HISTOPATHOLOGICAL SURVEY OF CULTURED SHRIMPS IN MODIFIED EXTENSIVE SYSTEMS OF COCHIN, KERALA

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Dedicated to

My Parents and sister

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I C A R

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CERTIFICATE

Certified that the thesis entitled "**HISTOPATHOLOGICAL SURVEY OF CULTURED SHRIMPS IN MODIFIED EXTENSIVE SYSTEMS OF COCHIN, KERALA**" is a record of independent bonafide research work carried out by **Ms. Liya Ambipillai** during the period of study from September 1999 to August 2001 under our supervision and guidance for the degree of **Master of Fisheries Science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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DECLARATION

I hereby declare that the dissertation entitled "HISTOPATHOLOGICAL SURVEY OF CULTURED SHRIMPS IN MODIFIED EXTENSIVE SYSTEMS OF COCHIN, KERALA" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

मार्च, 2001 से जून, 2001 के बीच ऊतकीय-विकृति-जन्य साधनों का प्रयोग करते हुए कोचीन के झींगा पालन क्षेत्रों का रोग सर्वेक्षण किया गया. 26 फार्मों से एकल किए गए झींगा प्रतिदर्शों में पीनिअस इंडिकिस, पीनिअस मोनोडोन एवं मेटापीनिअस डोबसोनी शामिल थे. सर्वेक्षण के दौरान 42% फार्में में मुख्य रोग लक्षण दैहिक जीवाणु के संक्रमण की सूचक दीर्घकालीय शोथन क्षति दर्शित हुई. 19% फार्मों में मुखीय / आँत की वाइब्रिओसिस का सूचक यकृदग्नाशय रोग देखा जा सका. एम.बी.वी. एवं वाइब्रिओसिस के मिले जुले संक्रमण की वजह से एक फार्म में पीनिअस मोनोडोन की मृत्यु प्रेक्षित हुई. पक्ष्माभी प्रोटोज़ोअन, जुथेम्नियम के कारण झींगों में परिदूषण केवल 8 प्रतिशत प्रकरणों में देखा जा सका. 36 प्रतिशत फार्मों में असामान्य लक्षण जैसे मांसपेशियों का स्वत:ऊतकक्षय, गहरे रंग के क्लोम व भूरी अपवर्णता दर्शित हुई. अधिक अमोनिया स्तर वाले भीषण यकृदग्नाशय रोग प्रकरण को छोडकर जल-गुण प्राचलों तथा रोगीय लक्षणों के आपतन में कोई सहसंबंध स्थापित नहीं किया जा सका. अध्ययन के दौरान मुख्य प्रेक्षणों में से श्वेत बिंदु विषाणु की प्रत्येक लक्षित ऊतक में अनुपस्थिति मुख्य थी. सर्वेक्षित फार्मों में से 27 प्रतिशत में कोई आभासी रोग लक्षण नहीं देखे गए.

ABSTRACT

A disease survey was conducted using histopathological tools in a cross section of shrimp farming area in Cochin during March to June, 2001. Shrimp samples collected from 26 farms comprised of *Penaeus indicus*, *Penaeus monodon*, and *Metapenaeus dobsoni*. The major pathological condition recorded was the presence of chronic inflammatory lesions characteristic of systemic bacterial infection and was observed in 42% of the farms surveyed. In 19% of the farms hepatopancreatic pathology, typical of oral/enteric vibriosis was recorded. Mortality of *P.monodon* due to mixed infection of MBV and vibriosis was recorded in one farm. Fouling of shrimps by the ciliate protozoan, *Zoothamnium* could be detected only in 8% of the cases. Abnormal conditions such as spontaneous muscle necrosis, dark coloured gills and brown discolouration of the shell were recorded in 36% of the farms. Absence of white spot viral inclusions in any of the target tissues of the samples examined was one of the most important observations during the study. No apparent pathological conditions were recorded in 27% of the farms surveyed.

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INTRODUCTION

1. INTRODUCTION

The farming of penaeid shrimp is practised in several countries around the world. But the industry is concentrated mainly in the Southeast Asian and the Latin American countries. The production is dominated by the two species, the black tiger shrimp (*Penaeus monodon*) in Indo-pacific region and the pacific white leg shrimp (*Litopenaeus vannamei*) in the Western hemisphere. In the year 1998, global production of farmed marine shrimp was 7,48,460 mt, with the Asian continent contributing about 71% of the world's shrimp production through aquaculture (Aquaculture magazine, 1999).

India has got vast potential for development of commercial shrimp farming, with a coastal brackishwater area of 1.2 million ha. Traditional shrimp trapping systems have been in practice for several years in the pokkali prawn filtration fields of Kerala, bheries of West Bengal, gasani lands of Karnataka and khar lands of Goa. Autostocking aided by tidal action was mostly being practised with minimal managerial inputs. Later, improved practices like pond preparation, screening the water source and stocking with desired species were introduced. Subsequently, more intensive culture practices evolved in response to evolution of scientific farming technologies, easy availability of hatchery raised seed in adequate quantities and also due to the high profitability and increasing demand for shrimps in the export market.

There was a rapid expansion of the industry between 1988 and 1994. Realising the enormous potential for investment opportunities and taking advantage of the liberalisation policies of the government, several national and international corporate houses, private companies and individual entrepreneurs ventured into the field. About 0.18 million ha. has been brought under shrimp culture until 1995 –'96 (Karunasagar and Karunasagar, 1999).

With the expansion of the industry, aquaculture waste management became a very serious issue. Majority of the farms were forced to use intake water source for waste discharge. Raw effluents rich in organic matter and waste feed were released directly into the water source without any

treatment or settlement. This has led to disease problems connected with bad water quality, where little attention only was being given to pond drying, disinfection and waste removal between crops (Mohan, 1996). The unorganised and unregulated expansion with lack of scientific consideration resulted in the outbreak of diseases and the industry collapsed between 1994 and '96. During 1994-'95 alone the loss was estimated at 10,000 – 12,000 tons valued at Rs. 250 – 350 billion (Karunasagar and Karunasagar, 1999).

World over, the growth of shrimp culture industry was accompanied with increased incidence of various types of diseases. Viral diseases in particular have severely impacted many leading shrimp farming nations of the world causing significant production and economic losses. Among the shrimp viruses recorded, the white spot virus is the most serious pathogen. Since 1993, this disease has been causing significant production losses in cultured shrimps all over Southeast and South Asia. This epizootic probably began in China in 1993 and subsequently spread from there to Japan, Taiwan and the rest of the Asia as far as India (Flegel and Alday-Sanz, 1998). In India this epidemic first erupted in Andhra Pradesh, in October 1994, and caused mass mortalities of *Peneaus monodon* and *Peneaus indicus*. In November – December 1994, heavy shrimp mortality reaching up to 100% was noticed in shrimp culture systems of Nellore and East Godavari districts (Mohan and Shankar, 1995). The disease then spread along the entire East coast and also in the West coast (Krishna *et al.*, 1997).

Diseases are usually related to the type and management of the culture systems, which influence the health and environment of the shrimps being cultured. Disease outbreaks occur, when a susceptible host comes in contact with a pathogenic organism in a stressful environment. Many diseases in cultured shrimp result from a combination of environmental and infectious components. The principal agents causing diseases are viruses such as White spot virus (WSV), Yellow head virus (YHV), Monodon baculovirus (MBV), Hepatopancreatic parvo-like virus (HPV) *etc.*, bacteria like vibrios and filamentous bacteria, fungi, rickettsia and parasites like microsporidians and gregarines (Lightner, 1996).

Many a times, outbreak of the disease may be the sole factor limiting the success of the culture. Often these outbreaks can be avoided by proper management measures. Rapid detection of a disease condition before reaching the epizootic level will help to contain it by appropriate management strategies. Analysing the disease history of an area will also be helpful in planning proper management measures. In order to formulate useful control strategies, it is essential to identify the diseases prevalent in an area. Knowledge of current disease problems is also essential if the changing pattern of disease associated with widespread intensification is to be monitored and controlled (Turnbull *et al.*, 1994).

Crop losses due to disease outbreaks have been reported at several farms in Cochin area during the past few years. However detailed studies regarding diseases affecting farmed shrimp along the brackish water farms in this area are limited. Histopathology is an ideal tool for routine health monitoring and for routine diagnosis, where the changes at the cellular and tissue level due to the pathogen is interpreted to arrive at diagnosis. The present histopathological survey on cultured shrimps in Cochin area was undertaken with the following objectives :

1) To identify the occurrence of disease conditions and pathogens involved, in a cross section of shrimp farming area at a given period during peak culture season and also to study
2) the tissue level pathological changes caused and host responses in different disease conditions
3) the relationship between case history, clinical signs and pathological conditions
4) the occurrence of disease problems in relation to environment.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In less than 30 years, the penaeid shrimp culture industries of the world developed from their experimental beginnings into major industries providing immense job opportunities, billions of U.S dollars in revenue and augmentation of the world's food supply with a high value crop. Concomitant with the growth of shrimp culture industry has been the recognition of the ever increasing importance of disease, especially those caused by infectious agents.

Conventional methods for the disease diagnosis include traditional methods of morphological pathology (light microscopy, histopathology, electron microscopy), traditional microbiology and the application of serological methods (Lightner and Redman, 1998). The industry now has modern diagnostic genomic probes with non radioactive labels for viral pathogens like IHHNV (Infectious hypodermal and hematopoietic necrosis virus), HPV (Hepatopancreatic parvo-like virus), TSV (Taura syndrome virus), WSV (White spot virus), MBV (Monodon baculovirus) and BP (Baculovirus penaei), for bacterial pathogens like NHP (Necrotising hepatopancreatitis) and certain *Vibrio* spp. and also for microsporidia (Lightner and Redman, 1998; Karunasagar and Karunasagar, 1999).

Disease investigations in shrimps have gone much ahead of other crustaceans, obviously due to their economic value and high demand world over. Diseases in shrimps have been reviewed a number of times by various authors (Overstreet, 1973; 1983; Lightner, 1977; 1983; Couch, 1978; 1983; Johnson, 1978; Johnson, 1983; Otta *et al.*, 1998). The most significant pathogens that are commonly associated with cultured shrimps belong to viruses, bacteria, fungi and parasites. Apart from these, non-infectious diseases like those caused by epicommensals, environmental stress factors, nutritional deficiencies and toxic factors are also reported to cause mortalities in shrimp ponds.

2.1.0 Viral diseases

Among the diseases of intensively cultured shrimp, viruses are the most important pathogens, characterised by high infection rates and ability to

cause high mortalities. Viruses spread from one host to another through horizontal and vertical transmission. Infection and disease development in the host depends on viral load in the environment (infectious dose). More often animals surviving infection become assymptomatic carriers. Viruses being obligatory pathogens, cell culture is essential for their study and propagation. But at present shrimp virus infection is mostly detected by histopathology, electron microscopy and PCR.

Viral aetiology is being attributed to nearly 20 diseases in crustaceans (Lightner, 1983) and fourteen of these viruses have been reported for penaeid shrimps (Otta *et al.*, 1998). Based on the type of nucleic acid, shrimp viruses can be broadly grouped as RNA and DNA viruses.

2.1.1 White spot syndrome virus

Mass mortalities due to white spot virus had occurred in the cultured kuruma shrimp, *Penaeus japonicus*, in Japan and *Penaeus monodon* in Taiwan during 1992 (Inouye *et al.*, 1994; Nakano *et al.*, 1994; Chen, 1995) and the virus caused a wide spread destruction of shrimp culture in Asia (Flegel, 1996). Disease was characterised by the presence of white spots on the inside surface of the carapace and was suggested to be caused by a ds-DNA virus (Chou *et al.*, 1995). Later it was named as WSDV (Chen, 1995). Similar disease, variously called as SEMBV, systemic ectodermal and mesodermal baculovirus was reported in Thailand (Wongteerasupaya *et al.*, 1995) and in China as HHNBV, hypodermal and haematopoietic necrosis baculovirus (Cai *et al.*, 1995). PAV (Penaeid acute viremia) reported to cause mass mortality in farm raised kuruma shrimps in Japan (Nakano *et al.*, 1994; Takahashi *et al.*, 1994; Inouye *et al.*, 1996) was also a similar disease. The causative agent being designated as RV-PJ, rod shaped nuclear virus of *Penaeus japonicus* (Inouye *et al.*, 1994) and later as rod shaped DNA virus (PRDV) (Inouye *et al.*, 1996). The clinical signs of white spot disease included many white spots on the inside surface of the carapace, in severe cases of infection, spots were also seen on the appendages. An abnormal red colouration or discolouration was usually seen in the diseased shrimp. WSV affected shrimps were found lethargic and inappetant. The white spots were representing a protrusion on the inside surface of the carapace and

its composition was similar to that of cuticle (Wang *et al.*, 1997). Histopathology demonstrated degenerated cells characterised by basophilic inclusion bodies in hypertrophied nuclei in various tissues including cuticular epidermis, gills, connective tissue, haemocytes, haemopoietic tissue, lymphoid organ, cuticular epithelium of foregut and hindgut, heart, antennal gland, nervous tissue, striated muscle (Momoyama *et al.*, 1997; Wang *et al.*, 1997). The disease shows a wide host range (Wongteerasupaya et al., 1995; Otta *et al.*, 1998). The environmental stressors such as ammonia may enhance the severity of white spot disease virus infection in cultured shrimp (Wang *et al.*, 1997).

2.1.2 Baculovirus penaei (BP)

Baculovirus penaei, was the first virus pathogen to be reported from penaeids. It was reported from the pink shrimp (*Penaeus duorarum*) by Couch (1974). Infection was by horizontal transmission to all life stages. Epizootics were chronic to acute with high cumulative mortality but presence of virus did not always result in disease. Feeding and growth rates reduced; gill and surface fouling increased with the occurrence of the disease. The target organs of BP virus were the epithelial cells of hepatopancreas and anterior midgut gland of larval and adult stages of several species of penaeid shrimps and caused mass mortalities in larval stages (Couch, 1974; Lightner, 1983). Single or multiple eosinophilic intranuclear polyhedral inclusion bodies were seen in the affected cells (Couch, 1978; Lightner, 1983). It was reported from species like *P. duorarum, P. aztecus, P. setiferus, P.vannamei, P. stylirostris, P. marginatus, P.pencillatus, P.schmitti* and *P. subtilis* over a wide geographic range (Couch, 1974; Brock *et al.*, 1986a; Overstreet *et al.*, 1988; Lightner *et al.*, 1989; Sergioluiz *et al.*, 1990; Leblanc *et al.*, 1991).

2.1.3 Monodon baculovirus (MBV)

Monodon baculovirus was named so because of its first detection from *Penaeus monodon* by Lightner and Redman (1981) in Taiwan. From 1987, serious mortalities up to 35-70% of the cultured populations of *Penaeus monodon*, occurred in Taiwan due to this virus (Rosenberry, 1988). The apparent target organs and tissues for MBV were the hepatopancreatic tubule

and duct epithelium of postlarvae, juveniles and adults and the anterior midgut epithelium of very young postlarvae (Lightner *et al.*, 1983a). MBV occlusion bodies appeared as prominent eosinophilic, single to multiple round bodies within the hypertrophied nuclei of hepatopancreatic tubule or midgut epithelial cells. Affected shrimps showed lethargy, anorexia, dark colouration and heavy surface fouling. Acute MBV caused loss of hepatopancreatic tubule and midgut epithelium and consequently, dysfunction of these organs, often followed by secondary bacterial infections (Bower *et al.*, 1994). Environmental stressors such as crowding and biological stressors, including the effect of other pathogens have been demonstrated to enhance the severity of MBV infection in *Penaeus monodon* (Chen *et al.*, 1989; Fegan *et al.*, 1991).

2.1.4 Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

Infectious hypodermal and haematopoetic necrosis virus, a parvovirus, causative agent for another viral disease in shrimps was first reported from *P. stylirostris* (Lightner *et al.*, 1983b). Affected shrimps showed reduced food consumption, cannibalism and increased mortality. Shrimp repeatedly rose slowly to surface, rolled over and sank to the bottom. Mottled colouration was a diagnostic feature. Disease was particularly severe in high density culture. The histopathology of acute and subacute IHHNV was dominated by conspicuous eosinophilic intranuclear inclusion bodies of the cowdry type A variety in ectodermally and mesodermally derived tissues that were undergoing necrosis (Lightner *et al.*, 1983b). The inclusion bodies were common in acute infections, later decreasing in number and followed by necrosis and inflammation of target tissue. Affected cells also had highly vacuolated cytoplasm with cytoplasmic bodies that range from eosinophilic to basophilic (Bower *et al.*, 1994). Distinctive histopathological lesion patterns were observed between species (Bell and Lightner, 1984) and within the same species the disease prevalence as number of inclusion bodies decreased with increase in size (Bell and Lightner, 1987). Similar pattern in disease prevalence was found in the case of BP in *P. vannamei* (Leblanc and Overstreet, 1990) and MBV in *Penaeus monodon* (Fegan *et al.*, 1991). RDS (Runt deformity syndrome),

characterised by reduced growth and deformities of the rostrum, anterior appendages or other parts of shrimps, a frequent disease problem of cultured *Penaeus vannamei* was found to be caused by IHHN virus (Kalagayan *et al.*, 1991).

2.1.5 Hepatopancreatic parvo-like virus (HPV)

A second parvovirus, found to affect shrimps is the hepatopancreatic parvo-like virus. It was reported from *P. merguiensis* and *P. semisulcatus* by Lightner and Redman (1985). It was found to affect other species like *P. chinensis, P. esculentus, P. monodon, P. indicus, P. penicillatus and* P. vannamei. Affected shrimps showed poor growth rate, anorexia, reduced preening activity, increased surface fouling and occasional opacity of tail musculature. Signs were accompanied by mortalities during juvenile stages after apparently normal development through larval and post larval stages. Histopathology showed necrosis and atrophy of the hepatopancreas, typically of mid-juvenile stages. Single prominent basophilic spherical intranuclear inclusion bodies were present in the affected epithelial cells of hepatopancreatic tubules and epigastric caecal epithelial cells with lateral displacement and compression of the host cell nucleolus and chromatin margination of the nucleus. Early in their development, HPV inclusions were seen as small eosinophilic bodies centrally located within the nucleus and closely associated with the nucleolus (Bower *et al.*, 1994).

2.1.6 Baculoviral midgut gland necrosis virus (BMNV)

Baculoviral midgut gland necrosis virus (BMNV) disease was first reported by Sano *et al.* (1981). The disease was also reported as midgut gland cloudy disease, white turbid liver disease and white turbidity disease. Disease was characterised by sudden onset of white turbid midgut gland in larvae and postlarve with associated high mortality. Larvae float inactively on the surface and exhibit a white midgut line through the abdomen (Bower *et al.*, 1994). The virus belong to baculoviridae do not form occlusion bodies. Midgut and intestine were the target organs. Diagnosed by greatly hypertrophied nucleus within hepatopancreatic epithelial cells that were undergoing necrosis (Sano *et al.*,

1981). Latently infected spawners and cultured animals were suggested to be the vertical and horizontal sources of infection respectively (Momoyama, 1988). BMNV generally infected larvae and early post larval stages causing high mortalities.

2.1.7 Reo-like virus (REO)

A non-enveloped, paraspherical virus, affecting *P. japonicus* was reported by Tsing and Bonami (1987). It belong to Reoviridae group and was a double stranded RNA virus. The external signs of this viral disease involved behavioral change (the shrimps did not hide themselves in the sand) and color change (the telson, uropods and hepatopancreas became reddish). Affected shrimps showed poor feeding, growth, anorexia, lethargy, reduced preening and increased surface fouling. Gut and nerve syndrome (GNS), an idiopathic condition found in chronically ill populations of *P. japonicus* cultured in Hawaii was suspected to be caused by this virus. Occasionally eosinophilic cytoplasmic inclusions were seen in hepatopancreatocytes (Bower *et al.*, 1994).

2.1.8 Rhabdovirus of penaeid shrimp (RPS)

Rhabdovirus of penaeid shrimp was the first reported rhabdovirus to be isolated from cultured penaeid shrimp populations (Lu *et al.*, 1991). RPS was found to infect *P.stylirostris* with the viral infection being localised in the lymphoid organ (Oka organ) and resulting in cellular alteration and necrosis (Nadala *et al.*, 1992).

2.1.9 Yellow head virus (YHV)

The yellow head disease of the black tiger shrimp, in Thailand since 1990, was reported to be caused by a baculo-like virus (Boonyaratpalin *et al.*, 1993). Affected shrimp had pale body and light yellow colouration of the hepatopancreas and gills. The virus particles observed in necrotic tissues of the gills and lymphoid organ were bacilliform in shape (Boonyaratpalin *et al.*, 1993). Viral particles were found in the cytoplasm of the infected cells and were contained within the cytoplasmic vacuoles (Lu *et al.*, 1994). Gross observations showed an increased feeding initially, followed by a reduction in later stages of

the disease (Bower *et al.*, 1994). The disease was found to be an acute and lethal one. Massive systemic necrosis were seen in numerous tissues, apparently of ectodermal and mesodermal in origin, of the affected shrimps and nearly spherical basophilic cytoplasmic inclusions were present in those cells (Chantanachookin *et al.*, 1993; Bower *et al.*, 1994).

2.1.10 Lymphoid Organ vacuolisation virus (LOVV)

Lymphoid Organ vacuolisation virus also known as the Lymphoid organ parvo-like virus, LOPV, is a togavirus. LOPV showed a similar histopathology as those reported from the cultured penaeid shrimps in Australia and Taiwan by a parvo-like virus (Bonami *et al.*, 1992). In histology the cytoplasm of the lymphoid organ cells were with highly vacuolated and intracytoplasmic inclusion bodies, that ranged in characteristic from eosinophilic to basophilic (Lightner *et al.*, 1992).

2.1.11 Taura Syndrome virus (TSV)

First noticed in Penaeid shrimp farms located near the mouth of Taura River in Ecuador (Jimenez, 1992). Taura syndrome virus (TSV) had been the causative agent of economically disastrous epizootics in *P. vannamei*, causing mass mortalities of 40-95% in affected post larval and juvenile populations (Lightner *et al.*, 1995). TSV caused three distinguishable phases in infected shrimps, the peracute and acute phase, characterised by an overall pale reddish coloration and a recovery phase characterised by multifocal, melanised cuticular lesions (Lightner, 1996).

2.2.0 Bacterial diseases

Mortalities due to bacterial diseases were observed in all life stages, larvae, juveniles and adults of penaeids (Lavilla-Pitogo, 1995). Most of the bacterial disease outbreaks were caused by species of *Vibrio*, *Pseudomonas* and *Aeromonas* (Lightner, 1977).

Bacterial diseases had been limiting factors in penaeid culture systems, their effects becoming directly proportional to the growth of the industry

in terms of severity and impact. Although eight genera had been reported to be associated with these problems, only two groups occur quite commonly, the filamentous bacteria and vibrios, with the later being more important (Lightner, 1988). Though many *Vibrio* species had been reported in penaeids, *V. vulnificus*, *V. parahaemoliticus*, and *V. harveyi*, were the most important species. Bacterial infections in shrimp took two forms: Localised pits in the cuticle (shell disease) or localised infections within the body and generalised septicemia (Lightner, 1983).

Diagnosis is mostly done by conventional bacteriology and histopathology. More rapid detection methods like indirect fluorescent antibody technique and other enzyme immunoassays are being developed to improve monitoring and surveillance.

2.2.1 Vibriosis

Vibriosis, the disease condition associated with the genus *vibrio*, was found to cause mass mortality in shrimp (Linsuwan, 1988; Karunasagar *et al.*, 1994; Leano *et al.*, 1994). Pathological changes associated with vibriosis were extensive necrosis by severe bacterial invasion and multiple formation of melanised nodules in the lymphoid organ (Egusa *et al.*,1988). Significant necrosis and inflammation occured especially in the oka/lymphoid organ, frequently but usually less severe on gills, heart, hepatopancreas and some times other tissues (Nash *et al.*, 1990; Jiravanichpaisal and Miyazaki, 1994). A number of *Vibrio* species were found associated with vibriosis and the major ones reported were *V. parahaemolyticus, V. harveyi, V. vulnificus, V. damsela, V. alginolyticus, V. anguillarum and V. campbelli* (Lightner, 1977; 1983; Nash *et al.*, 1990; Karunasagar *et al.*, 1994; Lavilla-Pitogo, 1995). But prior to the outbreak of vibriosis, a decrease in the diversity of the vibrio community was observed (Lavilla-Pitogo *et al.*, 1998; Sung *et al.*, 1999). *Vibrio* was found to affect all developmental stages from larvae in hatchery tanks to juveniles and brood stock in grow out. However bacterial strains responsible for vibriosis in successive stages were usually considered to be different and the virulence also changed both at the species and at the developmental stage levels (Gorant *et al.*, 1998).

Vibriosis of larvae and post larvae

Larval vibriosis was caused by luminescent forms like *V. harveyi*, *V.vulnificus* and *V. parahaemolyticus* and also by related nonluminescent forms like *V. alginolyticus* (Karunasagar *et al.*, 1994; Leano *et al.*, 1998). In luminous vibriosis, hepatopancreas was the target organ where severe inflammatory responses were seen in the intertubular sinuses (Lavilla-Pitogo *et al.*, 1998). Diseased postlarvae displayed cloudiness of the hepatopancreas or midgut gland (Takahashi *et al.*, 1984). Virulence of the strains and susceptibility of hosts were important factors leading to larval mortalities by luminous bacteria in hatcheries. Epibiontic infestation of luminous bacteria, *V. harveyi* was also observed in hatchery reared larvae of *P. indicus* (Abraham *et al.*, 1997). Bacterial flora associated with larvae was not very stable and is influenced by the bacterial flora of the administered food and by the environment (Vandenberghe *et al.*,1998).

Vibriosis of Juvenile and adult shrimp

In juveniles the infection displayed cloudiness of muscle in the sixth abdominal segment and brown spots in the gills and lymphoid organ (Takahashi *et al.*, 1985). Often affected shrimps were small and stunted, swimming lethargically at the water surface or lying motionless at the pond edges. Such severely affected shrimps lost their escape reflex, had a darkened cuticular colour and heavy fouling by epibionts. Early signs included body reddening, extended gill covers and slight melanised erosions of the uropods, pleopods, periopods (Anderson *et al.*, 1988). In localised cuticular vibriosis, the infections were typically well circumscribed by haemocytes forming nodules which later became melanised. Systemic vibriosis showed extensive necrosis and bacterial invasion of the lymphoid organ, with multiple melanised or nonmelanised haemocytic nodules. These nodules were most commonly composed of a bacterial colony in the center surrounded by a melanised zone, and multiple layers of haemocytes attempting to encapsulate the bacterial colony. While such nodules were found in other tissues such as the heart, gills, hepatopancreas, gonads, and muscle, necrosis equivalent to that observed in the lymphoid organ were not observed (Egusa *et al.*, 1988). Enteric vibriosis or septic

hepatopancreatitis included the characteristic features like haemocytic infiltration, melanisation and basophilic masses of bacterial colony and tissue debris in the hepatopancreatic tubule lumen (Lightner et al.,1992).

2.2.2 Filamentous bacterial disease

Filamentous bacterial disease, caused by *Leucothrix* infestation was often associated with poor water quality (Lightner, 1977). Filamentous growth were observed on setae of uropods and pleopods on gill filaments and on tip of epipodites (Lightner, 1977). The bacteria may cause mortality due to hypoxia and impairment of molting process (Karunasagar and Karunasagar, 1996).

2.2.3 Necrotising hepatopancreatitis (NHP)

First described in penaeid shrimps cultured in Texas (Krol *et al.*, 1991), later from Peru in 1993 (Lightner and Redman, 1994). It was caused by a small, highly pleomorphic, intracellular, Gram-negative bacterium. Gross signs included reduced feed intake, elevated food conversion ratios, reduced growth, poor length-weight ratios, soft shells, black or darkened gills, expansion of chromatophores of the pleopods and uropods, empty guts, fouling and lethargy. Moderately affected individuals showed a moderate atrophy of the hepatopancreas and a white colouration when dissected. Severely affected individuals showed a markedly atrophied hepatopancreas and contained more fluid than tissue (Jimenez et al., 1997).

2.3.0 Rickettsial Diseases

Rickettsia or rickettsial like organisms (RLO) had been reported to cause diseases in cultured shrimp (Brock *et al.*, 1986b; Anderson *et al.*, 1987). But these agents were not isolated, cultured or characterised from shrimps. Shrimps lightly infected were assymptomatic carriers while heavily infected ones became lethargic, went off feed and show pale colouration and atrophy of hepatopancreas.

Rickettsial infections were diagnosed by the demonstration of RLO in the cytoplasm of infected cells (Anderson *et al.*, 1987). Rickettsial microcolonies were intracytoplasmic, membrane bound and basophilic. Infected shrimps displayed gross signs of disease that included lethargy, inappetence, poor escape responses and white coloured hepatopancreas. In *P.marginatus, P.merguiensis*, and *P.stylirostris*, the infection occurred in the hepatopancreas epithelium. In *P. monodon*, the infection was wide spread in mesodermally and ectodermally derived tissues. It formed large membrane bound basophilic cytoplasmic masses in hypertrophied hepatopancreatocytes. Systemic Rickettsia like organisams localised within the fixed phagocytes, connective tissue cells and lymphoid organ. Rickettsial infection evoked marked haemocytic response.

2.4.0 Fungal diseases

Fungal pathogens invaded shrimps that have been injured or exposed to stressful conditions(Lightner and Fontaine, 1973). It affected the larval stages, juveniles and adults. Primary mycosis also was reported from shrimps. *Laginidium* and *Sirolpidium* were reported to be responsible for major epizootics of the larvae of most species of penaeid shrimps through out the world (Lightner 1977; Chin and Ching, 1985). Diagnosis was by wet mount or histological demonstration of hyphae with in the body and appendages and sporangia with discharge tubes and motile zoospores. Diagnosis can also be done by isolation and culture of the fungus from infected shrimp. The *Fusarium* caused black gill disease was a serious problem in adult penaeid shrimps (Ishikawa, 1968; Egusa and Ueda, 1972). The causative agent was identified as *Fusarium solani* (Hatai *et al.*, 1978). The inflammatory responses of *Penaeus japonicus* against *Fusarium solani* were haemocytic infiltration and collagen like fibre deposition. These lesions were more pronounced in the exoskeleton than in the gill lamellae (Bian and Egusa, 1981). The hyphae and tissue destruction due to black gill disease were observed in the gills and also in the maxillipeds, periopods, thoracic body wall, thoracic central nerve and occasionally in the ventral thoracic artery (Momoyama, 1987). *Fusarium moniliformae* was another species, isolated from the gills of *Penaeus japonicus*, that showed similar symptoms to that of *Fusarium solani* (Rhoobunjonde *et al.*, 1991).

Laginidium callinects infections in larval tiger shrimp was the first described larval mycosis in shrimps in India(Ramasamy *et al.*, 1996). Mycelium invaded and embeded itself in tissues or alternatively replaced the muscle tissues.

2.5.0 Microsporidiasis

In shrimp, microsporidiasis causes a condition known as milk or cotton shrimp. Common microsporidians which cause this disease condition were *Agmasoma spp., Amesoma spp.* and *Pleistophora spp.*. On the infected shrimps the developing trophozoites replaced the striated muscle causing it to become opaque and white. The gonands enlarged and became opaque and white. Microsporidians while replacing the host tissue did not invoke any host inflammatory response. Depending on the type of microsporidian, the site of infection was through out the musculature or in particular organs and tissues. Microsporidians were present in the affected shrimp in the form of spores (Overstreet, 1973; Johnson, 1978). It was found to affect a number of species like *Penaeus duorarum, Penaeus setiferus, Penaeus aztecus, Penaeus breziliansis, Pandalas jordani, Pandalus borealis, Crangon alaskensis* and *Penaeus monodon* (Lightner, 1977; Olson and Lannan., 1984; Parsons and Khan, 1986; Anderson *et al.*, 1989).

2.6.0 Epicommensals

Surface and/or gill fouling was very common in shrimp reared in high density culture systems or in systems with poor water quality (Lightner, 1988). These fouling organisms were called epicommensals because they use shrimp as a substrate for attachment. These organisms caused problems indirectly by attaching to gills or cuticular surfaces. They kill the shrimps by interfering with water flow over gill surface, moulting, feeding and locomotion. Diagnosis of epicommensals were usually done by examining whole animal wet mounts or wet mounts prepared from biopsied gill or appendages under microscope. Serious losses in cultured penaeids occured when the gills of the host became fouled by heavy infestations of epicommensal ciliates such as *Zoothamnium sp., Epistylis sp., Vorticella sp.* An increase in the intensity of

infestation with *Zoothamnium sp.* was found with an increase in stocking density (Overstreet, 1973). Different species of epicommensals prefered particular body parts of shrimp as sites of attachment (Johnson, 1978).

2.7.0 Non-infectious Disease

The rapid worldwide development of the shrimp culture industry has been accompanied by the occurrence of numerous significant diseases of non-infectious aetiologies. Some non-infectious diseases had simple aetiology while others had complex aetiologies in which the affected animals became susceptible to infection by opportunistic pathogens after initial insult by other predisposing factors that may be environmental or intrinsic. Several nutritional disease syndromes had also been reported such as ascorbic acid deficiency syndrome called black death (Lightner, 1993); cramped muscle syndrome (CMS) or cramped tail presumed to be due to mineral imbalance / physiological or nutritional factors that were enhanced by physical or environmental stressors (Lightner, 1988).

2.7.1 Chronic soft shell syndrome

Frequently observed in cultured penaeid shrimps (Baticados *et al.*, 1986; Baticados and Tendencia, 1991). Affected shrimps displayed thin and persistently soft, rough and wrinkled shells. Soft shelled shrimps were lethargic, weak, susceptible to wounding and cannibalism, showed poor growth rate and eventually died (Baticados *et al.*, 1990). It was suggested that soft shell syndrome is a metabolic disease involving calcium and phosphorous metabolism, but with multiple etiologies (Baticados and Tendencia, 1991). Inadequate feed and feeding practices such as improper storage of feeds, rancid or low quality feeds and exposure to certain pesticides had been linked to soft shell syndrome in penaeids (Baticados *et al.*, 1986; Baticados and Tendencia, 1991). Studies had indicated that occurrence of soft shelling was more under conditions of high p^H, low phosphate in the water and low organic matter in the soil (Baticados *et al.*, 1990).

2.7.2 Spontaneous necrosis (Idiopathic muscle necrosis)

Muscle necrosis was the name given to a condition in all species of penaeid and caridean shrimp that was characterised by whitish opaque areas in the striated musculature, especially of the distal abdominal segments (Lightner, 1993). The condition typically followed periods of severe stress from over-crowding, low dissolved oxygen levels, sudden temperature or salinity changes and rough handling (Lakshmi et al., 1978). It was reversible in its initial stages, but lethal if large areas were affected (Lightner, 1993).

2.8.0 Disease in Indian shrimp farming

Information regarding diseases affecting commercially important penaeid shrimp of India is limited. Rao and Soni (1988) had reviewed the diseases and parasites of penaeid prawns of India. There were also some published reports on incidence of disease problems (Soni, 1986; Felix and Devaraj, 1993; Abraham and Shanmugam, 1994; Karunasagar *et al.*, 1994; Sahul hameed and Rao, 1994; Shankar *et al.*, 1994; CIBA, 1995; Ramasamy *et al.*,1995; Ruby *et al.*, 1998; Shankar and Mohan, 1998; Sudha *et al.*, 1998). Mortalities due to luminous vibriosis and filamentous bacterial infections and larval mycosis were reported in hatcheries (Karunasagar *et al.*, 1994; Abraham *et al.*, 1997 and Felix, 2000).

Panchayuthapani (1997) had published a report on survey of shrimp diseases in India. It covered both *P. monodon* and *P. indicus* and was based on observations of a large number of shrimp farms practising both extensive and semi-intensive culture along both coasts of India, for a period of around two months. He had recorded mass mortalities due to viral diseases like MBV, IHHNV, YHV and WSV. Bacterial diseases due to pathogenic vibrios and filamentous bacteria, fungal infection (*Lagenidium*) and epicommensal infestation, abnormal conditions such as black gill and tail rot had also been reported.

Jasmin and Manissery (2000) had reported prevalence of white spot virus disease in shrimp farms around Cochin area. Deepa (1997) and Nisha (1997) had studied the occurrence of epicommensal ciliates in relation to water quality parameters in penaeid shrimps, *Metapenaeus dobsoni* and *P. indicus* in culture ponds. The ciliate protozoans such as *Zoothamnium, Epistylis, Vorticella*

and the suctorian, *Acineta* were recorded in both the species. *Zoothamnium* was recorded as the most dominant species. They could observe correlation between the incidence of epicommensal ciliates and the water quality parameters such as total suspended solids and dissolved oxygen. Ciliate infestations were more at higher levels of total suspended solids and low levels of dissolved oxygen.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Shrimp sample collection

The survey was conducted over a period of four months from March to June, 2001. Samples were collected from 26 farms mainly comprising of the *modified extensive systems in and around Cochin*. The period was coinciding with the peak culture season of the prawn filtration fields when the biomass of the culture system is believed to be the highest. The samples collected comprised of *Penaeus indicus*, *Penaeus monodon* and *Metapenaeus dobsoni*.

Shrimp samples were collected randomly by operating cast net. Both healthy looking shrimps and those appeared to be suffering from disease (those observed to be weak or with abnormal gill or shell coloration) were collected. A minimum of five shrimps was collected from each farm. Following gross examination, the samples were fixed using neutral buffered formalin or Davidsons fixative (Bell and Lightner, 1988), either at the farm site or carried live and fixed in the laboratory. The specimens were fixed as whole and larger specimens were injected with the fixative in the cephalothorax and abdomen.

At the time of sample collection, information regarding water source, pond preparation details, seed source, stocking rate, feed composition, *feeding schedule*, *management* practices followed, history of disease occurrence and current state of the culture system were gathered either from the farmers or by direct observation.

3.2. Water quality analysis

Hydrological parameters like salinity and temperature were measured at the farm site using a portable refractometer and an ordinary mercury thermometer respectively. Water samples were collected from each farm to analyse the water quality parameters like pH, dissolved oxygen, total alkalinity, ammonia, nitrite, nitrate, chemical oxygen demand and particulate *organic matter*.

pH was measured using a digital pH meter after standardising the instrument with a buffer solution of known pH. Other water quality parameters were determined following standard procedures (Strickland and Parsons, 1972).

3.3. Histological techniques

The shrimps fixed in Davidson's fixative were stored in 50% ethanol after 48hrs of fixation. The tissues fixed in neutral buffered formalin were given overnight washing in tap water.

Shrimps were cut in to four sections for further processing. The routine method of tissue cutting was followed, which ensured representation of all major tissues and organ systems in the sections. Longitudinal sections of cephalothorax, cross section of abdomen and slant section of the last two abdominal segments were used. The tissues were then properly labelled and processed following standard procedures (Bell and Lightner, 1988). Tissues were passed through ascending grades of alcohol for dehydration and then cleared in two changes of xylene. Tissues were then transferred to molten paraffin wax for infiltration. Three changes were given in molten wax. The processed tissue samples were embedded in molten paraffin wax and blocks were prepared. Sections were cut at 5 – 6 μm thickness in a rotary microtome. The deparaffinised sections were stained with Haematoxylin and Eosin (Bell & Lightener, 1988).

Photomicrographs were taken using a Carl Zeiss Axiostar 1061 – 130 trinocular research microscope with digital camera attachment.

3.4. Bacteriology

Shrimp samples showing clinical signs of disease were subjected to bacteriological examination of the haemolymph and hepatopancreas (Elston, 1989). The body surface was swabbed with alcohol. Haemolymph was then withdrawn using a sterile syringe and streaked on to thiosulphate citrate bile salt sucrose agar (TCBS). The carapace was then opened asceptically and a loopful of inoculum from the hepatopancreas was streaked on to TCBS agar and incubated, for presumptive identification of vibrios.

RESULTS

4. RESULTS

4.1. Sample details

Samples were collected from 26 farms having populations reported to be suffering from disease, with a previous history of disease outbreak or having reportedly healthy populations. *P. indicus* accounted for 70% of the samples, while *P. monodon* formed 24% and *M. dobsoni* 6% of the total. The samples varied in size from 8 -10 cm, 12 - 16 cm and 3 - 5 cm for *P.indicus*, *P. monodon* and *M. dobsoni* respectively.

4.2. Water quality

The data pertaining to the water quality parameters recorded from the farms surveyed are presented in Table1. Air and water temperature fluctuated between 30 – 34°C and 31 – 36°C respectively. Salinity values ranged between 15 – 27 ‰. The values of pH recorded were mostly in the range 7.0 - 8.5. However, pH values below 7.0 were also recorded in two farms. Dissolved oxygen varied between 1.5 -7.5 ml/l, but majority were in the range of 2 - 5 ml/l. Alkalinity was in the range of 20 - 30 ppm except for the farms having pH below 7.0, where the alkainity values fell below 12 ppm. Ammonia values showed wide variations and ranged from 0 - 4.6 µg atom N as ammonia/l. However, in three farms ammonia values reached 13.37, 15.93 and 26.25 µg atom N as ammonia/l. Nitrate levels ranged between 0.13 -1.66 µg atom N as nitrate/l. Nitrite was undetectable in most of the farms except for three farms where levels of 0.02, 0.06 and 0.08 µg atom N as nitrite/l were recorded. Particulate organic matter and the chemical oxygen demand values ranged from 12 - 52 mg/l and 40 -162 mg/l respectively.

4.3. Management practices

Most of the farms surveyed are following crop rotation with paddy and only seven farms *viz.* farm no.5, 6, 9, 10,11, 12 and 16 are perennial fields. Modified extensive system of farming practices is followed in all the farms. All farms are either connected directly or through tidal creeks to the Vembanad

backwaters. The farms surveyed are mostly tide fed and in ten farms pumps are also used when tidal amplitude is not sufficient. Pond preparation measures followed in the farms are almost similar. The drainable ponds are dried for one week before application of lime. In farms, which cannot be drained completely, mahuva oil cake or *Croton tiglium* is applied to eradicate predatory and weed fishes. Pond fertilization is mostly done with cow dung and three farms *viz.* farm no. 1, 13 and 16 also use chemical fertilizers like super phosphate and urea. On development of sufficient plankton bloom, stocking is done with post-larvae (PL 18 - 25). Hatchery raised seed is used in all the farms except in farm no. 17 where only seed collected from wild is used. Stocking density used in the farms ranged from 2000 -10,000/acre. The larvae are initially fed with Higashi starter feed (Higashimaru Feeds Pvt. Ltd., Cochin) and replaced in the later stages, either with farm made feeds or Higashi grower along with or without clam meat. In farms no.1 and 13 antibiotics is given in feed as a prophylactic measure. Feeding was either by dispensing in feeding trays (only in farm no.1 and 15.) or by casting by hand. Water is exchanged once daily in all the farms except in farm no. 1 and 16 where it is done twice a day. The timing of water exchange varied depending on the location of the farms and tidal amplitude in the area.

4.4. Case history and clinical signs

Most of the samples collected were appearing normal on gross examination (94%) and only 6% were showing clinical signs of disease or abnormal conditions. Nineteen percent of the farms were reported to have history of disease outbreak during the previous crop. Samples collected from farms no. 4, 8 and 15 were from the stock surviving disease outbreak. Shrimp mortality during the period of study was reported from farms no. 16 and 26. In farm no. 16, mortality was reported in *P. monodon* juveniles ranging in size from 3 - 5 cm, after one month of stocking. Affected shrimps were surfacing at the pond edges during daytime. No other external clinical signs were observed except for the breaking of the tip of right antenna. In farm no. 26 *P. indicus* ranging in size from 4 - 6 cm were affected. Affected shrimps were coming to pond margins and had reddish colouration of cephalothoraxes. Another case of stunted growth was observed in farm no. 14. Brown discolouration of gills was

noticed in the samples from farm no. 3 and darkened gills in samples from farms no. 9, 10,12 and 13. Samples from about 36% farms had several whitish, opaque areas in the muscle resembling instantaneous muscle necrosis (Plate1). There were no apparent histopathological features in samples examined from 27% of the farms surveyed.

4.5. Histopathology

The details of observations from the histopathological studies are summarised in Table 2.

Chronic inflammatory lesions in the form of melanised or non-melanised haemocytic nodules were identified in the gills, connective tissue, muscle and haemocoel spaces in samples from 42% of the farms surveyed (Plates 2 & 3). Pathology of hepatopancreas showing severe necrosis, loss of structure, atrophy of tubule epithelial cells, vacuolation and rounding and sloughing of cells into the lumen were recorded in 19% cases (Plates 4 to 7). No marked haemocytic infiltration was noticed in any of these cases. Normal hepatopancreas (midgut gland) in shrimps contain numerous tubules with star shaped lumen lined with various types of epithelial cells (Plates 8 and 9).

Presence of eosinophilc, spherical, intranuclear occlusion bodies characteristic of MBV was recorded in the hepatopancreatic tubule epithelial cells (Plate 10) of samples from farm no.16, where mortality was recorded during the period of study. The samples also showed hepatopancreatic pathology of severe necrosis, rounding and sloughing of cells into the lumen, vacuolation and loss of structure (Plate 11). Midgut epithelium of samples from all other cases appeared normal (Plate 12). Other than this, there was no histopathological evidence of viral inclusions in any of the samples examined. The target tissues of WSV, such as cuticular epidermis, connective tissue, gills, lymphoid organ, haematopoietic tissue, ventral nerve cord *etc.* were free from inclusion bodies characteristic for WSV (Plates 13 to 18).

Infestation by the epicommensal ciliate protozoan, *Zoothamnium* in the gills and cuticular surface was observed in histological sections of samples collected from 8% of the farms surveyed (Plates 19 and 20).

4.6. Bacteriology

Bacteriological investigations were carried out only for samples from farms facing mortality during the survey period, i.e., in farm no. 16 and 26. Haemolymph and hepatopancreas from both cases were positive for pathogenic vibrios.

Table: 1. Summary of water quality parameters recorded in the farms surveyed during March-June 2001

Farm No.	Salinity(ppt)	Temperature (°C)		PH	Do (ml\l)	Alkalinity(ppm)	NH3 (µg at/l)	Nitrate (µg at/l)	Nitrite (µg at/l)	POM (mg/l)	COD(mg/l)
		Air	Water								
1	26	33	35	7.5	3.015	22	4.06	1.36	ND	40.0	40.8
2	25	33	35	7.6	4.38	20	2.69	1.66	ND	44.0	122.4
3	25	33	35	7.7	3.70	20	3.69	0.20	0.057	42.0	81.6
4	24	33	35	7.4	5.84	20	1.32	1.49	ND	52.0	102.0
5	27	34	36	8.2	4.09	22	3.12	1.46	ND	25.2	90.0
6	26	34	36	7.9	2.14	22	3.36	1.40	ND	21.9	180.0
7	17	32	35	8.0	5.16	24	1.09	1.01	ND	26.4	108.0
8	17	32	35	7.5	3.70	20	0.33	0.42	ND	28.3	126.0
9	26	32	31	7.6	5.54	26	15.93	1.07	ND	19.2	90.0
10	25	32	31	7.8	7.59	32	26.25	2.24	ND	20.4	126.0
11	21	33	35	7.7	4.18	28	2.46	0.65	ND	21.6	72.0
12	21	33	35	8.3	6.52	32	1.99	0.88	ND	56.0	90.0
13	15	30	36	7.0	4.18	12	0.76	0.72	ND	15.2	54.0
14	16	30	36	6.3	3.11	8	5.34	1.04	ND	12.0	36.0
15	16	30	36	6.6	2.24	10	13.37	1.20	0.02	12.8	90.0
16	18	30	33	7.6	2.92	30	0.43	1.27	ND	30.0	105.0
17	15	30	33	7.0	1.56	30	0.95	0.23	ND	23.2	86.0
18	15	30	33	7.0	6.71	30	0.9	0.2	ND	33.2	98.0
19	15	30	33	7.0	3.21	28	0.0	0.23	ND	23.2	102.0
20	17	33	36	7.9	4.09	24	0.14	0.23	ND	20.4	93.0
21	17	33	36	7.8	3.11	24	0.28	0.26	ND	20.8	63.0
22	16	33	36	7.8	3.40	24	0.38	0.29	ND	38.4	74.0
23	18	33	36	7.8	3.11	22	0.52	0.26	ND	26.0	59.0
24	18	33	36	7.9	3.70	24	0.24	0.13	ND	16.8	56.0
25	17	33	36	7.6	3.99	26	0.43	0.16	ND	44.4	61.0
26	15	33	34	7.7	2.04	15	0.14	0.13	0.08	98.0	52.0

ND-Not detected

TABLE: 2. SUMMARY OF THE MAJOR DISEASE / HISTOPATHOLOGICAL FEATURES IDENTIFIED IN THE SHRIMP SAMPLES EXAMINED DURING THE SURVEY.

Farm No.	SPECIES	DISEASE HISTORY	HISTOPATHOLOGICAL OBSERVATIONS	SUSPECTED AETIOLOGY
1.	Pm, Pi	Not reported.	Haemocytic infiltration in connective tissue, muscle necrosis.	Systemic bacterial infection.
2.	Pi	Reported in the previous crop.	No apparent pathological changes.	—
3.	Pm	"	"	—
4.	Pm	Sample from disease survived stock	"	—
5.	Pm, Pi	Not reported.	"	—
6.	Pm	"	Melanised haemocytic nodule formation in the gills and connective tissue.	Systemic bacterial infection.
7.	Pi	"	Infestation by *Zoothamnium* on cuticular surface, with no apparent pathological changes.	—
8.	Pm	Sample from disease survived stock.	No apparent pathological changes.	—
9.	Pm, Pi	Not reported.	Severe pathology of hepatopancreas with necrosis, atrophy of epithelial cells and no inflammatory response.	Oral bacterial infection.
10.	Pm, Pi	"	Haemocytic nodules in gills, connective tissue. *Zoothamnium* infestation on gills.	Mixed infection: systemic bacteria and *Zoothamnium*.

Farm No.	SPECIES	DISEASE HISTORY	HISTOPATHOLOGICAL OBSERVATIONS	SUSPECTED AETIOLOGY
11.	Pm, Pi	Not reported.	Melanised haemocytic nodules in connective tissue and haemocoel spaces in the cephalothorax and abdomen.	Systemic bacterial infection.
12.	Pm, Pi	"	Haemocytic nodules in gills and connective tissue, gill pathology.	"
13.	Pm	"	No apparent histopathological changes.	—
14.	Pm, M	Reported in the previous crop.	Melanised haemocytic nodules in muscle and connective tissue.	Systemic bacterial infection.
15.	Pm	Samples from disease survived stock.	Melanised nodules in connective tissue and muscle.	"
16.	Pm, Pi	Diseased stock.	Eosinophilic, intranuclear, spherical occlusion bodies characteristic of MBV in the tubular epithelial cells of hepatopancreas, severe hepatopancreatic pathology, necrosis, rounding and sloughing of cells; loss of structure.	Mixed infection: MBV and Oral / Enteric bacteria.
17.	Pi	Not reported.	No apparent pathological changes.	—
18.	Pi	"	"	—
19.	Pi	"	Melanised haemocytic nodule formation in muscle and connective tissue.	Systemic bacterial infection.

Farm No.	SPECIES	DISEASE HISTORY	HISTOPATHOLOGICAL OBSERVATIONS	SUSPECTED AETIOLOGY
20.	Pi	Not reported.	No apparent pathological change.	—
21.	Pi	"	Melanised haemocytic nodules in gills, connective tissue and haemocoel spaces in cephalothorax.	Systemic bacterial infection.
22.	Pi	Reported in the previous crop.	Hepatopancreatic pathology with necrosis and loss of structure, melanised nodule formation in connective tissue.	Enteric and systemic bacterial infection.
23.	Pi	"	Hepatopancreatic pathology, melanised haemocytic nodule in gills, connective tissue and muscle.	Enteric and systemic bacterial infection.
24.	Pi	Not reported.	No apparent pathologicalchange.	—
25.	Pi	"	"	—
26.	Pi	Diseased stock.	Pathology in hepatopancreas, severe necrosis, loss of structure, atrophy of epithelial cells, tubules with basophilic inner margins, indicating presence of bacterial plaques.	Oral / Enteric bacterial infection.

Pi – *Penaeus indicus*
Pm – *Penaeus monodon*
M – *Metapenaeus dobsoni.*

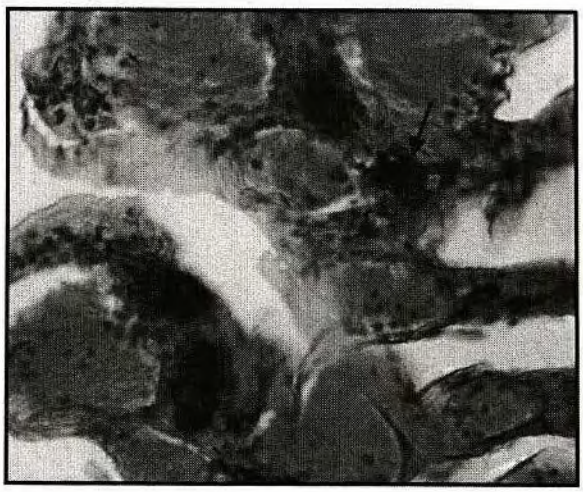
Plate 1 : Spontaneous muscle necrosis (arrow) in *P.indicus*.

Plate 2 : Chronic inflammatory lesions in the form of melanised/non-melanised haemocytic nodules (arrow) in the gills. H&E: X 400.

Plate 3 :Haemocytic nodules in the connective tissue. H&E:X 1000.

Plate 4 : Pathology of hepatopancreas in *P.monodon* showing severe necrosis, rounding and sloughing of cells, indicating bacterial infection. H&E:X 1000.

PLATE 1



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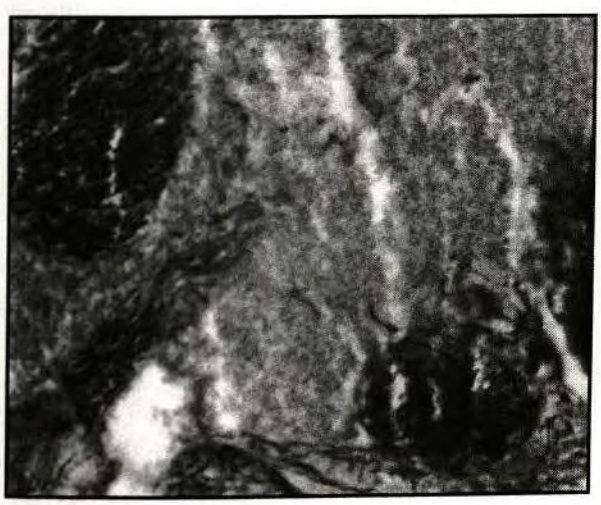

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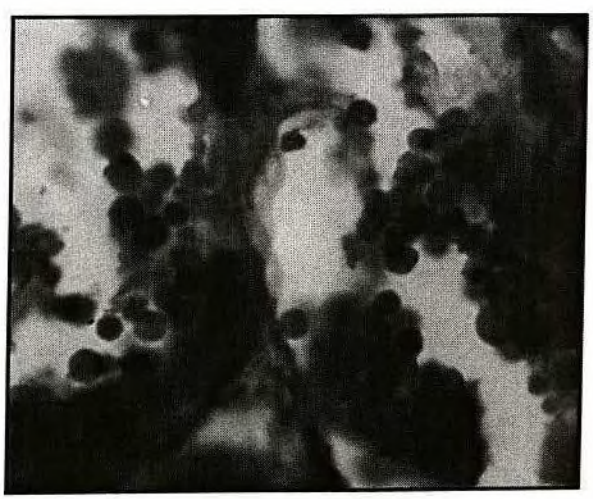


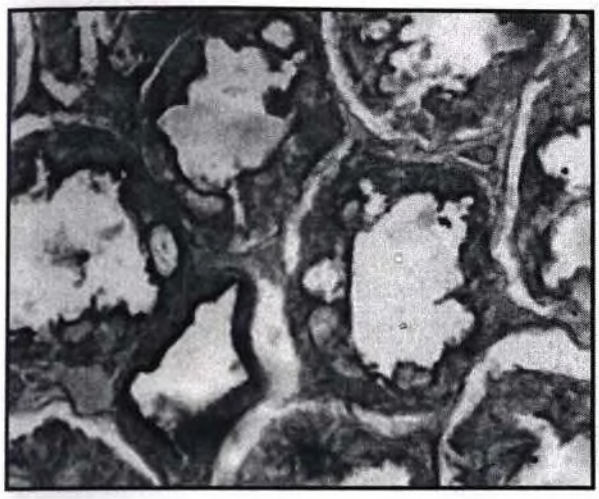
PLATE 4

Plate 5 :Hepatopancreatic tubules showing severe necrosis and atrophy of epithelial cells. Basophilic inner margins indicate presence of bacterial plaques suggesting extension of oral bacterial infection to enteric region. H&E: X 400.

Plate 6 :Section of hepatopancreas showing pathology, severe necrosis, atrophy of cells and sloughing into the lumen (arrow), with no inflammatory cells indicating oral bacterial infection. H&E: X 400.

Plate 7: Hepatopancreas pathology, with extensive necrosis and loss of structure. H&E: X 400.

Plate 8: Hepatopancreas section of normal shrimp showing tubules with starshaped lumen. H&E: X 100.



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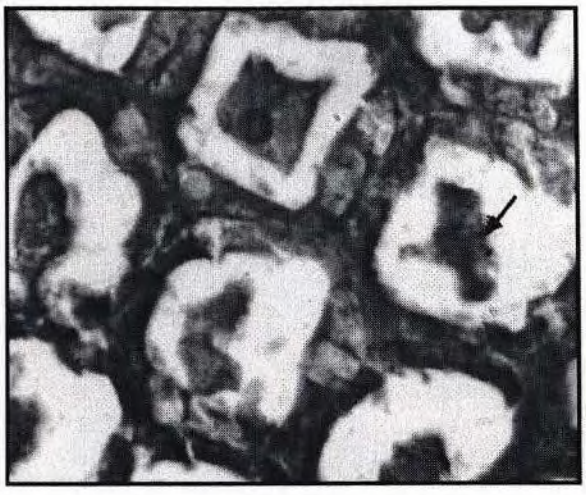

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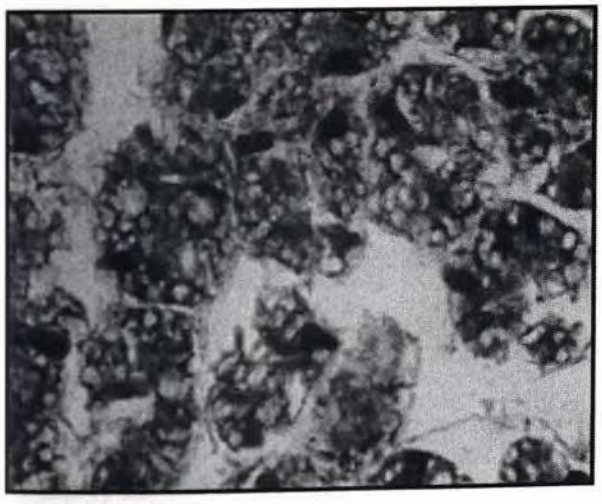

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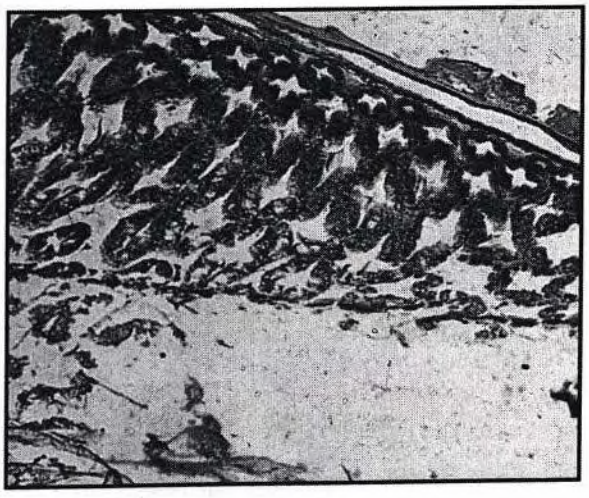

PLATE 8

Plate 9 : Tubular epithelial cells of the hepatopancreas from normal shrimp H&E: X 1000.

Plate 10 : Eosinophilic, intranuclear occlusion bodies (arrow), characteristic of MBV in the tubular epithelial cells of hepatopancreas from diseased *P.monodon*. H&E: X 1000.

Plate 11: Pathology of hepatopancreatic tubules showing necrosis, and vacoulation. H&E: X 400.

Plate 12: Section of midgut of normal shrimp showing the epithelial cells. H&E: X 1000.

PLATE 9

PLATE 10

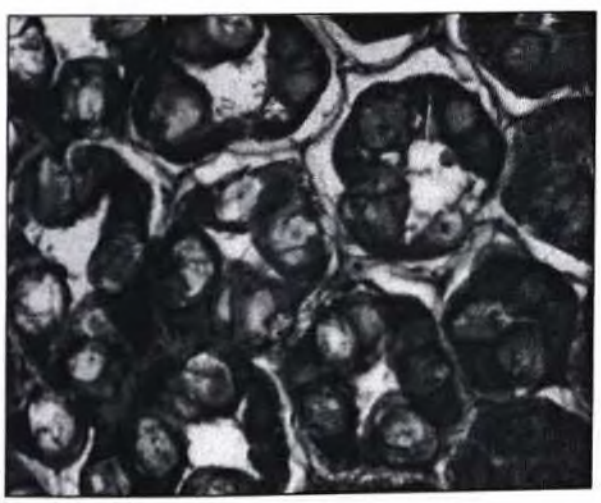

PLATE 11

PLATE 12

Plate 13: Cuticular epidermis (arrow) of normal shrimp with underlying connective tissue(arrowhead): H&E. X 1000.

Plate14: Section of gill from normal shrimp showing secondary gill Filaments. H&E: X 1000.

Plate 15: Lymphoid organ from normal shrimp showing tubules with simple lumen and surrounding stromal matrix of cells. H&E: X 1000.

Plate 16: Haematopoeitic tissue from normal shrimp showing cells at different stages of development. H&E: X1000.

PLATE 13

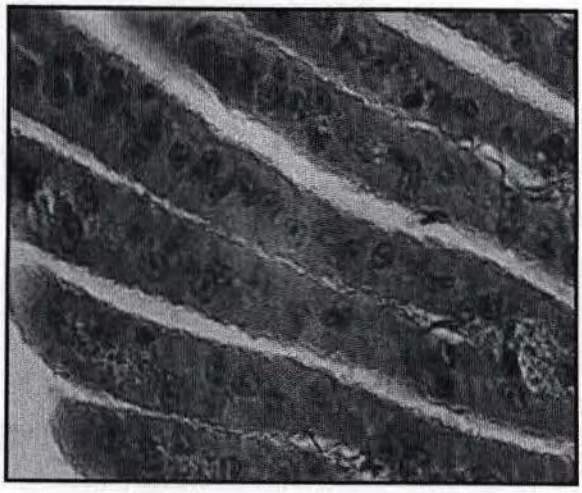

PLATE 14

PLATE 15

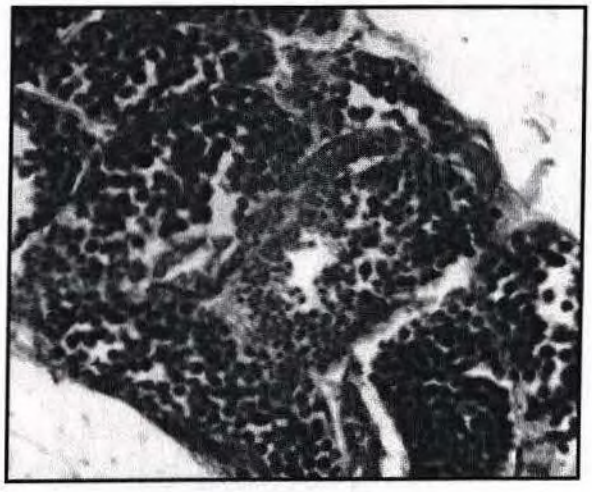

PLATE 16

Plate 17: Haematopoeitic tissue around the lateral arterial vessel. H&E: X 400.

Plate 18: Histological section showing ventral nerve cord in the abdominal region of normal shrimp. H&E: X1000.

Plate 19: Infestation by *Zoothamnium* in the gills of *P.indicus*. H&E: X 400.

Plate 20: *Zoothamnium* infestation on the cuticular surface of *P.indicus*. H&E: X 400.

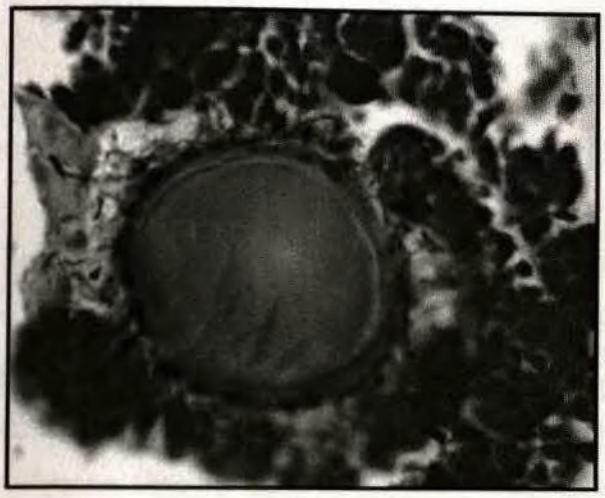

PLATE 17

PLATE 18

PLATE 19

PLATE 20

DISCUSSION

5. DISCUSSION

Epizootics of both infectious and non-infectious aetiology have continuously plagued the various sectors of shrimp farming industry. For efficient monitoring and control of diseases, information on various pathogens prevalent and their changing pattern resulting from change in culture practices is required. Epidemiological surveys, which are essential for managing the spread of shrimp disease agents, need urgent attention. The availability of standardised diagnostic methods with predictable results will make surveillance and diagnostic work easier. The epidemiological work calls for co-operation between farmers and pathologists in data gathering to assess situations pre-empting a disease outbreak. These data will be the basis for rational decisions for the prevention and/or control of disease.

Although it is generally recognised that intensive culture systems often encounter serious disease problems, more recent experiences have shown that low-density culture systems can also be severely affected. The present investigation was carried out to understand the range of disease conditions commonly encountered in the shrimp culture systems of Cochin area. Though industrial scale shrimp farming as practised along the East Coast of India is not common in the study area, disease outbreaks leading to mortality and crop loss have become a regular phenomenon here.

The various types of disease/pathological conditions of infectious and non-infectious aetiology, encountered during the study are discussed below in relation to water quality parameters recorded in the farms.

5.1. Water quality parameters

The environment in which the animal is cultured plays a critical role in the degree to which the animal is susceptible to pathogens and the occurrence of clinical disease (Flegel and Sriurairatana, 1993). An understanding of the relationship between the host, pathogen and environment

is important for understanding the cause, prevention and treatment of most aquatic animal diseases. In general, diseases affecting aquatic animals may be grouped in to:

- disease, resulting from poor environmental conditions leading to direct effects (*e.g.* low dissolved oxygen and toxins).
- disease, resulting from stress leading to infection by opportunistic pathogens (*e.g.* vibrios).
- pathogens causing disease only when the animals are stressed (*e.g.* MBV)
- primary pathogens causing disease without environmental stress. (*e.g.* some recently reported viral infections in shrimps such as YHV). However, environment management may be required to control entry of such pathogens to the culture systems.

The relationship between the environment and the occurrence of aquatic animal diseases are poorly understood, particularly the relations between stress and disease occurrence. For example, it is recognised that shrimps are 'stressed' by poor environmental conditions, but the interactions between disease occurrence and stress in shrimp is unclear (AAHRI, 1995). However enough is known of the general conditions under which aquatic animals remain healthy. Water quality, pond bottom conditions, feed and plankton bloom management are widely recognised as critical for disease prevention in shrimp culture, particularly with the case of commonly occurring opportunistic pathogens (Flegel and Sriurairatana, 1993). Unconsumed feed and metabolic wastes contribute significantly to the deterioration of water quality. In shrimp farming, any characteristic of water that affects the survival, growth, production or management of shrimps in any way is a water quality variable. It is well established that, successful culture of shrimps depends on maintaining pond salinity, pH and dissolved oxygen in appropriate ranges.

Though there are many water quality variables in pond culture, only a few of these normally play an important role. These are the variables that the culturist should concentrate on and attempt to control to some extent by

management techniques. Keeping this in view, some of the important water quality parameters were monitored in the farms surveyed during the study period. Most of the farms surveyed fall within the category of modified extensive system where stocking densities does not go beyond 10,000 nos. /acre and supplementary feeding level varies between 0.4-2.0 kg/day. The water quality parameters recorded were also within the range conducive to the growth of shrimps, with few exceptions.

5.1.1. Temperature

Temperature has pronounced effect on several physiological and biological processes in the cultured organisms. The aquatic productivity is considerably influenced by temperature. In the tropics, the water temperature shows only a little seasonal variation during the year. Temperature of water generally depends on climate, sunlight and depth of ponds. The air and water temperature recorded during the survey was slightly above the conducive temperature range (28 - 30°C) reported for tropical shrimp by Boyd (1990). However, the effect of such an elevated temperature was not reflected in the clinical signs or pathological conditions observed in the samples.

5.1.2. Salinity

The optimum salinity for aquaculture varies with the osmotic pressure requirements of the species cultured (Boyd, 1990). Shrimps are sensitive to sudden changes in salinity (Boyd and Pillai, 1984). Change in salinity is said to have an effect on spontaneous muscle necrosis (Lakshmi *et al.*, 1978). During the present study spontaneous muscle necrosis was observed in the samples from farms surveyed soon after onset of rains and subsequent decline in salinity. Discolouration of gills was also recorded mostly during the period following onset of rains, which could be attributed to the increased load of suspended matter in water following heavy rains.

5.1.3. pH

Water with pH values above 6.5 and below 9.0 at daybreak is considered best for aquaculture (Zingde, 1997). During the present study, pH recorded from the farms remained neutral or slightly alkaline and was within the best range suggested. Only two farms had pH values below 7.0. Effect of pH on

the condition of cultured shrimps was not that apparent except for the stunted growth in one farm with pH below 6.5.

5.1.4. Dissolved oxygen:

Dissolved oxygen is probably the most critical water quality parameter in shrimp farming. Prolonged exposure to substantially low concentrations of dissolved oxygen is harmful to the cultured organisms (Boyd, 1990). The optimum range suggested for penaeid shrimps is about 3 to 5 mg/l. However they can temporarily tolerate oxygen concentrations less than 3 mg/l. At oxygen levels below 4 mg/l shrimps may live but may not feed or grow (Zingde, 1997). In the present study, 5 farms recorded DO values below 3 ml/l. However, no significant pathological changes could be observed in relation to the low DO values recorded.

5.1.5. Total Alkalinity

Alkalinity is a measure of productivity of water. Waters of lower alkalinity are poorly buffered against fluctuations in pH. Desirable levels of total alkalinity depend on the carbon dioxide level, pH, photosynthesis and pond soil. Waters of total alkalinity value 40 mg/l or above are considered as hard water and are generally more productive (Boyd, 1990). The alkalinity values recorded during the present study, mostly ranged between 20 – 32 mg/l. In three farms the values were below 15mg/l. Stunted growth was observed in the farm that recorded the lowest alkalinity value, i.e., 8 mg/l.

5.1.6. Ammonia

Ammonia reaches pond water as a product of fish metabolism and decomposition of organic matter by bacteria. As ammonia concentrations increase in the water, ammonia excretion by fish diminishes and levels of ammonia in blood and the other tissues increase. This will result in elevation of blood pH leading to adverse effects on enzyme catalysed reactions and membrane stability. 24 hr LC 50 of ammonia to *P. monodon* PL is reported as 5.71 mg/l and 0.13 mg/l of ammonia is considered safe under pond production conditions (Boyd, 1990). Values beyond this range were not observed in any of

the farms during our study. Farm no. 9 and 10 had slightly high ammonia level. In these farms crab was also cultured along with shrimp and the raised ammonia level could be due to the feeding of trash fish to the crab. Hepatopancreatic pathology and histopathological evidence of bacterial infection was recorded in the samples collected from these farms.

5.1.7. Nitrite

The most likely source of nitrite is the reduction of nitrate to nitrite in aerobic ponds. A safe nitrite concentration of 4 to 5 mg/l was suggested for shrimp post larvae (Chen and Chin, 1988). High ammonia concentration was found to increase the toxicity of nitrite to *P. monodon* and sublethal concentration of nitrite was found to increase the susceptibility of fish to bacterial diseases (Hanson and Grizzle, 1985). Nitrite was not detected in most of the farms during the study. In three farms, very low concentration was recorded. Samples from these farms showed chronic inflammatory lesions or pathology typical of systemic/enteric bacterial infection.

5.1.8 Nitrate

Presence of nitrate is an indication of productivity of water. High nitrate concentration was reported in farms following heavy feeding rate (Boyd, 1990). Nitrate values ranging between 0.1 - 4.5 mg/l is considered as the favourable range in culture waters (Chandraprakash, 1997). No apparent relation between nitrate level and the health status of the cultured population could be delineated from the present study.

5.1.9. Organic matter

Chemical oxygen demand (COD) and particulate organic matter (POM) are the indicators of organic load in water. Uneaten feed, dead plankton and shrimp excreta increase the organic load in ponds. Therefore overfeeding can lead to high organic load. High organic load is conducive to parasitic infestation. Heavy ciliate infestation is reported in farms with high organic load (Lightner, 1988; Deepa, 1997; Nisha, 1997). During the present study ciliate protozoan infestation was observed only in 8% of the farms surveyed.

5.2. Disease/pathological conditions

5.2.1. Bacterial diseases

Results of the histopathological examinations showed chronic inflammatory lesions in the form of haemocytic nodules typical of systemic bacterial infections in gills, haemocoel spaces and in the loose connective tissue in majority of the cases. Such multifocal nodules were melanised or non-melanised and in some cases showed presence of fibrous tissue.

Severe hepatopancreatic pathology typical of oral or enteric vibriosis, such as necrosis of tubule epithelium and rounding and sloughing of cells into the lumen was the second major problem encountered. However in most of these cases with hepatopancreatic pathology, no marked haemocytic response was noticed. This indicates bacterial colonisation of the mouth and foregut region, which can lead to lot of pathology in the hepatopancreas probably due to action of bacterial toxins without causing any inflammatory response. Heavy bacterial colonisation of the mouthparts or the cuticular lining of the oesophagus and foregut can lead to rounding up and sloughing of hepatopancreatic tubule and midgut epithelial cells into their lumens leading to typical enteritis. Following cuticular colonisation, bacterial invasion of the midgut and hepatopancreas may occur. At this stage haemocytic inflammation is very commonly seen.

In samples from 8% of the farms, the hepatopancreatic pathology resembled that of necrotising hepatopancreatitis (NHP) described by Jimenez *et al.* (1997) *in cultured penaeid* shrimp in Ecuador, caused by an intracellular bacterium. However, further studies by using special staining techniques and electron microscopy are required for confirming the aetiology.

Due to the economic losses encountered following epizootics, bacteria are considered as one of the most economically significant disease agents of shrimp (Lavilla- pitogo, 1995). Bacterial disease in cultured shrimp

usually occurs in conjunction with other disease processes or reflects an outcome of a breakdown in the ecological balance within the culture system. Many of these bacteria are normal inhabitants of the marine and brackishwater environment. As majority is generally regarded as secondary opportunistic pathogens, problems related to vibriosis can be traced to stress, poor water quality and bad management.

Around 11 species of vibrios have been reported in penaeid shrimps of which most dominant ones are *V.vulnificus, V. parahaemolyticus* and *V.harveyi,* all non-sucrose fermenters (Lavilla-pitogo, 1995). In nursery and growout situations in India, *V.parahaemolyticus, V.alginolyticus, V.harveyi and V.vulnificus* are frequently encountered (Karunasagar *et al.*, 1994). Diagnosis is done mostly through conventional bacteriology and histopathology. During the present study, bacteriological investigations were carried out only for samples showing clinical disease. Twenty seven percent of the cases were positive for pathogenic vibrios (further biochemical characterisation for species level identification was not carried out). However, in several samples with no clinical disease, pathology typical of systemic or enteric vibriosis could be observed on histopathological examination.

Development and use of more rapid detection methods like enzyme immunoassays and fluorescent antibody techniques using monoclonal antibodies will be helpful in monitoring potentially pathogenic *Vibrio* strains before symptoms and mortalities occur in farms.

5.2.2. Viral diseases

Viruses are considered as the most important infectious disease agents of shrimp because of the limited means for control, treatment or even diagnosis.

Disease outbreak due to the dreaded white spot virus still prevails in many regions and poses a major threat to Indian shrimp farming. Mortalities due to this disease have been reported throughout the East and West coasts since October 1994 (Mohan and Shankar, 1995; Krishna *et al.*, 1997). Even now

the problem continues to be very severe in many areas. This disease affects shrimps of all sizes and species, in all types of culture system. The disease is caused by a systemic ectodermal and mesodermal non-occluded baculovirus. The virus replicates in the nucleus of cells of ectodermal and mesodermal origin tissues producing the characteristic basophilic intranuclear inclusion bodies (Wongteerasupaya *et al.*, 1995). Even in samples without clinical white spots, WSV inclusions have been recorded in the hypertrophied nucleus of target tissues (Sudha *et al.*, 1998)

Jasmin and Manissery (2000) have reported prevalence of WSV from the shrimp culture systems of Cochin area. During the present study, WSV inclusions were not observed in any of the shrimp samples examined. Histological sections of the important target tissues showed normal appearing nucleus without any hypertrophy or inclusion bodies. However, due to the limited number of samples taken and examined, it cannot be concluded that the shrimp farms in this area are free of WSV. However, even in samples taken from the stocks surviving disease outbreaks, WSV inclusions were not present. Further investigations following statistical sampling protocol and use of more sensitive diagnostic techniques like PCR, are required for confirmation of the observation.

Eosinophic, intranuclear, spherical occlusion bodies characteristic of MBV were detected in the hepatopancreas of shrimps sampled from one farm, where mortalities were recorded in one month old *P.monodon*. The hepatopancreas was badly affected showing severe pathology. The samples were also positive for pathogenic vibrios. The affected animals showed signs of reduced feeding, reduced growth, coming to pond margins and heavy mortality.

MBV is widely distributed in cultured penaeid shrimp population in India (Panchayuthapani, 1997). There were reports of mass mortality of *P.monodon* larvae in hatcheries (Felix and Devaraj, 1993; Ramasamy *et al.*, 1995). Lightner *et al.* (1983) recorded presence of occlusion bodies typical of MBV infection in apparently healthy post larval samples. The observed mortality may be due to the combined effect of bacterial infection. MBV affected shrimps are always reported to be infected with pathogenic *Vibrio* sp., filamentous

bacteria and protozoans (Felix and Devaraj, 1993; Karunasagar *et al., 1998*). Mass mortality due to MBV and luminescent bacteria was also reported in hatcheries (Felix and Devaraj, 1993).

MBV infections are common in wild brooders and elimination is not practical. However, contamination of PL could be easily overcome by good sanitary practices in hatchery. Control methods include screening of broodstock for presence of MBV occlusion bodies, individual spawning of gravid females, separation of eggs and larvae from brooders followed by rinsing in seawater and chemical disinfectants.

5.2.3. Epicommensal infestation

Surface or gill fouling by protozoan ciliates like *Zoothamnium, Epistylis* and *Vorticella* are very common in shrimp farming (Lightner, 1988; Turnbull *et al.*, 1994). These organisms do not cause any damage to the shrimps directly, but can cause problems indirectly by attaching to the gill or cuticular surfaces. Heavy infestations on gills and oral cavity can lead to accumulation of debris, discolouration of the affected areas and fouling. Such a condition can result in mortality by interfering with water flow over gills, gas exchange over the gill surfaces, moulting, feeding and locomotion, especially when the pond water quality is not good (Lightner, 1988). However no mass mortality due to epicommensal ciliate infestation has been reported in India (Panchayuthapani, 1997). Deepa (1997) and Nisha (1997) have reported the prevalence of ciliates like *Zoothamnium, Epistylis* and *Vorticella* in cultured penaeid shrimps in Cochin area.

In the present study, infestation with *Zoothamnium* was identified in the histopathological sections of samples from only 8% of the farms. Mixed infection of *Zoothamnium* and bacteria (as evidenced by presence of chronic inflammatory lesions) was observed in one case. Infections of mixed aetiology are serious but less conspicuous. It usually involves viruses, bacteria (principally vibrios), ciliates such as *Zoothamnium,* microsporidians *etc.*, coupled with nutritional and environmental stresses. In the case of viral diseases, infectious agents such as Vibrios, *Zoothamnium* and factors leading to stress such as high

stocking density, wide fluctuations in pH, temperature and salinity can become component causes for disease outbreak. Eradication of the virus or the susceptible shrimp species from the culture environment is impractical. Hence the rational approach would be to monitor and control component causes such as epibiont *Zoothamnium* infestation and proper feed and water quality management.

5.2.4. Abnormal conditions

Abnormal conditions such as discolouration of gills and spontaneous muscle necrosis were observed in 35% of farms, without any histopathological evidence of infectious aetiology. Hence it is suggested that these are all non-specific clinical signs due to some unknown factors. The occurrence of spontaneous/idiopathic muscle necrosis, characterised by the presence of whitish opaque areas in the striated musculature, especially in the distal abdominal segments have been reported by several authors (Rigdon and Baxtar, 1970; Lakshmi *et al.*, 1978; Akiyama *et al.*, 1982; Lightner, 1988). It has been reported that the condition typically follows periods of severe stress from overcrowding, low dissolved oxygen levels, sudden temperature or salinity changes and rough handling. It is reversible in its initial stages, if the stress factors are reduced, but it may be lethal if large areas are affected. When irreversible change occurs and opportunistic bacteria invade the necrotic areas of the abdomen, the condition is called tail rot (Lightner, 1993).

In 27% of the farms surveyed, no histopathological evidence for any disease conditions were identified. In the present study, statistical sampling protocol could not be followed and due to the limited number of samples examined from each farm, absence of any disease/pathological condition in the samples need not necessarily mean that it is from a disease free population.

Although shrimp culture has advanced in leaps and bounds, production could become unpredictable because of an array of poorly understood processes in the ponds. It is obvious, however, that the shrimp farmers have become more concerned with the environment in general, and its

effects on the cultured organisms in particular. With this realisation, the farmers began to demand for more effective, accurate, rapid and environment friendly solutions to his production problems. Research has to come up with novel ideas and user-friendly products for disease prevention, surveillance, diagnosis and control.

6. SUMMARY

- A histopathological survey was conducted to study the occurrence of diseases and pathogens, in relation to environmental quality in a cross section of shrimp farming area in Cochin during March to June 2001.
- Samples of *Penaeus indicus, Penaeus monodon* and *Metapenaeus dobsoni* collected from 26 farms following the modified extensive type of farming practices were used for the study.
- Chronic inflammatory lesions characteristic of systemic bacterial infection was the most prevalent pathological condition observed. This was recorded in 42% of the farms surveyed.
- Hepatopancreatic pathology typical of oral/enteric vibriosis was also a major problem and was recorded in 19% of the cases.
- The absence of WSV inclusions in any of the target tissues, in the samples examined was an important observation during the study.
- Mortality of *P.monodon* due to mixed infection of MBV and vibrios was recorded in one farm.
- Infestation by epicommensal ciliate, *Zoothamnium* was detected in 8% of the farms.
- Abnormal conditions such as spontaneous muscle necrosis, dark coloured gills, brown discolouration of the shell *etc.* were recorded in 36% cases.
- In 27% of the farms surveyed, no apparent pathological conditions were recorded.

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