the pond water.

In this condition, the rostrum acquired a reddish appearance, remained hard and did not loose its rigidity. The condition did not seem to have any significant impact on the prawns as they showed normal behaviour. Etiology of the red rostrum condition is unknown.

A similar condition of red rostrum condition had been reported earlier in P. indicus from Cochin by Mahadevan et al. (1978). These authors isolated the bacterium, Pseudomonas fluorescence from the affected prawns and named the syndrome as "haemorrhagic septicaemia".

3.6 CILIATE INFESTATION

(Plate V, Figs. 1 to 3)

Host: P. indicus (47 to 112 mm TL), P. monodon, (93 to 149 mm TL), P. semisulcatus (88 to 134 mm TL), Metapenaeus affinis (51 to 78 mm TL) and M. dobsoni (39 to 71 mm TL), juveniles and adults of both the sexes.

External symptoms: Prawns with heavy infestation possessed a fuzzy appearance on the surface of the gills, appendages and occasionally the carapace.

Material studied: Several specimens collected from prawn culture ponds and paddy fields in Vypeen island. Sometimes also

encountered in the wild population caught off the coasts of Cochin and Mandapam.

Date of collection: 4th February, 19th March, 17th May, 8th and 10th June, 24th August and 5th November in 1983.

Incidence: Low to moderate in cultured populations and rare in the individuals of wild population.

Season: Moderate during pre-monsoon (April to June) and rare during the other months of the year.

Environmental information: S = 31.2 ppt; DO = 2.36 ppm; T = 30.0°C; pH = 8.3 - data collected on 8th June, 1983.

Observations: Microscopic examination of the wet mounts of fresh gill lamellae of infested prawns revealed large number of dichotomously branching contractile colonies of peritrich ciliate attached to the surface of the thin gill cuticle (Pl. V, Fig. 1). Ciliate was most abundant on the bifurcated tips of each gill filament. Several trophonts of inverted bell shape with contractile stalk were found in each colony. rophonts were 40 to 45 µm X 20 to 35 µm in size and the diameter of stalk was 10 to 12 µm. A central contractile fibril or myoneme was always present inside the stalk. The myoneme was continuous throughout the branched stalk (Pl. V, Fig. 1). Each trophont - the adult feeding stage of attached peritrich - possessed

adoral ciliature, one or more vacuoles and a large horse-shoe shaped macronucleus located near the centre (Pl. V, Figs. 2 and 3). The basal part of the stalk attached to the gill cuticle was apparently a superficial attachment and there was no mechanical damage to the cuticle or underlying tissues. Host haemocytic response to the infestation was totally absent. Colonies of the peritrichous ciliate were less numerous on the body appendages of the prawn and only occasionally they were found on the carapace.

Usually infested prawns were in live condition and low infestation did not seem to be fatal to the them. However, one incidence of mortality of large number of stocked P. indicus from the prawn culture fields at Valappu in Vypeen island, Cochin was reported on 8th June, 1983. The live, moribund and dead prawns collected from the field were found to be heavily infested with this peritrichous ciliate. The gills of the infested prawns were observed to be the most favourable site of attachment. Besides, filamentous bacteria were also found offen accompanying these ciliates. These filamentous bacteria were non-branching and were seen attached singly to the gill cuticle superficially. They measured an average diameter of 2 µm and consisted of septate chains of almost square shaped bacteria. The water in the ponds and adjacent canal (S = 31.2 ppt; DO = 2.36 ppm; T = 30.8°C;

PLATE V

Fig. 1. The contractile colonies of peritrich ciliate, Zoothamnium sp. attached to the surface of the gill cuticle of an adult Penaeus indicus. This is a light infestation. Note the stalk (SK) with myoneme (MY) and apparently undamaged gill cuticle (GC). Wet mount.

Fig. 2. Penaeus indicus: Prophonts of Zoothamnium sp. with horse-shoe-shaped macronucleus(N). Wet mount.

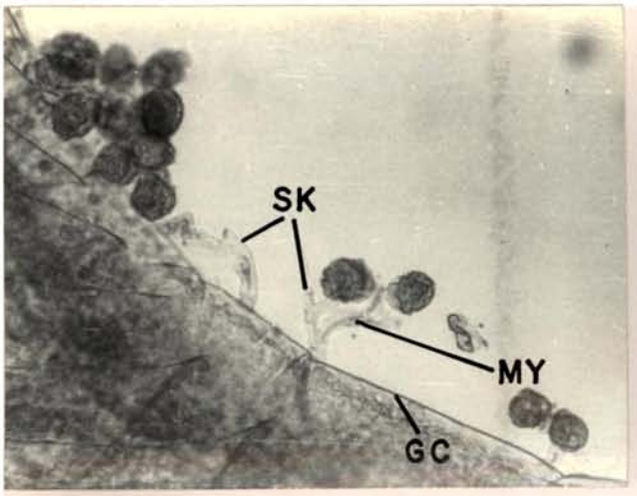
Fig. 3. Same, enlarged. X 320

Fig. 4. Penaeus semisulcatus: Microsporidian infected (above) and normal (below).

Fig. 5. Deheaded Penaeus semisulcatus: (A) normal prawn; (B) microsporidian infected prawn with opaque white, cotton-like appearance.

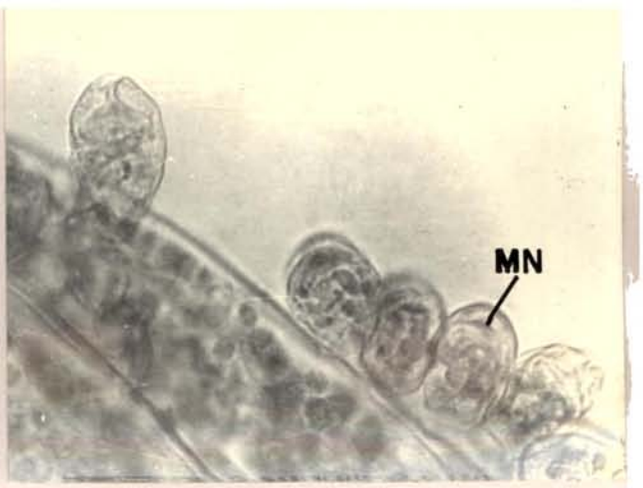
Fig. 6. Female Penaeus semisulcatus cut across the abdomen to show the opaque white discolouration of ovary (OV) due to microsporidian infection. Note the apparently normal abdominal muscle(arrows)

PLATE V



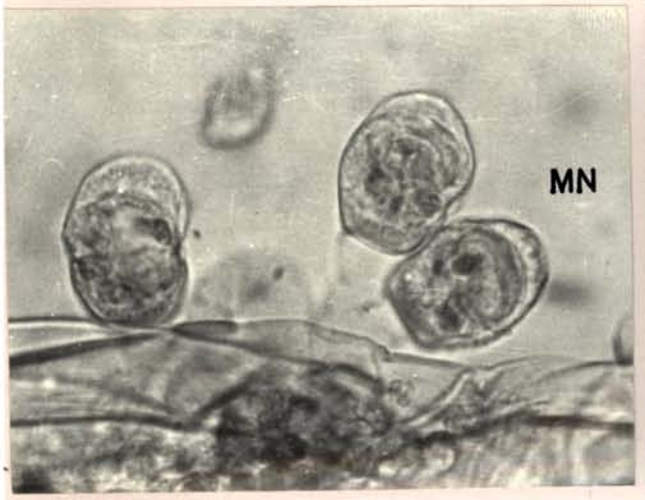
150 µm

1



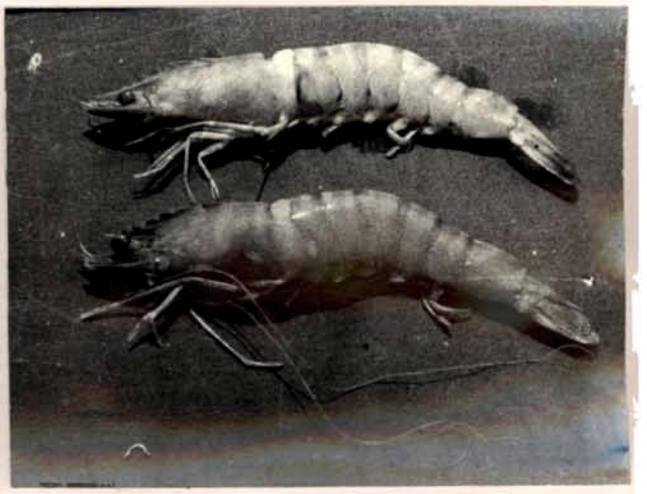
50 µm

2



40 µm

3



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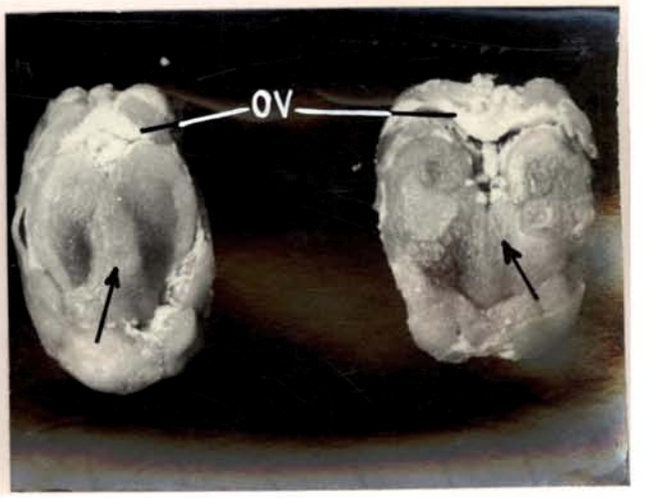


20 mm

6

pH 8.3) was found to be polluted and was abundant with atleast three types of blue-green alagae, one of which was identified as Anabenaeopsis sp.

In other instance, where sudden outbreak of "soft" prawn syndrome in stocked sub-adult P. indicus and P. monodon was observed in a rearing pond at NPCL of CMFRI on June 10, 1983, gills of both dead and moribund "soft" prawns were found to be infested from light to moderate levels with the same peritrichous ciliate, some specimens being accompanied with filamentous bacteria.

Remarks: Stalked pritrichs of Genera Zoothamnium, Epistylis and Vorticella have been reported infesting several penaeid prawns (Overstreet, 1973; Johnson, 1972, 1974a; Lightner, 1975, 1977, 1978a; Couch, 1978; Issac Rajendran et al., 1982; Santhakumari and Gopalan, 1980). The peritrichous ciliate observed in the present case apparently belongs to the Genus Zoothamnium since it possesses a characteristic continuous myoneme that connects stalks of each trophont within the colony so that the colony may contract as an unit. The Genus Epistylis lacks a contractile stalk. Although the Genus Vorticella is often colonial and possesses a contractile stalk, it does not have continuous myoneme connecting individual members of the colony, hence the Vorticella colonies do not contract as a unit as does the species belonging to Zoothamnium (Lightner, 1978a.

Zoothamnium sp. is a free living ciliate (epicommensal) and not a true pathogen (Lightner, 1978a. In a healthy environment, penaeids can tolerate a large number of these epicommensals with no apparent detrimental effect (Foster et al. 1978). However, heavy infestation of Zoothamnium sp. on gills of prawns stocked at high density in ponds many result in mass mortalities (Johnson et al., 1973; Overstreet, 1973). Overstreet (1973) found that an increase in the density of hosts held in captivity was paralleled by an increase in density of peritrichs on gills. Death occurs when the effective respiratory surface of the gills is reduced by presence of numerous colonies of Zoothamnium sp. and subsequently, the suffocation of the animals (Lightner, 1975). Death usually coincides with periods of low concentration of dissolved oxygen in the water, a common condition following several warm overcast days or following decomposition of large algal blooms (Overstreet, 1973). In the present observations, the dissolved oxygen level in the culture pond at Valappu, where the mortality occurred, was as low as 2.36 ppm and the gills of the affected prawns were found to be heavily infested with Zoothamnium sp. Johnson et al. (1973) reported the loss of an estimated 2000 pond-held brown and white shrimp in a single day due to the presence of large numbers of Zoothamnium sp. on the gills and to a reduction in dissolved oxygen level. Mortality was attributed to anoxia as the mortalities occurred when the infestation of ciliate became

heavy enough to restrict oxygen exchange and when the dissolved oxygen level in the ponds dropped below 3 ppm to a low level of 2.6 ppm. These authors noted that in the ponds where no Zoothamnium sp. infestation was observed on the shrimp, no mortalities occurred despite the low dissolved oxygen level. Lightner (1975) pointed out that in normal conditions, when Zoothamnium sp. was absent, dissolved oxygen level of 2.6 ppm was not lethal as good survival was experienced with P. aztecus in culture ponds even when the dissolved oxygen level fell to 1.0 ppm.

Issac Rajendran et al. (1982) noticed infestation of Zoothamnium sp. and Epistylis sp. in P. monodon reared in a pond at Madras which was not flushed with tidal waters for 20 days resulting in depletion of dissolved oxygen level to a lowest value of 1.0 ppm. They, however, found that the infestation could be controlled by improving water quality by frequent exchange of fresh tidal water over a period of 7 to 15 days.

3.7 MICROSPORIDIOSIS

(Plate V, Figs. 4 to 6)

Host: P. semisulcatus (65 to 168 mm TL) and M. affinis (97 to 143 mm TL) of both the sexes.

External symptoms: Opaque white discolouration of the entire body muscle giving a cotton-like appearance to the animal (Pl. V, Figs. 4 and 5); sometimes, in the female prawns, only medial dorsal line of the body turns opaque and white (Pl. V, Fig. 6).

Material studied: Several specimens of prawns collected from the commercial catches of the Gulf of Mannar and Palk Bay and landed at Mandapam, Rameswaram, Tuticorin and nearby fish landing centres.

Date of collection: A number of collections were made during September-October in 1982 and during March, June-July and November-December in 1983.

Incidence: Low and continuous.

Season: Throughout the year.

Environmental information: Data collected from inshore sea - S = 29 ppt to 34 ppt; DO = 3.64 to 4.37 ppm; T = 28.3 to 31.4°C; pH = 7.7 to 8.1.

Observations and remarks: Opaque and white prawns with cotton-like appearance, and sometimes only the dorsal medial line turning white, were encountered in the commercial catches landed at the fish landing centres at Mandapam, Rameswaram, Tuticorin and surrounding localities during different seasons of the year. The affected prawns were easily distinguishable from the normal prawns due to the apparent symptoms (Pl.V,

Figs. 4 and 5). On dissecting the animal, the abdominal muscle and gonad (ovary or testes) were found to turn opaque and white whereas the midgut showed white patches of infection. Occasionally, female prawns with only the ovary turning opaque and white, which ould be easily seen through the medial dorsal line of carapace and abdomen, were also seen (Pl. V, Fig. 6).

When smears from such infected prawns were prepared and observed under a compound microscope, there were large numbers of oval shaped spores ranging in size from 2.0 to 5.5 µm in length and 1.0 to 3.5 µm in width. In some cases, the spores were also seen in the group of eight. The number of spores were very high in the smears. Further study revealed these spores to be the microsporidian parasites The variations in the size, shape and structure of these spores pointed out that they belonged to three different species of Microsporidia producing more or less similar symptoms in their penaeid hosts. A detailed study undertaken on the microsporidiosis is dealt with in the Chapter 4.

3.8 HELMINTH PARASITISATION

(Plate VI, Figs. 1 to 6)

Host: P. semisulcatus, female; 156 mm and 169 mm TL.

External symptoms: No noteworthy external symptoms observed.

Material studied: Two specimens, caught off the coast of Mandapam in the Palk Bay at a depth of about 30 meters.

Date of collection: 30th November, 1983.

Incidence: Rare.

Season: Not clearly demarcated.

Environmental information: Data not available.

Observations: During the course of routine examination of histological sections of hepatopancreas of apparently normal P. semisulcatus collected from off the coast of Mandapam camp, a helminth parasite was observed in large numbers. No external symptoms of parasitisation were visible, but on dissecting the prawns, however, the hepatopancreas appeared to be relatively larger in size as compared to the hepatopancreas of other prawns of similar size group.

Following the detection of the parasites in the sections of the hepatopancreas, the midgut of these prawns preserved in Bouin's fixative were dissected and the contents of the midgut were examined in wet mount preparation when a few slender, unsegmented worms, ranging in size from 0.35 mm to 2.2 mm were encountered (Pl. VI, Fig. 1). In the hepatopancreas, the number of parasites varied from 4 to 13 and they were usually seen in between the tubules of the

middle part of the organ (Pl. VI, Figs. 2 and 3). The parasites, as those observed in the gut content, were slender and unsegmented, but their size varied from 530 µm to 900 µm in length and 150 µm to 230 µm in width in the transverse sections. Each worm possessed a thick cuticular body wall with minute cilia all over its exterior surface. Body of the worm was filled with oval shaped mesenchymal cells and a few muscle fibres arranged longitudinally in the middle. In certain cases, the body of the parasite was seen divided into two parts, the posterior part filled with mesenchymal cells and muscle fibres, and the anterior part with several rounded fibres, probably the coiled hooks. Digestive tract was totally absent. Subcuticular cells were present in large numbers on the periphery just beneath the cuticle. The cuticular wall although formed several collar-like folds in the middle and posterior parts, internally the body was unsegmented. Occasionally, some worms possessed strobila consisting of 4 to 5 dorsoventrally flattened proglottids, each with a small reproductive organ.

The effect of the parasitisation on the structure of the hepatopancreas, and the host response to the presence of these parasites were quite conspicuous in the histopathological alterations in the hepatopancreas. The gut, which passes through the hepatopancreas in normal cases, was not visible in the sections, and instead, large amount of

PLATE VI

Fig. 1. Penaeus semisulcatus: Slender unsegmented helminth parasite from the midgut. Wet mount.

Figs. 2-3 Penaeus semisulcatus: Transverse section of hepatopancreas with helminth parasites (arrows) located in between the hepatopancreatic tubules (HT). Bouin-Mallory's triple stain.

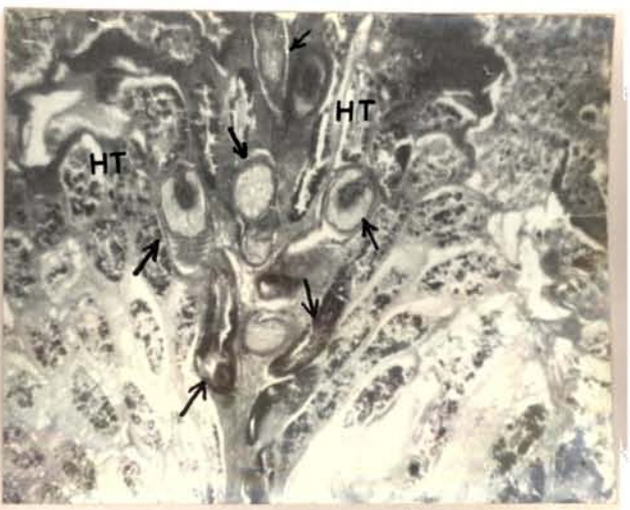
Fig. 4. Penaeus semisulcatus: Transverse section of hepatopancreas showing the heavy deposition of collagenous fibres (CF) due to helminth parasitisation; except the lumen (HL), no recognisable part of the gut is seen. Partly and totally damaged tubules (arrows) are also visible. Bouin-Mallory's triple stain.

Fig. 5. Penaeus semisulcatus: A higher magnification view of the parasite in the transverse section of hepatopancreas. The hepatopancreatic tubules (HT) are seen involved in the cyst wall formation (arrows) around the parasite. Bouin-Mallory's triple stain. X 32.

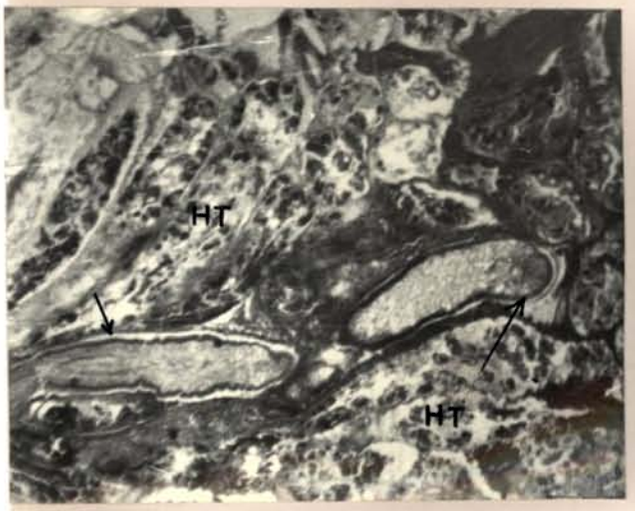
Fig. 6. Penaeus semisulcatus: Host response to helminth parasitisation in the hepatopancreas by means of encapsulation. Arrows show the cyst which has enclosed the parasite by means of encapsulation. HT=Hepatopancreatic tubules. Bouin-Harris hematoxylin and eosin.

PLATE VI

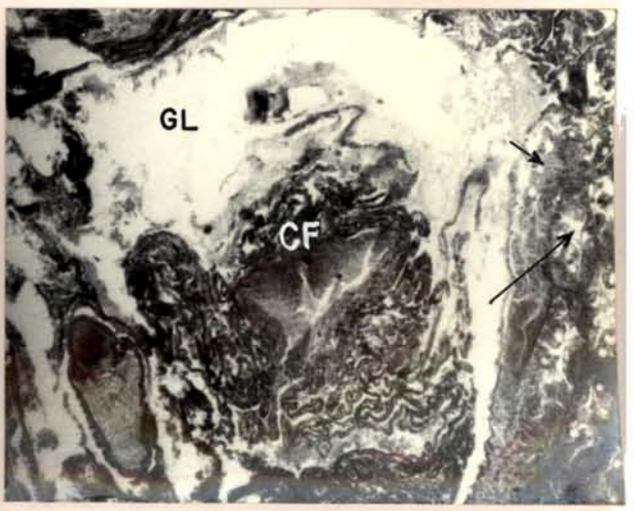
700 µm 1



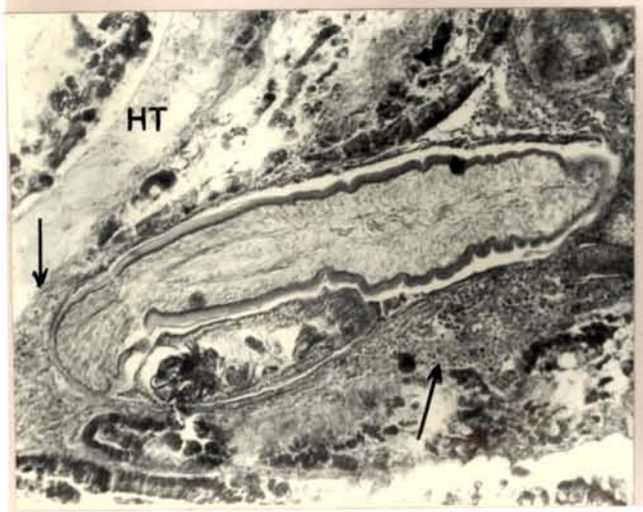
700 µm 2



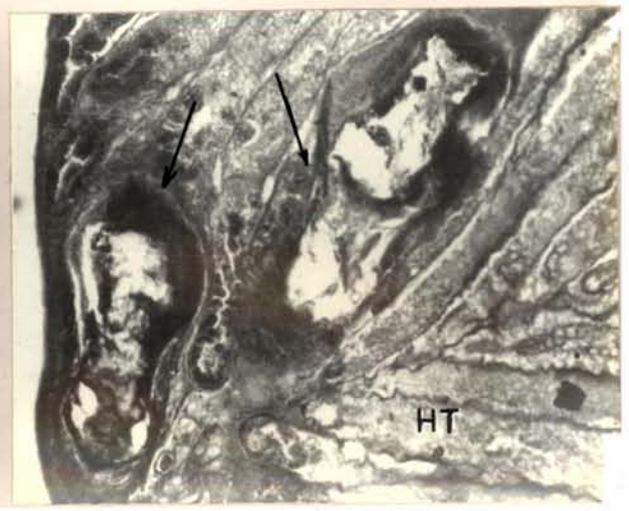
500 µm 3



500 µm 4



200 µm 5



200 µm 6

collagenous fibre deposition was seen at the original location of the gut (Pl. VI, Fig. 4). The gut wall and lumen were not discernible. Hepatopancreatic tubules in the vicinity of the parasite were crowded, reduced in size, intensely basophilic on staining than the normal tubules and were frequently incorporated into the cyst wall where they were partly or totally damaged (Pl. VI, Figs. 4 and 5).

There was considerable host response to the parasite which was encapsulated by the host tissue to varying degrees. The range of host response in the hepatopancreas was from a thin encapsulating cyst (Pl. VI, Fig. 5) to complete destruction of the parasite (Pl. VI, Fig. 6). The cyst formation involved infiltration of a large number of haemocytes, fibroblasts and thin but densely packed connective tissue fibres which appeared to be collagenous in nature because of their staining property with Mallory's triple stain. These fibres, haemocytes and fibroblasts were prominently oriented parallel to the cyst wall. The innermost haemocytes in the cyst wall were necrotic with pyknotic nuclei and at some places, these cells were melanised, forming a thick brown inner nodule where the parasite was almost completely destroyed and resorbed leaving a dense fibrous capsule in the hepatopancreas (Pl. VI, Fig.6).

Remarks: Several species of helminth parasites belonging to digenetic trematodes, cestodes and nematodes have been reported from penaeid prawns by several workers (vide Chapter 1, page 15-16). The helminth parasite observed in the present case although provided certain information as to its internal structure, the external morphology of the parasite could not be studied adequately because of the limited number of specimens. Further, these parasites were first recognised only when the histological sections of the hepatopancreas were examined. Despite attempts to examine the worms in the wet mounts of the midgut, detailed observations could not be made on its structural aspects, especially on the nature and pattern of hooks, spines or suckers. Due to these reasons, the identity of the parasite at hand could not be established positively.

Several workers (Hutton et al., 1959a Kruse, 1959; Overstreet, 1973; Feigenbaum, 1975; Couch, 1978) have reported an unidentified small, pyriform cestode larval stage commonly found in the intestine of penaeid prawns from the Gulf of Mexico and Atlantic coasts of Florida. Description of this unidentified cestode larva given by Couch (1978), could not be compared with the present species due to lack of sufficient information regarding the hooks/spines and suckers in the present material. However, the length and width of present worm as

determined from the sections of the hepatopancreas were calculated to be 0.53 mm to 0.9 mm and 0.15 mm to 0.23 mm, respectively, which agree to some extent with the length and width of the unidentified cestode worm (0.61 mm to 0.81 mm long and 0.12 mm to 0.22 mm wide) as given by Couch (1978). Couch (1978) stated that large number of this cestode worm might occulude the intestinal lumen or cause abnormal thickening of the intestinal wall in the penaeid hosts. On the basis of the observations, it may be presumed that the parasite presently observed embedded in the hepatopancreas and causing abnormalities of the midgut wall in P. semisulcatus, may be similar to that observed by Couch and others (Couch, 1978) in P. duorarum, P. aztecus, P. setiferus and P. brasiliensis from the Gulf of Mexico and Atlantic coasts of Florida. However, further detailed studies on the identity and the biology of the parasite described in present are essential to establish the nature of pathogenicity and its effect on the host.

3.9 MICROSPORIDIAN INFESTATION

(Plate II, Figs. 1 to 5)

Host: P. indicus (11 to 33 mm TL) and P. semisulcatus (9 to 42mm TL), postlarvae and juveniles.

External symptoms: Small, oval-shaped microsporidial cysts attached externally on the antennae (Pl. II, Fig. 1) and

other appendages of the host.

Material studied: Several specimens collected from the mudflat near Pamban in the Rameswaram island on the southeast coast of India.

Date of collection: 18th October, 1982.

Incidence: Moderate.

Season: Data not available.

Environmental information: S = 35.13 ppt; DO = 3.97 ppm; T = 28.5°C; pH = 7.9.

Observations: Small, encysted digenean trematode metacercaria were found attached on the antennae (Pl. VII, Figs. 1 to 3) and other anterior appendages of the prawn. In certain cases, as many as 63 cysts were noted from a single antenna of a 21 mm TL postlarva of P. semisulcatus.

The encysted metacercaria were oval in shape with an average size of 413 x 258 µm. The wall of the cyst was thin and rigid but ruptured easily when pressed between a microscopic slide and a cover slip (Pl. VII, Fig. 4). Each cyst possessed a metacercaria tightly packed within it. Flange of the cyst wall facilitated its attachment on the epicuticle of the appendages of the prawn(Pl. VII, Figs. 2 and 3). There were no morphological changes or any damage

PLATE VII

Fig. 1. Penaeus semisulcatus: Metacercarial cysts (arrows) attached to the antenna of a postlarva. Wet mount.

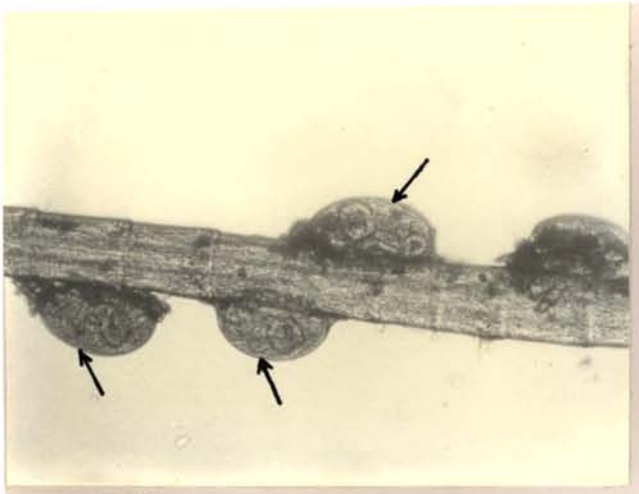
Figs. 2-3. Penaeus semisulcatus: Higher magnification views of the metacercarial cyst attached to the antenna; arrow indicates a tightly packed larva inside the cyst wall (CW). The flange of cyst (FC) facilitates the cyst attachment on the epicuticle(EC) of the antenna. Wet mount.X 50.

Fig. 4. Penaeus semisulcatus: A ruptured metacercarial cyst showing the cyst wall (CW) and metacercaria (arrows). Wet mount.

Fig. 5. Same, enlarged showing the structural details. OP=Oesophagus; OS=Oral sucker; PH=Pharynx; PS=Posterior sucker or acetabulum. Wet mount. X 80.

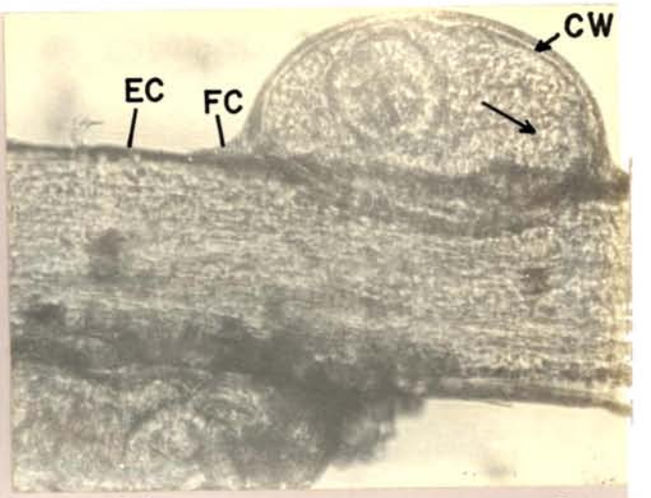
Fig. 6. Penaeus semisulcatus with a characteristic swelling on the lateral side of the carapace due to bopyrid isopod infestation in the brenchial chamber (arrows).

PLATE VII



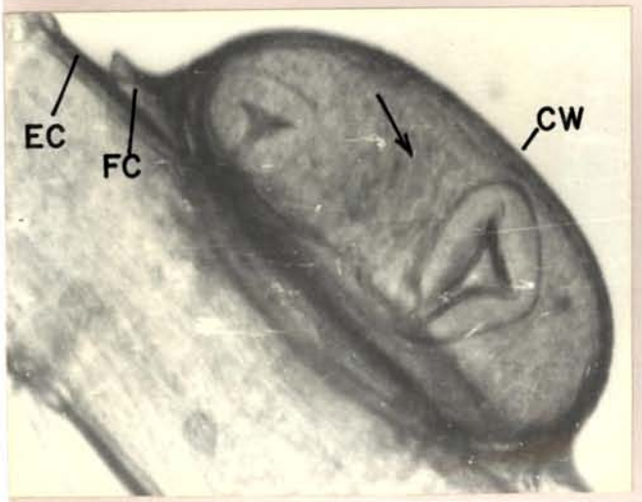
500 µm

1



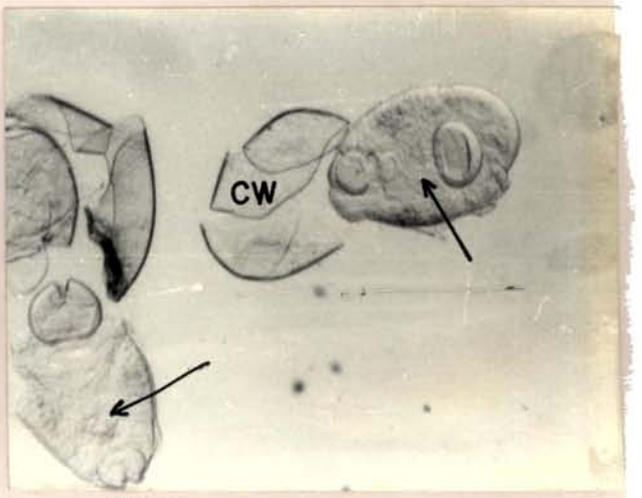
150 µm

2



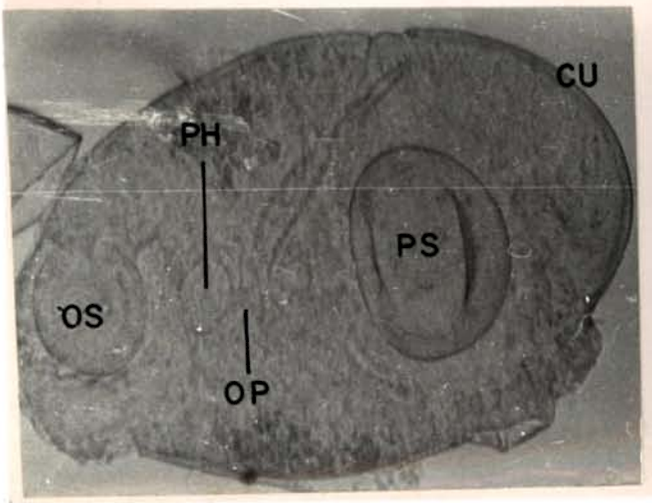
100 µm

3



300 µm

4



100 µm

5

6

noticed in the antennae or other appendages on which the cysts were attached. Each of the encysted metacercaria was provided with an adhesion apparatus consisting of an anterior or oral sucker and a large central or posterior sucker called acetabulum (Pl. VII, Fig. 5). Hooks and spines were absent. This kind of adhesion apparatus lacking hooks and spines was thought to be less developed and was found in digenean trematodes (Lyman, 1951). Examination of wet mounts and histological sections prepared from the hepatopancreas, gut and muscle of the infested hosts revealed absence of any stage of the life-cycle of the digenean in these tissues of the host.

Remarks: The presence of the oral and ventral suckers is a characteristic feature of Order Digenea (Phylum Platyhelminthes: Class Trematoda)(Erasmus, 1972). Digeneans usually have a complicated life-cycle with several larval stages and an alternation of hosts where the encysted stage is often described as "resting stage" and termed as metacercaria. Metacercarial stage may be regarded as resistant stage which permits the infectivity of the life-cycle to be extended over a longer period of time. The distribution of metacercaria within a particular environment (on vegetation, on shells or within a secondary intermediate host) is very closely linked to the food chain and feeding patterns of the final host of the digeneans (Erasmus, 1972). In this regard,

prawns harbouring the digenean may play important role as an intermediate host in the life-cycle of this digenean trematode metacercaria.

The correct identity of the metacercarial cysts reported and described at present has not been possible at this juncture due to the non-availability of other stages of its life-cycle. Kamalingam (1960) studied the morphology and life-history of Echinochasmus baculai found in the cercarial stage in snail, Natica marochinensis and in metacercarial (cyst) stage in the bivalve, Katelysia opima, collected from the same tidal mudflat at Pamban during 1954 and 1955, from where the present material has also been collected. The metacercarial cysts of E. baculai were spherical, and measured from 0.32 mm (or 320 µm) to 0.39 mm (or 390 µm) in diameter and were double walled (Kamalingam, 1960). The features such as shape, size and collection locality recorded for E. baculai agree well with the present observations on the metacercaria described for the prawn. On the basis of this information and circumstantial evidence, it is presumed that the present material may belong to E. baculai, and in the absence or scarcity of the intermediate bivalve host, E. baculai metacercaria (cyst) may attach on the external surface of the prawns as noticed in the present case. However, this observation needs further study for confirmation.

3.10 BOPYRID INFESTATION

(Plate VII , Fig. 6)

Host: P. semisulcatus, adults of both the sexes ranging in size from 131 to 173 mm TL.

External symptoms: Large swelling with pale brown colouration on the lateral side of the carapace (Pl. VII, Fig. 6).

Material studied: Thirteen specimens collected from the Gulf of Mannar off the coast of Mandapam.

Date of collection: 16th October, 1982, 11th March, 23rd June and 7th October, 1983.

Incidence: Low.

Season: Throughout the year.

Environmental information: (anges) - = 29.32 ppt to 34.18 ppt; 1 3.98 ppm to 4.47 ppm; T = 28.7°C to 30.1°C; pH = data not available.

Observations: The branchial chamber of infested prawns was greatly dilated and developed a characteristic bulging so as to accommodate the large bopyrid parasite which had almost completely occupied the branchial chamber (Pl. VII , Fig. 6). Infestation was found only in one of the branchial chambers.

Upon close examination, the parasite was found between the exoskeleton and the branchiae. The parasite adhered closely to the gill tissue of the host prawn. It was found to occur in a pair and only rarely more than one pair of parasites was found to infest a single host prawn. The pair consisted of a large female (seen with naked eyes) and a small male parasite. Body of the female parasite was large, ovoid and slightly assymetrical. Head was distinct from the thorax, a brood pouch was attached to the underside of thorax and contained enormous numbers of spherical eggs. On the ventral side of the female, a short-legged male parasite was attached.

The gill lamellae of the host's branchial chamber were slightly suppressed. The endopodites of the first pair of pleopods of infested male prawns which form the petasma were partially fused. Further, the petasma was found to be poorly developed in male prawns. In all the infested female prawns examined, the ovary was creamy white to yellow in colour and its posterior lobes were comparatively very thin indicating either undeveloped or developing stage. In none of the specimens examined, dark green ripe ovaries were found although the infested prawns were measuring over 130 mm TL. The growth of the other body parts of the infested prawns appeared to be normal

Remarks: The morphological characteristics of the presently studied bopyrid parasite are found to be similar to those of Epipenaeon ingens reported by Thomas (1977) from P. semisulcatus. Epicarideans (Crustacea:Isopoda) are well known parasites of decapod crustaceans and several genera occur parasitising penaeid prawns. Two families are important: the Bopyridae, which live principally in the gill chambers, and the Entoniscidae, which invade the haemocoel (Kindermann, 1970; Owens and Glazebrook, 1985).

Published information shows that two species of bopyrid, P. elegans (Chopra, 1923; Dawson, 1958; Abu-Hakima, 1984) and P. ingens (Thomas, 1977; Owens and Glazebrook, 1985) are found infesting the branchial chamber of P. semisulcatus.

There seems to be no gross effects of infestation on the normal body growth of the host. However, examination of the gill lamellae and reproductive organs of the infested prawns suggest indirect affects on the normal respiratory and reproductive activities of the host. Thomas (1977) found that in female prawns, ovaries were always in undeveloped condition irrespective of the size of the host and the season. Percentage infestation was more in female prawns. The petasma of the infested males failed to develop to normal shape and size. The observations made in the present case also are consistent with those of

Thomas (1977). Recently, Abu-Hakima (1984) made preliminary observations on the effect of E. elegans on the reproduction of P. semisulcatus. He found that although infestation did not inhibit the growth of the host, it affected considerably the reproductive characteristics of the prawn and was found to be typical to that of parasitic castration. Abu-Hakima (1984) found that gonadosomatic index (GSI) was considerably low (≤ 0.5) in both infested male and female prawns. The petasma was not formed in infested male prawns. The testes were also reduced in size and only few spermatozoa were found. The ovaries were small in infested females and the oocytes did not develop beyond the stages of pre-vitellogenesis and primary vitellogenesis. He (Abu-Hakima, 1984) observed that if the parasite was lost, the GSI of female host increased to 2.66 and ovaries contained oocytes in active vitellogenesis. Infestation did not prevent ecdysis in the host and sometimes the infested hosts reached a larger size than the uninfested prawns. Owens and Glazebrook (1985), who conducted a survey on bopyrid isopod parasites of commercial penaeid prawns from the northern Australia, observed that P. semisulcatus carried more than 90 per cent of the population of P. indens.

As was observed in the present study, the changes in the secondary sexual characters of infested male hosts lead to the improper formation of petasma which hinders the true

identification of the male prawns. Since the secondary sexual characters are important in competition and mate finding behaviour, the "feminisation" or "juvenilisation" of the host may reduce competition in such males(Abu-Hakima, 1984).

GENERAL REMARKS

Among the diseases encountered during the present survey and those described earlier by various workers, it appears that none of them, except that described as "soft" prawn syndrome and microsporidiosis, cause any serious mortalities at present in the penaeid prawn population of the country caught from the wild or those subjected to farming in the coastal waters. The "soft" prawn syndrome occurs in the brackishwater prawn culture systems in Central Kerala during the peak season (April-May) when the salinity and the temperature of the pond water reach the maximum or during the onset of south-west monsoon when these parameters decrease considerably. Although several instances of this phenomenon resulting in considerable loss of stocked prawns are reported, its reliable estimation is not available at present. The Central Marine Fisheries Research Institute, realising the importance of this syndrome, has launched a comprehensive research project entitled, " tudies on pathobiology of soft prawns" to study the biological, ecophysiological and histological aspects of this phenomenon.

The other most important disease which affects the penaeid prawns both in the capture and culture fisheries, as observed in the present survey and from the reports available, is microsporidiosis or "cotton" prawns disease. It is reported that about 2 to 3 per cent of the penaeid prawn population landed by the mechanised fishing operations at Mandapam are affected by this disease. This is also commonly found in the catches landed at Dhanushkodi, Rameswaram, Pamban, Devipattanam, Mandapam Camp, Vilakarai, Kayalpatnam, Tuticorin and Manappad. At Tuticorin and Rameswaram, about 2 to 4 kg. of "cotton" prawns are landed by each unit during February-April and August-November. It has been observed that in September, 1982, one of the fishing vessels engaged in prawn fishing off Manappad near Tuticorin landed about 75 kg. of P. semisulcatus infected by microsporidian parasite. Similarly, mass mortality of stocked prawns due to this disease has been observed in one of the culture operations carried out at Muthukad farm of C.M.F.R.I near Madras in 1983. These prawns are generally rejected for export purposes and bring no values even in the local market. Thus "microsporidiosis" is considered as one of the serious diseases encountered in penaeid prawns of the country, bringing in considerable loss to the production as well as to the fishermen. In view of this and since no detailed investigations on the disease have so far been made from India, it has been taken up for detailed studies and the results obtained are presented and discussed in the following Chapter.

CHAPTER 4

STUDIES ON THE MICROSPORIDIAN PARASITES OF PENAEID PRAWNS OF INDIA

Microsporidia constitute a remarkable group of protozoan parasites occuring in almost all the major animal phyla. They are intracellular parasites. They live in the cells of various organs and multiply by spore formation. The great work of Louis Pasteur, over a century ago, on the destructive disease known as "pebrine" in silkworks caused by the microsporidian, Nosema bombycis, brought to light the importance of these parasites. However, the credit goes to Balbiani (1882) who, for the first time, assigned this and some other related organisms to Protozoa and included them in the Order "Microsporidies" under the Class Sporozoa. Following this, Thelohan in 1892 elaborated further the taxonomy of the group by including a single Family "Glugeidees", comprising of three genera, namely, Glugea Thelohan,1891, "parasites des muscles du Cottus" (Leistophora Gurley, 1893) and Thelohania Henneguy, 1892. But Thelohan considered the group under "Myxosporidies" rather than in "Microsporidies". Although Gurley (1893) proposed a new name "Cryptocystes" for the Order Microsporidies of Balbiani (1882), this was later rejected.

In 1899, Labbe attempted the first review of the group. In persuance of law of priority, Labbe (1899) in his review proposed the Family Nosematidae in suppression of Glugeidae, and included the Genera Nosema, Pleistophora and Thelohania under Nosematidae. And, based the distinction of these three genera on the absence of the pansporoblastic membrane as a character of Nosema - Glugea complex and its presence in Pleistophora and Thelohania. In the same year, Doflein(1899) however, abandoned the conventional system of classification and arbitrarily separated Gurley's "Cryptocystes" into "Oligosporogenea" and "Polysporogenea" without specifying their systematic position. Yet, Doflein's influence on this classification did not persist for long, and it has now only a historical interest. His only positive contribution to the group was the creation of the Genus Glugea.

The first 25 years of this century witnessed a rapid increase in the knowledge of the Microsporidia. Among the earlier workers of this era, the contributions by Minchin (1903, 1909), Perez (1905, 1908) and Auerbach (1910) are noteworthy. Perez (1905) made a significant contribution by distinguishing for the first time the difference between Nosema and Glugea.

As the new taxa were delimited and ranked, a classification in the form of the classical Linnaean hierarchy was gradually evolved and paved the way for a modified classification by Stempell (1909). Recognising

the Families Glugeidae Thelohan, 1892 and Nosematidae Labbe, 1899, he created a new Family, Pleistophoridae, and distinguished a total of eight genera . Stempell (1909) used the form and development of vegetative stages as family characters, the mode of spore formation as generic characters and the form of spore as species characters.

In 1922, Leger and Hesse made a drastic revision of the classification in which they abandoned all criteria for distinguishing families excepting spore form. This system excluded the Families Nosematidae and Pleistophoridae, as all the genera with pyriform spore were combined in the Family Glugeidae, but inclueed two new sub-orders and three families.

Kudo(1924) compiled all the widely scattered published information on Microsporidia in a comprehensive morograph, where he adopted the classification of Leger and Hesse(1922) with slight modifications. This modified classification was universally adopted and perpetuated for about half a century, although this was considered to be a period of great confusion in microsporidian taxonomy.

Kenyon (1926) also adopted the classification of Leger and Hesse (1922) but improved it by recognising both Family Glugeidae and Nosematidae following Stempell (1909).

The history of the classification of the Microsporidia since 1924 recorded essentially the modifications suggested

over that of Kudo (1924) and those included to satisfy the needs arising from the expanding knowledge of the Microsporidia. Thus, Poisson (1953), Weiser (1961) and Corliss and Levine (1963) attempted certain modifications in the taxonomy, but these did little to advance the evolution of the classification. It was in 1971, that Tuzet et al. completely revised the group and abandoned entirely the system proposed by Leger and Hesse (1922) and presented a new system of classification under the Class Microsporidea Corliss and Levine, 1963. They (Tuzet et al., 1971) created two new sub-orders, namely, Pansporoblastina and Apansporoblastina under the Order Microsporida Balbiani, 1882 and distinguished the different genera under these two sub-orders on the basis of presence or absence of the pansporoblastic membrane around the developing sporoblasts. They also made the important generalisation that genera that have the pansporoblastic membrane (sub-order: Pansporoblastina) have a single nucleus while sporoblasts of the genera without such a membrane (Sub-order: Apansporoblastina) are binucleate.

While many authors were concerned with problems of classification under the group Microsporidia, the relationship of this group with other groups of Protozoa, particularly with Myxosporidia was also controversial since the time of Balbiani (1882). It was only recently that Lom and Vavra (1962), Lom and Corliss (1967) and Vavra(1966) concluded that Microsporidia are unrelated to

Myxosporidia. Sprague (1969) agreed with this view on the contention that Haplosporidia, like Microsporidia, have unicellular spores and proposed a new Sub-phylum Microspora to include Classes Microsporea Corliss and Levine, 1963 and Haplosporea Caullery, 1953. However, the idea of relating these two groups (Microsporidia and Haplosporidia) became no longer tenable when Ormieres et al., (1973) and Perkins (1976) found evidence that some haplosporidian spores are multicellular and have a type of development fundamentally quite different from that of microsporidian spores.

Since the publication of the scheme of classification for Microsporidia by Tuzet et al. (1971), a number of papers were published carrying new descriptions and proposals of new taxon (Weissenberg, 1970; Sprague et al., 1972; Ormieres and Sprague, 1973; Canning et al., 1974; Overstreet and Weidner, 1974; Hazard and Oldacre, 1975; Vinicker, 1975; Vivier, 1975). A series of papers by Hazard and his associates (Hazard and Anthony, 1974; Hazard and Fukuda, 1974; Hazard and Oldacre, 1975) have also brought a large amount of new data on the ultrastructure of Microsporidia together with proposals of new taxa.

Recently, Sprague (1977) felt that the classification given by Tuzet et al. (1971), although had certain meritorious features, contained some inaccuracies and was

incomplete. He, therefore, proposed a modified classification of Microsporidia in which he elevated the Sub-phylum Microspora Sprague, 1969 to the rank of an independent phylum and created the Phylum "Microspora". In this modified classification, Sprague (1977) synthesised all the previous contributions with such corrections, additions and other modifications as seemed necessary to accommodate all the known microsporidia within a natural system. He included about 525 named and about 200 unnamed species and arranged the taxa starting with the most "primitive" forms and proceeded in the general direction of the least "primitive" forms. Sprague (1977) has the distinction of being the first author to elevate Microsporidia upto the rank of a phylum and to speculate on its phylogeny.

Weiser (1977) proposed a similar classification using the number of nuclei in spores, synchrony of nuclear divisions, structures of polar tube and polaroplast, sporogonial dimorphism and general shape of the spore as taxonomic criteria. He (Weiser, 1977) considered the Order Microsporidia Stempell, 1909 as phylum and divided it into two Classes: Metchnikovellidea (Weiser, 1977) and Microsporididea Corliss and Levine, 1963.

Recently, Sprague (1982) has again modified his earlier system of classification to accommodate the new families which were created during the last five years, and

replaced the Order Chytridiopsida Weiser, 1974 to the Order Minisporida Sprague, 1972. The classification of Microsporidia as given by Sprague (1982) is as follows.

Sub-Kingdom PROTOZOA

Phylum MICROSPORA Sprague, 1977

Class NEOMICROSPOREA Sprague, 1977

Order METCHNIKOVELLIDA Vivier, 1975

Family METCHNIKOVELLIDAE Caullery and Mesnil, 1914

Genera Metchnikovella Caullery and Mesnil, 1897

Amphiacantha Caullery and Mesnil, 1914

Amphiamblys Caullery and Mesnil, 1914

Class MICROSPOREA Corliss and Levine, 1963

Order MINISPORIDA Sprague, 1972

Family Hesseidae Ormieres and Sprague, 1973

Genus Hessea Ormieres and Sprague, 1973

Family CHYTRIDIOPSIDAE Sprague, Ormieres and Manier, 1972

Genera Chitrydiopsis Schneider, 1884

Stenhausia Sprague, Ormieres and Manier, 1972

Family BURKEIDAE Sprague, 1977

Genus Burkea Sprague, 1977

Family BUXTEHUDEIDAE Larsson, 1980

Genera Buxtehudea Larsson, 1980

Jiroveciana Larsson, 1980

Order MICROSPORIDA Balbiani, 1882

Sub-order PLEISTOPHOROBLASTINA Couzet, Maurand, Pize, Michel and Fenwick, 1971

Family PLEISTOPHORIDAE Stempell, 1909

Genera Pleistophora Gurley, 1893

Mitoplistophora Codreanu, 1966

Vavraia Weiser, 1977

Family PSEUDOPLEISTOPHORIDAE Sprague, 1977

Genus Pseudopleistophora Sprague, 1977

Family DUBOSCQUIIDAE Sprague, 1977

Genera Duboscqia Perez, 1908

Trichoduboscqia Leger, 1926

Family THELOHANIIDAE Hazard and Oldacre, 1975

Genera Thelohania Henneguy, 1892

Agmasoma Hazard and Oldacre, 1975

Chapmanium Hazard and Oldacre, 1975

Cryptosporina Hazard and Oldacre, 1975

Heterosporus Schubert, 1969

Indosporus (=Orthothelohania) Overstreet and Weinder, 1974

Ormieriesia Vivares, Bouix and Manier, 1976

Pegmatheca Hazard and Oldacre, 1975

Pilosporella Hazard and Oldacre, 1975

Systenostrema Hazard and Oldacre, 1975

Toxoglugea Leger and Hesse, 1924

Family BURENNELLIDAE Jouvenaz and Hazard, 1978

Genera Vairimorpha Pilley, 1976

Burenella Jouvenaz and Hazard, 1978

Family AMBLYOSPORIDAE Weiser, 1977

Genera Amblyospora Hazard and Oldacre, 1975

Hyalinocysta Hazard and Oldacre, 1975

Parathelohania Codreanu, 1966

Family CULICOSPORIDAE Weiser, 1977

Genera Culicospora Weiser, 1977

Hazardia Weiser, 1977

Family CURLEYIDAE Sprague, 1977

Genera Curleya Doflein, 1898

Pyrotheca Hesse, 1935

Stempellia Leger and Hesse, 1910

Family LOMYXIDAE Leger and Hesse, 1910

Genus Lolomyxa Leger and Hesse, 1910

Family [illegible] Sprague, Duzet and Maurand, 1977

Genus Duzetia Maurand, Fize, Fenwick and Michel, 1971

Sub-order PLEISTOPHORINA Duzet, Maurand, Fize, Fenwick and Michel, 1971

Family GLUGEIDAE Thelohan, 1892

Genera Glugea Thelohan 1891

Encephalatozoon Levaditi, Nicolau and Schoen, 1923

Baculea Loubes and Akbarieh, 1978

Loma Morrison and Sprague, 1981

Family [illegible]QUIDAE Weissenberg, 1976

Genus Spraguea Weissenberg, 1976

Family PEREZIIDAE Loubes, Maurand, Camps and Lampillo, 1977

Genera *Perezia* Leger and Duboscq, 1909

Ameson Sprague, 1977

Family COUGOURDELLIDAE Poisson, 1953

Genus *Cougourdella* Hesse, 1935

Family CAUDOSPORIDAE Weiser, 1958

Genera *Coudospora* Weiser, 1946

Weiseria Doby and Naguez, 1964

Colbercia Weiser, 1977

Culicosporella Weiser, 1977

Octosporea Flu, 1911

Family NOSEMATIDAE Labbe, 1899

Genera *Nosema* Naegeli, 1857

Ichthyosporidium Caullery and Mesnil, 1905

Issia Weiser, 1977

Family MARTEKIIDAE Leger and Hesse, 1922

Genera *Marzekia* Leger and Hesse, 1922

Sirovecia Weiser, 1977

Although, the taxonomic consideration of Microsporidia formed the subject matter of study for over a hundred years, and the destructive effects of these parasites on silkworms and honey bees were known long back, it was only from the middle of this century that economic importance of the group was recognised. Consequently, several investigations were taken-up on the biology and pathology of microsporidians.

Increasing interest in the invertebrate pathology coupled with the application of modern tools of research, especially electron microscopy (Weiser, 1959; Huger, 1960; Lom and Vavra, 1961; Vavra, 1964, 1965, 1972, 1974, 1976; Sprague and Vernick, 1968; Lom and Weiser, 1972; Weidner, 1972; Hazard and Anthony, 1974), tissue culture techniques (Sen Gupta, 1964; Ishihara and Sohi, 1966; Ishihara, 1968; Vavra et al., 1972; Undeen, 1975), methods in cytochemistry (Vavra, 1959; Huger, 1960; Erickson and Sprague, 1970) and immunological techniques (Chalupsky et al., 1971, 1973; Cox et al., 1972; Pakes et al., 1972; Jackson et al., 1973; Kalalova and Weiser, 1973) gave new dimension to the study of these minute organisms. Their use as possible biological control agents against certain invertebrate vectors of diseases has also attracted considerable attention (Steinhaus, 1954, 1957; Canada, 1959, 1963, 1967, 1976; Cameron, 1963; Kramer, 1968; Weiser, 1970; McLaughlin, 1971, 1973).

Subsequent to the first electron microscope study of sectioned spores of Nosema larbiomae by Weiser in 1959, several investigations have been carried out on the ultrastructural aspects of Microsporidia. The results of these investigations were excellently reviewed and comprehensively presented in two volumes edited by Bulla and Cheng (1976, 1997).

Microsporidia infect both land and aquatic animals. Among aquatic animals of commercial importance, they are encountered in fishes, molluscs and crustaceans. In Crustacea, over 140 species have been described from hosts belonging to almost all orders of Crustacea (Couch, 1983). There are also indications of their involvement in epizootics in feral crustacean populations (Pixell-Godrich, 1923, 1956; Viosca, 1943; Laborn, 1976; Couch, 1983). Of the 140 species of Microsporidia recorded from crustaceans, about 37 species belonging to 10 genera are listed from Decapoda. The microsporidian belonging to genera Pleistophora, Thelohania, Agmasoma Perezia and Ameson are commonly found in the natural population of decapods, especially in crabs and prawns. They are found to infect mainly the skeletal muscle. Excellent reviews pertaining to the microsporidian parasites of decapods have been given by Sprague (1965, 1970, 1977, 1978), Sprague and Couch (1971) and Couch (1983). Table 1 gives a list of decapods and their respective microsporidian pathogens.

Four species of microsporidia have been found in penaeid prawns, and the disease they cause is collectively known as "cotton" or "milk shrimp" disease or "microsporidiosis" (Lightner, 1975, 1983; Iversen and Kelley, 1976). The pathogen usually infects muscle, gonad, gut, hepatopancreas, heart and nerve cord and the acute infection causes discolouration of muscle giving the prawn a whitish or

Table 1. Microsporidian parasites recorded from decapod crustaceans

Host	Pathogen	Tissue	Locality	Reference
Astacus fluviatilis (Astacus astacus)	Thelohania contejeani	Muscle	France, Finland and U.S.S.R.	Sumari and Westman (1970), Voronin (1971).
Astacus pallipes (Austropotamobius(Atlanto-astacus) pallipes pallipes)	Thelohania contejeani	Muscle, heart, brain, connective tissue surrounding the gut and envelopment of ovary	France, Germany and England	Schaperclaus(1954), Vey et al.(1971), Vey & Vago (1973), Cossins(1973).
Astacus nitescens	Thelohania sp. Nouvel & Nouvel, 1935	Muscle	France (Roscoff)	Nouvel & Nouvel (1935), Sprague (1977).
Atyephira spp.	Gurleya miyairii	Muscle	Japan (Fukuoka)	Sprague (1970).
Atyephira sp.	Pleistophora miyairii	Digestive tract	Japan	Kudo(1924), Sprague (1970).
Callinectes sapidus	Ameson michaelis	Early stages in haemopoetic organs and sporulation in skeletal muscle	U.S.A.(Atlantic and Gulf coasts)	Sprague (1970, 1977).
Callinectes sapidus	Nosema sapidi	Muscle	USA(North Carolina)	Sprague (1970).
Callinectes sapidus	Pleistophora cargoi	Muscle	U.S.A.(Maryland)	Sprague(1966, 1970).
Callinectes sapidus	Pleistophora sp. Johnson, 1972	Muscle	U.S.A.(North Carolina)	Johnson(1972), Sprague (1977).
Cambarellus puer	Pleistophora sogandaresi	Muscle	U.S.A.(Louisiana)	Sprague(1966), Sprague & Couch (1971).
Cambarus shufeldti	Thelohania sogandaresi	Muscle	U.S.A.(Louisiana)	Sogandares-Bernal (1962) 1965), Sprague (1977).
Cambarus bartoni	Thelohania cambari	Muscle	U.S.A.(Georgia)	Sprague(1950), Sprague & Couch (1971), Hazard & Oldacre (1975).
Carcinus maenas	Ameson pulvis	Skeletal muscles	France (Arcachon)	Perez(1905), Sprague (1970, 1977).
Carcinus maenas	Thelohania maenadis	Skeletal muscles	France (Arcachon)	Perez(1904), Hazard & Oldacre (1975).
Carcinus mediterraneus	Thelohania maenadis	Skeletal muscles	France (Arcachon)	Perez (1904), Sprague (1977).

4.1 DESCRIPTION OF MICROSPORIDIANS COLLECTED DURING THE STUDY

THELOHANIA SEMISULCATA SP. NOV.

Host and site: The green tiger prawn, Penaeus semisulcatus de Haan, ranging in size from 65 mm to 168 mm total length measured from tip of rostrum to tip of telson. Infection found in muscle, hepatopancreas, ovary, testes, midgut wall, heart, gills and eye stalks. Infected prawns appear opaque and white throughout the body due to the infected muscles.

Locality: Southeast coast of India - Tuticorin and Mandapam in the Gulf of Mannar and Palk Bay side of Rameswaram.

Vegetative stages: Vegetative stages are observed in the colonies of compactly arranged spherical polygonal to irregularly shaped cells. Three types of cells are observed in the colony: (1) small spherical cells, 3 to 4 µm in diameter; each cell with a single nucleus, about 2 µm in diameter; (2) large diplokaryotic cells ranging in diameter from 4 to 7 µm, and (3) large uninucleate cells, each cell measuring about 6 to 8 µm and nucleus about 4 to 5 µm in diameter. The first type of cells are meronts resulting from merogony which represents the vegetative phase. The third type of cells are sporonts

PLATE VIII

Thelohania semisulcata sp. nov. in Penaeus semisulcatus

Figs. 1-3. Cross section of the terminal ampoule of the prawn infected by Thelohania semisulcata. DY=Diplokaryotic cells; ME=Meronts; SP=Sporonts. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 4. Binary and multiple fissions in the meronts (arrows). NU=Nucleus. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 5. Sporonts with large nucleus (arrow). Glutaraldehyde-Giemsa.

Fig. 6. Early sporont(arrow) with characteristic chromosomal profile. Glutaraldehyde-Giemsa.

PLATE IX

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Figs. 1-2. Transverse section of the terminal ampoule of the prawn to show the initial stage of sporogony of Thelohania semisulcata (arrows). DY=Diplokaryon; SP=Sporont. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 3. Karyokinesis in Thelohania semisulcata during sporogony. The cytoplasm is not yet divided. NR=Nuclear material. Glutaraldehyde-Giemsa.

Figs. 4-5. Semi-thin sections of the infected abdominal muscle to show two-cell stage resulting from the first sporogonic division (arrow). PM=Pansporoblastic membrane. Glutaraldehyde-Toluidine blue.

Fig. 6. Four-cell stage (arrow) resulting from the second sporogonic division. PM=Pansporoblastic membrane. Glutaraldehyde-Toluidine blue.

paper-white appearance and hence the name of the disease. These microsporidian parasites form one of the most destructive groups of pathogens to penaeid prawns and it has also been reported that infection prevails in about 10 percent of prawns from nature and aquaculture (Couch, 1978).

With the increasing interest in the aquaculture of penaeid prawns in recent years, these parasites have attracted the attention of several marine pathologists and protozoologists particularly from the United States of America. Sprague (1950) named and described Nosema nelsoni and Pleistophora penaei from Penaeus aztecus and P. setiferus respectively. Viosca (1943) reported about a protozoan disease affecting the reproductive organs of about 90 percent of P. setiferus along the Louisiana coast in 1919, which Sindermann (1970) and Sprague (1970) believed to have been caused by P. penaei. Hutton et al. (1959a) also found P. penaei in the muscle of P. setiferus from Louisiana waters. In 1959, Iversen and Manning described a new species of microsporidian, P. duorara, infecting the muscle of P. duorarum from the coast of Florida. This species was also found to infect P. aztecus (Kruse, 1959; Hutton, 1964) and P. brasiliensis (Iversen and Van Meter, 1964) off the coast of Florida. The other microsporidian reported from penaeid prawns of U.S.A. is Pleistophora sp.(Kruse, 1959) Baxter et al., 1970; Constransitch, 1970; Overstreet, 1973).

Transmission experiments on microsporidian parasites in penaeid prawns were conducted by Roth and Iverson (1971) and Iversen and Kelley (1976). Kelley (1975) studied the structure of normal and microsporidian infected pink shrimp, P. duorarum while Hazard and Oldacre (1975) presented the ultrastructure of Agmasoma penaei which was earlier described as T. penaei by Sprague (1950). Sprague and Vernick (1969) made light and electron microscopic study of N. nelsoni.

Outside U.S.A., very few studies have been carried out on microsporidians infecting the penaeid prawns. H.B.F. Champion (as reported by Sprague and Couch, 1971) found a microsporidian similar to T. penaei from the ovary of P. indicus in the Republic of South Africa. Baticados (1980) worked on the histopathology of "microsporidiosis" in P. merguiensis in Philippines.

In India, information on microsporidian parasites infecting the decapods has been meagre. Subrahmanyam (1974) and Santhakumari and Gopalan (1980) found spores of microsporidia resembling to those of N. nelsoni in the muscles of Metapenaeus monoceros. Thomas (1976) reported still another microsporidian similar to T. duorara from P. semisulcatus in the Gulf of Mannar and Palk Bay. In these studies, the identity of the microsporidian by these authors was limited to the shape and size of the spores. The important aspects such as life-cycle of the

pathogen, their ultrastructure and infection characters are not available.

In the present investigation, two species of Microsporidia were encountered in P. semisulcatus and one species in M. affinis from off the coasts of Mandapam, Rameswaram and Tuticorin on the southeast coast of India. Detailed light and electron microscopic studies of these parasites and the comparison of their structural characteristics with the other species already described in the decapods revealed that all the three species are new to science and one belongs to a new genus. In the following account, the taxonomic descriptions of these species and the salient features of the different life stages and characteristics of their infection in the host are presented and discussed.

4.1 DESCRIPTION OF MICROSPORIDIANS COLLECTED DURING THE STUDY

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Host and site: The green tiger prawn, Penaeus semisulcatus de Haan, ranging in size from 65 mm to 168 mm total length measured from tip of rostrum to tip of telson. Infection found in muscle, hepatopancreas, ovary, testes, midgut wall, heart, gills and eye stalks. Infected prawns appear opaque and white throughout the body due to the infected muscles.

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Figs. 1-3. Cross section of the terminal ampoule of the prawn infected by Thelohania semisulcata. DY=Diplokaryotic cells; ME=Meronts; SP=Sporonts. Bouin-Heidenhain's haematoxylin and eosin.

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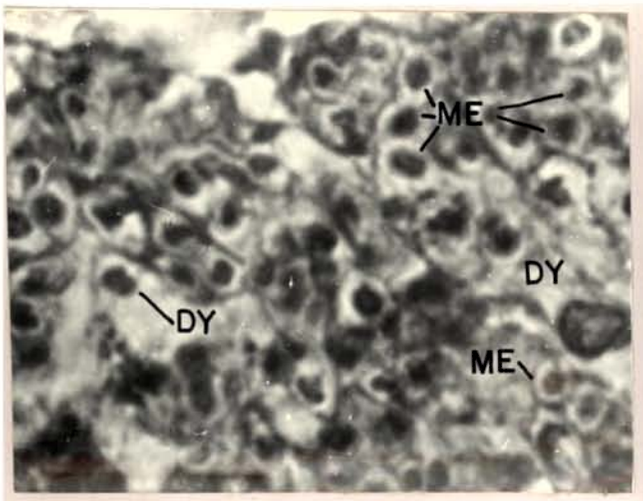
Fig. 6. Early sporont(arrow) with characteristic chromosomal profile. Glutaraldehyde-Giemsa.

PLATE VIII



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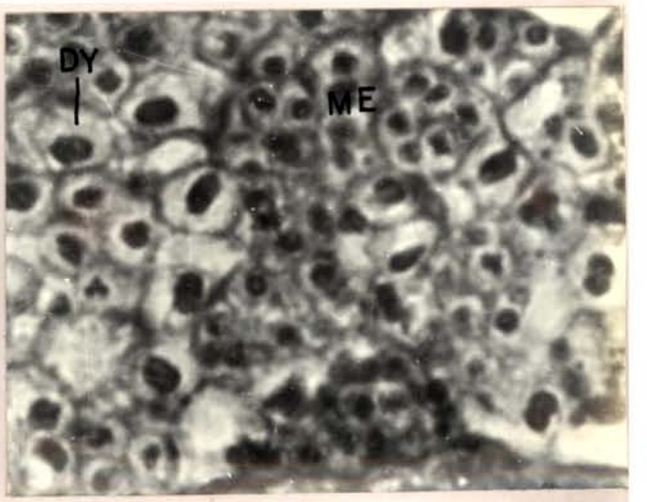

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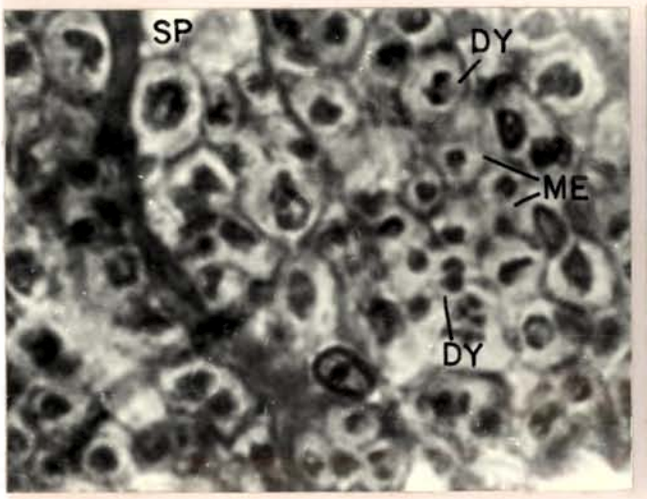

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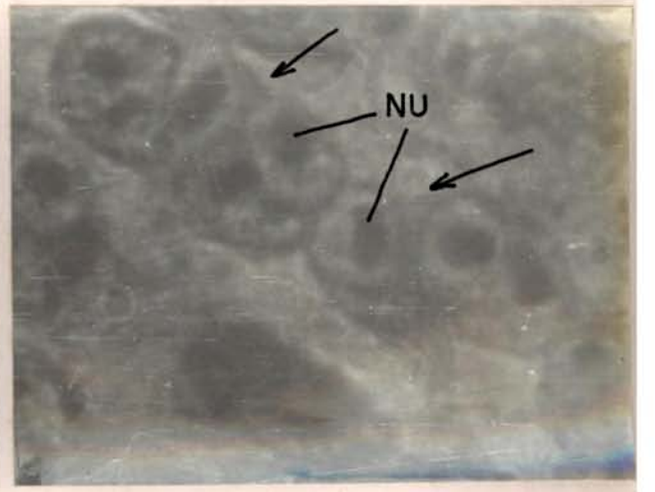

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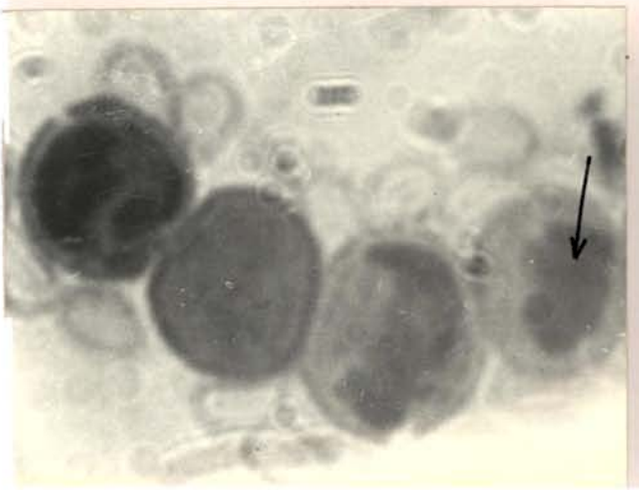

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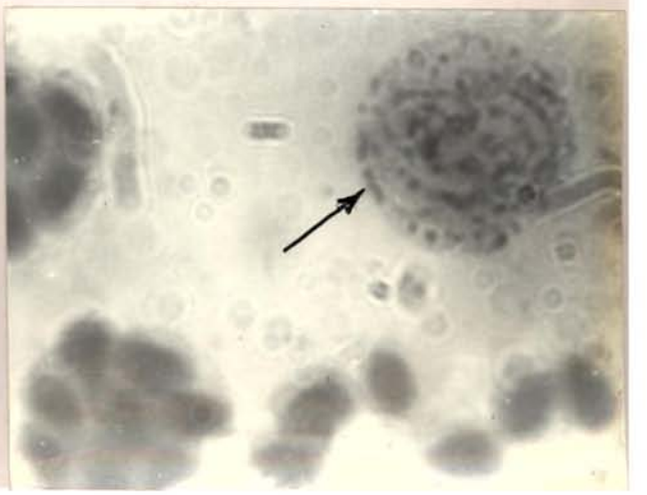

10 µm 6

PLATE IX

Thelohania semisulcata sp. nov. in Penaeus semisulcatus

Figs. 1-2. Transverse section of the terminal ampoule of the prawn to show the initial stage of sporogony of Thelohania semisulcata (arrows). DY=Diplokaryon; SP=Sporont. Bouin-Heidenhain's haematoxylin and eosin.

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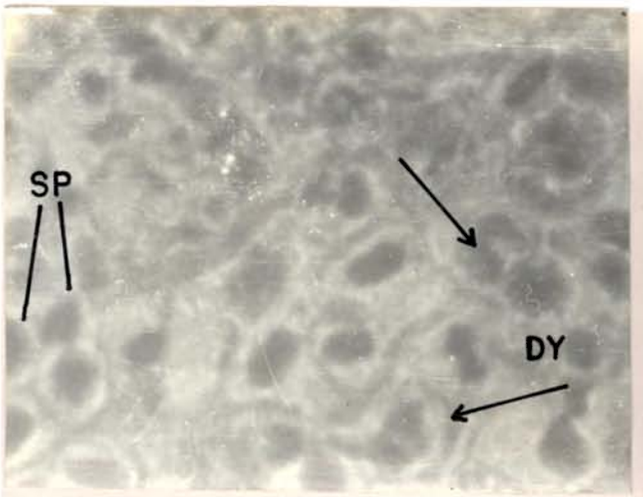
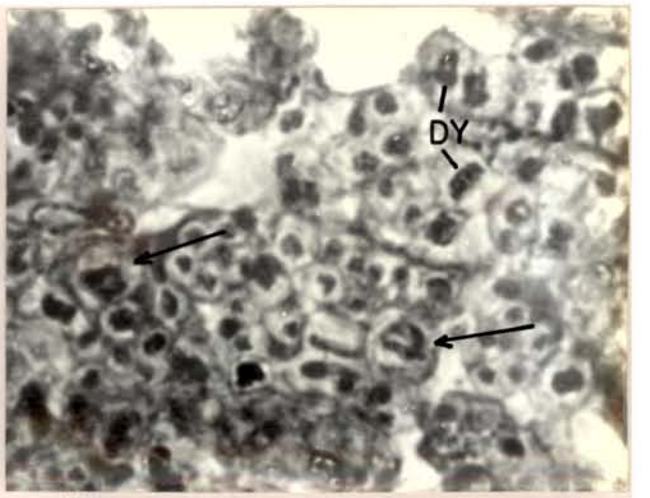
PLATE IX



10 µm

V



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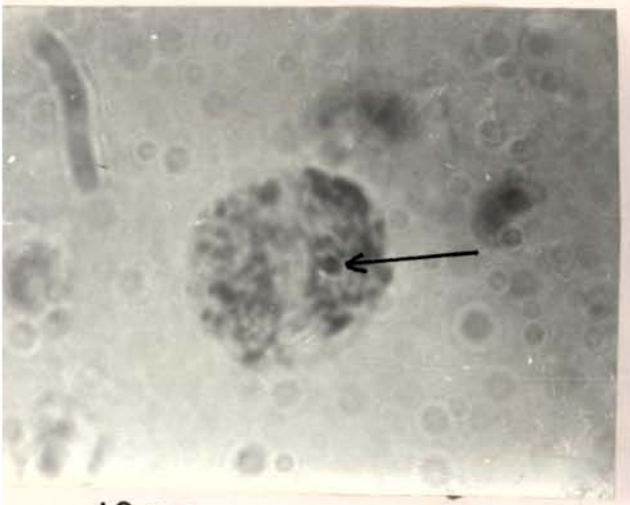

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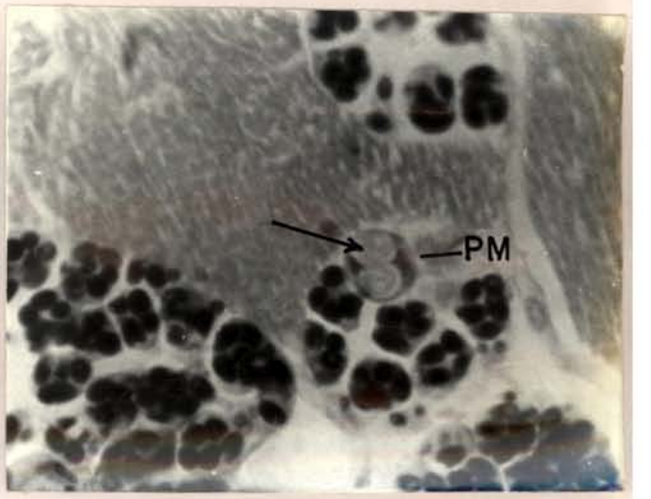

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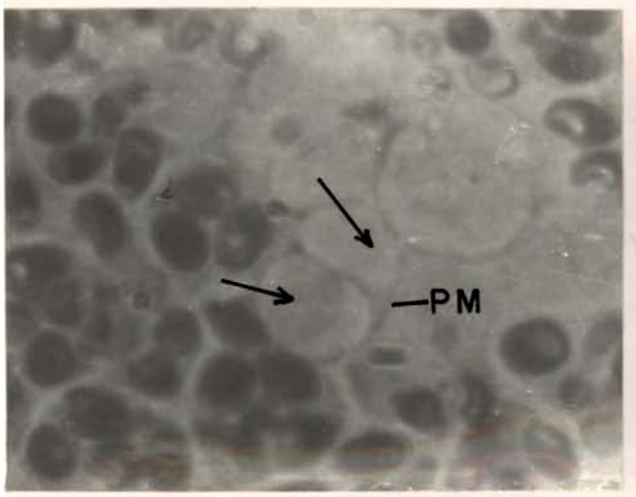
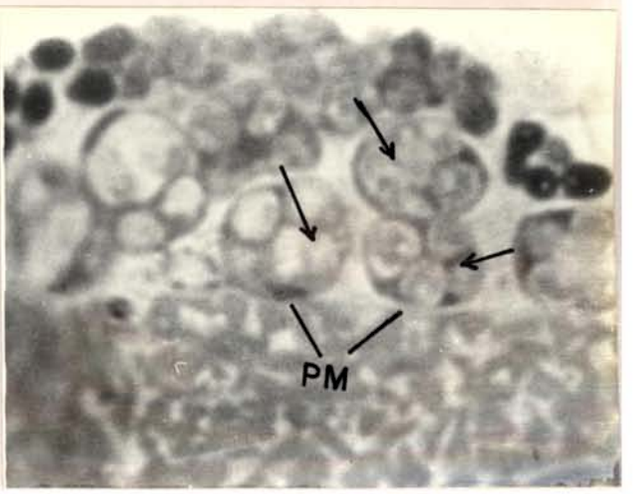


10 µm

5



15 µm

6

which are formed after the fusion of two nuclei in the diplokaryotic cell, the second type. The sporonts represent the sporogonial phase of the life-cycle of the pathogen. In transverse section of the terminal ampules of the testes of an infected P. semisulcatus, all the three cell types, namely, meronts, diplokaryotic cells and sporonts, are distinctly seen (Pl. VIII, Fig. 1 to 3).

Meronts increase in number by multiple and binary fission (Pl. VIII, Fig. 4). The nucleus of the cell undergoing merogony is small and compact whereas in sporont the nucleus is relatively large (Pl. VIII, Fig. 5) which exhibits characteristic chromosomal profiles during mitosis, especially in the early stages (Pl. VIII, Fig. 6). At the end of merogony, uninucleate meronts transform into diplokaryotic cells (Pl. IX, Figs. 1 and 2). This development of diplokaryotic cells can be considered as a transitional stage between merogony and sporogony.

Sporulation stages: Early sporont possesses a slightly thick wall, a large nucleus and irregularly spaced patches of dense material deposited on the inner side of the wall. The sporont functions as the sporogonial mother cell and divides three times (Pl. IX, Figs. 3 to 6; Pl. X, Figs. 1 to 4). Thus, sporogony is a series of three successive binary divisions of the sporogonial mother cell which ultimately gives rise to eight uninucleate sporoblasts

PLATE X

Thelohania semisulcata sp. nov. in Penaeus semisulcatus

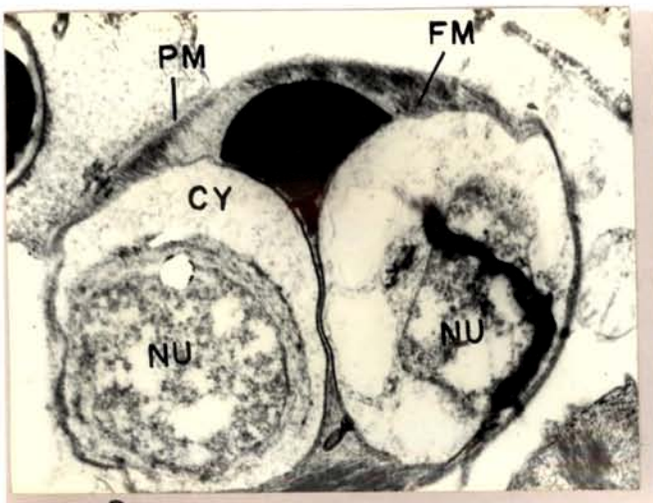
Figs. 1-3. Electron micrographs showing four-cell stage of Thelohania semisulcata. CY=Cytoplasm; FM=Electron-dense fibrous material; NU=Nucleus; PM=Pansporoblastic membrane.

Fig. 4. Eight-cell stage or Octosporoblast(OB) resulting from third sporogenic division. Arrow indicates immature sporoblasts covered in pansporoblastic membrane (PM). Free spores (SR) liberated from other pansporoblast are also visible. Glutaraldhyde-Giemsa.

Fig. 5. Mature eight spores (arrows) of Thelohania semisulcata resulting from metamorphosis of sporoblasts. Formalin fixed.

Fig. 6. Electron micrograph showing a part of the pansporoblast with mature spores. PM=Pansporoblastic membrane; SR=Mature spores.

PLATE X



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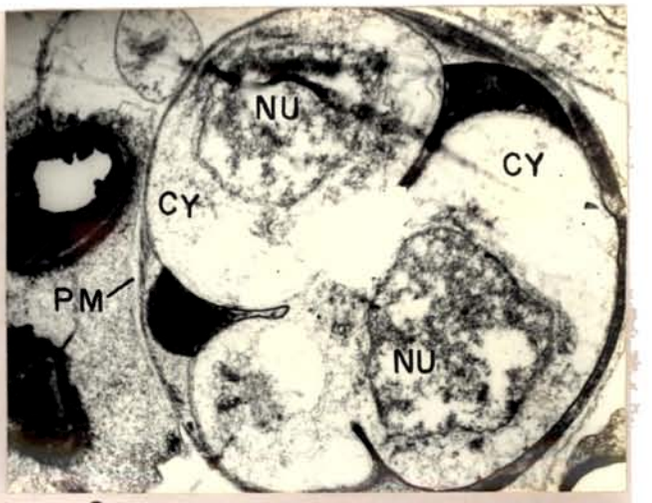
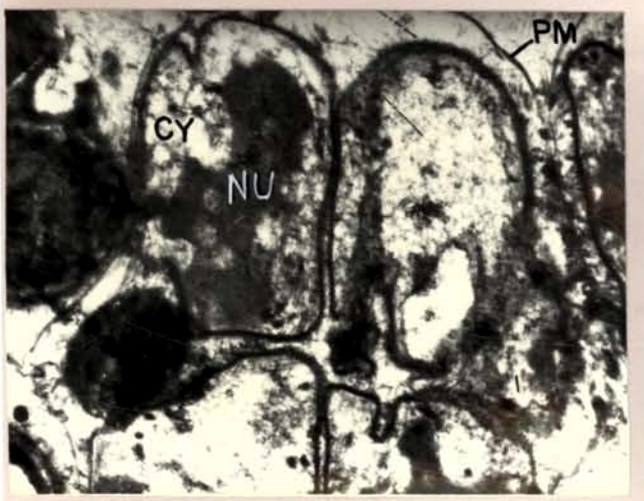
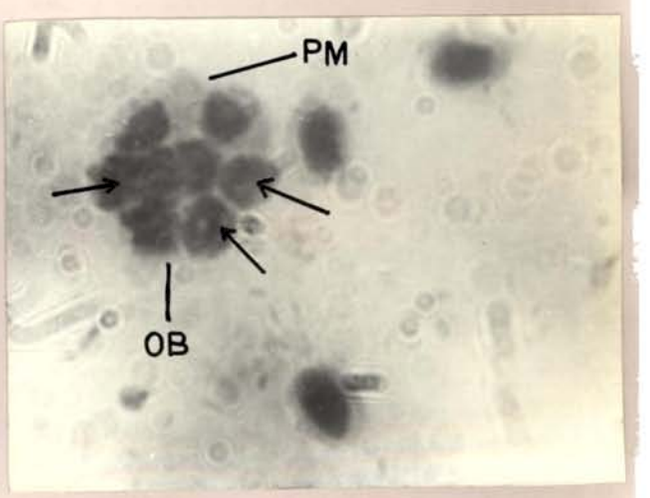


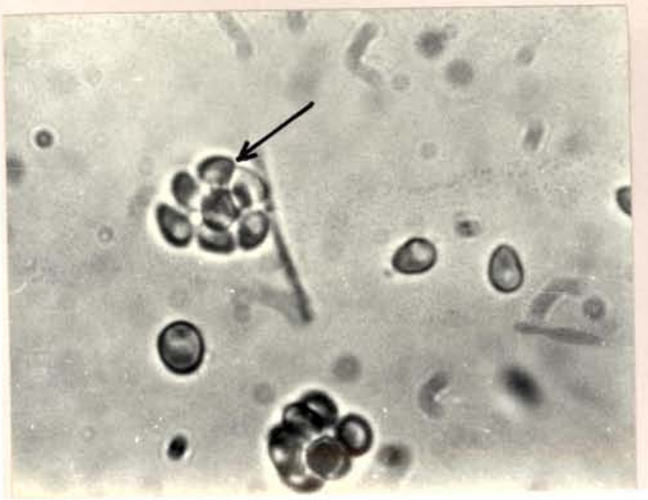
2 µm 2



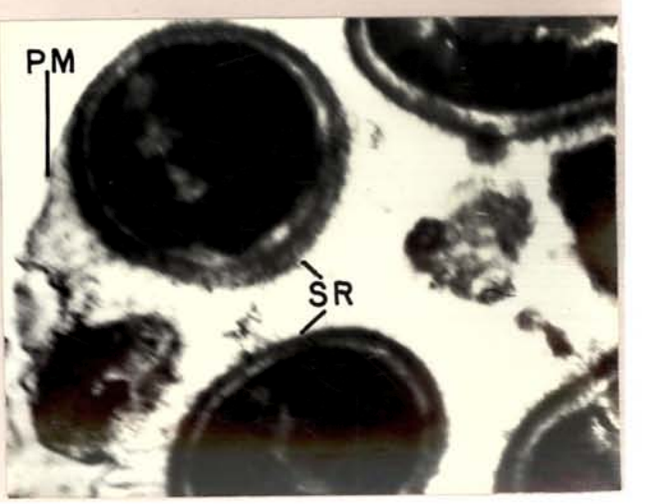
2 µm 3



10 µm 4



20 µm 5



1 µm 6

covered by a pansporoblastic membrane. During sporogony the cytoplasm and the nucleus divide synchronously.

Pansporoblast is a group of eight sporoblasts of equal size covered by a thin, sub-persistent, single layered pansporoblastic membrane (Pl. X, Fig. 4). Later, during metamorphosis, these sporoblasts transform into spores (Pl. X, Figs. 5 and 6). The mature pansporoblast is spherical and measures about 12 µm in diameter. The space between the pansporoblastic membrane and sporoblasts or spores is filled up with electron-dense fibrous material (Pl. XI, Fig. 1). This material has a stratified appearance with fibres of different layers often running in different directions (Pl. XI, Fig. 2). Several pansporoblasts are again surrounded by a membrane which separates them from the host tissue (Pl. XI, Fig. 3). Whether this membrane originates from the host tissue is not confirmed.

Spore: Spores are ovoid with rounded posterior and slightly pointed anterior end (Pl. XI, Fig. 4) and measure 5.0 to 5.5 × 2.5 to 3.5 µm in size in the fresh material. They are uninucleate. At the posterior region of the spore, there is a vacuole which occupies about 40 percent of the total volume of the spore. It has a characteristic shape and appears flattened with a straight anterior margin. In the light photomicrograph, one dot is seen on the either

PLATE XI

Thelohania semisulcata sp. nov. in Penaeus semisulcatus

Fig. 1. Electron micrograph showing the stratified, electron-dense, fibrous material(arrows) filled up between the sporoblasts (SB) and pansporoblastic membrane (PM). Basal part of the developing polar tube (BP) is seen in one sporont whereas in the other, the developing polar tube is visible in cross section (PT).

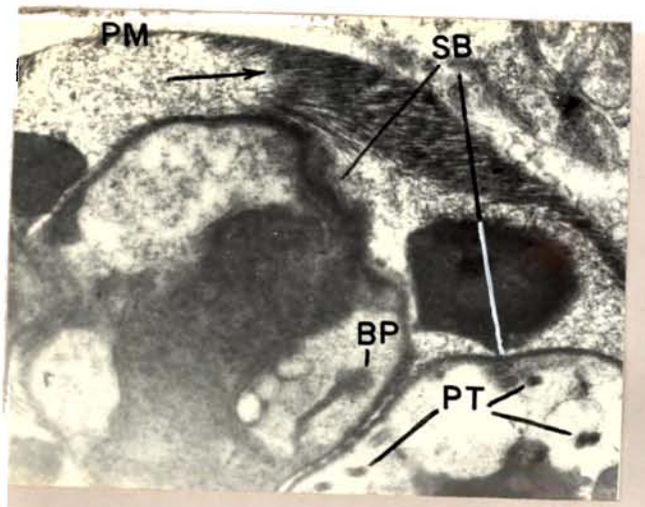
Fig. 2. Electron micrograph showing pansporoblast with mature spores (SR). Note the fibrous material (arrows) running in different directions inside the pansporoblastic cavity. On the right side (below), part of another pansporoblast is seen; several such pansporoblasts are surrounded by a membrane(MB) which separates them from the host tissue.

Fig. 3. Electron micrograph showing the membrane(MB) surrounding the group of pansporoblasts. MT=Muscle tissue of the host; OB=Octosporoblasts; PB=Pansporoblast with mature spores; SP=Sporont.

Fig. 4. Spores of Thelohania semisulcata exhibiting the characteristic appearance of the posterior vacuole(PV). Arrows show presence of dot-like structure probably representing the coiled polar tube. Wet mount.

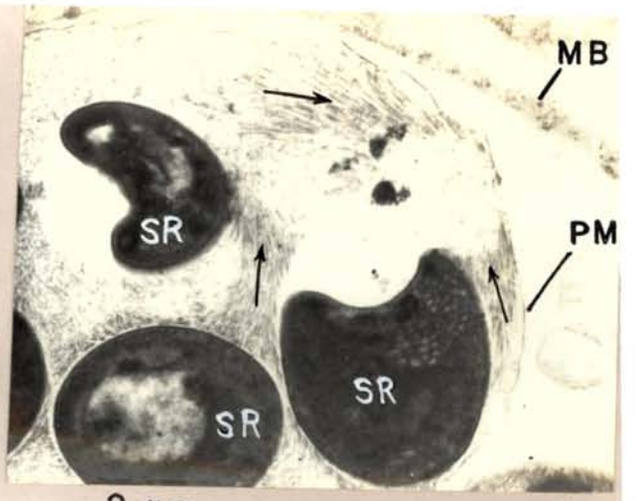
Fig.5-6. Electron micrographs of spores of Thelohania semisulcata from ovary. The spores in this organ possess thick exospore(EX) ornamented with appendages (AD) while the endospore (EN) is comparatively thin. PM=Pansporoblastic membrane.

PLATE XI



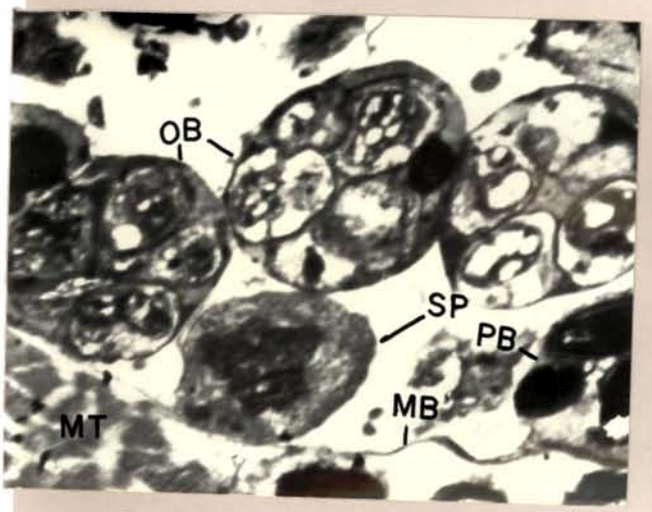
1 µm

1



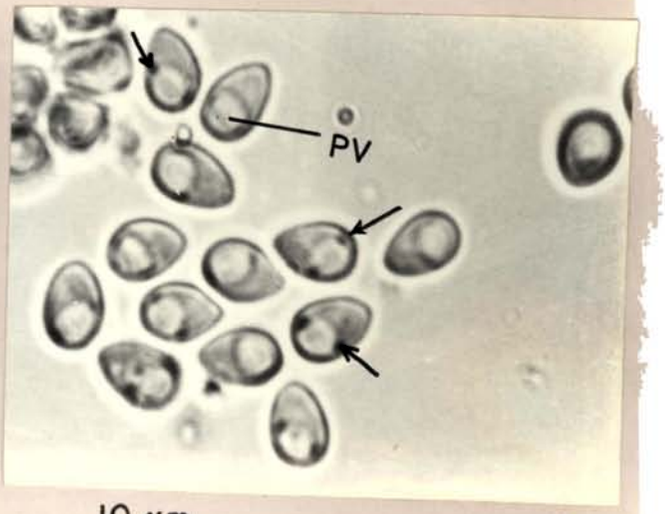
2 µm

2



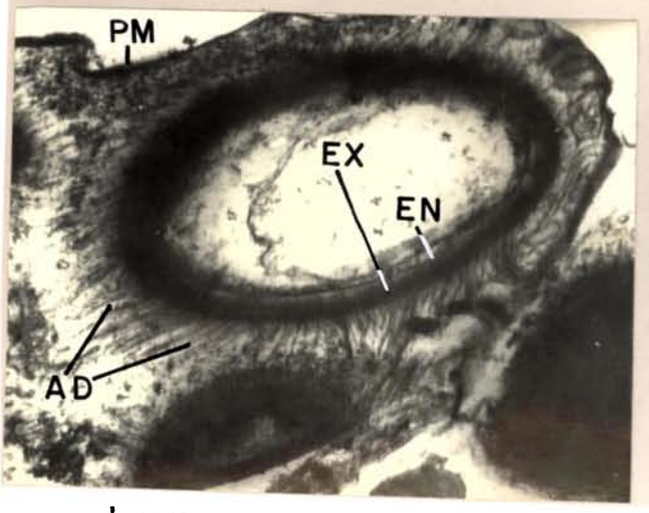
5 µm

3



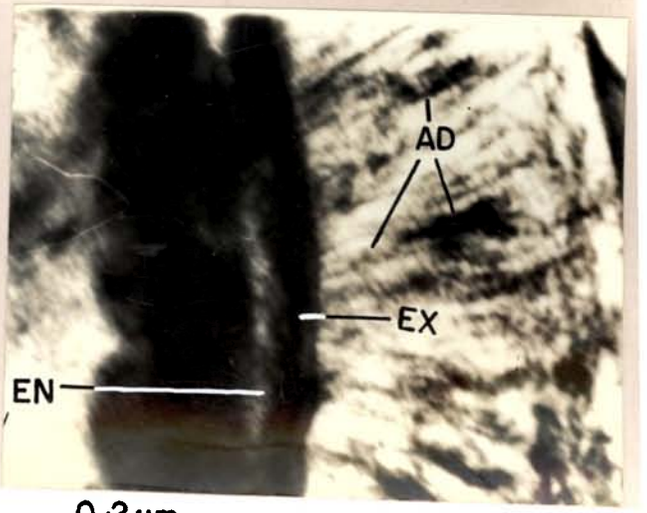
10 µm

4



1 µm

5



0·2µm

6

side of the vacuole on the outer margin (Pl. XI, Fig. 4) which probably represents the coiled polar tube.

The spore wall is trilaminar in structure consisting of an outer electron-dense layer, the exospore; an electron-transparent middle layer, the endospore, and an inner plasma membrane bounding the cytoplasmic contents of the spore. The structure of the exospore and endospore is found to be different in ovarian and muscle tissues. In ovary, the exospore is smooth, thick and ornamented with appendages and the endospore is comparatively thin (Pl. XI, Figs. 5 and 6), while in muscle, the exospore is finely corrugated and void of appendages and the endospore is comparatively thick (Pl. XII, Figs. 1 to 3). Each spore possesses a small, spherical nucleus in the centre but sometimes its position may change. The nucleus appears dark grey to black when stained with Heidenhain's haematoxylin. The spores are PAS positive and show a clear small polar cap atop at the anterior end.

The polar tube is about 14 to 22 µm in length (extruded with hydrogen peroxide treatment) and is isofilar, nearly uniform in diameter from base to distal end (Pl. XII, Fig.4). In ultrathin section it is visible forming about eight coils around the posterior vacuole (Pl. XII, Fig. 2).

In histological preparations of some lightly infected ovaries of P. semiculcatus, oval, round or bean-shaped

PLATE XII

Thelohania coniculata sp. nov. in Lonagus scoliculatus

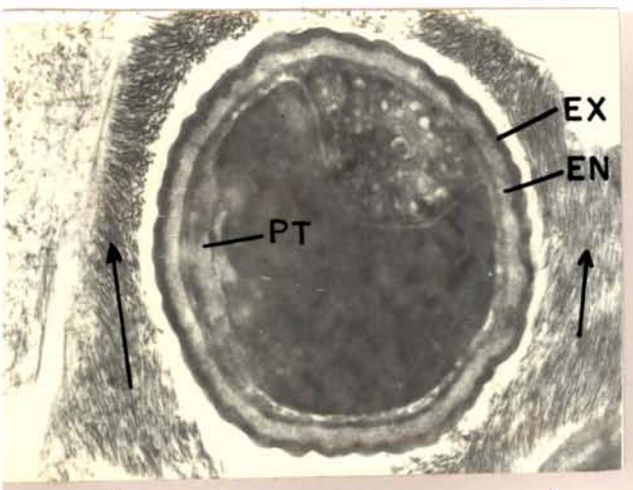
Figs. 1-2. Electron micrographs of Thelohania coniculata spores from the abdominal muscle. Note the fairly constricted exospore lacking the [illegible] and comparatively thick endospore. Note also the fibrous material of the sporoblastic cavity. EN= endospore; EX= exospore; PT=Polar tube.

Fig. 3. An enlarged view of Fig. 2 showing the thin exospore (EX), endospore (EN) and polar tube ( ).

Fig. 4. [illegible] showing [illegible] extruded polar tube. [illegible]; = [illegible]. [illegible].

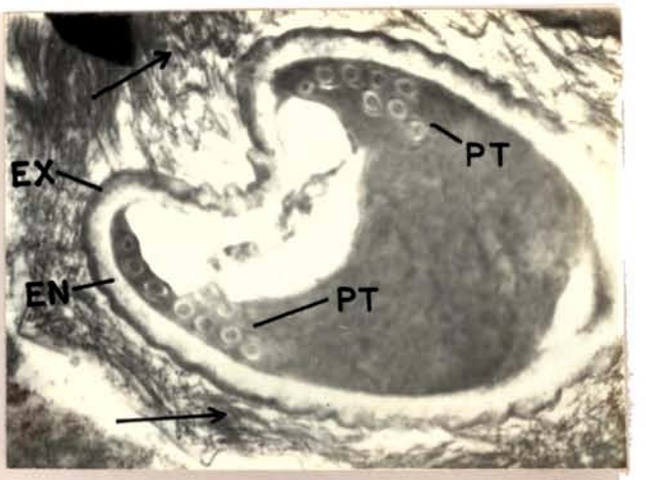
Figs. 5-6 [illegible] of lightly infected ovary of Lonagus scoliculatus: the ovum, young and an advanced stage (I) [illegible] lying in the cytoplasm ( ) of the ova. = clear membrane; [illegible]. [illegible]. Mallory's triple stain.

PLATE XII



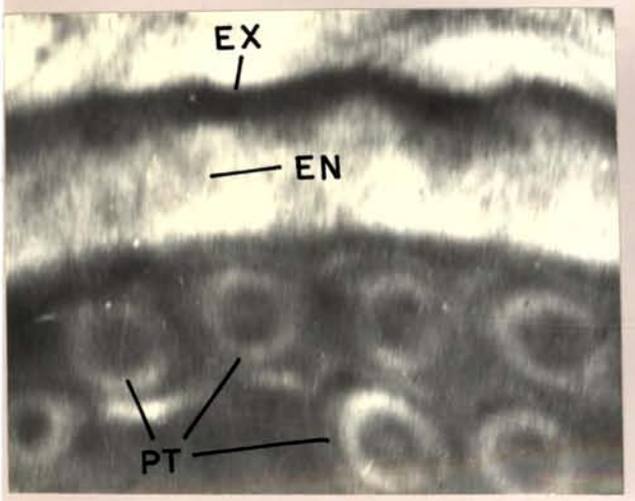
1 μm

1



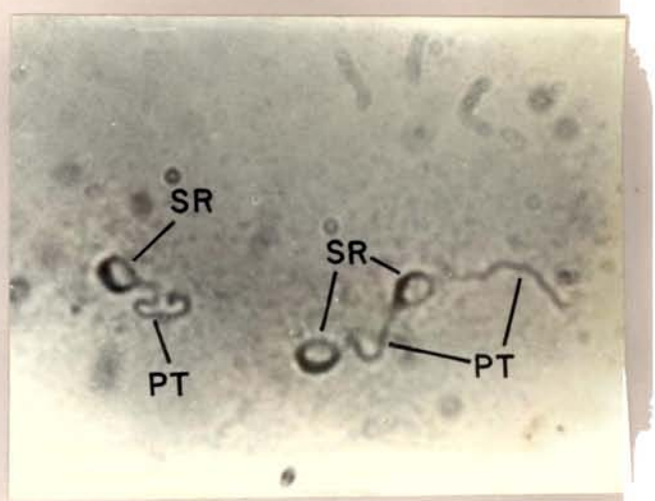
1 μm

2



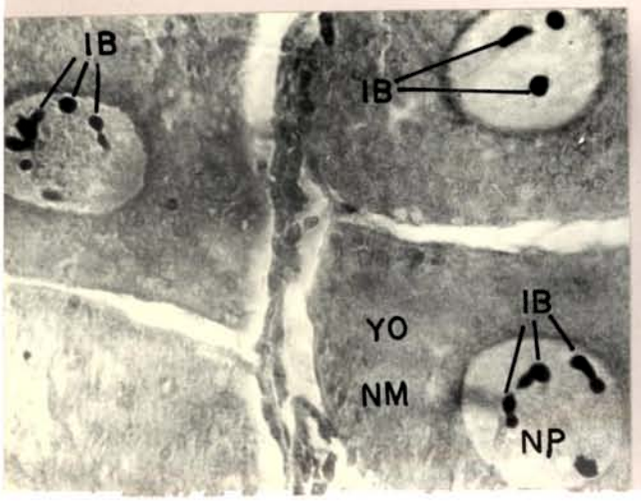
0·1 μm

3



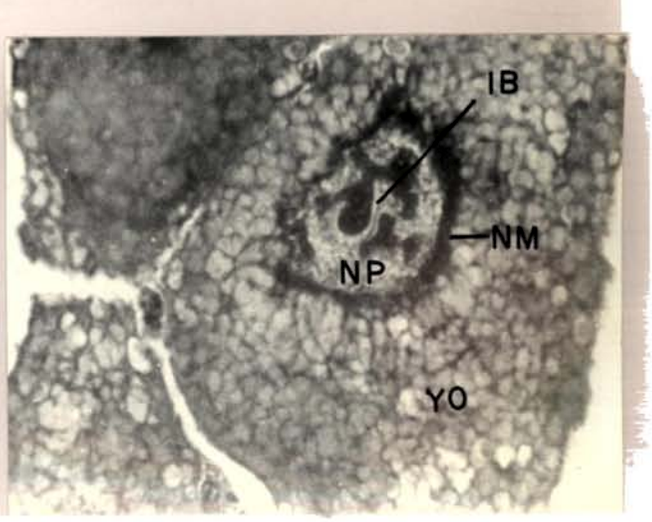
20 μm

4



50 μm

5



20 μm

6

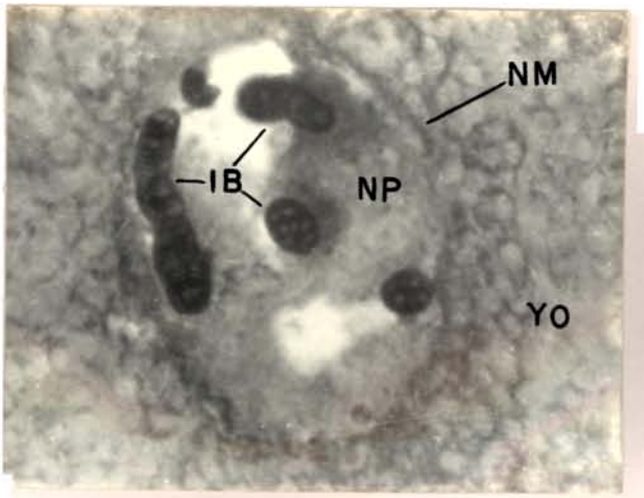
PLATE XIII

Thelohania semisulcata sp. nov. in Penaeus semisulcatus

Figs. 1-2. Transverse sections of lightly infected ovary of Penaeus semisulcatus with unidentified inclusion bodies (IB) in the nucleoplasm (NP) of ova. NM= Nuclear membrane; YO=Yolk. Formalin-Heidenhain's haematoxylin and eosin.

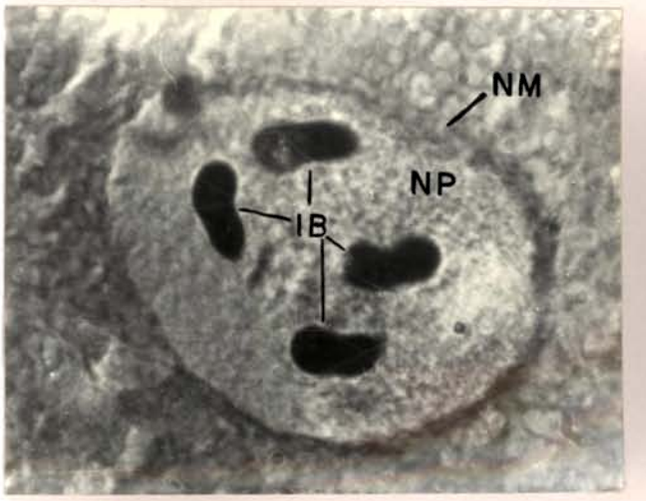
Fig. 3. Semi-thin section of lightly infected ovary: diplokaryotic meront(DY) is seen in one of the degerating ova. Methylene blue, azure II and basic fuchsin combination.

PLATE XIII



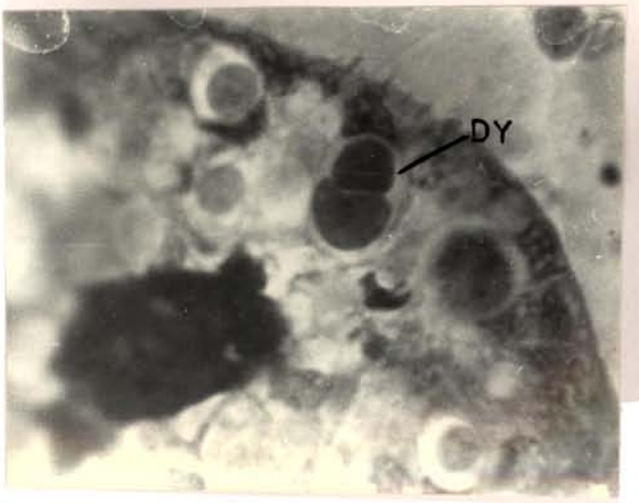
20 µm

1



20 µm

2



10 µm

3

structures of different sizes are found lying in the karyoplasm of the ova of various maturity stages (Pl.XII, Figs. 5 and 6; Pl. XIII, Figs. 1 and 2). In some ova these structures appear to be spores (Pl. XIII, Fig. 2). Diplokaryotic meronts are also been sometimes in the cytoplasm of some of the degenerating ova (Pl. XIII, Fig. 3). These structures, named here as unidentified inclusion bodies (IBB), are basophilic and are stained grayish black with Heidenhain's haematoxylin and magenta pink with Mallory's triple stain.

Remarks: Various species of microsporidia invade the tissues of many crustaceans, most notably the shrimps, prawns and crabs. Sprague (1977) has listed 34 species of microsporidians belonging to 8 genera from decapod crustacean hosts. In marine shrimps and prawns, 17 species of microsporidia belonging to 7 genera, namely, Pleistophora (Family: Pleistophoridae), Thelohania, Nosema, Indosporus, Chapmanium (Family: Thelohaniidae), Burleya (Family: Burleyidae) and Perezia (Family: Perezidae) are known to occur.

The present species shows the characteristic features of the Genus Thelohania. In decapod crustaceans, 11 named and 6 unnamed species of Thelohania have been reported. In the shape and size of the spores, the present species exhibits a general resemblence to Thelohania duorara

Iversen and Manning, 1959, T. maenadis Perez, 1904, T. paguri Perez, 1927, T. butleri Johnston et al., 1978 and Thelohania sp. Vivares, 1973. However, it differs from these species in having different host species and geographical distribution. Johnston et al. (1978) have used such criteria for distinguishing T. butleri from the other species of Thelohania. T. semisulcata could be clearly distinguished from T. giardi Henneguy, 1892 by the shape, size and structure of the spores. In the former species, the spores are ovoid with slightly pointed anterior end while in the latter, they are pyriform with very fine longitudinal striations. The present species also differs from T. ceccaldi Vivares, 1974, T. contejeani Henneguy, 1892, T. octospora Henneguy, 1892, T. petrolisthis Sprague, 1970 and T. sogandaresi Sprague, 1977 in the nature of the pansporoblast as well as spore and the nucleus. T. ceccaldi is characterised by a fusiform pansporoblast, T. contejeani by a non-persistant pansporoblastic membrane with very small nucleus and horse-shoe shaped nucleus, T. octospora by distinctly small spores with U-shaped nucleus, T. petrolisthis by a persistant pansporoblastic membrane and T. sogandaresi by very persistant pansporoblastic membrane and very small spores. In T. semisulcata, the three successive binary fissions of the sporont result in the formation of eight uninucleate, ovoid spores measuring 5.0 to 5.5 × 2.5 to 3.5 µm in size which are covered in a sub-persistant pansporoblastic membrane during sporulation. The pansporoblast is spherical

and measures an average of 12 µm in diameter in fresh condition.

In the shape and size of the spore, structure of the pansporoblast and sites of attack in the host, the present species is found to be closely related to T. duorara. However, a close examination reveals that the spore in the present species, although shows an ovoid shape, its anterior end is more pointed than that of T. duorara. The structure of the vacuole also differs in that the anterior margin of the vacuole is straight - a characteristic feature of the present species - unlike the spherical nature in T. duorara. The polar tube is only 14 to 22 µm long in the present species, whereas in T. duorara it is relatively longer (32 µm). Another significant difference is that the spores inside the pansporoblast of T. duorara are unequal in size whereas in the present material, spores are equal in size. Thelohania sp. described by Thomas (1976) comes from the same locality and the host from where the present species is also described. While the incomplete description given by Thomas (1976) makes it difficult to attempt a detailed comparison with that species, the size of the sporont and the structure of the posterior vacuole in the spores differ between these two species. The salient features of T. duorara Iversen and Manning, 1959 and Thelohania sp. Thomas, 1976 along with the characters noticed in the present species are given in Table 2. From this comparison and the differences noticed

Table 2. Comparison of different species of Thelohania described in panaeid prawns with the present species

Characters	Thelohania duorara Iversen and Manning, 1959	Thelohania sp. Thomas, 1976	Present species
1. Host	Penaeus duorarum, P. brasiliensis and P. aztecus.	Penaeus semisulcatus.	Penaeus semisulcatus.
2. Site of infection	Musculature, heart, gonads, brain and musculature.	Gonad and muscles.	Body muscle, hepatopancreas, gonad, midgut, heart, eyes and gills.
3. Locality	Coast of Florida, U.S.A.	South India (Mandapam).	Southeast coast of India-Tuticorin and Mandapam in the Gulf of Mannar and Palk Bay side of Rameshwaram.
4. Vegetative stages:			
Meront	Data not available.	Data not available.	Meronts are small sperical cells, 3 to 4 µm in diameter; each cell with a single nucleus about 2 µm in diameter; merogony by multiple and binary fission.
Diplokaryon	Data not available.	Data not available.	Large diplokaryotic cells, about 4 to 7 µm in diameter.
5. Sporulation stages:			
Sporont	Data not available.	Sporonts exhibit different sizes and stages of development.	Sporont, a big round cell, 6 to 8 µm in diameter; with a large nucleus, 4 to 5 µm in diameter. Early sporont possesses a slightly thick wall with irregularly spaced patches of dense material deposited on the inner side of the wall.
Sporogony	Data not available.	Sporonts exhibits different sizes and stages of development.	Sporogony by a series of three successive binary fissions of the sporont (the sporogonial mother cell) giving rise to eight uninucleate sporoblasts covered by a pansporoblastic membrane. During sporogony cytoplasm and nucleus divide synchronously.

Characters	*Thelohania duorara* Iverson and Manning, 1959	*Thelohania* sp. Thomas, 1976	Present species
Pansporoblast	Live sporonts rounded measuring about 11 µm in diameter; the eight spores are apparently surrounded by a thin pansporoblastic membrane.	Sporonts round, measuring 3 to 13 µm (preserved) in diameter; each pansporoblast with eight spores of equal size enclosed in a membrane.	Mature pansporoblast spherical, about 12 µm in diameter, with eight spores of equal size covered by a thin, sub persistent, single layered pansporoblastic membrane; space between the membrane and spores filled up by dense fibrous material which does not obscure the spores; spores readily visible in the pansporoblast.
6. Spore:			
Shape	Ovoid, anterior end rounded (based on the diagram given by Iversen and Manning, 1959).	Ovoid, free spores, slightly more pointed than those of *T. duorara*.	Ovoid, with rounded posterior and slightly pointed anterior end.
Size	5.4 X 3.6 µm (live) 5.7 X 4.2 µm (preserved).	4.5 to 5.5 X 3.13 to 3.75 µm (preserved).	5.0 to 5.5 X 2.5 to 3.5 µm (live).
Posterior vacuole	Rounded.	Rounded.	Posterior vacuole occupies about 40 per cent of the total volume of spore, has a characteristic shape and appears flattened with a straight anterior margin.
Polar cap	Data not available.	Data not available.	Present, PAS positive.
Nucleus	Data not available.	Data not available.	Single, small, spherical nucleus present.
Polar tube	About 38 µm long, uniform in diameter.	Data not available.	About 14 to 22 µm long, isofilar (uniform in diameter).
Spore wall	Spore membrane shows no striations.	Data not available.	Spore wall trilaminar in structure consisting of an outer electron-dense exospore, electron-transparent endospore and an inner plasma membrane. Spores from infected ovary possess smooth, thick exospore ornamented with appendages; endospore comparatively thin. Spores from infected muscle have finely corrugated exospore lacking appendages; endospore thin.

from the other microsporidian species described in decapod crustaceans, it is clear that the present species could be considered as new to science and is named as Thelohania semisulcata, the specific name being assigned on its occurrence in the host, Penaeus semisulcatus, which supports a fishery on the southeast coast of India.

Type specimen: Holotype slide is being deposited in the Zoological Survey of India, Calcutta.

SULCOVARIA MANNARENSIS GEN. ET SP. NOV.

Host and site: The green tiger prawn, Penaeus semisulcatus de Haan, ranging in size from 152 mm to 166 mm in total length measured from tip of rostrum to the tip of telson. The site of infection is found to be restricted to the ovary of the adult female prawns (Pl. V, Fig. 6; Pl. XIV, Fig. 1).

Locality: Rameswaram in the Gulf of Mannar and Palk Bay on the southeast coast of India. The infected prawns were collected from the shrimp trawl net operation at 14 to 20 meter depth off Rameswaram.

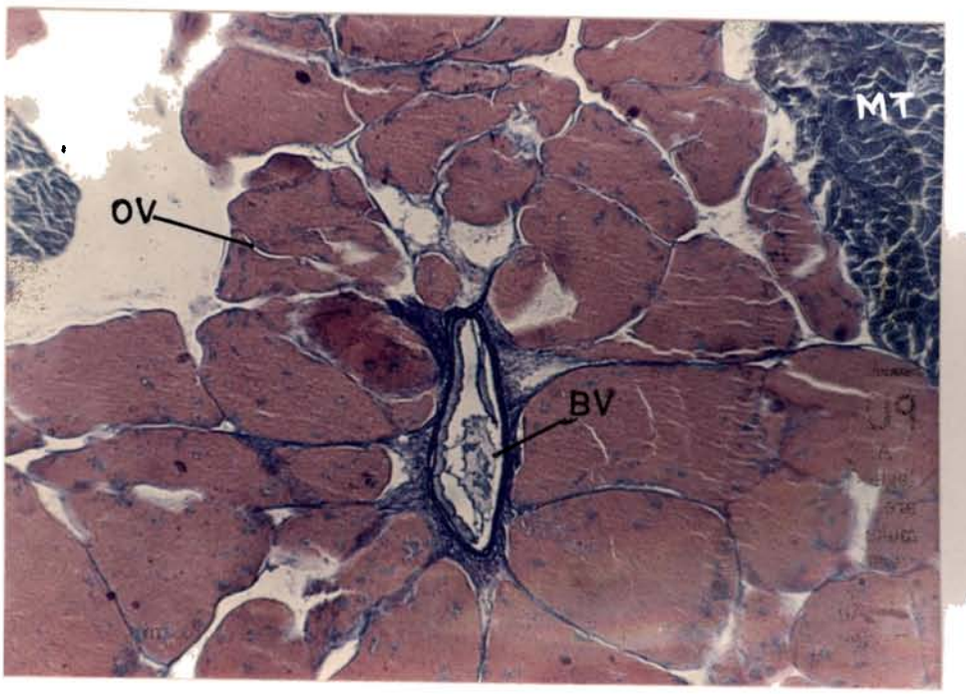
Vegetative stages: The earliest stage of development observed is a meront or a schizont, a vegetative cell which undergoes binary or multiple fission. Meronts are usually spherical. Polygonal meronts are also encountered occasionally

PLATE XIV

Sulcovaria mannarensis gen. et sp. nov. in Penaeus semisulcatus

Fig. 1. Penaeus semisulcatus: Cross section of the abdominal region along with the ovary. Note the heavy infection by Sulcovaria mannarensis in the ovary (OV) which is hypertrophied and has surrounded the blood vessel (BV) from all the sides. The muscle (MT) is apparently uninfected. Infected region is yellow-orange while the blue stained regions are uninfected. Modification to the technique of Mallory (1944).

PLATE XIV



I

(Pl. XV, Figs. 1 to 3). Meronts measure 5 to 6 µm in diameter in preserved material. When stained with Heidenhain's haematoxylin and eosin, the cytoplasm is stained deep pink with eosin. A small, spherical nucleus is discernible in the centre with dark grey to black colour. Meronts divide by binary or multiple fission with direct nuclear division. However, the nuclear division is not followed by immediate constriction of the cytoplasm. Prior to the onset of sporogony, in each meront, two nuclear divisions occur which finally give rise to four nuclei. In the subsequent stage, the tetranucleate cell divides into two daughter cells, each containing two nuclei. These diplokaryotic cells are the final product of merogony (Pl. XV, Fig. 4). Before entering into the sporulation phase, the chromatin of these two nuclei breaks up into fine granules and the fusion of both the nuclear substances follows (Pl. XV, Fig. 5) and results in the formation of sporogonial mother cell or sporont with a single, large, spherical nucleus in the centre (Pl. XV, Fig. 6; Pl. XVI, Fig. 1).

Sporulation stages: Early sporont is spherical and measures 6 to 8 µm in diameter. It is thick walled, unlike the thin walled nature of the meront. The sporont possesses a large, distinct nucleus in the centre surrounded by granular cytoplasm (Pl. XV, Fig. 6; Pl. XVI, Fig. 1). The sporont then grows in size, and undergoes three binary

PLATE XV

Sulcovaria mannarensis gen. et sp. nov. in *Penaeus semisulcatus*

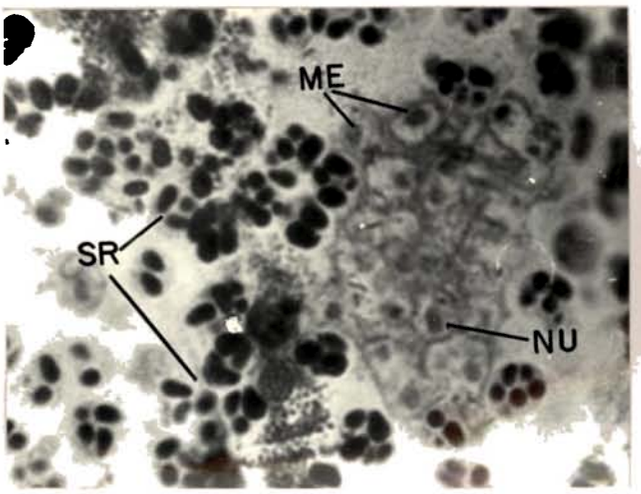
Figs. 1-3 Transverse section of ovary of the prawn to show the spherical and polygonal meronts (ME). The process of nuclear division prior to the onset of sporogony is continued in some of the meronts in Figs. 2 and 3(arrows). NU=Nucleus; SP=Mature spores. Semi-thin section-Toluedine blue.

Fig. 4. Semi-thin section of the ovary of the prawn. A diplokaryotic cell, the final product of merogony, is seen in the centre (arrow). SP=Mature spores. Semi-thin section-Toluidine blue.

Fig. 5. Electron micrograph showing the early stage of sporogony. Arrow shows the fusion (karyogamy) of the chromatin material of a diplokaryotic cell.

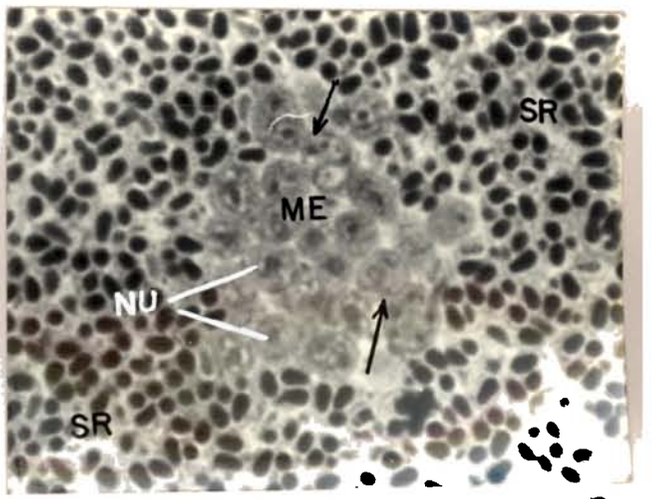
Fig. 6. Sporonts (SR) of *Sulcovaria mannarensis*. GR=Granular cytoplasm; NU=Nucleus; SP=mature spores.

PLATE XV



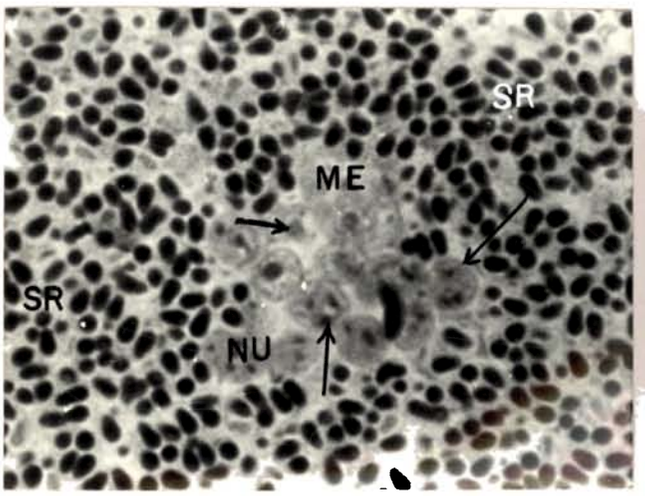
20 µm

1



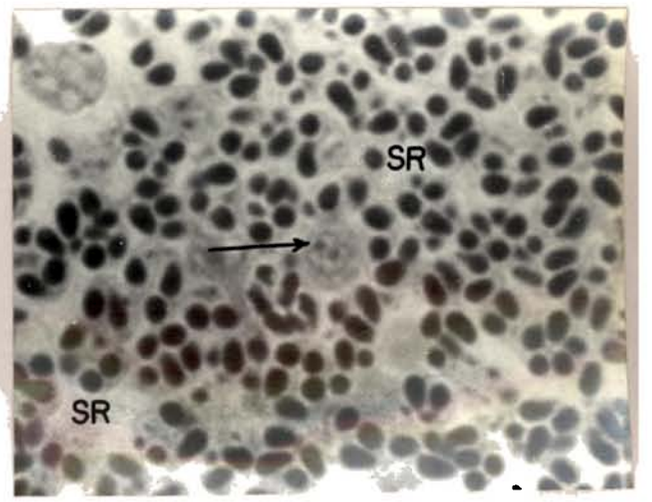
20 µm

2



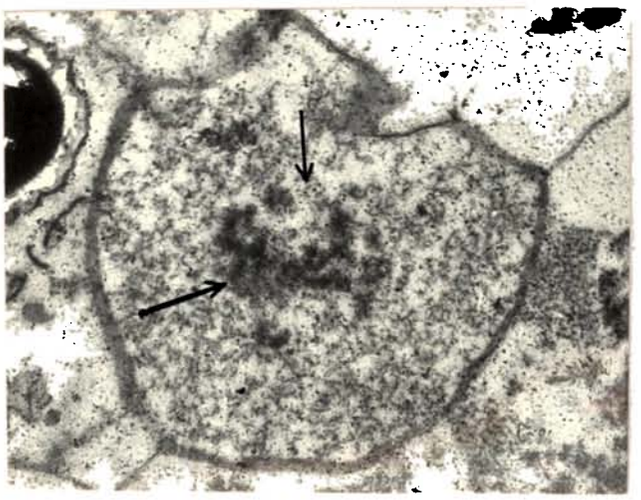
20 µm

3



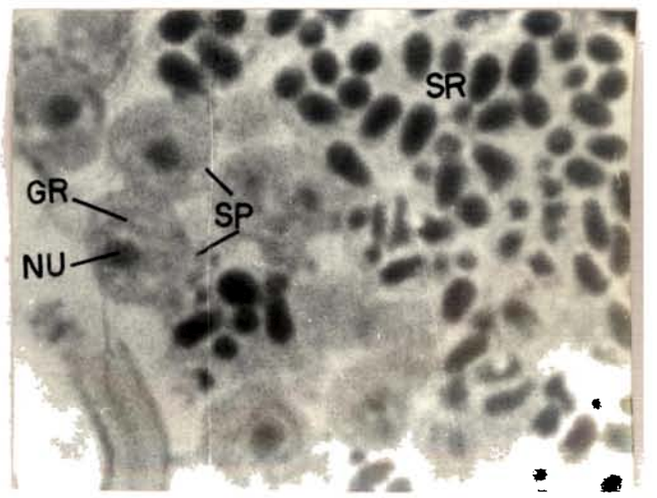
20 µm

4



1 µm

5



10 µm

6

PLATE XVI

Sulcovaria mannarensis gen. et sp. nov. in Penaeus semisulcatus

Fig. 1. Electron micrograph showing ultrastructural view of the sporont (SP) of Sulcovaria mannarensis GR=Granular cytoplasm; NU=Nucleus.

Figs.2-6. Different stages of sporogony in Sulcovaria mannarensis:

Fig. 2. Semi-thin section of the ovary to show the first division in the sporont (arrows). Toluidine blue.

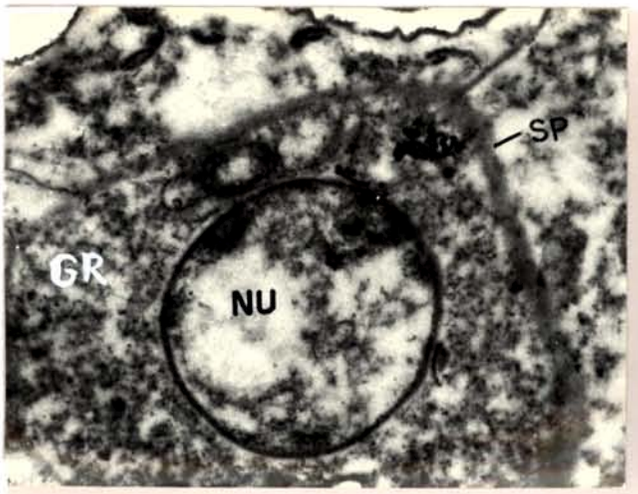
Fig. 3. Semi-thin section of the ovary to show the two-cell stage (arrow) following the first division. Toluidine blue.

Fig. 4. Electron micrograph of the two-cell stage. MG=Metabolic granules; PM=Pansporoblastic membrane.

Fig. 5. Smear preparation showing the four-cell stage (arrow). Methanol-Giemsa.

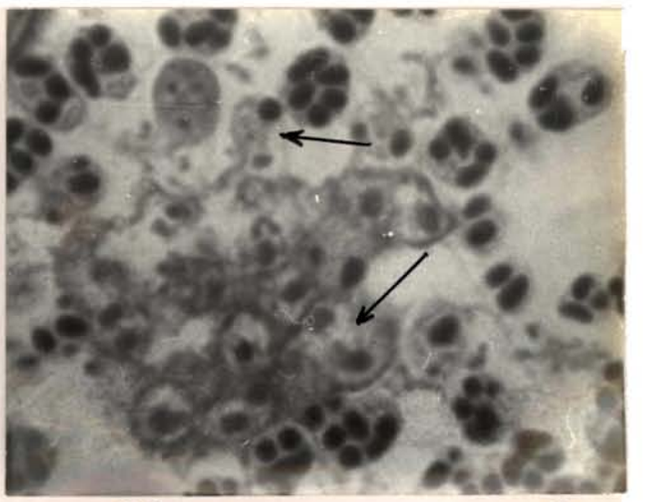
Fig. 6. Electron micrograph of four-cell stage. Abbreviations same as Fig. 4.

PLATE XVI



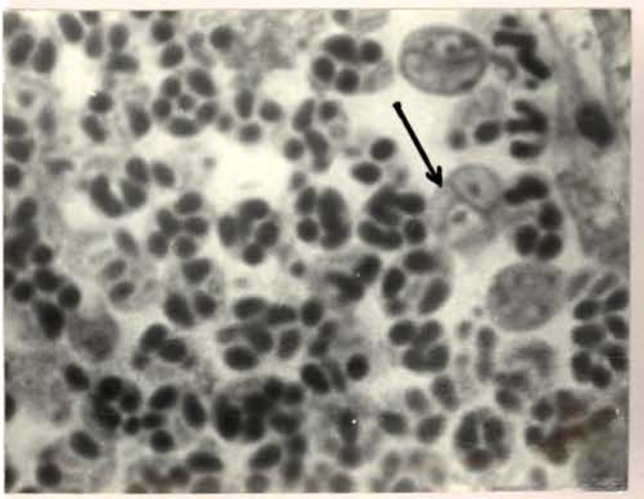
1 µm

1



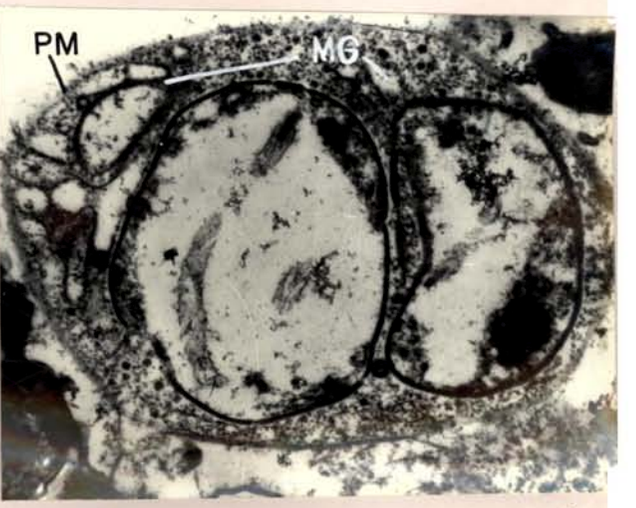
20 µm

2



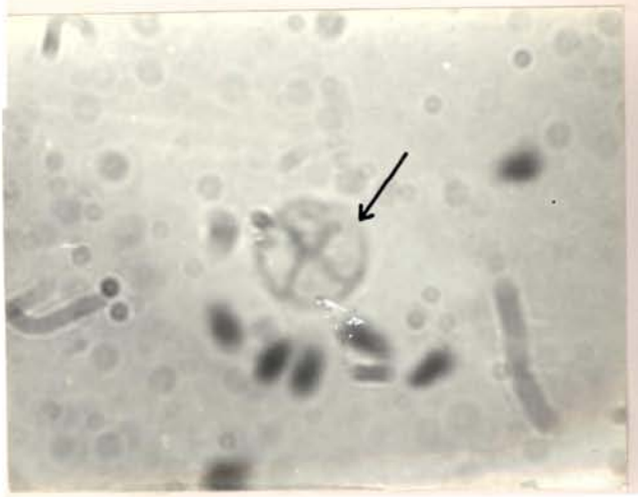
20 µm

3



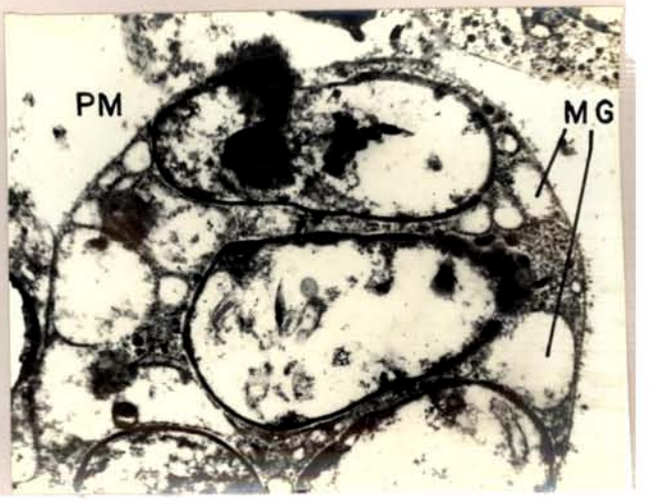
2 µm

4



10 µm

5



2 µm

6

divisions ultimately producing eight uninucleate sporoblasts enclosed within a pansporoblastic membrane (Pl. XVI, Figs. 2 to 6; Pl. XVII, Figs. 1 to 3). In this process, the cytoplasm and the nucleus are seen to divide synchronously. Dividing sporonts secrete metabolic products in the form of granules inside the pansporoblastic membrane (Pl. XVII, Fig. 4). These granules often clump together to form large dense masses which subsequently assume the shape of microtubules during sporulation (Pl. XVII, Figs. 5 and 6). Pansporoblast is sub-spherical or oval (Pl. XVII, Fig. 3) and has a fragile membrane that soon ruptures and frees mature spores readily.

Spore: Spores are pyriform, usually measuring 3 to 4.2 X 1.5 to 2.0 µm in fresh material. Occasionally, larger spores measuring about 5.5 X 3.0 µm are also encountered. Both these micro- and macro spores have identical sturctures but differ in size (Pl. XVIII, Figs. 1 and 2). In the light microscopic examination, each spore is found to possess a single, small, dot-like nucleus situated near the centre. Phase contrast microscopic observations of unstained, Giemsa stained and PAS stained smears reveal the presence of a spherical vacuole at the posterior end of the spore(Pl. XVIII, Fig. 3). A similar, but comparatively smaller, vacuole-like bright area is noticed at the anterior end of the spore which is presumbaly the polaroplast. A polar cap at the anterior end is also seen.

PLATE XVII

Sulcovaria mannarensis gen. et sp. nov. in Penaeus semisulcatus

Figs. 1-3. Stages of sporogony in Sulcovaria mannarensis:

1. Electron micrograph of octosporoblast; only six cells could be seen in the ultra-thin section. Scattered black patches are staining artifacts. MG=Metabolic granules; PM=Pansporoblastic membrane.

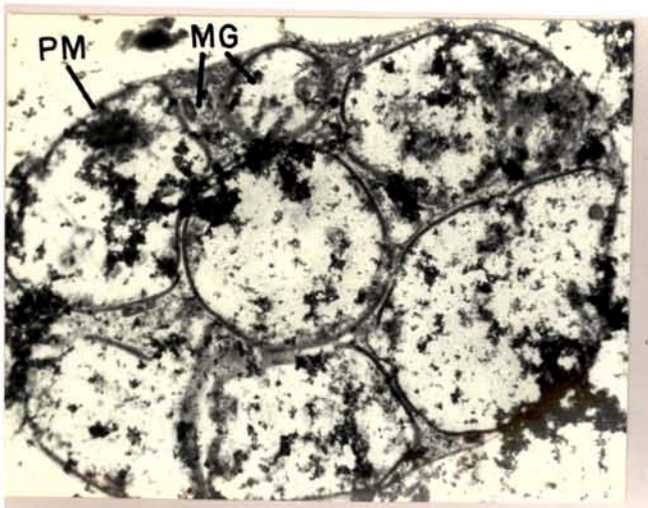
(2) Semi-thin section of the ovary showing octosporoblasts (OB) with pansporoblastic membrane. SR=Mature spores.

(3) Immature (OB) and mature (M) pansporoblast Note the nucleus (NU) in the sporoblasts. Wet mount.

Fig. 4. Electron micrograph showing the presence of granules (arrows) inside the pansporoblastic membrane (PM). S =Mature spores.

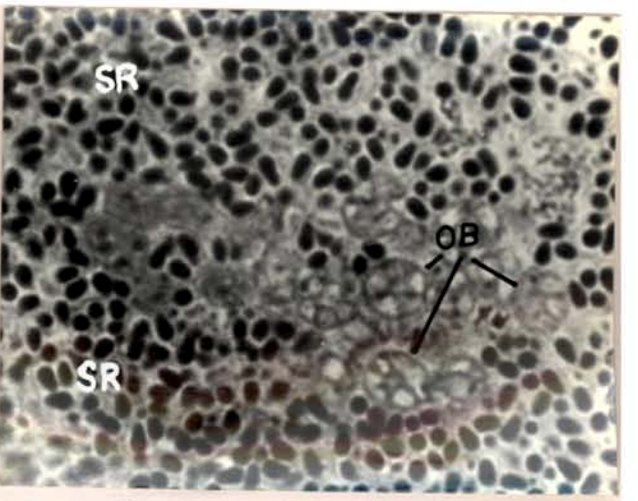
Figs. 5-6 Electron micrographs of mature pansporoblasts. Note the microtubules(arrows) inside the pansporoblasts derived from clumping of metabolic granules. PM=Pansporoblastic membrane; SR=Mature spores.

PLATE XVII



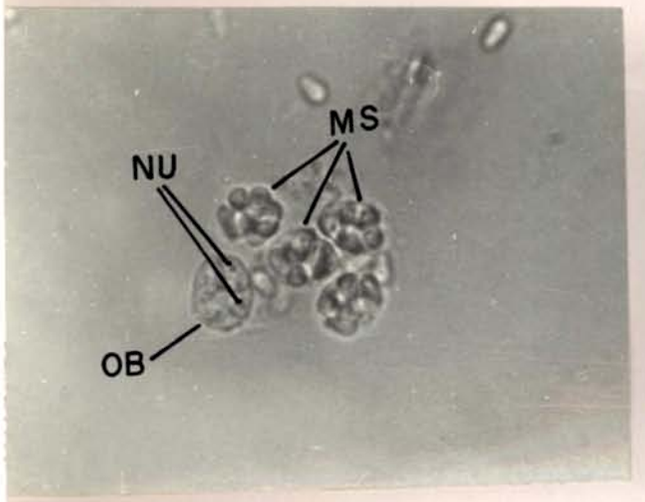
2 µm

1



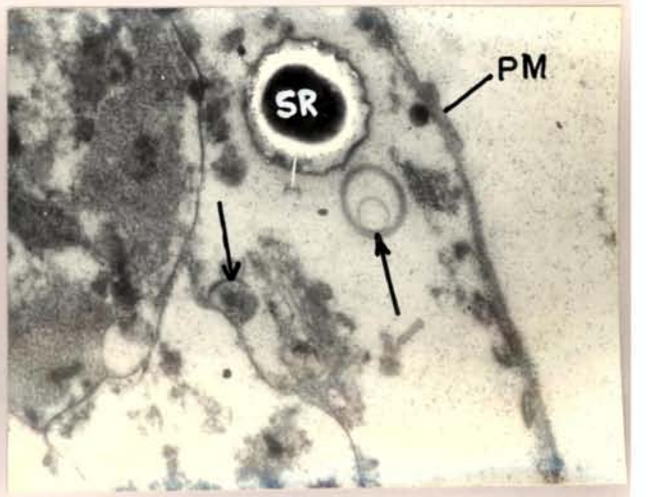
20 µm

2



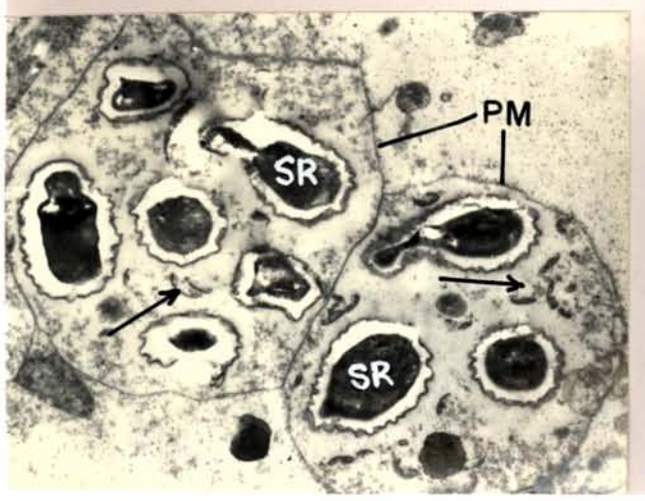
20 µm

3



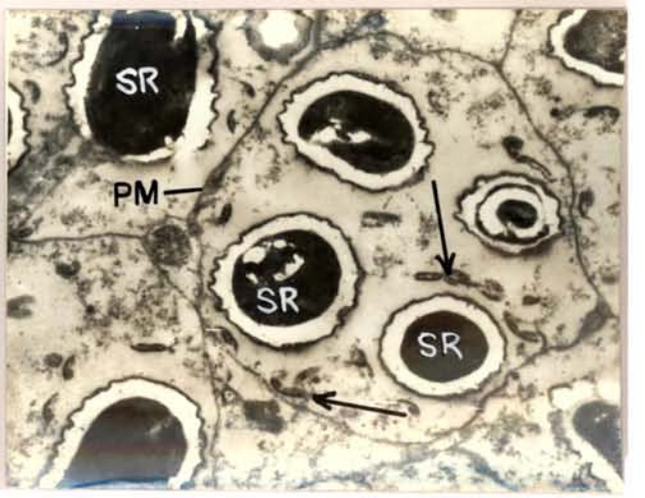
2 µm

4



5 µm

5



5 µm

6

The spore has an indistinct polaroplast and a long anisofilar polar tube. The proximal portion of the polar tube is stumpy and forms 2½ to 3 coils inside the spore; at this region the polar tube absuptly constricts to a very much narrow distal portion and forms about 9 to 10 coils (l. XVIII, Figs. 4 and 5). The spore wall has a thick, corrugated, electron-dense exospore and a thinner electron-lucent endospore (l. XVIII, fig. 6).

Remarks: The structure of meront, sporont, and mode of sporogony as well as the structure of the spores of the present species reveal that it belongs to the Family Thelohaniidae, where sporulation always occurs within a pansporoblastic membrane, usually resulting in eight uninuclear spores (Sprague, 1982). The Family Thelohaniidae contains 11 genera, namely, Thelohania, Tomasoma, Chermanium, Cryptosporina, Heterosporus, Indosporus (= Orthothelohania), Ormieriosia, Pegmatheca, Pilosporella, Octenostroma and Toxoglugea (Sprague, 1982). The only genera to which the present species is conceivably closely related are Thelohania and Tomasoma.

Thelohania is one of the oldest genera of microsporidia, established as early as 1892 by Henneguy. In the same year, Thelohan (1892) placed this genus in a new family, Glugeidae (Glugeidees). Since then, this genus was assigned differently by different authors to Family Glugeidae (Hurley, 1893; Leger and Hesse, 1922),

PLATE VIII

Sulcovaria mannarensis gen. et sp. nov. in *Penaeus semisulcatus*

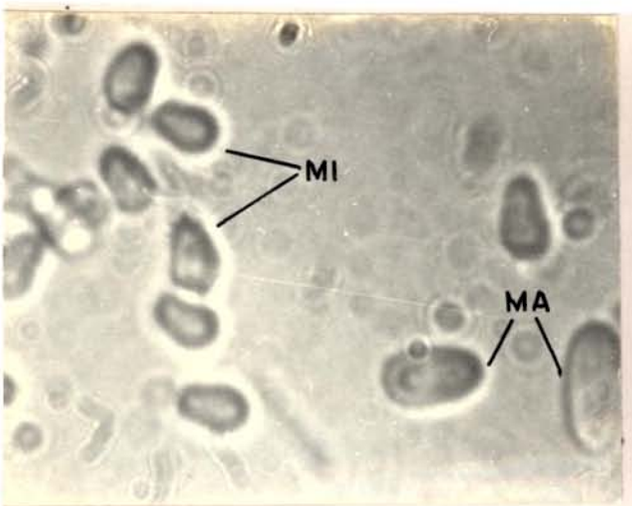
Figs. 1-2. Free spores of *Sulcovaria mannarensis*. Note the presence of macrospores (MA) along with the microspores (MI). Wet mount.

Fig. 3. Phase contrast photomicrograph of spores showing spherical vacuole at the posterior end (arrows). Crossed arrow shows the polaroplast at the anterior end of spore. Wet mount.

Figs. 4-5 Electron micrographs of *Sulcovaria mannarensis* spores. DL=Distal portion of polar tube; EX=Exospore; PP=Proximal portion of polar tube; SA=Shrinkage artifact in the region of endospore.

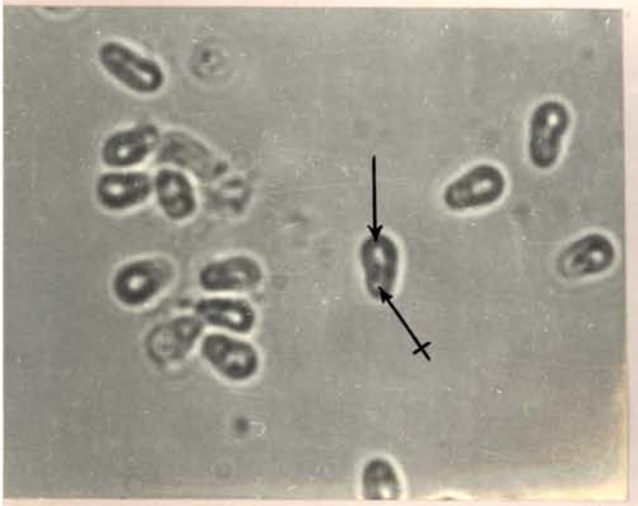
Fig. 6. Electron micrograph of *Sulcovaria mannarensis* spore showing the spore wall. EX=Exospore; EN=Endospore.

PLATE XVIII

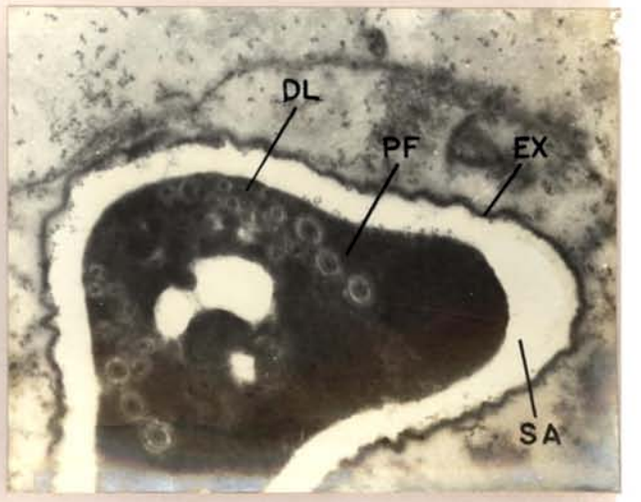


10 µm 1

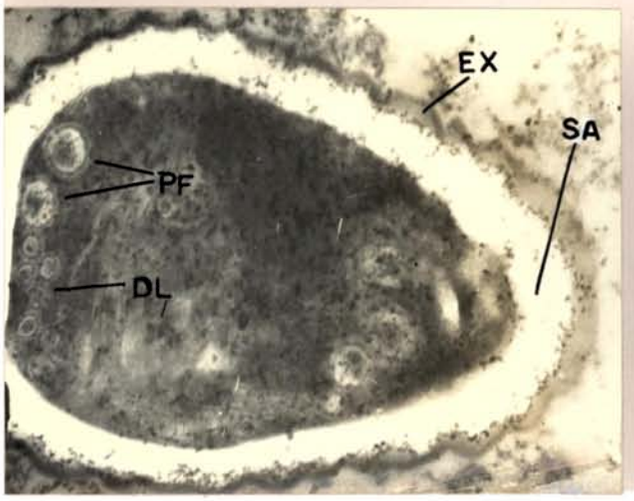
10 µm 2



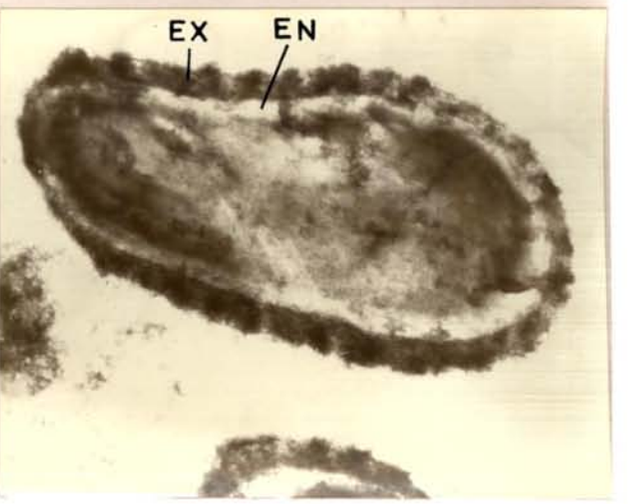
10 µm 3



2 µm 4



1 µm 5



1 µm 6

Nosematidae (Labbe, 1899; Stempell, 1909; Kudo, 1924) and Polysporidae (Tuzet et al., 1971). It was only recently that Hazard and Oldacre (1975) revised the Genus Thelohania and other closely related genera and erected a new Family, Thelohaniidae.

Species of the Genus Thelohania have only one known developmental sequence which produces eight spores. Sporogony is accomplished by means of endogenous budding which is sometimes accompanied by secretion of small dense granules. The pansporoblasts are sub-spherical, having a sub-persistent pansporoblastic membrane. Spores are oval or pyriform, without tails and possess a distinct posterior vacuole, a thin exospore wall, one isofilar polar tube and a polaroplast composed of tightly compressed lamellae.

The other Genus Agmasoma, to which the present species also resembles in certain characters, was created by Hazard and Oldacre (1975) after they re-examined the species, Thelohania penaei Sprague, 1950 infecting the gonads of P. setiferus inhabiting the coast of Louisiana and Florida (U. . ...). These authors found that the mode of sporogony and the polar tube in T. penaei were different from those of the Genus Thelohania. On the basis of these differences they transferred T. penaei to a new genus, namely, Agmasoma with the only known Species A. penaei (Sprague, 1950) (Hazard and Oldacre, 1965). The anisofilar type of polar tube and

sporogony by fragmentation of sporogonial plasmodium were the taxonomic criteria employed to distinguish Agmasoma from Thelohania. The name Agmasoma, meaning "fragmentary body" was referred to the type of cytoplasmic division at the time of sporulation. There is only one development sequence known in Agmasoma which produces eight spores. The cytoplasmic division in the sporont is delayed and is initiated only after the nuclei undergo three nuclear divisions forming octonucleate plasmodia. These micro-portions do not secret any metabolic substance inside the pansporoblast. Spores of Agmasoma are oval or pyriform, without any surface structure or mucous envelope and possess a thin, smooth exospore, a polar tube abruptly constricting near the middle to form a thick proximal and a thin distal portion, and an indistinct polaroplast.

The important characters thus used to distinguish the species at generic level by Hazard and Oldacre (1975), Sprague (1982) and Weiser (1977), are the mode of development of the stages in sporogony, presence or absence of the metabolic granules inside the pansporoblast, nature of the pansporoblastic membrane, shape of the spore, variation in the diameter of the polar tube and structure of the polaroplast. When such criteria are used in determining the generic position of the present species and a comparison is made with the generic characters of the closely related genera (Table 3), the present species

Table 3. Comparison of the Genera *Thelohania* Hennegyy, 1892 and *Agmasoma* Hazard and Oldacre, 1975 with the present genus

Character	*Thelohania* Hennegyy, 1892	*Agmasoma* Hazard and Oldacre, 1975	Present genus
1. Sporulation sequence	Only one sporulation sequence known producing octospores.	Only one sporulation sequence known producing octospores.	Only one sporulation sequence known producing octospores.
2. Pansporoblast	Pansporoblast sub-spherical containing 8 small oval or pyriform spores; pansporoblastic membrane persistent or may rupture shortly after the pansporoblast is dissected from the host.	Pansporoblast subspherical or oval containing 8 small oval to pyriform spores; pansporoblastic membrane fragile which ruptures soon after the pansporoblast is dissected from the host.	Pansporoblast subspherical or oval containing 8 small pyriform spores; pansporoblastic membrane fragile which ruptures soon after dissecting from the host and frees the mature spores readily.
3. Sporogony	Sporogony by endogenous budding producing 8 sporoblasts within a pansporoblastic membrane.	Sporogony by fragmentation of sporogonial plasmodium producing 8 sporoblasts within a pansporoblastic membrane.	Sporogony a series of 3 successive binary fission producing 8 sporoblasts within a pansporoblastic membrane.

Table 3. continued

Character	Thelohania Hennegy, 1892	Agmasoma Canard and Oldacre, 1975	Present genus
5. Secretion	Dividing sporonts secrete metabolic substances that form granules or crystalliform particles. No data on the transient phenomens.	Dividing sporonts do not secrete any metabolic substance. Giving octospores without mucous envelope.	Dividing sporonts secrete metabolic substances inside the pansporoblastic membrane that form granules. No data on the transient phenomens.
5. Spore	Spores ovoid with large, tightly compressed polaroplast; spore wall (exospore) thin, smooth and without any surface structure.	Spores pyriform with indistinct polaroplast; spore wall (exospore) thin, smooth and without any surface structure.	Spores ovoid with indistinct polaroplast; spore wall (exospore) rather thick, corrugated and without any surface structure.
6. Polar tube	Polar tube of nearly uniform diameter from base to the distal end or only gradually narrowing to the distal end (isofilar).	Polar tube abruptly constricts near the middle to form a much narrower distal end (anisofilar).	Polar tube forms a proximal stumpy and a distal very much narrow portion (anisofilar).

shows similarities with Thelohania in having sporogony by three successive binary divisions (endogenous budding) and secretion of metabolic granules during the sporulation inside the pansporoblast. However, it differs from Thelohania in having an anisofilar polar tube, a fragile pansporoblastic membrane and indistinct type of polaroplast.

On the other hand, the present species agrees with Acmasoma in having an anisofilar polar tube, a fragile pansporoblastic membrane and indistinct polaroplast. Nevertheless, sporogony by endogenous budding and secretion of metabolic granules inside the pansporoblast during the sporulation are the two major characters of the present species in which it apparently disagrees with Acmasoma. The sporulating sporont of A. penaei, as shown in an electron micrograph by Hazard and Oldacre (1975), is squarish with the wall of the developing spores not very clearly distinguishable. However, the sporulating sporont in the present species is spherical wherein the wall of the sporoblasts or spores is discernible at 2, 4 and 8 cell stages (Pl. XVI, Figs. 2 to 6; Pl. XVII, Figs. 1 to 3). The other similarities and differences noticed are given in Table 3.

Hazard and Oldacre (1975) considered the possibility of artifacts resulting from long storage of material in ice causing abnormalities in specimens of Acmasoma. This possibility as the cause of differences observed was also

considered by the present author. However, a careful evaluation of characters and their critical comparisons and the fact that the present material was never stored for periods longer than 5 or 6 hours after its catch and the careful preservation on the material in ice during the period, ruled out such possibilities of artifacts.

In view of the above differences in the present material from those of Thelohania and Agmasoma, a new genus, designated as Sulcovaria, is created here in the Family Thelohaniidae Hazard and Oldacre, 1975. The generic nomenclature Sulcovaria refers to the species of the host from which the microsporidian has been reported and the site of attack (Penaeus semisulcatus; ovary). The generic characters of this new genus are as follows.

Only one sporulation sequence known. Pansporoblast sub-spherical; sporogony by a series of three binary divisions producing eight sporoblasts within a fragile pansporoblastic membrane. Sporulation accompanied by secretion of metabolic granules within the pansporoblastic membrane. Spores usually ovoiform, sometimes oval, with a thick, corrugated exospore wall and an indistinct polaroplast; polar tube anisofilar, having a thick, stumpy proximal and a thin, narrow distal portion. Parasite of penaeid prawns' ovary.

New Species, Sulcovaria mannarensis sp. nov. is also created here to accommodate the present material. Name

of the species, mannarensis, has been derived from the name of one of the two localities from where the pathogen was collected. This species is characterised by the following characters.

Host species: Adult female Penaeus semisulcatus.

Lesion: Infected prawns exhibit opaque and white area along the median dorsal line.

Host tissue infected: Ovary.

Type locality: Gulf of Mannar and Palk Bay off the coast of Rameswaram (southeast coast of India).

Vegetative stages: Meronts spherical, 5 to 6 µm in diameter (fixed) with single nucleus. Merogony by binary or multiple fission. The final stage, a diplokaryotic cell.

Sporulation stages: Sporogony by three binary divisions producing eight sporoblast within a pansporoblastic membrane. Each sporoblast becomes a spore. Spores pyriform, 3 to 4.2 × 1.5 to 2.0 µm, refractile and thick walled, uninucleate, with a small vacuole at the posterior end and polar cap at the anterior end. Polar tube anisofilar, with a stumpy proximal portion forming 2 to 3 coils and a very much narrow distal portion forming about 9 to 10 coils anterio-posteriorly inside the spore.

Type specimen: Holotype slide is being deposited in the Geological Survey of India, Calcutta.

PEREZIA AFFINIS SP. NOV.

Host and site: The jinga shrimp, Metapenaeus affinis (H. Milne Edwards, 1837) of both the sex ranging in size from 97mm to 143 mm in total length measured from tip of rostrum to tip of the telson. Infection found in the body muscles, gonads and digestive tract. Occasionally, the infection also found in the body muscles of the green tiger prawn, Penaeus semisulcatus de Haan.

Locality: Gulf of Mannar and Palk Bay off the coasts of Pamban and Rameswaram on the southeast coast of India. The infected prawns were caught operating a shrimp trawl net unto a depth of about 20 to 25 meters.

Vegetative stages: Not known.

Sporulation stages: Small spherical cells, about 5 µm in diameter are found singly or in pair. They are thick walled sporonts with inner side of the wall having irregularly spaced patches of dense material. Sporonts are found having one, two or four nuclei located usually in the centre (Pl. XI., Figs.1 to 3). In some sporonts, the

PLATE XLV

Perezia affinis sp. nov. in *Metapenaeus affinis*:
Diagrammatic representation of different developmental stages.

Fig. 1. Sporont with one nucleus.

Fig. 2. Sporont with two nuclei.

Fig. 3. Sporont with four nuclei.

Fig. 4. Sporont with eccentric nucleus.

Fig. 5. Sporont with eccentric nuclei.

Fig. 6-7. Sporonts with semicircular body.

Fig. 8. Paired sporoblast with cytoplasmic bridge.

Fig. 9. Sporoblasts after the loss of cytoplasmic bridge, lying close to each other.

Fig. 10. Independent sporoblasts with immature spore inside.

Fig. 11. Thread-like structure inside the spores.

Fig. 12. Micro- and macrospores.

Fig. 13. Spores with posterior vacuole.

Fig. 14. Spore with extruded polar tube.

PLATE XIX

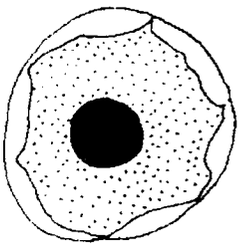


Fig. 1

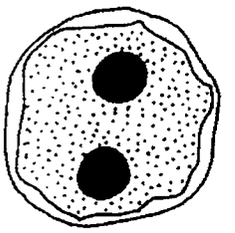


Fig. 2

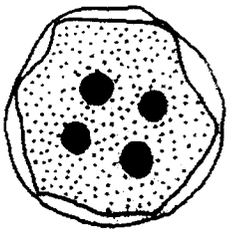


Fig. 3

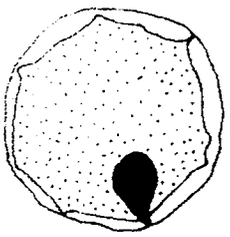


Fig. 4

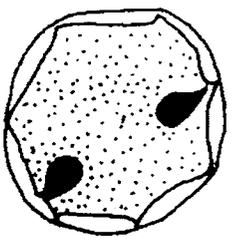


Fig. 5

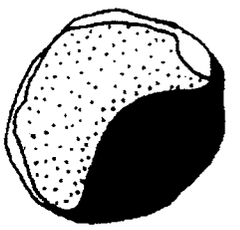


Fig. 6

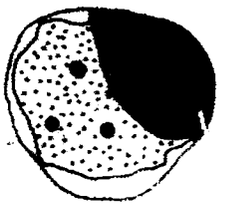


Fig. 7

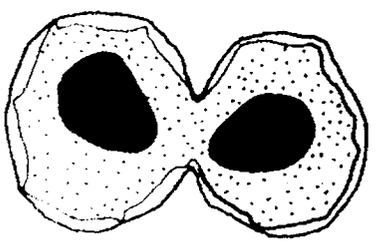


Fig. 8

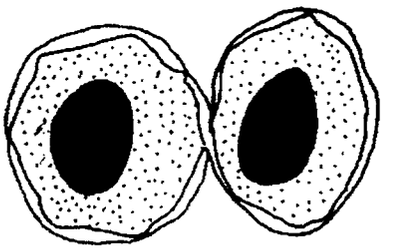


Fig. 9

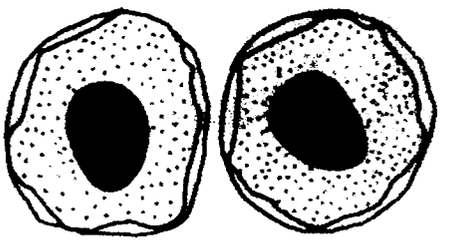


Fig. 10

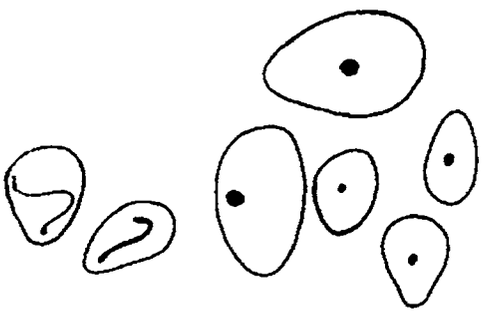


Fig. 11

Fig. 12

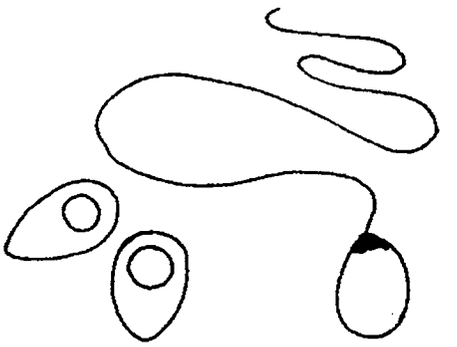


Fig. 13

Fig. 14

5 μm

nuclei exhibit eccentric position, near the periphery (Pl. [illegible] Figs. 4 and 5). This may, quite possibly, be due to the optical error. However, the location of these nuclei to as the peripheral part of the sporont cannot be ignored. Some sporonts possess a large body occupying about 30 percent. of the inner area of the sporont. This body is semi-circular and appears to be the nucleus when stained with Heidenhain's iron haematoxylin (Pl. XI[illegible], Figs. 6 and 7).

Paired sporoblasts with a clear passage connecting each [illegible] by a cytoplasmic bridge are often noticed in the smears [illegible] infected ovary stained with Heidenhain's haematoxylin (Pl. XI[illegible], Fig. 8). In each sporoblast, there is a single, small, ovoid body measuring about 1.5 to 2.0 µ in length bearing close resemblance to the spore in shape and size. Perhaps, they are immature spores derived from the division of sporogonial mother cell into two sporoblasts, each of them giving rise to a single spore. In the advanced stage, the cytoplasmic bridge disappears and the two sporoblasts appear close to each other as if they are glued (Pl. XI[illegible], Fig. 9). Eventually, the two sporoblasts separate from each other (Pl. XI[illegible], Fig. 10). The parasite is found to develop in direct contact with the host cell cytoplasm (Pl. [illegible], Figs.1 to 6) and no parasporoblastic membrane was observed at any stage of its development. The sporoblast and spore of the microsporidian under consideration, are uninucleate and no appendages are found on their surface.

PLATE XI

Perezia affinis sp. nov. in Metapenaeus affinis

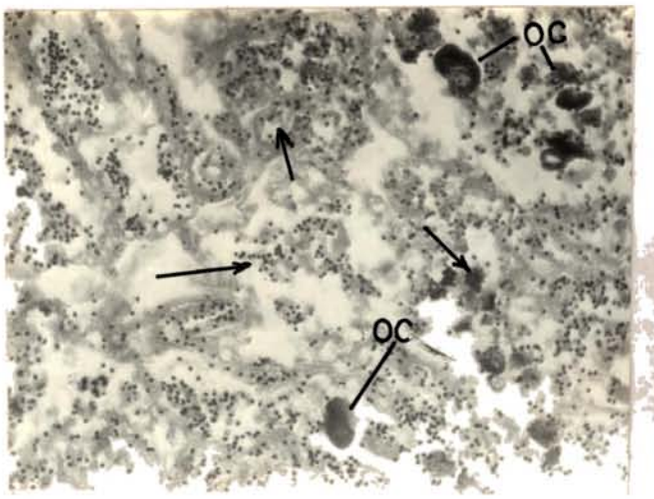
Figs. 1–2. Developing stages and spores of Perezia affinis (arrows) in the ovary. MT=Muscle; OC=Degenerating oocyte(s). Bouin–Heidenhain's haematoxylin and eosin.

Figs. 3–4. Same, in the body muscle.

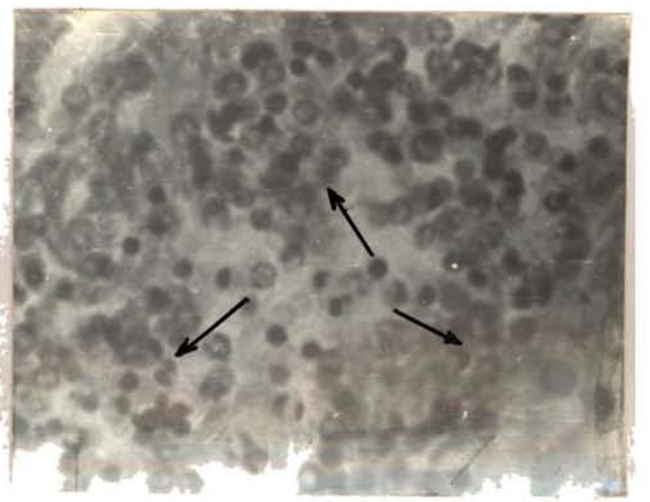
Fig. 5 Electron micrograph of sporont (SP) in the host muscle (MT). PT=Traces of polar tube.

Fig. 6. Electron micrograph showing the spores (arrows) in the host muscle (MT).

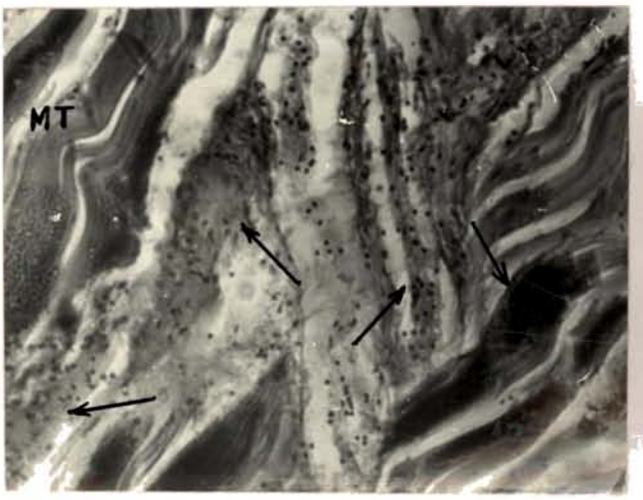
PLATE XX



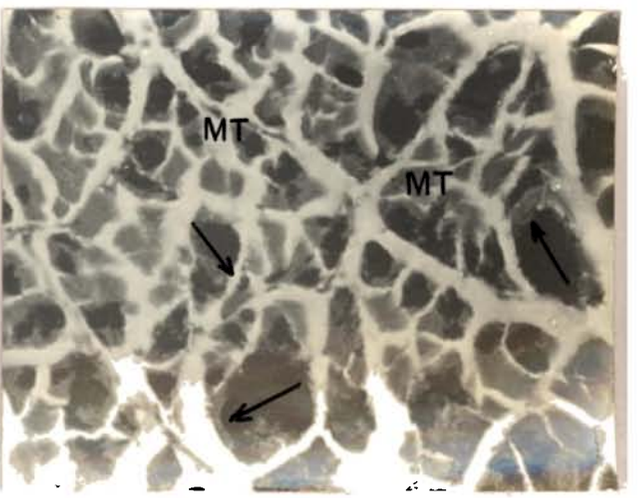
100 µm 1



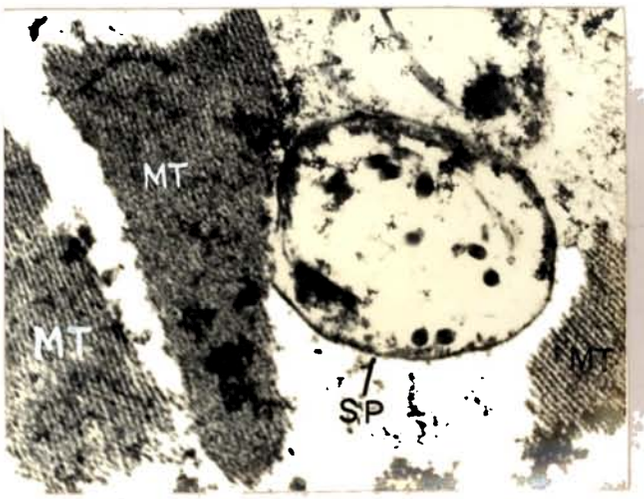
20 µm 2



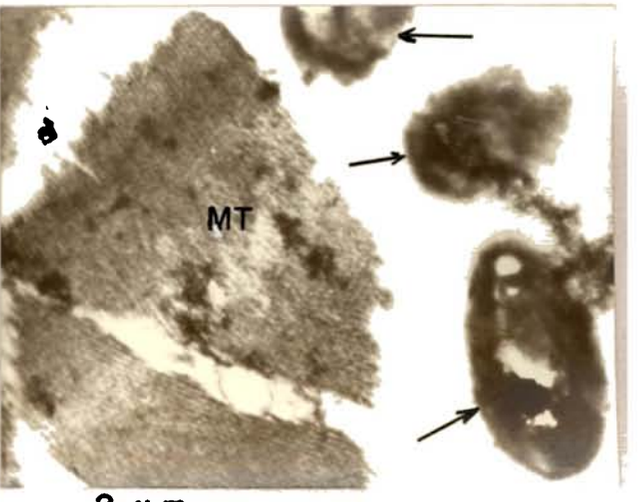
100 µm 3



100 µm 4



2 µm 5



2 µm 6

In some independent spores, a thread-like structure, presumably the polar tube, is seen connecting the two opposite ends (Pl. XIX, Fig. 11). In ultrathin section of the sporont, traces of polar tube are clearly visible (l. X, ig. 5).

Spores: ost of the spores are essentially uniform in shape and size. They are somewhat pear-shaped being broadly round d posteriorly and sharply rounded anteriorly. pores are very small, measuring 2 to 2.5 1 to 1.5 /um when erved in fresh condition. few relatively very large spores, representing apparently a distinct size range, as large as 4.5 X 2.5 to 3.0 /um are also observed (Pl. X , ig. 12). This type of spores are called as macrospores and their presence among the usual spores indicate the spore dimorphism.

spores are found singly in masses in the infected muscle and ovary. A very small, rounded posterior vacuole is seen in the spore when observed under the phase contrast microscope (Pl. XI , Fig. 13). The extruded polar tube is isofilar, about 25 /um long (Pl. XIX Fig. 14). In electron micrograph, a coiled polar tube occupies almost entire inner space of the spore (Pl.XXI, Fig. 1). The tube originates from the anterior end of the spore and shortly thereafter, it forms spiral coils inside the spore surrounding the ultrasporal space. It

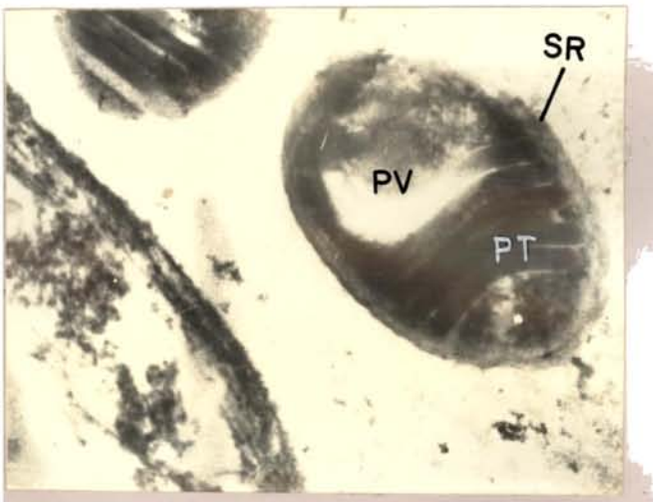
PLATE XXI

Perezia affinis sp. nov. in Metapenaeus affinis

Fig. 1. Electron micrograph of the spore (SP). Note the coiled polar tube (PT) which occupies almost entire inner space of the spore. The space at the posterior end of spore indicates poorly fixed posterior vacuole (PV).

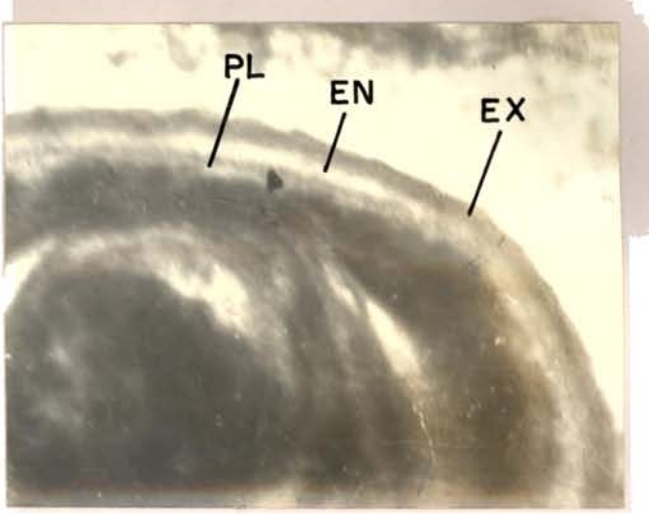
Fig. 2. Electron micrograph of a part of the spore at higher magnification showing exospore (EX), endospore (EN) and plasma membrane (PL).

PLATE XXI



1 µm

1



0·2µm

2

makes about 12 to 14 turns and ultimately terminates at the posterior end.

In smears, stained with Heidenhain's haematoxylin, a small dot-like nucleus is found situated near the centre but somewhat anteriorly. The spore wall shows two distinct layers: (1) the outer layer or exospore which is smooth, without any appendages and electron-dense, and (2) the inner layer or endospore which is electron-translucent (Pl. 1, fig. 2). The innermost layer or plasma membrane is also visible.

Remarks: The absence of pansporoblastic membrane during the spore development in the present species indicates that it belongs to the Sub-order Apansporoblastina (Order: Microsporea). Perusal of the earlier studies shows that the characteristics of the present species have similarities with some of the species which were earlier assigned to the Genus Nosema and reported from Decapod crustaceans.

Sprague and Couch (1971) listed seven species of Nosema found in Decapod crustaceans. Three of these were listed as hyperparasites and the other four species were Nosema pulvis, N. sanidi, N. nelsoni and N. michaelis. Sprague (1977) considered N. sanidi as synonym of N. michaelis and transferred it to a new Genus, Ameson from Nosema Naegeli, 1857 in the Family Nosematidae on the basis of polysporoblastic

sporogony and staining properties of the polarplast in N. michaelis. At the same time, N. pulvis and N. nelsoni, which showed similar pattern of sporoblast formation in chain, were also transferred to the newly created Genus Ameson by Sprague (1977). Family Nosematidae, to which Ameson was assigned, is characterised by the presence of diplokaryotic sporoblast. Vivares and Sprague (1979) demonstrated that Ameson was uninucleate and there was no diplokaryon in the sporoblast stage. This observation lead them to exclude Ameson from Family Nosematidae and place it in the Family Unikaryonidae which is characterised by uninucleate sporoblast. Since the Genus Perezia of this family showed the mode of sporogony identical to that of Ameson, Vivares and Sprague (1979) distinguished Ameson from Perezia by the presence of hair-like appendages in the former and its absence in the latter during the sporogony. They also pointed out that A. nelsoni did not possess hair-like appendages during sporogony unlike in A. michaelis and A. pulvis, and based on this character, transferred A. nelsoni to P. nelsoni. In this recent classification, Sprague (1982) has placed Genera Perezia and Ameson in the family Pereziidae.

When a comparison is made between the present species and those mentioned above as well as described earlier under the Genus Nosema from decapod crustacean hosts (Table 4), it appears that the species under consideration has more resemblance with P. nelsoni than with the other ones. Both

Table 4. Comparison of present species with the reported species of *Ameson*, *Perezia* and *Nosema* infecting decapod crustaceans

Characters	*Ameson michaelis* (Sprague, 1970)	*Ameson pulvis* (Perez, 1905)	*Perezia nelsoni* (Sprague, 1950)	*Nosema* sp. Walker and Hinsch, 1972	*Nosema* sp. Subrahmanyam, 1974	*Nosema* sp. Santhakumari and Gopalan, 1980	Present species
Host	*Callinectes sapidus*	*Carcinus maenas*	*Penaeus aztecus*, *P. duorarum*, *P. setiferus*	*Libinia dubia*	*Metapenaeus monoceros*	*Metapenaeus monoceros*	*Metapenaeus affinis* and *Penaeus semisulcatus*
Site of infection	Early stages in haematopoietic tissues and sporulation stages in skeletal muscles	Skeletal muscle	Skeletal Muscle	Epithelium of vas deferens	Muscle	Muscle	Body muscle, gonad and digestive tract of *M. affinis* and body muscle of *P. semisulcatus*.
Locality	Atlantic and Gulf coasts of U.S.A.	Arcachon, France	Southern coast of U.S.A.	Biscayne Bay, Florida, U.S.A.	Pulicat Lake, Adyar estury and Enmur Estury in Tamil Nadu, India	Backwaters of Cochin, India	Southeast coast of India-Gulf of Mannar and Palk Bay
Vegetative stages	Binary and multiple fission within haematopoietic organs involving small cylindrical or spherical plasmodia with four nuclei; merogony terminated with diplokaryotic cells. Occur within the haemocytes in the submucosa of the host midgut.	Multiplication by binary fission. Late meront(?) diplokaryotic	No Data	No data	No data	No data	No data
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)

Spore	Sporulation stages	(1)
Ovoid, 2.2 X 1.7 µm (fresh), probably uninucleate(Vivares and Sprague,1979), covered with fine projections or bristles; polaroplast a bipartite structure with compactly laminated anterior part; polar tube 40 µm long with about 11 turns, irregularly distributed in outer and inner coils	Early sporont a diplokaryotic cell. Sporogony involves delayed cytokinesis resulting in formation of uninucleate sporoblasts in pairs or chains; numerous short bristles emanate from the surface of sporoblast. All stages extracellular.	(2)
Ovoid, 1.3 X 1.0 µm (stained), probably uninucleate (Vivares and Sprague,1979), covered with hair-like projections; bipartite polaroplast with distinct lamellar and vesicular portions; polar tuble typically with 8 turns arranged in a distinct pattern.	Late sporogonial stages monilliform plasmodia which divide into several (3 to 5) uninucleate sporoblasts in chains. Sporoblast entirely covered with short hair-like projections. Each sporoblast develops in iosla-tiox into a spore.	(3)
Ellipsoidal, sometimes ovoid, 2.5 X 1.5 µm (fresh). Internal structure similar to that of A. michaelis spores (Sprague, 1977). Polar tube 23 µm long.	Sporogony unknown. Sporoblasts devoid of hair-like appendages; develops into isolated spore.	(4)
Ovoid, about 5 X 3 µm	Sporont with diplokaryon which divides into two diplokaryotic sporoblasts each of which transforms into spore	(5)
Ovoid with a central nucleus; single polar tube present.	No data	(6)
No data	No data	(7)
Egg-shaped, usually 2.0 to 2.5 X 1.0 to 1.5 µm (fresh) but occasionally 4.5 X 2.75 µm (fresh); polaroplast not observed; found singly in masses; posterior vacuole round; polar tube isofilar, 25 µm long forming 12 to 14 undulations. Spore wall with an outer smooth, electron dense exospore and an inner, electron lucid endospore.	Sporont spherical, 5 µm in diameter, possess small nuclei ranging in number from 1 to 4; sporont wall thick; sporogony disporoblastic producing uninucleate sporoblasts which are devoid of any hair-like appendages. Each sporoblast develops into an isolated spore. All stages develop in direct contact with the host cell cytoplasm.	(8)

(the present species and _. nelsoni) are found to infect penaeid prawns, possess similar spore shape with identical spore size. The length of polar tube and the number of its undulations also do not differ quite significantly in _. nelsoni and the present species. The electron micrograph of the sporulation stage of the present species, though not very clear perhaps due to the poor fixation of the material, does indicate the absence of any hair-like appendages on the sporonts. This is one of the distinguishing features of Perezia from Ameson and thus brings the present microsporidian more closer to Perezia. Both P. nelsoni and the present species again show a similarity in having monosporoblastic sporogony. However, the mode of sporogony in _. nelsoni follows the family pattern, that is, sporogony polysporoblastic by multiple fission of moniliform plasmodia (Sprague, 1977) whereas the present species shows a disporous nature, that is, sporont develops into two spores which remain joined together during the spore morphogenesis. Nevertheless, the disporous nature in other species of Perezia has been described by Leger and Duboscq (1909) (in Sprague, 1977) and Youssef (1974). In P. lankesteriae, the type species of the family Perezildae Doubes et al., 1977, each sporoblast divides into two products each of which becomes a spore and usually remain joined until they become essentially mature spores and then separate (Leger and Duboscq, 1909 in Sprague, 1977).

A similar pattern of spore formation has also been noticed in the present species. It is, therefore, reasonable to place the present species in the Genus Perezia (Family : Perezidae).

Although it has been discussed above that the present species bears many similarities with P. nelsoni, the difference in mode of sporogony, different host species and different geographical locations provide justifiable reasons for the creation of a new species. Subrahamanyam in 1974 described Nosema sp. from the Muscles of M. monoceros from Pulicat Lake, Adyar estuary and Ennur estuary in Tamil Nadu and recently, Santhakumari and Gopalan (1980) reported Nosema sp. affecting the same from the Cochin backwaters. However, these authors have not given detailed structure of the pathogen except the spore shape and gross symptoms in the host and have not assigned the pathogen to any species. It thus becomes difficult to compare the present species with these materials.

The new species observed at present is given the name of its major host and named as Perezia affinis sp. nov.

Type specimen: Holotype slide is being deposited in the Zoological survey of India, Calcutta.

4.2 SYMPTOMS OF MICROSPORIDIOSIS CAUSED BY THELOHANIA SEMISULCATA

Body deformities and changes in the normal colouration, texture, body pigmentation and the general appearance form the main external characters to distinguish the abnormalities in a prawn. Deviations from the normal behavioural pattern also serve to indicate the abnormalities of the animal.

The body of a normal, adult, live Penaeus semisulcatus is characterised by a light or pale brown colour with alternating dark bands of brown grey colour on the dorsal side of the abdomen and a pale yellow on the ventral side. The carapace often possesses two yellow cream coloured transverse bands. Among the appendages, the antennae are banded white and brown; the pereopods and pleopods dull red; the uropods yellowish distally and bluish brown or greenish brown proximally. The tips of the uropods are tinged with blue or red, while the fringe of setae are usually brownish red. The live prawn appears translucent. In the adult females, the ovary is visible through the exoskeleton as a dark band on the dorsal aspect of the abdomen extending up to the sixth abdominal segment.

When the prawn is infected by Thelohania semisulcata, the disease produces quite conspicuous symptoms. In the

initial stage , however, it is very difficult to distinguish externally the infected prawns from the normal ones with unaided eyes. According to Kruse (1959), early and light infections of microsporidians in prawns cannot be detected with unaided eyes. Degree of infection by P. semisulcata in P. semisulcatus can qualitatively be recognised as light, moderate and heavy (Pl. XVII, fig. 1). In the light infection stage, the abdominal musculature contains a few scattered streaks of infected muscle tissue which appear opaque and white; in the moderate infection, about half of the abdominal musculature becomes white and opaque; and in the heavy infection stage, nearly the entire muscle tissue of the abdomen appear white and opaque.

External symptoms become discernible when the prawn is heavily infected. At this stage, the exoskeleton becomes thin, delicate and semi-transparent through which the muscle can be seen clearly. The muscle appears chalky white with cotton-like texture, lacking the firmness of the normal tissue. This results in an opaque condition (Pl. XIII, Fig. 1) with muscle fibrin lost their translucency. According to Overstreet (1978), muscle in this condition gives the appearance of having been cooked. This symptom is conspicuous on the ventral membrane and the sternum where the muscle is clearly visible. The joints of the abdominal segments, the pleopods and carapace also appear white due to infection in the muscle. It is for this reason, the infected prawns are locally known as "chunambu eral" meaning, lime coloured

PLATE XVII

Fig. 1. Symptoms of the microsporidiosis caused by *Thelohania semisulcata* in *Penaeus semisulcatus* showing light, moderate and heavy infection stages.

Fig. 2. Diagrammatic representation of an intermittently infected ovary from a moderately infected *Penaeus semisulcatus* by *Thelohania semisulcata*.

Fig. 3. The midgut of a heavily infected *Penaeus semisulcatus*.

PLATE XXII

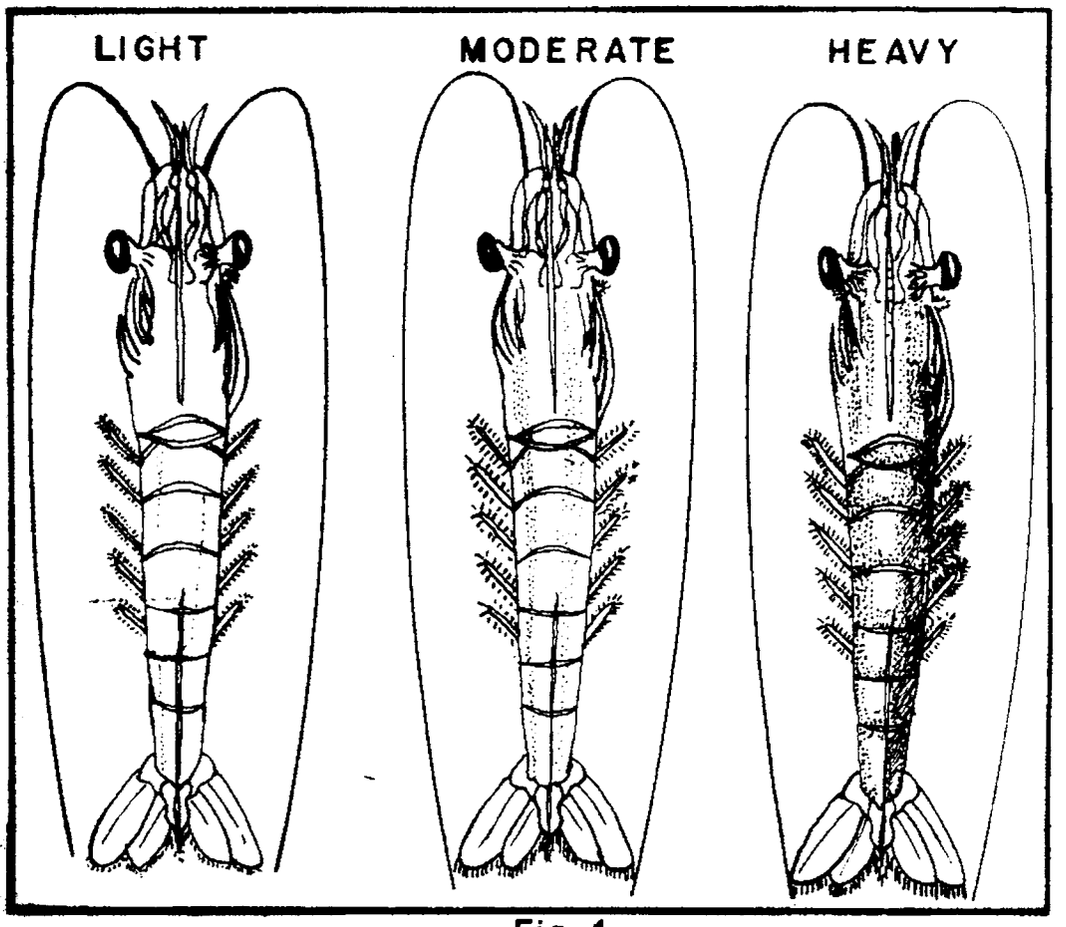


Fig. 1

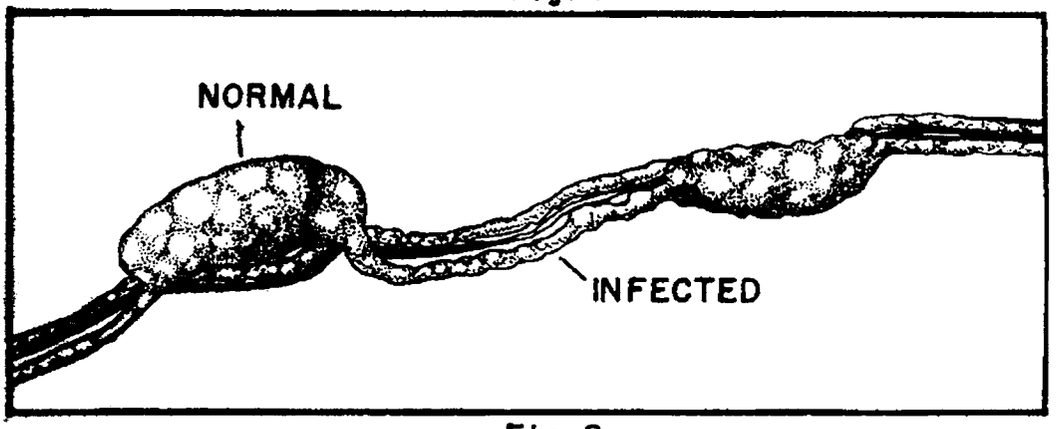


Fig. 2

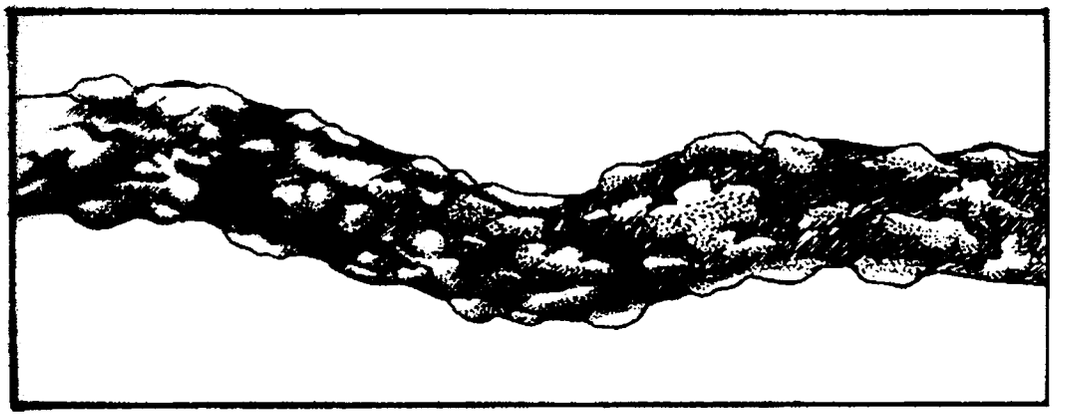


Fig. 3

prawns among the fishermen of the Tamil Nadu coast. It is otherwise referred to as the "milk prawn" or "cotton prawn" due to opaqueness in the muscle as seen through the exoskeleton (Kruse, 1959; Lightner, 1977; Johnson, 1978; Overstreet, 1978).

The symptoms begin to appear as the infection advances. During the early stage of moderate infection, white streaks of infected muscle fibres are seen between the still uninfected flexor and extensor muscles of the abdomen. When the body is cut into two halves medially, the ovary is also found to be infected intermittently. The infected areas appear narrow, thin and opaque. Thus at this stage, the ovary appears in clumps of normal green lobes interconnected by white, thin, ribbon-like infected regions (Pl. II, fig. 2). The outer epithelial surface of the midgut usually has very thin white patches of infection. Other organs do not usually exhibit any such symptom which can be seen with the naked eyes at this stage of infection.

In the highly infected prawns, the ovary turns from the normal yellow green or dark green to an opaque white colour. Thomas (1972) observed that the ovary of the prawn, P. semisulcatus infected by Thelohania sp., becomes very thin and contains no normal eggs. Small or large white patches occur on the outer epithelial wall of the midgut when P. semisulcata infection is heavy (Pl. VIII,

PLATE XXIII

Fig. 1. *Penaeus semisulcatus* infected by *Thelohania semisulcata*. Heavily infected prawn (above), normal prawn (below).

PLATE XXIII

1

Fig. 3). These patches represent colonies of pathogen. The hepatopancreas usually retains its dark, reddish brown colour even when heavily infected. The nervous system, foregut and heart usually do not show any prominent visible symptom, although infected by the pathogen.

Microscopic examination of squash preparation of infected ovary or muscle reveals the presence of enormous numbers of small, oval-shaped spores which may either be clumped together or found freely. In highly infected prawns, it is sufficient to merely touch the infected tissue on the surface of the glass slide to observe thousands of spores under the compound microscope.

The disease caused by *P. semisulcata* gradually debilitates the host which becomes less resistant to stress and more susceptible to predation. Besides, infected prawns also do not withstand handling, as in the case of uninfected prawns. However, infection does not affect the feeding habits of the animal as the gut of even the heavily infected prawns collected from the natural population was found to be filled with detritus.

Few heavily infected adult specimens of *P. semisulcatus*, which were collected alive from the nature and reared in the laboratory in a 100 l capacity glass aquarium tank, showed lethargic movements and sluggish behaviour. In one observation, the animal did not move from its place for about 15 hours. The swimming activities of the prawns were also found to be very much reduced, but feeding was normal especially during the night.

4.3 HISTOPATHOLOGICAL STUDIES OF PENAEUS SEMISULCATUS INFECTED BY THELOHANIA SEMISULCATA

Histopathological investigations of fixed body fluids or tissues form an important and powerful research area in facilitating proper diagnosis of the diseases, their effect on the various systems and in the understanding of the functional organisation of the affected organisms. Where the infection is not heavy enough to be detected by the macroscopical examination of the material, it forms one of the essential techniques for determination of the diseases. A study of the pathological changes occurring at the tissue and cellular levels also helps considerably in the clarification of the physiological functions of the host organism. The degree of divergence from normal cell structure indicates the relative health of the host. When the changes are detrimental, they interfere with normal physiological functions, reproductive capability and survivability of the host organism.

Most of the published information on microsporidian diseases of prawns deals with the structure and identification of the pathogen rather than the histopathological changes in the host tissues. Among the workers emphasising

the latter aspect, mention may be made of the studies by Baxter *et al.* (1970), Overstreet and Weidner (1974), Street and Sprague (1974), Lightner (1975), Iversen and Kelley (1976) and Breed and Olson (1977). Baxter *et al.* (1970) reported on the infection of muscle fibres and intramuscular space by the cysts, spores and developing stages of *Pleistophora* sp. in *Penaeus aztecus* and *P. setiferus*. Overstreet and Weidner (1974) found that the microsporidian, *Indosporus sprague*i, infecting the grass shrimp, *Palaemontes pugio*, attacks the muscle fibres and spreads throughout the host musculature, while Street and Sprague (1974), working on the same species of shrimp infected by *Pleistophora* sp., observed that the muscle cells of the host get hypertrophied and replaced by the microsporidian. Similar attack of the striated muscle fibres of the penaeid shrimps of North America by *Nosema* (=*Perezia*) *nelsoni* and of the postlarvae of *P. duorarum* by *Thelohania* (=*Agmasoma*) *penaei* was also reported by Lightner (1975) and Iversen and Kelley (1976) respectively. In *Crangon franciscorum*, *C. nigricauda* and *C. stylirostris*, infected by *P. crangoni*, Breed and Olson (1977) found that the muscle tissue was the main target of attack in these shrimps.

Specific studies on the histopathology of microsporidian infection in penaeid prawns were carried out by Constransitch (1970), Kelley (1975, 1979) and Baticados

(1980). Constransitch (1970) studied the pathological changes in the commercial penaeid shrimps, P. aztecus and P. setiferus from Louisiana, infected by P. pensei. Tissues infected were tail muscle, cardiac muscle, hepatopancreas, and intestinal and stomach walls. Kelley (1975, 1979) differentiated the histological structure of the normal and infected pink shrimp, P. duorarum by T. duorara, Agmasoma Pensei and Pleistophora sp. and discussed the pathogenicity and tissue affinity of the parasite in the host. Recently, Baticados (1980) studied the histopathological changes in the ovaries of P. merguiensis infected by an unidentified microsporidian parasite.

In India, no study has so far been made on the histopathology of the tissues affected by any microsporidian in prawns. In the present study, the histopathology of P. semisulcatus infected by T. semisulcata is presented by studying the different tissues affected by the pathogen. An attempt is also made to compare the structure of the tissues of the prawns affected by the microsporidian with those of the normal prawns so as to assess the impact of infection by the microsporidian on the host tissues and their functions.

The study was based on the prawns of size above 70 mm in total length, collected from the wild population by trawl net operations in the waters off Mandapam in October, 1982

and March, June, July, November and December, 1983. The most common sites of the microsporidian attack in the prawns were found to be the body muscles, gonad, hepatopancreas and midgut. To a lesser extent, infection was also found in the heart eyes and the gills.

OBSERVATIONS

Body muscles

In the lightly infected prawns, the pathogen was found concentrated in small foci throughout the entire musculature of the abdomen. In the longitudinal section, it was apparent that the muscle bundles were not completely infected by the pathogen (Pl. XXIV, Fig. 1); only individual muscle fibres were seen affected. In the early stages of infection, _. semisulcata was seen propagating by merogony inside the muscle fibres. The meronts dividing by successive binary fission, formed a chain of cells along the length of the muscle fibres (Pl. XXIV, Fig. 2). In some cells, still advanced stage of infection was seen where some meronts developed into sporonts and sporoblasts (Pl. XXIV, Fig. 3). Thus, in the longitudinal section of the abdominal muscle lightly infected by T. semisulcata, meronts and different sporulation stages of the pathogen were seen arranged longitudinally along the length of the invaded muscle fibres.

PLATE XXIV

Fig. 1. Longitudinal section of the abdominal muscle of Penaeus semisulcatus. Arrow shows infected area in the muscle (MT). Bouin-Mallory's triple stain.

Fig. 2. Longitudinal section of abdominal muscle of Penaeus semisulcatus showing Thelohania semisulcata in the early stage of infection. The meronts (ME) form a chain along the length of the muscle fibres (MF). Formalin-Heidenhain's haematoxylin and eosin.

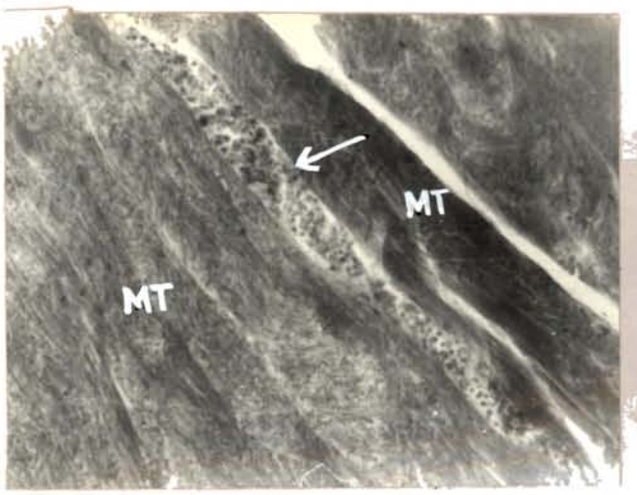
Fig. 3. Longitudinal section of lightly infected abdominal muscle of Penaeus semisulcatus where meronts are developing into sporonts and sporoblasts (arrows). MF=Muscle fibres. Davidson's fixative-Heidenhain's haematoxylin and eosin.

Fig. 4. Transverse section of lightly infected abdominal muscle of Penaeus semisulcatus showing the location of the multiplying microsporidian (intensely dark) in the muscle fibres; (A). Infection in the central part of a muscle fibre; (B). Infection near the periphery. Bouin-Heidenhain's haemotoxylin and eosin.

Fig. 5. The longitudinal section of abdominal muscle of Penaeus semisulcatus to show the advanced stage of infection. The areas parasitised (arrows) are more numerous and larger. MF=Muscle fibres. Bouin-Heidenhains haematoxylin and eosin.

Fig. 6. Longitudinal section of moderately infected abdominal muscle of Penaeus semisulcatus to show the mass of released spores and other developing stages of the microsporidian (arrow). MF=Muscle fibres. Bouin-Heidenhain's haematoxylin and eosin.

PLATE XXIV



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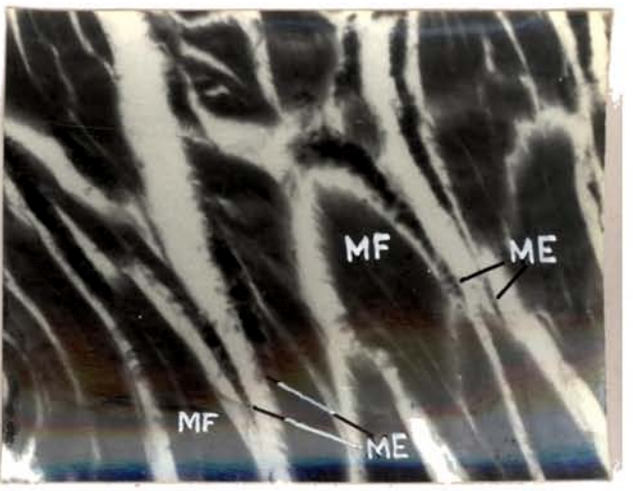

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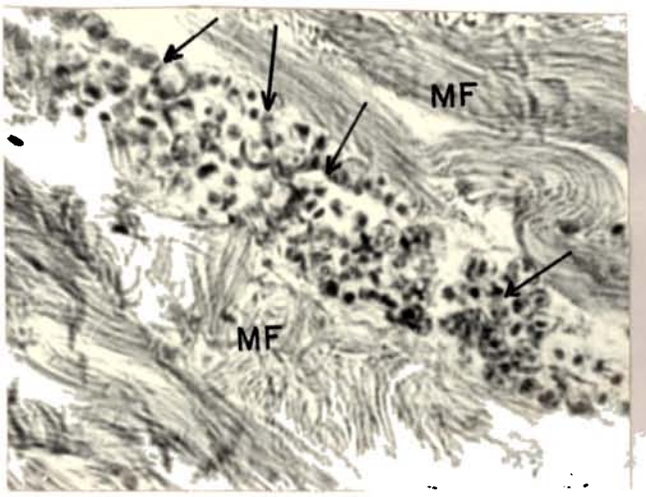

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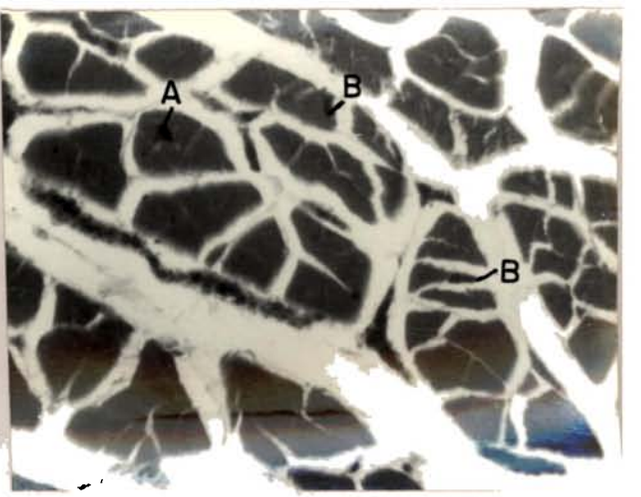

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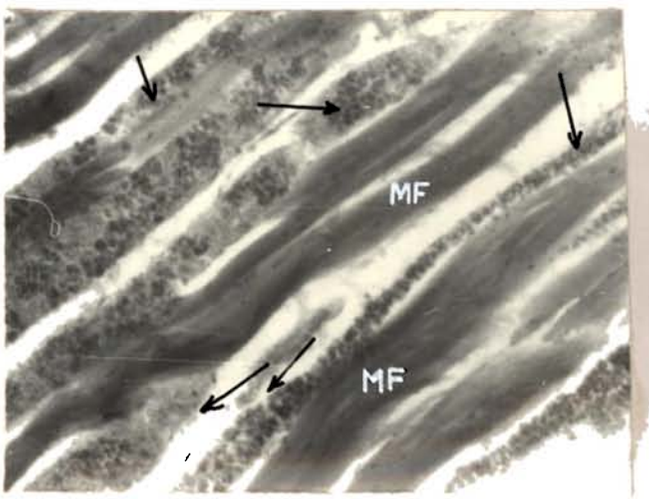

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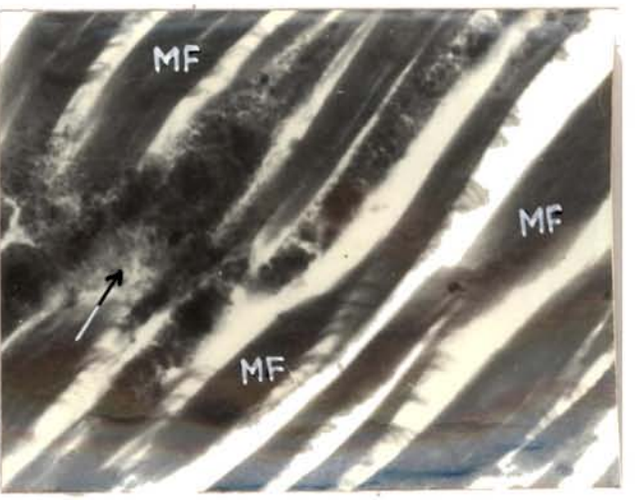

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Cross section of the lightly infected abdominal muscle revealed that the location of the multiplying microsporidian varied initially from cell to cell. In some muscle cells, infection was found in the central part whereas in others, it developed near the peripheral part of the cell (Pl. XXIV, Fig. 4).

In advanced stage of infection, the areas parasitised were more numerous and larger. The multiplying pathogen occupied the entire muscle fibre which was filled-up with round pansporoblasts and oval spores (Pl. XXIV, Fig. 5). Myofibrils, sarcoplasm and nuclei of infected muscle fibres completely disappeared. The only remanent of the muscle fibre visible at this stage was the endomysium which was intact and retained the long spindle shape. However, the great pressure developed by the continuously multiplying microsporidian inside the endomysium caused its expansion and eventual rupturing, thus releasing the spores and other developing stages (Pl. XXV, Fig. 6) which infected neighbouring cells of the tissue, presumably by means of autoinfection.

In the heavily infected prawns, fibres of most of the muscle bundles were found to be infected, lysed and replaced by the microsporidian (Pl. XXV, Fig. 1). The infection was easily detectable as black masses of spores against the pink uninfected muscle fibres which were very

few in number, being scattered at places. These fibres, although uninfected, were often contorted and twisted around the infected areas.

The nature of microsporidian infection in the muscles in the cephalothoracic region, locomotary organs and in the abdomen was found to be more or less homogeneous.

Ovary

The infection of the ovarian tissue by the microsporidian was not restricted to anyparticular stage of development of the ovary; complete or partial destruction by the pathogen was observed in the immature and maturing as well as the mature ovaries.

In the longitudinal section of a maturing ovary, which was partially infected at places (Pl. XXII, Fig. 2), the nucleoli of several developing oocytes were invisible, and instead, the nucleus of these oocytes possessed very conspicuous inclusion bodies (IBs) in the karyoplasm (Pl. XII, Fig. 5 and 6; Pl. XIII, Fig. 1 and 2). At low magnification, the IBs appeared as though they were the nucleoli of the oocytes, which in the normal cases are spherical and are found attached to the inner side of the nuclear membrane (Pl. XIV, Fig. 2). However, at higher magnification, these structures were found to be entirely different from the

PLATE XXV

Fig. 1. Longitudinal section of the abdominal muscle of Penaeus semisulcatus showing heavy infection stage. Note the black masses of spores (arrows) replacing the muscle fibres (MF). Bouin-Heidenhain's haematoxylin and eosin.

Fig. 2. Semi-thin section of a normal early developing stage of ovary of Penaeus semisulcatus. FW=Follicle membrane; NI=Nucleioli; NU=Nucleus; OC=Oocytes. Toluidine blue.

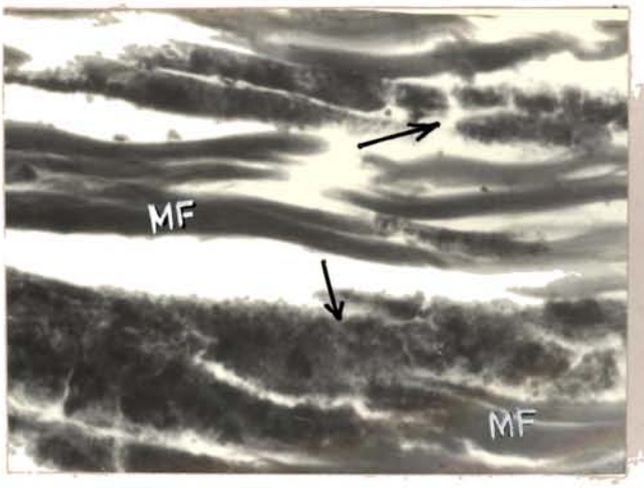
Fig. 3. Transverse section of a partially infected ovary of Penaeus semisulcatus. Note the variation in the number and arrangement of inclusion bodies (IB) in each oocyte (OC). Bouin-Heidenhain's haematoxylin and eosin.

Fig. 4. Semi-thin section of a lightly infected ovary of Penaeus semisulcatus: few free spores (arrow) are seen lying between the oocytes (OC). Toluidine blue.

Fig. 5. Transverse section of a lightly infected ovary of Penaeus semisulcatus. Some of the oocytes have been completely replaced by meronts of Thelohania semisulcata and a compact colony of the pathogen has been formed (arrows). CT=Connective tissue membrane; DY=Diplokaryon; FB=Fibroblasts; FW=Follicle membrane; HC=Haemocyte. Bouin-Mallory's Triple stain.

Fig. 6. Transverse section of a lightly infected ovary of Penaeus semisulcatus. Note the round to polygonal cells of microsporidian forming colony. Abbreviations same as in Fig. 5. Bouin-Mallory's triple stain.

PLATE XXV



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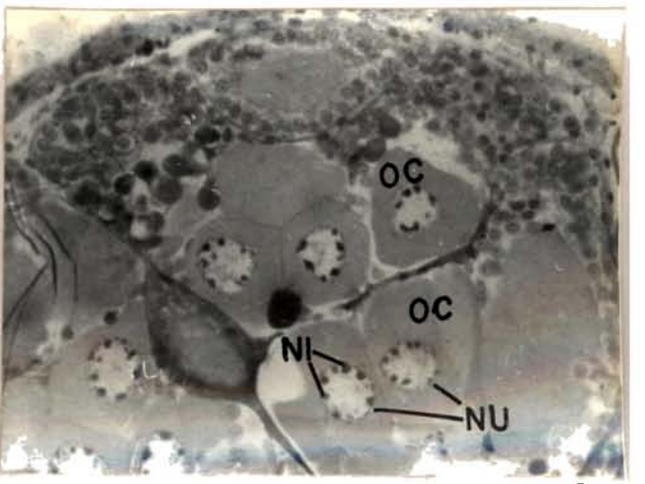

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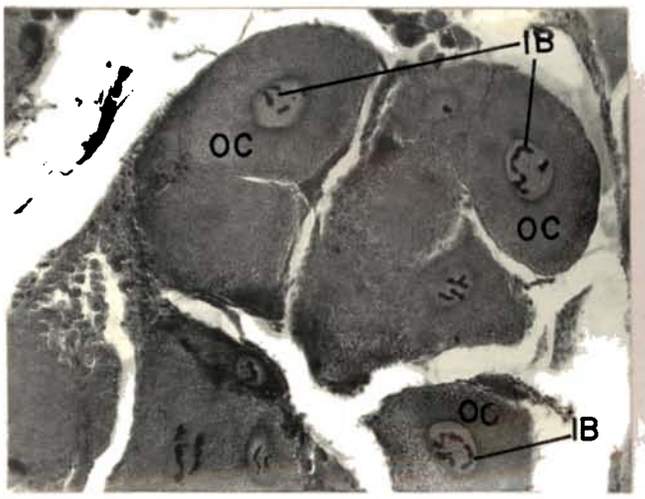

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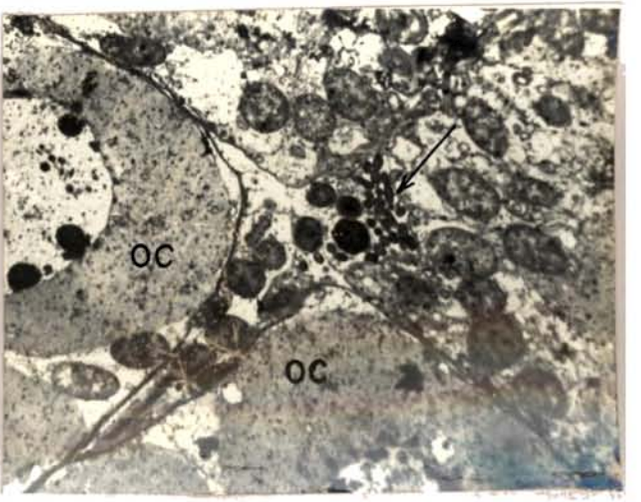

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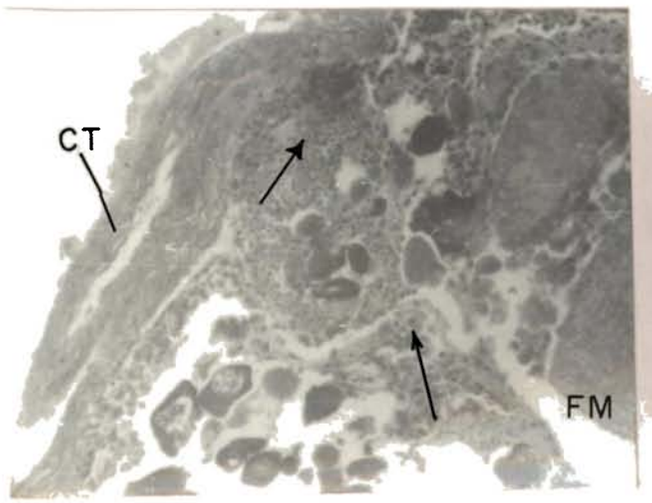

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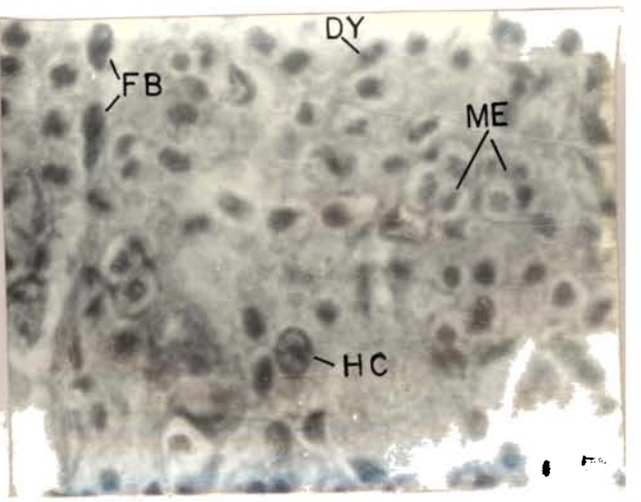

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6

nucleoli of a normal oocyte nucleus. The shape, size, number and arrangement of the IBs varied in each oocyte (Pl. XII, Fig. 5 and 6; Pl. XIII, Fig. 1 and 2; Pl. XXV, Fig. 3). They were round or ovoid or pear-shaped or amoeboid structures scattered sparsely or sometimes arranged in circular array. In the latter case, the IBs were seen connected with each other giving the appearance of a garland. The size of the ovoid and pear-shaped IBs ranged from 3 um to 9 um in diameter. IBs were locular structures without any distinct membrane and stained intensely with basic dyes. They stained dark grayish black with iron haematoxylin and deep red with acid fuchsin. Although the nuclear membrane in most of the oocytes having theIBs appeared intact as in the normal oocytes, occasionally it was seen to undergo degeneration. The other organelles such as cytoplasm, yolk and cortical bodies in the mature ovum were found to be unaffected, as evidenced by their slightly basophilic nature (in developing oocytes) or strong acidophilic nature (in mature ova) when stained with haematoxylin and eosin.

A few spores were seen lying freely in between the oocytes in the maturing ovary when lightly infected by _. semiglobata (Pl. XXV, Fig. 4). Some of the oocytes in the follicle were completely replaced by meronts of the microsporidian which formed a compact colony of small round to polygonal cells (Pl. XXV, Fig. 5 and 6). Some of the cells in the colony were in diplokaryotic stage (a

characteristic stage in the microsporidians which is usually followed by sporulation stage forming the spores). Some follicle cells, haemocytes and fibroblasts were also seen in the invaded follicle. The follicle wall and the outer most connective tissue of the ovary were, however, intact and uninfected (Pl. XXV, Fig. 5).

In transverse section of a heavily infected ovary, oocytes in almost all the follicles were replaced by meronts, sporonts and spores of T. semisulcata (Pl. XXVI, Fig. 1). The cell constituents of the infected oocytes could barely be distinguished as these oocytes were filled with the microsporidian. Although cytoplasmic disintegration was very prominent, cell hypertrophy was never observed. Each of the oocytes, fully invaded and destroyed by the pathogen, was seen as small group filled by the mass of spores and the other developing stages and each of the follicles was represented by larger groups formed of several smaller groups (Pl. XXVI, Fig. 1 and 2). Thus, the entire ovarian lobe in the transverse section was found to be filled up with spores and developing stages which appeared as grey to black masses when stained with Heidenhain's haematoxylin and eosin. Thin strands of connective tissue representing the follicle wall and the outer connective tissue membrane surrounding the ovary, stained pink to red with eosin indicating the unchanged components of the ovary (Pl. XXVI, Fig. 3). Follicle cells,

PLATE XXVI

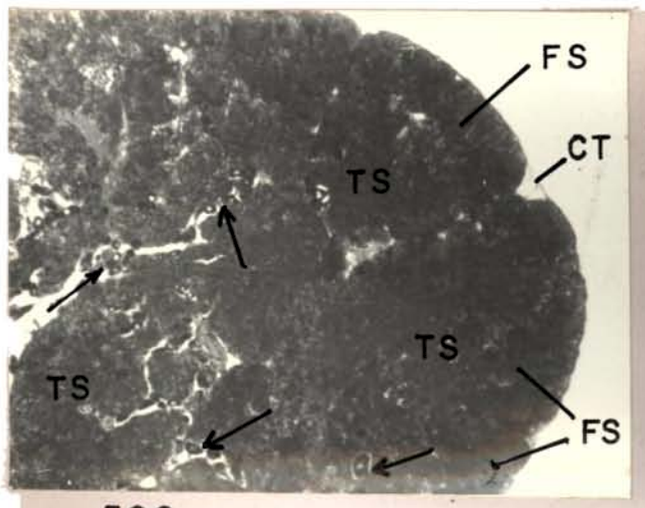
Figs. 1-3 Transverse sections of a heavily infected ovary of Penaeus semisulcatus. Note the degenerating oocytes scattered at places (arrows). The follicles (FS) are filled with the microsporidian(TS). CT=Connective tissue membrane; FM=Follicle membrane. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 4. Longitudinal section of a testicular lobe of Penaeus semisulcatus infected by Thelohania semisulcatus: the normal(NT) and infected tubules (IT) show apparent differential staining. Relatively larger number of infected tubules are located towards the peripheral part. CV=Connective tissue. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 5. An enlarged view of Fig. 4. Abbreviations same. 500.

Fig. 6. Transverse section of proximal vas deferens of testes of Penaeus semisulcatus. The glandular epithelial wall (GE) is slightly hypertrophied at some places (arrow) where the microsporidian forms compact colonies. CV=Connective tissue; LT=Lumen of the tubu SZ=Spermatozoa. Bouin-Mallory's triple stain.

PLATE XXVI



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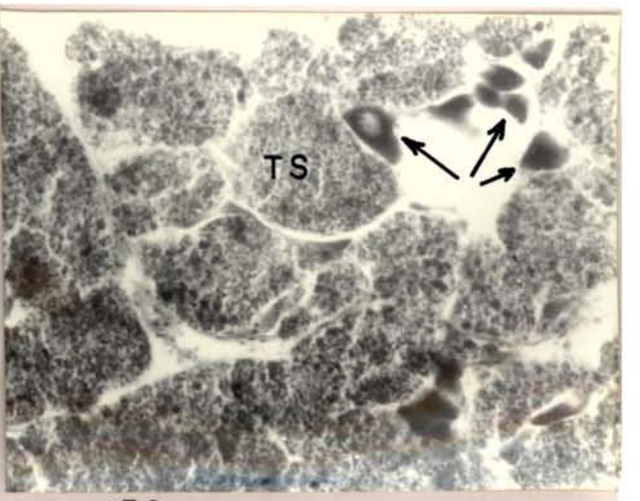

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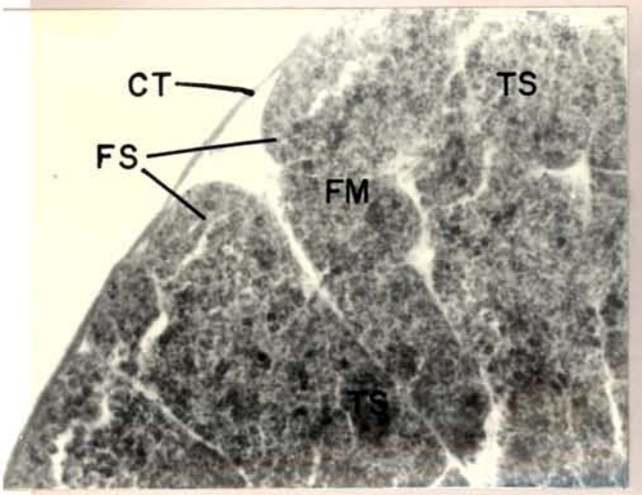

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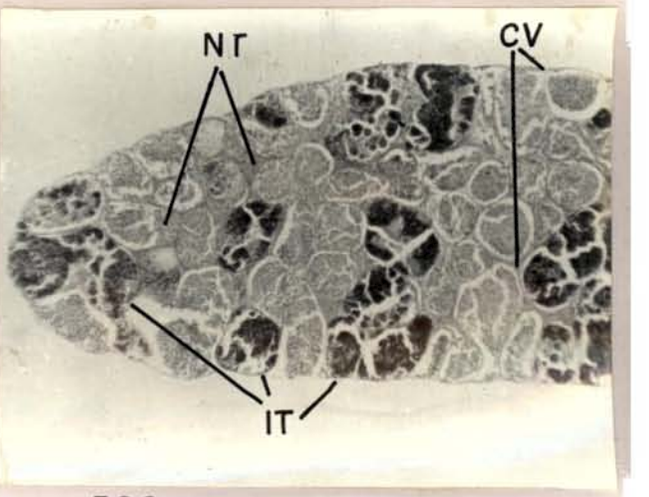

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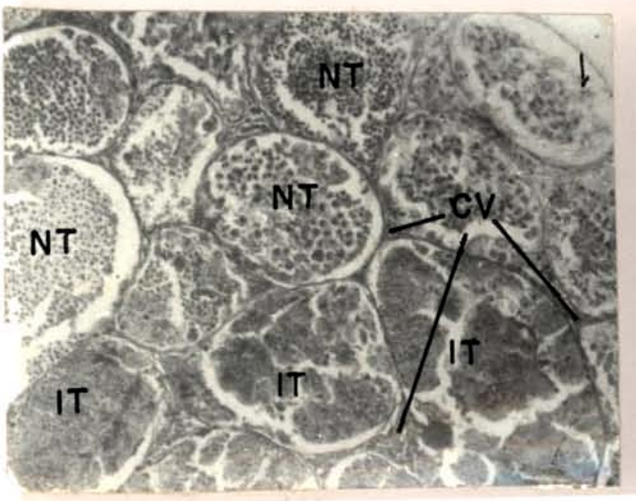

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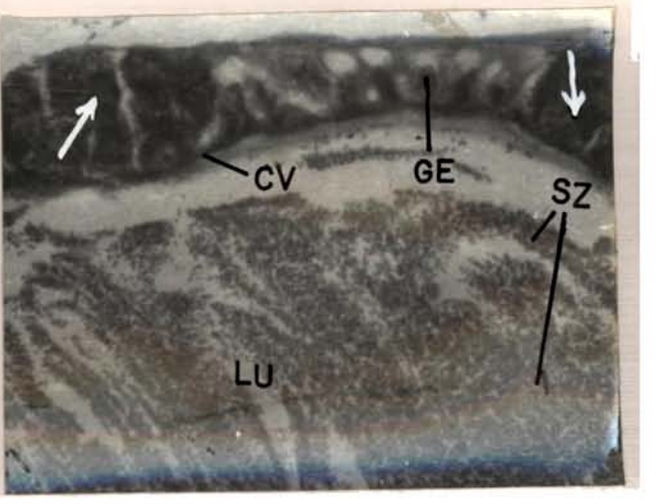

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6

haemocytes and fibroblasts were relatively few in number and found only near the follicle walls. Some cells in this region, probably the wandering phagocytes, were comparatively larger in size and contained darkly stained one, two or three nuclei. There were only a few shrunken oocytes scattered at places in the heavily infected ovary (Pl. XXVI, Fig. 1 and 2). These oocytes were degenerating and showed highly basophilic reaction upon staining with haematoxylin and eosin.

In certain cases, infection was seen in only one lobe of the ovary while the other lobe was still unaffected and contained normal and mature oocytes. In such cases, the affected lobe was relatively very thin as compared to the other unaffected one.

Male reproductive system

The entire male reproductive system comprising of testis, vasa deferentia and terminal ampoule was found to be infected by *T. semiglobata*. In the longitudinal section of a testicular lobe from an adult infected prawn, the infection was seen localised in some seminiferous tubules. When stained with Heidenhain's haematoxylin and eosin, infected tubules were clearly distinguishable from the normal tubules (Pl. XXVI, Fig. 4); the former appeared as darkly stained foci with haematoxylin, while the latter and the connective tissue stained deep pink with eosin. Although the infection was observed to spread irregularly in the seminiferous tubules in

testicular lobe, relatively larger number of infected tubules were found to be located towards the periphery. Each infected seminiferous tubule showed almost complete and distinct invasion by T. semisulcata. Neverthless, the thin connective tissue membrane binding the tubules was unaffected and appeared similar to those of the neighbouring normal tubules (Pl. XLVI, Fig. 5). Infected tubules contained compact masses of spores and other developing stages of the pathogen. The germinal epithelium of the tubule was replaced by compact colony of meronts whereas the tubule lumen was filled with dense mass of pansporoblasts, spores and empty spore cases. A few spermatogonia and spermatocytes were also seen occasionally in the infected tubules.

In the transverse section of the proximal vas deferens, the glandular epithelial wall contained several foci of infection protruding towards the lumen of the vas deferens (Pl. XLVI, Fig. 6). These foci appeared as slightly hypertrophied epithelial cells densely packed with meronts, sporonts and spores. Only a thin connective tissue membrane was visible in the affected area surrounding the developing microsporidian colony. As the disease advanced, invasion of several adjacent glandular epithelial cells led to the development of large colonies of the microsporidian which were lined at the inner side by a comparatively thick membrane of collagenous fibres (Pl. XLVII, Fig. 1). Meronts were the most abundant cell

types found in these colonies. The lumen of the tubule was filled with spermatozoa ensheathed in a mucous-like eosinophilic substance (Pl. XXVI, Fig. 6; Pl. XXVII, Fig. 1). The spermatozoa were free from infection. However, in a longitudinal section of the distal part of the vas deferens, spermatozoa were found to be replaced by meronts at several foci(l. XXVII, Fig. 2).

Infection was most pronounced in the terminal ampoule. The main tissue affected in this region was the tall, multinucleated glandular epithelium. Both in the transverse and longitudinal sections of terminal ampoule, the glandular epithelial cells were found to be greatly hypertrophied and filled with the compact colonies of meronts (l. XXVII, Fig. 3). In the advanced stages, the glandular epithelium was observed almost completely replaced by the microsporidian (Pl. XXVII, Fig. 4); only clumped nuclei of destroyed epithelial cells being seen occasionally. There were, however, a few individual epithelial cells which were found either unaffected or having only a few multiplying meronts (Pl. XXVII, Fig. 5). The spermatophore in the terminal ampoule was slightly compressed due to the enlarged glandular epithelial cells as a result of infection (Pl. XXVII, Fig. 6). In a longitudinal section of another infected terminal ampoule, there were two thick layers of strongly acidophilic gelatinous material and the growing meronts and sporonts

PLATE XVII

Fig. 1. Transverse section of the proximal vas deferens of testes of Penaeus semisulcatus showing large colonies of the microsporidian(arrows) in the glandular epithelial layer (GE) lined by a membrane (MB) of collagenous fibres. LU=Lumen of the tubule; MU=Mucous-like eosinophilic substance; SZ= Spermatozoa. Bouin-Mallory's triple stain.

Fig. 2. Longitudinal section of the distal vas deferens of testes of Penaeus semisulcatus. The spermatozoa have been replaced by meronts at many places (arrows). GE=Glandular epithelium; SZ=Spermatozoa. Bouin-Mallory's triple stain.

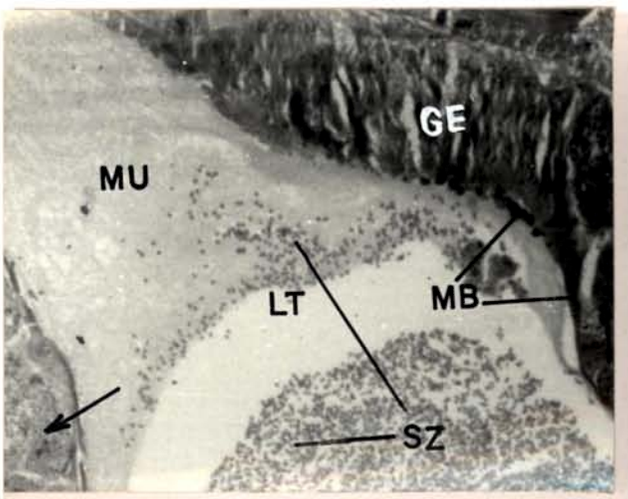
Fig. 3. Cross section of the terminal ampoule of the testes of Penaeus semisulcatus. The glandular epithelial cells are greatly hypertrophied (arrow) where the meronts (ME) form compact colonies. Unaffected glandular epithelial cells (UG) are also visible. Bouin-Mallory's triple stain.

Fig. 4. Transverse section of the terminal ampoule of the testes of Penaeus semisulcatus showing advanced stage of infection. The glandular epithelial cells have been completely replaced by Thelohania semisulcata. Arrow=Compact colonies of the microsporidian; GM=Gelatinous material; SZ=Spermatozoa. Bouin-Mallory's triple stain.

Fig. 5. Transverse section of the terminal ampoule of testes of Penaeus semisulcatus to show the multiplying meronts in the glandular epithelial cells (ME). Bouin-Mallory's triple stain.

Fig. 6. Longitudinal section of the terminal ampoule of testes of Penaeus semisulcatus. The spermatophore (SF) is compressed as a result of the enlargement of the glandular epithelial cells (GE) due to microsporidian infection. GM=Gelatinous material; MC=Muscular layer of terminal ampoule; SZ=Spermatozoa. Bouin-Mallory's triple stain.

PLATE XXVII



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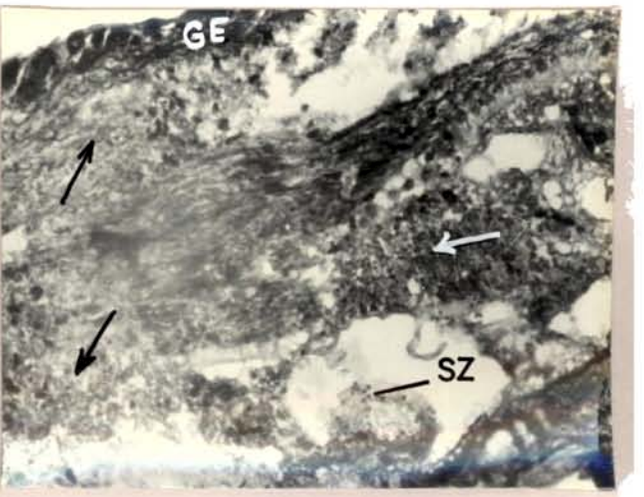

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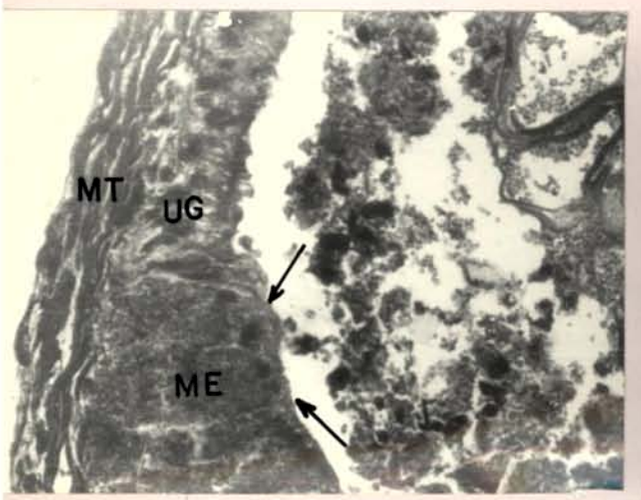

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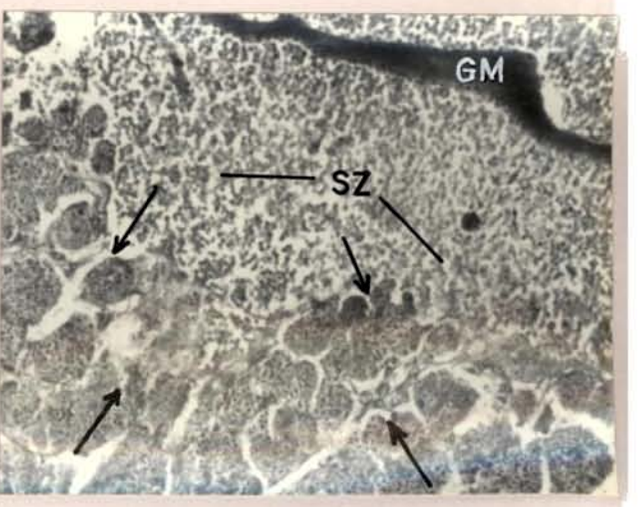

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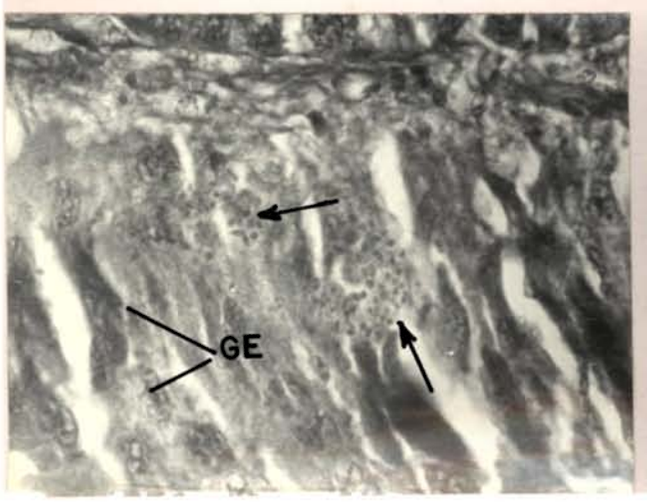

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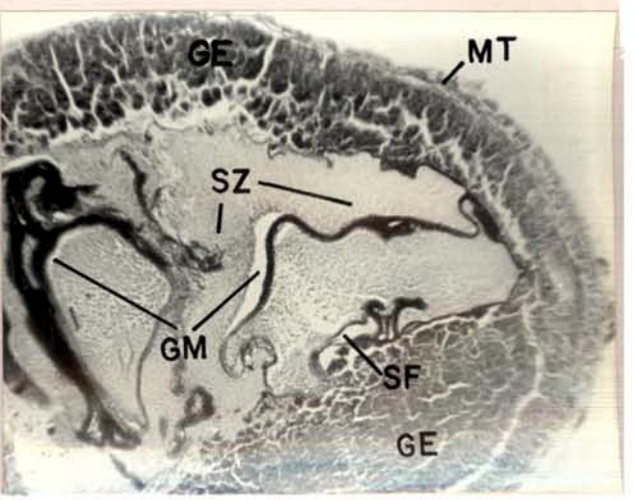


700 µm 6

were observed in the space between these two layers (Pl. XXVIII, Fig. 1). However, the spermatozoa ensheathed with a thin film of gel-like eosinophilic substance in the spermatophore were not infected. Similarly, the connective tissue and longitudinal and circular muscle fibres overlying the glandular epithelium were not infected except at the basal part of terminal ampoule around the external opening. In this region, compact colonies of meronts were observed in the connective tissue. Occasionally, wandering (phagocytic) cells were found carrying the infection.

Hepatopancreas

In the transverse section of hepatopancreas of a moderately infected prawn, most of the peripheral tubules were seen filled with spores and sporulation stages of T. semisulcata (Pl. XXVIII, Fig. 2). The connective tissue membrane bounding hepatopancreas was intact and unaffected (Pl. XXVIII, Fig. 3). Most of the epithelial cells of the infected bubules were replaced by spores and pansporoblasts of the pathogen. A few unaffected cells were found detached from the basophilic membrane of the tubule and lay as clumped debris in the tubule lumen (Pl. XXVIII, Fig. 4). At may places, the thin basophilic membrane supporting the epithelial cells was not visible and the basophilic connective tissue strands by which individual tubules are held together in normal hepatopancreas, were not very

PLATE XXVIII

Fig. 1. Longitudinal section of the terminal ampoule of testes of Penaeus semisulcatus showing the growing meronts and sporonts of microsporidian(TS) between the two layers of the gelatinous material(GM). GE=Glandular epithelium; MT=Muscular layer of terminal ampoule; SZ=spermatozoa. Bouin-Mallory's triple stain.

Fig. 2. Transverse section of hepatopancreas of a moderately infected Penaeus semisulcatus. Darkly stained areas show infected tubules which are replaced by the microsporidian. Bouins-Heidenhain's haematoxylin and eosin.

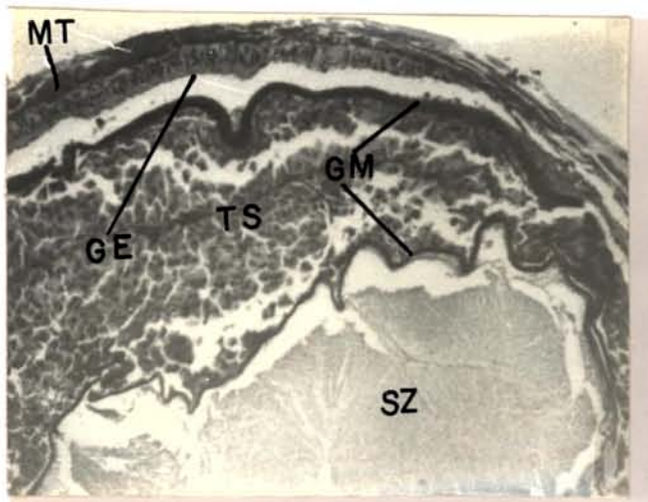
Fig. 3. Transverse section of hepatopancreas of a moderately infested Penaeus semisulcatus. The connective tissue membrane of the hepatopancreas (arrows) is still intact and unaffected. HT=Hepatopancreatic tubules; TS=Infected regions.

Fig. 4. Transverse section of hepatopancreas of a moderately infected Penaeus semisulcatus. Most of the epithelial cells of the hepatopancreatic tubules have been replaced by the microsporidian. Debris of unaffected epithelial cells (EP) are seen lying in the lumen of the degenerating tubules. Compact black masses are spores and pansporoblasts of Thelohania semisulcata.

Fig. 5. Transverse section of hepatopancreas of a moderately infested Penaeus semisulcatus. The basophilic membrane and connective tissue strands have almost disappeared. Arrow indicates the remnants of the membrane and strands. TS=Microsporidian spores.

Fig. 7. Transverse section of hepatopancreas from a heavily infected Penaeus semisulcatus. The pathogen has occupied most of the tubules (darkly stained areas) and those not filled up with the pathogen, are undergoing degeneration (arrow). Bouins-Mallory's triple stain.

PLATE XXVIII



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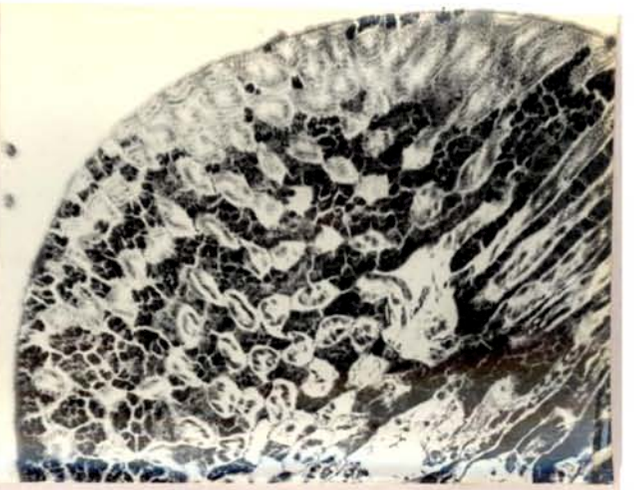
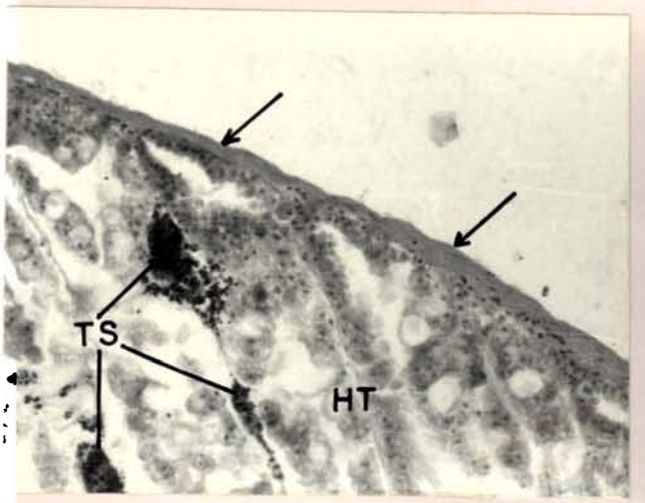
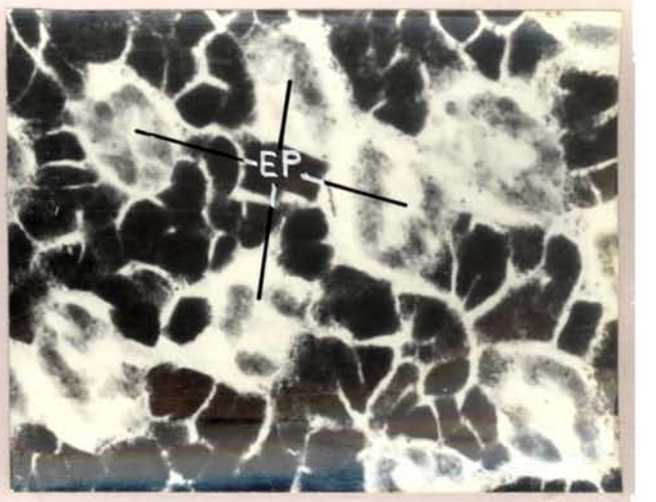


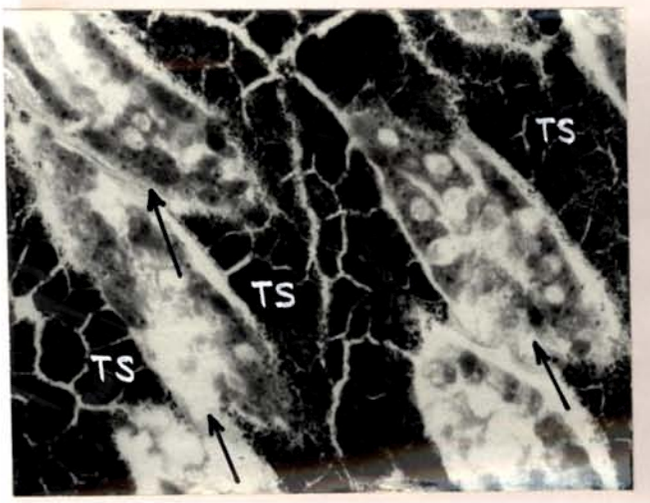
700 µm 2



150 µm 3



150 µm 4



150 µm 5

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conspicuous (Pl. XXVIII, Fig. 5).

The epithelial cells of some of the unaffected tubules contained one to many vacuoles and the cell cytoplasm stained pink when haematoxylin and eosin stain was used. However, the nucleus of these cells showed slight variation from what was generally observed in normal hepatopancreatic epithelial cells. The nuclear membrane bounding the nucleus in most of these cells appeared granular. Further, there were usually more than one nucleoli, sometimes upto six, which were strongly basophilic staining dark with Heidenhain's haematoxylin. In the nucleus of some epithelial cells, scattered chromatin material in the karyoplasm was clearly visible.

Transverse section of a hepatopancreas from a heavily infected prawn showed almost total replacement of the hepatopancreatic tubules by spores and pansporoblasts; tubule lumen was also filled with the pathogen (Pl. XXVIII, Fig. 6). The thin connective tissue membrane supporting the tubular epithelial cells completely disappeared and the inter-tubular space, which in the moderately infected material contained thin connective tissue strands, was generally replaced by spores and pansporoblasts. Occasionally, a few wandering cells were visible at places.

The spores and pansporoblasts were usually not freely dispersed but were arranged compactly in large

masses. The connective tissue membrane bounding the entire hepatopancreas was infected and possessed small or large groups of spores (Pl. XXIX, Fig. 1) randomly distributed throughout. However, the membrane was still intact. This membrane, encircling the colonies of microsporidian at certain places, was seen in hypertrophic condition.

Alimentary canal*

Of the three parts of the alimentary canal, namely, foregut, midgut and hindgut, infection in the midgut was more pronounced. When examined under the dissecting microscope, infection appeared as thick, white patches of irregular shape and variable sizes scattered all over the outer surface of the midgut (Pl. XXII, Fig. 3). The foregut was devoid of such symptoms whereas in the hindgut, the patches were either only a few or absent.

In the transverse section of an infected midgut, the outer and inner margins appeared uneven due to the infection by T. semisulcata (Pl. XXIX, Fig. 2). Infection was pronounced in the sub-mucosa, but rarely found in the muscularis. It was also not observed in the mucosa which forms the inner most layer of the midgut and consists of simple columnar epithelial cells facing the lumen of the

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* Refers here to the gut excluding hepatopancreas. The terminology used for different layers of the gut is after Lightner (1978b).

PLATE XXIX

Fig. 1. Transverse section of the peripheral part of the hepatopancreas of a heavily infected prawn. Note the infection in the hepatopancreatic membrane (arrow) bounding the hepatopancreas. Darkly stained areas are compact colonies of the pathogen. HT = Hepatopancreatic tubules. Bouin-Heidenhain's hematoxylin and eosin.

Fig. 2. Transverse section of the infected midgut of Penaeus semisulcatus. Note the blebing of the gut at the foci of infection (arrows) giving a characteristic uneven appearance to the outer margin of the organ. Bouin-Mallory's triple stain.

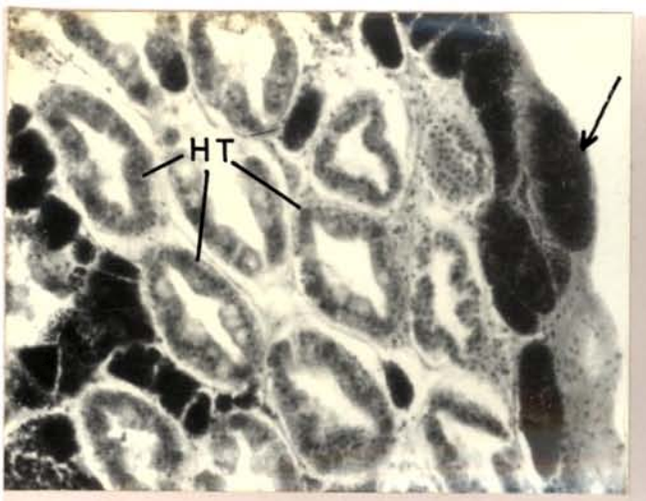
Fig. 3. Transverse section of a part of the infected midgut of Penaeus semisulcatus showing the compact colonies of the pathogen (TS) in the sub-mucosa (SU). The mucosa is not visible in the figure of it was sloughed. BL= Basal lamina; LG=Lumen of the gut. Bouin-Mallory's triple stain.

Fig. 4. Cross section of the heart of a heavily infected prawn. Two small, round to oval shaped aggregations of the pathogen (TS) are seen. CM=Cardiac muscle; HL=Haemolymph. Bouin-Heidenhain's hematoxylin and eosin.

Fig. 5. A blood vessel (BV) from a heavily infected prawn. Note the spores released from the vessel. Wet mount.

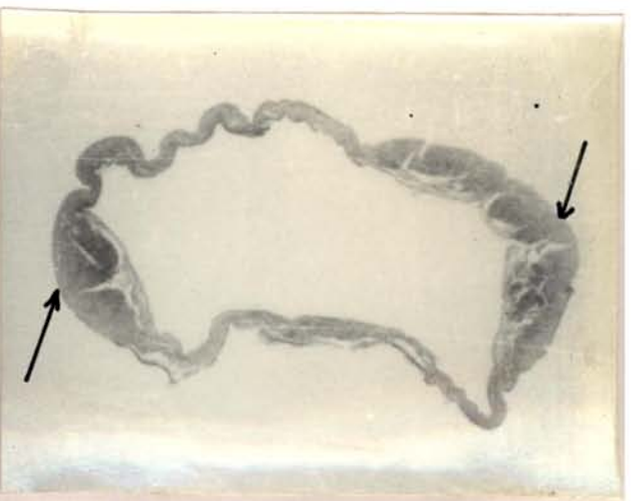
Fig. 6. Longitudinal section of eye ball of a heavily infected prawn. The sporulating stages of the pathogen (arrows) are seen located in the retinal region of the eye. Bouin-Heidenhain's haematoxylin and eosin.

PLATE XXIX



150 µm

1



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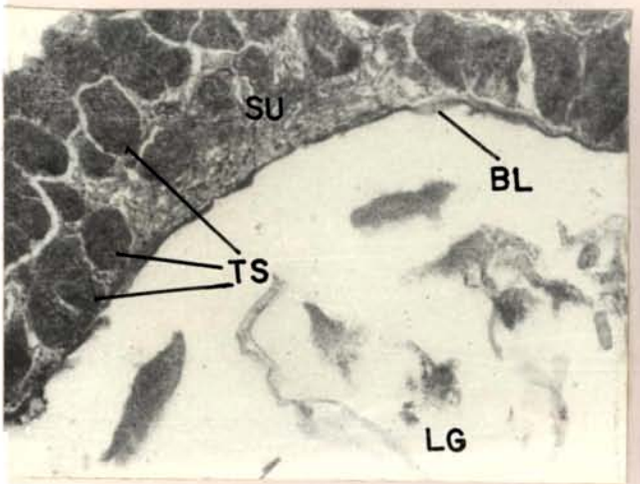

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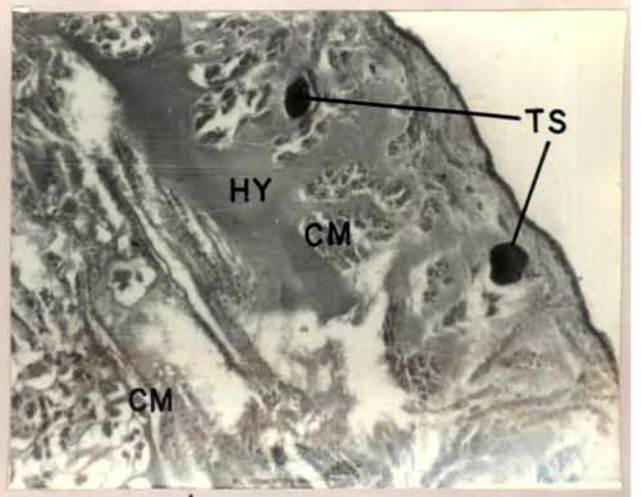

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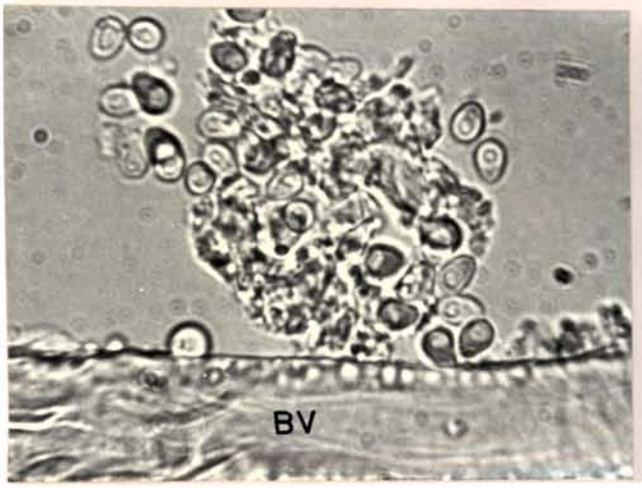

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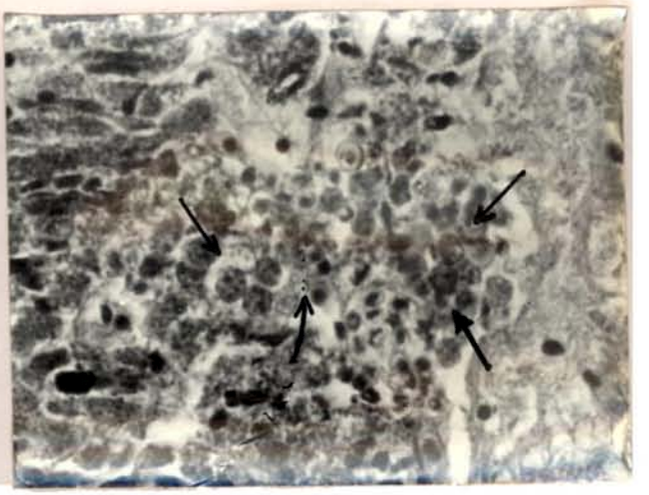
5



40 µm

6

midgut. This layer was usually sloughed rather than invaded. The basal lamina (basement membrane), overlying the mucosa and composed of thin reticular fibres, was also devoid of infection.

The sub-musoca, consisting of loosely arranged (areolar) connective tissue, was the main target of the microsporidian attack where the pathogen multiplied and produced pronounced infection. The multiplying _. semisulcata formed compact colonies which were localized at places in this layer (Pl. XXIX, Fig. 3). Growth of these colonies, irrespective of the circular border of the sub-mucosa, resulted in blebing at the foci of infection which gave the characteristic appearance as mentioned earlier, to the outer margin of the midgut (Pl. XXIX, Fig. 2). Each colony contained a large number of meronts, developing pansporoblasts and mature spores. The foci of infection were often surrounded with interlaced collagenous fibres and flat, branching fibroblasts. The formation of such a membranous layer around the growing foci of pathogen was presumably due to the inflammatory response of host tissue to the infection to delimit the growth of the pathogen. However, connective tissue layer around many of the pathogen masses was found ruptured, perhaps due to the excessive pressure exerted by continuously multiplying _. semisulcata. Numerous fibroblasts, phagocytes and haemocytes were present in the areolar connective tissue of sub-mucosa. Further, there was excessive deposition of

densely arranged collagenous fibres overlying the sub-mucosa; this appeared like a thick membrane which stained blue purple with aniline blue of Mallory's triple stain. Several individual spores and pansporoblasts were found to be embedded in this membrane. The thin membranous layers surrounding the developing colonies of microsporidian in the sub-mucosa were connected with this thick membrane of collagenous fibres at several places. The muscularis and serosal layers confluent with collagen fibres, were relatively thin and occasionally contained a very few spores and/or pansporoblasts.

Heart

Microscopic examination of the transverse section of the heart tissue of a heavily infected prawn showed a few small, round to oval aggregations consisting of spores and other developing stages in between the cardiac muscle fibres (Pl. XIV, Fig. 4). Haemolymph and the wall of the heart did not contain these aggregations. There was no apparent damage to the host tissue, and host response by accumulation of large number of haemocytes was not observed. Infection was also not seen in the wall of the blood vessels. However, free spores and other stages of the pathogen were occasionally observed in large numbers in the haemolymph in the blood vessels (Pl. XIV, Fig. 5).

Other tissues/organs

There were apparently no visible lesions on the ventral nerve cords removed and examined from at least 10 prawns heavily infected by T. semisulcata. In transverse and longitudinal sections of ventral nerve cord, none of the stages of life cycle of T. semisulcata were observed. However, in the optic nerve, cut longitudinally along with the eye-stalk of a heavily infected female prawn, spores were observed as small rounded masses entangled in between the axons and dendrites forming a complicated network in this region.

Infection was also found in the eye-ball; a small colony of pathogen consisting mostly of sporulating stages was seen located in the retinal region where numerous optic nerve-fibres, arising from the optic nerve ganglion and sub-retinal blood vessels form a complex (Pl. XLIX, Fig. 6). However, the overlying ommatidia were free from any such colonisation of the pathogen. The optic ganglion in the eye-ball was also devoid of infection, yet, lower in the eye-stalk, optic nerve was found infected as mentioned earlier. Besides, clumped spores and pansporoblasts were also seen in the haemolymph of the optic artery in the longitudinal section of the eye-stalk. Muscles present in the eye-stalk were much affected and damaged; several muscle fibres were replaced by pansporoblasts and spores of T. semisulcata.

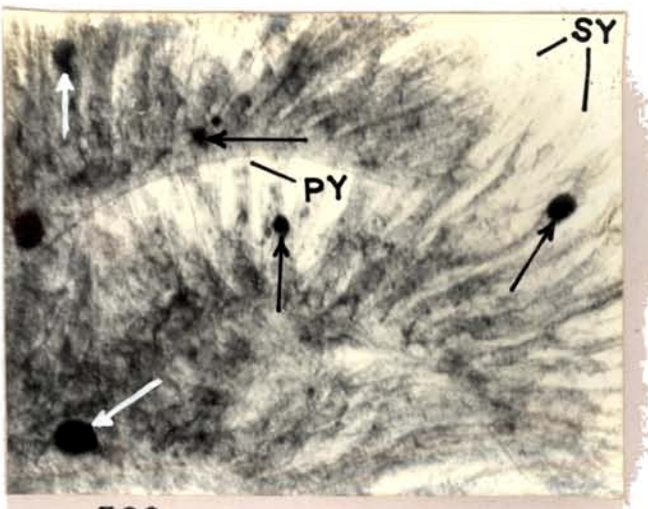
PLATE

Fig. 1. Gill lamella from an infected Penaeus semisulcatus showing colonisation of the microsporidian (arrows) in the primary (PY) and secondary lamellae(Y). Wet mount.

Fig. 2. Transverse section of normal developing stage ovary of Penaeus semisulcatus. CV=connective tissue; FS=Follicles; IZ=Inner germinal zone; OC=Oocytes; OE=Outer epithelium; OG=Oogonia; SC=Septa of connective tissue. Bouin-Mallory's triple stain.

Fig. 3. Transverse section of normal developing stage ovary of a Penaeus semisulcatus to show the maturing oocytes (OC) and the round nucleioli(NI). Bouin-Heidenhain's haematoxylin and eosin.

PLATE XXX



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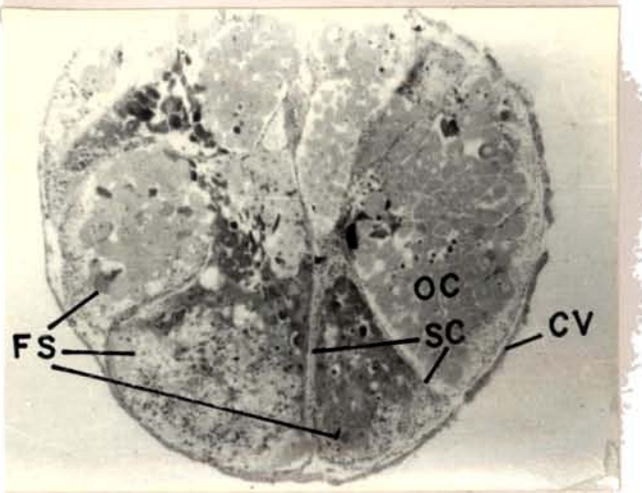
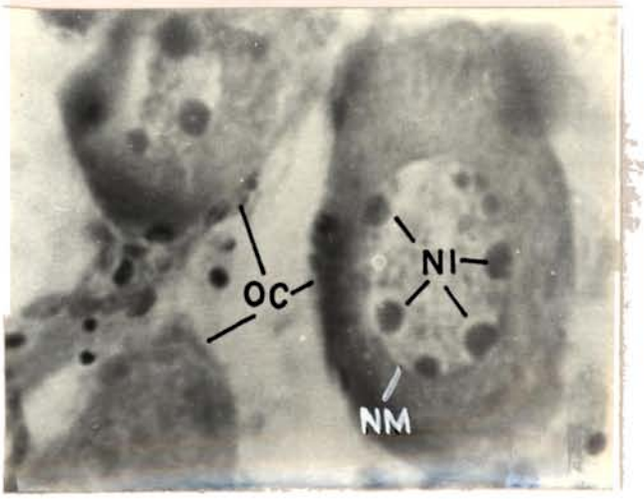


500µm 2



20µm 3

In the gills, infection was not much pronounced, yet, colonisation of the microsporidian was observed at certain places, usually, in the secondary lamellae (Pl.XXX, Fig. 1).

DISCUSSION

The histopathological investigations on the microsporidian, *P. semisulcata* indicate that it is an intracellular parasite and its tissue affinity is not restricted to a particular tissue of the host, *P. semisulcatus*. It produces more or less pronounced infection in the musculature, gonad, hepatopancreas and midgut. Infection was also seen, although not extensively, in the connective tissue, optic nerve , retina and gills. Weiser (1976) pointed out that microsporidia have several target tissues in their hosts where they concentrate and develop. Generally, they use one of the widely distributed tissues, such as connective tissue of the host, to produce the initial infection and subsequently spread to other adjacent tissues such as gonad, muscle and nerve ganglion. However, infection of more than one tissue/organ in prawns by the microsporidians is not uncommon as reported by Iversen and Manning (1959), Kruse (1959), Iversen and Van Meter (1964), Constransitch (1970), Overstreet (1973), Lightner (1975), Iversen and Kelley (1976) and Thomas (1976).

The initial site of infection by *P. semisulcata* in *P. semisulcatus* is the midgut (See Sub-Chapter 4.4 on transmission

experiments). The conditioned spores ingested by the host discharge their infective sporoplasm into the sub-mucosa below the gut epithelium and initiate merogony. The haemocytes present in the sub-mucosa eventually engulf the meront. These haemocytes probably serve as carriers of infection, transporting the pathogen to the different tissues of the host through haemolymph. Weiser (1976), while discussing the host-parasite relationship of the microsporidian parasites of the invertebrates, opined that infection beyond the gut wall needs the transportation of vegetative stages of the parasite by haemolymph to other parts of the body. He (Weiser, 1976) further pointed out that the primary host cells (probably haemocytes) which absorb the vegetative stages by phagocytosis are unable to destroy the parasite. In contrast, they feed and protect these stages and finally burst, thus liberating the various stages of the microsporidian which are subsequently transported to other body parts of the host through the haemolymph. Weidner (1970, 1972) found that infection of Nosema sp. in the blue crab, Callinectes sapidus is carried by the haemocytes in the sub-mucosa through the haemolymph to the muscle proximal to the haemocoel where sporogenesis occurs. These observations as well as those made in the present study indicate that after initial infection in the sub-mucosa of the gut, the pathogen spreads to the other tissues of different organs of the host through the haemolymph and, after successful establishment in the concerned tissues, proliferates by

merogony and sporulation.

Among the different organs of P. semisulcatus, affected by T. semisulcata, the midgut presents the typical lesions more precociously, hence its examination is presently the most suitable one for identifying the initial stage of infection in the individual.

The presence of empty spore cases of T. semisulcata in different organs of host, which are partly or completely infected, suggests a possible mechanism of autoinfection. In this process, the spores that are released by the degeneration of an infected cell (Pl. XLIV, Fig. 6), discharge their sporoplasm into the adjacent cells of the host organ and germinate, thereby playing a significant role in the spread of infection in the host body. The mechanism of autoinfection by microsporidian parasites has been reported by several workers (Lom, 1970; Summerfelt and Warner, 1970; Maticados, 1980; Sprague and Vussey, 1980).

In the body muscles, the meronts of T. semisulcata infiltrate the sarcoplasm of the muscle cells and develop colonies which later undergo sporogony; the sarcoplasm itself gets reduced and finally replaced almost completely by spores and other developing stages of the pathogen. The myofibrils in the affected muscle cells gradually disappear. Studies on other species of prawns infected by Pleistophora

spp. (Overstreet and Sprague, 1974; Breed and Olson, 1977) reveal that the repeated merogonic divisions followed by sporogonic divisions, and the resultant increase in spore numbers within the muscle sarcoplasm most likely cause host cell hypertrophy and subsequent lysis of the fibres. In the present study, however, such cell hypertrophy in the muscles is not observed but cytoplasmic disintegration is seen in several cells. Weissenberg (1976) has pointed out that cytoplasmic disintegration occurs through a possible lysis of cell structures by proteolytic enzymes which are believed to be released by meronts and/or maturing spores and catalyze the dissolution of the host cell cytoplasm.

In normal P. semisulcatus, the mature ovaries are paired organs, situated dorsally, extending from the base of the rostrum to the last abdominal segment. In general morphology, they are similar to those described in other penaeids (King, 1948; Rao, 1968). The transverse section of a developing normal ovary at maturing stage (Pl. XIX, Fig. 2) reveals that it is surrounded by an outer epithelium, a layer of connective tissue and the inner germinal zone. The septa of connective tissues give rise to large number of follicles in the ovary. The follicles contain oogonia and oocytes of different sizes in various stages of development. The immature and maturing oocytes possess finely granular basophilic cytoplasm which gradually becomes

acidophilic as the oocytes reach maturity. Their nuclei possess numerous round nucleoli arranged peripherally on the inner side of the nuclear membrane (Pl. XX, Fig. 3). The ratio of cytoplasm to nuclear material and the number of nucleoli vary with the development of oocyte. In the mature ovary (Pl. XXV, Fig. ?). follicles are not very distinct as the oocytes are fully packed inside the ovary. The mature oocytes are comparatively larger and surrounded by attenuated follicle cells. At the peripheral region, the oocyte possesses characteristic cortical bodies. There is dense accumulation of yolk in the cytoplasm.

Comparison of the histological characteristics of an uninfected ovary (Pl. XXV, Fig. 2; Pl. , Fig. 2) with that of the infected ones (Pl. XXV, Fig. 3 and 5; Pl. XXVI, Fig. 1, 2 and 3) illustrates the drastic cytological changes occurring in ovarian structure due to infection. The disappearance of normal nucleoli and the presence of "inclusion bodies" (IBs) inside the karyoplasm of oocytes are the conspicuous features observed in the lightly infected ovary. The IBs are of different size and shape. Their staining property reveals them to be basophilic in nature since they stain deeply with basic dyes. The presence of IBs in the karyoplasm was first thought to be an artefact, but their regular occurrence in the carefully preserved, prepared and processed ovaries of several specimens rules

out this possibility and indicates that they are associated with the infection of the ovary by the microsporidian. The presence of IBs only in the karyoplasm of oocytes of partly or fully infected ovaries, and their absence in other tissues, has alternatively prompted to suspect them to represent one of the dimorphic forms of T. semisulcata, where one form which represents the most common, may be present in all the tissue types of the host and the other form, represented here by the IBs, may be present only in the nucleus of the oocytes. According to Sprague (1976), dimorphism in microsporidia is characterised by the occurrence of two types of development and two types of morphology within a species. Further detailed studies are essential to understand the exact nature of origin of the IBs and their role in the infection of T. semisulcata.

In several specimens, partly infected ovaries are encountered. The affected parts of the ovarian lobes do not contain developing or mature oocytes, but contain dense colonies of meronts. This pattern of infection gives the ovary a characteristic nodular appearance (Pl. XXII, Fig. 2), where the infected parts are shrunken, relatively narrow and appear whitish. The other parts of the ovarian lobes are, however, turgid, light to dark green in colour and contain oocytes of different maturity stages with conspicuous IBs in their karyoplasm. When the parasite destroys and replaces the contents of the oocytes, it obviously utilises the cytoplasmic constituents of the host cell. Summerfelt and

Warner (1970), in their studies on Plistophora (=Pleistophora) ovariae infecting the golden shiner, Notemigonus crysoleucas, reported that the parasite seemed to be physiologically dependent on the phosphoproteins (vitellin) and the phospholipids (lipovitellin) present in the oocytes.

The male reproductive system of the normal and healthy P. semisulcatus is similar to that of other penaeid prawns (King, 1948; Subrahmanian, 1965; Haq, 1981), consisting of a pair of testes, vasa deferentia, terminal ampoules and a petasma. The testes are unpigmented, translucent organs located at the cardiac region, dorsal to the hepatopancreas. Each testis is comprised of several testicular lobes opening into the vas deferens of its side through a tubular duct. The vas deferens of each side traverses through the muscles of the cephalothorax and opens at the base of the fifth pereopod of the respective side through the bulbous terminal ampoule. The petasma in P. semisulcatus, which is formed by the modified endopodites of the first pair of pleopods, has been well described by Mohamed (1969).

Histologically, each testicular lobe has a thin outer membrane without any kind of muscle layer. The testis is composed of a number of seminiferous tubules in which the male reproductive cells are produced. The spermatogonia and spermatocytes are larger cells which are compactly packed and having a single, large nucleus. These cells are usually

found along the margin of the tubules. The spermatids and spermatozoa are smaller and are found in the lumen of the tubules. Nutritive or nurse cells are found scattered with developing germ cells. In tubules containing spermatocytes and spermatids, these nurse cells are confined to the peripheral region of the tubule. The vas deferens in transverse section has an outer thin membrane and an inner layer of columnar epithelial cells which are glandular in nature. The lumen is filled with spermatozoa mixed with mucous-like substance. The vas deferens at its distal end joins with the terminal ampoule which has two chambers. In one of the chambers the spermatophoric mass is found and in the other, a thick, sticky, gelatinous substance. Terminal ampoule possesses a thick muscular wall which is lined with tall columnar epithelial cells. These cells are secretory in nature and form numerous folds and partitions by extension.

Comparison of the infected male reproductive system with that of the normal one indicates the highly pathogenic nature of *L. semisulcata* impairing the production capacity of healthy spermatozoa by the host as the seminiferous tubules, vas deferens and terminal ampoules are found affected by the pathogen. As the infection spreads, the pathogen multiplies and replaces the host reproductive cells as well as the glandular epithelial cells. The latter cells, in the advanced state of infection,

are found heavily invaded by the growing colonies of the pathogen, which affects considerably the secretion of nutritive mucous by the cells. Similarly, the secretion of the thick, gelatinous sustance, which forms the outer covering of the spermatophore, is also affected as the glandular epithelial cells of vas deferens and terminal ampoules are replaced by the colonies of the pathogen. However, the connective tissue membrane surrounding the testes and the vas deferens as well as the muscular and the connective tissue layers of the terminal ampoules are not much affected.

In normal P. semisulcatus, hepatopancreas forms a large, compact, paired glandular mass occupying much of the cephalothoracic cavity. It is ensheathed by a thin connective tissue membrane. Usually, the colour of the hepatopancreas is brown to orange red. However, it varies considerably in the individuals of the same species with different maturity and moulting stages. Histological structure of the hepatopancreas of P. semisulcatus is similar to that of other penaeids. It consists of numerous blindly-ending tubules which are lined by simple columnar epithelial cells. Each of the tubules is connected to secondary ductules which, in turn, join the primary duct of the respective side. The primary ducts open into the gut at the junction between the pyloric stomach and

the midgut. Each hepatopancreatic tubule has a lumen in the centre. The epithelial lining of the tubules, except at the distal blind end, is only a single cell layer thick. Individual tubules are loosely held together by basophilic connective tissue strands. Wandering cells are present in the connective tissue and blood spaces between the hepatopancreatic tubules.

In the hepatopancreas, the infection by T. semisulcata is initiated in the tubules where the main target of attack is the tubular epithelial cells. The different cell types which are involved in the secretory, absorptive and metabolic activities (Gibson and Parker, 1979) as well as the characteristic vacuoles present in the cells, except in the undifferentiated ones, are found greatly affected by the infection, thus impairing the vital functions of the hepatopancreas. As the infection progresses, the lumen of the tubules are found almost packed with the spores and other developing stages. However, a few tubules of the hepatopancreas are found to be unaffected. The epithelial cells of these tubules contain rich vacuoles in their cytoplasm, indicating their functional nature. However, these cells also exhibit abnormal condition in the form of varying numbers of nucleoli and granular nuclear membrane. Nevertheless, whether these changes are due to infection or due to the normal process of mitotic division is not clear.

The connective tissue strands found between the tubules are also affected with the advancement of the infection and finally disappear. The hepatopancreatic membrane ensheathing the organ is the least affected tissue. However, localised foci of infection are occasionally seen (Pl. XXIX, Fig. 1) when the prawn is heavily infected.

The foregoing comparisons of the normal and affected organs of the prawn reveal that the infection of P. semisulcata, though mild in the initial stage, spreads gradually to all the vital organs of the prawn and becomes highly pathogenic, interfering with the normal functions of the different organs, in the advanced state of the disease. The histopathological investigation carried out at present further reveals that the host response to the microsporidian infection appears to be least developed or effective as the pathogen does not apparently elicit any significant inflammatory response in the host. When the microsporidian is initially encountered by a healthy prawn, the host neither shows any external clinical signs nor apparent internal lesions. This may be due to the lack of initial pathogenicity of the microsporidian and physiological stress response of the host before the pathogen is able to manifest its opportunism. The fact that the gut sub-mucosa gets infected first and atrophies, as has been observed in the present study as well as in fishes (Canning, 1976) and blue crab

(Weidner, 1970, 1972), supports this view as the infection in sub-mucosa leads to the reduction in immunological barrier against the invasion by the pathogen. With the failure of the immunological barrier, the infection spreads to other organs. As the infection becomes chronic, the prawn gets gradually enfeebled and when the density of the pathogen is maximum, almost replacing the cells of the host tissue, it becomes lethal and results in the death of the host. Histological examinations of cells in the different tissues of other organs such as musculature, hepatopancreas, gonad, heart and gills also reveal the absence of any significant host response.

Although all the important systems of the prawn are affected by the pathogen, the infection of the gonad (both testes and ovary) and their complete degeneration in the highly infected prawns, brings the greatest damage to the individual as well as the population. The degeneration process of the gonad directly affects the reproductive functions, as oocytes in all the stages of maturity as well as the testes and the vas deference are attacked by the pathogen and replaced almost completely by it in the advanced stage, thus impairing the production of viable ova and sperms. This annihilation of the reproductive capacity of the prawn affects the recruitment and replenishment of the prawn population. It has been reported that a protozoan infection, suspected to be caused by the

microsporidian, _. penaei_, destroyed the reproductive organs of about 90% of the white shrimp, _P. setiferus_ population in 1919 in Louisiana waters (Moseo, 1943). In the present investigation also, considerable number of prawns with their gonads seriously affected by _. **semisulcata** were collected.

Besides the gonads, the pathogen considerably damages the hepatopancreas as well as the abdominal and other locomotory muscles. While the infection of the former impairs the vital metabolic processes of the prawn, the latter, due to the development of characteristic white or cotton-like appearance leads to commercial rejection, resulting in heavy economic and production loss. Thus, _P. semisulcata_ affecting the population of _. semisulcatus_ can be considered as a highly pathogenic and most destructive parasite. The total effect of the pathogen on the prawn not only encompasses acute damage to the different organ systems, the metabolic and physiological processes, and propagation of the animal, but also the economics of their production. In fact, Couch (1973) considers microsporidia as highly pathogenic to shrimps and as one of the most destructive groups of pathogens to penaeid hosts.

4.4 LABORATORY EXPERIMENTS ON THE TRANSMISSION OF THELOHANIA SEMISULCATA

Experimental methods to study the life-cycle of the pathogens, symptoms and nature of the diseases introduced in the middle of the nineteenth century, gave a great impetus to the investigations in parasitology and enabled to unravel the complexity of several diseases caused by the parasites, particularly those completing their life-cycle in or through the secondary or intermediate hosts.

Experimental attempts to transmit microsporidians in decapods are only a few. Weidner (1970) studied the development of Perezia (=Nosema) nelsoni in Callinectes sapidus and succeeded in experimentally infecting the crabs. Another microsporidian, Ameson (=Nosema) michaelis was experimentally transmitted in the same crab by Overstreet and his associates (Overstreet, 1978). Breed and Olson (1977) were, however, unable to transmit Pleistophora crangoni in the sand shrimps, Crangon franciscorum, C. nigricauda and C. stylirostris, in their laboratory experiments.

In penaeid prawns, transmission experiments on microsporidians were carried out by Roth and Iversen (1971) and Iversen and Kelley (1976). The former attempted to transmit the microsporidian, Thelohania duorara to the uninfected pink shrimp, Penaeus duorarum in the laboratory.

Although they were unable to transmit the disease by feeding the heavily infected prawn tissue to the experimental animals, the work gave an insight to the possible modes of transmission in nature. They (Poth and Iversen, 1971) pointed out the possibility that the spores of *P. duorara* found between the old and new cuticle at the time of moulting could infect prawns that feed on cast off exoskeleton.

Iversen and Kelley (1976) successfully transmitted the microsporidian, *Amasoma* (=*Chelohania*) *pensei* to postlarvae of *P. duorarum* by feeding the faeces from spotted sea trout, *Cynoscion nebulosus* which was fed with infected pink shrimp. Besides these works, there has been no worth mentioning published information on the transmission experiments of microsporidian disease in penaeid prawns. The present experimental study was conducted with an aim to understand how the microsporidian, *P. semisulcata*, is transmitted from prawn to prawn and at what stage the prawns become infected. Three experiments on transmission of *P. semisulcata* to normal, healthy *P. semisulcatus* were conducted in the laboratory at the Regional Centre (RC) of Central Marine Fisheries Research Institute (CMFRI), Mandapam Camp during March, July and November-December, 1983. In the third series of experiments, *P. indicus* was also included. The results of these experiments are presented and discussed.

EXPERIMENT I

This experiment was carried out for 8 days from 19th March to 26th March, 1983.

Experimental set-up

Live and healthy adult specimens of *P. semisulcatus* ranging in size between 80 and 145 mm in total length (TL) were obtained from the bottom trawl net operated off the coast of Mandapam in the Palk Bay at about 30 meters depth on 16th and 17th March when 8 and 22 prawns were collected respectively. The prawns thus collected on each day were carefully transferred immediately to a fibreglass tank of 150l capacity on board the vessel containing about 75 l of sea water collected from the same area where the prawns were caught. The tank was transported as such to the wet laboratory of CMFRI at Mandapam Camp in a mobile van. During transportation, the water in the tank was agitated vigorously. In the laboratory, the prawns collected on both the days were transferred to a fibreglass tank of 500 l capacity provided with continuous running sea water. The source of sea water was from the adjacent Palk Bay, pumped in and supplied through the taps arranged in the laboratory. A double folded, very fine mesh cloth was wrapped to the tap's mouth in order to obtain filtered sea water. The tank had an outlet pipe at the middle of the tank for maintaining the water level. All

the animals were kept for acclimatization to the laboratory conditions in this tank for two to three days.

On 18th March, eight prawns were transferred from this tank to another fibreglass tank of 100 l capacity, which was provided with a sandy substratum of about 3 inches in thickness and a regulated, continuous flow of running sea water. This tank had an outlet near the surface to maintain the water level. In this environment, the prawns were seen to remain burried in the sand. Since the burrowing behaviour of the prawns in the sandy substratum posed difficulty for visual examination of animals as well as in the collection of faeces and unconsumed food without disturbance, sand was removed from the tank and the animals were maintained without any substratum.

During the period of acclimatization to laboratory conditions, prawns were starved to ensure complete digestion of already ingested food and to induce starvation stress as this would further increase the susceptibility to infection by microsporidians (Vavra and Maddox, 1976).

The experiments were commenced on 19th March, 1983 and continued to 26th March, 1983. All the experiments were carried out in ambient temperature (27 ± 2°), salinity (32 ± 2 ppt) and pH (8.1 ± 0.05) of the water in the tanks. The scheme of experiments carried out was as follows.

Tank 1. Control

Healthy prawns ranging in size from 92 mm to 137 mm TL were kept in a 500 l capacity tank containing about 350 l of running sea water filtered through a fine muslin cloth. The prawns were fed ad libitum with fresh clam (Meretrix sp) meat. ive clams were collected from the Gulf of Mannar and maintained in the laboratory in plastic troughs of 25 l capacity supplied with running sea water. efore feeding, it was ensured that the clam meat was not contaminated with any microsporidian or the microbes. It was also ensured that the sea water supplied to tank was clean and devoid of any pathogen.

Tank 2. Transmission by injection of fresh T. semisulcata

The main purpose of this experiment was to study the transmission through injection of the pathogen collected directly from already infected wild prawns into the body of the healthy experimental prawns. Four numbers of healthy prawns ranging in size between 87 and 131 mm TL were selected from the main stock and placed in a tank of 100 l capacity containing about 70 l of clean sea water. Continuous aeration with aerators was provided.

Specimens of P. semisulcatus infected by T. semisulcata (which was diagnosed by the white discolouration of abdominal

and thoracic muscle) were collected from Mandapam fish landing centre and brought to the laboratory in chilled sea water in an ice box. Infected prawns were examined by crushing a piece of abdominal muscle in a drop of physiological saline between a clean glass slide and a cover glass. This smear, when examined under an Olympus compound microscope, showed a large number of non-motile vegetative cells, spores and sporulation stages of *P. semisulcata*.

Spore suspension for injection was prepared by removing about 1 g of abdominal muscle from an infected prawn. It was homogenized and diluted with 3 ml of sterile distilled water. The sample thus obtained was centrifuged at 1000 r.p.m. for 1 minute. One drop of supernatant was taken on a clean glass slide covered with a glass cover slip and examined under a compound microscope. The sample was found to contain free spores and a few pansporoblasts containing octospores. 0.25 ml of supernatant was then drawn into a 1.0 ml glass syringe, which was earlier sterilized, and injected using a No. 24 hypodermic needle into the prawn in the lateral side between the second and third abdominal segments. All the four prawns in the tank were thus injected once with the pathogen. During injecting the pathogen, care was taken not to expose the prawns to the air for more than 30 seconds so as to minimize the stress. The prawns were fed once daily *ad libitum* with fresh clam meat. Every morning the unconsumed food material was removed and the water was changed with fresh, clean and filtered sea water.

Tank 3. Transmission by feeding

The main objective of this experiment was to study the transmission by feeding heavily infected tissue to the host. As in the experiment in tank 2, four healthy prawns (80 to 124 mm TL) were placed in a 100 l capacity tank, containing about 70 l of filtered, fresh and clean sea water. Small pieces of abdominal muscle of heavily infected prawns with T. semisulcata procured fresh from the fish landing centre, were fed ad libitum once in a day in the morning. After 24 hours, the unconsumed food was removed from the tank. The water in the tank was changed daily with clean and fresh sea water. Continuous aeration was provided to ensure adequate supply of oxygen.

Tank 4. Transmission by injection of defaecated T. semisulcata

The aim of this experiment was to find out whether the pathogen which was passed through the gut of the same species of prawn can produce infection if injected into the body of another prawn. As in previous experiments, 4 healthy prawns (110 to 145 mm TL) from the main stock were maintained in 100 l capacity fibreglass tank containing about 70 l of fresh, filtered and uncontaminated sea water. The faecal matter from the prawns in the experimental tank 3 was carefully collected in a centrifuge tube, diluted with sterile distilled water and centrifuged at 1000 r.p.m. for 1 minute. From the

supernatant, which was found to contain T. semisulcata. 1.0 ml was drawn out in a 1.0 ml sterilized glass syringe and of this solution, 0.25 ml was injected into each of the four prawns in the tank 4. This treatment was given only once on 20th March. The prawns were fed daily ad libitum with fresh and uncontaminated clam meat. As in the experiment in the tank 2 and 3, the sea water in the tank 4 was changed daily by fresh and clean sea water. Aeration was also provided as in the case of previous experimental tanks.

Faeces from all the four tanks were carefully collected everyday in separate embryo cups with the help of blunt forceps and a brush. These faeces were examined with a compound microscope by preparing wet mounts.

After 6 days from the commencement of the experiment, that is, on 25th March, 1983, haemolymph samples from one prawn in each of the tanks were drawn with the help of a 1.0 ml syringe fitted with No.24 hypodermic needle, which was pretreated with 2 percent sodium tricitrate to prevent coagulation of the haemolymph. The haemolymph samples thus obtained were immediately smeared on clean glass slides and were air dried. These smears were stained with Giemsa stain and mounted in DPX through xylene.

After drawing the haemolymph, these prawns along with other specimens were sacrificed and cut along the median dorsal line to allow better penetration of fixative and were

fixed in 10 percent neutral buffer formalin. Other sets of 2 prawns from each of the four tanks were sacrificed, dissected and fixed in the same manner on the next day, that is, 26th March, 1983. Later, the muscle, gonad and hepatopancreas were cut for histological observations at 5 to 7 µm thickness using a manual rotary microtome. Sections were stained with Heidenhain's haematoxylin and eosin as given in Chapter 2.

RESULTS

Tank 1. Control

The prawns were very active throughout the period of experiment and were found to feed normally on the clam meat. Examination of the faeces collected on various days revealed the complete absence of any stage of microsporidian. Similarly, the histological structures of the ovary and muscle tissues were similar to those of the normal prawn.

Tank 2. Transmission by injection of fresh *T. semisulcata*

The examination of the haemolymph smears, faeces collected from the tank and the histological sections of the abdominal muscle, gonad and hepatopancreas showed no infection by the pathogen after 6 days.

Tank 3. Transmission by feeding

Prawns were found to feed actively on the infected muscle tissue offered for feeding and were observed to defaecate thin strands of faecal matter after 12 to 18 hours of the initiation of the experiment. The colour of faeces was white as compared to that of brown faeces of control prawns. Examination of these faeces in the wet mount preparation under the compound microscope revealed the presence of clumps of spores of T. semisulcata. On close examination, it was found that the internal structure of the spores in the faeces was quite different from those in the infected prawn tissue which was offered as food (Pl.XXXI, Fig. 1). One of the differences observed was that the pansporoblastic membrane surrounding the octospores in the defaecated spores was completely absent. The other structural differences noticed were the absence of posterior vacuole and presence of one small granule which stained dark blue to light magenta when stained with dilute Giemsa stain. The granule was found at varying position in these defaecated spores. In haemolymph smears, however, none of the developmental stages of T. semisulcata was recognisable. Similarly, in the histological examination of abdominal muscle, ovary and hepatopancreas of the experimental prawns, no recognizable stage of the microsporidian was observed.

PLATE XXXI

Fig. 1. Characters of spores of Thelohania semisulcata before ingestion (A) and those collected from the faeces of the experimental prawns (B) in Experiments I, II and III.

Fig. 2. Meronts and sporonts of Thelohania semisulcata from the abdominal muscle of a postlarva of Penaeus semisulcatus fed with fresh infected prawn muscle on the fourth day of Experiment II.

PLATE XXXI

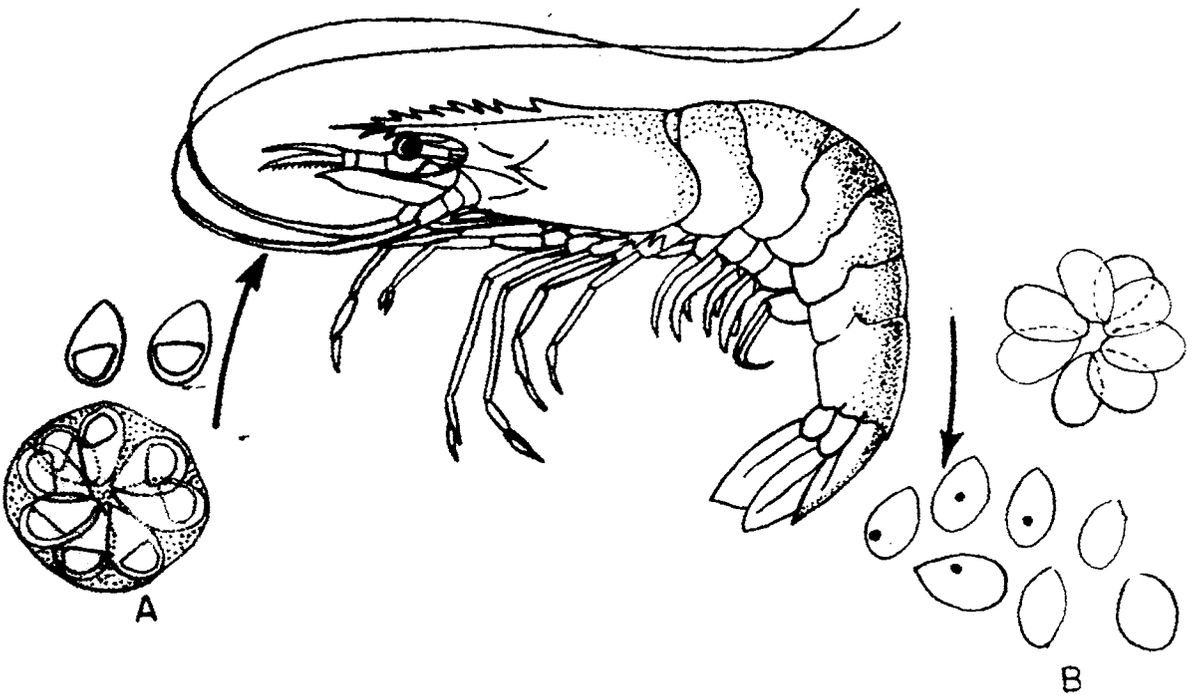


Fig. 1

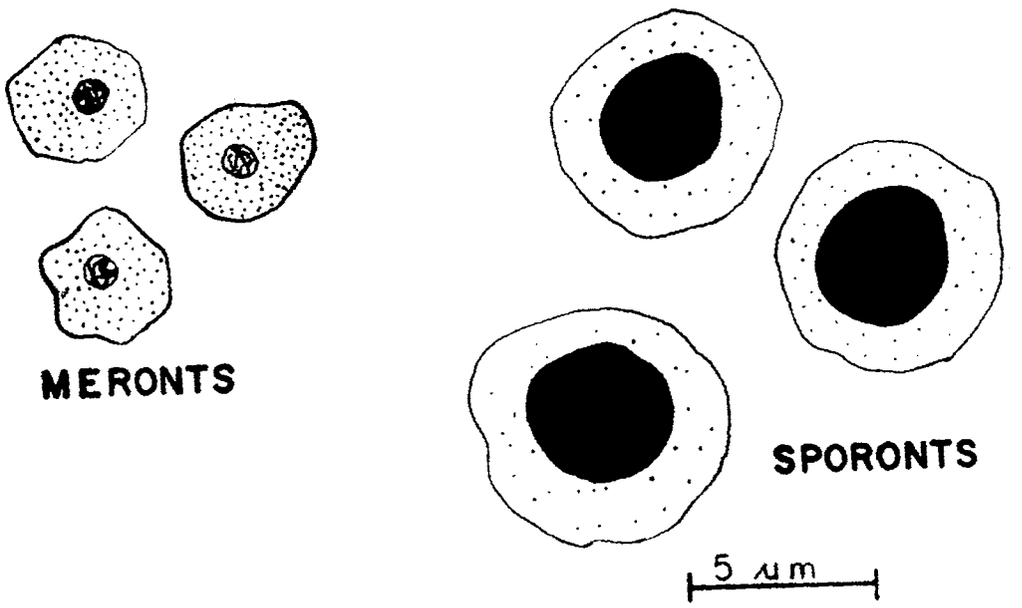


Fig. 2

Tank 4. Transmission by injection of defaecated *T. semisulcata*

As in the case of prawns in the experimental tank 2, the examination of the haemolymph, faeces and histological preparations of the muscle tissue, ovary and hepatopancreas did not show any sign of infection by the pathogen during the period.

EXPERIMENT II

Iversen and Kelley (1976), while carrying out experiments on the transmission of *T. duorora*, *Pleistophora* sp. and *P. penaei* to the pink shrimp, *P. duorarum*, observed that for the infection of the host tissue, it was essential that the microsporidian, *P. penaei*, is pre-conditioned by passing through the gut of the spotted sea trout, *C. nebulosus*. The structural changes observed in spores during the first experiment (tank 3) indicated this possibility in the present species also. In the light of this result, the second experiment was planned to transmit the pathogen by different methods to the different stages such as postlarvae, juveniles and adults of *P. semisulcatus*. The experiment was carried out for 14 days from 10th to 23rd July, 1983 at the MC of CMFRI, Mandapam camp.

Experimental set-up

Postlarvae and juveniles of P. semisulcatus ranging in size from 8 to 40 mm TL, and adults ranging in size from 95 to 195 mm TL, were used in this experiment. The postlarvae and juveniles were collected from the feeder canal of the fish farm of the MC of CMFRI, Mandapam camp, by using scoop net. They were transported to the laboratory in air-tight polyethylene transportation bags of 10 l capacity, half of which was filled with the water collected from the same area of the canal from where the postlarvae and juveniles were collected, and the other half with oxygen.

In the laboratory, 30 prawns were kept in each of the four plastic troughs of 10 l capacity containing about 8 l of filtered and fresh sea water. This was done in order to avoid overcrowding and occurrence of cannibalism. Adequate aeration was given by connecting the troughs with aerators. Animals in all these troughs were starved till the commencement of the experiment on 10th July, 1983.

Adult P. semisulcatus were caught off Mandapam coast at about 30 meters depth by mechanised boat operating a trawl net on 5th, 6th and 7th July, 1983. Live and healthy prawns from the catch were sorted out and transferred immediately to a rectangular fibreglass tank of 150 l capacity arranged on board the vessel. The tank was filled with fresh sea water collected from the same area where

the net was operated. The prawns were transported to the laboratory as described in Experiment I. In the laboratory, these prawns were maintained in four rectangular fibreglass tanks of 100 l capacity with open running sea water facility. Source of sea water in the laboratory was same as described in the experiment I. In each of the tanks, 10 prawns were kept for acclimatization. Food was not given to the prawns till the commencement of the experiment on 10th July.

From each of the four glass troughs, in which the postlarvae and juveniles were maintained, two prawns were sacrificed on the second day of their collection and dissected to examine for the presence or absence of the microsporidian parasite. Before and after use, the forceps and other instruments employed for dissecting the prawns and maceration of the tissue were carefully cleaned to avoid the possibility of contamination of the pathogen. Wet mounts were prepared from the gills, hematopancreas, midgut and abdominal muscle tissues as described in Experiment I. Detailed and careful examination of these tissues gave no clue as to the presence of microsporidian and revealed that all the animals were in normal condition. Similarly, from the adult prawn stock, four prawns, two males and two females, were examined and it was found that they were free from the microsporidian infection and were in a healthy state. Again on the day of starting the experiment, two postlarvae from each glass trough were

sacrificed and examined in the same manner to confirm that they were free from any earlier microsporidian infection. That the adult prawns were free from microsporidian was ascertained before starting the experiment by frequently examining their faeces under the compound microscope. In no case microsporidian spores or any developmental stages were observed. Further, the prawns showed all the natural colouration and texture of the exoskeleton and muscle, and exhibited normal behaviour in ieating that they were healthy and normal.

The experiment was started on 10th July, 1983 and terminated on 26th July, 1983. The experiment was carried out in a ient temperature (30 ± 2°C), salinity (35 ± 2 ppt) and p (8.25 ± 0.05) of the water in the tanks and troughs. The experiments described from to deal with the postlarvae and juveniles and those described from E to I with the adult prawns. The arrangements and treatment of troughs and tanks were as follows.

. Control

Two glass troughs, each 5.5 l capacity and filled with about 4.5 l of filtered and fresh sea water, were taken in duplicate and treated as control. In each of these two troughs, 30 postlarvae of P. semisulcatus, 8 to

15 mm TL were introduced. The control troughs were placed away from the other experimental units. The troughs were cleaned daily and refilled with fresh and clean sea water. The water was continuously aerated with aerators. Prawns were fed ad libitum with minced meat of fresh clam (Meretrix sp.). The unconsumed food was removed every morning on the next day.

B. Transmission through branchial chamber

The aim of this experiment was to find out whether the suspended microsporidian spores in the medium could infect the prawn through the branchial chamber. For this purpose, two groups of 30 postlarvae (10 to 15 mm TL) were kept in similar glass troughs of 5.5 l capacity containing about 4.5 l of filtered sea water.

On the same day, dead specimens of P. semisulcatus heavily infected by P. semisulcata, caught from the Palk Bay by trawl nets and landed at Rameswaram, were brought to the laboratory in chilled sea water in an ice box.

About 2 g (wet weight) of the abdominal muscle from a heavily infected prawn was cut into small pieces and homogenized in a glass homogenizer and was diluted with 5 ml of fresh and filtered sea water. The supernatant containing large numbers of spores of P. semisulcata was poured into the two glass troughs each receiving half the quantity of the

supernatant. This process was repeated daily using fresh abdominal muscle of I. semisulcata - infected prawns after changing the water in the cleaned glass troughs. The prawns were fed with fresh clam meat ad libtim and continuous aeration was provided to both the troughs. Unconsumed food was removed regularly on the next day morning.

C. Transmission by feeding fresh infected prawn muscle

This experiment was planned in the light of the results obtained in experiment I (tank 3) and in continuation to test the transmission of microsporidian infection by feeding the infected prawn tissue for a longer duration. Two glass troughs (each 5.5 l capacity) filled with about 4.5 l of fresh and filtered sea water were arranged and 30 postlarvae ranging in size from 10 to 15 mm TL were introduced in each of the troughs. The prawns infected by P. semisulcata collected fresh from the trawl catches were brought to the laboratory in the same manner as described for the experiment I. The abdominal muscle of one of the heavily infected prawns was cut into small pieces and offered as food to the postlarvae in each of the troughs ad libitum. Every morning on the next day, the unconsumed food was removed, the troughs were cleaned and refilled with fresh and filtered sea water and fresh infected muscle tissue was given for feeding to the postlarvae. Aeration to both the troughs was provided with aerators.

D. Transmission by feeding frozen infected prawn muscle

This experiment was designed to test whether the frozen spores have the capability to infect healthy prawns. For this purpose, 30 juveniles of P. semisulcatus ranging in size between 30 and 40 mm TL, were kept in a glass trough of 10 l capacity. The trough was filled with about 9 l of fresh and filtered sea water and continuously aerated with aerators. The adult P. semisulcatus heavily infected by E. semisulcata, which was collected from Tanjapam fish landing centre and kept in frozen condition for one week, was used for feeding. The abdominal muscle from this prawn was removed, cut into small pieces and fed to the experimental prawns ad libitum. On every next day morning, the unconsumed food was removed, the trough was cleaned and filled with fresh and filtered sea water and frozen infected muscle was fed to the prawns once a day.

E. Transmission in adult prawns by feeding fresh infected prawn muscle

The objective of this experiment was the same as that of D. However, the prawns used in the present experiment were adults. Eight prawns (4 male and 4 females ranging in size from 104 to 195 mm TL) were kept in a 100 l capacity fibreglass tank containing about 80 l of continuously running fresh and filtered sea water. The prawns were fed ad libitum with small pieces of fresh abdominal muscle of

P. semisulcatus heavily infected by *P. semisulcata* once a day in the morning. As in the previous experiments, the infected prawns were obtained fresh from the Mandapam fish landing centre.

F. Control

One fibreglass tank of 100 l capacity containing about 80 l of continuously flowing filtered sea water with eight adult *P. semisulcatus* (4 males and 4 females of size 95 to 173 mm TL) served as control for the experiment F. The prawns were fed *ad libitum* with fresh clam meat daily in the morning after cleaning the tank. The tank was kept away from the experimental sets to avoid the chances of contamination.

RESULTS

E and F. Control

The postlarvae and adults in the control experiments were active, feeding normally with the clam meat. The faecal matter collected on different days and examined, contained no pathogen or any of its developmental stages. Similarly, the histological preparation of the muscle, midgut, hepatopancreas and ovary showed normal structural characteristics of these tissues and did not show any sign of the infection by the pathogen.

B. Transmission through branchial chamber

Detailed and critical examination of the histological sections of the gills of the postlarvae revealed the absence of the pathogen in the gill lamellae indicating that the transmission is not occurring through the gills. The histological structure of gill lamellae were similar to those of the normal postlarvae. However, the examination of the faecal matter collected on different days showed the presence of very few spores. It is probable that the spores offered in a suspended state in the medium would have been ingested by the prawns along with the clam meat and ejected out with faeces. The histological examination of muscle, midgut and hepatopancreas of the animals at the end of the experiment has also showed the absence of any recognizable stage of the pathogen.

C. Transmission by feeding fresh infected prawn muscle

The faeces collected on different days of the experiment showed the presence of large number of spores. While the internal structure of some of the spores examined were similar to that of the spores in the infected muscle given as food, most of the spores were found to have lost their refractivity and posterior vacuole. However, they were found to retain their original shape. These spores, stained with 5 percent toluidine blue or dilute Giemsa stain showed the presence of a small granule at varying

positions which stained bright purplish to violet (Pl. XXXI, Fig. 1). Further a few empty spores with only spore cover or with their spore wall having a small opening at the anterior end were also encountered in the faecal matter.

Histological examination of the abdominal muscle, midgut and hepatopancreas, prepared from the prawns preserved at the end of the experiment, did not show the infection of these tissues by the microsporidian spores or any of its recognisable stages. However, the examination of the wet mount of the abdominal muscle of the postlarvae sacrificed on the fourth day of the experiment revealed the presence of different recognisable stages of P. semisulcata (Pl. XXXI, Fig. 2) in between the muscle fibres. Two types of cells were observed, some of the cells (meronts) were relatively smaller with granular cytoplasm and the other, larger spherical cells (sporonts) with conspicuous nucleus.

b. Transmission by feeding frozen infected prawn muscle

The examination of the gut content and faecal matter of the experimental prawns revealed that they were feeding actively on the frozen infected tissue containing P. semisulcata spores, as large number of spores were encountered both in the faeces as well as in the gut contents. However, the spores in the faeces and those in the gut had the same structural characteristics as those

found in the frozen muscle tissue. This indicated that the frozen spores passed through the gut without any structural change.

E. Transmission in infected prawns by feeding fresh infected prawn muscle

The faecal matter collected on different days from this experimental tank contained large number of spores. On closer examination of these spores, most of them were found to have undergone changes in the structure as observed in the experiment D. The posterior vacuole was absent and spores were not refractile. However, the histological preparation of muscle, midgut, hepatopancreas and ovary did not show any sign of infection by the spores of the parasite or any of its developmental stages.

EXPERIMENT III

Reed and Olson (1977) conducted experiments to transmit P. crangoni in three species of crangonid sand shrimps and, on the basis of the results of their experiments as well as the field observations, suggested that only very young shrimps were susceptible to microsporidian infection during a relatively short period in summer months in U.S.A.

The presence of different stages of P. semisulcata in one postlarva of P. semisulcatus during the Experiment II(C) also suggested the possibility of its occurence in the young prawns. In the light of the above observations, this experiment was designed to transmit the pathogen, P. semisulcata, to the postlarvae of two species of penaeid prawns, namely, P. semisulcatus and P. indicus through feeding with the infected prawn muscle. This experiment was conducted at the C of I, Mandapam Camp for 37 days from 20th November, 1983 to 27th December, 1983.

Five postlarvae of P. indicus ranging in size between 10 to 20 mm TL were collected from the feeder canal of fish farm of the Regional Centre of CMFRI, Mandapam Camp and those of P. semisulcatus (10 to 15 mm TL) from the tidal mud flat near Pamban, about 7 km away from Mandapam Camp. The postlarvae from both the places were collected by operating scoop net and were transported to the laboratory as described in Experiment II. In the laboratory, the postlarvae of each of the species were intiailly held in two separate fibreglass tanks of 100 l capacity. Both the tanks were filled with about 80 l of fresh and filtered sea water aerated with aerators. The prawns were starved for 48 hours with a view to induce starvation stress. By random sampling, five postlarvae from each of the tanks were taken out and sacrificed, and wet mounts were

prepared as described in Experiment II. The wet mounts were critically examined under a compound microscope to ensure that the prawns collected for the experiment were free from any microsporidian infection. Besides, the faecal matters from both the tanks were carefully collected and examined under the compound microscope. The faecal matter samples were found to be devoid of any microsporidian spores.

On 20th November, 1993, the experiments were commenced and extended upto 27th December, 1993. The experiments were carried out in ambient temperature (26 ± 2 °), salinity (33 ± 2 ppt) and pH (8.2 ± 0.05) of the water in the glass troughs. The scheme of the experiments was as follows.

1. Experiment with P. semisulcatus

Control

Twenty postlarvae of P. semisulcatus (9 to 15 mm, TL) were introduced to a 5.5 l capacity glass trough filled with 4.5 l of filtered sea water which was provided with continuous aeration. The postlarvae were fed ad libitum with minced meat of fresh clam (Meretrix sp.) for 10 to 12 hours, once in five days. Every fifth day, the trough was cleaned and refilled with fresh, filtered sea water. To avoid any possibility of contamination, the control trough was kept separate from the other experimental troughs. The cleaning and feeding procedures as described above, were done only once in five days.

Experiment

Duplicate sets of 20 postlarvae (9 to 15 mm TL) were maintained in two glass troughs of 5.5 l capacity, filled with fresh and filtered sea water and aerated continuously. The experiment was designed to feed the postlarvae with infected prawn tissue and to change the water in the glass troughs once in five days with a view to test whether the spores which were consumed by the postlarvae and conditioned after passing through the gut were again ingested by the postlarvae and thus the transmission of the microsporidian could be affected. Minced muscle tissue of infected prawns collected afresh from the Mandapam fish landing centre was fed ad libitum to the postlarvae for 10 to 12 hours in the beginning of the experiment. After 12 hours, the consumed food was removed from the troughs. The water in the troughs was not changed for the next five days although continuously aerated. The faecal matter from the troughs were also not removed during these days. On the fifth day, the troughs were cleaned and refilled with fresh, filtered sea water and postlarvae were fed with fresh, infected prawn muscle tissue for 10 to 12 hours. This feeding and cleaning schedule once in five days was continued till the end of the experiment.

2. Experiment with P. indicus

Both the control and experimental desions as well as the feeding and cleaning of the troughs were similar to

those set-up and worked out for *P. semisulcatus*. Twenty postlarvae of *P. indicus* (10 to 20 mm TL) were introduced to each of the three glass troughs, retaining one as control and the other two as experiments. The control trough was kept away from the experimental troughs.

During the experiment, samples of faeces from all the troughs, including controls, were collected periodically and examined under the compound microscope. On the 9th day of the experiment (29th November), one postlarva from each glass trough was sacrificed and wet mounts of the abdominal muscle and gut contents were prepared for critical microscopic examination. Another set of sample of one postlarva from each glass trough was taken on the twenty fifth day of the experiment(15th December) and wet mounts were prepared and examined. At the termination of the experiment, five postlarvae from each glass trough were randomly selected, measured, dissected and fixed in Bouin's fixative for light microscopy. Later, the fixed specimens were processed for routine histology as described in Chapter 2. Tissues embedded in paraffin wax were cut at 5 to 7 µm thickness and after deparaffinization, sections were stained either with Heidenhain's iron haematoxylin and eosin, dilute Giemsa stain or Mallory's triple stain. Permanent histological preparations, mounted in DPX, were critically examined and photographed with Carl Zeiss ERGOVAL binocular compound microscope attached with camera unit.

RESULTS

1. P. semisulcatus

Control

The postlarvae in the control tank were observed to feed normally on the minced clam meat, whenever food was given. Often, the postlarvae were also noticed feeding on their own faeces. Microscopical examination of the faeces revealed the complete absence of P. semisulcata. Similarly, in the transverse sections of the carapace, abdomen, hepatopancreas and the gut were found devoid of microsporidian and these tissues showed similar structures of normal and healthy prawn. In the histological preparations of abdomen, the muscle did not contain any stage of the life-cycle of the microsporidian.

Experiment

The postlarvae in the experimental tanks were found to accept the heavily infected prawn muscle tissue which was given as food. Through the translucent body of the postlarvae, this infected muscle tissue was seen throughout the gut. After 20 hours of the initiation of the experiment, the faeces were collected and examined under the compound microscope. The structural changes in the spores after passing through the gut were quite conspicuous. The changes such as the absence of the pansporoblastic membrane and posterior vacuole, loss of

refractivity, presence of small granule inside the spores and a small opening at the anterior end of the spores were similar to those observed in Experiment I (tank 3) and II (C and). The postlarvae were observed to feed on their own faeces on the third day of experiment. This habit of the postlarvae was observed several times during the experiment.

Examination of faeces on the fourth day showed enormous number of empty spores with a slightly bulging structure protruding from the anterior end of the spore (Pl. XXXII, Fig. 1). These were probably incompletely extruded polar tubes suggesting the possible failure of spores to fully extrude their polar tubes.

Examination of the wet mounts of abdominal muscle, hepatopancreas and gut contents of two postlarvae on the ninth day showed that the microsporidian had not yet established the infection in the muscle or in the hepatopancreas. However, in the gut contents, some of the spores were seen with fully extruded polar tubes (Pl. XXXII, Fig. 2). In another observation of the wet mounts of the abdominal muscle only on twenty fifth day, a few empty spores were encountered in between the muscle fibres.

Throughout the duration of the experiment, the experimental postlarvae did not show any apparent behavioural

PLATE XLIII

Fig. 1. Spores of Thelohania semisulcata in the faeces of Penaeus semisulcatus on the fourth day of Experiment III. Note the slightly bulging structure (arrows) protruding from the anterior end of the empty spores. Wet mount.

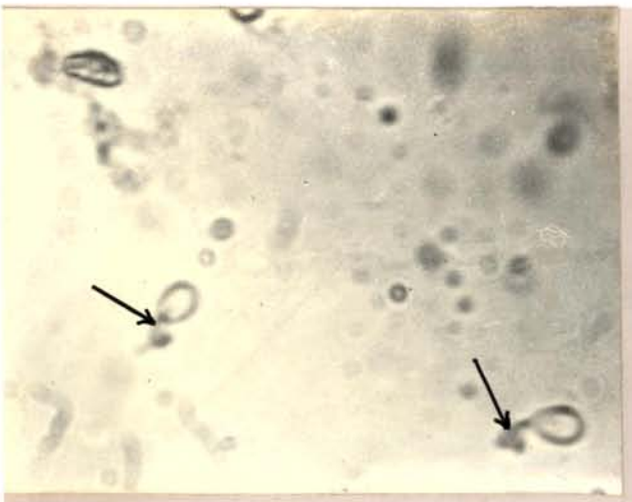
Fig. 2. Spores from the gut content of Penaeus semisulcatus on the ninth day of Experiment III. Arrow=Fully extruded polar tube. Wet mount.

Fig. 3. Transverse section through the abdominal region of an experimental Penaeus semisulcatus postlarva from Experiment III. Darkly stained area (TS) around the midgut (MD) shows the initiation of Thelohania semisulcata infection after 37 days. Arrows indicate infection in the abdominal muscle (AM) in the form of chains of meronts. ES=Exoskeleton. Bouin-Heidenhain's haematoxylin and eosin.

Fig. 4. A portion of the infected abdominal muscle from Fig. 3 at higher magnification to show the early developmental stages of Thelohania semisulcata (arrows). MF=Muscle fibres. ×800.

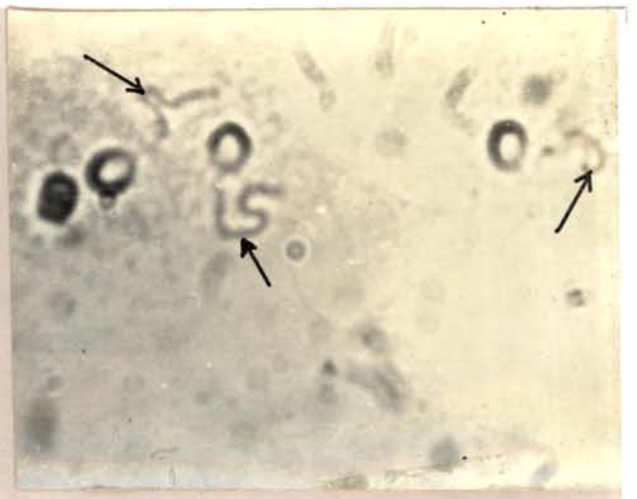
Fig.5-6. Transverse section of the abdominal muscle surrounding the midgut of Penaeus semisulcatus from Experiment III after 37 days. Arrows show the immature octosporoblasts. Bouin-Heidenhain's haematoxylin and eosin.

PLATE XXXII



20 µm

1



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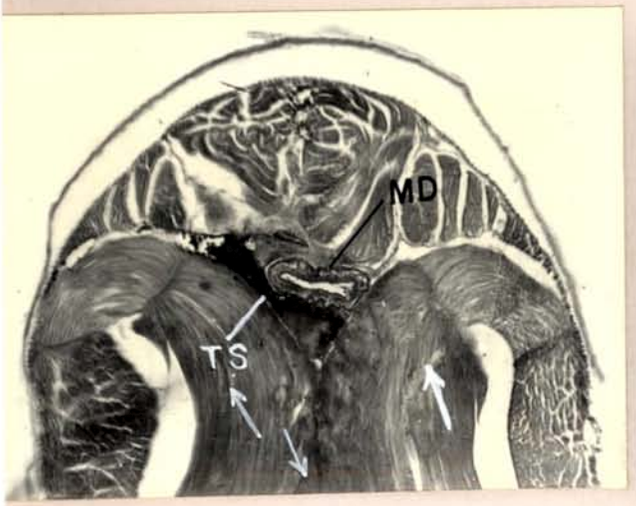
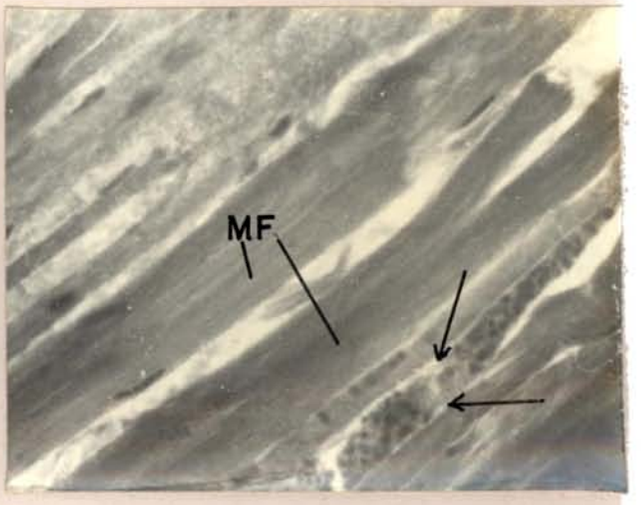


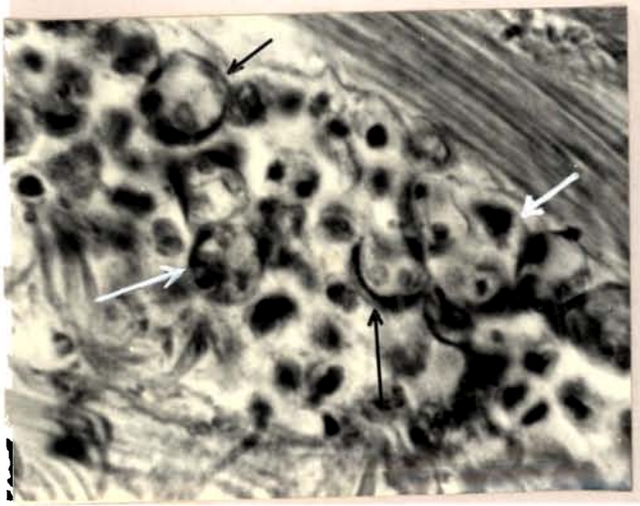
500 µm

3



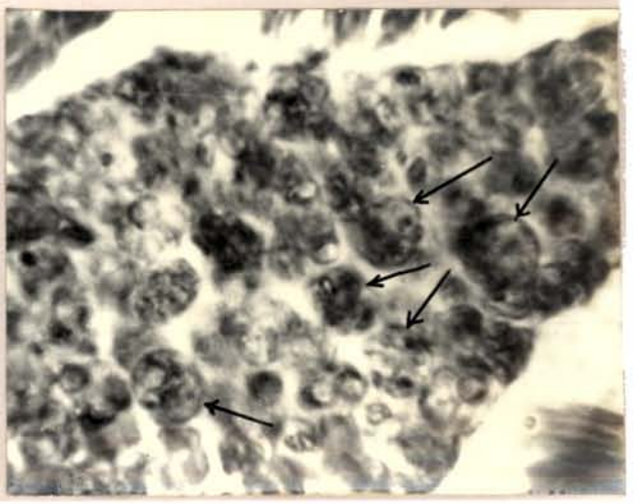
50 µm

4



20 µm

5



20 µm

6

abnormality nor did they show any visible symptom of microsporidian infection, such as whitening of the muscle.

Johnson (1983d) suggested that the histological examination may be helpful in the cases where microbial infection is not heavy enough to be detected by examination of fresh material. With this in view, histological sections of the thoracic and abdominal region of these postlarvae, which were fixed in Bouin's fluid, were prepared and stained with Heidenhain's hematoxylin and eosin or dilute Giemsa stain. Sections cut across the cephalothorax region did not show any sign of microsporidian infection. However, the sections from the abdominal region showed in certain slides (Pl. II, fig. 3) the presence of small colonies of vegetative cells of _P. semisulcata_ and some sporulating stages with two or four-celled stages (Pl. II, fig. 4). In certain other slides (Pl. II, fig 5 and 6) plasmodia containing eight immature sporoblasts were also seen. These were encountered in the abdominal muscles which were directly in contact with the midgut. Thus the presence of different stages of _P. semisulcata_ in the abdominal muscles indicated the successful transmission of the pathogen by feeding the infected muscle tissue or the defaecated spores for over 35 days.

2. P. indicus

Control

The postlarvae were observed to feed actively on the minced meat of clam and occasionally on their own faeces. The examination of the faeces collected on different days showed complete absence of microsporidian spores. Further, the transverse sections of postlarvae revealed the normal structure of the different organ tissues with no sign of infection.

Experiment

The experimental P. indicus showed similar feeding behaviour as mentioned for experimental P. semisulcatus. The faeces of the P. indicus postlarvae contained large number of spores which were found to have been changed structurally as in the case of defaecated spores of P. semisulcata. However, wet mounts of abdominal muscle, hepatopancreas and gut contents prepared and examined on ninth and twenty eighth day did not give any positive clue as to the presence of the pathogen in these tissues. Similarly, the histological examination of the transverse sections of postlarvae cut through the carapace and the abdominal region, did not reveal the presence of spores or any other recognizable stage of P. semisulcata and the different tissues in the sections appeared quite normal, though the lumen of the gut contained spores clumped with the food.

DISCUSSION

Among the various factors such as affinity of the pathogen for some tissue (tissue affinity), abnormal sensitiveness of certain tissues to pathogens(hypersensitivity), number of pathogens required to produce the disease (infection dosage), capability of the pathogen to escape from the host and infect the susceptible organisms (communicability) and the route or the portal of entry influencing infection, the latter forms an important factor. In several cases the portal of entry and selectivity against the natural barriers determine the nature of infection, development of the pathogen in the host's body and its pathogenicity. These criteria are quite applicable for microsporidia also. Ishihara (1963) pointed out that the pathogenicity of microsporidian infection could be related to the manner in which the pathogen invades the host, while Weiser (1976) opined that the route of invasion changes entirely the pattern of infection.

According to Kramer (1970, 1976), three natural routes of invasion provide microsporidian to have access to the host tissues and to produce infection. These are the oral route, the cuticular route and the ovarian route. The portal of entry through oral route involves ingestion of the pathogen or one or the other stage of its life-cycle in the food consumed by the host. Generally, such stages

constitute those excreted in the faeces of the host of origin. Entry through cuticular portal involves transmission of spores through the originally intact integument by a parasitoidal wasp engaged in oviposition; this phenomenon is recognised only among microsporidian associated with insects and their parasitodes. Ovarian transmission involves the incorporation of spores and non-sporulated forms into developing ova or embryos within the female reproductive system. Generally, the oral transmission of microsporidia, according to Canning (1976), is more pathogenic than the ovarian transmission.

The results of the present transmission experiments carried out in P. semisulcatus with _. semisulcata through injection of spores through exoskeleton of the prawns (Experiment I, tank 2 and 4) and through spore suspension in the medium (Experiment II, B) showed that the transmission of the pathogen does not occur through cuticular route. The absence of spores or any of the developmental stages in the gill lamellae even after 14 days of experiment with spore suspension lends further support to the view that _. semisulcata does not enter the host through the delicate gill cuticle also. Similarly, the absence of spores or any of the developmental stages in the gonads in the experimental prawns (Experiment I, Tank 2, 3 and 4; Experiment II, B) subjected to transmission through cuticular or oral route showed that the pathogen does not enter through the ovarian route either.

The results of the experiments carried out by feeding of muscle tissue infected by T. semisulcata to postlarvae, juveniles and adult P. semisulcatus (Experiment II, C and E; Experiment III, experimental set 1) revealed the oral route of transmission of this pathogen. This is evidenced by loss of refractivity and the changes in the internal structure of the spores after passing once through the gut of the prawns (Experiment , tank 3; Experiment II, C and E; Experiment III, experimental set 1 and 2) as well as the extrusion of the polar tube of the spores inside the midgut upon re-ingestion of defaecated spores by the prawn (Experiment III, experimental set 1). Presence of different recognisable stages of T. semisulcata in the muscle (Experiment II, C; Experiment III, experimental set 1) and histological demonstration of infection in the muscle surrounding the midgut (Experiment III, experimental set 1). However, the pathogen is not transmitted at the first instance during the first feeding, although heavily infected muscle tissue offered as food to the experimental uninfected prawns is ingested by them. This is quite evident by the results of the Experiment III involving P. semisulcatus postlarvae. The results of present transmission experiments also showed that no intermediate host is needed for the transmission of T. semisulcata to P. semisulcatus and the infection could be achieved by feeding the infected prawn tissues and defaecated spores.

The observations and examinations of the faecal matter from the prawns fed with fresh infected muscle tissue revealed that the spores, as they pass through the gut of the prawn, undergo some structural changes. As mentioned earlier, the changes are related to the internal structure and loss of refractivity of the spores. It is obvious that for the successful transmission it is necessary that the spores undergo these changes in the gut environment, as such conditioned spores could only transmit infection to the host tissue. This view is supported by the fact that only defaecated spores when ingested again by the prawn could produce the infection. Kramer (1969) observed that in Phormia regina (Diptera:Trachycera) 100 percent of the spores of Octosporea muscaedomesticae did not germinate, but the excreted spores, when fed to other adults, germinated and produced infection. Overstreet and Hatley (1975) had experimentally infected the blue crab, P. sapidus by feeding P. michaelis spores mixed with the food. Later, Overstreet (1978) successfully infected both the young and the old crabs without much difficulty by the feeding method. Iversen and Kelley (1976) found that spores of P. penaei, conditioned by passing through the gut of the spotted sea trout and the trout faeces then fed to the postlarval pink shrimp, P. duorarum, could transmit the disease in the latter. Conversely, Toth and Iversen (1971) were unable to transmit T. duorara in P. duorarum by feeding the heavily infected prawn tissue to the uninfected experimental prawns.

Although, P. semisulcata is successfully transmitted to the healthy P. semisulcatus postlarvae in the present experiments, the attempt to transmit the microsporidian through feeding in P. indicus postlarvae was not successful.

It is quite probable that all the fresh spores ingested by the prawn may not extrude their polar tube and transmit the disease. Feeding in prawns is a continuous process in which the gut shows peristaltic movements. These peristaltic movements help in pushing the undigested gut contents from foregut to midgut and from that to the hindgut. Spores ingested along with the food are perhaps actively involved in this process of movement of the gut contents. It may be assumed that most of the spores ingested by the prawn are unable to stand against this continuous movement of the gut contents as, often, quite a large number of ingested spores are excreted along with the faeces (experiment I, tank 3; experiment II, C and D; experiment III, experimental set 1 and 2). For the same reason, most of the spores thus excreted showed normal structure. However, some of the spores when subjected to gut environment may undergo some structural changes in which posterior vacuole disappears while a single or two conspicuous small dark staining body appears in some of the spores are represented only by empty spore cases. The presence of these types of spores only relatively in a few numbers suggests that although the gut

of the prawn is suitable for T. semisulcata, the speed by which the undigested food as well as the ingested spores are pushed behind allows only a little time for the spores to get conditioned to the gut environment and to extrude their polar tubes and discharge the infective sporoplasm into the host cell. Overstreet and Whatley (1975) pointed out that the age of a spore, the number of times a spore passes through the alimentary tract and the initial concentration of spores, possibly all contribute to the rate of infection. Presence of empty spore cases would indicate that they are eigher the undigested partsof the spores or remnants which have discharged or emptied their sporoplasm into the host midgut epithelium.

Extrusion of the polar tube inside the midgut of P. semisulcatus was noticed in some of the spores in the Experiment III. According to Lom and Vavra (1963), microcinematographic recordings of some microsporidian spores showed that the extruding polar tubes retract within a few seconds after the extrusion is completed.

Information on the nature and pattern of spore germination within the gut wall and on the physiological processes involved, are scanty and observations are inconsistent. Weiser (1970) pointed out that autonomy of these processes is independent of the germ within the spore. Further, Weiser (1976) and Canada (1976) indicated that success or failure of transmission of microsporidians through

oral route depends on the gut environment and relates to the factors such as rate of flow of ingested food, pH, enzymes, osmotic pressure and adequate digestion of the seal covering the polar tube. However, in some cases, extrusion of the polar tube is not induced, even in spores with viable germs, by a variety of stimuli as shown by Iverson and Kelley (1976) who attempted to transmit I. duorara, A. penaei and Pleistophora sp. in the pink shrimp, P. duorarum by direct feeding of infected shrimp muscle, fresh and aged free-floating spores, spores conditioned with Versenol acetate buffer, spores conditioned by bathing in shrimp digestive juices and using fish as intermediate or conditioning hosts but without any success. In other case, such as in Nosema bombycis, Ishima (1970) found that spores with dead germs were able to extrude their polar tubes merely by the change in the osmotic pressure. Weidner (1976) pointed out that the energy release associated with spore extrusion is due to an osmotic shift; presumably, the collapse of the spore aperture allows an inflow of water producing a sudden build up in the osmotic pressure within the spore. Lom and Vavra (1963) also that the discharge intensity is directly proportional to the osmotic condition or the viscocity of the medium exterior to the spore.

The extrusion of polar tubes of spores of N. bombycis (Ishima, 1964) and of N. fumiferanae (Ishihara, 1967) are affected by the pH and certain cations. According

to Weidner (1976), there is some evidence that spore walls are selectively permeable to certain ions or molecules that may effectively trigger the extrusion. Spraguea (=Nosema) lophii, Encephalitozoon cuniculi and N. bombycis resist noxious concentrations of NaOH and HCl; however, these species often discharge their sporoplasm when exposed to certain osmotic or ionic shifts (Weidner, 1976). Such observations indicate that the extrusion of polar tube through oral transmission depends on the gut environment and the types of secretions of digestive juice by the host.

Toth and Iversen (1971) reported that spores of N. duorum, found between the old and new cuticle at the time of moulting, could infect shrimps that feed on cast off cuticle. Myers and Cox (1976) indicate that starvation prior to moulting makes some insects to be more susceptible to infection by microsporidia.

What happens to the spores which are defaecated by the prawn is not known. However, the spores which passed out of the gut of the prawn but have not yet extruded the polar tube, may possibly survive in the extracorporeal environment within the bound faeces. The problem of survival assumes great proportions for spores using oral portal of entry since the survival time in the external environment would vary and depend upon their chance of ingestion by a suitable host. According to Kramer (1976) there are no data

pertaining to spore life in the extracorporeal environment since more than 90 percent of the known species of microsporidians and published accounts concerning the longevity and survival of spores are related to the laboratory investigations of species whose hosts are economically important terrestrial hosts. From the available evidence, Kramer (1970) has opined that naked spores of some microsporidians may survive for 7 to 10 days in a cold, clean aqueous medium. Canada (1976) supported this finding and pointed out that the microsporidian spores could survive in external environment, and the spores generally favour the moist conditions, low temperatures, and being protected in faeces of carriers. However, Kramer (1976) emphasised the possibility that spores from some marine hosts are sensitive to variations in osmotic pressure and cannot withstand the stress imposed by the environment for extended periods. In the present study, spores once conditioned and defaecated in the bound faeces were found at the bottom of the experimental tanks in troughs. As the prawns were fed with infected prawn muscle tissue only once in 5 days and that too for a few hours (maximum 10 to 12 hours), the prawns consumed their own faeces during the inter-feeding period, thereby reconsumed the conditioned spores. In nature, it is possible that the faeces of prawns containing the spores are ultimately mixed with the bottom sediments or detritus and are consumed by the prawns along with other organisms while feeding. It has been indicated that prawns in their younger stage (about 20 to 40 mm TL) are detritus feeders.

Thomas (1972) has observed that P. semisulcatus feeds on detritus.

The occurrence of initial infection in experimental P. semisulcatus postlarvae only in the abdominal muscle, especially those which are directly in contact with the midgut, provides evidence to the possibility that T. semisulcata enteres through the midgut. Weiser (1976) has pointed out that the site in the host's gut where spore opens, differs among the various microsporidian species, but generally, the first cysts with developing stages are found in the middle part of the abdomen. In prawns, the inner wall of the foregut and hindgut are lined with a thin layer of cuticle whereas in the midgut this is absent (Satwardhan, 1937; Young, 1959). Thus the chances of invasion through the midgut are more probable since it provides a non-cuticulised area where the pathogen can easily invade.

In the midgut, spores discharge the infective sporoplasm through the extruded polar tube into the midgut epithelial cells thereby initiating infection. The extrusion process has been studied in vitro with electron microscopy by Petri (1969) in T. cuniculi, by Ishihara (1968) in the silkworm pathogen N. bombycis, and by Weidner (1972) in N. michaelis from the blue crab and T. lophii from the goosefish. The route of invasion to the other host tissues from the gut epithelium has not been determined, but it is likely that there is a passive transfer in blood or in

migratory host cells (macrophages) to the final site of the infection (Canning, 1977).

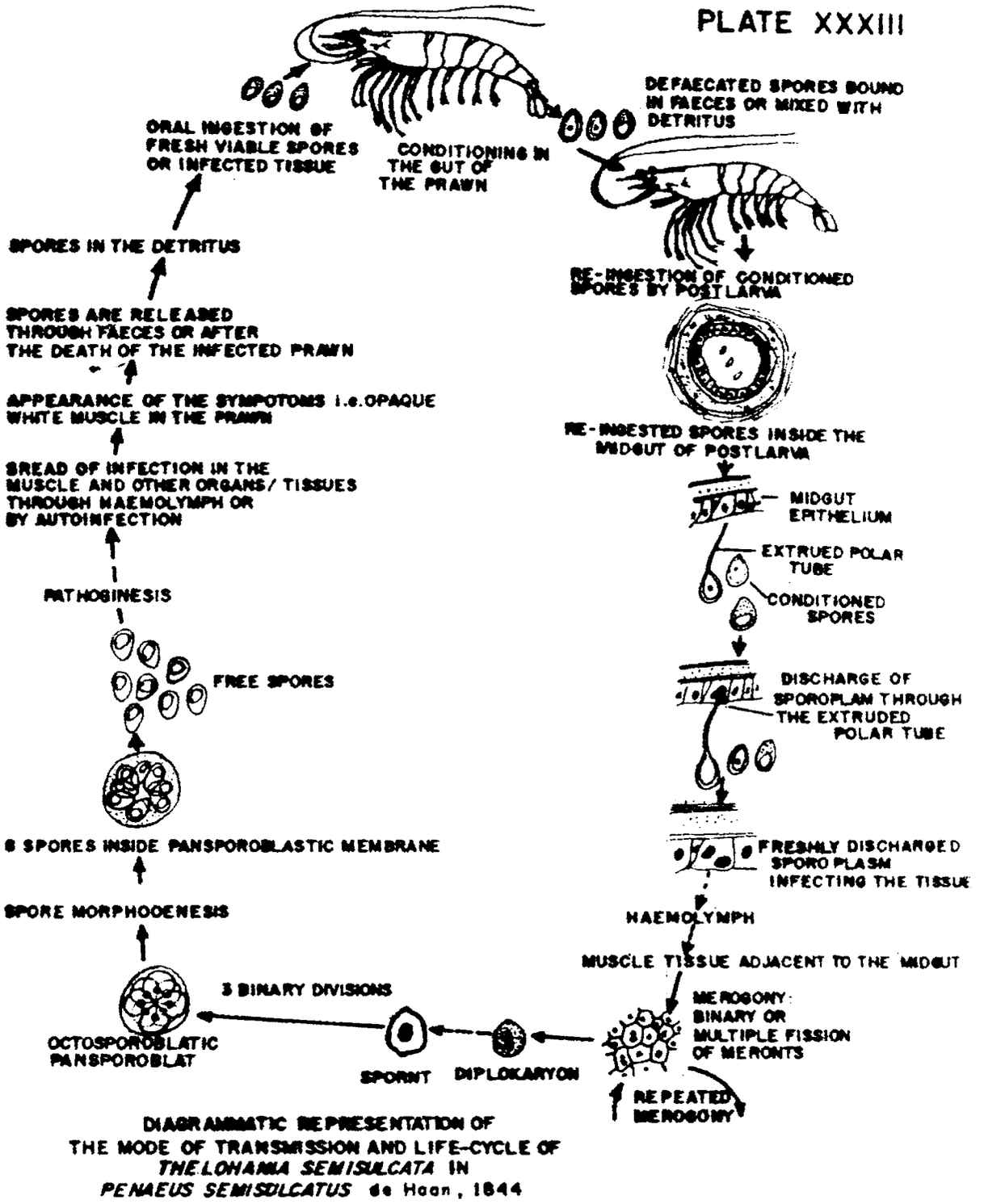
It is also possible that the spores may invade the host tissues through the openings of the hepatopancreatic ducts at the junction of foregut and midgut. However, this possibility was not evidenced in the histological sections of this region in the present prawns subjected to experimental transmission through the oral route. Weiser (1976) opined that this mode of transmission seems unlikely because spores are passive migrants and the flow of secretions from hepatopancreas through the ducts is against them. He (Weiser, 1976) pointed out that only microsporidians infecting the gut wall are able to reach the definite site for development directly by injecting the sporoplasm through their polar tube. Roth and Iverson (1971) observed in the naturally infected shrimps that the spores in the abdominal muscle adjacent to the gut mature first. They (Roth and Iverson, 1971) speculated that the spores entering new hosts leave the spore case, penetrate the gut and then move directly to the site of infection.

In the present experiments, the postlarvae of P. semisulcatus are found to be more susceptible to infection by P. semisulcata rather than juveniles or the adults. Successful experimental transmission of the microsporidia in the postlarvae, but not in juveniles or adult prawns, here and elsewhere (Iverson and

Kelley, 1976) indicates that the prawns in their very early stage of life are more vulnerable to infection. Breed and Olson (1977) have pointed out that only very young shrimps are susceptible to microsporidian infection and emphasised the possibility that the age of the shrimp is a critical factor. On the other hand, microsporidian-infected prawns with visible symptoms found in the nature are either juveniles or adults (Sprague, 1950; Hutton et al., 1959; Iversen and Manning, 1959; Kruse, 1959; Overstreet, 1973; Lightner, 1975). Such difference in the laboratory and field observations suggests that in the very young prawns although the infection exists, the disease produced by the microsporidian may not be discernible for a long duration of time. The pathogenesis of microsporidian seems to be rather slow producing the apparent symptoms during which time, the prawn grows to larger size. Lightner (1975) has considered that microsporidian infection in penaeid prawns becomes chronic which, as Finne (1980) has defined, usually proceeds more slowly, attains less intensity, but persists over long periods of time.

On the basis of the observations made during the experimental transmission of the infection and the study of the different stages of _. semisulcata, a diagrammatic representation of the life-cycle of the pathogen is given in Plate IIII.

PLATE XXXIII



DIAGRAMMATIC REPRESENTATION OF THE MODE OF TRANSMISSION AND LIFE-CYCLE OF *THELOHANIA SEMISULCATA* IN *PENAEUS SEMISULCATUS* de Haan, 1844

4.5 PROXIMATE COMPOSITION IN *MACROBRACHIUM* [illegible]-INFECTED [illegible]

It is well known that parasites, if they infect, bring forth several alterations in the physiochemical, nutritional and metabolic pattern, and in endocrine functions in their host species. In an excellent review, Thompson (1983) comprehensively discussed the diverse and complex nature of these interactions between the metazoan parasites on their several invertebrate or vertebrate hosts, their role in the host-parasite relationship and its success or ultimately the fate of the host. Considerable information on the biochemical effects of parasitism on the host is available in the terrestrial animals and insects (Wilhon *et al.*, 1951; Ormerod, 1957; Roberts, 1960; Chang and Geller, 1970; Geiser and Lysenko, 1972; Newton *et al.*, 1973; Thompson, 1983). In marine animals, however, there have been only a few studies on the subject. This is particularly so in the case of euryhaline species, which regulate their physiology and metabolism according to the external environmental changes (Scaldi, 1972).

In decapod crustaceans, Morieux *et al.* (1976) and Reinhardt *et al.* (1978) studied the effect of *Sacculina* parasitism on haemolymph protein concentrations

in the crabs. These authors observed an additional protein fraction in the haemolymph of parasitised crabs. Recently, Andrieux et al. (1980) and Herberts et al. (1980) found inhibition of moulting in the parasitised crabs. Alterations in the glucose, total carbohydrate and lactic acid (Stewart and Cornick, 1972) and in the glycogen content (Stewart and Arie, 1973) in the haemolymph were reported in the lobster, Homarus americanus infected with Aeromonas viridans var. homari.

Biochemical and physiological impact of microsporidian parasites on decapod crustaceans were recently studied by Vivares and his associates. Martin et al. (1977), Vivares et al. (1977) and Vivares and Cuq (1981) observed that the levels of haemolymph protein and glucose, composition of fatty acids and the activities of various enzymes in the crabs, Carcinus maenas and C. mediterraneus were modified when they were infected by the microsporidian, Thelohania maenadis. Further, Vivares et al. (1978, 1980a) found that the free amino acids of microsporidians were inversely proportional to those in their crustacean hosts. The influence of T. maenadis infection on the free amino acid content of the haemolymph and muscle tissue of the crab, C. mediterraneus, as well as the influence of low and high salinities, and the combined influence of salinities and temperatures on the free amino acid concentration in the haemolymph of healthy and parasitised crabs were studied by Vivares et al. (1980b).

Analysing the free amino acids, these authors found four additional, non-identifiable compounds in the haemolymph and eleven in the muscle of the infected crabs. Ceccaldi (1982) pointed out that the amino acids, which were found to exist at high levels in th microsporidian parasites, were similar to the essential amino acids of their crustacean hosts. Erickson and Sprague (1970) indicated that microsporidian infection, in general, produced hypoaminocidemia and a high ratio of saturated to unsaturated fatty acids in the host.

In the present study, the proximate composition of abdominal muscle, ovary and hepatopancreas of normal and T. semisulcata-infected Penaeus semisulcatus were studied and the results presented and discussed.

MATERIAL AND METHODS

The adult specimens of P. semisulcatus, both normal and infected by T. semisulcata, were collected regularly during October-December, 1983 from the fresh catches landed at Mandapam fish landing centre. These specimens were caught by the mechanised fishing vessels operating bottom shrimp trawl in the Gulf of Mannar and Palk Bay off the coast of Mandapam. The normal and infected prawns thus collected were immediately preserved in ice in separate ice boxes and transported to the laboratory at the Regional

Centre of Central Marine Fisheries Research Institute, Mandapam Camp. The time lapse between the collection of the samples and their biochemical analysis was from 3 to 6 hours throughout the study.

In the laboratory, the prawns were analysed for size, sex and maturity stages. The normal and infected prawns, within the size range of 110 to 185 mm total length in the intermoult stage, were selected for biochemical analysis. Infection by P. semisulcata in the infected specimens was confirmed by examining the squash of a small piece of abdominal muscle on a microscope slide with a compound microscope. Based on the degree of infection revealed by the external gross symptoms (as described earlier in the Sub-chapter 4.2) and the microscopic examination of the muscle, the parasite burden was divided into moderate and heavy infections. Both normal and infected prawns were dissected separately and tissues such as the abdominal muscle and hepatopancreas from male and female prawns and ovary from the females were removed. In the case of ovary from normal prawns, care was taken to select only maturing or mature ovary in order to get consistent results. In the ovary of infected prawns, however, it was often difficult to identify the maturity stage as the infection resulted in emaciation and whitening of the ovary. Therefore, the infected ovaries were divided into moderately and heavily infected groups depending on the visual degree of infection. For analysis,

the muscle and ovary were cut into small pieces, blotted in the folds of a tissue paper to remove the external adhering water and weighed separately using a pre-weighed aluminium foil. The wet weight of whole hepatopancreas was similarly determined. All the weights were taken using a Metler monopan balance with 0.001 g accuracy. The tissues were analysed for moisture, ash content, total protein, total lipid and total carbohydrate.

Moisture

Moisture content of abdominal muscle, hepatopancreas and ovary was determined by keeping the pre-weighed wet samples at 60°C in a hot air oven for 48 hours, cooled in a desiccator using silica gel as desiccant and re-weighed till a constant dry weight was obtained. The percent moisture content in the samples was calculated as follows.

$$\text{Percent moisture} = \frac{\text{Difference in wet and dry weight of sample}}{\text{Wet weight of the sample}} \times 100$$

The moisture values thus obtained were used to convert the values of protein, lipid and carbohydrate obtained in terms of wet weight into dry weight.

Ash content

Dried samples of abdominal muscle, hepatopancreas and ovary with their known wet weight were individually taken in

pre-weighed silica crucibles and were ashed in a muffle furnace at a constant temperature of 550° for 6 hours, cooled in a desiccator with phosphorus pentoxide as desiccant and weighed. The 6 hours' duration was taken after initial standardisation. The percent ash in the samples was calculated as follows.

$$\text{Percent ash} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

Total lipid

Lipid was extracted as per the method of Folch et al. (1957). Pre-weighed wet samples weighing around 2 to 5 g were homogenised in a clean glass mortar for 5 minutes with 2 ml of 2:1 (v/v) ratio of chloroform and methanol. The homogenised samples were transferred into stoppered centrifuge tubes and the residue on the mortar and pestle was washed with 8 ml of chloroform and methanol mixture (2:1 v/v). The mixed samples were stored in dark at 10°C overnight. The samples were then centrifuged at 800 r.p.m. for 5 minutes keeping the centrifuge in a cold storage room, and the supernatant was collected. To the supernatant was added 2.5 ml of doubly distilled water and the mixture was allowed to stand in a separating funnel for a few hours to separate into the upper phase (methanol) and the lower phase (chloroform). The lower phase containing the lipid

was carefully collected into a clean, dry, pre-weighed container and the chloroform was allowed to evaporate at 30°C in a vacuum desiccator for two days till a constant weight of the dried lipid was obtained. The percent of lipid was calculated as follows.

$$\text{Percent lipid} = \frac{\text{Weight of lipid}}{\text{Weight of wet tissue}} \times 100$$

Total carbohydrate

To the delipidised residues in the centrifuge tubes, 10 ml of cold 5 percent trichloracetic acid was added and homogenised thoroughly. Then, the sample was stored at 10°C for 3 hours and the supernatent was collected by centrifuging at 1000 r.p.m. for 10 minutes. The collected supernatent was then analysed for total carbohydrate by Anthrone method (Roe, 1955) wherein glucose was used as standard. The absorbance (A) of samples were determined spectrophotometrically at 620 nm in an ICI Spectrophotometer. The percent carbohydrate was calculated as follows.

$$\text{Percent carbohydrate} = \frac{\text{A of sample}}{\text{A of standard}} \times \frac{\text{Concentration of standard}}{\text{Wet weight of sample}} \times 100 \times \text{Dilution factor}$$

Total protein

The delipidised and cold trichloracetic acid-treated residue was dissolved in 5 ml of 1N sodium hydroxide. An

Table 5. Comparative proximate composition* of normal** and *Thelohania semisulcata*-infected*** *Penaeus semisulcatus*

	Muscle			Hepatopancreas			Ovary		
	Normal	Infected		Normal	Infected		Normal	Infected	
		Moderate	Heavy		Moderate	Heavy		Moderate	Heavy
Moisture	73.59 ±1.56	71.74 ±0.41	71.04 ±0.59	67.04 ±1.59	64.89 ±2.88	64.40 ±2.92	68.27 ±1.07	69.69 ±1.57	70.37 ±1.37
Ash	10.23 ±3.15	6.95 ±0.80	6.63 ±1.20	16.42 ±2.14	15.17 ±1.90	14.98 ±2.17	9.65 ±1.46	9.73 ±1.06	9.81 ±0.45
Protein	66.03 ±3.85	66.50 ±3.68	66.78 ±3.60	26.16 ±2.44	36.40 ±5.14	35.86 ±5.07	47.94 ±2.93	58.60 ±1.63	58.59 ±0.99
Lipid	16.43 ±2.28	18.15 ±2.74	19.57 ±2.10	40.28 ±2.37	34.11 ±3.50	34.85 ±3.22	27.80 ±2.05	21.69 ±1.0	21.61 ±1.28
Carbohydrate	7.43 ±1.72	7.87 ±1.92	7.49 ±1.49	16.61 ±2.19	14.84 ±2.32	14.76 ±2.52	14.56 ±1.93	10.70 ±1.29	10.10 ±1.71

Note: * All the values, except for the moisture are expressed as % dry weight.
** Each value represents a mean of 24 samples of normal prawn tissue.
*** Each value represents a mean of 12 samples of infected prawn tissue.

Table 6. Calculated t values for comparison of means of proximate composition of normal and Thelohania semisulcata-infected Penaeus semisulcatus*

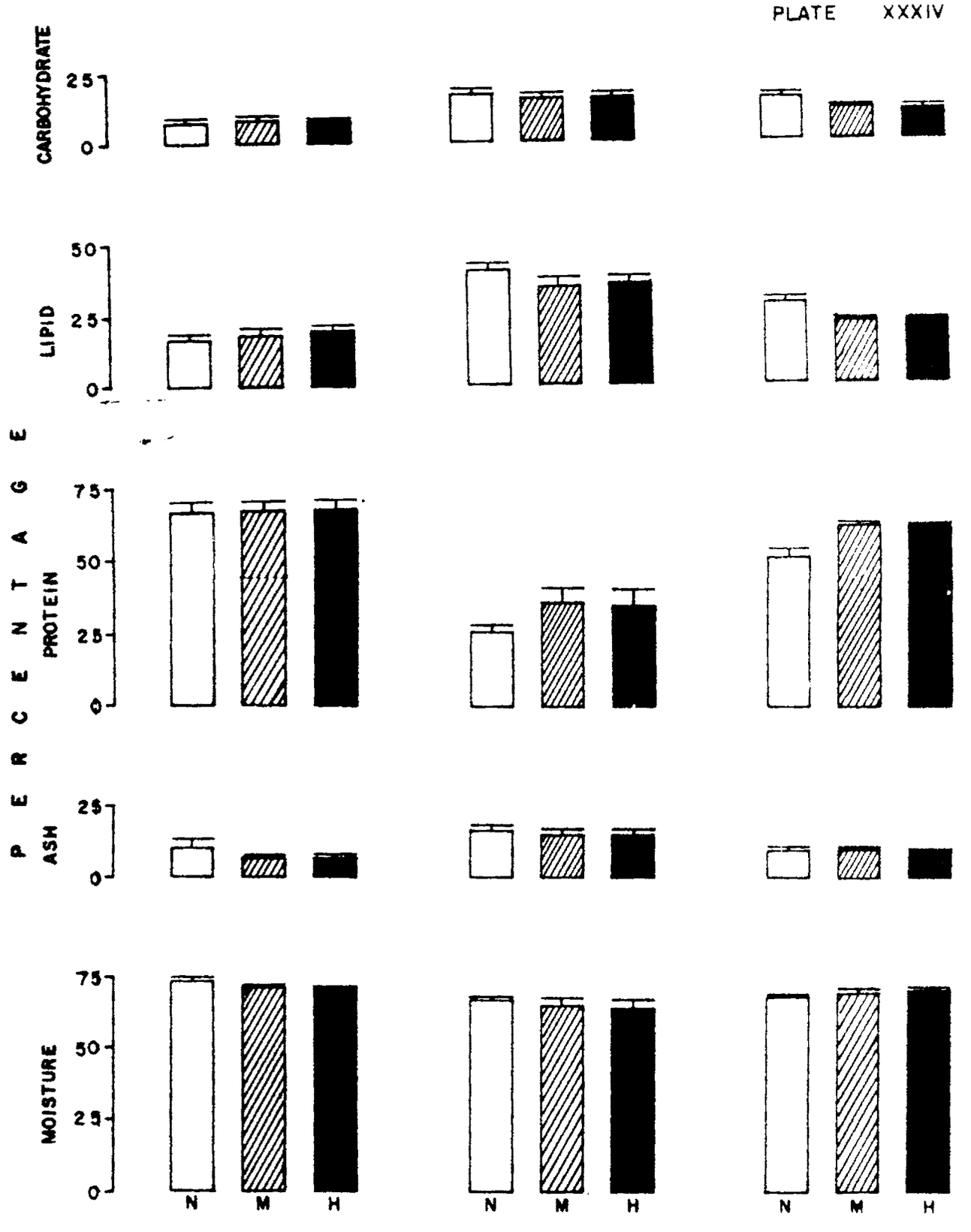
	Muscle		Hepatopancreas		Ovary	
	Normal-Moderate	Normal-Heavy	Normal-Moderate	Normal-Heavy	Normal-Moderate	Normal-Heavy
Moisture	4.03*	3.86*	3.80*	4.72*	3.20*	5.06*
Ash	3.52*	3.80*	1.70	1.88	0.17	0.38
Protein	0.35	0.56	2.17	7.81*	11.69*	12.18*
Lipid	1.99	3.99*	6.26*	5.74*	15.48*	9.53*
Carbohydrate	0.69	0.11	2.25*	2.27*	6.25*	6.80*

Note: * Differences between the mean values of normal-moderately infected and normal-heavily infected tissues are significant at 5% level ($P \leq 0.05$)

PLATE XXXIV

Histrograms showing proximate composition of abdominal muscle, hepatopancreas and ovary of normal and Thelohania semisulcata - infected Penaeus semisulcatus.

PLATE XXXIV



N = NORMAL , M = MODERATELY INFECTED, H = HEAVILY INFECTED

aliquot of 1.0 ml was pipetted out and analysed for total protein following the method of Lowry et al. (1951). Bovine serum albumin was used as standard. The absorbance(A) was read at 540 nm in NCIL Spectrophotometer. The percent total protein in the tissue samples was calculated as follows.

$$\text{Percent protein} = \frac{\text{A of sample}}{\text{A of standard}} \times \frac{\text{Concentration of standard}}{\text{Wet weight of sample}} \times 100 \times \text{Dilution factor}$$

Statistical analysis

The data were statistically analysed using student's t-test to evaluate the significance of differences between means. Differences between means were considered significant when P values were ≤ 0.05.

RESULTS

The data on the proximate composition of moisture, ash, total protein, lipid and carbohydrate contents in the abdominal muscle, hepatopancreas and the ovary of the normal as well as moderate and heavily infected prawns are given in Table 5 and presented in Plate XXXIV. The percent ash, protein, lipid and carbohydrate have been calculated in terms of dry weight. Table 6 gives the significance of differences between the mean values of normal and infected prawns at 5% level.

Abdominal muscle

Moisture: In the normal prawns, the moisture content in the abdominal muscle was 73.6% and was low in the moderately and heavily infected prawns, being 71.7% and 71.0% respectively. When statistically tested, a significant difference was observed between the normal and infected prawns (Table 6).

Ash: As in the case of the moisture content, the ash content in the abdominal muscle of the normal prawnswas higher (10.2) than that of moderately (7.0%) and heavily (6.6%) infected prawns. These observed values were significant at 5% level (Table 6).

Protein: The values of the protein content in the abdominal muscle of the normal prawns ranged between 58.4% and 75.1% with a mean value of 66.0%. Though the protein content in the infected prawns was slightly higher than in the normal prawns, the difference was statistically not significant (Table 5 and 6).

Lipid: The lipid content in the normal and heavily infected prawns showed significant difference (Table 6) whereas the variations was not significant between the normal and moderately infected prawns. The lipid content was highest in the abdominal muscles of heavily infected prawns (19.6%) as compared to the moderately infected (16.2) and normal (16.4) prawns (Table 5).

Carbohydrate: No significant variation was observed in the carbohydrate content of abdominal muscle of three groups of prawns. The highest percentage of carbohydrate content (7.9%) in the abdominal muscle was recorded in the moderately infected prawns. In the normal and heavily infected prawns, the carbohydrate level was more or less similar, being about 7.4%. These variations, however, were found to be not significant as in the case of protein.

Hepatopancreas

Moisture: The moisture content in the hepatopancreas of normal, moderately and heavily infected prawns showed significant variations (Table 6). The highest moisture content in the hepatopancreas was recorded in the normal prawns (67.%), whereas in the moderately and heavily infected prawns, the moisture content was almost the same (Table 5).

Ash: No significant differences in the ash content of the hepatopancreas of the normal, moderately and heavily infected prawns were observed. However, in the normal prawns, the ash content of the hepatopancreas was slightly higher (15.4%) than that in the moderately (15.2%) and heavily (15.0%) infected prawns.

Protein: The protein content in the hepatopancreas showed significant variations at 5 percent level (Table 6) between the normal and infected prawns. The highest protein content

in the hepatopancreas was recorded in the moderately infected group of prawns (36.4%) and the lowest in the normal prawns (26.2). In the heavily infected prawns, the protein content in the hepatopancreas (35.9%) was slightly less than that recorded in the moderately infected prawns.

Lipid: The hepatopancreas of the infected prawns showed significant variations in their lipid content when compared with that of normal prawns (Table 6). The total lipid content was highest in the normal hepatopancreas (.3), while in the infected conditions, it was relatively less (Table 5).

Carbohydrate: The carbohydrate content in the hepatopancreas of the normal prawns and that of the moderately and heavily infected prawns was observed to show significant variations. As in the case of the lipid content, the observed value of carbohydrate in the normal prawns was higher (16.6) (Table 5).

Ovary

Moisture: The moisture content in the ovary significantly varied in both the moderately and heavily infected prawns from that of the normal (Table 6). In the case of heavily infected ovary, the moisture content was highest (70.4) followed by the moderately infected ovary (69.7). In the normal ovary, the moisture content was comparatively low (68.3).

Ash: No significant variation was observed in the ovary of normal and infected prawns. The ash content in the ovary of normal, moderately and heavily infected prawns ranged between 9.7% and 9.8% (Table 5).

Protein: Protein content in the ovary showed significant variations in the normal as well as moderately and heavily infected prawns (Table 6). In the latter two groups, the protein content in the ovary was almost the same (58.6%) but in the normal prawns, it was much lower (47.9%) than that in the infected ones.

Lipid: The ovary of normal prawns showed significant differences in the total lipid content to that of the ovaries of the infected prawns (Table 6). The lipid content in normal ovary was higher (27.8%) than that of moderately and heavily infected prawns (21.7%).

Carbohydrate: The carbohydrate content in the ovary of the normal and infected prawns showed significant variations at 5% level (Table 6). This parameter was higher in the normal ovary (14.6%) than that of the moderately (10.7%) and heavily infected (10.1%) prawns.

From the above analysis of the different biochemical parameters in the normal and infected prawns it can be inferred that: (1) the moisture content in the abdominal muscle and hepatopancreas was always at a higher level in the normal prawns as compared to that of infected prawns, while in the

ovary of the normal prawns, the moisture content was lower than in the infected ones; (2) the ash content, similarly, was at a higher level in the abdominal muscle and hepatopancreas in the normal prawns, but in the ovary, the values were comparable; (3) the difference in the protein level between the normal and infected prawns was almost similar in the abdominal muscle, while in the hepatopancreas and ovary of the normal prawns it was at a lower level, compared to the infected ones; (4) in the case of the lipid content, it was found to be lower in the abdominal muscle of the normal prawns as compared to the infected ones, but in the hepatopancreas and ovary of the normal prawns, the level of lipid content was always higher; (5) the carbohydrate level in the abdominal muscle was more or less same while it was higher in both hepatopancreas and ovary of the normal prawns as compared to the infected ones. Thus the infection brings forth appreciable changes in the moisture content in the abdominal muscle, hepatopancreas and ovary, and in the ash content in abdominal muscle and hepatopancreas. Significant changes in the total protein, lipid and carbohydrate contents were also observed as a result of infection in the hepatopancreas and ovary.

DISCUSSION

There is very little information on the proximate composition of different tissues of P. semisulcatus. In

the present study, the abdominal muscle of normal P. semisulcatus has shown a protein content of about 66% and lipid about 16.4%, and these values fall within the range reported for Cancer magister (Allen, 1971), P. aztecus (Dewhart et al., 1972), Metapenaeus affinis (Pillay and Nair, 1973) and for P. indicus and P. monodon (Sriraman and Reddy, 1977). The protein and lipid content in the hepatopancreas of the decapod crustaceans have been reported to range between 23.8% and 36.0% (Vonk, 1960; Allen, 1971; Pillay and Nair, 1973), and between 10.5% and 52.0% (Schafer, 1968; Pillay and Nair, 1973; Middleditch et al., 1980) respectively. In P. semisulcatus also, the protein (26.2%) and lipid (40.3%) content of hepatopancreas have been found to be well within these reported ranges of other decapods. The lipid content in the normal ovary of P. semisulcatus has been recorded to be 27.8%, which is comparatively higher than that reported in the mature ovary of M. affinis (14.8% to 21.0%) by Pillay and Nair (1973), presumbbly, due to the larger size of ovary of the former as compared to that of the latter. However, the protein content in the normal ovary of P. semisulcatus was 47.9%, which agrees well with the values recorded for the ovary of C. magister (Allen, 1971) and Portunus pelagicus (Pillay and Nair, 1973). Thus, the proximate composition of the abdominal muscle, hepatopancreas and ovary in respect of the major nutrients of protein, lipid and carbohydrate in the normal P. semisulcatus, does not show wide variations and almost

agrees with the proximate composition of other related decapod crustacean species.

Earlier investigations on the biochemical changes occurring in different tissues of the host due to parasitic attack and its manifestation have shown that they greatly depend on the density of the parasite in the host tissue, and the susceptibility, internal defence capability and the age of the host. All these factors also significantly influence the host-parasite relationship. This relationship is highly complex involving both metabolic and physiological alterations, and according to Ceccaldi (1972), it necessitates a number of adaptations on the part of the parasite to the internal body composition of the host. So close is this relationship that Thompson (1953) refers to the parasite and the host as an integrated complex. Mead (1970) attributes the occurrence of disease to the failure of this host-parasite interaction.

Histopathological study of *P. semisulcatus* infected by *P. semisulcata* (refer Sub-chapter 4.3) has shown that the ovary, hepatopancreas and the abdominal muscles were the major organs harbouring large quantity of *P. semisulcata* spores. In the present study, therefore, these organs were observed for the gross biochemical changes in the proximate composition as a result of infection.

Poorer changes in the proximate composition of *P. semisulcatus*, as revealed by the variations in the moisture

content, protein, lipid and carbohydrate in the normal and infected ones, were observed in the ovary and the hepatopancreas (Table 5). In the abdominal muscle, however, notable variations were observed mainly in the mean values of the moisture and ash content and total lipid levels between the normal and infected prawns (Table 5). The difference in the total protein content between the normal and infected ovary has been to the tune of about 11% and that of lipid about 6 . imilarly, the protein content of the hepatopancreas showed an increase of about 10% while the lipid decreased by 6%. his significant drop in the lipid and rise in protein levels in the infected ovary and hepatopancreas indicates that the parasite largely depends on the easily accessible lipid from the host and multiplies faster in these organs. he increased level of total protein in the ovary and hepatopancreas appears to be due to higher concentration of spores. he results of the present study based on the biochemical changes thus showed that the most preferred site of infection by *L. semisulcata* is the ovary and hepatopancreas followed by abdominal muscle. his observation further confirms the earlier conclusion arrived at by the histological studies on the sites of infection.

Several studies have shown that the hepatopancreas in decapod crustaceans stores large quantities of fat (Gibson and arker, 1979) and provides rich supply of absorbed nutrients. Lipid also plays a major and significant role in the metabolism of crustaceans (illay and air, 1973).

In the present study, high lipid constituting about 40% is recorded in the normal *P. semisulcatus*. Similarly, the ovary of the normal *P. semisulcatus* also contains relatively high lipid . These rich nutrients obviously attract the microsporidian and afford the most favourable conditions for growth. That the lipid and carbohydrate nutrients are greatly utilised by the pathogen is evident both by the biochemical changes observed in these tissues and also by the histological examination. It may be referred here that the histological examinations of the hepatopancreas and the ovary (vide-chapter 4.3) have shown that the tissues in these organs are undergoing lysosomal digestion or autophagy under conditions of advance nutritional stress as a means of physiological survival mechanism. The rise in the moisture content observed in these organs is due to the state of lysis, as normally observed in such situations. Thus, these observations and the depleting trend of lipid and carbohydrate in these tissues along with the progress of infection, and at the same time, increase of protein, suggests that the changes could be due to parasite induced lysis or lysis as the survival mechanism of the host due to starvation induced by parasitisation or combination of these factors. It may be pointed out that the metabolic alterations is an ongoing process which is affected by the parasites that could stimulate hosts' enzymatic activities to suit their need for nutrients and energy sources. The parasite can modify host's

metabolism by secreting metabolic regulators (Rutherford and ebster, 1978). Marshall et al. (1974) have shown that enzymatic activities of the parasite are much greater than those of the hosts, whereby parasites are effectively able to compete with the host for the available nutrients. Ishak et al. (1975) have reported parasite induced glycogen depletion in the host snails.

yrons and Jones (1974) observed increase in lipid level in the liver of rats infected by a nematode. unn et al. (1977) recorded that the collagen synthesis is higher in the in ecte animals than those in the normal ones. This could be the reason for the slight increase observed in protein in the mo ely infected prawns.

It is interesting to note that in the abdominal muscle, the biochemical changes are rather insignificant although it contains a reciable levels of observed moisture and protein. The lipid level is found to be relatively less as compared to that of hepatopancreas and ovary. Nevertheless, the muscle also forms an active site for _. semisulcata infection probably due to its high proteinaceous nature. However, it appears that the abdominal muscle does not provide an optimum environment for the parasite to develop and multiply as efficiently and successfully as the ovary and the hepatopancreas which contain higher levels of lipid.

The degree of infection, age and moulting behaviour of prawn and environmental conditions impose a significant influence on the microsporidian. Ceccaldi (1982) pointed out that in order to survive and develop, the parasites must adopt to the composition of the internal medium of the host to the extent that the characteristics and variations in the host correspond to their physiological needs. On the other hand, the physiology and metabolic activities of the host may also be disturbed to certain levels as a result of infection. According to Thomson (1963), the disturbance in the metabolism of the host, which can be indicated by changes in tissue metabolite levels are intimately associated with the establishment and success of parasitic relationship. Ceccaldi (1982) opined that parasite could modify the internal medium of the host and lead directly to changes in the cell or tissue structure or indirectly through changes in the hormonal equilibrium. It can also lead to changes in the body composition of the host, causing changes in water content or the circulation of minerals and organic elements of the haemolymph and the tissues (Ceccaldi, 1982). That the degree of infection as such does not change the overall proximate composition once the infection is established in the host is evidenced by the fact that the proximate composition between the moderately and heavily infected prawns remains more or less same with little variations between the normal and moderately infected group of prawns (Table 5; Plate XXXIV).

CHAPTER 5

DISEASE CONTROL

During the last decade, appreciable advances have been made on the control of diseases affecting the farmed organisms. This has been possible through the use of histological techniques, better understanding of the diseases and the causative organisms, and the progress made in the chemical treatments. In recent years, greater emphasis has also been given to prophylactic measures and prevention of diseases through better water quality management and minimising the environmental and handling stresses. Immunology and methods of vaccine application are also fast emerging as important means of disease control.

Relative to the studies on prawn diseases, very little work has been done on their control. This is particularly so in the case of penaeid prawn diseases. Shigueno (1975) reported on various methods of control of bacterial and fungal diseases encountered in the larval population in hatcheries and in the prawns cultured in the tanks in Japan. Similarly, the treatments developed and used to control the diseases encountered in the larvae and adult penaeid prawns in America was discussed by Lightner (1975, 1977, 1983). Some information on the control of diseases during larval

rearing is also available from the works carried out at the Southeast Asian Fisheries Development Centre (SEAFDEC) at Philippines and at Tahiti by the AQUACOP team.

In India, although the work on the preventive and curative measures of parasites and diseases of fishes, especially the freshwater fishes has been carried out by several workers (Khan, 1939, 1944; Tripathi, 1954, 1957; Saha and Sen, 1955; Hora and Pillai, 1962; Gopalakrishnan, 1963, 1964, 1968; Ghosh and Pal, 1969; Pal and Ghosh, 1975; Srivastava, 1975; Ghosh, 1978; Mandaloi, 1982; Seenappa and Manohar, 1982; Seenappa et al., 1982; Srivastava, 1982), there has been no work worth mentioning on the control of diseases and parasites of penaeid prawns from this country. Pandian (1982) conducted experiments on the rearing of Penaeus indicus and Metapenaeus dobsoni in the medium treated with antibiotic tetracycline and antifungal agent acriflavin and observed that tetracycline at a concentration of 1 to 3 ppm did not affect the survival rate of the larvae while the acriflavin was not suitable in penaeid larval rearing, although it was found to be a suitable fungicide in the culture of juvenile lobster, Homarus americanus(Abrahams and Brown, 1977).

In view of the little information available, an attempt is made here to briefly summarise the disease control measures developed and practiced in the culture of penaeid prawns.

Although several viral diseases have been identified in penaeid prawns, there has been very little progress in their control (Lightner et al., 1983d).

Several methods of treatment for bacterial diseases have been reported. Drying, cleaning and disinfection of spawning, hatching, larval rearing and nursery tanks considerably reduce bacterial infection (ACUACOP, 1977; Lightner, 1977; Lightner et al., 1980). Supply of good and quality water, filtration and sterlisation of water and alleviation of stress factors have also been suggested for reducing or preventing bacterial diseases (Lightner, 1977; Johnson, 1983b). Use of certain antibiotics such as furacin, furanace, chloramphenicol and oxytetracycline, either by direct addition to the medium in the tank or by incorporating in the feed, has been found as successful thereapy (Chan and Lawrence, 1974; Delves-Broughton, 1974; Lightner, 1975, 1977, 1983; ACUACOP, 1977; Corliss et al., 1977; Lightner et al., 1977; Corliss, 1979; Lightner et al., 1980). Feeding the prawns with compounded feed mixed with sulphizozole, nifurstyric acid and chloramphenicol is found to cure the bacterial disease caused by Vibrio spp. in P. japonicus (Shigueno, 1975). Similarly, immersion in 2 to 3 ppm furazolidon is also reported to be effective in treating the disease caused by certain bacteria which discolour the gills (Shigueno, 1975). The filamentous gill

disease caused by Leucothrix infestation could be treated effectively using a sea water soluble copper compound, commercially known as cutrine-plus (Lightner and Supplee, 1976). Potassium permangnate is also found to be effective in treating the filamentous bacterial infestation (Lightner, 1977).

Methods of chemotherapy for Lagenidium infection in penaeids have been reported by Bland et al. (1976), AQUACOP (1977) and Lightner (1977), and application of chemicals such as malachite green oxylate at 0.006 ppm (static) or trifluralin at 0.01 ppm concentration have been found to be effective in preventing the disease. At SEAFDEC, this fungal disease is being controlled by furanace, while antibiotic such as gallymycin and fungicide trifluralin are used to control infection in larval rearing at Tahiti. Lio-Po et al.(1982) studied in vitro the sensitivity of Lagenidium spp. isolated from P. monodon and Scylla serrata,to 34 antimycotic chemical compounds. Similarly, pure cultures of Haliphthoros philippinensis isolated from P. monodon larvae were exposed for 24 hours to varying concentrations of the antifungal agents and efficiency of each compound in inhibiting sporulation and mycelial growth of the fungus was measured in a recent study conducted at SEAFDEC by Lio-Po et al. (1985). Practical methods of chemotherapy for Fusarium infection are lacking. Several

fungicides have shown some promise in *in vitro* studies with this fungus (Hatai *et al.*, 1974; Hatai and Egusa, 1978; Lightner *et al.*, 1979a) but none has been effective in treating the established *Fusarium* infection under culture conditions (Johnson, 1983b).

Chemical control of ciliate and other protozoan infestations has been suggested by different workers (Johnson *et al.*, 1973; Johnson, 1974a, 1974c, 1976b; Schnick *et al.*, 1979). Formalin at the rate of about 25 ppm is reported to be effective in controlling ciliate infestations. The other important chemicals found to be effective in treating ciliates and other protozoan epicommensals in the culture tanks are glutaraldehyde at 2 ppm, chloramine T, quinine sulphate or quinine bisulphate at 5ppm and quinacrine hydrochloride at 0.6 ppm concentration (Johnson, 1976b).

The black death disease caused by ascorbic acid deficiency in prawns cultured in tanks with artificial diet, is controlled by providing appropriate feed having 2000 to 3000 mg of vitamin per kilogram of feed (Deshimaru and Kuroki, 1976; Lightner *et al.*, 1979b; Magarelli *et al.*, 1979) or by feeding a supplement of fresh algae to the affected prawns (Lightner, 1977).

Studies on the control of microsporidian parasites are limited. Overstreet (1975) and Overstreet and Whatley (1975)

conducted
a series of experiments in order to observe the effect of various drugs for prevention of microsporidiosis in the blue crab, Callinectes sapidus caused by Ameson (=Nosema) michaelis. These authors used several drugs such as Benomyl, Buquinolate, Clopidol, Fumagillin, Furazolidone, Nitrofurazone, Sulphamethazine and Zoalene in their experiments where only one dose of a particular drug or combination of two drugs was orally administered to the normal crabs along with the diced portions of fish inoculated with infective spores of A. michaelis. The control crabs for each experiment were fed with fish tissue along with the A. michaelis spores but without the addition of the particular drug. At the end of these experiments, which lasted for 1 to 3 months, Overstreet and Whatley (1975) found that only "Buquinolate" proved reliably effective in reducing the number of infected crabs when compared with controls which were given no drug. Furazolidon, the combination of Buquinolate and Clopidol, and Benomyl showed much less effectiveness in that order. Couch (1978) suggested that if Buquinolate is used for treating microsporidian infected prawns in the culture system, depuration of the drug from the prawn tissue might be necessary before the treated prawns are used for human consumption.

In another experiment, Overstreet and Whatley(1975) found that when spores of A. michaelis treated either with a commercial bleach or a disinfectant containing iodine, were

fed along with the diced fish to the normal experimental crabs, none of the tested crabs produced infection. The controls, in contrast, revealed about 48 percent infected crabs after a period of 30 days. Disinfection of the closed systems, where prawns are cultured, with a commercial bleach (Purex-Fleecy White^{R} Bleach with 5.25 percent sodium hypochlorite) or a disinfectant containing iodine (Wescodyne^{R} with 9.1 percent polyethoxy polypropoxy polyethoxy ethanol-iodine complex and 8.74 percent nonylphenoxypoly (ethyleneoxy) ethanol-iodine complex) has, therefore, been recommended by Overstreet and Whatley (1975) to prevent or treat microsporidian contamination.

SUMMARY

1. The thesis embodies the results of the studies carried out on certain diseases affecting the commercially important penaeid prawns in the capture and culture fisheries of the southwest and southeast coasts of India during October, 1981 to April, 1985.

2. Initially, a survey is conducted to obtain information and to understand the common diseases and abnormalities occurring in the penaeid prawns in nature and those farmed, in the study area. The pattern of the infection/infestation, symptoms and of the pathogenicity of each of the cases encountered is studied macro- and microscopically and by employing histopathological techniques.

3. As a result of the survey, ten cases of diseases and abnormalities are reported. These include tumour-like growth, "soft" prawn syndrome, tail necrosis, brown spot disease, red rostrum, ciliate infestation, microsporidiosis, helminth parasitisation, metacercarial infestation and bopyrid infestation in the penaeid prawns such as Penaeus indicus, P. monodon, P. semisulcatus, Metapenaeus dobsoni and M. affinis.

4. The symptoms, occurrence and incidence of each of the above cases are provided along with the information on environmental factors such as salinity, dissolved oxygen, temperature and pH of the water from the collection sites. The nature of the disease, the tissues of the host that are affected by the infection or infestation or by the pathogen, and the factors influencing the infection in each of the ten cases are studied histopathologically and discussed.

5. The disease caused by the microsporidian parasites, commonly known as "cotton" or "milk" shrimp disease, and encountered in the wild juvenile and adult population of P. semisulcatus and M. affinis exploited off Rameswaram, Mandapam and Tuticorin on the southeast coast of India is selected for detailed investigation.

6. The nature, structure and characteristics of the different developmental stages and spores as studied by the light and electron microscopy and histological techniques of the microsporidian parasites collected from P. semisulcatus and M. affinis have revealed that they belong to three species, two of them assignable to the Family Thelohaniidae (Order:

microsporida; Sub-order: Pansporoblastina) and the other one to the Family Perezilidae (Order: Microsporida; Sub-order: Apansporoblastina). Further detailed studies and comparisons with the described and known microsporidian species have revealed that they are new to Science.

7. The sporont of one of the two species belonging to the family Thelohaniidae is found to undergo a series of three successive binary divisions producing eight sporoblasts in a thin, sub-persistent pansporoblastic membrane. These sporoblasts metamorphose into free, mature spores which are ovoid, uninucleate and measure 5.0 to 5.5 X 2.5 to 3.5 µm in size and possess isofilar polar tube measuring 14 to 22 µm in length. This species is described as Thelohania semisulcata sp. nov. and is found to infect mainly the body muscle, hepatopancreas, gonad and midgut of E. semisulcatus. The other organs affected to lesser extent are the heart, eyes and the gills.

8. The other microsporidian assigned to the Family Thelohaniidae, shows a combination of characters of the Genera Thelohania and Agmasoma. This microsporidian is characterised by three successive binary divisions of the sporont resulting in the formation of eight sporoblasts covered in a fragile pansporoblastic membrane. The mature and free spores are pyriform,

uninucleate and measure 3.0 to 4.2 × 1.5 to 2.0 µm in size. The polar tube is anisofilar and forms about 9 to 10 undulations anterio-posteriorly inside the spore. In view of these characters, this microsporidian is assigned to a new genus, namely, Sulcovaria and the species described as Sulcovaria mannarensis. This species, unlike T. semisulcata, is site specific and infects only the ovary of P. semisulcatus.

9. The third microsporidian attributed to the family Perezziidae is apansporoblastic and disporous in nature and has ovoid, uninucleate spores which measure 2.2 to 2.5 × 1.0 to 1.5 µm in size and possess isofilar polar tube measuring about 25 µm in length. This is described as Perezia affinis sp. nov. and is found to infect the body muscle, gonad and digestive tract of M. affinis and P. semisulcatus.

10. Symptoms of microsporidiosis caused by T. semisulcata are studied by qualitatively categorising the infection as light, moderate and heavy.

11. The histopathological investigation on T. semisulcata - infected prawns revealed that T. semisulcata is an intracellular parasite and highly pathogenic in

nature. The effect of the pathogen on the cellular structure of the important organs such as gonad, hepatopancreas, body muscle, midgut, heart, optic nerves, retina and gills is studied. The host response to the infection appears to be least developed or effective as the pathogen does not apparently elicit any significant inflammatory response in the host. The nature of infection initially through the sub-mucosa of the midgut, its subsequent spread to the other organs and finally leading to the death of the host are studied and discussed.

12. The laboratory experiments are carried out to transmit _. semisulcata to the normal and healthy prawns by contaminating the rearing medium with the pathogen, injecting the pathogen into the body of the prawns and feeding the normal, healthy prawns with the heavily infected prawn muscle tissue. The results indicate that the pathogen follows oral route of transmission and does not require any intermediate host. However, the spores of _. semisulcata have to undergo a process of conditioning by passing through the gut of the prawn before being capable of infecting the prawn. The initial site of infection by _. semisulcata is found to be the

midgut. The process of infection, the successful establishment of the pathogen and its spread to the various tissues of the host, and the transmission of infection in nature to the healthy prawns on the basis of the observations made, are presented and discussed.

13. Proximate composition of abdominal muscle, hepatopancreas and ovary of normal and those of moderately and heavily infected P. semisulcatus by I. semisulcata are studied and satistically compared.

14. The infection by I. semisulcata in P. semisulcatus is found to bringforth appreciable changes in the moisture content in the abdominal muscle, hepatopancreas and ovary. The variation in the ash content is, however, generally observed in the abdominal muscle and hepatopancreas. ignificant changes in total protein, lipid and carbohydrate content in the hepatopancreas and ovary of the normal and infected prawns are also observed as a result of infection.

15. In the light of the available published information, the control measures for the different diseases of penaeid prawns are presented and discussed.

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